Combined foliar application of Zn and Fe increases grain micronutrient concentrations and alleviates water stress across diverse wheat species and ploidal levels

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This study aimed to examine the reaction of several wheat species with different ploidy levels to foliar application of zinc (Zn) and iron (Fe) under different water regimes. Thirty-five wheat genotypes, including nineteen tetraploids from ten different species, ten hexaploids from five species, and six diploids from three species, were evaluated in the field over two moisture regimes with the following four treatments: control, foliar Zn application, foliar Fe application, and foliar Zn + Fe application. The experiments were conducted according to a split-plot scheme in a randomized complete block design with two replications in each moisture regime. Water stress negatively affected all measured traits, except grain Zn and Fe content. Combined foliar application of Zn + Fe significantly increased yield and alleviated yield reduction caused by water stress. Applying Zn and Fe significantly increased both micronutrient content in grains under both moisture conditions. Tetra and hexaploid species yielded nearly four times as much grain as unimproved diploid species and were less affected by water stress. All ploidy levels responded almost similarly to Zn and Fe treatments, with the combined application being as effective as each element separately. The highest yield increase in response to combined application of Zn + Fe under the two moisture conditions and the highest grain Zn content in response to Zn application under water stress was observed in hexaploid wheat. Combined foliar application of Zn and Fe increases grain Zn and Fe and alleviates water stress’s adverse effects on all wheat ploidy levels, making biofortification cost-effective.

Globally, wheat occupies a central place in human nutrition, and its grain yield has been continuously improved through breeding and management efforts over the past several decades. However, yield improvement was associated with a substantial decline in grain protein and mineral contents, especially micronutrients such as zinc (Zn) and iron (Fe).

Generally, commercial wheat cultivars grain has 20–35 mg/kg Zn and Fe, which is inadequate for human diets constituting wheat as the primary source of essential minerals. Besides plant factors, almost half of cereal-cultivated soils are globally soils with inadequate concentrations of available Zn (i.e., chemically soluble Zn) further cause reducing grain Zn concentration. Dietary Zn and Fe deficiency have been associated with diverse health problems such as stunted growth, anemia, depressed immune function, brain function, development impairments, and vulnerability to deadly infectious diseases due to immune dysfunction, especially in children.

Among strategies to combat such deficiencies is biofortification, which increases the mineral nutrient content in crops, either by agronomic practices such as fertilization or genetic manipulation to obtain cultivars with higher mineral absorption and accumulation capabilities. Crop improvement activities for Zn/Fe dense wheat focus first on exploring the available genetic diversity for Fe and Zn. To identify useful variability for genetic biofortification, emphasis has been placed on screening progenitor species including *Triticum monococcum*.
Results

Analysis of variance. For all studied traits, the combined analysis of variance showed significant effects (P*0.01) for moisture regimes, ploidy levels, genotypes, and micronutrient treatments. Genotype × moisture regime interaction was also significant for all traits, except for FLL, FLW, grain Zn, and Fe content. The interaction effect for genotype × micronutrient application was significant for GY, NKS, and PH (Table S3). Analysis of variance for the data collected from each moisture regime also showed a significant (P*0.01) variation among genotypes, tetraploid and hexaploid species concerning all studied traits under both moisture conditions. The diploid group was significantly (P*0.01) diverse for all traits except FLL, FLW, and grain Fe content in normal moisture conditions and for GY, NKS, and PH traits in water stress conditions, respectively (Table S4). All traits excluding grain Zn content were significantly different, comparing 2x with each of 4x and 6x genotypes under normal water conditions.

A significant variation was observed among genotypes for all traits, except for the grain Zn and Fe content under water stress conditions. Likewise, in normal water conditions, a comparison of genotypes with tetraploid versus hexaploid levels showed a considerable difference for PH, FLW, and grain Zn content and in water stress conditions for PH, Zn, Fe content, and FLW (Table S4). Genotype × micronutrient application interaction was significant for GY, TKW, PH, and grain Zn content in normal conditions and for GY, NKS, and PH traits in water stress conditions.

Water-stress effects on the studied traits. Water stress negatively affected most measured traits, including GY, NKS, KL, KD, and PH. TKW, FLL, and FLW were also negatively affected, but their mean values were not significantly different from those in normal conditions. The highest percent reduction (38%) was observed for GY due to water stress. Contrary to other traits, grain Zn and Fe contents were significantly increased under water stress conditions, with a higher percent increase (20%) for Fe content (Table 1, Fig. 1).

Zinc and iron application effects. Under normal water conditions, Zn and Fe applications improved grain yield, but their combined application significantly increased the mean value of this trait. Water stress significantly reduced grain yield, but Zn and Fe application improved it somewhat. However, the combination of Zn + Fe application significantly alleviated yield reduction caused by water stress compared to the control moisture regime. Thousand kernel weight (TKW) was more influenced by Zn application than other treatments under both moisture conditions, with a significantly higher mean value for Zn application under water stress conditions (Table 2). Zinc + iron application enhanced NKS slightly in normal irrigation conditions. Still, under water stress situations, the Zn + Fe application significantly affected NKS compared to other treatments and eased the water stress effect to some degree. Kernel length and diameter (KL and KD) were significantly reduced.
Response of wheat ploidy levels to water stress and foliar application of zinc and iron. Grain yield was nearly four times higher in tetra and hexaploid species than in diploids under normal moisture conditions. Water stress reduced grain yield to a different extent at different micronutrient treatments for all wheat ploidy levels (2x, 4x, and 6x) (Table 3). A higher yield reduction was observed in 2x, followed by 4x, and finally 6x species at control treatment of micronutrient application. For 2x and 4x species, Zn and its combination with Fe applications slightly increased grain yield under normal irrigation conditions and eased yield reduction under water stress. However, the mean yields for 6x species were significantly increased in both moisture conditions at Zn and Zn + Fe applications (Table 3).
| Moisture Env | Trait | PL | Control | Change (%) | Zn Change (%) | Fe Change (%) | Zn + Fe Change (%) | LSD (0.05) |
|------------|------|----|---------|------------|---------------|---------------|-----------------|-------------|
| Normal     | GY   | 2x | 212.71| -48.69     | 210.83        | -26.78        | 273.12         | 53.80       |
| Normal     | TKW  | 2x | 13.32| -7.96     | 15.83         | -10.80        | 15.87          | 1.13        |
| Normal     | NKS  | 2x | 4.09 | -23.45    | 41.34         | -15.31        | 33.48          | 0.24        |
| Normal     | KL   | 2x | 6.95 | -9.06     | 7.01          | -7.99         | 6.90           | 0.05        |
| Normal     | KD   | 2x | 2.26 | -1.32     | 2.35          | -4.68         | 2.29           | 0.15        |
| Normal     | PH   | 2x | 115   | -3.52     | 114.39        | -3.03         | 114.97         | 1.51        |
| Normal     | FLL  | 2x | 3.33 | -8.31     | 3.16          | -8.25         | 3.18           | 0.38        |
| Normal     | FLW  | 2x | 6.33 | -1.42     | 5.74          | -3.83         | 6.06           | 0.05        |
| Normal     | Zn   | 2x | 12.84| 0.93      | 13.14         | -0.53         | 13.15          | 0.38        |
| Normal     | Zn   | 4x | 12.28| 3.04      | 13.62         | -8.52         | 13.11          | 0.05        |
| Normal     | Zn   | 6x | 50.93| 18.64     | 60.55         | -16.43        | 58.06          | 6.69        |

Continued
### Table 3. Mean comparison of traits for different wheat ploidy levels in response to foliar application of Zn and Fe under two moisture environments. GY, Grain yield (g/m²); NKS, Number of kernel per spike; TKW, Thousand kernel weight (g); KL, Kernel length (mm); KD, Kernel diameter (mm); PH, Plant height (cm); FLL, Flag leaf length (mm); FLW, Flag leaf width (mm); Zn, Grain zinc content (µg/g); Fe, Grain iron content (µg/g).

*For each trait, means followed by the same letter are not significantly different, using LSD test at 5% level of probability.*

| Moisture Env | Trait | PL<sup>a</sup> | Control | Change (%) | Zn | Change (%) | Fe | Change (%) | Zn + Fe | Change (%) | LSD (0.05) |
|--------------|-------|----------------|---------|-------------|---|-------------|---|-------------|--------|-------------|------------|
| Normal       |       | 2x             | 82.70<sup>b</sup> | 18.85 | 85.27<sup>cde</sup> | 16.68 | 99.41<sup>de</sup> | 17.75 | 89.98<sup>de</sup> | 17.25 |
|              |       | 4x             | 68.07<sup>f</sup> | 26.15 | 74.82<sup>cde</sup> | 18.39 | 84.46<sup>c</sup> | 20.86 | 75.27<sup>c</sup> | 24.46 |
|              |       | 6x             | 73.80<sup>HI</sup> | 23.67 | 79.27<sup>k</sup> | 13.26 | 84.85<sup>c</sup> | 35.09 | 73.63<sup>HI</sup> | 26.09 |
| Stress       |       | 2x             | 98.29<sup>de</sup> | -     | 99.49<sup>cde</sup> | -     | 117.06<sup>c</sup> | -     | 105.50<sup>bc</sup> | -     |
|              |       | 4x             | 85.87<sup>fgh</sup> | -     | 88.58<sup>HI</sup> | -     | 102.08<sup>cd</sup> | -     | 93.68<sup>def</sup> | -     |
|              |       | 6x             | 91.27<sup>HI</sup> | -     | 89.78<sup>HI</sup> | -     | 114.62<sup>cde</sup> | -     | 93.22<sup>HI</sup> | -     |

**Similar to grain yield, TKW was about four times higher in 4x and 6x species than the 2x ones under normal moisture conditions. Water stress reduced TKW in all ploidy level species, with 2x being less affected. At this moisture condition, the lowest TKW mean was observed for the control treatment and the highest for Zn application. A comparable NKS was observed for all wheat ploidy levels at normal water conditions. Water stress significantly reduced NKS for all three ploidy level species, similarly. The lowest reduction of this trait due to water stress was observed for foliar application of Zn + Fe in hexaploid species. The highest KL belonged to 4x species, with the means for 2x and 6x genotypes being almost comparable under normal water conditions. The mean KL was reduced under water stress conditions for all ploidy levels, with 2x genotypes were more affected. Foliar application of Zn + Fe abridged the effects of water stress on KL at all ploidy levels to some degree (Table 3).**

In contrast to KL, 2x and 4x species had the lowest mean KD under normal moisture conditions, and 6x genotypes had the highest. At the same time, water stress influenced 6x species more than 2x, and 4x with percent KD reduction higher for hexaploids. Zinc, Fe, and their combined applications had no significant effect on this trait. The mean plant height (PH) was almost the same for all wheat ploidy levels under normal water conditions and was not significantly influenced by water stress. The treatments also had no significant effect on this trait. The mean values of FLL and FLW were almost two times higher for 4x and 6x than 2x genotypes under normal water conditions. Compared to other traits, the reduction rate of these two traits was non to minimal under water stress conditions. A meaningful pattern of change due to micronutrient treatments was not observed for these two traits. Compared to 4x and 6x species, the grain Zn and Fe content were higher for 2x genotypes under normal moisture conditions (Table 3). Unlike other traits, the grain Zn and Fe contents were increased under stress conditions regardless of the micronutrient treatments. However, Zn and Zn + Fe treatments significantly increased grain Zn content, with the mean value being highest at Zn application under water stress conditions. Correspondingly, the most elevated content of grain Fe was observed for Fe application, and the highest value was observed for this treatment under water stress conditions (Table 3).

**Species discrimination based on biplot analysis for micronutrient treatments.**

*Control treatment.* Under normal irrigation, in the control treatment (Fig. 2a) principal components PC1 and PC2 explained 47.5 and 17.4% of the variation, respectively. The PC1 was positively associated with GY, KD, and TKW, and negatively correlated with Zn. PC2 was positively loaded with KL. Most emmer genotypes (numbers 20, 33, 7, 21, and 29) and *T. polonicum* (#11) along with one spelt genotype (#4) were placed in a distinct group from other genotypes in the left quadrant of the biplot with a high value of PC2 and a low value of PC1. *T. turanicum* and negatively correlated with Zn. PC2 was positively loaded with KL. Most emmer genotypes (numbers 20, 33, 7, 21, and 29) and *T. polonicum* (#11), one spelt genotype (#4), and wild einkorns (#31, 32, 5, and 6) were placed in a distinct group from other genotypes in the left quadrant of the biplot with a medium value of PC1 and low to medium PC2. Compared with 4x and 6x, diploid species showed higher scores for grain Zn. Also, synthetic.
Figure 2. Visualization of biplot based on PCA and interrelationships between traits for 35 genotypes with different ploidy levels/species subjected to four treatments under normal irrigation (a = control, b = zinc, c = iron, and d = zinc + iron applications) and water stress (e = control, f = zinc, g = iron, and h = zinc + iron applications). Abbreviations: Grain yield (GY g/m²), Number of kernel per spike (NKS), Thousand kernel weight (TKW g), Kernel length (KL mm), Kernel diameter (KD mm), Grain zinc content (Zn µg/g), and Grain iron content (Fe µg/g).
Iron treatment. Under normal irrigation and Fe treatment (Fig. 2c), PC1 and PC2 described 50 and 16.1% of the variation, respectively. The PC1 was positively associated with GY, TKW, and KD, and negatively loaded with Zn and Fe. PC2 was positively loaded with KL. The position of the genotypes in the left quadrants of the biplot with a high value of PC2 was the same as with Zn treatment under normal conditions. Furthermore, genotypes 5 and 31 showed higher scores for grain Fe, and likewise, genotypes 7, 29, and 11 displayed higher scores for grain Zn. Synthetic crosses (#34 and 35) and T. aestivum (#18) exhibited higher scores for GY. PC1 was highest for genotypes 34 and 8 with a medium value of PC2. PC2 was highest for T. dicoccoides (#33) and 19, with a low to medium value of PC1.

Under water stress conditions (Fig. 2g), PC1 and PC2 presented 43.8 and 16.3% of the variation, respectively. The PC1 was positively associated with GY, TKW, and KD, and negatively loaded with Zn and Fe. PC2 was positively loaded with KL. Several emmer genotypes (#20, 33, 7, and 29), along with genotypes number 4, and 11 were grouped together with a high value of PC2 and low value of PC1 on the left quadrants of the biplot. Among them, genotypes 1 and 4 had the highest value of PC2 and low value of PC1. Moreover, emmer genotypes 20 and 33 showed the highest score for grain Fe whereas T. turanicum genotypes 5 and 6 presented a high score for grain Zn. Synthetic crosses (#35 and 34) and Chines spring (#9) registered the highest score for GY and a low value of PC2. Genotypes 19, 8, and 3 revealed a high value for both PCs.

Zinc + Iron treatment. Under normal irrigation and Zn + Fe treatment (Fig. 2d), PC1 and PC2 described 45.9 and 19.4% of the variation, respectively. The PC1 was positively associated with KD, TKW, and GY and negatively associated with grain Zn. PC2 was positively correlated with KL and Fe. In this condition, the position of genotypes on the left quadrants of the biplot with high PC2 was similar to Zn and Fe treatment under normal irrigation conditions, except for genotype 11. Emmer genotypes 33 and 20 showed a high score for grain Fe. Genotype 18 had the highest value of PC1 and a high PC2. The highest PC2 and a high value of PC1 belonged to T. turanicum.

Under water stress, (Fig. 2h), PC1 and PC2 described 46.2 and 18.8% of the variation, respectively. The PC1 was positively associated with GY, TKW, and KD, and negatively associated with grain Zn and Fe. PC2 was positively correlated with KL. Similar to non-stress conditions, mostly the same genotypes were grouped in the left quadrants of the biplot with high PC2 and low to medium PC1. Genotype 18 showed a high score for GY. Genotypes 20 and 33 showed top scorers for grain Zn while genotype 32 had a high value for grain Fe. Synthetic cross (#34) had the highest PC1 with a low value of PC2, and genotype 11 had the highest PC2 with a low value of PC1. Water stress increased PC1 and PC2 values for T. compactum (#10) compared to normal conditions. Congruent with other treatments, the dipsoids were separated from tetra and hexaploids on the left side of the biplot chart, while tetra and hexaploids species did not completely detach on the right side.
In addition to the nutrient concentration effect due to the smaller grain size under moisture stress conditions, Zn and Fe applications significantly increased the content of these micronutrients in grain. These results strongly support previous findings suggesting the efficacy of Zn and Fe foliar application in raising the grain Zn and Fe content, especially under water stress conditions, where soil application may limit their mobility and plant availability.\(^{27,28}\) It is also believed that due to the poor mobility of Fe in the phloem, Fe fertilization appears to be less effective than Zn fertilization in the enrichment of cereal grains\(^{8,9}\). However, this was not the case in our research, and Fe foliar application was even more effective in increasing the grain Fe content than the Zn application and grain Zn content (Table 2). The timing of foliar application is also a determining factor in increasing grain Zn and Fe content, with the late growth stages being the best\(^7\). In this research, Zn and Fe were foliarly applied at two late plant growth stages of booting and milky-dough.

Foliar application of Zn and Fe positively affected all traits except PH, FLL, and FLW, particularly in water-limited conditions, where the stress effect on traits was mitigated to some degree. Zinc + iron treatment increased grain yield and its components and Zn application improved NKS and TKW under both moisture conditions. Our finding was consistent with previous studies in this respect\(^{28,29}\).

Our results regarding the response of different wheat ploidy levels (2x, 4x, and 6x) to water stress and different micronutrients application were consistent with other reports\(^ {28}\). In this study, 4x and 6x species yielded nearly four times as much grain as 2x species at both moisture regimes. Diploid species are generally less developed than 4x and 6x species that have undergone many selection cycles to increase grain yield\(^ {31}\). This is evident in several superior attributes of 4x and 6x species, including T. aestivum, and T. durum compared to diploids, T. boeoticum, and T. urartu. Also, we found much lower values for TKW and GY for diploids, reflected by their smaller KL and KD, compared to higher wheat ploidy levels.

The impact of water stress on grain yield varied by ploidy levels (2x, 4x, and 6x), with a higher reduction (48%) observed for 2x at the control treatment of micronutrient application. All wheat ploidy levels responded almost similarly to Zn, Fe, and Zn + Fe treatments, resulting in an increased yield under normal moisture conditions or a lower reduction at water stress. At the same time, the application of Zn, Fe, and their combination significantly increased the concentration of these micronutrients in grain which is in agreement with previous reports\(^{32,33}\). This suggests that Zn and Fe foliar applications can simultaneously increase grain yield and Zn and Fe content under both normal and water-stressed conditions.

The combined application of Zn and Fe was as practical as the application of each element separately, which may make Zn and Fe biofortification cost-efficient. Interestingly, the highest grain yield increase in response to the combined application of Zn and Fe under the two moisture conditions and the highest grain Zn content in response to foliar application of Zn under water stress conditions was observed in 6x wheat. Hexaploid wheat (Triticum aestivum L.) accounts for around 95% of the world's wheat production and is a staple crop for an estimated 40% of the world's population\(^9\). Most modern wheat cultivars are inherently low in grain Zn and Fe, a severe human health concern.

Principal component analysis depicted in biplot charts (Fig. 2a–h) revealed discrimination of species in response to micronutrient treatments. Diploid species of T. boeoticum, T. monococcum, and T. urartu are considered wild or domesticated wild\(^ {35}\). As expected, these unimproved species reacted inconsistently to micronutrient treatments compared to more improved 4x and 6x counterparts. At both irrigation conditions and all micronutrient treatments, 2x had a much lower yield than 4x and 6x species. Compared to modern 4x and 6x cultivars, they grouped away on the left quadrants of the biplot, exhibiting higher grain Zn and Fe content. A total of ten species were studied at the 4x level. In both irrigation conditions and all treatments, mostly wheat landraces belonging to T. dicoccoides (wild emmer), T. dicoccum (emmer), T. ispahanicum (emmer), and one T. polonium genotype (#11) were separated from more improved species and mainly occupied the left quadrants on the biplot chart linked with higher grain Zn, Fe, and KL and lower GY, TKW, NKS, and KD. In contrast, the improved genotypes of tetraploids that are naturally poor in Zn and Fe were placed on the right quadrants of the biplot associated with low grain Zn and Fe, and higher GY, TKW, KD, and NKS. This points to the known fact that the landrace genotypes cannot compete with the modern bred varieties for yield, but they have many valuable characteristics, such as higher grain Zn and Fe content\(^ {19}\). Among the kinds of wheat tested so far, wild emmer, Triticum turgidum ssp. dicoccoides contain the highest concentrations of grain Zn and Fe\(^ {7}\). The Gpc-B1 allele located on chromosome six of this species has been well characterized and associated with higher grain protein, Zn, and Fe\(^ {36}\).

Hexaploids consisted of five species with different degrees of improvement. In both moisture conditions and all treatments, spelt genotype number 4 was placed in the left quadrant of the biplot, close to emmer and einkorn genotypes, and associated with higher grain Zn and Fe, and lower GY, TKW, NKS, and KD. Reports\(^ {37,38}\) indicate that among hexaploid wheat, T. spelta, despite having lower grain yield, has higher grain protein, Zn, and Fe. On the other hand, the majority of genotypes of improved species, including synthetic crosses and T. aestivum, were found in the right quadrants in both moisture conditions and all treatments. These genotypes had high GY, TKW, NKS, KD, and low grain Zn, Fe, and KL. This may further confirm that yield improvement was associated with a substantial decline in grain mineral contents\(^ {23,25}\) and, therefore, the better response of these genotypes to micronutrients application such as Zn and Fe.

Ploidy levels and species within each ploidy level responded differently to water stress concerning yield reduction, with 2x being more affected and becoming farther apart from 4x and 6x species mainly on the lower left quadrant of the biplot chart in all treatments. Even though 6x genotypes had higher yield and lower grain Zn and Fe than 4x genotypes under normal conditions, they showed a more pronounced decrease in yield under water stress. In general, 4x wheat is more tolerant to water stress than the 6x counterparts\(^ {19}\). As a result, in most treatments and both moisture conditions, the 4x species of T. turanicum and T. durum (#8) showed high GY, TKW, and KD and grain Zn and Fe.
Conclusion
Water stress negatively affected all measured traits except for grain Zn and Fe content, which was increased under water-limited conditions. Under normal and water stress conditions, foliar Zn and Fe applications somewhat improved grain yield, but their combined application significantly increased this trait compared to the control treatment. Application of Zn + Fe also alleviated grain yield reduction caused by water stress. In addition to the concentration effect due to the smaller grain size under moisture stress conditions, Zn and Fe applications significantly increased the content of these two micronutrients in grain. Consistent with other reports, our results showed that 4x and 6x species yielded nearly four times as much grain as unimproved 2x species at both moisture levels. The negative impact of water stress on grain productivity varied by ploidy levels, with a higher grain yield reduction observed in 2x wheat. All ploidy levels responded almost similarly to Zn, Fe, and Zn + Fe foliar fertilization. The combined application of Zn + Fe was as practical as each element separately, making Zn and Fe biofortification cost-effective. Interestingly, the highest increase of grain yield in response to combined application of Zn + Fe under both moisture conditions and the highest grain Zn content in response to foliar application of Zn under water stress conditions was observed in 6x wheat.

Consistent with the above findings, PCA analysis categorized different species in response to micronutrient treatments. At both water conditions, wheat landraces and wild species were separated on the biplot chart from high-yielding improved genotypes of 4x and 6x species. The less improved landraces naturally contain higher grain Zn and Fe since they have not been selected against in the process of yield improvement which is the case for most varieties of durum and bread wheat.

Materials and methods

Plant Materials and experimental method. In a preliminary study, a collection of 144 wheat genotypes consisting of different ploidy levels with a wide geographical distribution were tested for yield, and 86 higher yielding genotypes were selected for grain Zn and Fe measurements. Again, considering all ploidy levels and geographical origin, 35 genotypes with higher grain Zn and Fe concentrations were designated for the current study (data not shown). The collection comprised ten hexaploid genotypes from five species, 19 tetraploids from 10 species, and six diploids from three species (Table S1). A split-plot scheme in a randomized complete block design with two replication was used in each of two irrigation regimes. The experiment was conducted at the Research Farm of Isfahan University of Technology (IUT) (32°32’ N and 51°23’ E, with 1630 m altitude) during the 2019–2020 growing seasons. Each plot consisted of two rows, each 100 cm long, 20 cm apart (0.40 m²), and 1.25 cm plant spacing. The micronutrient application was considered as the main factor and the genotype as sub factor. The soil in the experiment site was clay loam with a pH of 7.5 (Table S2) and its average annual precipitation and temperature were 140 mm and 15 °C, respectively. All plots were uniformly irrigated until 50% of all genotypes were at anthesis. At this stage, two irrigation regimes were applied to the experimental plots using a volumetric counter and polyethylene pipes from a pumping station. To provide non-stress and water stress conditions, irrigation was applied when 40 and 80% of the total available water around the root zone were depleted, respectively. The foliar application of Fe and Zn was performed at two plant growth stages of booting (scale 40) and early milk stage (scale 73) suggested to be the most responsive stages. Fe-EDTA and Zn-EDTA were applied by spraying a 0.25% (w/v) solution containing 200 mg L⁻¹ of Tween 20 as a surfactant. The 0.25% w/v (2.5 g L⁻¹) solutions of Zn-EDTA and Fe-EDTA were prepared by dissolving appropriate amounts of Fe-EDTA (13.2% Fe) or Zn-EDTA (14.0% Zn) chelates (supplied by the TradeCorp AZ) in 250-L polyethylene tank containing 220 L tap water.

An equivalent amount of water was sprayed on plants in control plots. For Zn + Fe treatment, the Zn-EDTA was sprayed first, and three days later, the Fe-EDTA was applied. All sprayings were conducted to cover the leaf surface uniformly at the point of runoff late in the afternoon, considering the wind velocity. Except for irrigation and foliar treatments, all other field operations were done uniformly for all experimental plots. Our plant material complies with relevant institutional, national, and international guidelines and legislation.

Measurement of the traits and Data analysis. The traits, including the number of kernel per spike (NKS), plant height (PH cm), flag leaf length (FLL mm), and flag leaf width (FLW mm), were measured on five samples randomly selected from each plot. The PH, FLL, and FLW were measured at late anthesis. Grain yield per plot (GY g/m²) was the total grain weight harvested from each plot. Kernel length and diameter (KL, KD mm) were measured on 100 grains using a caliper and then averaged. One hundred grains were counted, weighed, and used for the calculation of thousand kernel weight (TKW g). The grain Zn and Fe concentrations were determined as follows. A grain sample (0.2 g) from each plot was ashed at 550 °C for three h. Inorganic ions were then extracted using 10 ml 2 N HCl, and the volume of each sample was standardized to 100 ml. The samples were analyzed using a Rayleigh, WFX-210 flame atomic absorption spectrometer. The detection limits for Fe and Zn were 0.01 and 0.007 mg L⁻¹, respectively. All grain micronutrient concentrations were expressed on a dry weight basis.

Before analysis of variance (ANOVA), the Kolmogorov–Smirnov test was conducted to examine the normality distribution of data. The Bartlett's test was used to test the homogeneity of residual variances. Subsequently, the differences among genotypes, between irrigation regimes, and their interactions were examined in a combined analysis of variance, using SAS release 9.4 (SAS Institute, Cary, NC, USA). Principal component analysis (PCA) was performed to reduce the multiple dimensions of data space and also used to identify the relationships between traits and dispersion of the treatments using the R package software.
Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Received: 11 September 2022; Accepted: 22 November 2022
Published online: 25 November 2022

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**Acknowledgements**
This research was supported by Isfahan University of Technology, Wheat Research Chair. The authors are very grateful to Dr. Mozhgan Abtahi for the careful and meticulous reading of the paper.

**Author contributions**
F.Sh. did the field and lab work, analyzed the data, and wrote the original manuscript draft; F.Sh., A.M., and G.S. designed the field experiment; A.M. managed the project. All authors read and commented on the final draft. All authors reviewed the manuscript.

**Competing interests**
The authors declare no competing interests.

**Additional information**

**Supplementary Information** The online version contains supplementary material available at [https://doi.org/10.1038/s41598-022-24868-1](https://doi.org/10.1038/s41598-022-24868-1).

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