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RESPONSE OF ONION GROWTH AND YIELD GROWN IN SOILS OF SEMI-ARID REGIONS TO FOLIAR APPLICATION OF IRON UNDER WATER STRESS CONDITIONS

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ABSTRACT. Iron (Fe) is one of the major micronutrient crucial for plant growth, yield and quality. A field experiment was conducted in Fallujah, Iraq during the autumn season in 2019 to study the effects of foliar application of iron and irrigation levels on growth characteristics and yield of onion (Allium cepa L.). A two-factorial experiment arranged as a randomized complete block design with three replications was conducted in loamy sand soil. The two factors were water stress (50, 75, and 100% consumptive use of water) and iron concentrations (0, 100 and 200 mg L^{-1}). The results show a significant decrease in plant height, number of bulbs, total chlorophyll content, dry mass, iron content in the leaves, the average weight of bulbs and total yield of bulbs by reducing irrigation levels from 100 to 50% of the water supply. Application of iron by foliar spraying significantly increased most of the aforementioned traits. The interactions between iron and irrigation levels were significant in most of the measured traits. The interaction between 100% water supply and 100 mg L⁻¹ of iron achieved the highest total yield value (4 332 Mg ha⁻¹) while the combination of 75% of water supply and 100 mg L⁻¹ of iron gave the highest value of water use efficiency (84.7%).

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Introduction

Water scarcity is one of the constraining factors for crop production, it plays a primary role in reducing the crop production more than the reduction resulting from all other environmental factors, hence irrigation has the priority in influencing the characteristics and quality of the crop through its effect on the formation of plant parts and its growth (Sharp et al., 2004; Al-Shammari et al., 2018). Consequently, limits the photosynthesis rate thereby cells partition, growth, and biogeochemical processes (Su, Shangguan, 2019; Abood et al., 2019). This consistent with the scarcity of water in arid and semi-arid regions that suffer from low precipitation and deep groundwater. Therefore, improving the understanding of soil moisture dynamics is very important to precisely determine the soil moisture status (Wang et al., 2013), which requires a revision of water resources

management for optimal use of water, by rationalizing its consumption in the agriculture sector by controlling the number of irrigation events per season, or supplying the sufficient amount of water through irrigation scheduling (El-Siddig et al., 1998). Besides water deficiency, the low availability of nutrients often controls the crop growth and production potential because most crops are sensitive to water and nutrients shortage during different growth stages. Microelements that applied to the soil are often dissolved too quickly and then drain into deeper layers of the soil profile, making nutrients unavailable to plants. Iron functions are very important in plant growth and development, such as take part in the biosynthesis of chlorophyll, respiration, chloroplast development and improves the performance of photosystems. Also, an iron element is an essential part of many enzymes (Barker, Pilbeam, 2007). Moreover, it participates in the oxidation



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process that releases energy from sugars and starches, and convert nitrate to ammonium in the plant, iron plays a vital role in nucleic acid metabolism (Havlin, 2014). Water available status in the soil profile determines the nutrient distribution in the soil solution, with limited soil water content, the nutrients absorption and the diffusion of nutrients between the soil and root decrease due to the low soil water potential (Marschner, 2011). Recent studies have pointed out the effect of iron in reducing the negative effect of water deficiency. In this regard, iron foliar spraying caused increasing in soybean yield when exposing the plant to water stress (Kobraee et al., 2011). In the same direction, the iron foliar spraying for sesame has increased the yield components and reduced the effect of water deficiency at the same time (Heidari et al., 2011). However, iron availability depends on soil moisture content, where the iron is more available under wet conditions due to its transformation to more soluble forms (reduced). Therefore, supply iron under dry soil conditions may give adverse results (Havlin, Beaton, 1999). Onion is a major bulbous crop among vegetables, falls in order of 15 among vegetables classified by FAO, onion next to tomato in terms of total global production (Pathak, 2000). It has moderate amounts of protein, fat, fibre and good amounts of calcium, phosphorous and potassium, vitamin C and B6 (Mitra et al., 2012). Therefore, around 175 countries cultivate onion (FAO, 2019). This study aimed to determine the effects of the irrigation levels and iron concentration applied using the foliar method in some growth characteristics and yield of onion.

Material and methods

Study area description

The study area was agricultural (33°17'26.5"N 43°48'56.1"E) located 8 km south of Fallujah, Iraq. It is a semi-arid area with a big difference in day and night temperature variation and the humidity is low. July and August are the hottest months with temperature rises to 49 °C and the temperature drops to 9 °C in January. The wind is northwesterly and southwesterly, sometimes with a maximum speed of up to 21 m s⁻¹. The average annual rainfall is less than 250 mm. Therefore, agriculture in the study area depends mainly on the irrigation water from the river Euphrates.

Field preparation and experimental design

A field experiment was conducted during the autumn season in 2019. The soil was classified as loamy sand. Soil samples were randomly collected and mixed to prepare a represented sample to be used in the analysis to determine the chemical and physical soil properties (Table 1). The percentage of sand, silt, and clay at 30 cm depth were recorded by using the pipette method after oxidizing soil organic matter with hydrogen peroxide. While pH and EC were measured using pH and EC meter from the suspension of 1:1 soil water ratio (Black *et al.*, 1965). The potassium was determined after treating the sample with ammonium acetate buffer (NH₄OAC) at pH 7.0 using 500801 PFP7/C

jenway flame photometer, while phosphorus was determined according to (Olsen et al. 1982), the total nitrogen was measured using Kjeldahl, where the sample was digested in sulfuric acid, in the presence of a catalyst (CuSO₄/TiO₂) that helps to convert the amine nitrogen to ammonium ions, which subsequently converted into ammonia gas, then heated, distilled and titrated with a standard solution for the required calculations. The micronutrients such as iron, zinc, manganese were extracted according to NH₄HCO₃-DTPA (dientilenetriaminepenta acetic acid as an extractant) modified method (Soltanpour et al. 1979) and the elements were measured using Atomic absorption spectroscopy (AOAC, 1999). Before planting, the field preparation involved ploughing, levelling and dividing the field into plots. A two-factorial experiment of randomized complete block design (RCBD) with three replications was conducted. The two factors were water stress ($W_1 = 50$; $W_2 = 75$ and $W_3 = 100\%$ of union water consumptions) and iron concentration (Fe $_0 = 0$, $Fe_1 = 100$, and $Fe_2 = 200 \text{ mg L}^{-1}$), where the iron source is Fe-EDTA 15%. The plots area were 3.6 m², during the period of vegetative growth. The T-Tape irrigation system was used, where three irrigation lines were used with the discharge of 1 L h⁻¹. The distance between irrigation lines and adjacent drippers were 0.75 and 0.1 m, respectively. Before planting, the field was irrigated liberally to prepare the plots for cultivation. The onion seeds (Texas Early Grano) were sowed on 30.11.2019 on both sides of drip irrigation lines (T-Tape). The application rates of fertilizer were 140, 60 and 120 kg ha⁻¹ for nitrogen, phosphor, and potassium respectively. The manually weeding was conducted when needed. The data was analyzed statistically at P = 0.05, by using Genstat software (GenStat, 2005). The Least Significant Difference (LSD) was used to compare the averages of measurements.

Calculations of supplied water

After the seeds have been germinated, the supplied water was calculated according to (Al-Janabi, 2005). The water amount was divided based on the number of irrigation events from the germinated to the maturity stage. A valve was fixed at the beginning of each drip line to control the amount of supplied water. The supplied water for each plot was calculated according to Eq. 1:

Supplied water,
$$m^3 = \frac{Water\ irrigation\ depth}{1000} \times Area, ha$$
 (1)

The plot area used in this equation was $3.6\ m^2$. The operation time for each drip line was calculated according to Eq. 2.

Operation time,
$$h = \frac{Supplied\ water\ to\ plot,\ L*No.\ of\ plots}{No.\ of\ drippers*Dripper\ discharge} \times 100$$
 (2)

The onion was harvested on 02.03.2020 when the onion bulbs reached the marketable size (2–3 cm diameter).

The traits under study

Five plants were randomly chosen in each plot to determine the plant height at the end of the season, the measurements were from the contact point between the stem and soil to the highest point of the plant using measurement tape. The total yield was counted according to Eq. 3.

Total yield,
$$kg \ ha^{-1} = \frac{Yield \ per \ plot*10\ 000}{Plot \ area}$$
 (3)

The number of leaves on the main stem was accounted manually. At the end of the season, five plants were randomly collected from each plot, then the roots and bulbs were removed before drying. The vegetative parts were dried at 70 °C for 72 hours until the constant weight is reached. Ten plants were randomly chosen to take the average reading of the total chlorophyll. The measurements were conducted by using a Chlorophyll meter (SPAD-502). The weight of the bulb was calculated after removing the vegetative part according to Eq. 4.

Weight of bulb,
$$g = \frac{Total\ yield\ per\ plot}{Number\ of\ plants\ per\ plot}$$
 (4)

The iron content of leaves was determined based on Jones *et al.* (1991), where extracts were made of plant samples, then iron content was measured using Atomic absorption.

Water use efficiency (kg m^{-3}) was calculated according to Wright (1988) Eq. 5.

Water use efficiency =
$$\frac{\text{Yield, kg}}{\text{Supplied water, } m^3}$$
 (5)

Table 1. Chemical and physical properties for the soil under study

| Chemical properties | | | Physical properties | | |
|---------------------|--------|---------------------|--------------------------|-------|------|
| Parameter | Value | Unit | Parameter | Value | Unit |
| EC 1:1 | 7.4 | ds m ⁻¹ | Sand | 83 | % |
| pH 1:1 | 7.35 | | Silt | 9 | % |
| Potassium | 165.97 | $mg kg^{-1}$ | Clay | 8 | % |
| Phosphor | 13.44 | $mg kg^{-1}$ | Soil texture: loamy sand | | |
| Nitrogen | 35.26 | $mg kg^{-1}$ | | | |
| Iron | 2.79 | $mg kg^{-1}$ | | | |
| Zinc | 1.72 | $mg kg^{-1}$ | | | |
| Manganese | 2.67 | mg kg ⁻¹ | | | |

Results

Characteristics of vegetative growth

The obtained results showed a reduction in the average of plant height associated with water content decline, where supplying 50% of water supply (W₁), caused a significant reduction in the average of plant height reached 26.37 cm compared to 100 and 75% of water supply that gave 29.18 and 30.09 cm respectively Table 3. Probably due to water stress causes many physiological and chemical changes in the plant that leads to growth limitation. In addition to water stress also led to a decrease in the frequency of plant cell division and elongation (Sharp et al. 2004; Zheng et al., 2013). Furthermore, the direct effect of dehydration lies in the expansion of the plant cell wall, where elongation involves cell wall to stretch under the influence of fullness effort, an effort decline as a result of the imbalance of the water plant content leads to decline or even to stop growth completely underwater deficiency (Cosgrove, 1989).

The plant height was significantly increased when the foliar feeding has been used, wherein the level of 200 mg L⁻¹ (Fe₂) had achieved the highest value of plant height reached 30.70 cm, with the increasing rate of 13.45 and 10.15% compared to 0 and 100 mg L^{-1} respectively, increasing of plant height was achieved as a result of iron spraying on the onion vegetative part that reflected positively on the studied traits owing to the role of iron in the process of cell division thereby formation of many cytochromes and viroxin compounds which has great importance in photosynthesis and respiration processes, thereby increases the plant efficiency to absorb nutrients and then increase in most growth traits (Rout, Sahoo, 2015). Also, the interaction effect between water supply and iron fertilizer was significant in the aforementioned trait, where the highest value of plant height was 33.84 cm for the combination of W₁Fe₂ whilst the lowest value was 21.40 cm for the combination of W₁Fe₀ with the increasing rate of 58.13%.

The results showed a significant reduction in the average number of leaves, reached to 4.21 and 4.71 leaf plant⁻¹ for the treatments 50 and 75% of water supply respectively, with a reduction rate 21.75 and 45.12% compared to 100% of water supply, which achieved 5.38 leaf plant⁻¹. Also Table 2 shows a significant difference in terms of dry weight when reducing the water supply of onion, where the treatments W₂ and W₃ gave the lowest value of dry weight reach 5.69 and 5.63 g respectively, compare to the treatment W_1 that gave 9.66 g plant⁻¹. The evidence indicated the soil moisture content was insufficient to supply the needs of the onion plants consequently, reduced growth and the development of smaller bulbs (Kandil et al., 2011; Tolossa, Yildiz, 2020). On the other hand, the evidence showed that the iron foliar spraying significantly differenced the average of dry weight, where the highest value was obtained from the treatment Fe2 reach 7.74 g plant⁻¹ and did not differ significantly from Fe₁, but achieved an increasing rate reached 27.72% compare to Fe₀ (6.06 g plant⁻¹). Due to the role of iron in the physiological process which subsequently leads to an increased dry weight matter (Kim et al., 2006).

Table 2. The plant height, leaves number, dry weight and total chlorophyll content of the onion receiving different combination of irrigation water and iron concentration levels

| Water | Iron | Plant | Number | Dry | Total |
|----------------|--------|---------|---------------------|-----------|--------------------|
| | | | | - | |
| supply | levels | height, | of leaves | weight, g | chlorophyll, |
| | | cm | plant ⁻¹ | | mg g ⁻¹ |
| \mathbf{W}_1 | Fe_0 | 21.40 | 5.10 | 6.07 | 26.03 |
| | Fe_1 | 32.31 | 5.43 | 11.43 | 34.38 |
| | Fe_2 | 33.84* | 5.60* | 11.47* | 35.17* |
| \mathbf{W}_2 | Fe_0 | 31.88 | 4.10 | 6.23* | 25.50 |
| | Fe_1 | 26.86 | 4.77 | 5.20 | 25.31 |
| | Fe_2 | 31.52* | 3.77 | 5.63 | 25.43 |
| W_3 | Fe_0 | 27.91 | 5.43* | 5.87 | 26.47 |
| | Fe_1 | 24.44 | 4.00 | 4.90 | 23.07 |
| | Fe_2 | 26.75* | 4.70 | 6.13 | 24.63 |
| LSD(0.05) | | 2.986 | 1.077 | 1.285 | 3.388 |

 $W_1-100,\,W_2-75$ and $W_3-50\%$ represent the water supply. While Fe0, Fe1 and Fe2 represent the iron concentration (0, 100 and 200 mg $L^{-1},$ respectively).

The interaction between water supply and iron fertilizer has significantly superior in the plant leaves trait, where the highest number of leaves was obtained from the combination of W₁Fe₂ reached 5.60 leaf plant⁻¹ while the lowest number of leaves was 3.77 leaf plant-1 obtained from the combination W₃Fe₂ with increasing rate 48.54%, the evidence showed that reducing water content leads to encouraging the physiological role of iron compared to full irrigation supply. Regarding the dry weight, the significance was obtained from the interaction effect between water supply and iron levels, where the highest and the lowest value was obtained from the combination W₁Fe₂ and W₃Fe₁ reached 6.23 and 4.90 g plant⁻¹ respectively, increasing the soil water tension from W₁ to W₃ reduced the dry weight matter because the iron in the combination of W₃Fe₁ was insufficient to reduce the negative effect of water stress. The results of the statistical analysis listed in Table 3. showed a significant reduction in the total chlorophyll content reached 25.41 and 24.72 mg g^{-1} in W_2 and W_3 respectively, compared to W1 that achieved 32.01 mg g⁻¹. However, the results showed significant differences when spraying the iron, where the highest value of chlorophyll content was obtained from the treatment Fe₂ reached 28.41 mg g⁻¹ compare to Fe₀ that gave 26.00 mg g^{-1} .

Similarly, the interaction effect was significant where the highest value was achieved from $W_1 Fe_2$ reached 35.17 mg g^{-1} while the lowest value of chlorophyll content was 23.07 mg g^{-1} resulted from $W_3 Fe_1$, the decrease in the total chlorophyll content was probably due to the water stress that led to a decrease in the number and size of chloroplasts thereby a reduction in the compounds needed to build chlorophyll such as water, nutrients and carbohydrates, consequently reduces the total chlorophyll content production (Berkowitz 1998).

Table 3. Plant height, leaves number, dry weight and total chlorophyll of onion receiving different irrigation water and iron concentration levels

| Averages | Plant height, | Number of | Dry weight, | Total |
|---------------------|---------------|----------------------------|-------------|--------------|
| | cm | leaves plant ⁻¹ | g | chlorophyll, |
| | | | | $mg g^{-1}$ |
| \mathbf{W}_1 | 29.18 | 5.38 | 9.66 | 32.01* |
| \mathbf{W}_2 | 30.09* | 4.21* | 5.69* | 25.41 |
| W_3 | 26.37 | 4.71* | 5.63* | 24.72 |
| LSD _{0.05} | 1.742 | 0.622 | 0.742 | 1.956 |
| Fe_0 | 27.06 | 4.88 | 6.06 | 26.00 |
| Fe_1 | 27.87 | 4.73 | 7.18* | 27.73 |
| Fe ₂ | 30.70* | 4.69 | 7.74 | 28.41* |
| LSD _{0.05} | 1.742 | N.S. | 0.742 | 1.956 |

 W_1 , W_2 and W_3 represent the water supply (100, 75 and 50% respectively); Fe_0 , Fe_1 and Fe_2 represent the iron concentration (0, 100 and 200 mg L^{-1} respectively).

Leaves iron content, yield traits and water use efficiency

The results listed in Table 4 showed that the W_3 treatment gave the lowest rate of leaves iron content reached 161.2 mg kg⁻¹ compared to W_1 and W_2 with 175.9 mg kg⁻¹ and 168.1 mg kg⁻¹ respectively, this can

be attributed to the lack of water in the leaves and stem that associated with a decrease in water irrigation depth and relative humidity (Heidari *et al.* 2011). While the foliar application of iron had achieved the highest concentration of iron reached 183.5 mg kg⁻¹ obtained from the treatment Fe₂ compare to the control treatment which gave 149.9 mg kg⁻¹, with an increasing rate of 22.4%. Since the foliar nutrients absorbed easily by the leaf cuticle and enter the cells, facilitating easy and rapid utilization of nutrients by the crop (Latha, Nadanassababady, 2003). Consequently, increasing its content in the plant tissue.

In terms of interaction, the combination of W₁Fe₂ gave the highest average of iron content reached 194.8 mg kg⁻¹ with an increasing rate of 32.52% compare to W₂Fe₀. In addition to Table 5 shows the significant effect of water stress on the average bulb weight, where the water deficiency caused a clear reduction in the bulb weight. In this regard, the treatment W_3 gave the lowest weight of bulb up to 5.57 g compare to 12.46 g resulted from the treatment W_1 with a reduction rate of 55.30%. At the same time, the spraying iron with the concentration of 100 mg L⁻¹ had achieved the highest weight of bulb up to 11.70 g with an increasing rate of 43.03 and 33.42% compared to the control treatment and Fe₂, wherein gave 8.18 and 7.79 g, respectively. While the significant increase was obtained from the interaction effect, where the combination W₁Fe₁ gave the highest weight of bulb reached 16.20 g compare to W_3Fe_3 with 5.03 g (Table 4).

Table 4. Interaction of iron foliar spraying and water supply on yield components and water use efficiency some vegetative growth traits of onion

| Water | Iron | Leaves iron | Bulb | Total | WUE, |
|----------------|--------|-------------|---------|--------------|--------|
| supply | levels | content, | weight, | yield, | % |
| | | $mg L^{-1}$ | g† | $Mg ha^{-1}$ | |
| \mathbf{W}_1 | Fe_0 | 152.40 | 9.43 | 2.552 | 49.00 |
| | Fe_1 | 180.40* | 16.20* | 4.332 | 84.20* |
| | Fe_2 | 194.80* | 11.73 | 3.129 | 61.00* |
| \mathbf{W}_2 | Fe_0 | 150.40 | 8.57 | 2.284 | 59.40 |
| | Fe_1 | 170.60* | 13.77* | 3.671* | 84.70* |
| | Fe_2 | 183.30* | 6.60 | 1.76 | 44.70 |
| W_3 | Fe_0 | 147.00 | 6.53 | 1.742 | 67.90* |
| | Fe_1 | 164.20* | 5.13 | 1.369 | 53.40 |
| | Fe_2 | 172.50* | 5.03 | 1.327 | 51.70 |
| LSD(0.05) | • | 11.94 | 2.572 | 0.685 | 15.71 |

 $\dagger Average$ weight of the bulbs; WUE – water use efficiency, $W_1,\,W_2$ and W_3 represent the water supply (100, 75 and 50% respectively).

The results listed in Table 5 showed that reducing the water supply significantly decreased the bulb yield. Where the yield was decreased from 3.32 to 1.47 Mg ha⁻¹ when the water supply was reduced from 100% to 50% of water supply, with a reduction rate of 55.74%. Probably due to water stress that caused an imbalance of nutrients, since the most available elements are present in the soil solution, which negatively affects the metabolic processes of the plant, including photosynthesis that considers the main source of the other physiological processes of the plant (Rout, Sahoo, 2015). On the other hand, the iron application had positively increased the yield, where the treatment

 Fe_1 gave the highest yield reached 3.12 Mg ha⁻¹ followed by Fe_0 and Fe_2 with 2.18 and 2.07 Mg ha⁻¹ respectively. This increase was due to the role of iron in controlling the negative effect of water stress in the aforementioned traits Table 5. Also, iron has a vital role in nucleic acid metabolism.

In terms of interaction, the highest yield was obtained from the combination W_1Fe_1 up to 4.33 Mg ha⁻¹, compare to W_3Fe_2 that gave 1.32 Mg ha⁻¹. Furthermore, the statistical analysis showed an insignificant reduction in the water use efficiency (WUE) when reducing the amount of applied irrigation water, while spraying with iron had a significant effect in increasing the efficiency of water use, where the concentration of 100 mg L^{-1} had achieved the highest water use efficiency with 77.1% compared to 200 mg L^{-1} and control treatment (0 mg L^{-1}) in which gave 52.5 and 58.8% respectively. Regarding the interaction, the combination W_2Fe_1 achieved the highest water use efficiency up to 84.7% compared to W_2Fe_2 which gave 44.7%.

Table 5. Means effect of water supply and iron concentration

| Averages | Leaves iron content, mg L ⁻¹ | Bulb average weight, g | Bulbs total yield, kg ha ⁻¹ | WUE, |
|---------------------------|---|---------------------------|---|-------|
| $\overline{\mathbf{W}_1}$ | 175.9* | 12.46* | 3.321* | 64.7 |
| \mathbf{W}_2 | 168.1* | 9.64* | 2.572* | 65.9 |
| W_3 | 161.2 | 5.57 | 1.479 | 57.7 |
| LSD _{0.05} | 6.89 | 1.485 | 0.396 | N.S |
| Fe ₀ | 149.9 | 8.18 | 2.181 | 58.8 |
| Fe_1 | 171.7* | 11.7* | 3.12* | 77.1* |
| Fe ₂ | 183.5* | 7.79 | 2.072 | 52.5 |
| LSD _{0.05} | 6.89 | 1.485 | 0.396 | 9.07 |

 $W_1,\ W_2$ and W_3 represent the water supply (100, 75 and 50% respectively); $Fe_0,\ Fe_1$ and Fe_2 represent the iron concentration (0, 100 and 200 mg L^{-1} respectively).

Conclusions

The results of this study showed that the foliar application of iron with a concentration of 100 mg L⁻¹ caused increasing in the traits under study (average of bulb weight, total yield of bulb, WUE) when the plant did not expose to the water stress, this increasing continues with applying the 200 mg L⁻¹ however, when the plant exposed to the water stress, the increasing achieved only when the foliar feeding of iron with the concentration of 100 mg L⁻¹ has been used, where the iron has reduced the negative effect of water stress, which positively reflected on all traits under study. While applying the second level of iron (Fe₂), gave adverse results in most traits when exposing the plant to water stress, therefore, iron foliar application with a concentrate of 200 mg L⁻¹ is recommended when the onion is grown under sufficient water condition. While the level of 100 mg L⁻¹ can be used under water stress conditions. The evidence showed that 25% of the water supply can be saved by applying iron with a concentrate of 100 mg L^{-1} similarly, the interaction (W₁Fe₁) showed increasing in weight bulb, total yield and WUE, these findings support the results obtained from foliar application of iron with a concentration of 100 mg L^{-1} . while when reducing the water content with constant of Fe in the combination of W₂Fe₁ leads to reduce the leaves iron content, weight bulb and total yield. These findings confirm the importance of using iron in reducing the negative effect of water deficiency with acceptable limits, where expose the onion to water stress more than 50% of water supply caused a significant reduction in the yield even though the iron foliar spraying has been used. Regarding the interaction, the combination W_2Fe_1 achieved the highest water use efficiency up to 84.7% compared to W_2Fe_2 .

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

LG 25%, BB 25%, MA 25%, JA 25% – study conception and design:

LG 50%, MA 50% – acquisition of data;

BB 25%, JA 25%, MA 25%, GH 25% – analysis and interpretation of data;

LG 50%, MS 25%, JA 25% – drafting of the manuscript; MA 50%, GH 50% – critical revision and approve the final manuscript.

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