



Nanochelated Iron Outperforms Ionic Iron in Kidney Bean (*Phaseolus vulgaris* L.) Productivity Under Drought Stress

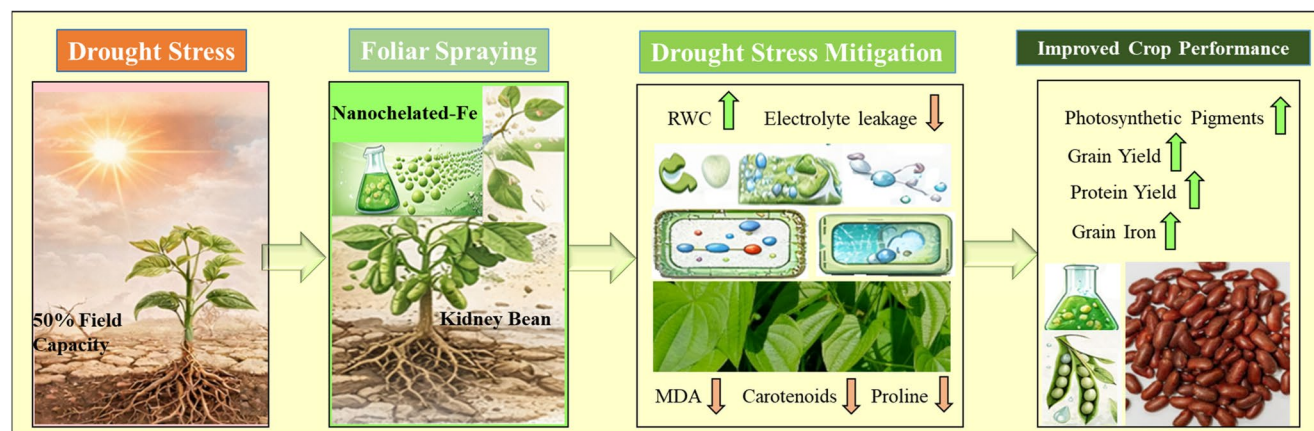
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Abstract

Drought stress poses a formidable abiotic challenge that severely curtails crop productivity. This study evaluated the effectiveness of foliar-applied nanochelated iron (nanochelated-Fe) compared to ionic Fe^{2+} in kidney bean (*Phaseolus vulgaris* L.) under both nondrought and drought conditions. A one-year field trial was performed following a randomized complete block design consisting of three replicates (six plants per treatment), and *P. vulgaris* grown under nondrought (70% field capacity) and drought (50% field capacity) stress was foliarly treated with equivalent concentrations of nanochelated-Fe or ionic Fe^{2+} (0, 50, 200, 500, and 1000 mg Fe L^{-1}) and compared with untreated control. Untreated plants under drought stress had significantly lower photosynthetic pigments, underscoring the vulnerability of the crop to drought. Remarkably, foliar application of 1000 mg L^{-1} nanochelated-Fe under drought resulted in significant increase in grain yield (47%), protein content (21%), protein yield (78%), and grain iron (36%) compared to untreated control. Under non-drought conditions, similar benefits were recorded with 27%, 22%, 55%, and 31% increases in grain yield, protein content, protein yield, and grain Fe concentration, respectively. Moreover, nanochelated-Fe treatment outperformed ionic Fe^{2+} by providing an extra 7% increase in protein content and an 8% enhancement in grain Fe concentration. These superior outcomes are attributed to the nanochelated-Fe's ability to mitigate oxidative stress, modulate proline and carotenoid levels, reduce electrolyte leakage, and improve relative water content. These findings demonstrate that nanochelated-Fe may serve as a sustainably effective strategy for improving kidney bean production and nutritional quality, particularly in regions experiencing moderate drought stress.

Graphical Abstract



Keywords Nano-chelated iron · Photosynthesis · Drought tolerance · Lipid peroxidation · Protein · Iron fortification

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1 Introduction

Drought stress profoundly impacts plant growth, development, and productivity. Drought severity adversely influences the morphological, physiological, and biochemical processes in plants, leading to diminished global crop yield (Buragohain et al. 2024; Tiwari et al. 2021). For example, drought stress can lead to decreased water absorption, increased stomatal closure and hormonal imbalance disrupting crucial metabolic pathways in plants (Buragohain et al. 2024). It can also result in reduced root growth, biomass (Hussein et al. 2022), and chlorophyll while increasing carotenoids, soluble sugars, proline, amino acids, and phenols in response to drought stress (Gullap et al. 2022; Hussein et al. 2022). To counter such abiotic stress, plants are known to produce abscisic acid and antioxidative enzymes in excess (Gullap et al. 2022). Drought can trigger a series of physiological and biochemical transformations in plants, ultimately leading to decreased growth, development, and crop yield. Comprehending these effects is vital for formulating strategies to enhance crop resilience and sustain agricultural productivity under water-limited conditions (Buragohain et al. 2024; Chauhan et al. 2024; Chandra et al. 2021).

Bean (*Phaseolus vulgaris* L.) is a crucial legume, holding significant importance in agroecology and human nutrition across various regions, including Iran. In 2022, bean cultivation globally covered 5.34 million hectares, yielding an average of 882 kg/ha, while Iran cultivated beans over an area of 105,113 hectares, achieving an average yield of 2100 kg/ha (FAO 2022). Bean grains are high in protein, typically containing 22–26% crude proteins (Rezende et al. 2017). They are also abundant in dietary fiber, minerals, and vitamins (Jarecki and Migut 2022; Rezende et al. 2017), with significant amounts of iron, zinc, phosphorus, potassium, and magnesium (Jarecki and Migut 2022). Consequently, beans not only supply proteins and energy but also offer a broad spectrum of essential nutrients beneficial for human health (Simões et al. 2022). Their high protein content, favorable amino acid profile, and rich mineral composition render it a vital element of sustainable diets, especially in addressing food security and malnutrition challenges (Katoch et al. 2023; Popoola et al. 2023). Drought stress is known to significantly hinder growth, development, and yield in beans (Polania et al. 2020). For example, drought can result in reduced leaf area, harvest index, and grain weight in common bean (Smith et al. 2019), and can negatively affect photosynthesis rate, chlorophyll fluorescence parameters, and intact chloroplast separation (Zadrazilnik and Šuštar-Vozlić 2020).

In addition to being essential for humans (Naz et al. 2022), iron (Fe) is critical for plant growth and development, serving as an essential micronutrient involved in various metabolic processes (Ramsey 2024; Gao and Dubos

2020). Fe is vital for photosynthesis, respiration, and amino acid biosynthesis, and functions as a cofactor for numerous enzymes (Gao and Dubos 2020). Despite its abundance in soil, Fe bioavailability is often limited, leading to deficiencies in plants (Shumayla and Upadhyay 2022; Zhang et al. 2019). Such deficiencies can impair plant health and function by disrupting physiological processes and negatively affecting nutrient concentrations in edible tissues (Naz et al. 2022). For instance, in soybean (*Glycine max* L.), Fe deficiency, particularly in alkaline soils, can worsen the impacts of drought stress on plant health. Furthermore, the simultaneous occurrence of Fe deficiency and drought stress can markedly impact photosynthetic performance, nutrient uptake, and plant biomass. This highlights the importance of maintaining adequate Fe nutrition for plants to effectively manage drought stress (Ramsey 2024). Foliar applications of chelated and magnetic liquid Fe fertilizers have demonstrated promising results in promoting drought tolerance in legumes, including soybean and velvet bean (*Mucuna pruriens*) (Ramsey 2022, 2024). The application of magnetic Fe chelates has been associated with the inactivation of interconnected gas exchange parameters linked to plant defense activities under water stress, thereby improving metabolic efficiency, lowering stress levels, and increasing plant resilience to abiotic stresses (Ramsey 2024).

Fe chelates are also utilized to address Fe deficiencies in plants. For example, foliar spraying (2000 mg L⁻¹) of Fe nanochelate increased leaf area, chlorophyll content, and catalase enzyme activity in quince (*Cydonia oblonga* Mill.) (Rahemi et al. 2019). Furthermore, Fe chelates enhance drought tolerance by increasing photosynthesis rate and water use efficiency in plants under water stress. A recent study in legumes indicated that foliar application of chelated and magnetic liquid Fe fertilizer yielded a 56% increase in photosynthesis and a 263% increase in water use efficiency compared to non-magnetic Fe treatments (Ramsey 2022). Likewise, foliar application of 0.2% ferrous sulfate or 0.5% Fe chelate has been shown to promote the uptake of nitrogen, phosphorus, potassium, and Fe in rice cultivars (Kumar et al. 2017).

The nutritional value of beans is critically important for people in developing regions. However, many of these areas, characterized by a semi-arid climate and low rainfall, also contend with calcareous soils with high pH and salinity. As a result, crops encounter not only periods of water scarcity but also limitations in the absorption of trace elements, such as Fe, due to unfavorable soil conditions. Even when water is adequately supplied, Fe may still be insufficiently available to plants, leading to reduced crop yield and nutritional quality. Prior research has indicated that certain concentrations of nanoparticles can enhance drought tolerance in plants (Rezayian et al. 2023; Linh et al. 2020). Considering the above, herein we investigated the comparative potential of

nanochelated iron (nanochelated-Fe) and ionic Fe^{2+} to mitigate drought stress and consequently improve physiological attributes, grain yield and nutritional quality (proteins and Fe uptake) in kidney bean. We hypothesized that nanochelated-Fe will outperform ionic Fe^{2+} to mitigate drought stress and significantly improve physiological attributes, grain yield and nutritional quality in kidney bean. The findings of this field study are novel, demonstrating that nanochelated-Fe has potential to serve as a sustainable and effective strategy for improving bean production and nutritional quality, particularly in regions impacted by moderate drought stress.

2 Materials and Methods

2.1 Material Characterization

Iron nanochelate (nanochelated-Fe) and ferrous sulfate (FeSO_4 or ionic Fe) were procured from Sodour Ahrar Shargh Knowledge-based Company (Khazra) and Kargozar companies, respectively. Iron nanochelate was produced via “self-assembly,” a process detailed in previous publications (Nazaran 2012; Fakharzadeh et al. 2020). Briefly, 2.8 g of iron chloride and 3.5 ml of chloric acid were dissolved in distilled water, then the mixture was placed on a shaker for 15 min. Subsequently, 3 g of citric acid and 2 g of urea were added to the mixture, agitated for 20 min, and warmed below 60 °C. The solution was left to cool for 2 h at room temperature and subsequently dried using a freeze dryer (Nazaran 2012; Fakharzadeh et al. 2020). The mean particle size and hydrodynamic diameter were determined by transmission electron microscopy (TEM) and dynamic light scattering (DLS), respectively. TEM analysis showed mean particle diameter of 80 ± 3.2 nm (see Supplementary Fig. S1), and DLS showed mean hydrodynamic diameter of 57 ± 2.0 nm and zeta potential of -13.7 ± 2.5 mV.

2.2 Experimental Design and Treatment

Comprehensive details regarding the study site's (50°49' N, 32°21' E, 2050 m asl.) climatic and soil characteristics are presented in Supplementary Tables S1 and S2. The study site experienced no rainfall during the research period, thereby allowing for the simulation of drought conditions. Furthermore, the low levels of organic matter and soil pH suggest a restricted availability of micronutrients, including Fe.

The experiment was designed as a factorial experiment employing a randomized complete block design (RCBD), with three replications. The initial factor included two levels of water status: no drought (70% field capacity; FC) and drought (50% FC). The second factor included the type of iron particle (nanochelated-Fe and ionic Fe^{2+}), while the

third factor examined varying nominal concentrations of Fe (0, 50, 200, 500, and 1000 mg Fe L^{-1}) (Table S3). Plastic pots, 29 cm in height and 28 cm in diameter, were wrapped in plastic bags and placed in 30 cm deep holes in the soil (Fig. S2a). The bottom of each pot was filled with washed sand, atop of which was added 13 kg of agricultural soil (Table S2). On June 20, 2022, five seeds of kidney bean (*Phaseolus vulgaris* var. Yaghot) were planted in each pot at a depth of 5 cm, spaced 2 cm apart. The Yaghot variety, characterized by type II (erect indeterminate bush), is a medium-sized grain bean type that exhibits a high level of yield and quality. This variety possesses a desirable plant structure, acceptable marketability, is considered resilient to drought, and is the most sought out kidney bean variety in Iran (Ghadiri 2017). Thinning was conducted after full establishment to ensure the health and uniformity of the seedlings. Once the pots reached soil capacity following initial irrigation, they were watered with tap water every 72 h, with each pot receiving 3000 ml of water per irrigation session based on the soil's agricultural capacity. Foliar spraying commenced before the onset of drought stress, with the first application on August 30, 2022, and the second two weeks later (September 13, 2022). Starting next day, half of the pots were subjected to drought stress, receiving 1500 mL of water each day (50% field capacity) until harvest on October 11, 2022, while the control pots were irrigated according to 70–100% field capacity until the end of the growing season on October 11, 2022.

2.3 Measurement of Traits

At 107-day post-planting, fully developed leaf samples, weighing between 2.5 and 3.0 g, were collected from each plant. Various traits were evaluated, including plant cell ultrastructure, photosynthetic pigments, leaf relative water content (RWC), proline content, malondialdehyde (MDA), and membrane electrolyte leakage, following established protocols. Additionally, upon grain maturity, at 126-day post-planting, grain yield, protein content and Fe uptake were measured.

2.3.1 Ultrastructure of Plant Cell

The investigation of leaf organelles and possible alterations in (sub)cellular structure was conducted using a transmission electron microscope (TEM) (Philips, EM208S, 100 KV, Netherlands). Initially, the leaves were immersed in a 1:1 solution of final Spurr resin and absolute acetone at room temperature for one hour. Subsequently, the samples were transferred to a 1:3 mixture of the final resin and absolute acetone for three hours, followed by an overnight immersion in the final resin solution. The samples were then heated at 70 °C in Eppendorf tubes containing Spurr resin for over 9 h. Afterward, the specimens were sectioned using

a microtome (Leica EM UC7), and the sections were stained with alkaline lead citrate and uranyl acetate for 10 min before being examined under the TEM.

2.3.2 Photosynthetic Pigments

The quantity of photosynthetic pigments was determined using the method outlined by Lichtenthaler and Buschman (2001). 100 mg of fresh leaf tissue was finely ground with 5 mL of 80% acetone in a mortar to form a homogeneous mixture. The extract was then filtered through filter paper and a funnel. The residue was re-grounded with 80% acetone and processed until the remaining leaf material turned white. The extract was subsequently diluted to a final volume of 10 mL with 80% acetone. The optical density of the extract (OD) was measured at wavelengths of 663.2, 646.8, and 470 nm using a UV-Vis spectrophotometer (AE-UV 1606), with 80% acetone used as a blank. The quantity of photosynthetic pigments (chlorophyll a [Chla], Chlorophyll b [Chlb], and total chlorophyll [Chlt]) was calculated using the following equations and expressed in mg g⁻¹ FW (fresh weight):

$$\text{Chla} \left(\frac{\text{mg}}{\text{mL}} \right) = 12.25 \times \text{OD}_{663.2} - 2.79 \times \text{OD}_{646.8} \quad (1)$$

$$\text{Chlb} \left(\frac{\text{mg}}{\text{mL}} \right) = 12.51 \times \text{OD}_{646.8} - 5.10 \times \text{OD}_{663.2} \quad (2)$$

$$\text{Chlt} \left(\frac{\text{mg}}{\text{mL}} \right) = \text{Chla} + \text{Chlb} \quad (3)$$

$$\text{Carotenoids} \left(\frac{\text{mg}}{\text{mL}} \right) = \frac{1000 \times \text{OD}_{470} - (1.82 \times \text{Chla}) - (85.02 \times \text{Chlb})}{198} \quad (4)$$

2.3.3 Leaf Relative Water Content (%)

The relative water content (RWC) in leaves was measured according to Martínez et al. (2007). The RWC was calculated using the following equation:

$$\text{RWC} (\%) = \frac{Fw - Dw}{Sw - Dw} \times 100 \quad (5)$$

FW=leaf fresh weight immediately after sampling.

DW=leaf dry weight after oven drying.

SW=leaf saturated weight after exposure to distilled water.

2.3.4 Proline Content

Proline was extracted from plant tissue following the protocol by Bates et al. (1973). Initially, the required solutions were prepared, and extracts were obtained from leaf samples and placed into a falcon tube. The samples were centrifuged and

the supernatant (plant extract) was transferred to a new falcon tube, where 2 mL of ninhydrin acid and 2 mL of glacial acetic acid were added and mixed thoroughly. The samples were heated in a water bath at 100 °C for 1 h, followed by a 10 min incubation in an ice bath. Finally, 4 mL of toluene was added to the resulting solution and vortexed for 20 s. The samples were left undisturbed for 15–20 min to allow complete separation of the two phases. The supernatant, containing toluene and proline, was measured at 520 nm using a UV-Vis spectrophotometer (AE-UV 1606) to determine proline concentration, with toluene serving as a blank solution.

2.3.5 Membrane Lipid Peroxidation

Membrane lipid peroxidation was assessed using the method described by Heath and Packer (1968). The extent of lipid peroxidation in leaf tissues was determined by quantifying malondialdehyde (MDA), a byproduct of lipid peroxidation, through the thiobarbituric acid reaction. The MDA concentration was calculated using an extinction coefficient of 155 mM⁻¹ cm⁻¹, with results expressed in millimoles per gram of fresh weight using the formula established by Narwal et al. (2009).

$$\text{MDA} \left(\frac{\text{mmol}}{\text{g FW}} \right) = \frac{A_{532} - A_{600}}{\epsilon dFW} \times V \quad (6)$$

A=absorption rate.

ε = extinction coefficient of malondialdehyde at 532 nm (155 mM⁻¹ cm⁻¹).

V=sample volume (L), FW= weight of fresh plant tissue (g).

d=width of cut (cm).

2.3.6 Membrane Electrolyte Leakage

The percentage of membrane electrolyte leakage (EL) in leaf cells was determined using the following formula (Dionisio-Sese and Tobita, 1998).

$$\text{EL} (\%) = \frac{C1}{C2} \times 100 \quad (7)$$

C1=electrical conductivity of the solution after exposure to a temperature of 32 °C.

C2=electrical conductivity of the solution after being subjected to a temperature of 121 °C.

2.3.7 Grain Yield

On October 22, 2021, the plants were harvested when 65–75% of the pods had matured. The grains from each plant were then separated from the chaff, and the yield was assessed by weighing in grams per plant.

2.3.8 Grain Protein and Protein Yield

To determine the crude protein content in the grains, the nitrogen percentage was first measured using the Kjeldahl method (Bremner 1996). The protein yield was subsequently calculated using Eq. 9 (Villwock et al. 2022).

$$\text{Crude protein (\%)} = \text{Nitrogen (\%)} \times 6.25 \quad (8)$$

$$\text{Crude protein yield} \left(\frac{\text{g}}{\text{plant}} \right) = \text{Crude protein (\%)} \times \text{Grain yield} \quad (9)$$

2.3.9 Iron Content

Dry burning technique was used to prepare plant extract for quantifying grain Fe content (Campbell and Plank 1998). Initially, 0.5 g of desiccated grain was weighed and placed in an electric furnace. The furnace temperature was gradually increased to 550 °C for 2 h, maintaining the samples at this temperature for a period ranging from 2 to 8 h until the sample turned into white ash. A small volume of distilled water was added to stabilize the resulting ash, followed by addition of 10 mL of 2 M hydrochloric acid. The mixture was then heated on an electric stove to 80 °C until the first white vapors appeared. Finally, the mixture was filtered through filter paper and transferred into a 50 mL volumetric flask. This extract was used to measure the grain Fe content using an ICP-OES (Spectro Genesis), and total Fe concentration expressed in mg Fe kg⁻¹ of grain.

2.4 Statistical Analysis

Data were organized in Excel and analyzed in SAS 9.1. Statistical difference in sample means were evaluated using ANOVA following the slicing method for drought and non-drought conditions (water status, and iron

fertilizer; 2 × 10) in a randomized complete block design. The “ANOVA with slicing method” refers to a statistical technique used to analyze simple effects within a multi-factor ANOVA model when a significant interaction is detected. It involves breaking down the interaction into a series of one-way ANOVAs or specific comparisons at each level of one factor to understand where the differences lie. Further, the means were compared between treatments using the LSD post-hoc test and $p \leq 0.05$ was considered statistically significant.

3 Results

ANOVA revealed that photosynthetic pigments, relative leaf water content, proline content, membrane lipid peroxidation, membrane electrolyte leakage rate, iron content, grain protein content and yield, and grain yield were significantly influenced by water status and applied Fe concentrations ($p < 0.01$). The particle form exhibited a significant effect on all traits measured ($p < 0.01$), except for proline content and membrane lipid peroxidation (Tables 1, 2 and 3). The interaction between water status and particle form was significant for chlorophyll-a, chlorophyll-b, total chlorophyll, and protein yield ($p < 0.05$; Tables 1, 2 and 3). All traits measured, except for grain Fe and protein contents, were influenced by the interaction of water status and Fe concentration ($p < 0.01$).

3.1 Cellular and Sub-Cellular Structure

The transmission electron microscopy (TEM) images indicated that the cell wall and plasma membrane remained intact in the control plants not subjected to drought stress (Fig. 1a). In control plants experiencing drought stress, slight irregularity in the cytosol was noted (Fig. 1b). Starch granules appeared

Table 1 Mean square (MS) from the analysis of variance (ANOVA) performed for photosynthetic pigments in kidney bean influenced by water status and iron fertilizer

S.O.V. ^a	DF ^b	Chlorophyll-a	Chlorophyll-b	Total chlorophyll	Carotenoids
R	2	0.02 ^{ns}	0.005 ^{ns}	0.01 ^{ns}	0.004*
W	1	21.2**	25.0**	92.3**	1.46**
Fe	9	0.30**	0.07**	0.64**	0.03**
W × Fe	9	0.06*	0.06**	0.23**	0.002 ^{ns}
Error	38	0.02	0.02	0.04	0.001
Non-drought					
Replication	2	0.02 ^{ns}	0.02 ^{ns}	0.08 ^{ns}	0.0002 ^{ns}
Fe	9	0.13**	0.07*	0.36**	0.0105**
Error	18	0.02	0.02	0.05	0.0004
Drought stress					
Replication	2	0.06*	0.04**	0.15**	0.009*
Fe	9	0.23**	0.06**	0.52**	0.024**
Error	18	0.02	0.01	0.02	0.002

Table 2 Mean square (MS) from the analysis of variance (ANOVA) performed for relative leaf water content, proline content, membrane lipid peroxidation and electrolyte leakage in kidney bean influenced by water status and iron fertilizer

S.O.V. ^a	DF ^b	Relative water content	Proline content	Membrane lipid peroxidation	Electrolyte leakage
R	2	1.09 ^{ns}	0.24 [*]	0.01 ^{**}	26.2 [*]
W	1	798 ^{**}	26.0 ^{**}	0.96 ^{**}	8074 ^{**}
Fe	9	248 ^{**}	0.20 ^{**}	0.01 ^{**}	215 ^{**}
W × Fe	9	35.7 ^{**}	0.51 ^{**}	0.01 ^{**}	126 ^{**}
Error	38	4.24	0.07	0.002	5.60
Non-drought					
Replication	2	23.4 ^{**}	0.06 ^{ns}	0.014 ^{**}	20.8 ^{**}
Fe	9	56.0 ^{**}	0.07 ^{ns}	0.001 ^{ns}	15.6 ^{**}
Error	18	2.92	0.05	0.002	2.82
Drought stress					
Replication	2	11.3 [*]	0.26 ^{ns}	0.002 ^{ns}	54.5 ^{**}
Fe	9	227 ^{**}	0.65 ^{**}	0.017 ^{**}	326 ^{**}
Error	18	2.29	0.09	0.002	3.53

^a S.O.V.: Source of Variation; R: Replication; W: Water status; Fe: Iron fertilizer (combination of particle form and concentration)

^b Degrees of Freedom

Table 3 Mean square (MS) from the analysis of variance (ANOVA) performed for iron content, protein content, grain and protein yield in kidney bean influenced by water status and iron fertilizer

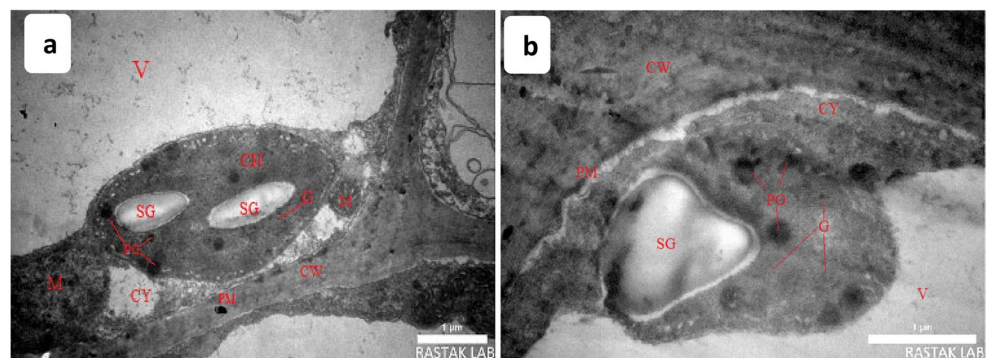
S.O.V. ^a	DF ^b	Iron content	Protein content	Grain yield	Protein yield
R	2	26.2 ^{ns}	0.25 ^{ns}	1.83 ^{**}	0.06 ^{ns}
W	1	16,613 ^{**}	403 ^{**}	358 ^{**}	42.8 ^{**}
Fe	9	1610 ^{**}	25.9 ^{**}	18.1 ^{**}	2.47 ^{**}
W × Fe	9	18.1 ^{ns}	0.52 ^{ns}	1.19 ^{**}	0.16 ^{**}
Error	38	12.8	0.46	0.27	0.02
Non-drought					
Replication	2	47.6 [*]	0.79 ^{ns}	1.36 [*]	0.04 ^{ns}
Fe	9	942 ^{**}	15.1 ^{**}	10.8 ^{**}	1.83 ^{**}
Error	18	9.77	0.56	0.25	0.02
Drought stress					
Replication	2	3.47 ^{ns}	0.21 ^{ns}	0.58 ^{ns}	0.02 ^{ns}
Fe	9	686 ^{**}	11.3 ^{**}	8.54 ^{**}	0.81 ^{**}
Error	18	14.6	0.34	0.31	0.02

larger and grana were more in number in control plants under nondrought condition. Plastoglobules were larger in control plants under nondrought compared to drought stress (Fig. 1a, b). Under drought stress, application of 1000 mg L⁻¹ nano-chelated-Fe resulted in increased number of mitochondria

and grana, including the number and size of starch granules in leaf cells, compared to untreated control and plants treated with 1000 mg L⁻¹ ionic Fe²⁺. Plastoglobules were also clearly visible with 1000 mg L⁻¹ nano-chelated-Fe treatment as in untreated control (Fig. 2a, b).

3.2 Chlorophyll Content

The interaction effect of water status with Fe fertilizers on chlorophyll levels was significant ($p < 0.05$; Table 1). Under both non-drought and drought conditions, chlorophyll-a and chlorophyll-b levels increased, on average, in plants treated with 500–1000 mg L⁻¹ nano-chelated-Fe compared to equivalent concentrations of ionic Fe²⁺ (Fig. 3a, b). Total chlorophyll in plants treated with different concentrations of nano-chelated-Fe (5.4–5.6 mg g⁻¹ FW) were significantly higher than ionic Fe²⁺ treatments (4.7–5.0 mg g⁻¹ FW) under nondrought condition (Fig. 4a). Under drought stress, 500 and 1000 mg L⁻¹ nano-chelated-Fe treatments had the highest total chlorophyll content; i.e., 43% higher than untreated control and 10% higher than 500 and 1000 mg L⁻¹ ionic Fe²⁺-treated groups ($p < 0.05$; Fig. 4a).

Fig. 1 Transmission electron microscope (TEM) images of untreated control kidney bean leaves under non-drought control (a) and drought stress (b). Cell wall (CW), plasma membrane (PM), cytosol (CY), chloroplast (CH), mitochondria (M), starch granules (SG), vacuole (V), plastoglobule (PG), grana (G)

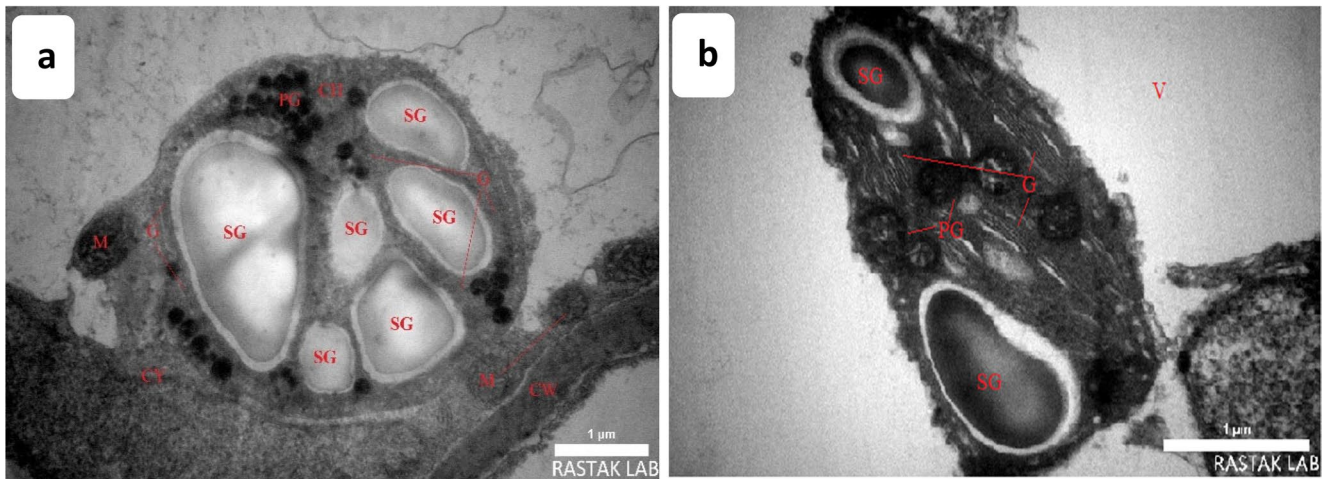


Fig. 2 Transmission electron microscope (TEM) images of kidney bean leaves under drought conditions and foliar application with 1000 mg L⁻¹ of nanochelated-Fe (**a**) and 1000 mg L⁻¹ of ionic Fe²⁺ (**b**). Cell

wall (CW), plasma membrane (PM), cytosol (CY), chloroplast (CH), mitochondria (M), starch granules (SG), vacuole (V), plastoglobule (PG), and grana (G)

3.3 Carotenoid Content

The effect of Fe fertilizers on leaf carotenoid levels under drought and non-drought conditions was significant ($p < 0.01$; Table 1) and dose-dependent, with increasing dose the carotenoid decreased, and more so for ionic Fe²⁺. Carotenoid levels in plants treated with 500 and 1000 mg L⁻¹ of nanochelated-Fe and ionic Fe²⁺ decreased by 22–30% and 33–35% under drought and non-drought conditions, respectively, compared to untreated controls ($p < 0.05$). Under non-drought condition, except for 50 mg L⁻¹ nanochelated-Fe, the carotenoid content of plants treated with nanochelated-Fe or ionic Fe²⁺ was significantly lower than untreated control (Fig. 4b). Overall, higher carotenoid levels were observed under drought stress compared to nondrought condition across all treatments (Fig. 4b).

3.4 Leaf Relative Water Content (RWC)

Under drought and non-drought conditions, RWC was significantly affected by Fe fertilizers ($p < 0.01$; Table 2), but more prominently by nanochelated-Fe. RWC in untreated plants under drought and non-drought conditions were 55–57% and 67–69%, respectively. The highest RWC was observed with 1000 mg/L nanochelated-Fe, which was 4% and 5% higher under drought and non-drought conditions, respectively, compared to equivalent concentration of ionic Fe²⁺ ($p < 0.05$; Fig. 5a). Under drought condition, RWC did not differ significantly between the two forms of Fe, particularly in the concentration range 50–500 mg L⁻¹ ($p > 0.05$; Fig. 5a).

3.5 Proline Content

Under non-drought condition, proline content was not affected ($p > 0.05$), but under drought conditions, proline content significantly increased, by Fe fertilizers ($p < 0.01$; Table 2). Under drought condition, proline levels in Fe-treated plants were significantly lower compared to untreated control, but were, on average, higher than equivalent Fe treatments under non-drought condition (Fig. 5b).

3.6 Membrane Lipid Peroxidation

Under non-drought condition, membrane lipid peroxidation (measured as MDA) was not affected by Fe fertilizers ($p > 0.05$; Table 2). The highest MDA level was observed in the untreated control under drought stress (0.77 mmol MDA g⁻¹ FW), whereas foliar application of both Fe fertilizers under drought stress significantly reduced the level of MDA in bean (Fig. 6a). Overall, MDA levels in plants treated with nanochelated-Fe (0.55–0.63 mmol g⁻¹ FW) were not statistically different from plants treated with ionic Fe²⁺ (0.58–0.62 mmol g⁻¹ FW) (Fig. 6a).

3.7 Membrane Electrolyte Leakage Rate

As shown in Table 2, the effect of Fe fertilizers on membrane electrolyte leakage under different water stress was significant ($p < 0.01$). Under non-drought condition, the range of membrane electrolyte leakage for plants treated with nanochelated-Fe was 27.4–30.5% but was higher for plants treated with ionic Fe²⁺ (range 30.8–33.9%) (Fig. 6b). Under drought for untreated plants, the range of membrane

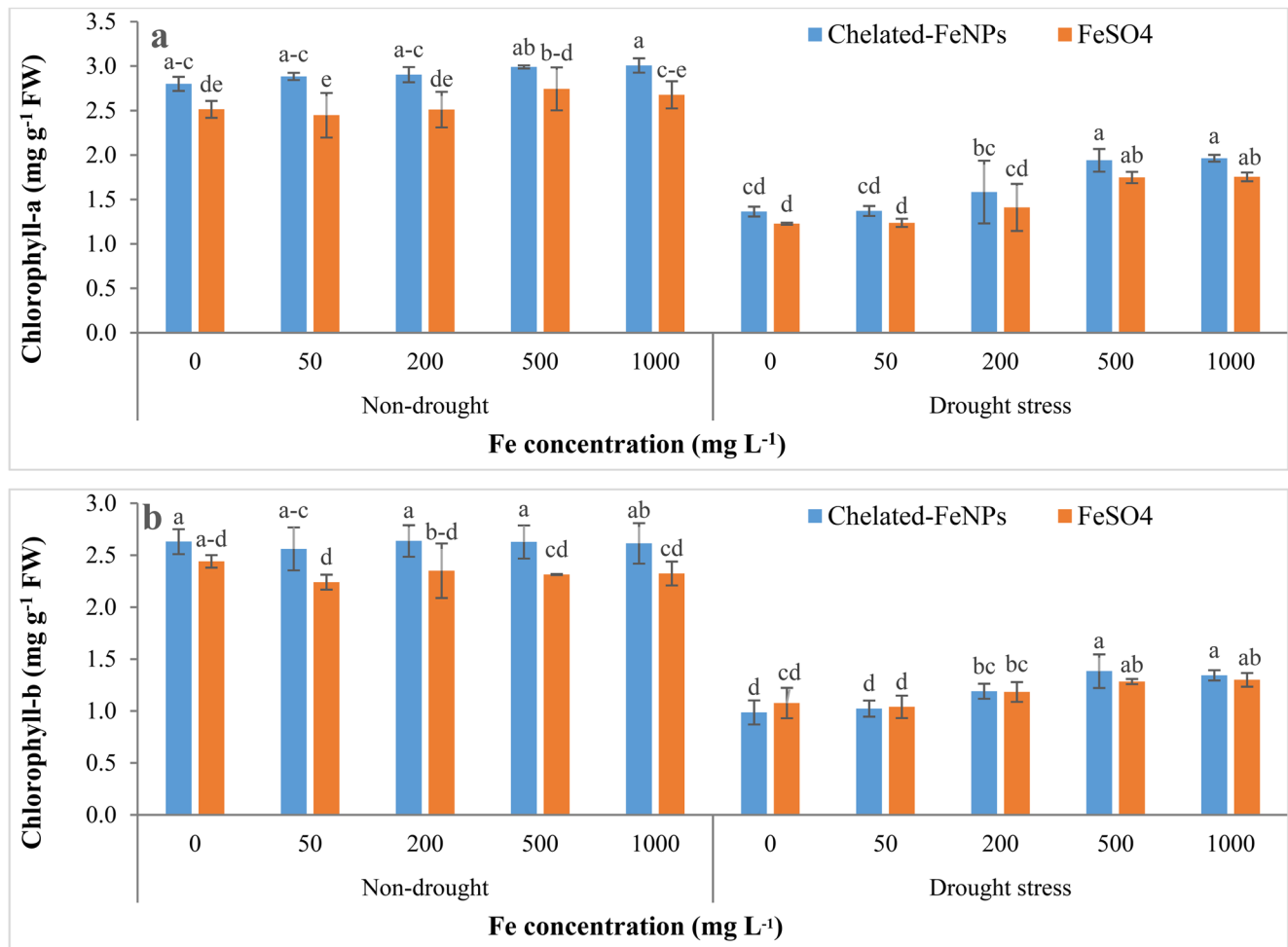


Fig. 3 Effects of nanochelated-Fe and ionic Fe²⁺ on chlorophyll-a (**a**), and chlorophyll-b (**b**) in kidney bean under drought and non-drought conditions. In each water status, bars with similar letter are not significantly different, according to the Least Significant Difference (LSD) post hoc test ($p < 0.05$). Error bars represent $\pm$ SD. Concentrations are shown in mg L⁻¹. Chelated FeNP denotes nanochelated Fe

electrolyte leakage was 39.4–66.9%. However, plants treated with 500 and 1000 mg L⁻¹ nanochelated-Fe had significantly lowest membrane electrolyte leakage (39.4% and 40.5%, respectively) under drought stress. Whereas, equivalent concentrations of ionic Fe²⁺ treatments showed 45% and 56% of membrane electrolyte leakage at 500 and 1000 mg L⁻¹, respectively. Nonetheless, membrane electrolyte leakage between 200 mg L⁻¹ nanochelated-Fe and 200 mg L⁻¹ ionic Fe²⁺ was not statistically different ($p > 0.05$; Fig. 6b).

3.8 Grain Iron Content

Drought stress significantly reduced grain Fe uptake ($p < 0.01$; Table 3). Under both drought and non-drought conditions, the highest grain Fe content was observed with 1000 mg L⁻¹ nanochelated-Fe (145 and 183 mg kg⁻¹,

respectively), followed by 500 mg L⁻¹ nanochelated-Fe and 1000 mg L⁻¹ ionic Fe²⁺ (Fig. 7a). In both water status, the Fe content in plants treated with 200 mg L⁻¹ nanochelated-Fe was not significantly different from 500 mg L⁻¹ ionic Fe²⁺ treatment ($p > 0.05$). While 50 mg L⁻¹ ionic Fe²⁺ did not improve grain Fe content ($p > 0.05$), 50 mg L⁻¹ nanochelated-Fe increased grain Fe content by 7% under drought conditions (Fig. 7a; $p < 0.05$).

3.9 Grain Protein Content

For untreated controls, the range of grain protein under non-drought condition was 21.4–28.2%, and 17–22% under drought condition (Table 3; Fig. 7b). Under drought and non-drought conditions, 1000 mg L⁻¹ nanochelated-Fe promoted grain protein content by 22% and 28.2%, respectively, and

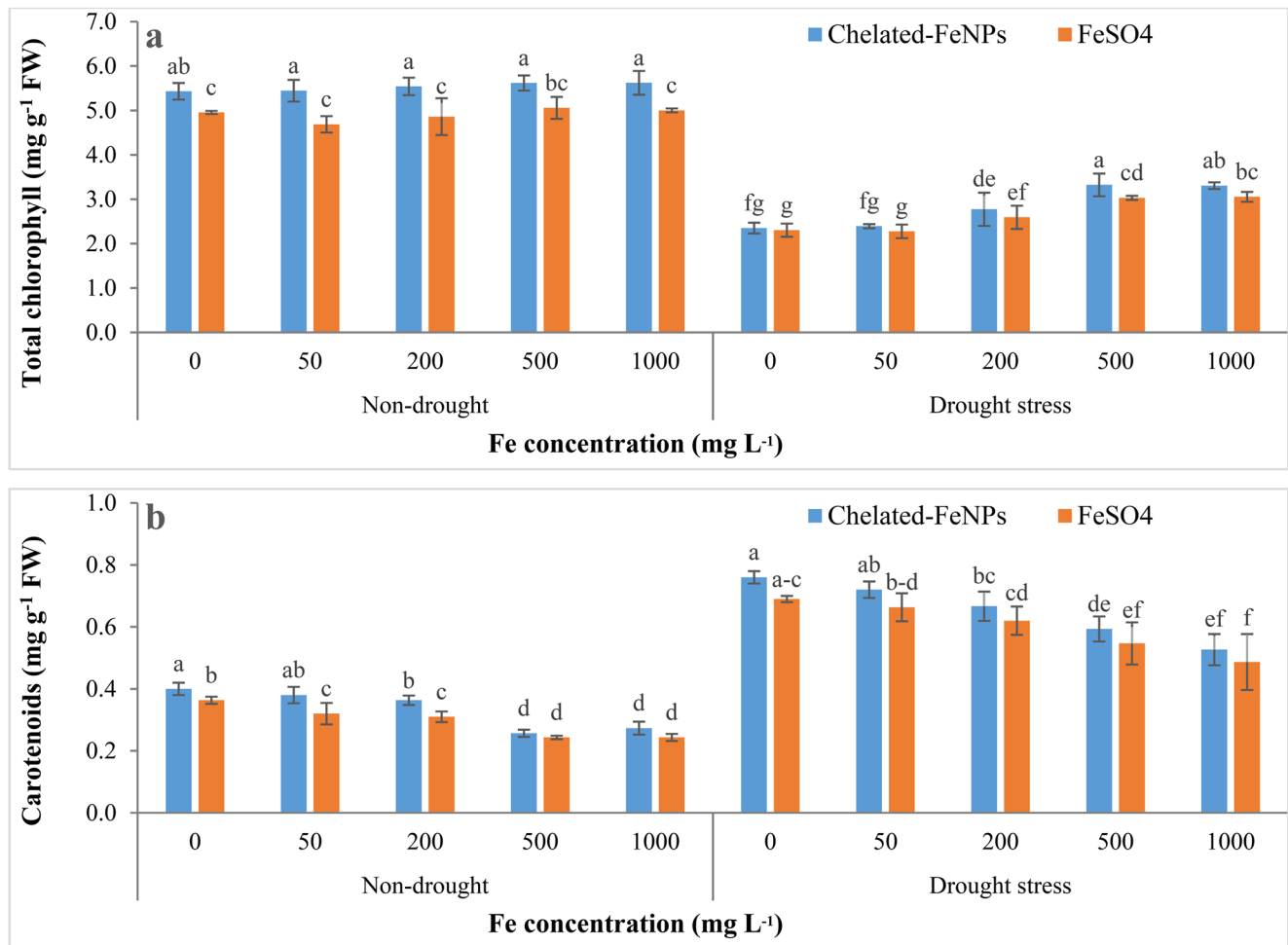


Fig. 4 Effects of nanochelated-Fe and ionic Fe²⁺ on total chlorophyll (a), and carotenoids (b) in kidney bean under drought and non-drought conditions. In each water status, bars with similar letter are not

significantly different, according to the Least Significant Difference (LSD) post hoc test ($p < 0.05$). Error bars represent $\pm$ SD. Concentrations are shown in mg L⁻¹. Chelated FeNP denotes nanochelated Fe

was not statistically different from 500 mg L⁻¹ nanochelated-Fe ($p > 0.05$). With 1000 mg L⁻¹ ionic Fe²⁺, grain protein content improved by 20.8% and 26.4% under drought and non-drought conditions, respectively, and was not statistically different from 500 mg L⁻¹ nanochelated-Fe or 500 mg L⁻¹ ionic Fe²⁺ ($p > 0.05$; Fig. 7b). Under drought condition, the protein content at 50 and 200 mg L⁻¹ of nanochelated-Fe was significantly higher at 18.5 and 18.9%, respectively, compared to equivalent concentrations of ionic Fe²⁺ (17 and 17.2%, respectively).

3.10 Grain Yield

Grain yield was affected by Fe fertilizer under drought and non-drought conditions ($p < 0.01$; Table 3). Under non-drought condition, 1000 mg L⁻¹ nanochelated-Fe had the highest grain yield (increased by 24.5 and 8.4%,

respectively) compared to the untreated control and equivalent concentration of ionic Fe²⁺ ($p < 0.05$). Grain yield at 50 mg L⁻¹ nanochelated-Fe did not significantly differ from 200 mg L⁻¹ ionic Fe²⁺ ($p > 0.05$).

Under drought conditions, 500 and 1000 mg L⁻¹ nanochelated-Fe showed comparable and yet the highest grain yield, followed by 1000 mg L⁻¹ ionic Fe²⁺ (Fig. 8a). Grain yield at 200 mg L⁻¹ nanochelated-Fe was not different from 200 to 500 mg L⁻¹ ionic Fe²⁺ treatments ($p > 0.05$; Fig. 8a).

3.11 Protein Yield

Table 3 illustrates significant effects of water status, Fe fertilizers and their interaction on protein yield ($p < 0.01$). Under non-drought condition, the highest protein yield was obtained in plants treated with 1000 mg L⁻¹ nanochelated-Fe; and under drought condition, 500–1000 mg L⁻¹

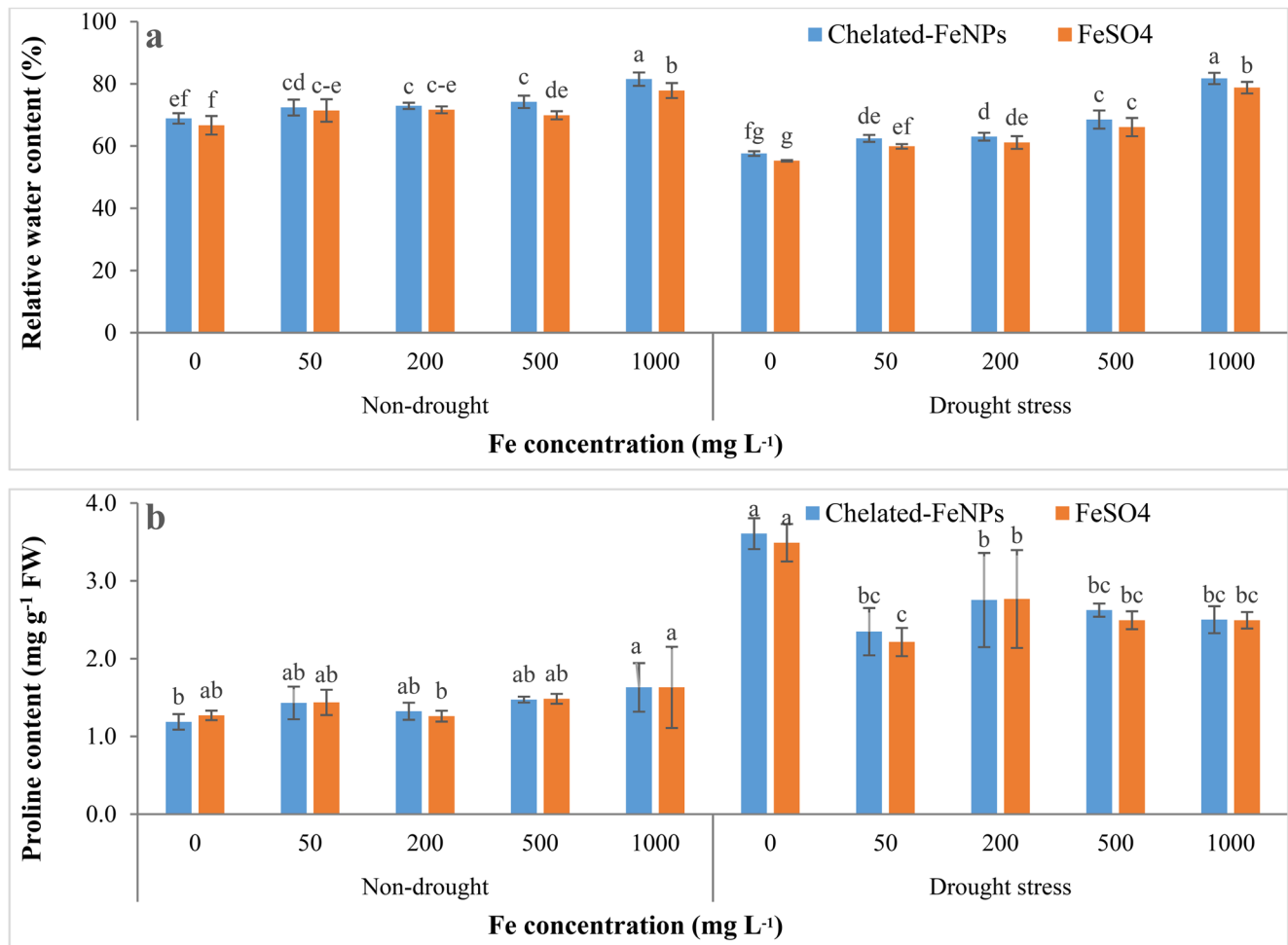


Fig. 5 Effects of nanochelated-Fe and ionic Fe²⁺ on relative water content (a), and proline content (b) in kidney bean under drought and non-drought conditions. In each water status, bars with similar letter are not

significantly different, according to the Least Significant Difference (LSD) post hoc test ($p < 0.05$). Error bars represent $\pm$ SD. Concentrations are shown in mg L⁻¹. Chelated FeNP denotes nanochelated Fe

nanochelated-Fe had the highest protein yield ($p < 0.05$; Fig. 8b), followed by 1000 mg L⁻¹ ionic Fe²⁺. Under non-drought condition, 200 mg L⁻¹ nanochelated-Fe and 500 mg L⁻¹ ionic Fe²⁺ had comparable protein yield ($p < 0.05$); while under drought, protein yield was significantly higher with 200 mg L⁻¹ nanochelated-Fe compared to equivalent concentration of ionic Fe²⁺ ($p < 0.05$).

4 Discussion

The demonstrated intracellular changes in drought-stressed plants underpin their response to water deficit (Fig. 1b). Chloroplast biogenesis and grana stacking are critical for optimizing light absorption during photosynthesis (Jiang et al. 2012) and safeguarding photosystem II (Gu et al. 2022).

The potential increase in grana and starch in leaves treated with 1000 mg Fe L⁻¹ nanochelated-Fe (Fig. 2a) suggests enhanced photosynthetic activity. The observed disorganization of the plasma membrane and cytosol in drought-stressed untreated plants compared to those treated with 1000 mg L⁻¹ nanochelated-Fe suggests the latter's protective role in maintaining cellular integrity under drought stress as confirmed by TEM analysis (Fig. 2a). The application of 1000 mg L⁻¹ ionic Fe²⁺ that led to a higher starch content compared to control plants also indicates potential for increased photosynthesis by ionic Fe²⁺. These findings point to the nanochelated-Fe role in boosting photosynthesis by promoting chlorophyll and grana development, thereby improving plant resilience to drought. Yuan et al. (2018) reported 0.05 mM L⁻¹ FeNPs significantly improved chloroplast and grana organization compared to untreated control

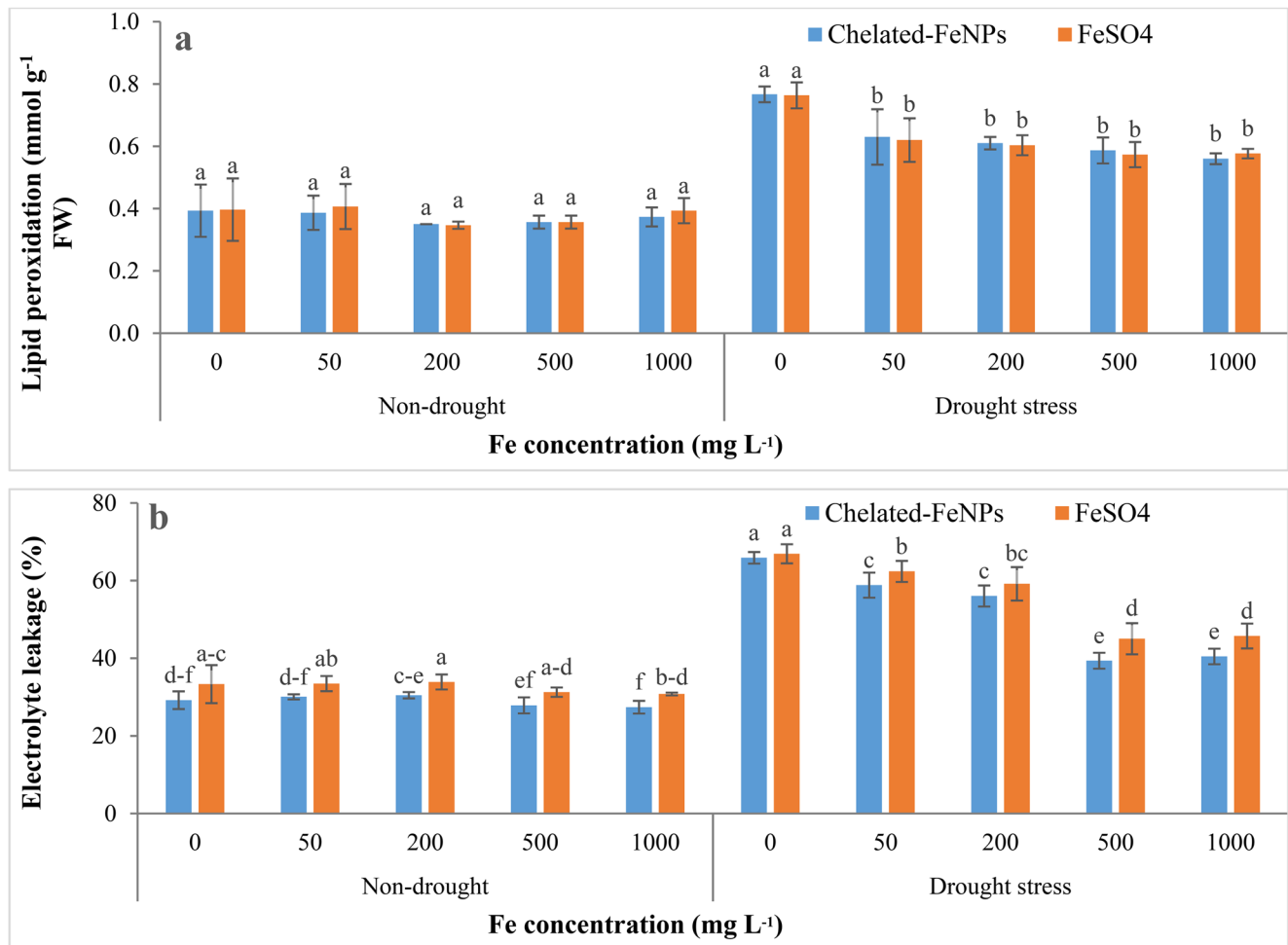


Fig. 6 Effects of nanochelated-Fe and ionic Fe²⁺ on membrane lipid peroxidation (MDA; **a**), and electrolyte leakage (**b**) in kidney bean under drought and non-drought conditions. In each water status, bars with similar letter are not significantly different, according to the

Least Significant Difference (LSD) post hoc test ($p < 0.05$). Error bars represent $\pm$ SD. Concentrations are shown in mg L⁻¹. Chelated FeNP denotes nanochelated Fe

and ionic Fe²⁺ treatments. Cai et al. (2020) reported absorption of nano-magnetite by tobacco leaf and its distribution through the vascular system to the roots. Ruan et al. (2023) found that FeNPs exhibited edge defects and oxidation, suggesting a potential risk for mitochondrial accumulation, yet no damage to intracellular compartments, including mitochondria, was noted in their study, which is consistent with our findings.

Chlorophyll is vital for photosynthesis (Liu and van Iersel 2021). The levels of chlorophyll and carotenoids in leaves serve as critical indicators of the physiological status of photosynthetic tissues, influencing photosynthesis rates significantly (Gao et al. 2024). Increased doses of Fe chelates, particularly Fe-EDDHA (Ethylenediamine di-2-hydroxyphenyl acetate ferric), could enhance chlorophyll content, particularly in Fe-deficient soils (Gao et al. 2022).

In *Ocimum basilicum* L., optimal Fe-EDDHA application improved both fresh and dry weight alongside chlorophyll content (Adiloğlu 2021). Moreover, Fe chelates elevate total chlorophyll, thereby enhancing photosynthesis and water use efficiency (Mahmoud et al. 2022). Mahmoud et al. (2022) reported that foliar application of Fe fertilizers increased chlorophyll levels by 4.4% and 3.6% in broad bean (*Vicia faba* L.) compared to untreated controls. The enhanced chlorophyll and carotenoid content with chelate treatment may stem from increased Fe oxygen reductase activity, aiding porphyrin metabolism for chlorophyll precursor synthesis (Jeong et al. 2024). Gutiérrez-Ruelas et al. (2021) found an 18% chlorophyll increase with Fe chelate and a 17% increase with ionic Fe²⁺ treatments compared to controls, linking the increased biomass to Fe role in enzyme functions, promoting plant growth (Feizi et al. 2013). Reduced

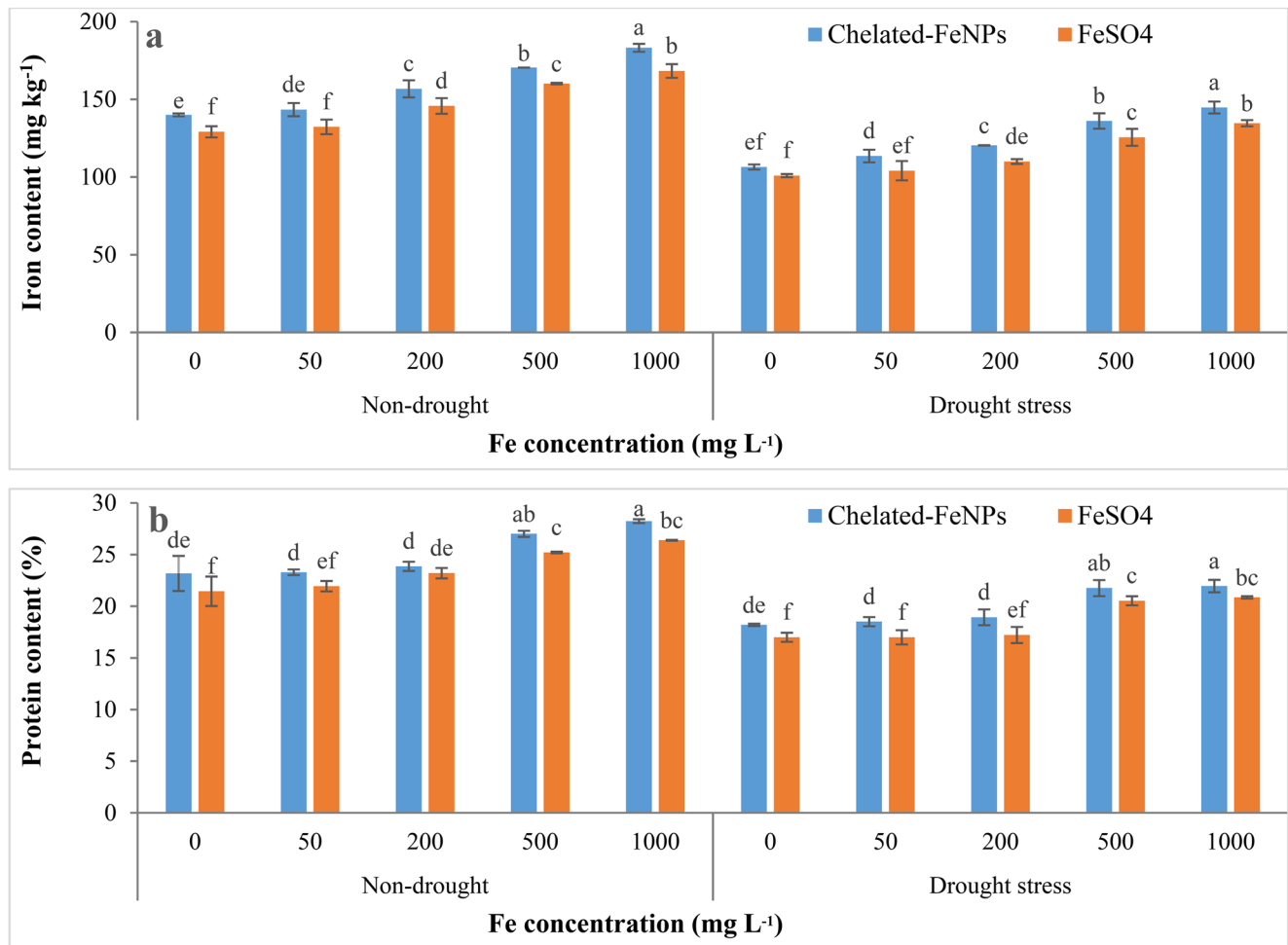


Fig. 7 Effects of nanochelated-Fe and ionic Fe²⁺ on iron content (a), and protein content (b) in kidney bean under drought and non-drought conditions. In each water status, bars with similar letter are not

significantly different, according to the Least Significant Difference (LSD) post hoc test ($p < 0.05$). Error bars represent $\pm$ SD. Concentrations are shown in mg L⁻¹. Chelated FeNP denotes nanochelated Fe

chlorophyll levels from Fe deficiency may arise as plants mature, reallocating nutrients towards fruit. Amanullah et al. (2012) observed that ferrous sulfate applications, both via soil and foliar, enhanced chlorophyll content in *Zea mays* L. by regulating delta-aminolevulinic acid, essential for chlorophyll synthesis. Therefore, Fe fertilizer application was predicted to increase chlorophyll concentrations in kidney bean.

Carotenoids are bioactive pigments that protect chlorophyll from photodegradation and oxidation (Pérez-Gálvez et al. 2020). This study's findings of increased carotenoid levels under drought stress compared to non-drought condition is consistent with Waqas-Mazhar et al. (2022), suggesting a protective role of carotenoid under water-deficit conditions. To counteract potential oxidative stress from drought, plant activates various protective mechanisms, including both enzymatic and non-enzymatic antioxidant systems, with carotenoids being non-enzymatic (Waqas Mazhar et al. 2022).

A notable decrease in leaf water content was observed in drought-stressed plants (Fig. 5a). Foliar 1000 mg L⁻¹ of nanochelated-Fe application helped maintain moisture levels in drought-stressed plants, showing no significant difference from non-stressed plants. The increased leaf water content may be linked to enhanced root growth and continued water absorption, supporting optimal plant growth. Improving relative water content in drought-stressed plants is critical for drought-stress tolerance, directly affecting growth and yield through efficient photosynthesis and nutrient uptake. Mechanisms for cellular water retention have evolved, driven by osmolyte accumulation, which improves soil water absorption under water-limited conditions (El-Esawi et al. 2018, 2019). In rice, Fe chelate application has shown to improve nutrient uptake and water content under drought stress (Rizwan et al. 2017). Enhanced Fe availability is associated with increased chlorophyll content, thereby boosting photosynthesis and transpiration rates, and

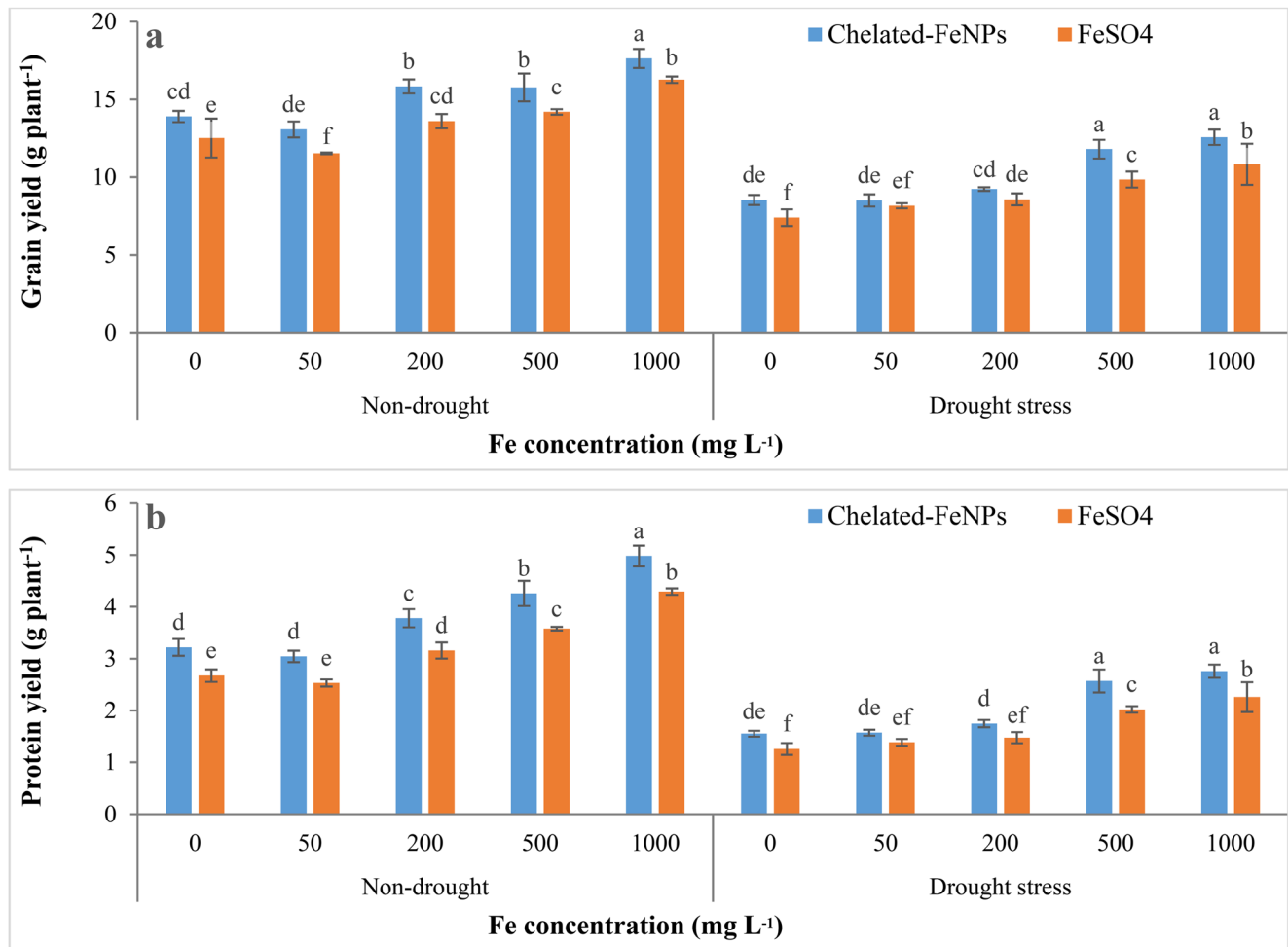


Fig. 8 Effects of nanochelated-Fe and ionic Fe²⁺ on grain yield (a), and protein yield (b) in kidney bean under drought and non-drought conditions. In each water status, bars with similar letter are not significantly different, according to the Least Significant Difference (LSD) post hoc test ($p < 0.05$). Error bars represent $\pm$ SD. Concentrations are shown in mg L⁻¹. Chelated FeNP denotes nanochelated Fe

positively impacting relative water content (Borowski and Michałek 2011).

Osmolytes for cellular osmotic regulation include amino acids, sugars, and other nutrients (El-Esawi et al. 2018; Jamil et al. 2015). This study found that drought stress led to a two-fold increase in proline content in kidney bean leaves, but Fe treatments suppressed the proline content effectively, illustrating its role in moderating drought stress. Inadequate proline leads to osmotic stress and oxidative damage, while excessive proline, as observed in control plants, can deplete glutamate, leading to chlorophyll deficiency and yellowing (Fig. S2c). Additionally, Fe is known to stimulate antioxidant enzyme defenses, which are sensitive to micronutrients, thereby managing drought stress before osmolyte requirements escalate (Shirvani-Naghani et al. 2024). Proline accumulation did not differ significantly between nanochelated-Fe and ionic Fe²⁺ in our study; however, under drought stress significantly

higher proline content was observed for untreated control plants and decreased with nanochelated-Fe and ionic Fe²⁺ treatments. Proline typically responds to abiotic stress, including nutrient deficiency. Foliar Fe applications can mitigate such stress, potentially lowering proline levels (Ahmad et al. 2024; Mahmoud et al. 2022). Iron chelates, such as Fe-EDDHA and Fe-Citrate, help alleviate chlorosis, improving overall plant health and potentially affecting proline levels (Gamble et al. 2014).

Iron-chelated fertilizers enhance membrane integrity by decreasing lipid peroxidation (Nurozi-Boldaji et al. 2024). Iron application in crops like *Nigella sativa* (L.) and broad bean boosts physiological and biochemical functions, notably reducing lipid peroxidation (Nurozi-Boldaji et al. 2024; Mahmoud et al. 2022). Foliar Fe applications reduce oxidative stress and prevent reactive oxygen species accumulation, mitigating lipid peroxidation and membrane damage (Nurozi-Boldaji et al. 2024). In drought-stressed

plants, increased Fe concentrations correlate with decreased proline and carotenoids, indicating reduced oxidative stress and lipid peroxidation. Increased electrolyte leakage suggests plasma membrane damage, as membrane lipid peroxidation stems from oxidative damage affecting cell membranes. Under drought conditions, simultaneous processes of lipid peroxidation and electrolyte leakage occur. Elevated H_2O_2 levels increase hydroxyl radical formation, leading to lipid degradation (Yanli et al. 2020). Drought-induced excessive reactive oxygen species and lipid peroxidation disrupt cell membrane integrity, resulting in cell death. This cascade leads to electrolyte leakage and organelle damage (Ravi et al. 2023; Mansoor et al. 2022; Sun et al. 2022). Iron also promotes chlorophyll levels, improving photosynthesis and nutrient uptake in drought-stressed plants. Antioxidant enzymes (Shirvani-Naghani et al. 2024) or soluble sugars may work with amino acids to prevent cell desiccation and maintain membrane structure during drought (Yanli et al. 2020). However, foliar Fe application can reduce electrolyte leakage and alleviate drought stress effects (Nurozi-Boldaji et al. 2024).

The significant decline in grain Fe content under drought highlights the need to promote Fe levels for the nutritional value of crops in developing regions. Foliar-applied Fe positively influenced grain Fe concentration, with higher dosages correlating with increased grain Fe content, indicating effective transfer of foliar-applied Fe to bean grain, particularly with nanochelated-Fe fertilizer. A study by Ahmadov et al. (2020) is consistent with these findings, noting improved grain quality and elevated Fe concentration due to foliar application in peas. A study on common bean found that 25 μ M Fe-EDDHA effectively increased grain Fe accumulation (Sida-Arreola et al. 2015). Iron supplements, especially Fe chelates, not only boosted grain yield but also mitigated drought stress, highlighting the importance of Fe supplementation for improved agriproduction (Nurozi-Boldaji et al. 2024).

Drought stress negatively impacts grain protein content; however, foliar spraying with nanochelate-Fe significantly improved grain proteins in this study. Mahmoud et al. (2022) documented that the use of Fe_3O_4 nanoparticles and chelated Fe as foliar fertilizers resulted in notable increase in proteins; 35% and 30%, respectively, compared to untreated broad bean grown in sandy soil with compost application (26% protein). However, no drought or other abiotic stress responses were studied. It is well established that drought stress can suppress grain protein due to stomatal closure, reduced CO_2 fixation, and lower nutrient absorption (Qiao et al. 2024). Enhanced photosynthetic systems and reduced oxidative damage suggest optimal conditions in kidney beans foliarly-treated with nanochelated-Fe in our study. Additionally, Fe is known to promote nitrogen

fixation in rhizobial nodules in leguminous plants such as beans (Groppa et al. 2022), potentially increasing protein production. EDDHA and nanochelated-Fe have been documented to improve plant growth and nutrient uptake in Fe-deficient, alkaline soils (Mirbolook et al. 2023; Zuluaga et al. 2023), leading to better grain quality and protein yield.

Foliar application of Fe boosts crop yield by promoting photosynthetic system, improving flower survival and pod conversion through increased assimilates. Mahmoud et al. (2022) and Gutiérrez-Ruelas (2021) documented increased yield and yield components in broad bean and green bean due to Fe foliar application, which increased leaf area and durability, resulting in greater assimilate production. Research shows that Fe chelates significantly boost yield, offering economic advantages when growing crops in Fe-deficient soils (Hergert et al. 2019). Moreover, foliar application of micronutrient fertilizers, including Fe chelates, is shown to promote antioxidant response, yield, and nutritional quality in *P. vulgaris* (Tripathi et al. 2018; Sida-Arreola 2015).

Protein yield is influenced by grain yield and protein content. This study showed that changes in protein yield closely followed grain yield. Iron supplied via nanochelated-Fe boosts assimilate accumulation, increasing both grain and protein yields. Molecular studies in *Pisum sativum* (L.) indicate that, although improving protein content may be easier than enhancing grain yield, both can be optimized through genomic selection, potentially increasing protein yield per unit area (Crosta et al. 2022). The observed consistency in improved protein and grain yields in this study upon nanochelated-Fe treatment supports the conclusion that grain yield significantly affects protein yield, which is supported by previous studies (Visha Kumari et al. 2023; Tripathi et al. 2018; Sida-Arreola 2015; Mahmoud et al. 2022; Ram et al. 2024; Nasar et al. 2022).

Our results supported the hypothesis that “nanochelated-Fe will outperform ionic Fe^{2+} to mitigate drought stress and significantly improve physiological attributes, grain yield and nutritional quality in kidney bean.” Overall, based on the findings, the following mechanism of drought stress mitigation and improved yield is proposed: nanochelated-Fe led to decreased oxidative stress (lowered MDA levels) and carotenoid levels that modulated electrolyte leakage, proline and water content (lowered electrolyte leakage and proline while retaining higher water content). This led to optimal growth under water-deficit condition, subsequently promoting grain and protein yields, including grain Fe levels, in kidney bean (Fig. 9). The findings of this field study underscore the potential of foliarly applied nanochelated-Fe to serve as a sustainably effective strategy for improving bean production and nutritional quality, particularly in regions experiencing moderate drought stress.

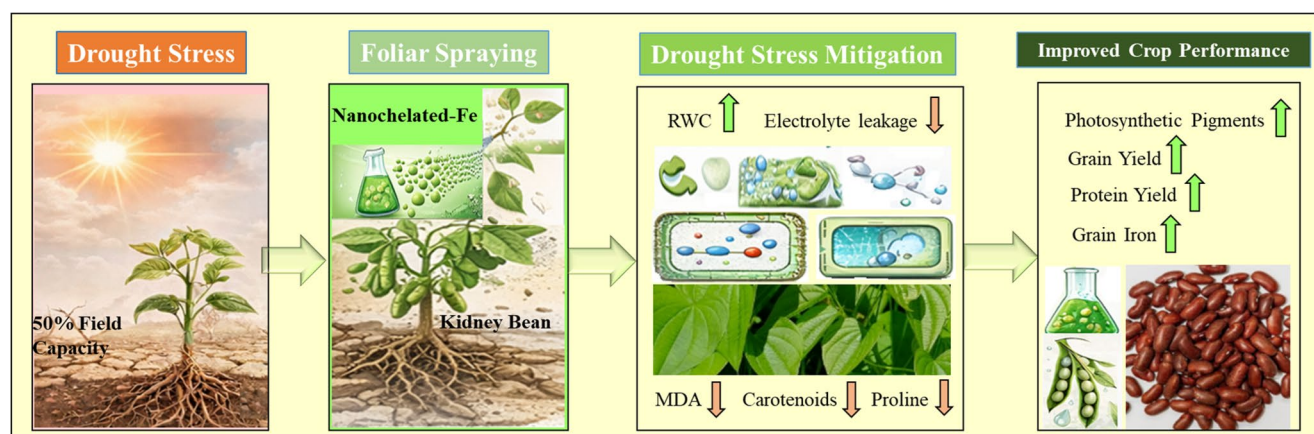


Fig. 9 A schematic depiction of the mechanism of drought stress mitigation by nano-chelated-Fe and overall improved performance of kidney bean

5 Conclusion

The findings of this study indicate that moderate drought stress (50% of field capacity) could significantly hinder kidney bean growth and photosynthetic performance, whereas foliar application of 1000 mg L⁻¹ nano-chelated-Fe may provide an inroad for increased grain yield, protein yield, and grain Fe under drought stress compared to conventional ionic Fe²⁺ fertilizers. Taken together, these findings underscore the importance of using novel nano-chelated-Fe as a sustainably effective strategy for improving bean production and nutrient quality in areas experiencing moderate drought stress. Multi-site large scale field trails will be needed in the future, possibly including other legumes (soybean, fava bean, mung bean, etc.) under different soil types, to determine potential agri-promoting role of nano-chelated-Fe. Nonetheless, the study findings are promising for improving agro-economy and may have positive implications to developing policies that support the use of novel micronutrient-based fertilizers such as nano-chelated-Fe.

Abbreviations

Fe	Iron
MDA	Malondialdehyde
Fe ²⁺	Ferrous ion
ANOVA	Analysis of variance
TEM	Transmission electron microscopy
RWC	Relative water content
FeNPs	Iron nanoparticles
Fe-EDDHA	Ethylenediamine di-2-hydroxyphenyl acetate ferric
Fe-Citrate	Iron citrate
CO ₂	Carbondioxide
FeSO ₄	Ferrous sulfate
DLS	Dynamic light scattering

RCBD	Randomized complete block design
FC	Field capacity
KV	Kilo volt
OD	Optical density (a.u.)
FW	Fresh weight of tissue (g)
Chla	Chlorophyll a
Chlb	Chlorophyll b
Chlt	Total chlorophyll
DW	Leaf dry weight after oven drying
SW	Leaf saturated weight after exposure to distilled water
A	Absorption rate
ε	Extinction coefficient of malondialdehyde at 532 nm (155 mM ⁻¹ cm ⁻¹)
V	Sample volume (L)
D	Width of cut (cm)
EL	Electrolyte leakage (%)
C1	Electrical conductivity of the solution at 32°C
C2	Electrical conductivity of the solution at 121°C
ICP-OES	Inductively coupled plasma-optical emission spectroscopy
LSD	Fisher's least significant difference

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Authors' Contributions FH: Conceptualized and designed the study, Investigation, Collected the data and analysis, Writing-original draft preparation. SF: Conceptualized and designed the study, Project administration, Supervision, Writing-original draft, Writing-review & editing, Funding acquisition. LRP: Conceptualized and designed the study, Analyzed data, Writing-review & editing, Project advisory committee.

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Data Availability The data generated or analyzed during this study are available from the corresponding author upon reasonable request.

Code Availability Not applicable.

Declarations

Competing interests The authors declare that they have no competing interests.

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