

Effects of Sodium Nitroprusside Applications on Antioxidant Defense System and Oxidative Stress in Leaves and Berries of the 'Horoz Karası' Grapevine (*Vitis vinifera* L.)

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Research Article

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Abstract

Background

Nitric oxide (NO) has been widely recognized as a multifunctional signaling molecule in plants, participating in the regulation of growth, developmental transitions, and stress adaptation processes. Its involvement in modulating cellular redox homeostasis is considered one of the key mechanisms underlying these effects. In experimental studies, sodium nitroprusside (SNP) is frequently employed as an exogenous NO donor, and numerous reports indicate that SNP treatments can activate antioxidant systems in different plant species. However, information regarding the temporal effects of repeated SNP applications under vineyard conditions and their integrated effect on leaf and grape berries antioxidant responses in grapevines remains limited.

Results

Foliar SNP applications significantly modulated antioxidant enzyme activities and oxidative stress indicators in the Horoz Karası grape variety. Among the tested concentrations, 300 ppm SNP consistently resulted in the highest activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and glutathione reductase (GR), while effectively suppressing malondialdehyde (MDA) and hydrogen peroxide (H₂O₂) accumulation in leaf tissues. The effects of SNP were more pronounced during the veraison and harvest periods than in the pre-veraison stage, indicating a stronger regulatory role under elevated metabolic activity. In grape berries tissue, SNP applications, particularly at 300 ppm, reduced MDA levels and promoted higher antioxidant enzyme activities, demonstrating a systemic leaf– grape berries interaction mediated by NO signaling.

Conclusions

Repeated foliar application of SNP effectively enhances antioxidant defense capacity and limits oxidative damage in Horoz Karası grapevines in a dose- and time-dependent manner. The 300 ppm concentration emerged as the optimal dose for maintaining redox homeostasis in both leaf and grape berries tissues. These findings highlight the potential of NO-based applications as an environmentally friendly strategy to improve oxidative stress management and quality preservation in sustainable viticulture practices.

Background

Grapes (*Vitis vinifera* L.), an important member of the Vitaceae family, possess considerable economic and nutritional value worldwide, both as fresh fruit and in processed products. Grape berries are characterized by their attractive aroma and flavor, as well as their rich nutritional composition. Grapes at harvest maturity represent an important source of dietary fiber, organic acids, vitamins, and minerals, and are particularly rich in phenolic compounds and antioxidant capacity. Owing to these bioactive components, grapes provide significant health benefits and are widely accepted as a functional food.

Horoz Karası, one of the important grape varieties endemic to Türkiye, stands out especially for its high adaptability and favorable fruit quality. Due to increasing consumer demand and the need to protect local genetic resources, the cultivation of this variety has become increasingly important. In viticulture, increasing both yield and quality is a fundamental goal for producers, and the development of environmentally friendly and sustainable practices plays a crucial role in achieving this goal. [1–3].

Today, various chemical treatments are used in agricultural production to increase yield and improve quality. However, the intensive use of pesticides and chemical fertilizers poses risks not only to the environment but also to product quality and human health. Therefore, there is increasing interest in lower-risk alternative applications that support the physiological and biochemical mechanisms of plants. [4]. In this context, the use of signaling molecules that regulate growth, development, and stress responses in plants is gaining prominence. Nitric oxide (NO), one of these signaling molecules, is an important compound that plays a role in regulating many physiological processes in plants, such as photosynthesis, respiration, cell division, and aging [5–7]. NO also plays a critical role in the development of tolerance to environmental stress conditions in plants. In particular, it contributes to the maintenance of cellular redox balance through its interaction with reactive oxygen species (ROS) [8, 9].

Sodium nitroprusside (SNP) is one of the compounds widely used for the external application of NO in plants, considered an effective donor for controlled NO release. Owing to its light and temperature-sensitive structure, small molecular size, and high diffusion capacity, it can easily cross cell membranes and effectively regulate intracellular signaling mechanisms in plant tissues [5, 10]. In plants, NO is synthesized via NO synthase-like enzymes during the conversion of L-arginine to L-citrulline and is widely distributed in different plant tissues. NO is known to exert significant effects on seed germination, stomatal movement, root development, defense responses against pathogens, and leaf physiology [11–13]. It also plays an important role in the regulation of cell division, chlorophyll metabolism, photosynthesis, and respiration processes [14, 15]. One of the most important functions of NO in plants is the control of oxidative stress. NO activates the antioxidant defense system, regulating the activities of enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), and glutathione reductase (GR); conversely, it limits the accumulation of hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) which are indicators of lipid peroxidation. The application of NO donors such as SNP has been reported to protect membrane integrity and reduce cellular damage in different plant and fruit species [16–18].

Recent studies have shown that SNP applications are effective not only in vegetative tissues but also in fruit development and veraison, with significant effects on quality characteristics. Pre- or post-harvest SNP applications have been reported to enhance antioxidant capacity in fruits, regulate phenylpropanoid metabolism, and reduce quality losses [17–21]. These findings demonstrate that SNP has the potential to improve fruit quality by maintaining oxidative balance in fruit tissues.

Although studies on NO and SNP applications in grapevines are limited, existing data indicate a regulatory role of SNP on the antioxidant defense system [19, 22]. However, studies evaluating the

combined effects of repeated foliar SNP applications on antioxidant enzyme responses in leaves and the reflection of these responses in fruit tissue, particularly under vineyard conditions, are quite insufficient.

This study aims to determine the effects of repeated applications of different SNP concentrations at specific intervals on antioxidant enzyme activities (GR, SOD, APX and CAT) and oxidative stress indicators (MDA and H₂O₂) in the leaves of the Horoz Karası grape variety, and to evaluate the relationship between these effects and biochemical responses in the fruit tissue following the final application. The originality of this study lies in the consideration of SNP applications in a temporal framework under vineyard conditions and their evaluation within the context of leaf–fruit integrity. It is expected that the findings will contribute to a better understanding of the physiological and biochemical effects of NO-based applications in viticulture.

Materials and methods

Plant material and experimental design

This study was conducted in a commercial vineyard in Kilis province, located in the Southeastern Anatolia Region of Turkey, during the 2025 growing season. The experimental material consisted of vines of the Horoz Karası grape variety (*Vitis vinifera* L.), commonly cultivated in the region. All vines in the vineyard were of the same age (15 years), at a similar phenological development stage, and grown under uniform conditions in terms of cultural practices such as irrigation, fertilization, pruning, and disease and pest control.

The experiment was designed with three treatment groups, each consisting of ten vines. At the beginning of the experiment, leaf samples were taken for control purposes before the SNP application. Subsequently, SNP solutions were applied to the treatment groups at concentrations of 100, 300, and 500 ppm respectively, using a foliar spray method; the control group was sprayed only with pure water.

Leaf samples were taken 15 days after the first SNP application (pre-veraison period), and a second SNP application was performed at the same doses. On the thirtieth day (veraison period), leaf samples were collected again and a third SNP application was performed. On the forty-fifth day (harvest period), both leaf and fruit samples were harvested for analysis. During this period, fruits from vines not treated with SNP were also collected as a control group.

All collected leaf and fruit samples were transported to the laboratory under cold chain conditions and stored under appropriate conditions until analysis. Biochemical analyses were performed to determine oxidative stress indicators and enzyme activities of the antioxidant defense system.

Biochemical Measurements and Analyses

Preparation of Enzyme Extracts

Leaf and grape berries tissues collected after SNP treatments were immediately immersed in liquid nitrogen, pulverized to a fine powder, and preserved at -80°C prior to biochemical analyses. For the preparation of crude enzyme extracts, 1 g of frozen material was thoroughly homogenized in a pre-chilled mortar using 100 mM phosphate buffer (pH 7.0) supplemented with 10 mM Na_2EDTA , 1 mM MgCl_2 , 1% (w/v) polyvinylpyrrolidone (PVP), 10 mM KCl, and 2 mM dithiothreitol (DTT). The homogenate was subsequently filtered and centrifuged at 14,000 rpm for 20 min at 4°C . The clear supernatant obtained after centrifugation served as the enzyme source.

Determination of Catalase (CAT; EC 1.11.1.6) Enzyme Activity

Catalase (CAT) activity was assayed by following the decomposition of hydrogen peroxide spectrophotometrically. The reaction mixture consisted of 30 mM $\text{KH}_2\text{PO}_4/\text{K}_2\text{HPO}_4$ buffer (pH 7.0) containing 10 mM H_2O_2 , and the assay was started by the addition of 300 μL enzyme extract, bringing the final reaction volume to 1.4 mL. The decline in absorbance at 240 nm, corresponding to H_2O_2 degradation, was recorded for 3 min at 30 s intervals. Enzyme activity was calculated using an extinction coefficient of $36\text{ mM}^{-1}\text{ cm}^{-1}$ for H_2O_2 and expressed as units per milligram of protein ($\text{U mg}^{-1}\text{ protein}$). The procedure followed the method of Aebi (1984) [23].

Determination of Ascorbate Peroxidase (APX; EC 1.11.1.11) Enzyme Activity

APX activity was assayed spectrophotometrically by monitoring the oxidation of ascorbate in the presence of hydrogen peroxide. The reaction mixture contained 0.8 mL of 50 mM potassium phosphate buffer (pH 7.0), 0.1 mL of 0.5 mM ascorbate, and 0.1 mL of enzyme extract. The reaction was started by the addition of 0.1 mL of 100 mM H_2O_2 . The decrease in absorbance at 290 nm, reflecting ascorbate oxidation, was recorded over a 3-min period. Enzyme activity was calculated using an extinction coefficient of $2.8\text{ mM}^{-1}\text{ cm}^{-1}$ and expressed on a protein basis ($\text{U mg}^{-1}\text{ protein}$). The assay procedure followed the method described by Nakano and Asada (1981) [24].

Determination of Glutathione Reductase (GR; EC 1.6.4.2) Enzyme Activity

GR activity was assayed by following the oxidation of NADPH during the reduction of oxidized glutathione. The reaction mixture (final volume 2.8 mL) consisted of 120 mM phosphate buffer (pH 7.2; 2.5 mL), 0.065 mM GSSG (0.1 mL), 0.015 mM Na_2EDTA (0.1 mL), 9.6 mM NADPH (0.05 mL), and 0.1 mL enzyme extract. The decline in absorbance at 340 nm, corresponding to NADPH consumption, was monitored for 3 min. Enzyme activity was calculated and expressed as $\text{U mg}^{-1}\text{ protein}$. The assay was carried out according to the procedure described by Goldberg and Spooner (1983) [25].

Determination of Superoxide Dismutase (SOD; EC 1.15.1.1) Enzyme Activity

SOD activity was determined by a method based on the inhibition of nitroblue tetrazolium (NBT) reduction by superoxide radicals under light. The reaction medium was prepared to contain 50 mM phosphate buffer (pH 7.8), 12 mM methionine, 75 μ M NBT, 3 mM Na₂EDTA, and 50 mM Na₂CO₃, with different volumes of enzyme extract added. The assay was initiated by adding 10 μ M riboflavin, after which the reaction mixtures were exposed to fluorescent light at 25°C to allow photochemical reduction. The formation of the blue formazan product resulting from NBT reduction was quantified spectrophotometrically at 560 nm. One unit of superoxide dismutase (SOD) activity was defined as the amount of enzyme required to cause 50% inhibition of NBT reduction under the assay conditions. Enzyme activity was expressed as U mg⁻¹ protein. The determination of SOD activity was carried out following the method of Giannopolitis and Ries (1977) [26].

Determination of Malondialdehyde (MDA) Content

MDA content, used as an indicator of lipid peroxidation, was quantified according to the thiobarbituric acid (TBA) assay [27]. For this purpose, 0.5 g of leaf tissue previously frozen in liquid nitrogen and stored at -80°C was homogenized in 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C, and the supernatant obtained was used for subsequent measurements. For color development, 1 mL of the supernatant was mixed with 4 mL of 0.5% (w/v) TBA prepared in 20% (w/v) TCA. The reaction mixture was heated at 95°C for 30 min, followed by rapid cooling in an ice bath to terminate the reaction. After cooling, samples were centrifuged again at 10,000 rpm for 10 min. The absorbance of the clear supernatant was recorded at 532 nm, and non-specific turbidity was corrected by subtracting the absorbance measured at 600 nm.

The MDA amount was calculated using a standard curve created with MDA standard solutions prepared in the range of 0–120 μ M (Fig. 1). The standard equation is $y = 0.0023x + 0.0241$, and determination coefficient $R^2 = 0.991$. Results are expressed in μ mol MDA g⁻¹ fresh weight.

Determination of Hydrogen Peroxide (H₂O₂) Content

H₂O₂ content in leaf and grape berries samples was determined using a colorimetric method described in the literature, adapted to the conditions of our study [28]. For analysis, 0.5 g of samples were taken from those powdered with liquid nitrogen and stored at -80°C. The samples were homogenized with a suitable extraction buffer under cold conditions. The resulting homogenates were centrifuged at 14,000 rpm at 4°C for 20 minutes, and the supernatant was used in the analyses. The determination of H₂O₂ content is based on the spectrophotometric measurement of the colored complex formed as a result of the reaction of the sample extract with the relevant reagents. At the completion of the reaction, absorbance was measured at 390 nm against a reagent blank. The concentration of H₂O₂ was quantified based on the calibration curve generated using known H₂O₂ standards (Fig. 2). The linear regression

equation ($y = 0.01x + 0.0359$; $R^2 = 0.992$) was used to calculate H_2O_2 content, and the results were expressed as $mmol\ g^{-1}$ fresh weight.

Statistical Analysis

All measurement data obtained from the experiments were expressed as mean $\pm$ standard deviation for each application. All data were subjected to statistical analysis using SPSS version 24.0. Differences among treatments were evaluated by one-way analysis of variance (ANOVA). When significant effects were detected, mean comparisons were performed using Duncan's multiple range test to determine the source of variation among groups. In statistical analyses, p -value ≤ 0.05 was accepted as the threshold value at which the differences between applications were significant.

Results

Effects of Sodium Nitroprusside (SNP) Applications on Antioxidant Defense System and Oxidative Stress Indicators in Grape Leaves

The effects of SNP applications on antioxidant defense system and oxidative stress indicators in the leaves of Horoz Karası grape variety showed significant dose-dependent changes throughout the phenological development process (pre-veraison, veraison, and harvest period). Compared to the control group, 100, 300, and 500 ppm SNP applications affected biochemical parameters to varying degrees in all periods.

Findings regarding the pre-veraison period are presented in Table 1. In this period, generally limited differences were observed between the SNP-treated and control groups in terms of GR, SOD, CAT, and APX activities. The one-way analysis of variance indicated that none of the evaluated parameters differed significantly among the treatments. Nevertheless, an examination of the mean values revealed a tendency toward higher GR and SOD activities in the 300 ppm SNP treatment compared with the control. In contrast, MDA and H_2O_2 levels were generally lower in the SNP-treated groups than in the untreated control. These trends point to the potential of SNP to regulate oxidative balance in the early phenological stage.

Table 1
Effects of SNP application at pre-veraison on antioxidant enzyme activities and oxidative stress markers in leaves

SNP doses	GR	SOD	CAT	APX	MDA	H ₂ O ₂
Control	3,75 ± 0,48 ^a	75,87 ± 2,48 ^a	6,77 ± 0,61 ^a	12,91 ± 1,07 ^a	5,35 ± 0,01 ^a	93,61 ± 0,99 ^a
100 ppm	4,74 ± 1,34 ^{ab}	81,93 ± 4,59 ^a	8,24 ± 0,52 ^a	15,47 ± 0,00 ^{ab}	5,30 ± 0,05 ^a	80,61 ± 0,42 ^a
300 ppm	6,74 ± 1,14 ^b	95,36 ± 6,78 ^b	8,40 ± 0,84 ^a	16,80 ± 1,70 ^b	5,55 ± 0,05 ^a	97,71 ± 0,28 ^a
500 ppm	4,82 ± 0,45 ^{ab}	86,32 ± 0,31 ^a	6,02 ± 0,60 ^a	14,29 ± 0,00 ^{ab}	5,55 ± 0,09 ^a	80,51 ± 0,85 ^a
a, b, c ↓: Different lower cases represent statistically significant differences among means						

Comparisons of pre-veraison period parameters depending on SNP doses are presented in Fig. 3. Graphical evaluation shows that, in particular, the application of 300 ppm SNP resulted in a numerical increase in antioxidant enzyme activities and had a suppressive effect on oxidative stress indicators.

Leaf traits measured at the veraison stage are presented in Table 2. The effects of SNP applications became more pronounced during this period, with statistically significant differences observed between applications, particularly in terms of GR and SOD activities ($p \leq 0.05$). The highest levels of GR and SOD activity were observed in the 300 ppm SNP application, while the 100 and 500 ppm doses exhibited intermediate values. With regard to the CAT and APX activities, the results of the ANOVA indicated no overall significance. However, it was observed that all groups treated with SNP exhibited higher averages in comparison to the control group. When the oxidative stress indicators were evaluated, it was determined that the contents of MDA and H₂O₂ decreased significantly, especially in the 100 and 300 ppm SNP applications, compared to the control group ($p \leq 0.05$).

Table 2

Effects of SNP application at veraison on antioxidant enzyme activities and oxidative stress markers in leaves

SNP doses	GR	SOD	CAT	APX	MDA	H ₂ O ₂
Control	3,75 ± 0,48 ^a	75,87 ± 2,48 ^a	6,77 ± 0,61 ^a	12,91 ± 1,07 ^a	5,35 ± 0,01 ^a	93,61 ± 0,99 ^a
100 ppm	5,93 ± 0,44 ^b	56,37 ± 3,29 ^b	9,85 ± 0,94 ^b	17,37 ± 0,98 ^b	4,56 ± 0,06 ^b	61,26 ± 0,49 ^b
300 ppm	9,67 ± 0,72 ^c	110,54 ± 9,39 ^c	9,62 ± 0,85 ^b	20,95 ± 0,80 ^b	4,79 ± 0,04 ^b	91,96 ± 0,49 ^b
500 ppm	6,67 ± 0,00 ^b	91,74 ± 6,29 ^b	9,17 ± 0,45 ^b	17,31 ± 1,17 ^b	5,18 ± 0,02 ^b	72,06 ± 2,19 ^b
a, b, c ↓: Different lower cases represent statistically significant differences among means						

The comparative effects of SNP doses during the veraison period are presented in Fig. 4. The graph shows that antioxidant enzyme activities reach maximum levels during the veraison period and that SNP applications enhance this response.

Leaf-related parameters measured at harvest are presented in Table 3. It was observed that the effects of SNP applications differed depending on the parameter. Statistically significant differences were identified between the applications in terms of GR and CAT activities ($p \leq 0.05$), with the highest values determined in the 300 ppm SNP application. Despite the absence of statistically significant findings for SOD and APX activities, it was observed that all SNP-treated groups exhibited higher mean values in comparison to the control group. With regard to the MDA and H₂O₂ content, the 100 ppm SNP application exhibited the lowest values, thus suggesting that a lower SNP dose may be more effective in limiting oxidative stress during the harvest period.

Table 3
Effects of SNP application at harvest on antioxidant enzyme activities and oxidative stress markers in leaves

SNP doses	GR	SOD	CAT	APX	MDA	H ₂ O ₂
Control	3,75 ± 0,48 ^a	75,87 ± 2,48 ^a	6,77 ± 0,61 ^a	12,91 ± 1,07 ^a	5,35 ± 0,01 ^a	93,61 ± 0,99 ^a
100 ppm	4,14 ± 0,98 ^{ab}	89,12 ± 9,60 ^a	8,47 ± 0,28 ^a	14,57 ± 1,08 ^a	4,34 ± 0,04 ^a	73,66 ± 0,78 ^a
300 ppm	8,30 ± 1,41 ^b	82,67 ± 3,22 ^a	12,40 ± 1,11 ^a	19,95 ± 5,23 ^a	5,02 ± 0,04 ^a	89,16 ± 0,49 ^a
500 ppm	6,53 ± 0,49 ^{ab}	81,98 ± 3,77 ^a	7,38 ± 0,39 ^a	17,60 ± 1,08 ^a	5,35 ± 0,05 ^a	89,86 ± 3,18 ^a
a, b, c ↓: Different lower cases represent statistically significant differences among means						

Harvest-related parameters were compared across SNP doses (Fig. 5). SNP applications suppress oxidative stress indicators and support the antioxidant defense system throughout phenological progression.

The results related to grape berries parameters are summarized in Table 4. Analysis of variance revealed no significant effect of SNP treatments on grape berries GR activity compared with the control ($p = 0.408$). Although the control group exhibited the lowest GR activity and the 300 ppm SNP treatment showed the highest value, these differences were not statistically meaningful. Similarly, SOD activity displayed some variation among treatments; however, the observed differences did not reach statistical significance ($p = 0.053$). The highest SOD activity was detected at 300 ppm SNP, while lower activities were observed in the control and 500 ppm treatments. The CAT activity varied among the treatments; however, the difference was not statistically significant ($p = 0.052$). The highest activity was recorded at 300 ppm SNP, while the control and 500 ppm treatments exhibited lower values. The effect of SNP applications on APX activity was not statistically significant. Although the highest APX value was observed in the 300 ppm application, no significant difference was observed between the applications. MDA content varied significantly depending on the treatments ($p < 0.05$). The lowest MDA values were determined in the 500 and 300 ppm SNP treatments, while the control group had the highest MDA content. Hydrogen peroxide (H₂O₂) content did not differ significantly between the SNP-treated groups and the control. Nevertheless, the lowest H₂O₂ level was observed in the 300 ppm SNP treatment, whereas the control group exhibited the highest value.

Table 4
Effects of SNP application on antioxidant enzyme activities and oxidative stress markers in in grape berries tissue

SNP doses	GR	SOD	CAT	APX	MDA	H ₂ O ₂
Control	1,564 ± 0,170 ^a	17,264 ± 0,480 ^a	1,874 ± 0,491 ^a	4,819 ± 0,757 ^a	2,129 ± 0,037 ^a	9,860 ± 0,212 ^a
100 ppm	1,645 ± 0,332 ^a	18,415 ± 0,815 ^a	2,237 ± 0,096 ^a	5,229 ± 0,739 ^a	1,981 ± 0,025 ^a	7,510 ± 0,141 ^a
300 ppm	1,833 ± 0,324 ^a	19,808 ± 0,929 ^a	3,237 ± 0,467 ^a	6,116 ± 0,000 ^a	1,755 ± 0,049 ^a	6,710 ± 0,283 ^a
500 ppm	1,682 ± 0,297 ^a	17,906 ± 0,338 ^a	1,759 ± 0,257 ^a	5,380 ± 0,331 ^a	1,720 ± 0,049 ^a	8,610 ± 0,424 ^a
a, b, c ↓: Different lower cases represent statistically significant differences among means						

A comparison of antioxidant enzyme activities and oxidative stress markers in grape berries among different SNP applications is presented in Fig. 6. No statistically significant differences were observed among treatments for GR, SOD, CAT, and APX activities; however, 300 ppm SNP consistently exhibited higher enzyme activities compared to the control and 500 ppm treatments. In contrast, MDA content differed significantly among treatments, with markedly lower values in the 300 and 500 ppm SNP applications relative to the control. Although H₂O₂ content did not differ significantly, the lowest levels were observed at 300 ppm SNP, whereas the control showed the highest values. Overall, the comparative graphical analysis indicates that SNP treatment, particularly at 300 ppm, is associated with enhanced antioxidant responses and reduced oxidative stress in grape berries.”

To provide an integrated overview of the antioxidant system and oxidative stress responses, a heatmap based on Z-score normalization was constructed (Figure X). The visualization revealed clear stage-dependent differences in the response to SNP applications.

At veraison, particularly under 300 ppm SNP, antioxidant enzyme activities (GR, SOD, CAT, and APX) showed pronounced increases compared to the control, as indicated by the intense red clusters. Importantly, this enzymatic activation was accompanied by a marked reduction in oxidative stress markers, with both MDA and H₂O₂ exhibiting lower relative values than the control treatment. This pattern suggests that SNP application, especially at 300 ppm, effectively enhanced antioxidant defense while simultaneously mitigating lipid peroxidation and hydrogen peroxide accumulation.

A similar but less pronounced trend was observed at pre-veraison and harvest stages, where elevated enzymatic activities were generally associated with reduced MDA and H₂O₂ levels relative to the control. In contrast, grape berries displayed comparatively lower antioxidant enzyme activities and a distinct oxidative profile, indicating tissue-specific regulation of redox metabolism during fruit development.

Overall, the coordinated increase in antioxidant enzymes together with the decrease in MDA and H_2O_2 under 300 ppm SNP highlights this dose as the most effective treatment for improving redox homeostasis across developmental stages.

Discussion

In this study, foliar SNP applications were found to dynamically modulate the antioxidant defense system throughout the phenological stages and to limit oxidative damage in the Horoz Karası grape variety. In particular, the observation that the 300 ppm SNP dose was the most effective concentration in both leaf and grape berries tissues supports current findings indicating that NO exhibits a hormetic effect in plants. While low doses act as signaling molecules that trigger defense responses, the reduced effectiveness observed at higher doses (such as 500 ppm) can be explained by the tightly regulated and delicate balance of NO metabolism [29]. The integrative heatmap analysis provides further evidence that SNP-mediated nitric oxide signaling enhances redox regulation in a stage-dependent manner. The coordinated increase in antioxidant enzyme activities (GR, SOD, CAT, and APX), together with the reduction of MDA and H_2O_2 levels compared to the control, indicates that SNP application effectively mitigates oxidative damage while strengthening the antioxidant defense system. Particularly, the 300 ppm dose appears to optimize redox homeostasis across developmental stages. These findings suggest that exogenous SNP plays a regulatory role in maintaining cellular oxidative balance during grapevine development.

The results indicated that SNP treatments led to a marked increase in the activities of major antioxidant enzymes, namely SOD, CAT, and GR, with the most pronounced effects observed during the veraison stage. Superoxide dismutase (SOD) acts as the primary enzymatic barrier against oxidative stress by catalyzing the dismutation of superoxide radicals. Subsequently, enzymes such as CAT and APX facilitate the conversion of the generated H_2O_2 into less harmful molecules, thereby contributing to the regulation of cellular redox balance. Consistent with these results, previous studies have reported that NO applications activate the Ascorbate–Glutathione (Asada–Halliwell) cycle in grape tissues, leading to increased activities of SOD, CAT, APX, and GR, while simultaneously suppressing ROS accumulation and lipid peroxidation [30]. In line with this, a molecular-level study conducted on grape berries in 2023 demonstrated that NO donors have been reported to stimulate phenylpropanoid pathway activity and to enhance the transcription of antioxidant defense–associated genes, including VvSOD and VvCAT [31]. In line with these findings, earlier studies have shown that the combined treatment of NO and H_2S alleviated oxidative damage by promoting the activities of major antioxidant enzymes such as APX, CAT, and GR. This coordinated upregulation was accompanied by reduced accumulation of MDA and H_2O_2 , indicating an improved cellular redox status [32]. These molecular insights provide a robust mechanistic explanation for the observation that the 300 ppm SNP application was associated with higher antioxidant enzyme activities compared to the other doses evaluated in the present study. When evaluated in terms of oxidative stress indicators, the significant reduction in MDA levels in both leaf and grape berries tissues following SNP applications reflects the protective effect of NO against membrane lipid peroxidation [6]. NO can stabilize free radicals through direct interactions with reactive oxygen

species and can also indirectly limit ROS accumulation by inducing the antioxidant defense system [13]. Current studies demonstrate that NO applications slow the oxidation of unsaturated fatty acids in cell membranes, preserve fruit tissue integrity, and reduce post-harvest quality losses [19, 33–35]. For Horoz Karası, which is among thin-skinned and oxidative stress–sensitive grape varieties, this protective effect is particularly important for the sustainability of fruit quality.

Another noteworthy finding of the study is that the effects of SNP applications became more pronounced during the veraison and harvesting periods compared to the pre-veraison stage. This suggests that exogenous NO applications act as a “buffer mechanism” against the increased production of ROS associated with elevated metabolic activity during veraison [36, 37]. Indeed, recent studies have demonstrated that NO can delay fruit ripening and softening processes. For example, a recent study reported that NO significantly enhanced the activity of key enzymes involved in the phenylpropanoid biosynthesis pathway, thereby increasing disease resistance and mitigating fruit softening in bitter melon [38]. In another study, the combined application of gibberellic acid and NO was shown to delay fruit ripening and color development in strawberry fruits by regulating chromatin-level processes [39]. Similarly, in tomato fruits, NO application delayed ripening and softening by inhibiting the expression of cell wall-softening genes [40]. In addition, S-nitrosoglutathione application has been reported to delay color change and slow the ripening process during post-harvest storage of tomato fruits [41].

The observation that foliar-applied SNP influenced antioxidant enzyme activities and MDA levels in fruit tissue indicates that the NO signal is systemically transported through the vascular system of the grapevine, thereby proactively regulating fruit quality within the framework of leaf–fruit integrity.

Conclusion

This study shown that foliar application of SNP effectively regulates the antioxidant defense system and limits oxidative damage throughout the phenological process in the Horoz Karası grape variety. Considering all evaluated physiological and biochemical parameters, the 300 ppm SNP dose emerged as the optimum concentration, maximizing antioxidant enzyme activities while minimizing MDA and H₂O₂ accumulation.

The more pronounced effects of SNP applications during the verasion and harvest stages indicate that exogenous NO supplementation acts as an efficient regulator, balancing the oxidative load associated with increased metabolic activity. Moreover, the influence of foliar SNP application on oxidative stress indicators and antioxidant responses in fruit tissue suggests that NO signaling operates systemically, maintaining leaf–fruit functional integrity.

Overall, these findings highlight the strong potential of NO-based applications as a sustainable strategy for oxidative stress management and grape quality preservation in viticulture.

Abbreviations

APX

Ascorbate peroxidase

AsA–GSH cycle

Ascorbate–Glutathione cycle

CAT

Catalase

GA

Gibberellic acid

GR

Glutathione reductase

H₂O₂

Hydrogen peroxide

MDA

Malondialdehyde

NO

Nitric oxide

NO + H₂S

Nitric oxide + Hydrogen sulfide

ROS

Reactive oxygen species

SNP

Sodium nitroprusside

SOD

Superoxide dismutase

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

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Author Contribution

İSG was solely responsible for the conceptualization and design of the study, experimental implementation, data acquisition and statistical analysis, interpretation of findings, and preparation and critical revision of the manuscript. The author approved the final version of the manuscript.

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Not applicable.

Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

References

1. Kamiloglu O. Influence of some cultural practices on yield, fruit quality and individual anthocyanins of table grape cv. 'Horoz Karasi'. *J Anim Plant Sci.* 2011;21(2):240–5.
2. Sucu Dağ S, Gün B, Şekeroğlu N. The Effects of Cluster Thinning Yield and Quality on 'Horoz Karasi' Cultivar of Grapevine. *Black Sea J Agric.* 2026;9(1):1–8.
<https://doi.org/10.47115/bsagriculture.1792498>.
3. Brás LP, Luís Â, Chatel G, Socorro S, Duarte AP. Stilbenes from Vine Extracts: Therapeutic Potential and Mechanisms. *Int J Mol Sci.* 2025;26(17). <https://doi.org/10.3390/ijms26178269>.
4. Devi PI, Manjula M, Bhavani RV, Agrochemicals, Environment, Health H. Annual Review of Environment and Resources. 2022;47(Volume 47, 2022):399–421.
<https://www.annualreviews.org/content/journals/10.1146/annurev-environ-120920-111015>
5. Khan M, Al Azzawi TNI, Ali S, Yun B-W, Mun B-G. Nitric Oxide, a Key Modulator in the Alleviation of Environmental Stress-Mediated Damage in Crop Plants: A Meta-Analysis. *Plants.* 2023;12(11):2121.
<https://www.mdpi.com/2223-7747/12/11/2121>
6. Fejes G, Bodor T, Szöllősi R, Kondak S, Kutasi K, Fotopoulos V, et al. Nitric oxide as an integral element in priming-induced tolerance and plant stress memory. *J Exp Bot.* 2025;76(13):3669–85.
<https://doi.org/10.1093/jxb/eraf033>.
7. Amooaghaie R, Nikram Z. Salicylic acid enhances photosynthesis in *Borago officinalis* plants exposed to water deficit by nitric oxide-mediated boosting carbonic anhydrase and antioxidant system. *BMC Plant Biol.* 2025. <https://doi.org/10.1186/s12870-025-07770-6>.

8. Kumar D, Ohri P. Say NO to plant stresses: Unravelling the role of nitric oxide under abiotic and biotic stress. *Nitric Oxide*. 2023;130:36–57. <https://doi.org/10.1016/j.niox.2022.11.004>.
9. Pande A, Mun BG, Methela NJ, Rahim W, Lee DS, Lee GM, et al. Heavy metal toxicity in plants and the potential NO-releasing novel techniques as the impending mitigation alternatives. *Front Plant Sci*. 2022;13:1019647. <https://doi.org/10.3389/fpls.2022.1019647>.
10. Sami F, Faizan M, Faraz A, Siddiqui H, Yusuf M, Hayat S. Nitric oxide-mediated integrative alterations in plant metabolism to confer abiotic stress tolerance, NO crosstalk with phytohormones and NO-mediated post translational modifications in modulating diverse plant stress. *Nitric Oxide*. 2018;73:22–38. <https://doi.org/10.1016/j.niox.2017.12.005>.
11. Rahim W, Khan M, Al Azzawi TNI, Pande A, Methela NJ, Ali S, et al. Exogenously applied sodium nitroprusside mitigates lead toxicity in rice by regulating antioxidants and metal stress-related transcripts. *Int J Mol Sci*. 2022;23(17):9729. <https://doi.org/10.3390/ijms23179729>.
12. Ahmad P, Abdel Latef AA, Hashem A, Abd_Allah EF, Gucel S, Tran L-SP. Nitric Oxide Mitigates Salt Stress by Regulating Levels of Osmolytes and Antioxidant Enzymes in Chickpea. *Front Plant Sci*. 2016;7–2016. 10.3389/fpls.2016.00347. <https://www.frontiersin.org/journals/plant-science/articles/>.
13. Alnusairi GSH, Mazrou YSA, Qari SH, Elkelish AA, Soliman MH, Eweis M et al. Exogenous Nitric Oxide Reinforces Photosynthetic Efficiency, Osmolyte, Mineral Uptake, Antioxidant, Expression of Stress-Responsive Genes and Ameliorates the Effects of Salinity Stress in Wheat. *Plants*. 2021;10(8):1693. <https://www.mdpi.com/2223-7747/10/8/1693>
14. Bhardwaj D, Sahoo RK, Naqvi AR, Lakhanpaul S, Tuteja N. Pea Gβ subunit of G proteins has a role in nitric oxide-induced stomatal closure in response to heat and drought stress. *Protoplasma*. 2020;257(6):1639–54. <https://doi.org/10.1007/s00709-020-01529-6>.
15. Chen WW, Yang JL, Qin C, Jin CW, Mo JH, Ye T, et al. Nitric oxide acts downstream of auxin to trigger root ferric-chelate reductase activity in response to iron deficiency in Arabidopsis. *Plant Physiol*. 2010;154(2):810–9. <https://doi.org/10.1104/pp.110.161109>.
16. Vergara R, Parada F, Rubio S, Pérez FJ. Hypoxia induces H₂O₂ production and activates antioxidant defence system in grapevine buds through mediation of H₂O₂ and ethylene. *J Exp Bot*. 2012;63(11):4123–31. <https://doi.org/10.1093/jxb/ers094>.
17. Saeedi M, Shirzad H, Noruzi P, Ghasemi G. Foliar application of sodium nitroprusside alters the physicochemical properties, antioxidant capacities, and enzymatic activities of strawberry cv. Camarosa *Sci Rep*. 2024;14(1):30943. <https://doi.org/10.1038/s41598-024-81936-4>.
18. Han J, Fan Y, Sun T, An J, Ding Y, Zhang W, et al. Sodium nitroprusside (SNP) treatment increases the postharvest resistance of apple fruit to *Alternaria alternata* by enhancing antioxidant enzyme activity. *Physiol Mol Plant Pathol*. 2024;129:102199. <https://www.sciencedirect.com/science/article/pii/S0885576523002540>.
19. Hu L, Zheng Q, Chen Z, Qin Y, Si H, Ji J, et al. Pre-harvest treatment with gibberellin (GA(3)) and nitric oxide donor (SNP) enhances post-harvest firmness of grape berries. *Food Chem (Oxf)*.

- 2025;10:100235. <https://doi.org/10.1016/j.fochms.2024.100235>.
20. Ge Y, Li X, Li C, Tang Q, Duan B, Cheng Y, et al. Effect of sodium nitroprusside on antioxidative enzymes and the phenylpropanoid pathway in blueberry fruit. *Food Chem.* 2019;295:607–12. <https://doi.org/10.1016/j.foodchem.2019.05.160>.
21. Liang F, Li X, Zhou C, Wang X, Wang H, Yuan J, et al. Short-term nitric oxide treatment enhances the antioxidant capacity and maintains the preservation quality of Meizao cherries. *Int J Food Sci Technol.* 2023;58(8):4354–65. <https://doi.org/10.1111/ijfs.16538>.
22. Kesen Ö, Yagci A, Hatterman-Valenti H, Kaya O. Enhancing Grape Seed Germination and Seedling Development Through Varietal Responses to Sodium Nitroprusside and Gibberellic Acid Applications. *Horticulturae.* 2025;11(7):754. <https://www.mdpi.com/2311-7524/11/7/754>
23. Aebi H. Catalase in vitro. *Methods Enzymol.* 1984;105:121–6.
24. Nakano Y, Asada K. Hydrogen Peroxide is Scavenged by Ascorbate-specific Peroxidase in Spinach Chloroplasts. *Plant Cell Physiol.* 1981;22(5):867–80. <https://doi.org/10.1093/oxfordjournals.pcp.a076232>.
25. Bergmeyer H-U. *Methods of enzymatic analysis.* Elsevier; 1983.
26. Giannopolitis CN, Ries SK. Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol.* 1977;59(2):309–. <https://doi.org/10.1104/pp.59.2.309>. 14.
27. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.* 1979;95(2):351–8. [https://doi.org/10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3).
28. Velikova M, Bankova V, Sorkun K, Houcine S, Tsvetkova I, Kujumgiev A. Propolis from the Mediterranean region: chemical composition and antimicrobial activity. *Z Naturforsch C J Biosci.* 2000;55(9–10):790–3. <https://doi.org/10.1515/znc-2000-9-1019>.
29. Calabrese EJ, Agathokleous E. Nitric oxide, hormesis and plant biology. *Sci Total Environ.* 2023;866:161299. <https://www.sciencedirect.com/science/article/pii/S0048969722084030>.
30. Zhang Z, Xu J, Chen Y, Wei J, Wu B. Nitric oxide treatment maintains postharvest quality of table grapes by mitigation of oxidative damage. *Postharvest Biol Technol.* 2019;152:9–18. <https://www.sciencedirect.com/science/article/pii/S0925521418311098>.
31. Shi J, Huang D, Du Y, Zhu S, Hussain Z, Haider MS, et al. Effects of Exogenous Nitric Oxide Treatment on Grape Berries Against Botrytis cinerea and Alternaria alternata Related Enzymes and Metabolites. *Plant Dis.* 2023;107(5):1510–21. <https://doi.org/10.1094/pdis-04-22-0928-re>.
32. Liu R, Ji N, Wang R, Li Y, Nie H, Chen C, et al. Transcriptomic and metabolomic analyses insight into the synergistic effects of nitric oxide and hydrogen sulfide fumigation on enhancing postharvest antioxidant defense and phenylpropane metabolism in ‘crystal’ grape. *Food Bioscience.* 2025;66:106110. <https://www.sciencedirect.com/science/article/pii/S221242922500286X>.
33. Li C, Yu W, Liao W. Role of Nitric Oxide in Postharvest Senescence of Fruits. *Int J Mol Sci.* 2022;23(17). <https://doi.org/10.3390/ijms231710046>.

34. Ghorbani B, Pakkish Z, Najafzadeh R. Shelf life improvement of grape (*Vitis vinifera* L. cv. Rish Baba) using nitric oxide (NO) during chilling damage. *Int J Food Prop.* 2017;20(sup3):S2750–63. <https://doi.org/10.1080/10942912.2017.1373663>.
35. Plakunmonthon T, Chutimanukul P, Matsui K, Seraypheap K. Integrated effects of pre-harvest high blue light and postharvest sodium nitroprusside on volatile oil composition and quality of cold-stored holy basil. *Food Chem X.* 2026;33:103493. <https://doi.org/10.1016/j.fochx.2026.103493>.
36. Mishra AK, Gupta S, Tiwari S. Differential antioxidant and metabolic responses to ozone stress in ozone-sensitive and tolerant wheat cultivars treated with nitric oxide donor. *Sci Total Environ.* 2025;1002:180588. <https://doi.org/10.1016/j.scitotenv.2025.180588>.
37. Ahsan M, Tufail A, Jamal A, Al-Yasi HM, Radicetti E, Raza MA, et al. Nitric oxide regulates water status, antioxidant enzymes, nutritional balance, and growth of gazania (*Gazania rigens*) under drought stress. *Funct Plant Biol.* 2025;52. <https://doi.org/10.1071/fp25092>.
38. Wang H, Li L, Ma L, Fernie AR, Fu A, Bai C, et al. Revealing the specific regulations of nitric oxide on the postharvest ripening and senescence of bitter melon fruit. *aBIOTECH.* 2024;5(1):29–45. <https://doi.org/10.1007/s42994-023-00110-y>.
39. Yang M, Hou G, Peng Y, Jiang Y, He C, She M, et al. editors. Exogenous GSNO and CPTIO treatment affected the ripening and glutathione metabolism of strawberry fruit. *III International Symposium on Fruit Culture along Silk Road Countries* 1401; 2023. <https://doi.org/10.17660/ActaHortic.2024.1401.34>
40. Yao Y, Yang Y, Ding Z, Yao K, Zhang J, Liu Z, et al. Nitric oxide delays tomato fruit softening by inhibiting SINAP2 (NAC-like, activated by *apetala3/pistillata2*) transcription factor-activated transcription of soften-related genes. *Int J Biol Macromol.* 2025;309:143148. <https://www.sciencedirect.com/science/article/pii/S0141813025037006>.
41. Liu Z, Huang D, Yao Y, Pan X, Zhang Y, Huang Y et al. The Crucial Role of SIGSNOR in Regulating Postharvest Tomato Fruit Ripening. *International Journal of Molecular Sciences.* 2024;25(5):2729. <https://www.mdpi.com/1422-0067/25/5/2729>

Figures

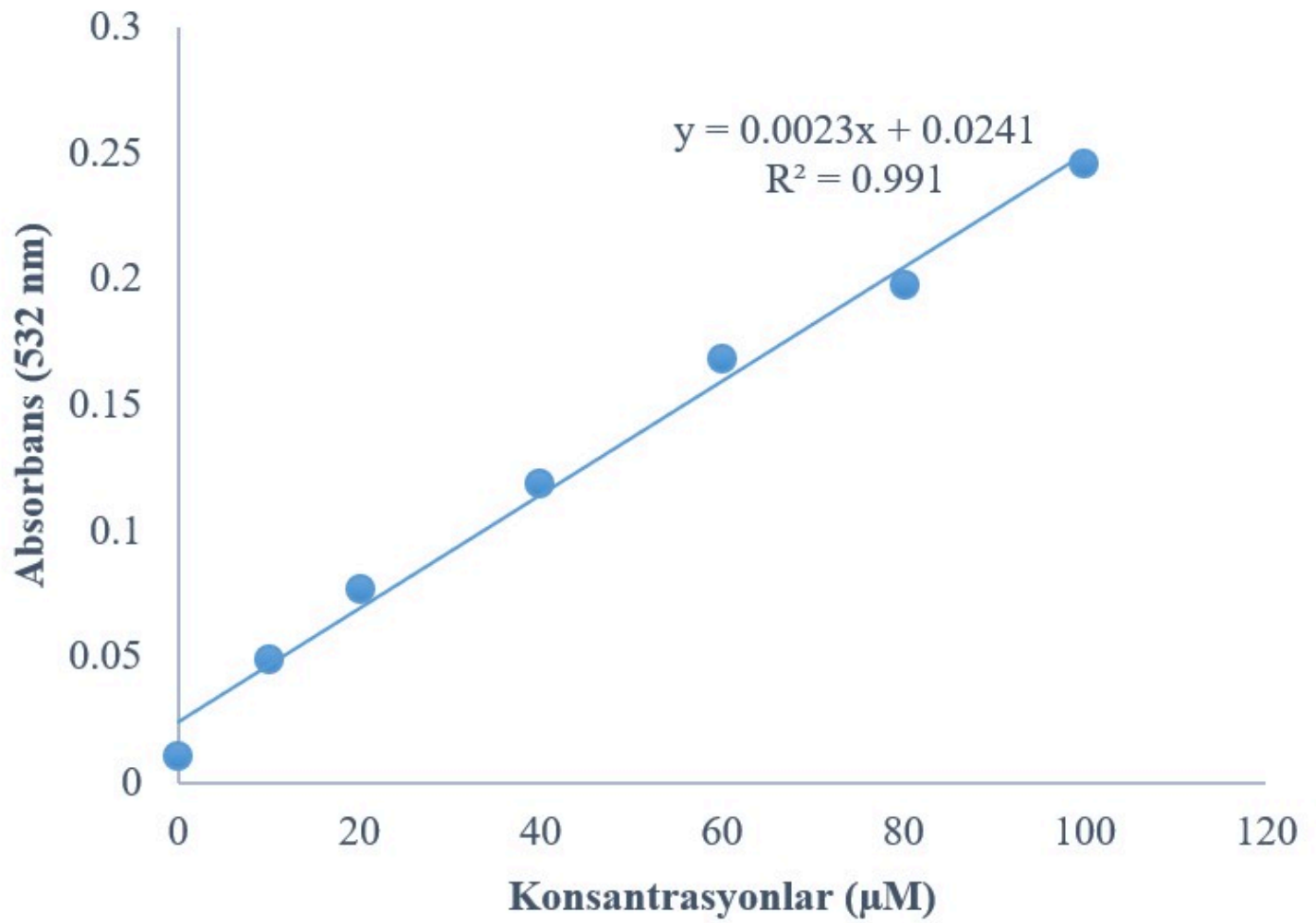


Figure 1

Standard curve of 1,1,3,3-Tetraethoxypropane (MDA).

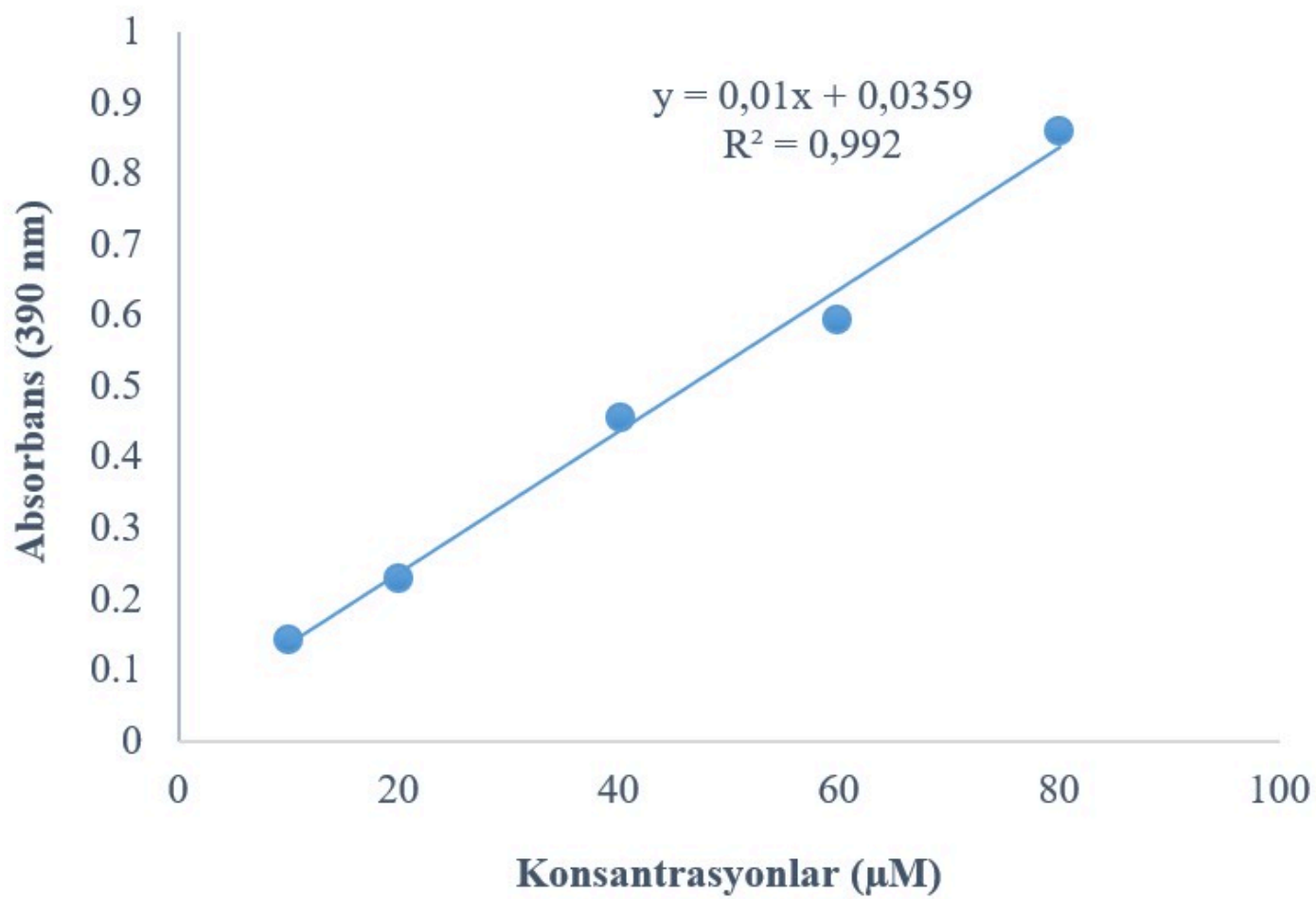


Figure 2

Standard curve of Hydrogen Peroxide (H₂O₂).

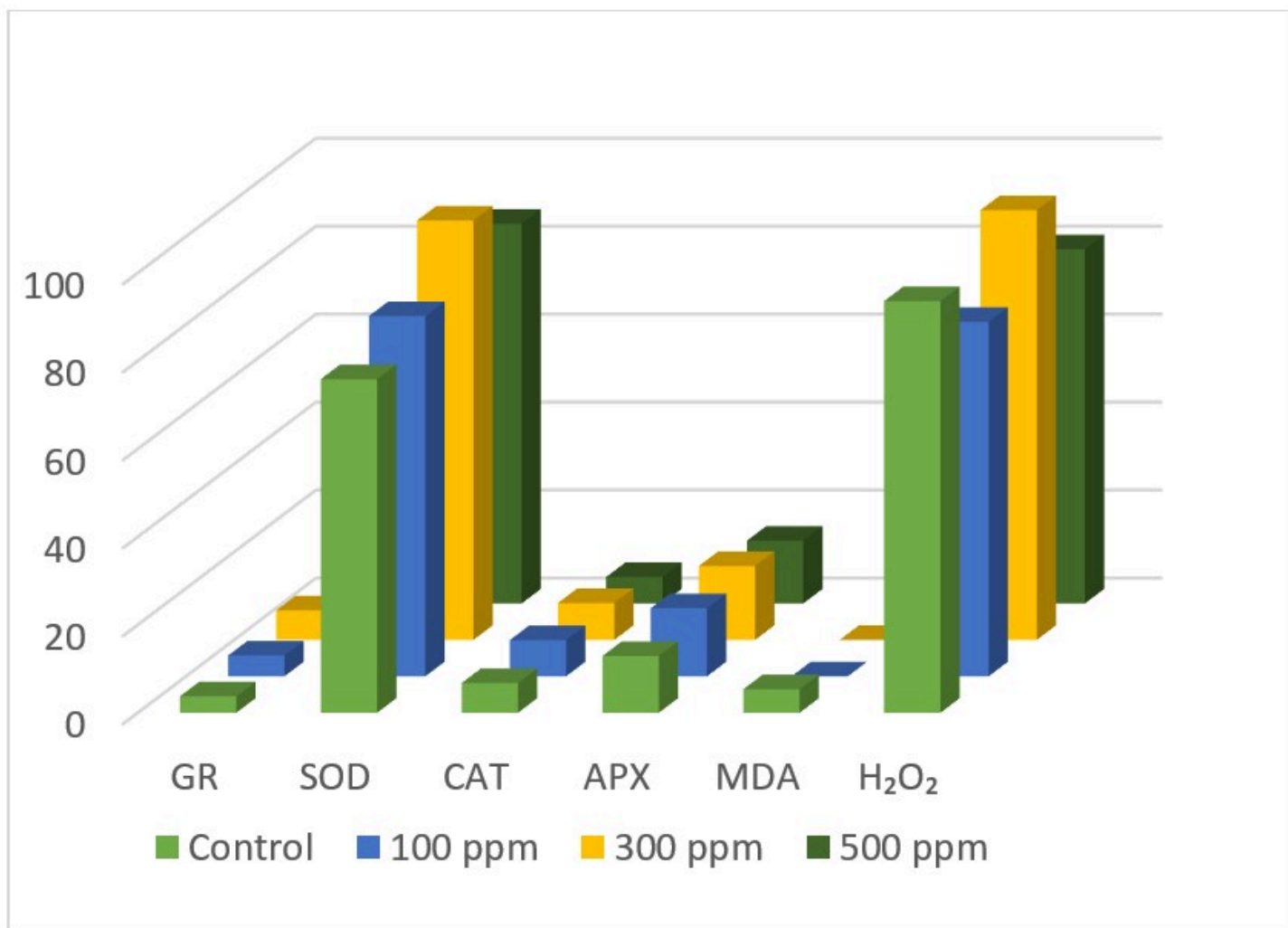


Figure 3

Changes in antioxidant enzyme activities and oxidative stress indicators in grape leaves during pre-veraison under different SNP doses

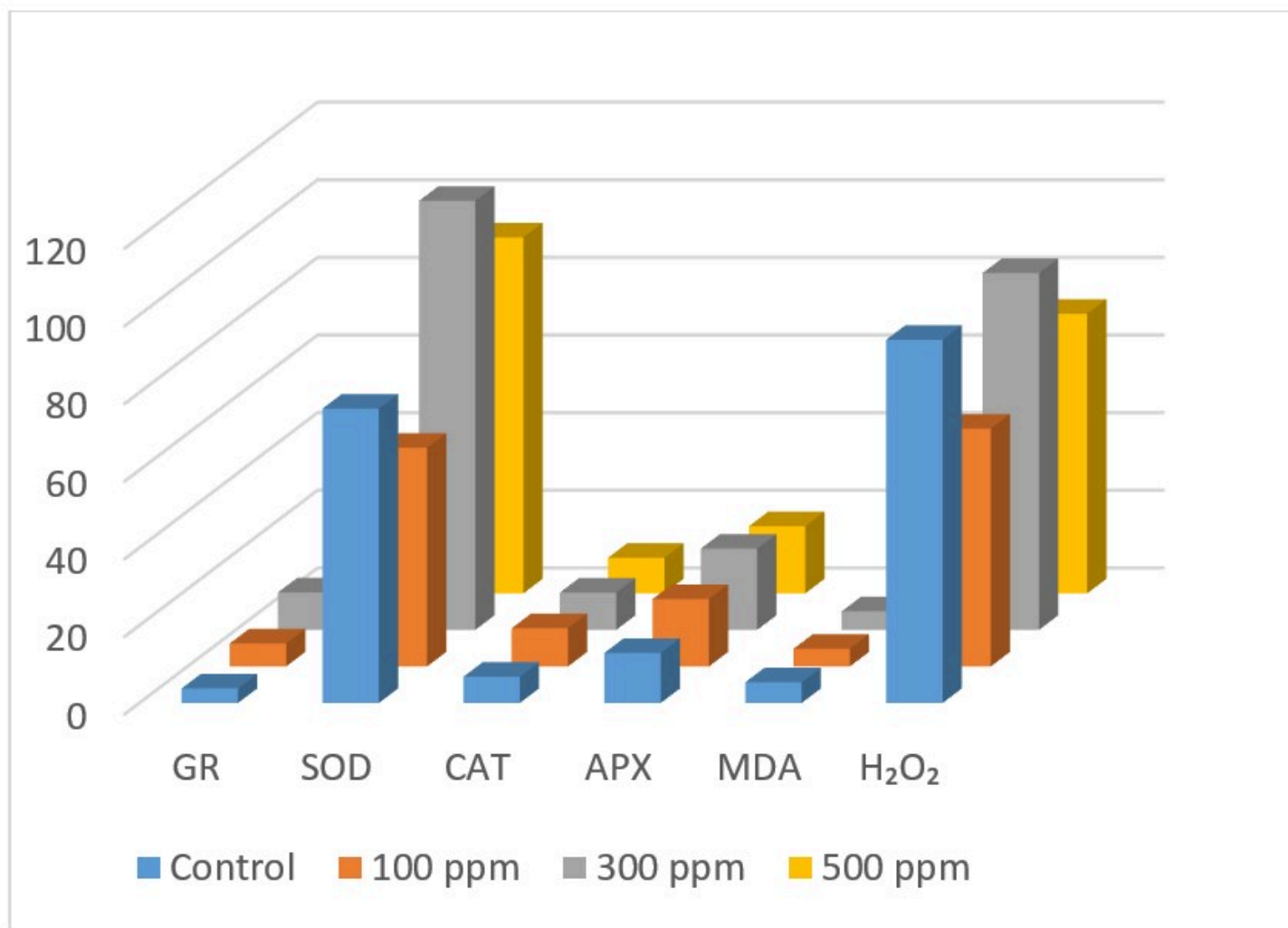


Figure 4

Changes in antioxidant enzyme activities and oxidative stress indicators in grape leaves during veraison under different SNP doses

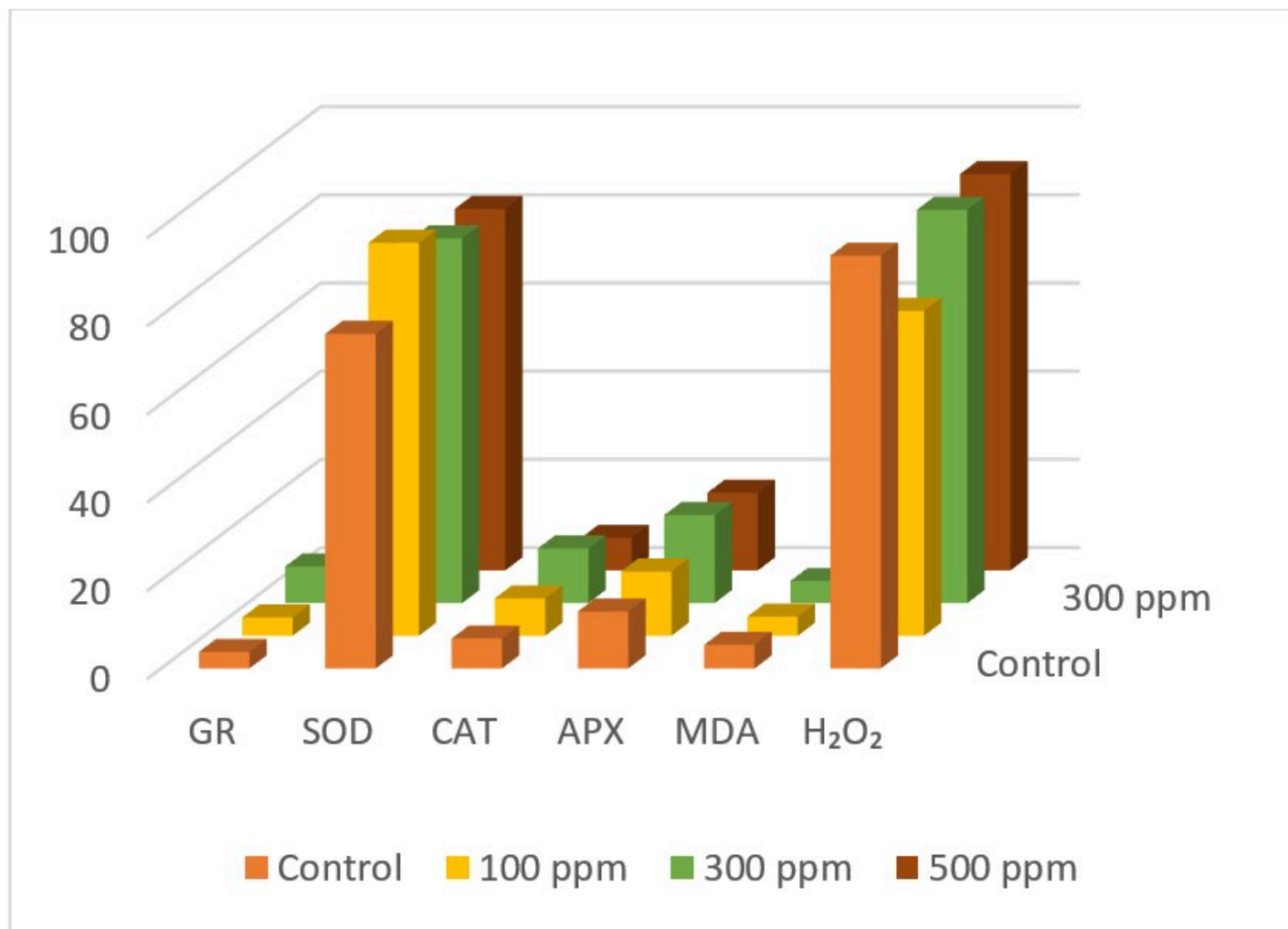


Figure 5

Changes in antioxidant enzyme activities and oxidative stress indicators in grape leaves at harvest under different SNP doses

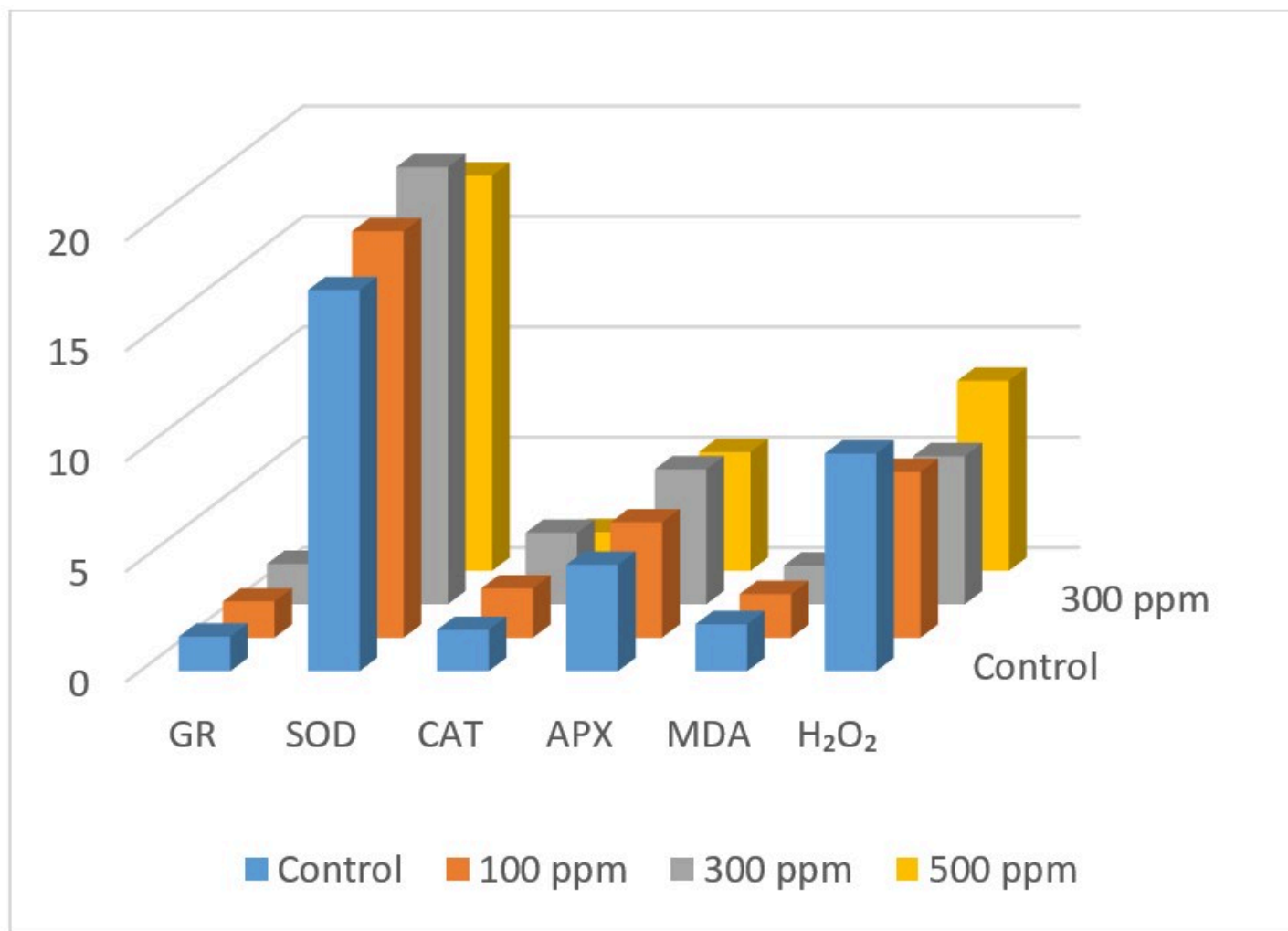


Figure 6

Changes in antioxidant enzyme activities and oxidative stress indicators in grape berries at harvest under different SNP doses

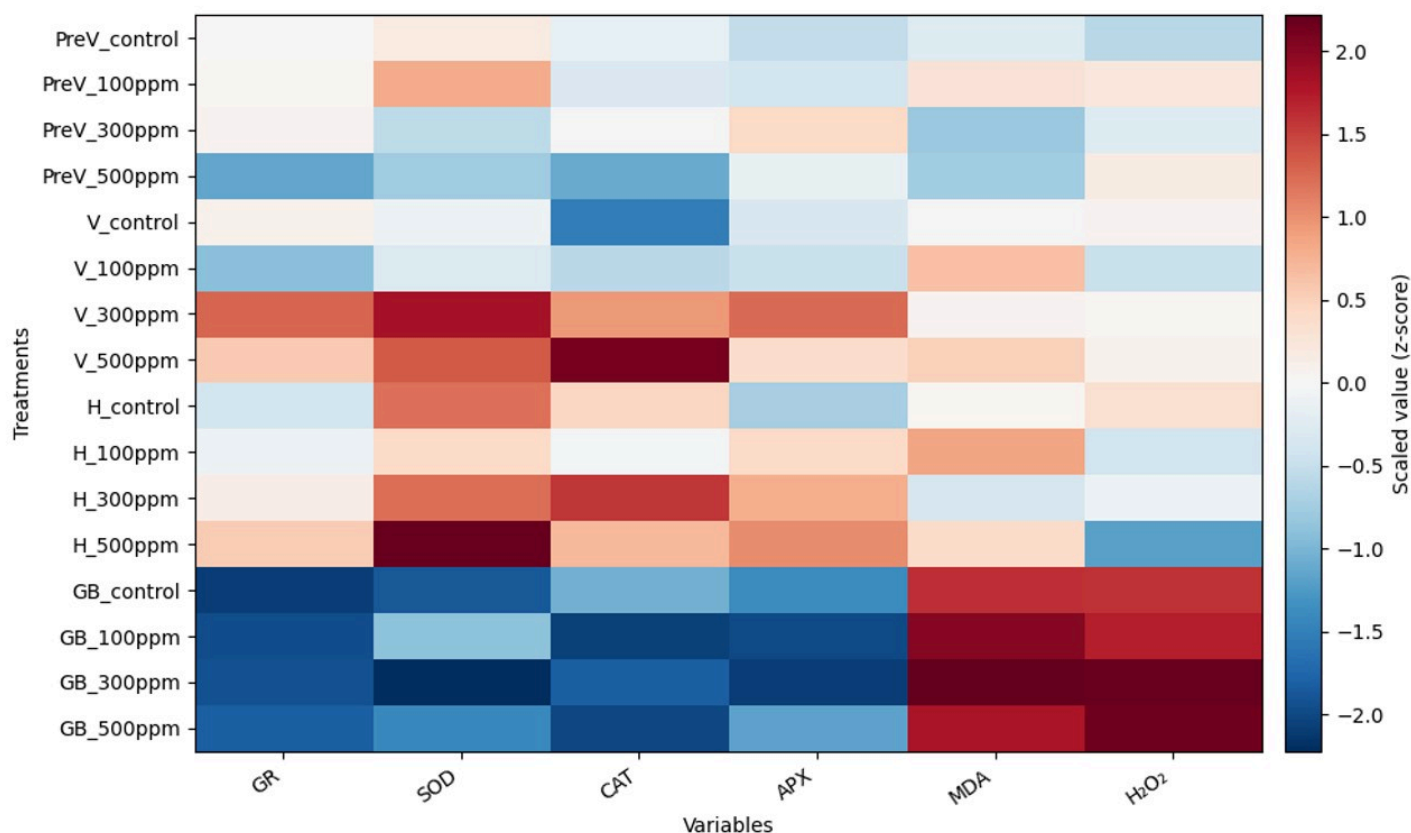


Figure 7

Heatmap illustrating the relative changes in antioxidant enzyme activities (GR, SOD, CAT, APX) and oxidative stress markers (MDA and H₂O₂) across developmental stages (pre-veraison, veraison, harvest) and grape berries under different SNP doses. Data were standardized using Z-score normalization to allow comparison among variables with different units and scales.

Supplementary Files

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