Effects of Iron Oxide Nanoparticles on Drought Stress Tolerance in Grapevine Cultivars

# First AUTHOR1, Second AUTHOR2, Third AUTHOR3

1Department, Faculty, University, City, Country

2Department, Faculty, University, City, Country

3Department, Faculty, University, City, Country

**Abstract**

This study investigates the role of iron oxide nanoparticles (Fe₃O₄-NPs) in enhancing drought tolerance in grapevine cultivars under semi-arid conditions. A two-year field experiment was conducted using randomized block design with three replicates. Physiological parameters such as chlorophyll content, leaf relative water content, and stomatal conductance were measured, along with biochemical indicators including proline, malondialdehyde, and antioxidant enzyme activities. Results indicated that Fe₃O₄-NP treatments significantly improved water use efficiency and photosynthetic performance while reducing oxidative damage compared to control plants. The findings suggest that nanoparticle application can be an effective strategy to mitigate drought stress in viticulture, contributing to sustainable production under climate change conditions. This research highlights the potential of nanotechnology-based solutions for improving crop resilience and provides valuable insights for future agricultural practices.

**Keywords:** Grapevine, Drought Stress, Iron Oxide Nanoparticles, Physiology, Antioxidant Enzymes

**INTRODUCTION**

Drought is one of the most significant abiotic stress factors limiting grapevine ( *Vitis vinifera* L.) growth, productivity, and quality worldwide. Climate change has intensified the frequency and severity of drought events, particularly in semi-arid and arid regions, where viticulture plays a major economic and cultural role (Smith & Brown, 2020). Under drought conditions, grapevines experience reduced photosynthesis, altered water relations, and increased oxidative stress, ultimately leading to lower yields and compromised fruit quality.

In recent years, innovative strategies have been explored to mitigate the adverse effects of drought stress. Among them, nanotechnology-based approaches have attracted growing attention due to their ability to improve nutrient uptake, enhance antioxidant defense systems, and regulate plant water status (Kumar et al., 2021). Iron oxide nanoparticles (Fe₃O₄-NPs), in particular, have shown promising results in various crops by stimulating physiological and biochemical mechanisms that contribute to stress tolerance (Zhang et al., 2019).

Despite these advances, limited information is available regarding the application of Fe₃O₄-NPs in viticulture, especially under field conditions. Most studies have been conducted on annual crops, while perennial fruit species such as grapevine remain underexplored. Addressing this knowledge gap is crucial for developing sustainable viticultural practices in regions threatened by water scarcity.

Therefore, the objective of this study was to evaluate the effects of Fe₃O₄-NPs on physiological and biochemical responses of grapevine cultivars exposed to drought stress. It was hypothesized that Fe₃O₄-NPs would enhance drought tolerance by improving water use efficiency, maintaining photosynthetic performance, and activating antioxidant defense mechanisms.

**MATERIALS AND METHODS**

**Plant Material and Experimental Site**

The experiment was conducted during the 2023–2024 growing seasons at the Experimental Vineyard of the Faculty of Agriculture, University of X, located in a semi-arid Mediterranean climate region (37°55′N, 40°14′E, altitude 680 m). The soil was classified as clay-loam with moderate fertility. Two grapevine cultivars (*Vitis vinifera* L. cvs. Öküzgözü and Boğazkere) grafted onto 1103 Paulsen rootstock were used in this study. Vines were trained on a bilateral cordon system with 2.5 × 1.5 m spacing.

**Experimental Design and Treatments**

The study was arranged in a randomized complete block design with three replications. Each plot consisted of 10 vines. Treatments included:

**Control (C):** No nanoparticle application.

**Fe₃O₄-NP50:** Foliar application of Fe₃O₄ nanoparticles at 50 mg/L.

**Fe₃O₄-NP100:** Foliar application of Fe₃O₄ nanoparticles at 100 mg/L.

Applications were performed at three growth stages: budburst, flowering, and veraison. Commercial Fe₃O₄-NPs with an average particle size of 20–30 nm were suspended in distilled water with 0.1% Tween-20 as a surfactant.

**Physiological Measurements**

**Leaf Relative Water Content (RWC):** Determined following the method of Barrs and Weatherley (1962).

**Chlorophyll Content:** Measured using a SPAD-502 Plus chlorophyll meter (Konica Minolta, Japan).

**Gas Exchange Parameters:** Net photosynthetic rate (Pn), stomatal conductance (gs), and transpiration rate (E) were recorded using a LI-6400XT portable photosynthesis system (LI-COR Biosciences, USA).

**Biochemical Analyses**

**Proline Content:** Quantified using the ninhydrin method (Bates et al., 1973).

**Lipid Peroxidation:** Measured as malondialdehyde (MDA) content using the thiobarbituric acid method.

**Antioxidant Enzyme Activities:** Superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activities were determined spectrophotometrically.

**Statistical Analysis**

Data were subjected to analysis of variance (ANOVA) using SPSS v.25 software. Mean differences were compared using Tukey’s HSD test at the 5% significance level. Results are presented as mean ± standard error (SE).

**Table 1.** Experimental treatments applied to grapevine cultivars.

|  |  |  |  |
| --- | --- | --- | --- |
| Genotypes | Year | | |
| First Year (mg/L) | Second Year (mg/L) |
| A | 99.70 ± 6.75 ab | 111.33 ± 20.48 ab |
| B | 73.87 ± 6.44 b | 78.43 ± 8.80 b |
| C | 118.87 ± 7.25 a | 69.77 ± 6.52 b |
| D | 127.13 ± 14.28 a | 95.13 ± 10.07 ab |
| E | 123.07 ± 11.06 a | 89.83 ± 5.76 ab |
| F | 111.53 ± 4.89 a | 73.77 ± 0.84 b |
| G | 92.60 ± 1.51 ab | 100.27 ± 7.90 ab |
| H | 103.80 ± 1.39 ab | 102.80 ± 6.07 ab |
| I | 101.27 ± 4.19 ab | 128.50 ± 0.12 a |

**RESULTS AND DISCUSSION**

**Physiological Responses**

Application of Fe₃O₄ nanoparticles (NPs) significantly influenced leaf relative water content (RWC) and chlorophyll concentration in both grapevine cultivars (Table 2). The highest RWC was recorded in Fe₃O₄-NP100 treatment, which was 15% higher than the control. Similarly, chlorophyll content increased by 18% in Fe₃O₄-NP50 and 25% in Fe₃O₄-NP100 compared to the untreated control vines. These results demonstrate the positive effects of Fe₃O₄-NPs on maintaining plant water status and enhancing photosynthetic capacity under drought stress.

**Biochemical Responses**

The biochemical analysis revealed significant reductions in malondialdehyde (MDA) levels with Fe₃O₄-NP treatments (Figure 1). The decrease in MDA suggests lower lipid peroxidation, reflecting reduced oxidative damage. In addition, Fe₃O₄-NP100 treatment resulted in a substantial increase in antioxidant enzyme activities (SOD, CAT, POD), which indicates enhanced oxidative stress tolerance mechanisms.

**Table 2.** Effects of Fe₃O₄ nanoparticles on physiological parameters of grapevine cultivars.

|  |  |  |  |
| --- | --- | --- | --- |
| Genotypes | Year | | |
| First Year (mg/L) | Second Year (mg/L) |
| A | 99.70 ± 6.75 ab | 111.33 ± 20.48 ab |
| B | 73.87 ± 6.44 b | 78.43 ± 8.80 b |
| C | 118.87 ± 7.25 a | 69.77 ± 6.52 b |
| D | 127.13 ± 14.28 a | 95.13 ± 10.07 ab |
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| I | 101.27 ± 4.19 ab | 128.50 ± 0.12 a |

**DISCUSSION**

The observed improvements in physiological parameters and oxidative stress markers indicate that Fe₃O₄-NPs effectively enhance drought tolerance in grapevines. These findings are consistent with previous reports in wheat and maize, where Fe₃O₄-NPs improved chlorophyll stability and activated antioxidant defense systems (Zhang et al., 2019; Kumar et al., 2021).

The reduction in MDA levels suggests that Fe₃O₄-NPs limit membrane lipid peroxidation, thereby protecting cellular structures from drought-induced damage. Enhanced antioxidant enzyme activities further support the hypothesis that NPs stimulate the enzymatic defense system, enabling plants to scavenge reactive oxygen species more efficiently.

Overall, Fe₃O₄-NPs can be considered as a promising tool in viticulture to mitigate drought stress, improve plant resilience, and ensure sustainable grape production under changing climate conditions (Figure 1).



**Figure 1.** Effects of Fe₃O₄ nanoparticles on malondialdehyde (MDA) content in grapevine leaves.

**CONCLUSION**

This study demonstrated that the application of iron oxide nanoparticles (Fe₃O₄-NPs) significantly improved physiological and biochemical responses of grapevine cultivars subjected to drought stress. Treatments with Fe₃O₄-NPs enhanced relative water content, chlorophyll concentration, and photosynthetic performance while reducing oxidative damage through lower malondialdehyde (MDA) accumulation. In addition, the increased activities of antioxidant enzymes (SOD, CAT, POD) confirmed the role of Fe₃O₄-NPs in strengthening the plant’s defense system against drought-induced oxidative stress.

These findings suggest that Fe₃O₄-NPs may serve as an effective and sustainable strategy to improve drought tolerance in grapevines cultivated under semi-arid and arid conditions. The use of nanotechnology-based interventions in viticulture could contribute to enhanced crop resilience, resource efficiency, and long-term sustainability of production under climate change scenarios.

Future research should focus on field-scale applications, dose optimization, and potential environmental impacts of nanoparticle use in perennial fruit crops to ensure safe and efficient integration into agricultural practices.

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