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Foliar application of iron and zinc improves morpho-physiological and agronomic grain biofortification of wheat (*Triticum aestivum* L.) under restricted irrigation conditions

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ABSTRACT

To increase the nutritional value of food crops and their endurance to water shortage circumstances, agronomic biofortification with zinc (Zn) and (Fe) may be used. By enhancing physiological and enzymatic antioxidant defense systems, Zn and Fe may help plants tolerate water stress. Major objective of this study was to investigate the effect of foliar applied Zn and Fe on grain biofortification and drought tolerance in wheat. Treatments include application of Zinc and Iron at terminal growth phases (tillering (20-40 DAS), booting (60-80) and earing stages (100-110) growth stages) with each three levels: [$Zn_{0\%}$, $Zn_{1.0\%}$, and $Zn_{2.0\%}$] [$Fe_{0\%}$, $Fe_{0.5\%}$ and $Fe_{1.0\%}$]), laid out in factorial randomized block design with three replications. From the results revealed that water stress significantly reduced relative water contents, gas exchange attributes, and morpho-physiological parameters (leaf area index at 30, 60, 90 DAS, chlorophyll intensity, Canopy temperature depression) and yield related attributes. In contrast to application of various levels of Zn and Fe significantly improved and were markedly increased under water stress condition leaf area index at 30, 60, and 90 DAS, chlorophyll intensity, canopy temperature depression, Zn uptake of seed, Fe uptake of seed and protein content of the seed. Foliar applied $Zn_{1.0\%}$ and $Fe_{0.5\%}$ predominantly reduced the damaging impact of water stress by improving the plant status in the form of leaf area index, chlorophyll intensity, canopy temperature depression, and Zn uptake of seed, Fe uptake of seed and protein content of the seed. Likewise, wheat plants treated with $Zn_{1.0\%}$ and $Fe_{0.5\%}$ under water stress conditions increased the grain yield by improving the number of grains per spike, 1000 grain weight, and biological yield compared with control. Moreover, increasing Zn levels also increased Zn concentration in grains and leaves. Overall, this study suggests that the optimum level of Zn (1%) might be promising for alleviating the adverse impacts of water stress and enhance the grain biofortification in wheat.

Keywords: *Foliar application, Micronutrient, Zinc, Iron, Wheat, leaf area index, and chlorophyll intensity.*

1. Introduction

The maintenance of global food security is severely hampered by the water deficit. In comparison to the preceding century, the world's growing population has raised food demand, necessitating the use of eight times more irrigated land [23]. Due to unexpected atmospheric changes, there is rising competition for water supplies across different regions, which could lead to increased restrictions and risks for global food security [1]. At all growth phases of wheat, a lack of water has negative effects. The most severe effect was seen at the reproductive stage, specifically at the grain filling stage, which results in shorter and smaller grains of wheat [15]. Water stress decreased assimilates partitioning, as well as hindered the activity of vital enzymes necessary for the synthesis of synthetic sucrose and starch, which decreased grain filling [8 &13].

According to [5] and [19], wheat is a crop that is relatively susceptible to iron and zinc deficiencies. The global production volume of wheat amounted to over 781 million metric tonnes, in an area of 222.7 million hectares [47].

The nation's demand for wheat is rising daily. Increased acreage devoted to wheat or higher yields per area will be required to meet the high demand for wheat in the upcoming years. India is the second largest wheat producer in the world. It contributes about 12% of wheat production in the world. Wheat output in India reached 112.74 mt and ranked next to the China in global wheat production during 2022–23, with an average productivity of 35.07 quintal/ha from an area of 30.47 million ha, accounting for 36% of the nation's total production of food grains [3]. With an area of 9.42 million ha, Uttar Pradesh produced 33.95 mt of wheat overall, with an average productivity of 36.04 q/ha. According to a review of state-by-state output, Uttar Pradesh is in first place with 33.95 mt, followed by Madhya Pradesh (22.42 mt), Punjab (14.82 mt), Haryana (10.45 mt), Rajasthan (9.48 mt) and Bihar (6.22 mt). About 92 per cent of the overall production was produced by these top six states together [4].

Micronutrients are necessary for plant growth and nutrient uptake. Agricultural soils frequently have micronutrient shortages, such as those in iron, copper, boron, and zinc. The deficiency may be caused by the low concentrations of micronutrients in the soil, [42]. Micronutrient deficiency, often known as Hidden Hunger, is one of the biggest problems the human race is facing right now. It affects more than 2 billion people globally and is caused by a lack of essential vitamins and minerals (mostly vitamin A, zinc, and iron) in the diet. [50] Zinc (Zn) and Iron (Fe) is an imperative nutrients serving as a physical, basic, or regulatory cofactor for a variety of enzymatic

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functions and regulates growth and development. In another study, it was documented that the optimum dose of Zn maintained the water status; stomatal regulation, and adjustment of guard cells in chickpeas under water stress [26, 18]. Furthermore, foliar applied Zn improved the crop growth which ultimately increased the yield because it has the ability to regulate stomatal openings, in the chlorophyll formations, and also increase in the leaf area of the plant [9, 39, 28, 32 and 29].

The micronutrient Zn and Fe deficiency is a severe threat around the world and about one-third population of developing countries is facing micronutrient deficiency symptoms [37]. Peoples of Pakistan, China, India, and Turkey are facing Zn deficiency problems [7, 12, 31, and 33]. The 43% population of these countries depends upon wheat nutrition; therefore, wheat bio-fortification with Zn and Fe would be beneficial for the human nutrition in these countries [11]. Several agricultural practices like agronomic biofortification are considered as cost beneficial and sustainable approaches to increase the micronutrients especially Zn in the wheat grains to overcome Zn shortage symptoms in the humans [17 & 6]. Biofortification of Zn and Fe through these agricultural strategies increases grain Zn and Fe contents and diminishes the Zn and Fe deficiency symptoms in humans [37& 22]. Several scientists have reported that applied Zn and Fe is an efficient agronomic strategy to achieve the required Zn and Fe contents in wheat grains for human nutrition [21, 6, 14 & 41].

Furthermore, various scientists have reported on the efficiency of Zn and Fe in the alleviation of negative impacts of water stress [9 & 35]. But there is a lack of knowledge on the effect of Zn and Fe to regulate the physiological and biochemical mechanism and Zn and Fe biofortification under water stress. Therefore, the major aim of this study was to investigate the potential of Foliar applied Zn and Fe to regulate morpho-physiological and grain biofortification of wheat under restricted water stress for sustainable wheat yield.

2. Materials and methods

2.1. Experimental treatments and design

Experimental treatments comprised of two levels of variety i.e., (K-1317 and K-1616) and two micronutrients each of three levels of foliar applied Zn i.e., 0 (control) water spray, 1.0%, 2.0%, and Fe i.e. 0 (control), 0.5% and 1.0%. These treatments were arranged according to a triplicate randomized block design (RBD) with the factorial arrangement. The Zn was applied as zinc sulphate ($ZnSO_4$.7H2O) and Fe was applied as Ferrous Sulphate ($FeSO_4$.7H2O) its application was done at (tillering (20-40 DAS), booting (60-80) and earing stages (100-110) growth stages) under water stress.

2.2. Experimental procedure

Breeder Seed of wheat cultivars K-1317 and K-1616 was collected from Chandra Shekhar Azad University of Agriculture and Technology, Kanpur (U.P.). The NPK fertilizers were applied 120, 60, and 40 Kg $ha^{-1,}$ and the standard procedure was followed during experimentation. Nitrogen, phosphorous, and potassium were applied in the form of urea, ammonium phosphate, and potassium sulphate respectively. The full amount of DAP, MOP, and 50% of the nitrogen were administered at the time of sowing, and the remaining nitrogen was top dressed in two equal portions at the CRI and tillering stages. The amounts were weighed separately for each treatment to apply varying levels of zinc and iron. Specific doses of zinc and iron were applied randomly on the experiment plots. Zinc was applied in the form of Zinc sulphate and iron in the form of ferrous sulphate. The plot size during experimentation was 6×2.4 m^2 and a total number of the plot was 54, and 12 lines in each plots were maintained for the subsequent studies.

2.3. Measurement the Leaf Area Index at 30,60 and 90 Days

The leaf area index was determined by using leaf area meter provided by the Section of Rabi cereals in CSAUA&T Kanpur. The formula provided by [49] was used for calculating the Leaf Area Index.

$$LAI = \frac{LA}{P}$$

Where,

LA= Leaf Area
P = Accupied Land Area

2.4. Determination of chlorophyll intensity

By using a chlorophyll metre SPAD 502, the amount of chlorophyll was measured and expressed as a percentage. The equipment provided by the Section of Rabi cereals in CSAUA&T Kanpur.

2.5. Determination of Canopy Temperature Depression

By using an infrared thermometer from Teletamp AG in the USA, the canopy temperature depression was calculated and expressed as ^{0}C.

2.6. Zn content of seed

The amount of zinc in the digested material was calculated using an AAS by [48]. A 100 ml conical flask was filled with a 0.5 gramme sample of ground plant material. The flask was heated on a hot burner after adding 10 cc of the di-acid mixture until the residue turned colorless. The liquid was then allowed to cool before being diluted with distilled water and put through Whatman No. 1 filter paper. By adding distilled water, the volume of the filtrate was increased to 50 ml. With the aid of an atomic absorption spectrophotometer and a hollow cathode lamp (HCL), the zinc concentration of the solution was determined. The following formula was used to calculate uptake using the zinc content (ppm) as reported by the AAS based on a computed standard curve:

Total zinc uptake (g ha^1) = Zn uptake in seed (g ha^{-1}) + Zn uptake in straw (g ha^{-1})

Zn uptake in Seed (g ha^{-1})

$$= \frac{\text{Zn content in seed (ppm)} \times \text{Seed Yield as dry weight (g ha}^{-1})}{10^6}$$

2.7. Fe uptake of Seeds

Using a Perkin Elmer 3110 Atomic Absorption Spectrometer, the available Fe contents of the grain samples were extracted using a treatment of 0.005 M Diethylene Triamine Penta Acetic acid (DTPA) + 0.01 CaCl2 + 0.1 M Triethanolamine (TEA) solution, pH = 7.3 [27].

2.8. Protein estimation of Seed

Wheat protein content was analyzed using the Kjeldahl technique. The total protein content of weight seedlings from various treatments was calculated using the nitrogen analyzer Kel plus (Pelican-ultima Deo Dist.). Using this procedure, the total protein and nitrogen contents were estimated. The total protein content was then multiplied by a factor.

Nitrogen (%) = [(1.4 x N x V) / W] x 100

Where,

N = Normality of HCl
V = Titre value of sample – titre value of blank
W = Weight of sample
[Protein (%) = Nitrogen% x 6.25]

2.9. Statistical analysis

Using Fisher's Analysis of Variance technique, all the data of the experiment was analyzed considering a two-factor complete factorial randomized block design **[36].** For the comparison of means was carried out by using statistical software (OP STAT). Moreover, figures were prepared by using Microsoft Excel 365.

3. Results

3.1 Morpho-physiological attributes

3.1.1. Leaf Area Index at 30 DAS

The effect of both years zinc and iron showed a significant effect on Leaf Area Index at 30 DAS. However, numerically it was found maximum (1.687) in the combination of Zn$_{1.0\%}$ × Fe$_{0.5\%}$ in wheat variety K-1317 from the table (i). In wheat variety K-1616, a combination of Zn$_{1.0\%}$ × Fe$_{1.0\%}$ (1.637) was found maximum from the table (i).

3.1.2 Leaf Area Index at 60 DAS

The effect of zinc and iron did not show a significant effect on Leaf Area Index at 60 DAS. However, numerically it was found maximum (2.553) in the combination of Zn$_{1.0\%}$ × Fe$_{0.5\%}$ in wheat variety K-1317 from the table (ii). In wheat variety K-1616, a combination of Zn$_{1.0\%}$ × Fe$_{1.0\%}$ (2.550) was found maximum from the table (ii).

3.1.3. Leaf Area Index at 90 DAS

The effect of zinc and iron did not show a significant effect on Leaf Area Index at 90 DAS. However, numerically it was found maximum (3.467) in the combination of Zn$_{1.0\%}$ × Fe$_{0.5\%}$ in wheat variety K-1317 from the table (iii). In wheat variety K-1616, a combination of Zn$_{1.0\%}$ × Fe$_{1.0\%}$ (3.483) was found maximum from the table (iii).

3.1.4. Zn uptake of Seeds

The effect of zinc and iron significantly affect on Zn Uptake in Seeds. However, numerically it was found maximum Zn Uptake in Seed (45.35) in the combination of Zn$_{1.0\%}$ × Fe$_{0.5\%,}$ and minimum Zn Uptake in Seed was found in Zn$_{0\%}$ × Fe$_{0\%}$ (42.32) in wheat variety K-1317 from the table (iv). In wheat variety K-1616, a combination of Zn$_{1.0\%}$ × Fe$_{1.0\%}$ (45.68) was found maximum and the minimum Zn Uptake in Seed found in Zn$_{0\%}$ × Fe$_{0\%}$ (42.63) from table (iv).

3.1.5. Fe uptake of Seeds

The effect of zinc and iron significant effect on Fe Uptake in Seed. However, numerically it was found maximum Fe Uptake in Seed (1.250) in the combination of Zn$_{1.0\%}$ × Fe$_{0.5\%,}$ and minimum Fe Uptake in Seed was found in Zn$_{0\%}$ × Fe$_{0\%}$ (1.083) in wheat variety K-1317 from the table (v). In wheat variety K-1616, a combination of Zn$_{1.0\%}$ × Fe$_{1.0\%}$ (1.233) was found to maximum and the minimum Fe Uptake in Seed found in Zn$_{0\%}$ × Fe$_{0\%}$ (1.100) from the table (v).

3.1.6. Protein content of Seeds

The effect of zinc and iron have a significant effect on the protein content in Seed. However, numerically it was found maximum protein content in Seed (12.403) in the combination of Zn$_{1.0\%}$ × Fe$_{0.5\%}$ and minimum protein content in Seed was found in Zn$_{0\%}$ × Fe$_{0\%}$ (10.153) in wheat variety K-1317 from the table (vi). In wheat variety K-1616, a combination of Zn$_{1.0\%}$ × Fe$_{1.0\%}$ (12.463) was found maximum and the minimum protein content in Seed found in Zn$_{0\%}$ × Fe$_{0\%}$ (10.367) from the table (vi).

Table (i): Effect of Zinc and Iron on LAI at 30 DAS in wheat variety K-1317 and K-1616 (Pooled)

Variety	**K-1317**			**K-1616**		
Zinc / Iron	**(Zn$_{0\%}$)**	**(Zn$_{1.0\%}$)**	**(Zn$_{2.0\%}$)**	**(Zn$_{0\%}$)**	**(Zn$_{1.0\%}$)**	**(Zn$_{2.0\%}$)**
(Fe$_{0\%}$)	0.618	1.657	1.385	0.712	1.307	1.432
(Fe$_{0.5\%}$)	1.300	1.687	1.457	1.362	1.568	1.475
(Fe$_{1.0\%}$)	1.400	1.405	1.413	1.322	1.637	1.457
SE (d)	**0.063**					
CD(p=0.05)	**0.128**					

Table (ii): Effect of Zinc and Iron on LAI at 60 DAS in wheat variety K-1317 and K-1616 (Pooled)

Variety	**K-1317**			**K-1616**		
Zinc / Iron	**(Zn$_{0\%}$)**	**(Zn$_{1.0\%}$)**	**(Zn$_{2.0\%}$)**	**(Zn$_{0\%}$)**	**(Zn$_{1.0\%}$)**	**(Zn$_{2.0\%}$)**
(Fe$_{0\%}$)	1.203	2.460	2.310	1.200	2.303	2.310
(Fe$_{0.5\%}$)	2.257	2.553	2.287	2.260	2.517	2.420
(Fe$_{1.0\%}$)	2.270	2.353	2.350	2.320	2.550	2.357
SE (d)	**0.089**					
CD(p=0.05)	**NS**					

Table (iii): Effect of Zinc and Iron on LAI at 90 DAS in wheat variety K-1317 and K-1616 (Pooled)

Variety	**K-1317**			**K-1616**		
Zinc **Iron**	**(Zn$_{0\%}$)**	**(Zn$_{1.0\%}$)**	**(Zn$_{2.0\%}$)**	**(Zn$_{0\%}$)**	**(Zn$_{1.0\%}$)**	**(Zn$_{2.0\%}$)**
(Fe$_{0\%}$)	1.300	3.403	3.273	1.287	3.283	3.317
(Fe$_{0.5\%}$)	2.883	3.467	3.387	2.883	3.467	3.433
(Fe$_{1.0\%}$)	3.013	3.370	3.397	3.273	3.483	3.363
SE (d)	**0.201**					
CD(p=0.05)	**NS**					

Table (iv): Effect of Zinc and Iron on Zinc Uptake in Seed in wheat variety K-1317 and K-1616 (Pooled)

Variety	**K-1317**			**K-1616**		
Zinc **Iron**	**(Zn$_{0\%}$)**	**(Zn$_{1.0\%}$)**	**(Zn$_{2.0\%}$)**	**(Zn$_{0\%}$)**	**(Zn$_{1.0\%}$)**	**(Zn$_{2.0\%}$)**
(Fe$_{0\%}$)	42.32	44.35	44.39	42.63	42.56	44.27
(Fe$_{0.5\%}$)	44.03	45.35	43.06	44.10	44.48	44.88
(Fe$_{1.0\%}$)	44.74	44.67	42.64	44.09	45.68	43.05
SE (d)	**0.191**					
CD(p=0.05)	**0.387**					

Table (v): Effect of Zinc and Iron on Iron Uptake in Seed in wheat variety K-1317 and K-1616 (Pooled)

Variety	**K-1317**			**K-1616**		
Zinc **Iron**	**(Zn$_{0\%}$)**	**(Zn$_{1.0\%}$)**	**(Zn$_{2.0\%}$)**	**(Zn$_{0\%}$)**	**(Zn$_{1.0\%}$)**	**(Zn$_{2.0\%}$)**
(Fe$_{0\%}$)	1.083	1.133	1.100	1.100	1.117	1.100
(Fe$_{0.5\%}$)	1.100	1.250	1.133	1.183	1.233	1.067
(Fe$_{1.0\%}$)	1.100	1.050	1.083	1.067	1.233	1.067
SE (d)	**0.049**					
CD(p=0.05)	**0.099**					

Table (vi):Effect of Zinc and Iron on Protein estimation in Seed in wheat variety K-1317 and K-1616 (Pooled)

Variety	**K-1317**			**K-1616**		
Zinc **Iron**	**(Zn$_{0\%}$)**	**(Zn$_{1.0\%}$)**	**(Zn$_{2.0\%}$)**	**(Zn$_{0\%}$)**	**(Zn$_{1.0\%}$)**	**(Zn$_{2.0\%}$)**
(Fe$_{0\%}$)	10.153	11.507	11.223	10.367	11.393	12.213
(Fe$_{0.5\%}$)	11.237	12.403	11.183	11.167	11.56	11.58
(Fe$_{1.0\%}$)	10.457	11.527	11.28	11.213	12.463	11.57
SE (d)	**0.080**					
CD(p=0.05)	**0.162**					

4. Discussion

Increased yield through intensive agriculture with high yielding crop varieties, use of NPK fertilizers, and limited use of manures along with restricted recycling of plant residues are some important factors that have accelerated the exhaustion of soil micronutrients. Micronutrients exist in very small amounts in both soils and plants, but their role is frequently as important as the primary or secondary nutrients. Important micronutrients include six elements, namely, Fe, Mn, Zn, Cu, B and Mo [45].

The function of zinc in plants is as activator of enzymes. It enters into the constituents of enzymes systems that regulate initial metabolic reactions in the plant body. Zinc catalaes the process of oxidation in the plant cells [2] and is vital for the transformation of carbohydrates. It regulates the consumption of sugar and increases the source of energy for the production of chlorophyll. Zinc also aids the formation of auxin and the synthesis of protein. It is involved in the biosynthesis of plant hormones [46] and in maintaining the normal auxin concentration in tissues. Zinc is constituted of several enzymes such as alcohol dehydrogenase, carbonic amylase, and protenase and act as a co-factor for several others. It plays a vital role in the synthesis of protein and nucleic acid and helps in the utilization of nitrogen and phosphorus in plants.

Iron has many important functions in plant growth and development, such as involvement in the biosynthesis of chlorophyll, respiration, and chloroplast development and improves the performance of photo systems. It is an essential part of many enzymes. Iron also participates in the oxidation process that releases energy from sugars and starches and in responses that convert nitrate to ammonium in plants. It plays an essential role in nucleic acid metabolism [38, 30, 10 and 20].

In the present investigation, all morpho-physiological parameters were found to be affected significantly due to the application of zinc and iron. The linear increase in values related to morpho-physiological parameters due to the application of zinc and iron was observed. Maximum leaf area index at 30, 60, and 90 DAS, chlorophyll intensity and canopy temperature depression were observed in the combination application of zinc 1.0% and iron 0.5% in variety K-1317. However, a dose of zinc 1.0% and iron 1.0% have been found to significantly improved leaf area index at 30, 60 and 90 DAS, chlorophyll intensity, and canopy temperature depression in variety K-1616 and statistically closed to the application of zinc 1.0% and iron 0.5%. It seems that application of zinc and iron interacts with the physiological activity of the plant that which helps better in the growth of the plants. Progressively increase in leaf area index due to the application of zinc earlier reported by [34, 24, 40, 43, 25 and 44] . The superior combination effect of zinc and iron on morpho physiologically characters of the wheat variety, is obvious that the ($Zn_{1.0\%} \times Fe_{o.5\%}$) and ($Zn_{1.0\%} \times Fe_{1.0\%}$).

5. Conclusion

Application of Zn and Fe improves morpho-physiological, antioxidant enzymatic activities and also biofortification (Zn and Fe enrichment) of variety. Moreover, by the application Zn and Fe, the concentration of Zn and Fe increased in grain which regulates the stomatal conductance and photosynthesis and improved the growth and development of wheat plants under drought conditions. Although improvement in spike related traits, grain yield, grain Zn contents, and grain Fe contents was observed in normal irrigated plants due to Zn fertilization, but the improvement in the traits was relatively more prominent under water stress conditions. Based on the results, an optimum dose of Zinc and Iron is ($Zn_{1.0\%} \times Fe_{o.5\%}$) and ($Zn_{1.0\%} \times Fe_{1.0\%}$) suggested along with NPK fertilization for alleviation of water stress and grain biofortification.

Declaration: The authors should declare that they do not have any conflict of interest.

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