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FOLIAR APPLICATION OF ASCORBIC ACID AND GREEN-SYNTHESIZED NANO IRON FOR ENHANCING DROUGHT TOLERANCE AND ANTIOXIDANT DEFENSE IN COMMON BEANS

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Abstract: This study evaluated the effects of foliar-applied iron nanoparticles (FeNPs, 100 mg L^{-1}) and ascorbic acid (AsA, 400 mg L^{-1}) on the growth, photosynthetic pigments, and antioxidant defense mechanisms of common beans under optimal (100% FC) and water-restricted (50% FC) conditions. Under drought stress, both FeNPs and AsA significantly alleviated the negative impacts of water deficit, improving plant height, chlorophyll content, and carotenoid accumulation. FeNPs increased chlorophyll a by 60% and carotenoid content by 83.5%, while AsA enhanced ascorbate peroxidase activity (APX) activity by 44.8%, demonstrating its role in reducing oxidative stress. Additionally, FeNPs boosted catalase (CAT) and superoxide dismutase (SOD) activities by 198.2% and 17.3%, respectively. These treatments also significantly reduced malondialdehyde (MDA) concentration, with FeNPs-treated plants showing a 54.7% reduction compared to the control (P<0.01), indicating lower oxidative damage. This study is the first to use green-synthesized FeNPs in the context of global climate change, highlighting their potential in enhancing drought tolerance. Future research should explore the long-term effects of nanomaterials on human health and environmental safety.

Keywords: Biostimulants, Defense mechanism, Drought stress, Global warming, Green-synthesized nanoparticles

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1. Introduction

Climate change and the depletion of water resources have increasingly become critical issues, threatening agricultural production and global food security (Gosai et al., 2024). Among the many environmental stresses that plants encounter, drought stress is one of the most severe, particularly affecting water-sensitive crops such as the common bean (*Phaseolus vulgaris* L.) (Tapia et al., 2022). Drought conditions significantly reduce plant growth, physiological functions, and yield, posing a major challenge to sustaining crop productivity (Iqbal et al., 2020; Benlioğlu et al., 2024). In regions with limited access to irrigation, particularly in developing countries, this problem is exacerbated, affecting the primary protein source for millions of people, especially through legume consumption (Yilmaz et al., 2022; Kashem and Hossain, 2023). As a staple crop, the common bean is not only a key component of diets in these areas but also a vital tool in addressing hidden hunger, a form of malnutrition caused by micronutrient deficiencies (Yilmaz et al., 2023a; Rasheed and Azeem, 2024). Given its nutritional value and functional properties, enhancing the resilience of the common bean under water-limited conditions is essential for combating global food insecurity (Khatun et al., 2021; Ngalamu et al., 2023). Drought stress triggers a variety of biochemical and physiological responses in plants. One of the primary consequences is the overproduction of reactive oxygen species (ROS), which can cause oxidative damage to cellular structures, including lipids, proteins, and nucleic acids (Mansoor et al., 2022). As a result, the levels of malondialdehyde (MDA), a marker of lipid peroxidation, increase significantly under drought conditions (Shahid et al., 2022). To counteract oxidative stress, plants activate various antioxidant defense mechanisms, including enzymatic antioxidants such as ascorbate peroxidase (APX), superoxide dismutase (SOD), and catalase (CAT), which work synergistically to detoxify ROS (Sharma et al., 2022). Additionally, drought stress adversely affects the photosynthetic apparatus, leading to reductions in chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents, thereby limiting the plant's ability to convert light energy into chemical energy (Sharma et al., 2020). These physiological disruptions ultimately result in a decline in crop yield and quality, necessitating the exploration of innovative strategies to mitigate drought-induced damage. Numerous studies have explored strategies for improving plant tolerance to drought stress. For example, Shemi et al. (2021) investigated the effects of the exogenous application of salicylic acid, zinc, and glycine betaine, finding that it enhanced drought tolerance in wheat by mitigating oxidative damage and improving water retention. Similarly, Tayyab et al. (2020) reported that the application of salicylic acid and methyl jasmonate in maize under drought stress improved antioxidant enzyme activities and maintained chlorophyll content, leading to better growth performance. In the case of the common bean, Ahmad et al. (2018) demonstrated that foliar application of potassium increased drought tolerance by enhancing root development and photosynthetic efficiency. Moreover, Imran et al. (2021) highlighted the role of exogenous melatonin in modulating the antioxidant defense system and improving drought tolerance in soybeans, suggesting that biochemical interventions can significantly alleviate stress-induced damage. Considering these findings, the exploration of additional biochemical interventions, particularly those involving nanotechnology and antioxidant compounds, has gained traction in recent years. Recently, nanotechnology has emerged as a promising tool for enhancing plant resilience under abiotic stresses. Nano-iron particles have shown the potential to promote plant growth and stress tolerance by improving nutrient uptake and facilitating metabolic activities (Dola et al., 2022; Mazhar et al., 2023; Türkoğlu et al., 2023a). Green synthesis methods for producing nanoparticles have gained attention due to their eco-friendly nature (Priya et al., 2021; Santhosh et al., 2022), with *Salvia officinalis* (sage) being a notable example of a plant used for such purposes (Alrajhi and Ahmed, 2023). Additionally, ascorbic acid, a potent antioxidant, plays a critical role in protecting plants from oxidative damage by scavenging free radicals and maintaining cellular redox balance (Akram et al., 2017). Previous studies have shown that the application of ascorbic acid (Gaafar et al., 2020) and nano-iron chelate fertilizers (Fatollahpour Grangah et al., 2020; Ghasemi et al., 2022) increased secondary metabolite production, antioxidant activity, yield, and growth in bean plants under water stress. In this study, the effects of 50% water restriction were investigated on common bean plants, focusing on key biochemical and physiological parameters such as MDA levels, APX, SOD, and CAT activities, as well as chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents. To alleviate the detrimental effects of drought stress, foliar applications of nano iron (synthesized via green synthesis from sage) and ascorbic acid were administered. Our findings revealed that these treatments significantly improved the plants' tolerance to drought stress, highlighting their potential as effective tools for enhancing crop resilience under water-limited conditions. This research contributes to the growing body of knowledge on sustainable agricultural practices and offers practical solutions for improving the productivity of common beans in regions facing water scarcity.

2. Materials and Methods

2.1. Plant Material

The dwarf common bean variety "Yunus 90," obtained from the Eskişehir Transitional Zone Agricultural Research Institute, was used as the plant material. The study was conducted in the research greenhouse of the Faculty of Agriculture, Bolu Abant İzzet Baysal University, from April to June 2024."

2.2. Green-Biosynthesis of Fe Nanoparticles

The green synthesis of nano iron from *Salvia officinalis* leaves was carried out based on the method of Wang et al. (2015), with a few modifications. The preparation of the plant leaf extract involved grinding 100 g of *Salvia officinalis* leaves (collected from Bolu, Türkiye) followed by heating them in 500 mL of Milli-Q water at 80°C for 1 hour. After the mixture rested for an additional hour, it was filtered using a Whatman filter (No:1). Separately, a 0.1 M $FeCl_3$ solution was made by dissolving 16.23 g of $FeCl_3$ (Sigma–Aldrich) in 1.0 L of Milli-Q water. The leaf extract was then combined with the $FeCl_3$ solution in a 1:2 volume ratio. The pH of the solution was adjusted to 11 by gradually adding 0.1 M NaOH, after which the mixture was stirred for an additional 10 minutes at 80°C. It was then allowed to cool to room temperature. The solution turned black, indicating the formation of Fe nanoparticles. The nanoparticles were washed thrice with water and centrifuged (4000 rpm) to remove water-soluble impurities. The FeNPs were left in the oven (70°C) for 24 hours. The dried FeNPs were ground into powder using a porcelain mortar and stored in an airtight colored glass bottle in the refrigerator (+4°C) until use.

2.3. Experimental Design and Irrigation Management

Each pot, with a capacity of 1 kg, was filled with a mixture of two-thirds soil and one-third peat. Three seeds were sown per pot, and after germination, the seedlings were thinned to retain only one plant per pot. The experimental design consisted of three treatments: Control, Ascorbic Acid (400 mg L^{-1}), and Nano-Fe (100 mg L^{-1}), along with two irrigation regimes (100% Field Capacity and 50% Field Capacity). The ascorbic acid dosage was determined according to the protocol outlined by Gaafar et al. (2020), while the dosage for nano-Fe was adapted from Mahmoud et al. (2022). The experiment followed a randomized parcel design with three replications. Field capacity was determined by saturating the soil with water and allowing it to drain for 24 hours. The water retained by the soil particles against gravitational forces was measured to define 100% field capacity (Özel et al., 2016). Depending on ambient temperature, the pots were weighed every 2–3 days to calculate the water lost through evaporation and transpiration, and water was added to restore the pots to their designated field capacity levels. The 50% field capacity irrigation treatment was gradually introduced after the common bean seedlings developed true leaves. Following the initiation of the water restriction treatment, foliar applications of ascorbic acid and nano-iron were conducted four times during the growing period. To ensure precise application, the soil surface in each pot was covered during spraying, and the plants were transferred to a wind-free environment. The plants were harvested after a 5-week growth period, and leaf samples were immediately stored at -80 °C for subsequent analysis.

2.4. Physical Analyses, Chlorophyll, and Carotenoid Contents

Plant growth parameters such as stem length (cm), was recorded for each plant as part of the physical analysis. Total chlorophyll, carotenoid content, and chlorophyll a and b were determined using Arnon's method (Arnon, 1949). For chlorophyll analysis, 0.1 g of leaf tissue was homogenized in 80% acetone, and absorbance was measured at 663, 645, and 470 nm using a UV-visible spectrophotometer. For carotenoid analysis, 100 mg of leaf tissue was homogenized in 80% (v/v) acetone and filtered through filter paper. The absorbance of the filtrate was measured at 470 nm to determine carotenoid content. Carotenoid, chlorophyll a, chlorophyll b, and total chlorophyll concentrations were expressed in mg/g fresh weight using the following formulas (Equation 1-4):

$$\text{Carotenoid (mg g}^{-1}\text{)} = [((1000 \times A470) - (2.27 \times Chla) - (81.4 \times Chlb)) / 227] \times V / g \quad (1)$$

$$\text{Chlorophyll a (mg g}^{-1}\text{ F.W.)} = (12.7 \times A663 - 2.69 \times A645) \times V / 1000 \times g \quad (2)$$

$$\text{Chlorophyll b (mg g}^{-1}\text{ F.W.)} = (22.9 \times A645 - 4.68 \times A663) \times V / 1000 \times g \quad (3)$$

$$\text{Total chlorophyll (mg g}^{-1}\text{ F.W.)} = (20.2 \times A645 + 8.02 \times A663) \times V / 1000 \times g \quad (4)$$

Here, V represents the extract volume, g refers to the sample weight, Chla stands for chlorophyll a, Chlb for chlorophyll b, and A represents absorbance at specified wavelengths.

2.5. Malondialdehyde (MDA) Analysis

Lipid peroxidation levels were determined by measuring malondialdehyde (MDA) content, a key indicator of lipid peroxidation. A 500 mg plant sample was homogenized in 10 mL of 0.1% trichloroacetic acid (TCA), and the homogenate was centrifuged at 15.000 rpm. From the supernatant, 1 mL was mixed with 4 mL of a reaction mixture containing 20% TCA and 0.5% thiobarbituric acid. The samples were incubated at 95 °C for 30 minutes and then rapidly cooled. Absorbance was measured at 532 and 600 nm (Sairam and Saxena, 2000).

2.6. Ascorbate Peroxidase (APX), Catalase (CAT), and Superoxide Dismutase (SOD) Analysis

Ascorbate peroxidase (APX) activity was measured by monitoring the decrease in absorbance at 290 nm. A 200 mg sample was homogenized with 2 mL of extraction buffer (0.1 M sodium phosphate, 0.5 mM sodium EDTA, and 1 mM ascorbic acid) and centrifuged at 15.000 rpm. The reaction mixture for APX consisted of 50 mM sodium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, and 0.1 mM EDTA. To this, 0.1 mL of extract and 0.1 mL of 0.1 mM H_2O_2 were added, and the reaction was allowed to proceed for 60 minutes. The activity was compared to a standard curve of ascorbic acid (Yilmaz and Kulaz, 2019). Superoxide dismutase (SOD) activity was determined by measuring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT), following the method of Beauchamp and Fridovich (1971). A 200 mg sample was homogenized in extraction buffer (0.1 M sodium phosphate and 0.5 mM sodium EDTA), and the mixture was centrifuged at 15,000 rpm. For SOD activity measurement, 0.1 mL of the supernatant was added to a reaction mixture containing methionine, NBT, EDTA, sodium phosphate buffer, sodium carbonate, and riboflavin. The reaction was initiated by exposing the mixture to light (75 mol m^{-2} s^{-1} (40 W)) for 15 minutes, and absorbance was read at 560 nm. Catalase (CAT) activity was assessed using a reaction mixture of 0.036% hydrogen peroxide and 50 mM sodium phosphate buffer (pH:7). Three mL of this solution was placed in a quartz cuvette, and 100 µl of the supernatant from the SOD extraction was added. Absorbance was recorded at 240 nm after 0 and 60 seconds (Beers and Sizer, 1952).

2.7 Statistical Analysis

The experiment was designed in randomized parcel with three biological replicates and three technical replicates per treatment. A one-way analysis of variance (ANOVA) was used to determine the effects of water restriction and treatments. Post-hoc comparisons between control and treatments were performed using the LSD test. Correlation analyses were conducted to explore the relationships between yield parameters and antioxidant enzyme activities under water stress, using Pearson's coefficient. Data were visualized with the 'corrplot' R package (Wei et al., 2017). Principal component analysis (PCA) was performed to examine the interactions between water-restricted treatments and measured variables, using the 'ggplot2' R package (Wickham, 2016).

3. Results and Discussion

3.1. Plant Growth and Photosynthetic Pigments

ANOVA results of FeNPs and AsA applications on common beans grown under different water regimes (100% and 50% FC) revealed significant differences among treatments across various parameters (Table 1). The study demonstrated that common bean plants subjected to water-restricted conditions (50% FC), showed significant reductions in plant height compared to those under a normal (100% FC) irrigation regime (Figure 1). Foliar applications of FeNPs and AsA had a notable impact on plant height across both irrigation treatments ($P<0.05$). Under the 100% FC treatment, plant height increased by 26.9%, from 19.16 cm to 24.33 cm, while under 50% FC irrigation, plant height rose by 34.7%, from 14.03 cm to 18.9 cm, compared to the control. Photosynthetic pigment analysis revealed significant differences ($P<0.05$) among all measured parameters. Under 100% FC conditions, chlorophyll a level ranged from 0.0030 to 0.0133 mg g^{-1} F.W., chlorophyll b levels ranged from 0.0024 to 0.0067 mg g^{-1} F.W., the chlorophyll a/b ratio varied between 2.33 and 1.25, total chlorophyll content ranged from 0.0056 to 0.0198 mg g^{-1} F.W., and total carotenoid content ranged from 146.37 to 329.76 mg g^{-1} F.W. Under water-restricted conditions, chlorophyll a value ranged from 0.010 to 0.016 mg g^{-1} F.W., chlorophyll b values ranged from 0.004 to 0.015 mg g^{-1} F.W., the chlorophyll a/b ratio fluctuated between 0.68 and 2.58, total chlorophyll content ranged from 0.015 to 0.025 mg g^{-1} F.W., and total carotenoid content varied between 249.27 and 457.47 mg g^{-1} F.W. The application of FeNPs under 100% FC conditions significantly enhanced the levels of chlorophyll a, chlorophyll b, chlorophyll a/b ratio, total chlorophyll, and carotenoid content in common bean plants compared to the control group. Specifically, chlorophyll an increased by 343.3%, chlorophyll b by 179.2%, chlorophyll a/b ratio by 56%, total chlorophyll content by 253.6%, and total carotenoid content by 125.2%.

Table 1. Effects of water-restricted conditions (100% and 50% FC), FeNPs, and AsA treatments on measured parameters and statistically significant differences

Trait	$F_{Half\ Irrigation}$	$F_{Full\ Irrigation}$	$F_{Irrigation*Treatment}$
Plant Height	20.43**	19.68**	0.04^{ns}
Chlorophyll a	96.53**	355.70**	20.13**
Chlorophyll b	196.48**	157.32**	229.38**
Chlorophyll a/b	59.46**	15.90**	7.13**
Total Chlorophyll	87.75**	687.69**	184.51**
Total Carotenoid	2652.99**	814.64**	27.19**
MDA	24.17**	14.01**	5.62*
SOD	17.52**	1454.15**	177.72**
CAT	3957.73**	19.83**	1368.86**
APX	31.44**	172.22**	4.07*

Indicate significant differences according to LSD test; ns= non-significant, *= (P<0.05), ** (P<0.01).

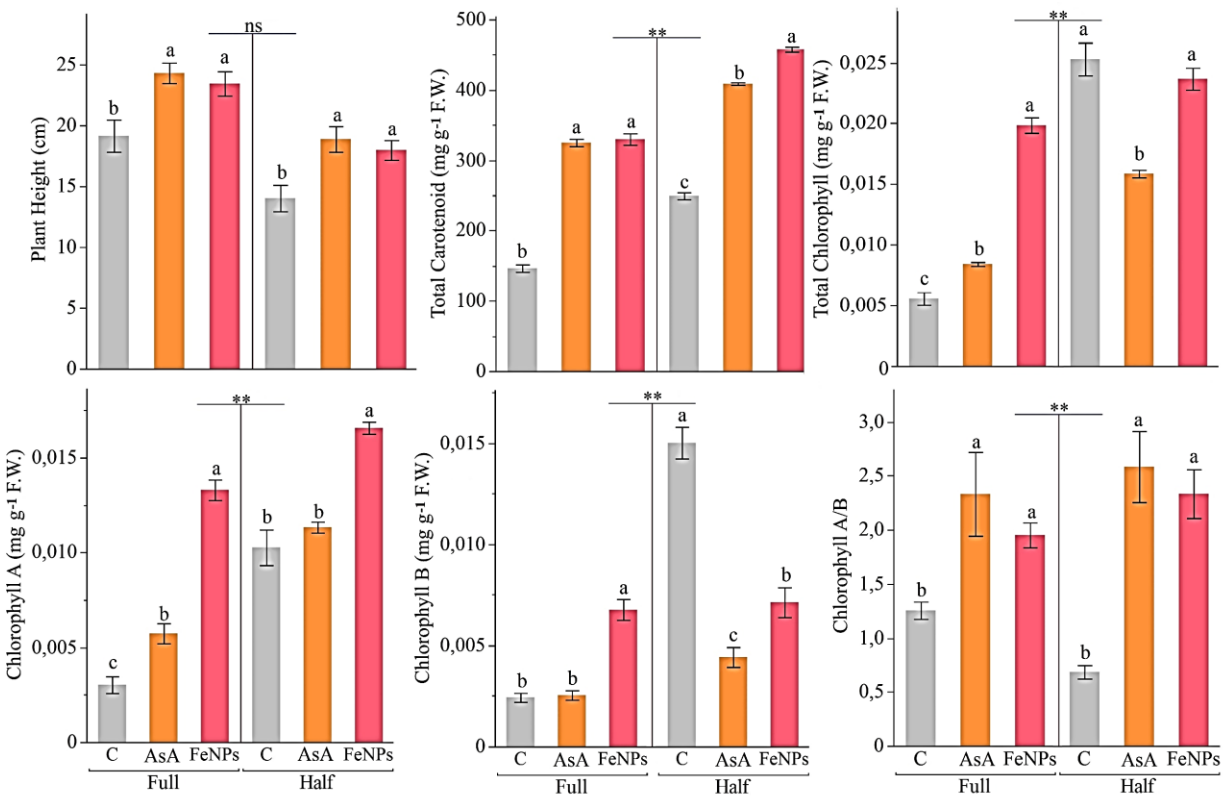

Figure 1. Effect of FeNPs (Iron Nanoparticles) and AsA (Ascorbic Acid) on plant height, chlorophyll a, b, a/b, total chlorophyll, and total carotenoid content of common bean under different irrigation regimes (100% FC- 50% FC) conditions. (C= control, different letters on the top of the bars indicate significant differences according to the LSD test, ns= non-significant, **= P<0.01.

Under water-restricted conditions, FeNPs treatment also led to significant improvements in chlorophyll a, the chlorophyll a/b ratio, total chlorophyll, and carotenoid content compared to the control group, with increases of 60% for chlorophyll a, 242.6% for the chlorophyll a/b ratio, 66.7% for total chlorophyll, and 83.5% for carotenoid content. Under water-restricted conditions, FeNPs treatment led to significant improvements in chlorophyll a, the chlorophyll a/b ratio, total chlorophyll, and carotenoid content compared to the control group, with increases of 60% for chlorophyll a, 242.6% for the chlorophyll a/b ratio, 66.7% for total chlorophyll, and 83.5% for carotenoid content. However, chlorophyll b content, which often serves as an indicator of plant stress, did not show a similar increase. Chlorophyll b levels were higher in the control group, and a significant reduction was observed with the treatments. The most pronounced decrease, 73.3%, occurred with the ascorbic acid treatment

In this study, the effects of FeNPs (iron nanoparticles) and ascorbic acid treatments on the physiological and biochemical responses of common beans under optimal and water-restricted conditions were investigated. Abiotic stress typically reduces chlorophyll content, which significantly impairs photosynthetic capacity. This decline is likely due to stress-induced inhibition of chlorophyll synthesis and its accelerated degradation (Chen et al., 2024). Water scarcity severely disrupts leaf gas exchange by causing stomatal closure, limiting tissue growth, reducing biomass production, lowering transpiration rates, and ultimately diminishing photosynthesis (Chieb and Gachomo, 2023). These findings align with the current study, where water-restricted conditions led to similar physiological responses in common bean plants. The significant reduction in plant height observed under drought stress is consistent with prior studies highlighting the detrimental effects of water deficit on plant growth and development (Ahmadikhah and Marufinia, 2016; Sofi et al., 2018; Torabian et al., 2018). Reduced plant height is often seen as an adaptive response in drought-tolerant species, as it helps minimize water loss and allows the plant to prioritize resources towards root development and other survival mechanisms (Seleiman et al., 2021). The increase in plant height following FeNPs and ascorbic acid treatments, even under drought stress, suggests that these treatments effectively mitigated the negative impact of water scarcity (Afshar et al., 2013; Khazaei et al., 2020; El Amine et al., 2024). Chlorophyll content, a key indicator of photosynthetic efficiency, showed significant improvement with FeNPs treatment under both irrigation regimes. The notable increase in chlorophyll-a and total chlorophyll content in FeNPs treated plants supports previous findings that nanoparticles enhance chlorophyll synthesis by improving iron bioavailability, which is essential for chlorophyll molecule formation (Manzoor et al., 2021; Alabdallah et al., 2021; Zia-ur-Rehman et al., 2023). Iron is a critical cofactor for the enzyme protochlorophyllide reductase, a key enzyme in the chlorophyll biosynthesis pathway (Kobayashi et al., 2019). Additionally, FeNPs treatment may enhance the overall photosynthetic apparatus, as evidenced by the observed increase in carotenoid content (Bidi et al., 2021). Nanoparticles can penetrate chloroplasts and reach the photosystem-II (PS-II) reaction center, enhancing electron transport and light absorption, particularly under drought stress. This improves photosynthetic efficiency and promotes plant growth (Maity et al., 2018). The role of nanoparticles in improving photosynthesis and chlorophyll production, and their ability to mitigate drought stress damage, is well-supported by the literature (Rasheed et al., 2022). Furthermore, the increase in carotenoid content underscores their protective function, as carotenoids play a key role in quenching reactive oxygen species (ROS) and maintaining membrane integrity, which is vital under stress conditions (Emiliani et al., 2018; Van Nguyen et al., 2022). These findings highlight the positive effects of FeNPs and ascorbic acid treatments on the photosynthetic capacity and growth of common bean plants under water-restricted stress conditions, emphasizing the beneficial role of green synthesis nanoparticles in plant physiology.

3.2. Plant Defense Mechanisms in Response to Drought Stress

In this study, MDA (malondialdehyde) accumulation in common beans under the normal irrigation regime ranged from 11.88 to 17.37 nmol g^{-1} F.W., with the highest levels recorded in the control group (Figure 2, Table 1). Under the water deficit irrigation regime, MDA levels increased, ranging from 17.53 to 27.13 nmol g^{-1} F.W. Significant differences ($P<0.01$) were observed between the control and the other treatments, although no statistical difference was found between the FeNPs and AsA treatments. MDA accumulation in the control plants was notably 54.7% higher than in plants treated with FeNPs. These results suggest that FeNPs and AsA foliar applications are highly effective in mitigating oxidative damage in common beans under drought stress. Ascorbate peroxidase (APX) activity under 100% FC ranged from 452.93 to 674.67 µmol g^{-1}, catalase (CAT) activity from 86.32 to 128.09 U mL^{-1}, and superoxide dismutase (SOD) activity from 662.11 to 1081.45 U mL^{-1} F.W. Statistically significant differences ($P<0.05$) were observed for all treatments.

FeNPs application boosted CAT activity by 48.4% compared to the control, while AsA treatment enhanced APX and SOD activities by 48.9% and 78.4%, respectively. Under 50% FC irrigation, APX activity ranged from 565.49 to 818.9 µmol g^{-1}, CAT activity from 247.54 to 738.08 U mL^{-1}, and SOD activity from 727.02 to 852.82 U mL^{-1} F.W. Significant differences ($p \leq 0.05$) were observed among all treatments. AsA application resulted in the highest increase in APX activity, with a 44.8% increase compared to the control.

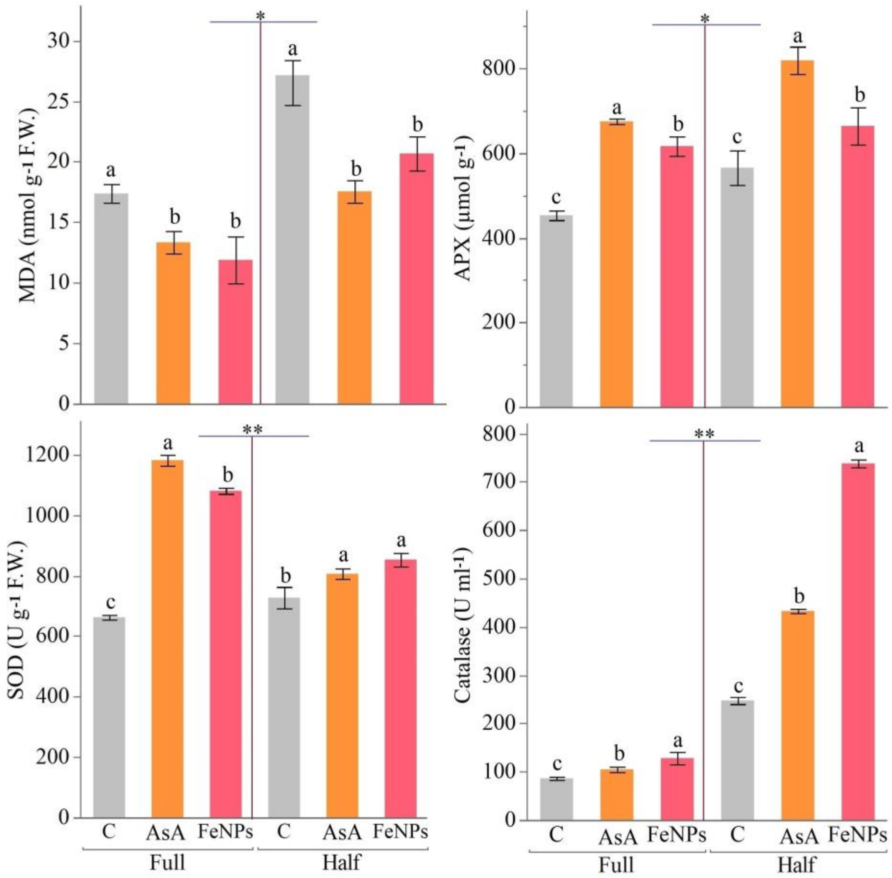

Figure 2. Effect of FeNPs and AsA on malondialdehyde (MDA) levels, superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) activities in common bean under different irrigation regimes (100%-50%) conditions. (C= control, different letters on the top of the bars indicate significant differences according to LSD test, *(P<0.05), ** (P<0.01).

FeNPs application resulted in significant increases in CAT and SOD activities, with rises of 198.2% and 17.3%, respectively.

The findings of this study highlight the potential of foliar applications of FeNPs and AsA to mitigate oxidative stress in common beans under drought conditions. MDA accumulation, an indicator of lipid peroxidation and oxidative damage, significantly increased under water deficit conditions (Canal et al., 2023; Yilmaz et al., 2023b; Kavas et al., 2013). However, applying FeNPs and AsA effectively reduced MDA levels, with FeNPs-treated plants exhibiting a 54.7% lower MDA concentration compared to the control group. This reduction in MDA is consistent with previous studies indicating that iron nanoparticles (FeNPs) can improve the oxidative stress tolerance of plants by enhancing the activity of the antioxidant system (Shah et al., 2022; Faizan et al., 2022; Manzoor et al., 2023). Similarly, AsA, known for its potent antioxidant properties, has been reported to play a critical role in scavenging reactive oxygen species (ROS) and reducing oxidative damage in plants exposed to abiotic stress (Noman et al., 2015; Akram et al., 2017). The enzymatic antioxidant system, including APX, CAT, and SOD, was significantly modulated by the treatments. Under optimal irrigation conditions, FeNPs application led to a 48.4% increase in CAT activity, while AsA treatment significantly enhanced APX and SOD activities by 48.9% and 78.4%, respectively. These enzymes are crucial in ROS detoxification pathways, with CAT being primarily responsible for decomposing hydrogen peroxide (H_2O_2) into water and oxygen (Anwar et al., 2024), and APX catalyzing the reduction of H_2O_2 to water using ascorbate as an electron donor (Anjum et al., 2016). The increases in enzyme activities observed in this study align with previous research, demonstrating the positive effects of FeNPs on enhancing CAT activity, thereby protecting plants from oxidative stress (Ngan et al., 2020). Additionally, the role of AsA in upregulating APX and SOD activities has been well-documented, as it not only functions as a substrate for APX but also stabilizes SOD in detoxifying superoxide radicals (Hassan et al., 2021; de Cássia Alves et al., 2022). Under drought conditions (50% FC), enzyme activities increased, reflecting the plants' heightened reliance on antioxidant defenses to combat stress-induced ROS. Ascorbic acid (AsA) application led to the highest rise in APX activity, with a 44.8% increase compared to the control, aligning with previous studies that highlight AsA's role in maintaining the efficiency of the ascorbate-glutathione cycle under stress (Semida et al., 2021). Additionally, FeNPs significantly enhanced CAT and SOD activities by 198.2% and 17.3%, respectively, under water deficit conditions. This supports Mahmoud et al. (2022), who reported that FeNPs alleviate drought stress by boosting antioxidant enzyme activities, aiding in ROS detoxification. FeNPs have also been shown to improve nutrient uptake and physiological health under stress, contributing to better growth and yield (Zia-ur-Rehman et al., 2023; Sun et al., 2023). These findings highlight the potential of FeNPs and AsA as effective strategies for enhancing drought tolerance in common beans.

3.3. Relationships between Principal Components and Correlation Analysis

Principal Component Analysis (PCA) was employed to reduce the dimensionality of the dataset and reveal its underlying structure, facilitating a more comprehensive understanding of the relationships among variables (Demirel et al., 2021; Türkoğlu et al., 2023b). The PCA analysis highlights significant physiological responses under different treatments (Figure 3). The first two components explained a substantial portion of the data variability, with PC1 (42%) and PC2 (39.1%). Antioxidant enzymes such as APX, CAT, and pigments like Total Chlorophyll and Carotenoids were positively correlated with FeNPs and 50% irrigation, suggesting these treatments enhance antioxidant defense and pigment accumulation, likely as protective mechanisms against drought stress. Ascorbic acid (AsA) treatment notably increased SOD and APX activities, supporting its role in mitigating oxidative stress, as previously reported in the literature. Full irrigation mainly promoted vegetative growth, reflected in the association with plant height (PH), while not significantly influencing antioxidant activity. In contrast, control plants showed higher MDA levels and reduced antioxidant activity, indicating greater susceptibility to oxidative damage. These findings underscore the effectiveness of FeNPs and AsA in improving drought tolerance through enhanced antioxidant systems and photosynthetic pigment protection, while untreated plants experience more oxidative stress and damage.

The correlation analysis revealed several important relationships between physiological and biochemical parameters (Figure 4). A strong negative correlation between plant height (PH) and MDA (-0.91) suggests that increased oxidative stress, as indicated by higher MDA levels, leads to reduced growth. Additionally, chlorophyll pigments (CHLA, CHLB, and TCHL) showed significant positive correlations with each other, highlighting the coordinated response of the photosynthetic system under drought stress. Antioxidant enzymes, particularly SOD and APX, were negatively correlated with MDA (-0.69 and -0.17, respectively), indicating their crucial role in mitigating oxidative damage and protecting cellular structures (Rajput et al., 2021). The positive correlation of APX with CHLA (0.47), CHLA/B (0.75), and total carotenoids (0.81) further suggests that ascorbate peroxidase activity helps maintain photosynthetic efficiency under stress conditions (Asgher et al., 2021). These results underscore the importance of antioxidant enzyme activity in reducing oxidative stress and preserving chlorophyll and carotenoid content, contributing to the plant's overall resilience under drought.

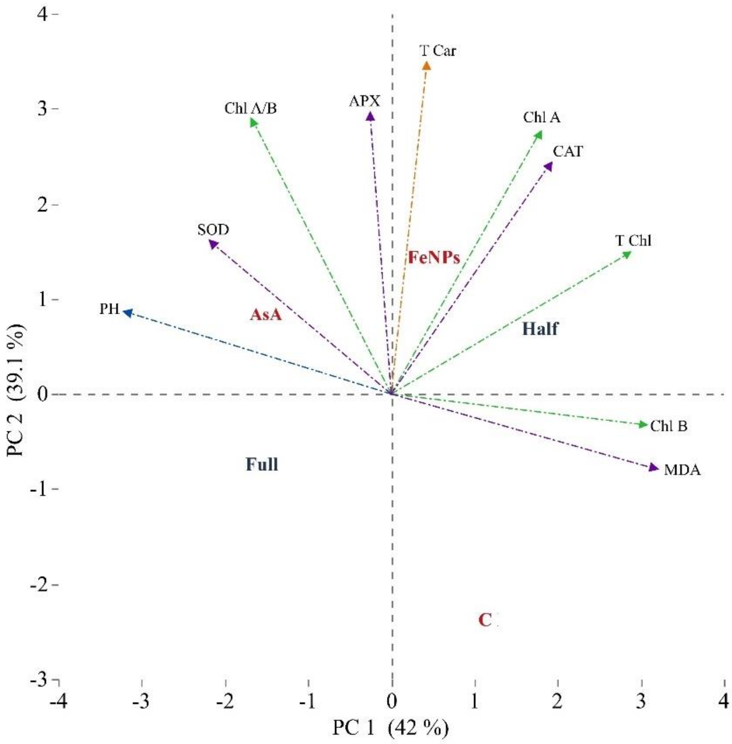

Figure 3. The biplot from PCA analysis illustrates the distribution of the FeNPs and AsA treatments. The variables included in the analysis are Full= 100% FC, half= 50% FC, C= control, PH= plant height, Chl A= chlorophyll a, Chl B= chlorophyll b, Chl A/B= chlorophyll a/b, T Chl= total chlorophyll, T Car= total carotenoid, SOD= superoxide dismutase, APX= ascorbate peroxidase, CAT= catalase, MDA= malondialdehyde.

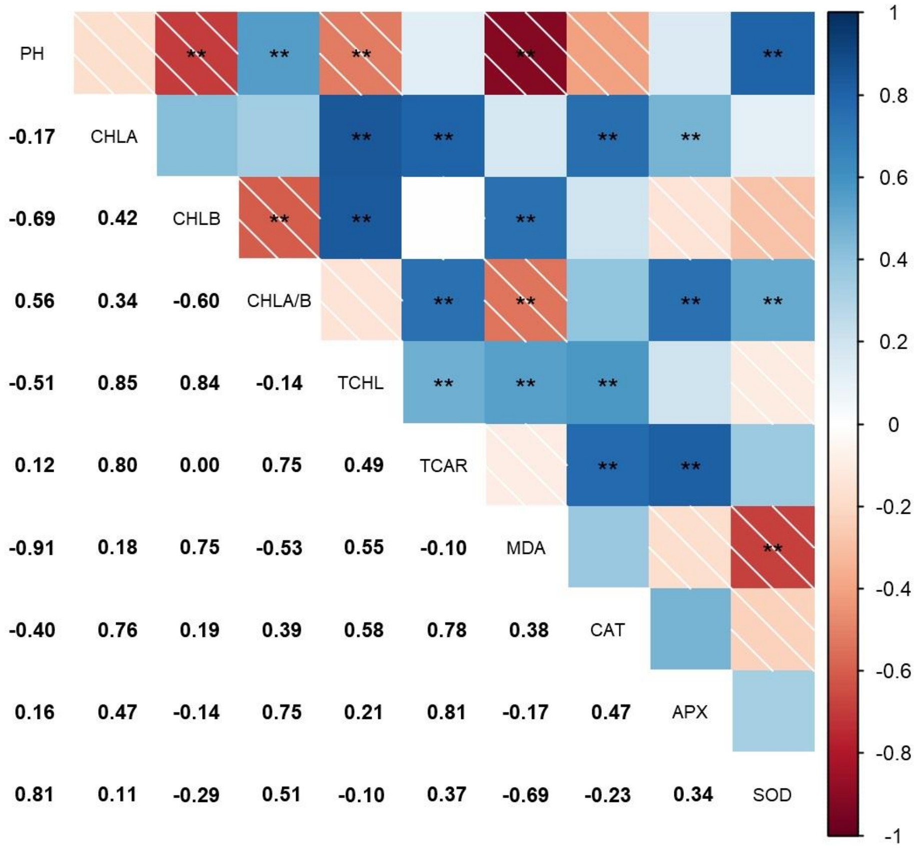

Figure 4. Correlations between the studied characteristics in common bean. *, and ** indicates significance at P<0.05, P<0.01, respectively (PH= plant height, CHLA= chlorophyll B, CHLB= chlorophyll b, CHLA/B= chlorophyll A/B, TCHL= total chlorophyll, TCAR= total carotenoid, SOD= superoxide dismutase, APX= ascorbate peroxidase, CAT= catalase, MDA= malondialdehyde.).

4. Conclusion

The findings of this study clearly demonstrate that the application of FeNPs and ascorbic acid (AsA) significantly mitigates the negative effects of water deficit in common beans. The FeNPs, particularly those synthesized through green methods, proved highly effective in enhancing plant resilience by promoting antioxidant activity and preserving photosynthetic pigments, resulting in improved growth and physiological stability under drought conditions. AsA further amplified the plants' antioxidant defenses, reducing oxidative stress and preventing damage caused by reactive oxygen species. These results underscore the potential of FeNPs and AsA as practical solutions for enhancing crop tolerance to drought, a critical benefit in the context of increasing water scarcity due to global climate change.

Moreover, this study represents the first comprehensive evaluation of green-synthesized iron nanoparticles in the context of drought stress, offering a sustainable and eco-friendly approach to agricultural management. While these findings are promising, the broader implications of nanomaterials, particularly their potential impacts on human health and environmental safety, warrant further investigation. Addressing these concerns in future research will be essential to fully harness the benefits of nanotechnology in sustainable agriculture while ensuring long-term safety and viability.

Author Contributions

The percentage of the author(s) contributions is presented below. The author reviewed and approved the final version of the manuscript.

	H.Y.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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