

Iron supplementation restores growth and physiological performance of drought-stressed soybean plants

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ABSTRACT

Drought stress severely limits soybean productivity by impairing plant growth, water relations and nutrient acquisition. Iron (Fe) is an essential micronutrient involved in metabolic process in plants; however, its role in alleviating drought-induced physiological disruption in soybean remains understood. This study investigated the influence of Fe supplementation on soybean plants exposed to drought stress under controlled growth room conditions. Soybean plants were cultivated for eight weeks under four treatment conditions: control, drought, drought + Fe, and Fe+. Drought stress markedly reduced chlorophyll score, relative water content, shoot height, shoot biomass, root growth, and Fe-chelate reductase activity compared with control plants. Fe supplementation partially mitigated drought-induced growth inhibition by improving chlorophyll content, water status, shoot growth, root development, and nitrogen accumulation. Rhizosphere siderophore levels were significantly elevated in drought + Fe-treated plants, suggesting enhanced rhizosphere-mediated Fe mobilization under stress conditions. In contrast, excessive Fe treatment alone reduced chlorophyll content, nitrogen accumulation, and root growth despite increased Fe-chelate reductase activity. The findings demonstrate that appropriate Fe supplementation can alleviate drought-associated physiological impairment in soybean by improving nutrient acquisition and maintaining photosynthetic performance. This study highlights the importance of Fe homeostasis in drought adaptation and provides insights into nutrient-assisted strategies for improving soybean resilience under water-limited conditions.

Introduction

Soybean (*Glycine max* L.) is one of the world's most economically important legume crops because of its high protein and oil content and its essential role in global food and feed production. However, soybean productivity is highly vulnerable to environmental stresses, particularly drought stress, which is becoming increasingly severe due to climate change and irregular precipitation patterns. Drought stress negatively affects photosynthesis, water balance,

nutrient uptake, nitrogen fixation, oxidative homeostasis, and biomass accumulation, ultimately leading to significant yield reduction (Farooq et al., 2009; Anjum et al., 2017).

Water deficit commonly disrupts mineral nutrient acquisition from soil by limiting nutrient diffusion and root uptake activity. Among micronutrients, iron (Fe) plays a central role in plant metabolism because it functions as a cofactor in chlorophyll biosynthesis, respiration,



electron transport chains, antioxidant enzymes, and nitrogen assimilation pathways (Kroh & Pilon, 2019). Despite its abundance in soil, Fe availability is often restricted under stressful environmental conditions, leading to impaired physiological performance and oxidative damage in plants (Kabir et al., 2012). Drought stress can also interfere with rhizosphere dynamics and reduce microbial activities associated with nutrient mobilization. Plants often respond to Fe deficiency by enhancing Fe-chelate reductase activity and increasing rhizosphere siderophore interactions to improve Fe acquisition (Sun et al., 2016). Fe availability is particularly important in legumes because biological nitrogen fixation requires adequate Fe for nitrogenase activity and nodule function (Schwember et al., 2019). Therefore, disruption of Fe homeostasis under drought may negatively affect nodulation and nitrogen metabolism in soybean plants.

Previous studies have demonstrated that micronutrient supplementation can improve drought tolerance by maintaining chlorophyll stability, antioxidant activity, and nutrient balance (Sadeghzadeh, 2013; Sinclair & Krämer, 2012). However, the interaction between drought stress and Fe-mediated physiological responses in soybean remains incompletely understood. Therefore, the objective of the present study was to investigate the physiological and biochemical responses of soybean plants exposed to drought stress with or without Fe supplementation. We hypothesized that Fe supplementation would partially restore drought-induced growth inhibition by improving water status, chlorophyll accumulation, Fe acquisition responses, and nitrogen metabolism.

Materials and methods

Soybean cultivation and stress treatments

Soybean plants were cultivated under controlled growth room conditions for eight weeks. During the first two weeks after transplantation, all plants were maintained under well-watered conditions to ensure uniform establishment prior to treatment initiation. After establishment, plants were subjected to four treatment conditions: control, drought, drought + Fe and

Fe+. Soybean plants were cultivated in soil pots containing a 1:1 mixture of commercial potting mix and natural field soil. Drought stress was imposed by supplying approximately 70% less water than the well-watered control plants at one-day intervals throughout the treatment period. Iron supplementation was applied at a rate of 0.1 g Fe per 500 g soil pot in the designated treatment groups. Plants were maintained in a controlled growth room under a 16 h light/8 h dark photoperiod at approximately 25 °C. The experiment was conducted using a completely randomized design with multiple biological replications per treatment.

Morphological measurements

Shoot height, stem diameter, root length, shoot dry weight, and root dry weight were determined after eight weeks of growth. Plant height and root length were measured using a measuring scale. Harvested shoot and root samples were oven-dried at 70°C for three days prior to dry weight determination. Root nodules were carefully harvested after washing roots thoroughly with water. Nodules were visually quantified and photographed for comparative analysis among treatment groups.

Chlorophyll score and relative water content

Leaf chlorophyll score was measured using a handheld chlorophyll meter. Relative water content (RWC) was determined to evaluate plant water status using the formula: $RWC = [(FW - DW)/(TW - DW)] \times 100$, where FW represents fresh weight, TW represents turgid weight, and DW represents dry weight.

Determination of leaf nitrogen content

Leaf nitrogen content was determined using an elemental analyzer. Briefly, dried leaf samples were finely ground into a homogeneous powder, and a known amount of each sample was weighed into tin capsules. Samples were combusted at high temperature in the elemental analyzer, and nitrogen concentration was quantified based on the released combustion gases. The results were expressed as $mg\ g^{-1}$ dry weight.

Determination of rhizosphere siderophore levels

Rhizosphere siderophore content was determined using the chrome azurol S (CAS) assay as previously described by Himpsl and Mobley (2019). Briefly, rhizosphere soil samples were homogenized with extraction solution and centrifuged to obtain the supernatant. An aliquot of the extract was mixed with CAS reagent and incubated at room temperature for color development. Absorbance was measured at 630 nm using a spectrophotometer, and siderophore units were calculated based on the reduction in blue color intensity of the CAS reagent.

Fe-chelate reductase activity

Fe-chelate reductase activity in roots was determined using the sodium bathophenanthroline disulfonate (Na-BPDS) assay based on the formation of Fe²⁺-BPDS complexes (Bienfait et al., 1983). Briefly, freshly harvested root samples were incubated in assay solution containing Fe(III)-EDTA and Na-BPDS under dark conditions. The reduction of Fe³⁺ to Fe²⁺ by root-associated ferric chelate reductase resulted in the formation of a colored Fe²⁺-BPDS complex. After incubation, absorbance was measured spectrophotometrically at 535 nm, and Fe-chelate reductase activity was calculated using the extinction coefficient of the Fe²⁺-BPDS complex. The activity was expressed as $\mu\text{mol Fe}^{2+} \text{ g}^{-1} \text{ fresh weight h}^{-1}$.

Statistical analysis

Data was analyzed using one-way analysis of variance (ANOVA) to determine the effects of treatments on the measured parameters. When significant treatment effects were detected, mean comparisons were performed using Duncan's multiple range test (DMRT) as a post hoc analysis at $P \leq 0.05$. Different letters above bars indicate statistically significant differences among treatment groups. All values are presented as mean \pm standard deviation (SD).

Results

Drought stress reduced soybean growth and chlorophyll accumulation

Drought stress caused severe reductions in soybean growth and visible chlorosis symptoms compared with control plants (Fig. 1). Plants exposed to drought exhibited stunted growth, reduced biomass accumulation, and lower leaf greenness. Fe supplementation partially restored plant vigor under drought conditions. Furthermore, leaf chlorophyll score was significantly reduced under drought stress compared with control plants (Fig. 2A). However, drought + Fe-treated plants exhibited significantly higher chlorophyll levels than drought-stressed plants. Fe⁺ treatment alone caused the lowest chlorophyll score among all treatments (Fig. 2A). In addition, relative water content in the shoot also significantly decreased under drought stress (Fig. 2B). Fe supplementation improved water retention in drought-treated plants, although values remained lower than the control. However, stem diameter declined significantly under drought stress and showed limited improvement with Fe supplementation (Fig. 2C).



Control Drought Drought+Fe Fe+

Fig. 1. Phenotype of soybean plants grown under different treatment conditions: Control, Drought, Drought + Fe, and Fe⁺ treatments. Plants were cultivated for 8 weeks under growth room conditions. During the initial 2 weeks after transplantation, all plants were maintained under well-watered conditions to ensure uniform establishment prior to the initiation of drought treatment.

Drought altered nitrogen status and nodulation

Leaf nitrogen concentration declined significantly under drought stress (Fig. 3A). Fe supplementation improved nitrogen accumulation in drought-treated plants, suggesting improved nutrient assimilation and metabolic recovery. Nodulation patterns also differed among treatments (Fig. 3B). Drought stress reduced nodule abundance and size, whereas drought + Fe plants showed improved nodulation compared with drought-stressed plants. These observations suggest that Fe availability may contribute to maintaining symbiotic nitrogen fixation under drought conditions (Fig. 3B).

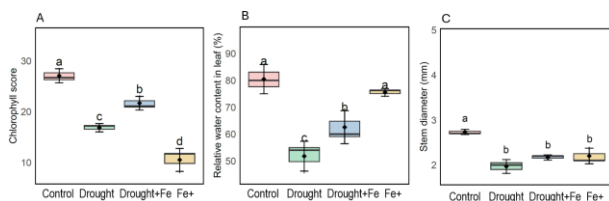


Fig. 2. Effects of drought and Fe treatment on (A) leaf chlorophyll score, (B) relative water content in leaves, and (C) stem diameter of plants after 8 weeks of growth. Different letters indicate significant differences among treatment groups at $P \leq 0.05$. Values represent mean \pm SD.

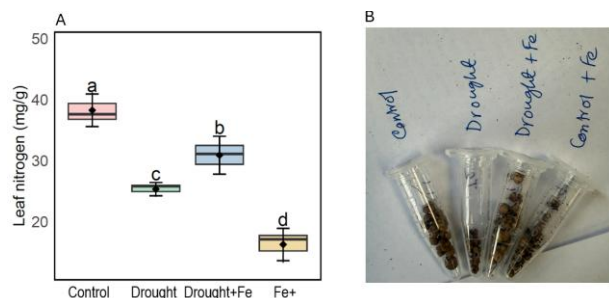


Fig. 3. Leaf nitrogen content (A) and nodulation (B) of plants grown under Control, Drought, Drought + Fe, and Fe+ treatments after 8 weeks of growth. Different letters indicate significant differences among treatment groups at $P \leq 0.05$. Values represent mean \pm SD.

Fe supplementation partially restored shoot and root growth

Drought stress significantly inhibited shoot height, shoot dry weight, root length, and root dry weight (Fig. 4). Control plants exhibited the highest shoot height and biomass accumulation, whereas drought-stressed plants showed substantial growth suppression. Fe supplementation under drought conditions significantly improved shoot height and shoot dry weight compared with drought alone. Similarly, root length and root dry weight were partially restored in drought + Fe-treated plants. Nevertheless, Fe+ treatment alone reduced root growth relative to the control (Fig. 3).

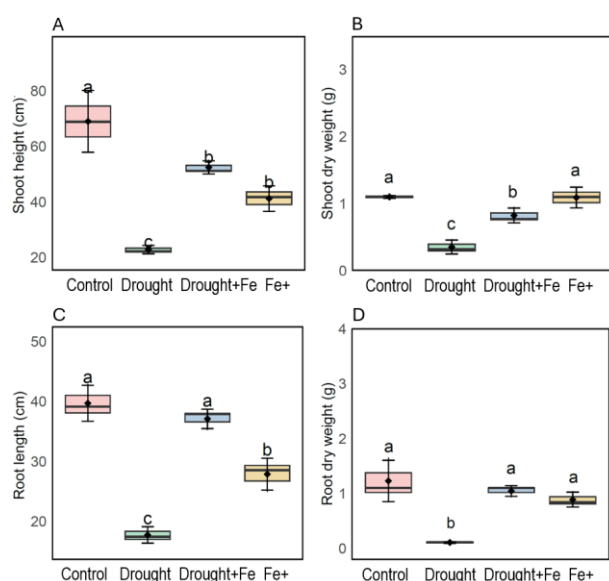


Fig. 4. Effects of drought and Fe treatment on (A) shoot height, (B) shoot dry weight, (C) root length, and (D) root dry weight of plants after 8 weeks of growth. Different letters indicate significant differences among treatment groups at $P \leq 0.05$. Values represent mean \pm SD.

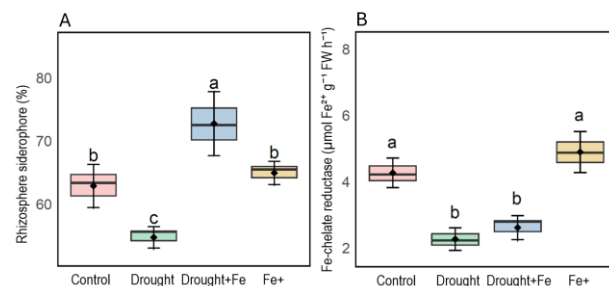


Fig. 5. Effects of drought and Fe treatment on rhizosphere siderophore (A) and Fe-chelate (B)

reductase activity in roots (B) in plants after 8 weeks of growth. Different letters indicate significant differences among treatment groups at $P \leq 0.05$. Values represent mean \pm SD.

Fe supplementation influenced rhizosphere siderophore production and Fe-chelate reductase activity

Rhizosphere siderophore levels were significantly reduced under drought stress (Fig. 5A). In contrast, drought + Fe treatment induced the highest siderophore accumulation among all treatments, suggesting enhanced rhizosphere-mediated Fe mobilization. Fe-chelate reductase activity also declined significantly under drought conditions (Fig. 5B). Fe supplementation partially improved Fe-chelate reductase activity under drought stress, while Fe⁺ treatment alone exhibited the highest activity overall (Fig. 5B).

Discussion

Drought stress severely impaired soybean growth, physiological performance, and nutrient-associated responses in the present study. The substantial reductions in shoot height, biomass accumulation, chlorophyll score, relative water content, root growth, and nodulation collectively demonstrate the sensitivity of soybean plants to prolonged water limitation. Water deficit is known to disrupt cellular hydration, reduce stomatal conductance, impair carbon assimilation, and suppress nutrient uptake due to restricted soil water movement and reduced root metabolic activity (Farooq et al., 2009; Anjum et al., 2017). The visible chlorosis and stunted phenotype observed in drought-treated plants further indicate that drought stress strongly interfered with nutrient homeostasis and photosynthetic machinery.

One of the most striking findings of this study was the partial restoration of drought-imposed growth inhibition following Fe supplementation. Iron is a fundamental micronutrient required for chlorophyll biosynthesis, electron transport chains, cytochromes, ferredoxins, and antioxidant enzymes involved in cellular redox

regulation (Kroh & Pilon, 2019). Therefore, the improved chlorophyll score and shoot growth observed in drought + Fe plants strongly suggest that Fe supplementation helped maintain photosynthetic competency under water-deficit conditions. Drought-induced chlorophyll degradation is often associated with oxidative damage to chloroplast membranes and impaired synthesis of chlorophyll precursors (Murchie & Niyogi, 2011). The improvement in chlorophyll content in Fe-supplemented plants may indicate stabilization of photosynthetic pigments and maintenance of electron transport activity, thereby sustaining carbon assimilation during stress exposure.

The reduction in relative water content under drought conditions demonstrates severe impairment of plant water balance. Relative water content is widely considered a reliable indicator of cellular hydration and drought tolerance because it reflects the ability of plants to maintain tissue turgor under stress (Farooq et al., 2009). The higher relative water content in drought + Fe-treated plants suggests that Fe supplementation improved physiological water retention mechanisms. This improvement may be linked to enhanced root growth and better maintenance of membrane integrity. Iron has been reported to contribute indirectly to osmotic adjustment and membrane stability through improved antioxidative metabolism and reduced lipid peroxidation (Nadarajah, 2020). Thus, the recovery of water observed in Fe-supplemented plants may reflect reduced oxidative damage and enhanced stress adaptation.

Root growth responses provide additional insight into the mechanisms underlying drought tolerance. Drought stress markedly reduced root length and root dry weight, indicating suppression of root elongation and biomass accumulation. Water deficit commonly limits root cell expansion and metabolic activity due to decreased turgor pressure and restricted nutrient transport. Interestingly, Fe supplementation partially restored root development under drought conditions. Improved root growth under Fe



supplementation could enhance soil exploration capacity and water uptake efficiency, thereby contributing to the improved physiological performance observed in drought + Fe plants. Since roots are the primary site of nutrient sensing and water acquisition, enhanced root growth may represent an adaptive mechanism enabling plants to better tolerate drought conditions.

Nitrogen metabolism appeared to be strongly influenced by both drought stress and Fe availability. Drought-treated plants exhibited significantly lower leaf nitrogen content and reduced nodulation compared with control plants. Legume nodules are highly sensitive to drought because biological nitrogen fixation is an energetically demanding process requiring adequate oxygen regulation, carbon supply, and micronutrient availability (Schwember et al., 2019). Iron is particularly important for symbiotic nitrogen fixation because nitrogenase enzymes and leghemoglobin proteins contain Fe as an essential component. Therefore, the reduction in nodulation and nitrogen accumulation under drought may partly reflect impaired Fe availability and reduced nodule functionality. The partial restoration of leaf nitrogen content and nodulation under drought + Fe treatment suggests that Fe supplementation helped sustain symbiotic activity and nitrogen assimilation under stress conditions.

Another important finding was the strong alteration of rhizosphere siderophore dynamics among treatments. Rhizosphere siderophore levels declined under drought stress but increased substantially following Fe supplementation during drought exposure. Siderophores are Fe-chelating compounds produced primarily by rhizosphere-associated microorganisms and play a major role in mobilizing insoluble Fe under limiting conditions (Sun et al., 2016). Increased siderophore accumulation in drought + Fe plants may indicate activation of rhizosphere-mediated nutrient acquisition mechanisms. Drought stress often suppresses microbial activity due to reduced soil moisture; however, Fe supplementation may

have stimulated beneficial rhizosphere interactions that enhanced Fe mobilization and nutrient availability. The elevated siderophore levels therefore suggest that drought + Fe plants maintained a more metabolically active rhizosphere environment compared with drought-stressed plants without Fe supplementation.

The changes in Fe-chelate reductase activity further support the involvement of Fe acquisition mechanisms in drought adaptation. Fe-chelate reductase is a key component of Strategy I Fe acquisition responses in dicot plants and is responsible for reducing ferric Fe^{3+} to the more soluble ferrous Fe^{2+} form prior to uptake by root transporters (Kabir et al., 2012). Drought stress significantly reduced Fe-chelate reductase activity, indicating suppression of active Fe acquisition mechanisms under water deficit conditions. Reduced root metabolic activity and impaired membrane-associated enzymatic processes may explain this decline. However, Fe supplementation partially restored reductase activity in drought-treated plants, suggesting reactivation of Fe acquisition pathways and improved nutrient homeostasis.

Interestingly, Fe+ treatment alone exhibited the highest Fe-chelate reductase activity but showed lower chlorophyll content, reduced nitrogen accumulation, and decreased root growth compared with control plants. This observation suggests that excessive Fe availability may disrupt cellular homeostasis and induce secondary stress responses. Excess Fe can catalyze the formation of reactive oxygen species through Fenton reactions, resulting in oxidative damage to cellular components (Halliwell, 2006). Therefore, while moderate Fe supplementation appears beneficial under drought conditions, excessive Fe availability may negatively affect plant growth due to redox imbalance and potential Fe toxicity.

The overall findings of this study indicate that Fe-mediated drought tolerance is multifactorial and involves coordinated regulation of photosynthesis, water balance, nutrient



acquisition, rhizosphere interactions, and nitrogen metabolism. The improvement in drought tolerance observed under Fe supplementation was not restricted to a single parameter but rather reflected integrated physiological recovery across multiple biological processes. This highlights the central role of Fe homeostasis in maintaining plant performance under environmental stress.

From an agricultural perspective, these findings have important implications for improving soybean resilience under drought-prone environments. Climate change is expected to increase the frequency and intensity of drought events worldwide, necessitating sustainable strategies to maintain crop productivity. Appropriate micronutrient management, particularly Fe supplementation, may represent a practical approach to enhancing drought adaptation in soybean cultivation systems. However, the contrasting responses observed under Fe⁺ treatment alone also emphasize the importance of optimizing Fe dosage to avoid nutrient imbalance or oxidative toxicity. Future studies should further investigate the molecular and microbiome-associated mechanisms underlying Fe-mediated drought tolerance in soybean.

Conclusion

Drought stress severely impaired soybean growth, chlorophyll accumulation, water status, nodulation, nitrogen content, and Fe acquisition responses, indicating strong disruption of physiological and nutrient-associated processes under water deficit conditions. However, Fe supplementation partially alleviated these adverse effects by improving chlorophyll score, relative water content, shoot and root growth, nitrogen accumulation, rhizosphere siderophore production, and Fe-chelate reductase activity. These findings suggest that Fe plays an important role in maintaining photosynthetic performance, nutrient homeostasis, and stress adaptation in soybean under drought conditions. Overall, the study highlights the potential of balanced Fe supplementation as a promising strategy to

improve drought resilience and physiological stability in soybean plants.

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Conflict of interest

The authors declare they have no conflicts of interest.

Author contributions

M.R.H. conceived and conducted the study, performed data collection and analysis, and prepared the original manuscript draft. A.H.K. conceptualized and supervised the research and revised the manuscript.

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