

Biofortification of Iron induced drought tolerance in mung bean (*Vigna radiata* L.) by modulating the antioxidant defense system

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Abstract

Purpose

Drought is a major abiotic stressor having a detrimental effect on morphology and development of plants. However, Iron (Fe) acts as an acute stress signal, enhancing nutrient absorption under drought stress. The objective of this study was to evaluate the impact of foliar application of Fe to mitigate the toxic effects of drought stress in mung bean.

Methods

A pot experiment was conducted by using two mung bean varieties, Pre-Mung 2018 and LS-442, four treatments: Control (No spray), WS (Distilled water spray), Iron (0.5% Fe), and Iron (1% Fe), and two moisture levels maintained: 50% FC and 100% FC.

Results

Drought stress reduced shoot length, shoot fresh weight, and root fresh weight up to 18%, 33%, and 18% while increased the root length up to 21.7% while foliar applications of Fe (1% Fe) improved these parameters up to 51%, 79%, 76%, and 63.3%, respectively. Among the antioxidants, Fe application improved superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) by 5.9%, 11.7%, and 6%, respectively. Furthermore, foliar application of Fe improved the accumulation of root Fe up to 12.6 and shoot Fe up to 81.8%, respectively. Among both varieties of mung bean, Pre-Mung 2018 outperformed LS-442 in terms of plant morpho-physiological, biochemical attributes, and nutrient absorption under drought-stressed conditions.

Conclusion

In crux, Fe applications in mung bean decreased the toxicity of drought stress by modifying the plant's antioxidant defense system.

Introduction

The mung bean (*Vigna radiata* L.), which is grown on more than 6 million hectares globally (about 8.5% of the world's pulse area) and mostly consumed in Asian homes, is one of the most important edible legume crops (Dikr, 2023). The mung bean is widely grown in many Asian countries (concentrated mainly in China, India, Bangladesh, Pakistan, and some Southeast Asian countries), as well as in dry regions of southern Europe and warmer parts of Canada and the United States, due to its characteristics of being a relatively drought-tolerant, low-input crop, and short growth cycle (70 days or so) (Haq et al. 2023). For many generations, the mung bean has been an essential part of China's and India's mostly cereal-based diets (Kagnew et al. 2022; Ul Haq et al. 2023).

Protein, dietary fibre, minerals, vitamins, and high concentrations of bioactive substances make up the mung bean's well-balanced nutritional profile (Gan and others, 2017). For those who are vegetarians or cannot afford animal proteins, the mung bean is a good and reasonably priced source of protein (Dhaliwal et al. 2023).

Prolonged insufficient water supply is a hallmark of drought stress (Ramzan et al. 2023; Haider et al. 2026b). Transpiration, cellular water content, and the plant's capacity to take in more water are all disrupted by drought (Ramzan et al. 2025; Ahmad et al. 2024). Additionally, drought stress impairs important plant metabolic processes, including photophosphorylation, electron transport, and protein synthesis, which eventually stunts growth and development (Naderi et al. 2020). Common signs of drought stress in plants include etiolation, turgidity, wilting, senescence, premature fall, rolling and brittleness of the leaves, burnt and limp leaves, sagging and closed blooms, and yellowing of the leaves (Haider et al. 2026).

Drought stress has also been demonstrated to lower yields in a number of crops, including broad beans (*Vicia faba*), mustard (*Brassica napus*) (Ramzan et al. 2023), wheat (*Triticum aestivum*) (Ishfaq et al. 2024; Zhao et al. 2020), and maize (*Zea mays*) (Danish et al. 2020; Batool et al. 2022b). To combat drought, plants can develop a variety of defence mechanisms. As part of their ROS-scavenging defence mechanisms, plants produce both enzymatic and non-enzymatic antioxidants to fend against the damaging effects of drought (Abdullah et al. 2025; Shahid et al. 2022).

Iron (Fe) is also important for plant germination and flowering, in addition to being necessary for many cellular functions. Moreover, it regulates the synthesis of nucleotides, mitochondrial respiration, plant photosynthesis, metal homeostasis, and other functions (Tripathi et al. 2018; Dhaliwal et al. 2023). Iron is a necessary micronutrient because of its importance for metabolic processes like respiration, photosynthesis, and DNA synthesis. Iron is a metabolic process activator that also stimulates several other metabolic processes and is a part of the prosthetic groups of many enzymes (Molnár et al. 2023).

By encouraging assimilate synthesis, iron (Fe) nutrition during drought conditions can increase plants' capacity to tolerate stress (Visha Kumari et al. 2020). Furthermore, Fe is essential for photosynthesis, nitrogen fixation, sulphur absorption, and plant respiration. It functions as a crucial component of proteins that aid in oxygen transport and control cell growth and differentiation. Fe shortage diet leads to reduced oxygen supply to cells resulting in weariness, impaired immunity, and an increased risk of blood anaemia (Distéfano et al. 2021; Chen et al. 2022). Additionally, a lack of iron can cause product to deteriorate and crops to develop chlorosis (Dissanayaka et al. 2021).

Regardless of previous studies on the role of Fe in alleviating drought stress, there remains a substantial gap in knowing the particular mechanism through which exogenous applications of Fe might ameliorate drought stress in mung bean. Based on the available information, it is hypothesized that applying Fe to mung bean plants may lessen the negative effects of drought stress by modifying morpho-physiological and ionic characteristics. Iron applied topically significantly improved all low-level factors, hence improving yield qualities under drought stress. The study's objective are to ascertain how foliar iron application affects the growth and physiology of mung beans under drought stress and to assess the significance of the morpho-physiological and biochemical characteristics of mung beans under drought stress.

Materials and methods

Experimental Site

Two mung bean varieties Pre-Mung 2018 and LS-442 were obtained from Ayub Agriculture Research Institute Faisalabad. A pot experiment was conducted to ascertain the effects of foliar sprayed iron on the physiology, biochemistry, and root properties of mung bean under drought stress. The three treatments Control (No spray), Ws (Distilled water spray), Iron (0.5% Fe), and Iron (1% Fe), were used on two different mung bean varieties, LS-4422 and Pre-Mung, during the vegetative period. There were two moisture levels maintained: 50% FC and 100% FC. Ten seeds of LS-442 and Pre-Mung varieties were sown in each pot containing 2 kg/pot sand. Field capacity was determined by following standard procedure described by Earl et al. (2003). After seedling establishment five seedlings were maintained in each pot, all pots were divide into two main sets first was kept under well water or 100% field capacity (control) second under 50% field capacity for 45 days as per treatment level. After the complete establishment of the seedlings, foliar application of Control (No spray), WS (Distilled water spray), Iron (0.5% Fe), and Iron (1% Fe), was applied. An aliquot (5 and 10 mL) of Fe solution from each treatment was applied to each transparent pot 14 days after drought stress imposition. The nutritional requirements of mung bean plants were fulfilled by applying Hoagland's nutrient. A completely randomized design (CRD) was used to set up the experiment with three replications.

Crop husbandry

In order to clearly examine the impact of iron application, all other agronomic and management practices were maintained and following parameters were recorded.

Harvesting and data collection

After 65 days, plants were harvested, and several growth indicators were recorded for later examination. Following the identification of the fresh plants' morphological features, the plants were stored for 15 days at 65 °C in an oven model (WFO-600ND Japan) in order to measure their dry weight and examine the ions in their roots and shoots. In order to assess the antioxidant and photosynthetic activity of several wheat kinds, the remaining plants were harvested, packed in plastic zipper bags, and promptly frozen at -15 °C.

Morphological attributes

The shoot and the root length of the plants of each replication was measured with a meter rod and the average of these values was taken. The fresh and dry weight of roots and shoots were measured with the help of an electronic balance. Roots and shoot samples were dried in an oven for 15 days at 65 °C in an oven and their dried weight was measured.

Photosynthetic pigments

Carotenoids and chlorophyll (*a* and *b*) were measured by the Arnon (1949) method. Tillers were collected at the time when fresh leaves were observed, that is, 0.1 g. The leaves were chopped, put in a 5 mL (80%) solution of acetone and left overnight at room temperature in the dark. The concentration of the carotenoids and chlorophyll *a* and *b* in the reaction mixture was then ascertained using a spectrophotometer (HALO-SB 10 UV-VIS single-beam) by measuring the optical density of the reaction mixture at 480, 663 and 645 nm respectively (Davies, 1976; Arnon, 1949).

Enzymatic antioxidant activities

Five milliliters of phosphate buffer (pH 7.8) were used to crush fresh leaves (0.5 g) in a chilled environment. The mixture was then homogenized for 12 minutes at 12,000 rpm using a centrifuge. To make it easier to detect antioxidant enzymes later, the separated mixture was stored in a freezer at -20 °C.

The catalase activity was measured using the technique described by Chance and Maehly (1955). The reaction mixture consisted of 0.1 mL of mung bean plant extract, 1 mL of H₂O₂, and 1.9 mM cold phosphate buffer. A spectrophotometer was then used to measure the combination at 240 nm, and the absorbance was noted every 30, 60, and 90 seconds.

Giannopolitis and Reis (1977), suggested a method of measuring the activity of superoxide dismutase. In order to prepare a 1 mL reaction mixture, triton x (250 µL), enzyme extract (mung bean) NBT, and riboflavin each of 50 µL, phosphate buffer solution (250 µL), and distilled water (400 µL) were added to the cuvettes. After 20 minutes of exposure of the mixture to strong white light, absorbance at 560 nm was measured using a spectrophotometer (HALO-SB 10 UV-VIS single-beam).

The Chance and Maehly (1955) protocol was followed in order to quantify the peroxidase activity. The cuvettes were filled with 0.1 mL of enzymatic extract (mung bean), 750 µL potassium phosphate buffer, guaiacol, and H₂O₂ each of 0.2 mL to make a 1 mL reaction mixture. Absorbance was measured at 470 nm for 30, 60, and 90 seconds using a spectrophotometer.

Water relation parameters

Leaf water potential

The same method used for sample selection in the collection of samples for the measurement of the relative water content was utilized to gather leaf samples in order to record the water potential of the leaves. The decision was made far in advance of sunrise so as to avoid transpiration losses. With the use of a pressure bomb that was available in the crop physiology lab, the water potential of the leaves was determined. A single leaf was cut with scissors, then it was placed in a pressure chamber so that the sliced surface would be predicted outside of the slot. A fluid from the xylem was formerly visible at the

leaf's sliced surface. Nitrogen gas from a dense-gas cylinder was used to apply pressure to the chamber. Once it reaches kg/pascal, note the value of the leaf water potential.

Leaf osmotic potential

We measured the leaf solute potential using a leaf that was also used to measure water potential and solidified at -200 °C for osmotic potential evaluations. As a hardened leaf sample was being crushed with a glass rod, the cell fluids were drawn out, and sap was then injected using a disposable syringe. Utilizing a Wescor 5500 osmometer, the liquid so extracted was used directly for the solute potential calculation.

Leaf turgor potential

The leaf of the turgor potential of the selected plants was calculated by the following formula:

Leaf turgor potential = Leaf water potential - Leaf osmotic potential.

Relative water content

Three plants from each treatment were used to collect mature leaf samples. Each sample was freshly weighed using a digital electrical scale, placed in disposable glasses filled with distilled water, and kept overnight to preserve turgid conditions. After nibbling the leaf with the tissue paper, the weight of the leaf was estimated. The sample was dried at 65 °C for 72 hours using its dry weight. The following formula (Karrou and Maranville, 1995) was used to calculate the relative water content for each treatment.

$$RWC = [(FW - DW) / (TW - DW)] * 100$$

FW-fresh weight DW-dry weight and TW-turgid weight

Gaseous exchange parameters

A portable photosynthetic system called the IRGA-CI-340 was used to measure the gas exchange parameters between 22.4 and 27.9 °C outside, 99.9 K Pa atmospheric pressure, 6.0 and 8.9 m bar water vapour pressure inside the chamber walls, 403.3 mmol m⁻² s⁻¹ air flow rate per unit area, 352 mol 5 mol⁻¹ ambient carbon dioxide, and an average leaf temperature of between 28.5 and 32.5°C (Haider et al. 2026b).

Root attributes

In order to analyses and visualize the root data, WINRHIZO'S Companion Software was used to record root traits.

Statistical analysis

The observed data was evaluated at a five percent significance level using Statistix 8.1 software. Additionally, Microsoft Excel (2016 edition) (Microsoft Corporation, Redmond, WA, USA) was used to construct the figures. To evaluate the link between the evaluated attributes, R-studio (V 4.3.3) was used to create a heatmap with a dendrogram using the "heatmap" package and a correlation matrix using the "corrplot" package. OriginPro2024 software was used to perform principal component analysis (PCA).

Results

Morphological parameters

Statistical analysis revealed that mung bean morphological characteristics were significantly affected under drought stress and iron application to the leaves. In both varieties, drought stress reduced shoot length, shoot fresh weight, root fresh weight up to 18%, 33%, and 18%, shoot dry weight, 10%, and root dry weight up to 8% respectively in contrast to control. The negative effects of drought stress are reduced by foliar applied iron. Application of iron significantly increased shoot length, shoot fresh weight, and root fresh weight up to 51%, 79%, and 76%, shoot dry weight and root dry weight up to 15% and 11% at (1% Fe), respectively. Under conditions of drought stress, foliar treatment of iron was more beneficial. Pre-Mung 2018 outperformed LS-442 in terms of morphological features among the two kinds when subjected to drought stress (Table 1).

Photosynthetic pigments

Statistical analysis revealed that photosynthetic pigments were significantly decreased in mung bean under drought stress while improved under iron application to the leaves. In both varieties, drought stress decreased Chl. *a* up to 14.5%, Chl. *b* 15.5%, and carotenoids up to 29.7% respectively. While Iron application (1% Fe) improved the Chl. *a* up to 39.6%, Chl. *b* 119.6%, and carotenoids up to 110.7% as compared to control under drought stress in both varieties respectively. Pre-Mung 2018 performed better under drought stress than LS-442 in terms of photosynthetic pigments between the two varieties (Table 2)

Oxidative stress determinants

Statistical analysis revealed that oxidative stress determinants were increased in mung bean under drought stress, while reduced under iron application to the leaves. In both varieties, drought stress increased hydrogen peroxide up to 13.5% and MDA up to 86%, respectively as compare to no stress. The negative

effects of drought stress are reduced by foliar applied iron. Iron application (1% Fe) decreased hydrogen peroxide up to 40%, and MDA up to 29.4%, respectively. Pre-Mung 2018 performed better under drought stress than LS-442 in terms of oxidative stress determinants between the two varieties (Fig. 1).

Biochemical parameters

In both varieties, drought stress increased superoxide dismutase, peroxidase and catalase up to 2.7%, 18.0% and 4.0%, respectively as compare to no stress. Iron application (1% Fe) further increased superoxide dismutase, peroxidase and catalase up to 5.9%, 11.7% and 6%, respectively. Foliar application of iron proved more advantageous in drought-stressed situations. Pre-Mung 2018 performed better under drought stress than LS-442 in terms of biochemical characteristics between the two varieties (Table 2).

Water relations parameters

Statistical analysis revealed that water relation parameters of mung bean were significantly affected under drought stress and iron application to the leaves. In both varieties, water deficit conditions increased (negative) leaf water potential and leaf osmotic potential, while reduced leaf turgor potential and relative water content up to 28%, 36%, 22.4% and 55%, respectively, in contrast to non-drought. LS-442, in contrast to Pre-Mung 2018, experienced a more drastic drop. Application of iron (1% Fe) enhanced (least negative) the leaf water potential, leaf osmotic and turgor potential, and relative water content up to 57%, 44%, 57.3% and 66%, respectively. During a drought, iron applied to the foliage is more beneficial. Pre-Mung 2018 outperformed LS-442 in terms of water relation when subjected to drought stress (Fig. 2).

Gas exchange attributes

Statistical analysis revealed that gas exchange attributes of the mung bean were significantly affected under drought stress and iron foliar application. In both varieties, water deficit conditions decreased the photosynthetic rate up to 10.6%, transpiration rate 12.4%, and stomatal conductance up to 22%, in contrast with control. While foliar application of Fe increased the photosynthetic rate up to 106.1%, transpiration rate 120.6 and stomatal conductance up to 110.7% respectively, under stress conditions. For gas exchange attributes, Pre-Mung 2018 performed better than LS-442 (Fig. 3)

Root morphological attributes

Statistical analysis was used to determine the root characteristics of the mung bean during drought stress and when iron was supplied to the leaves. In both varieties, drought stress increased root length, root surface area and average root diameter up to 21.7%, 211% and 65%, respectively as compare to control (no stress). LS-442, in contrast to Pre-Mung 2018, experienced a more drastic drop. Iron application (1% Fe) further increased the root length, root surface area and average root diameter up to 63.3%, 89% and 153% respectively. Iron applied to the leaves is more advantageous during a drought. Under drought stress, Pre-Mung 2018 performed better than LS-442 in terms of root morphological characteristics (Fig. 4).

Root and shoot iron concentration

Statistical analysis was used to determine the iron accumulation in the mung bean roots during drought stress and when iron was supplied to the leaves. In both varieties, drought stress decreased the accumulation of root Fe up to 1.77 and shoot Fe 12.9%. On the other hand, Fe foliar application (1% Fe) increased the root Fe up to 12.6 and shoot Fe up to 81.8% in both varieties, respectively. Under drought stress, Pre-Mung 2018 performed better than LS-442 in terms of root and shoot Fe accumulation (Fig. 5).

Heatmap analysis

A two-way heatmap with a dendrogram was made in order to investigate the effects of iron (Fe) on a mung bean observations under drought stress circumstances (Fig. 6). The connections between the groups were shown as colored squares. The color Maroon exhibited a strong positive association. In contrast, the color green demonstrated a strong negative correlation for various observations, impacted by Fe under drought stress (Fig. 6). The Heatmap has clustered into four groups. In the first group, gas exchange, length of the shoot fresh and dry weights of the root and dry weight of shoot, Chl. *a*, *b*, root Fe, carotenoids, relative water content, as well as photosynthetic and transpiration rate were clustered. These parameters are strongly positively correlated under control D0 and 1% Fe and exhibited a significant negative connection when exposed to drought stress and control (no Fe) respectively. This group indicated that using Fe improved the morphological and photosynthetic parameters as well as gas exchange attributes. In the second group, water and osmotic potential and shoot fresh weight were clustered. These measures showed strong positive correlations under control D0 and 1% Fe and exhibited a significant negative connection under drought stress and control (no Fe), among both types, respectively. The third group included SOD, CAT, root surface area, root length, shoot Fe and POD, which were strongly positively correlated under control D1 and 1% Fe, as well as showed a significant negative correlation under control (D0) and no Fe application among both types, respectively. The fourth group contained H₂O₂, and MDA. These attributes were strong positively correlated at D1 and under control (no Fe) and exhibited a significant negative connection under control (D0) and 1% Fe, respectively. These observations demonstrated that the Fe application increased the growth parameters and, gas exchange and photosynthetic pigments of mung bean varieties under drought stress (Fig. 6).

PCA analysis

The PCA analysis revealed that PCA 1 and PCA 2 accounted for 92.09% of the accumulated variations, with 75.44% and 16.65% respectively (Fig. 7). However, the morphological, photosynthetic, water relation, gas exchange and root attributes differed significantly under different drought (D0=100% FC) and (D1=50% FC) stress, iron applications, I₀ (No spray), I₁ (Distilled water spray), I₂ (0.5% Fe), and I₃ (1% Fe), A strong connection was noted among different photosynthetic, gas exchange and morphological attributes in the same treatment (100% FC and 0.5% and 1% Fe), while root length, root surface area, projected area, root and shoot Fe content indicated close relationships with antioxidants (SOD, POD, and CAT) in the same (50% FC and TU 1500 ppm)

treatments and water and osmotic potential respectively in the same (50% FC and 0.5% Fe) treatment. In our experiment, Fe treatment was shown to be beneficial in alleviating drought stress through enhancing morpho-physiological characteristics, photosynthetic attributes, gas exchange, and water relation parameters (Fig. 7).

Correlation analysis

The correlation matrix demonstrates both high positive and strong negative relationships between the different mung bean attributes under drought stress (Fig. 8). The results of the correlation analysis demonstrated that the growth parameters, such as shoot and root length, fresh weight, and dry weight, root surface area, root diameter, Chl. a, Chl. b, total chlorophyll, and carotenoids, were positively correlated with water and osmotic potential, RWC while negatively correlated with H_2O_2 , and MDA (Fig. 7). There was a positive correlation found between the CAT, SOD, and POD and gas exchange attributes including photosynthetic and transpiration rate as well as root and shoot Fe, respectively. Furthermore, the correlation was strongly positive among morphological and physiological attributes (Fig. 8).

Discussion

Abiotic stresses, such as drought stress, limit plant performance by affecting plant morpho-physiological, biochemical, and root attributes (Sabir et al. 2024; Ramzan et al. 2023; Haider et al. 2026a). The goal of the current study was to investigate how applying iron can lessen the detrimental effects of drought stress on mungbean development and physiological, biochemical, and root characteristics. The current study's findings support our theory that mung bean plants were negatively impacted by drought stress, and that applying iron significantly increased photosynthetic activity as well as morphological and enzymatic activities.

Mung bean morphological characteristics were considerably reduced by drought stress (Table 1). Similar findings were observed in black gram and mustard (Ahmad et al. 2024; Ramzan et al. 2023). Morphological traits appear to be reduced during drought stress due to reduced cell turgor and growth, which results in lesser leaf area, stomatal closure, and limited photosynthesis (Akbari & Maleki, 2018). Furthermore, one of the primary causes of reduced plant development under drought stress circumstances is decreased plant nutrient intake from the soil (Ahmad et al. 2024). Both sources of iron treatment improved shoot and root fresh weight, and dry weight (Table 1). By promoting fundamental developmental processes, iron helps mitigate the impacts of drought stress on plant growth. Balanced Fe nutrition increases root and shoot biomass during drought conditions by improving nutrient absorption and increasing the capacity to convert resources into growth mechanisms. This suggests that Fe promotes growth in unfavourable drought conditions by improving nutrient uptake and metabolic activity (Ram et al. 2024; Naz et al. 2023).

Under water deficit conditions, chlorophyll pigments of mungbean decreased compared to the controlled treatments (Table. 2). Drought stress is the cause of these reductions, which can be linked to a drop in transpiration rate and cell turgor. Under drought stress, stomatal closure limits CO₂ absorption, which lowers concentrations of photosynthetic pigments, and stops water loss through transpiration, allowing the plants to sustain hydrostatic activity (Batool et al. 2022a; Ahmad et al. 2024). As a result, cell elongation and growth are reduced. Additionally, it severely damages the photosynthetic machinery, dehydrates plants, suppresses metabolism, closes stomata, restricts gas exchange, and increases reactive oxygen species (Haider et al. 2026; Batool et al. 2022a; Ramzan et al. 2023). In drought-stressed mungbean plants, the application of iron greatly enhances photosynthetic activity and plant development that is linked to the energy acquisition and more carbon uptake (Ding et al. 2025).

Drought stress significantly decreased the plant water relations parameters in our study (Fig. 2). The plant's relative water contents (RWC) were lowered under stressful conditions, it gradually reduces transpiration rate, photosynthetic efficiency, and stomatal conductance (Fig. 3). Haider et al. (2026) and Ahmad et al. (2023) also observed similar findings. Stomatal closure is a plant's initial response to drought stress and is widely regarded to be the main source of the drought-induced decline. Reduced water availability alters plant metabolism, which has a detrimental effect on growth and cell turgidity and water balance (Haider et al. 2026). In contrast to stressed plants without Fe supplementation, improved pigment stability helps with improved light harvesting, energy transfer, which translates to improved physiological performance and relative water content, photosystem II stability, and improved gaseous exchange (Ram et al. 2024).

In the current experiment, water stress increased the oxidative stress determinants. ROS generation, which causes oxidative damage in plants, is the main reaction of plants grown under abiotic stress (Raja et al. 2017; Haider et al. 2026; Ramzan et al. 2025). Nucleic acids are adversely affected by this oxidative damage, which may result in plant death through interactions with the regulatory processes on cell membranes (Smirnoff and Arnaud, 2018; Ramzan et al. 2026a). The use of iron in this study reduced the increased ROS generation under drought stress conditions (Fig. 1). Fe has the ability to strengthen antioxidant defence systems to eliminate the excess reactive oxygen species (ROS) caused by drought stress, which lead to the disintegration of macromolecules and cell structures. Enzymes like catalase (CAT), peroxidases (POD), and other redox-containing iron proteins that eliminate ROS and stabilise membranes under stressful circumstances depend heavily on iron (Kong et al. 2023; Ram et al. 2024). Increased antioxidant enzyme activity improves tolerance to Fe-supplemented mungbean plants by lowering the redox balance and lipid peroxidation (MDA buildup) of the stressed tissues. In plants exposed to drought stress utilizing inadequate Fe, these biochemical advantages support cell homeostasis and lower oxidative stress indicators (Fareed et al. 2024; Zia-ur-Rehman et al. 2023; Ram et al. 2024).

In line with previous studies, we found that root diameter, length, and surface area all increased during drought (Haider et al. 2026). The results showed that when roots were stressed by drought, root length increased and root weight dropped as a result of more slender roots. It was shown that root development was significantly reduced during drought stress due to limited growth in low-water situations. A deep and plentiful root system is necessary to function well against drought stress. This might have slowed the growth of the plants (Ors et al. 2016; Ahmad et al. 2024).

Conclusion

Under drought stress circumstances, both varieties of mung bean demonstrated a deterioration in morpho-physiological properties, including a considerable loss in photosynthetic pigments and the accumulation of reactive oxygen species (ROS). However, the detrimental effects of drought stress were mitigated by adding iron (1%) as a foliar spray. By encouraging the synthesis of enzymatic antioxidants, iron therapy was essential for preserving osmotic equilibrium, mineral ion uptakes, and ROS detoxification. When it came to reducing the consequences of water stress, 1% iron was more effective than iron (0.5%). Additionally, Pre-Mung 2018 outperformed LS-442 in terms of morpho-physiological traits. More research should be done to examine the use of iron on a broader scale in field settings, utilizing various techniques. Iron has the potential to be used to improve spinach growth during drought conditions because it is cheap and widely accessible. Field testing are necessary to confirm the findings' application in the real agricultural context, even though the pot experiment provides some controlled information.

Declarations

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Author's Contributions

Conceptualization: Ejaz Ahmad Waraich, Methodology: Arslan Haider and Tahrim Ramzan, Software: Arslan Haider, Formal analysis: Aiman Sikandar, Investigation: Tahrim Ramzan Data curation: Arslan Haider Writing-original draft preparation: Arslan Haider Writing-review and editing, Tahrim Ramzan, Mohamed M. El-Mogy and Sundas Qadeer. All authors have read and agreed to the published version of the manuscript.

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Data Availability The data that support this study will be shared upon reasonable request to the corresponding author.

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Conflict of interest Work included in this manuscript has no conflict of interest.

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Tables

Table 1 Effect of drought stress and iron (Fe) treatment on the morphological characteristics of mung bean varieties

Drought & Varieties	Iron (Fe) treatments	RL (cm)	SL (cm)	RFW (g)	SFW (g)	RDW (g)	SDW (g)
Ls-442	100% FC	Control	12.28±0.14g	21.2±0.65d-f	1.11±0.009bc	1.79±0.019c-e	0.317±0.009fg
		WS	13.5±0.57e-g	23.8±1.13b-e	1.30±0.09a-c	2.01±0.28b-e	0.36±0.021ef
		0.5% Fe	17.44±1.31b-f	26.05±0.73a-c	1.52±0.22a-c	2.32±0.16a-d	0.423±0.03b-e
	50% FC	1% Fe	18.39±0.39a-e	28.3±0.67ab	1.66±0.012ab	2.52±0.21a-c	0.48±0.029ab
		Control	13.55±0.54e-g	18.9±0.36f	0.96±0.088c	1.52±0.04e	0.27±0.015g
		WS	14.89±1.56d-g	21.2±0.65d-f	1.18±0.308bc	1.68±0.06de	0.307±0.012fg
		0.5% Fe	19.83±0.19a-c	23.1±1.20c-f	1.39±0.138a-c	2.07±0.07b-e	0.38±0.006d-f
		1% Fe	20.83±0.09ab	25.9±0.91a-c	1.54±0.18a-c	2.29±0.15a-d	0.427±0.019b-e
Pre-Mung 2018	100% FC	Control	15.44±1.14c-g	23.7±1.33b-e	1.37±0.124a-c	2.01±0.05b-e	0.383±0.007d-f
		WS	16.67±0.66b-g	25.8±1.26a-d	1.46±0.052a-c	2.46±0.001a-c	0.41±0.006b-e
		0.5% Fe	18.3±0.34a-e	27.1±0.79ab	1.66±0.07ab	2.62±0.11ab	0.47±0.02a-c
	50%FC	1% Fe	18.9±0.06a-d	29.6±0.49a	1.88±0a	2.98±0.05a	0.52±0.01a
		Control	18.9±2.32a-d	20.1±1.16ef	1.22±0.1a-c	1.51±0.1e	0.32±0.01fg
		WS	19.6±1.03a-d	23±0.48c-f	1.33±0.1a-c	1.65±0.2de	0.36±0.02ef
		0.5% Fe	21.4±0.59ab	25.2±0.55a-d	1.48±0.18a-c	1.95±0.15b-e	0.4±0.01c-e
		1% Fe	22.6±0.2a	28±0.84ab	1.62±0.1a-c	2.39±0.25a-d	0.45±0.01a-d

Values are the means ± standard error of three replicates. Control (No spray), Ws (Distilled water spray), Iron (0.5% Fe), and Iron (1% Fe), V1= LS-4422 and V2= Pre-Mung 2018, D0= 100% FC and D1=50% FC.

Table 2 Effect of drought stress and iron (Fe) treatment on the photosynthetic pigments and enzymatic antioxidants of mung bean varieties

Drought & Varieties	Iron (Fe) treatments	Chl a (mg g ⁻¹ F.W)	Chl b (mg g ⁻¹ F.W)	Total Chlorophyll	Carotenoids (mg g ⁻¹ F.W)	SOD (Units mg ⁻¹
Ls-442	100% FC	Control	0.006±0.001a	0.006±0.001b	0.012±0.003a	0.14±0.003ef
		WS	0.006±0.001a	0.006±0.001ab	0.012±0.001a	0.16±0.006d-f
		0.5% Fe	0.006±0.001a	0.009±0.001ab	0.016±0.001a	0.188±0.006c-e
	50% FC	1% Fe	0.007±0.001a	0.011±0.003a	0.018±0.004a	0.21±0.006b-d
		Control	0.005±0a	0.006±0b	0.011±0a	0.113±0.019f
		WS	0.006±0a	0.006±0.001	0.012±0.001a	0.127±0.015f
		0.5% Fe	0.006±0a	0.008±0ab	0.014±0a	0.167±0.012d-f
		1% Fe	0.007±0a	0.009±0.001ab	0.015±0.001a	0.197±0.015b-e
Pre-Mung 2018	100% FC	Control	0.006±0.001a	0.007±0ab	0.013±0.002a	0.167±0.012d-f
		WS	0.007±0.001a	0.007±0.001ab	0.014±0.001a	0.19±0.006c-e
		0.5% Fe	0.01±0a	0.01±0ab	0.02±0a	0.22±0.01a-c
	50%FC	1% Fe	0.01±0a	0.02±0ab	0.02±0a	0.27±0.01a
		Control	0.01±0a	0.01±0ab	0.01±0a	0.14±0.02ef
		WS	0.01±0a	0.01±0ab	0.01±0a	0.16±0.02d-f
		0.5% Fe	0.01±0a	0.01±0ab	0.02±0a	0.2±0.01b-e
		1% Fe	0.01±0a	0.01±0ab	0.02±0a	0.25±0.02ab

Values are the means ± standard error of three replicates. Control (No spray), Ws (Distilled water spray), Iron (0.5% Fe), and Iron (1% Fe), V1= LS-4422 and V2= Pre-mung 2018.

Figures

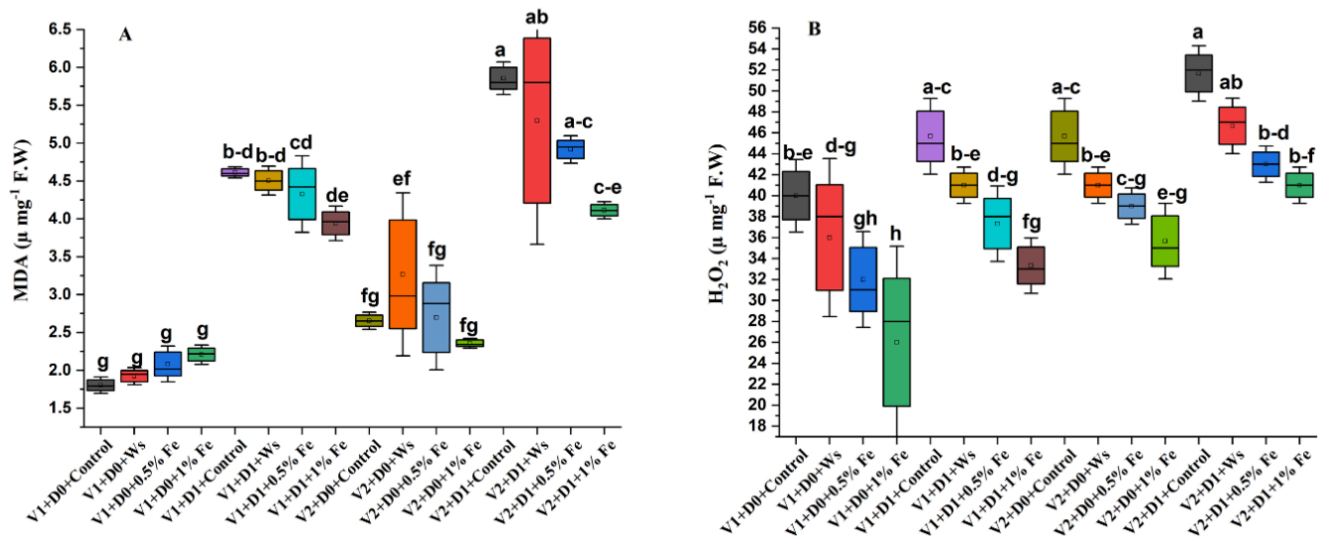


Figure 1

Impact of drought stress and iron applications on A) MDA ($\mu \text{ mg}^{-1} \text{ F.W}$) and B) H_2O_2 ($\mu \text{ mg}^{-1} \text{ F.W}$) of mung bean. Error bars above indicate the $\pm$ SE of three replicates. Control (No spray), Ws (Distilled water spray), Iron (0.5% Fe), and Iron (1% Fe), V1= LS-4422 and V2= Pre-Mung 2018, D0= 100% FC and D1=50% FC.

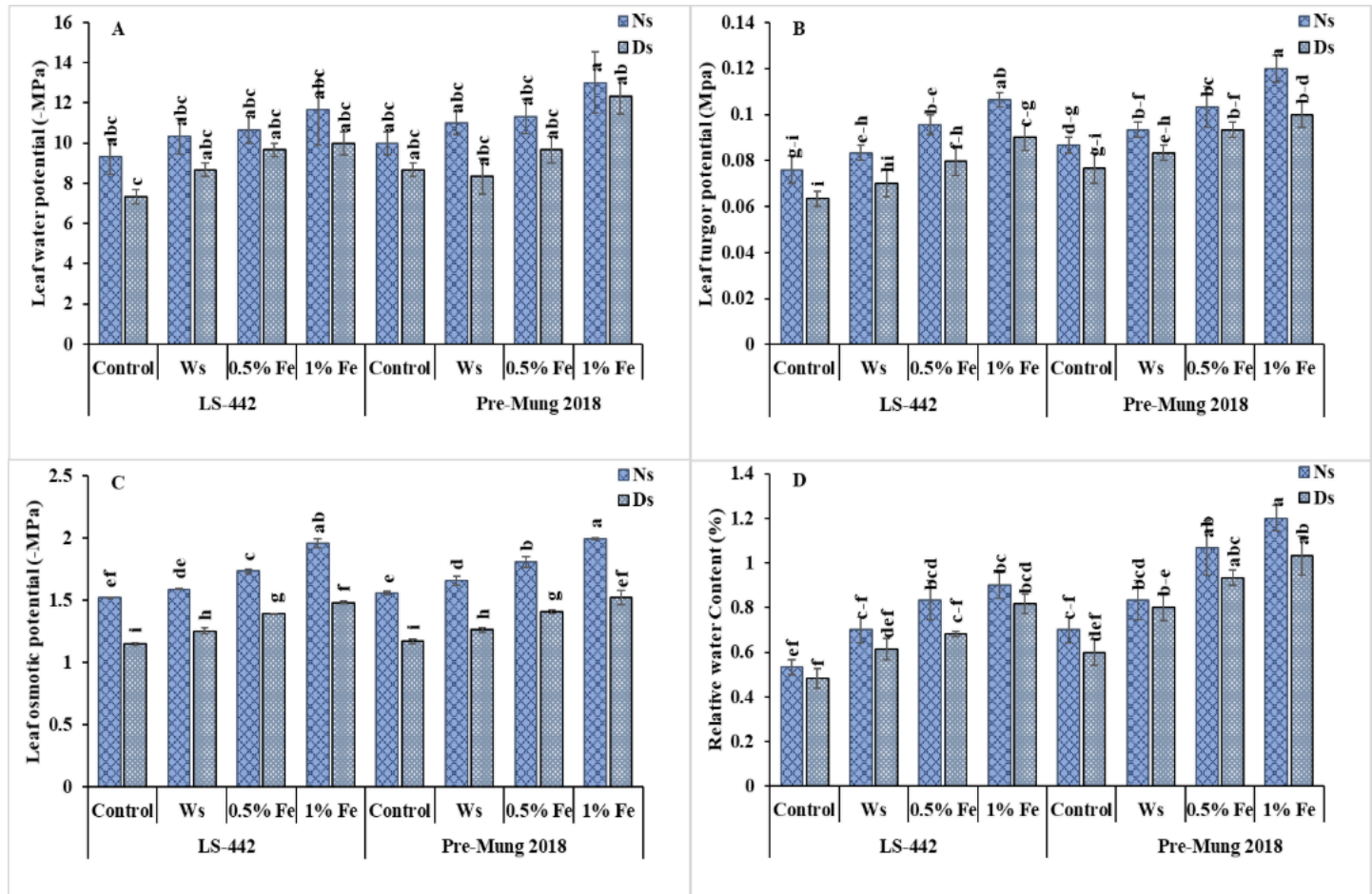


Figure 2

Impact of drought stress and iron applications on A) leaf water potential, (-MPa), B) leaf turgor potential (MPa), C) leaf osmotic potential (-MPa), and D) relative water contents (%) of mung bean. Error bars above indicate the $\pm$ SE of three replicates. Control (No spray), Ws (Distilled water spray), Iron (0.5% Fe), and Iron (1% Fe), V1= LS-4422 and V2= Pre-Mung 2018, D0= 100% FC and D1=50% FC.

and Iron (1% Fe), V1= LS-4422 and V2= Pre-Mung 2018, No stress (NS)= 100% FC and drought stress (DS)=50% FC.

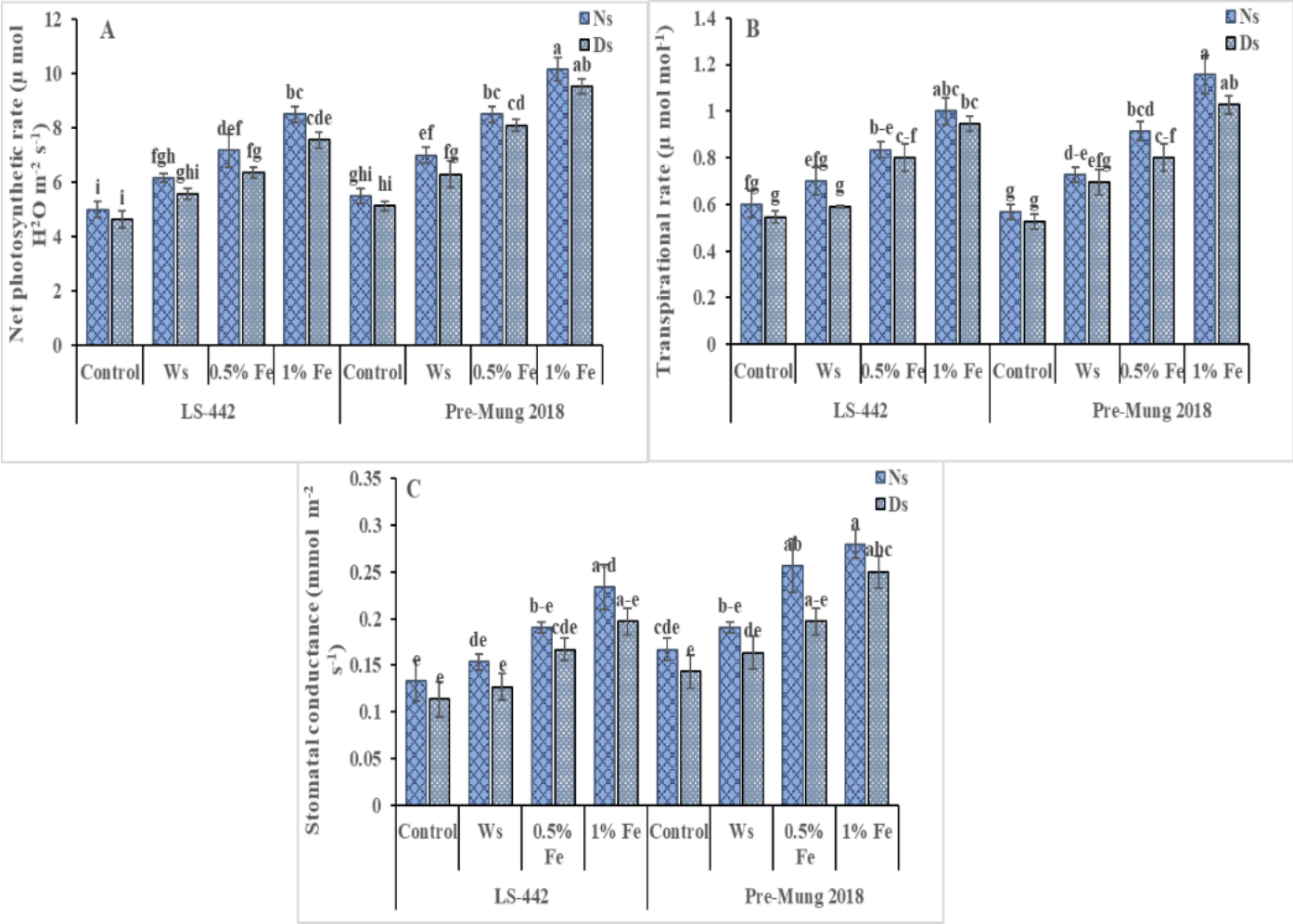


Figure 3

Impact of drought stress and iron applications on A) net photosynthetic rate ($\mu\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$), B) transpirational rate ($\text{mmol m}^{-2} \text{s}^{-1}$), and C) stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) of mung bean. Error bars above indicate the $\pm$ SE of three replicates. Control (No spray), Ws (Distilled water spray), Iron (0.5% Fe), and Iron (1% Fe), V1= LS-4422 and V2= Pre-Mung 2018, No stress (NS)= 100% FC and drought stress (DS)=50% FC.

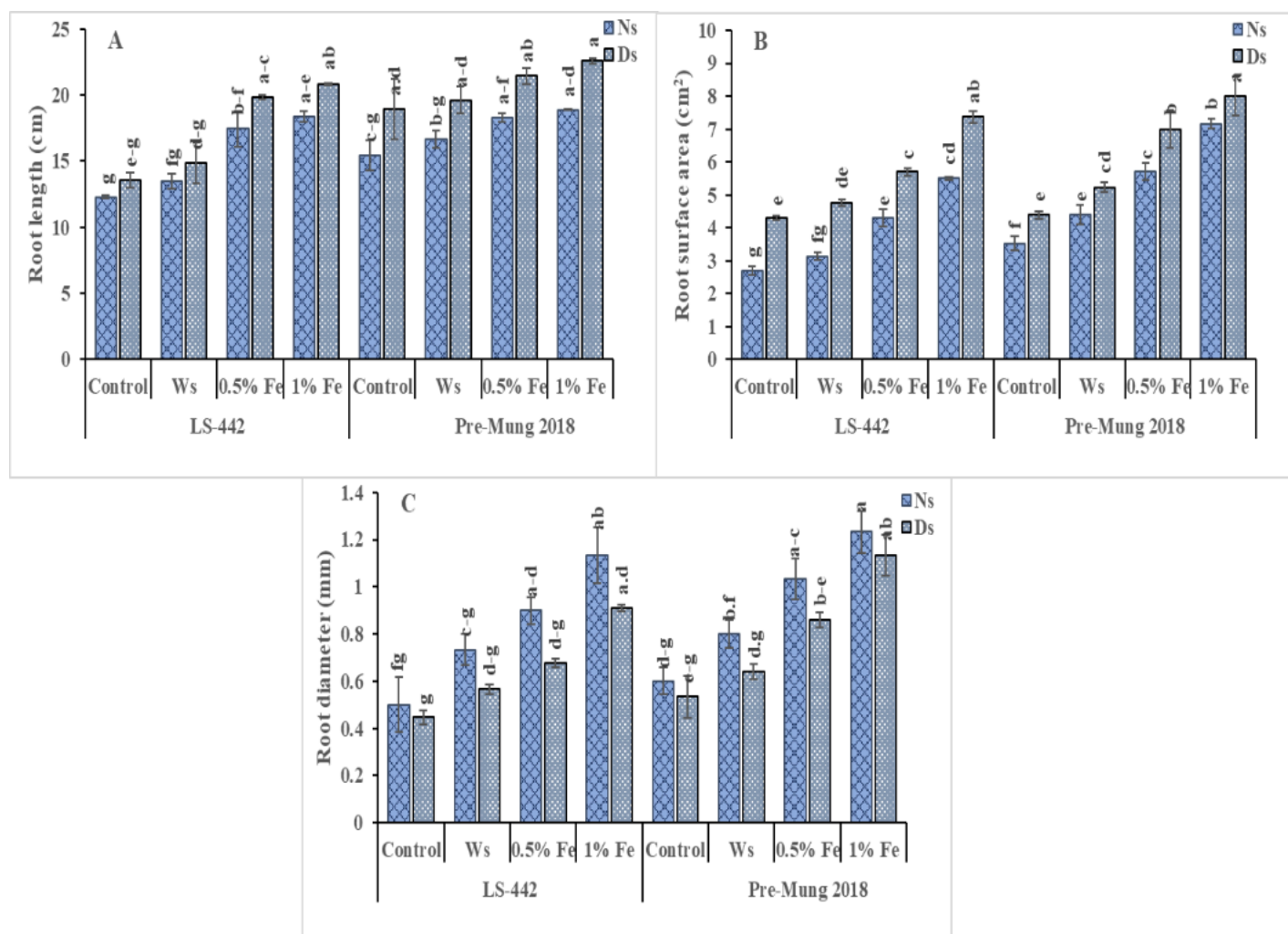


Figure 4

Impact of drought stress and iron applications on A) root length (cm), B) root surface area (cm²), and C) root diameter (mm) of mung bean. Error bars above indicate the $\pm$ SE of three replicates. Control (No spray), Ws (Distilled water spray), Iron (0.5% Fe), and Iron (1% Fe), V1= LS-4422 and V2= Pre-Mung 2018, No stress (NS)= 100% FC and drought stress (DS)=50% FC.

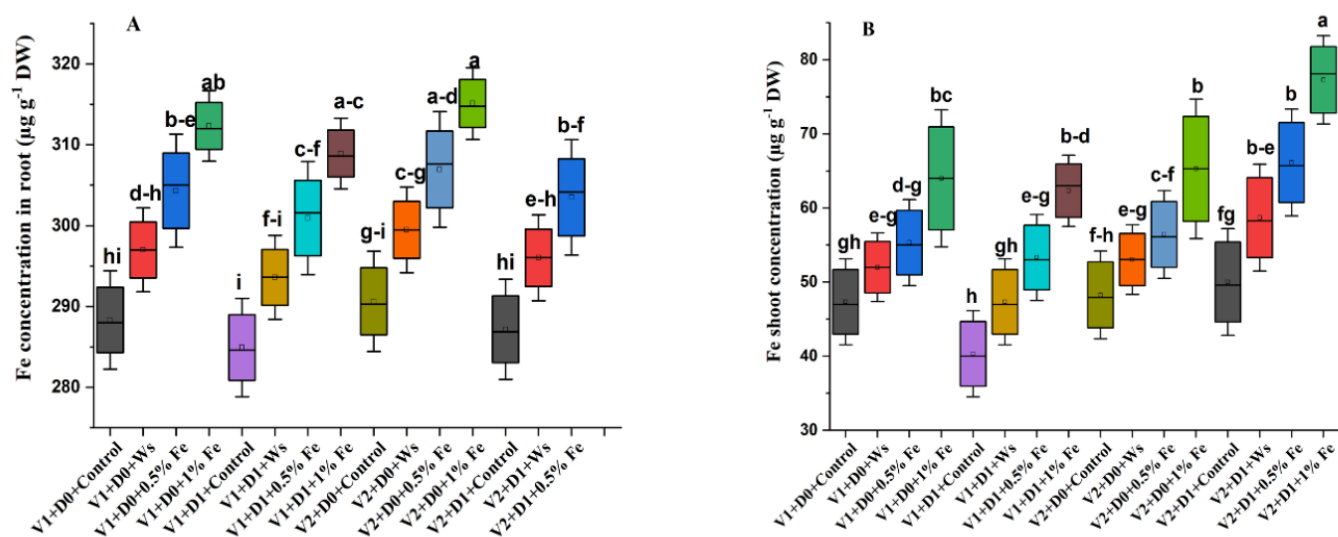


Figure 5

Impact of drought stress and iron applications on A) Fe concentration in root and B) Fe concentration in shoot, of mung bean. Error bars above indicate the $\pm$ SE of three replicates. Control (No spray), Ws (Distilled water spray), Iron (0.5% Fe), and Iron (1% Fe), V1= LS-4422 and V2= Pre-Mung 2018, No stress (NS)= 100% FC and drought stress (DS)=50% FC.

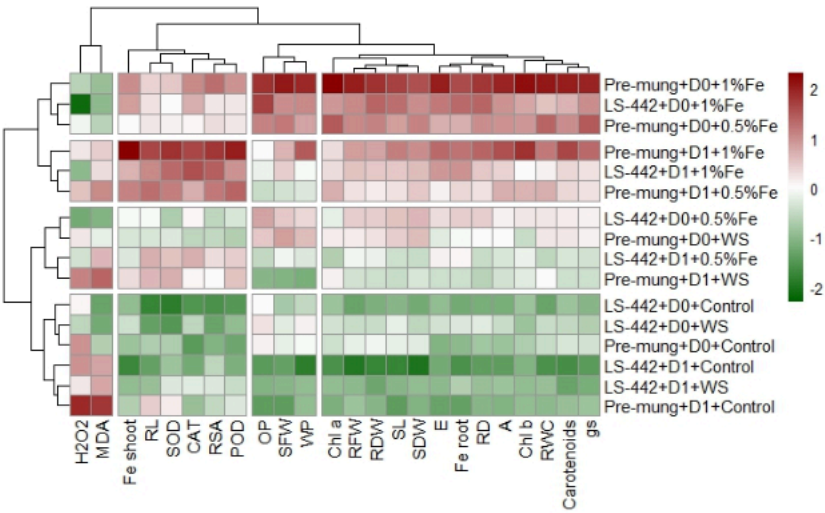


Figure 6

Heatmap with two way dendrogram analysis of mung bean under drought stress and iron applications on morpho-physiological, water relation, gas exchange, oxidative stress determinants, enzymatic antioxidants, and Fe concentration in root and Fe concentration in shoot, of mung bean. Error bars above indicate the $\pm$ SE of three replicates. Control (No spray), Ws (Distilled water spray), Iron (0.5% Fe), and Iron (1% Fe), V1= LS-4422 and V2= Pre -Mung 2018, D0= 100% FC and D1=50% FC.

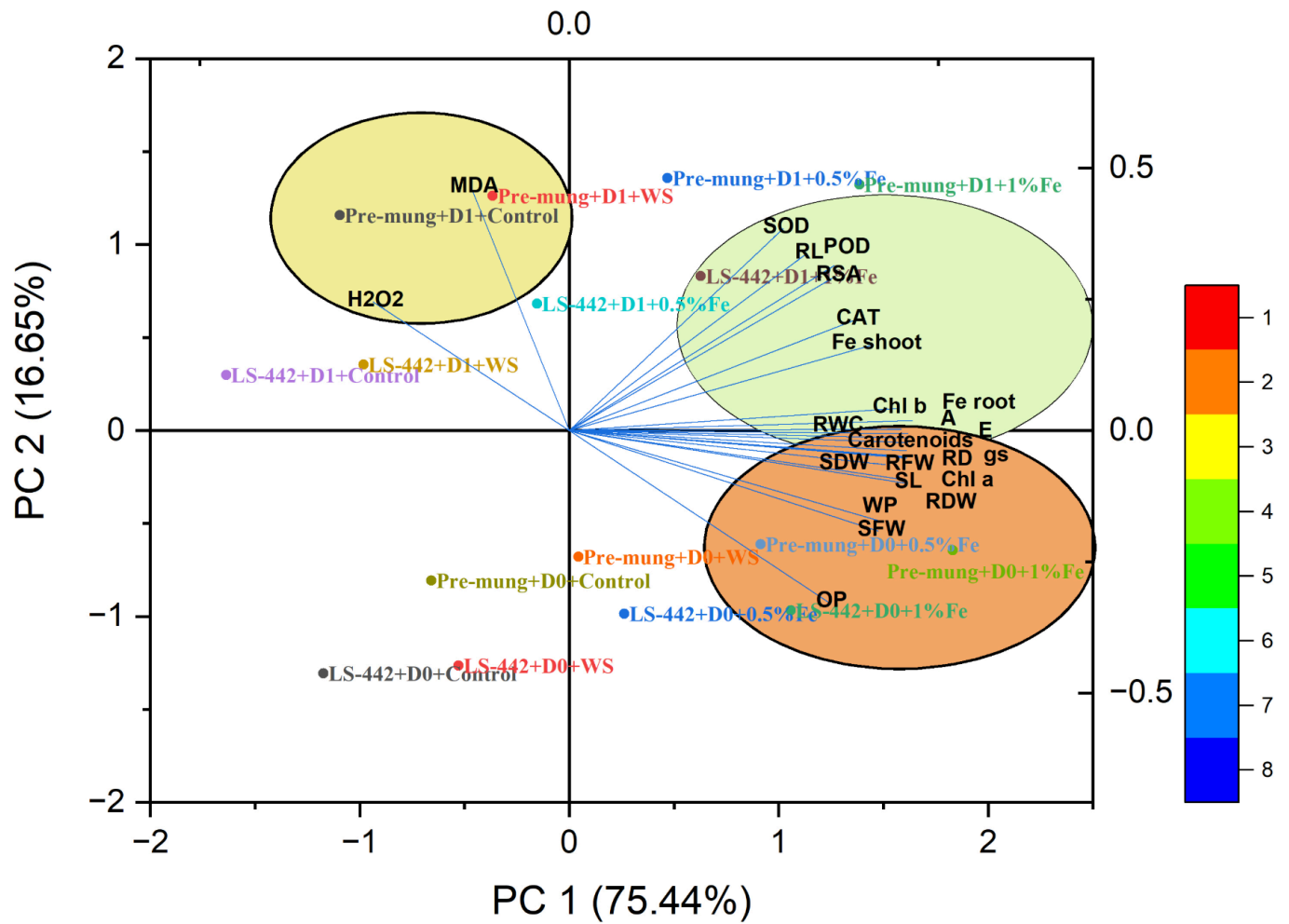
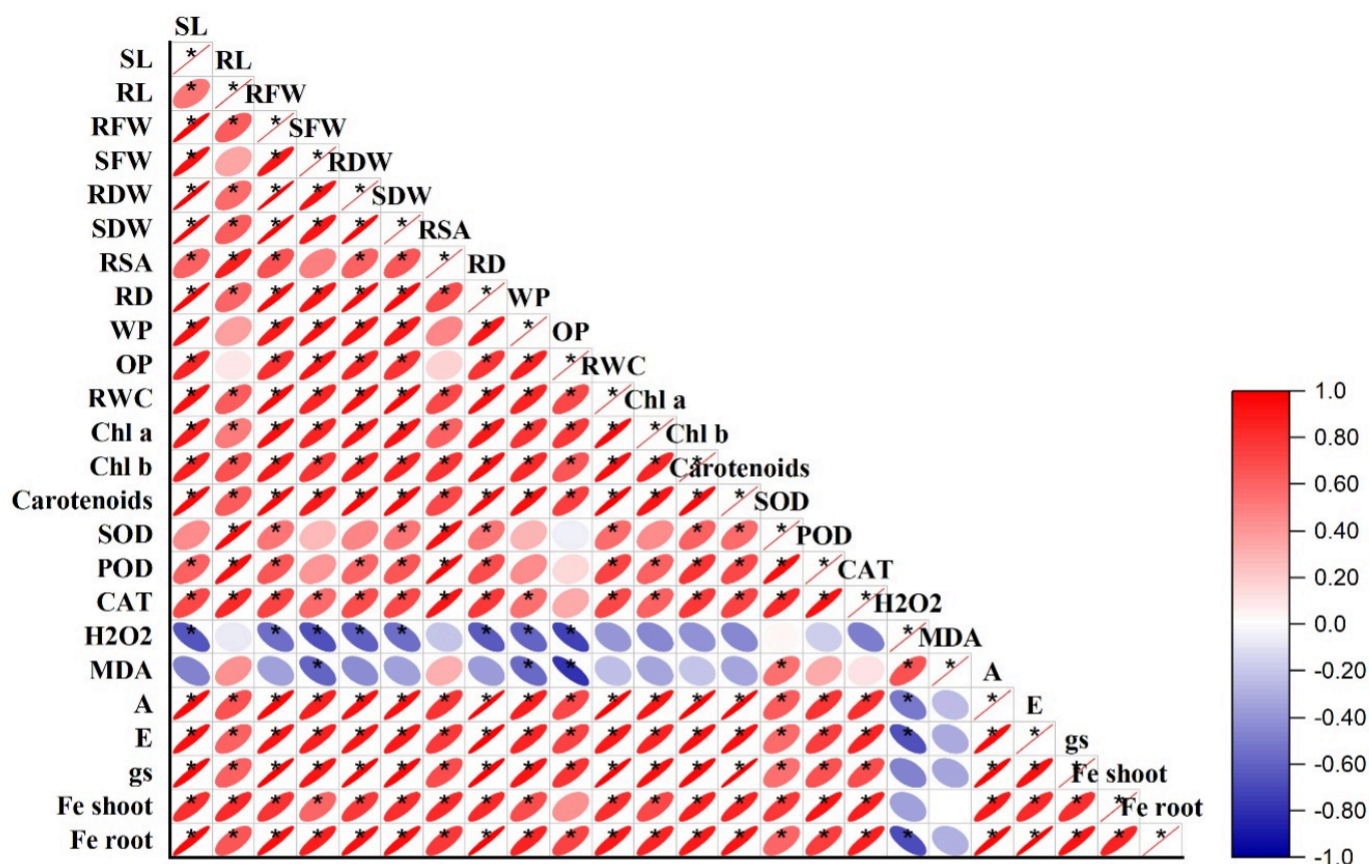


Figure 7

Principal component analysis of mung bean under drought stress and iron applications on morpho-physiological, water relation, gas exchange, oxidative stress determinants, enzymatic antioxidants, and Fe concentration in root and Fe concentration in shoot, of mung bean. Error bars above indicate the $\pm$ SE of three replicates. Control (No spray), Ws (Distilled water spray), Iron (0.5% Fe), and Iron (1% Fe), V1= LS-4422 and V2= Pre-Mung 2018, D0= 100% FC and D1=50% FC.



* $p \leq 0.05$

Figure 8

Pearson correlation analysis of mung bean under drought stress and iron applications on morpho-physiological, water relation, gas exchange, oxidative stress determinants, enzymatic antioxidants, and Fe concentration in root and Fe concentration in shoot, of mung bean. Error bars above indicate the $\pm$ SE of three replicates. Control (No spray), Ws (Distilled water spray), Iron (0.5% Fe), and Iron (1% Fe), V1= LS-4422 and V2= Pre-Mung 2018, D0= 100% FC and D1=50% FC.