



Agronomic Biofortification with Zinc and Iron Improves Yield and Nutrient Content of Bread Wheat

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ABSTRACT

The rapid progress in developing high yielding wheat varieties during the past also led to significant decline in Zn and Fe content in the grain due to the dilution effect. The Zn and Fe deficiency in cereal grain posed serious health issues in the human being. Zn and Fe content of the grain can be increased either by genetic or agronomic biofortification. A field experiment was carried out to evaluate the effects of agronomic bio-fortification with Zn and Fe on yield, yield attributes, and grain Zn and Fe content of normal and biofortified wheat varieties during winter season of 2021-2022 at Rampur, Chitwan. The experiment was laid out in strip-split plot design with twelve treatments and three replications. The treatments consisted of two varieties of wheat (Zinc Gahun 2 and Vijay) in vertical plots, two iron levels (0 and 10 kg Fe ha⁻¹ soil application) in horizontal plots and three levels of zinc (0, 10, and 20 kg Zn ha⁻¹ soil application) in sub plots. The results revealed that Zinc Gahun 2 produced higher grain yield (4821 kg ha⁻¹), grain zinc content (40.47 mg kg⁻¹) and grain Fe content (39.29 mg kg⁻¹) followed by Vijay (4271 kg ha⁻¹, 31.42 mg kg⁻¹ and 32.61 mg kg⁻¹). Similarly, soil application of Fe at 10 kg ha⁻¹ and Zn at 20 kg ha⁻¹ produced significantly higher grain yield ($P < 0.05$) (10.04% and 10.11%, respectively) than control. The Zn content increased with increasing Zn levels but decreases with an increase in Fe levels and vice-versa due to their antagonistic effect. Therefore, fertilization of micronutrients (zinc and iron) in both biofortified and non-biofortified wheat varieties is one of the best approaches for farmers to harvest higher yield with enriched Zn and Fe content in their wheat grains.

Keywords: Biofortification, Zinc and Iron, Soil application, Wheat

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INTRODUCTION

Micronutrients, such as vitamins and minerals, are crucial for overall health, particularly for the growth and development of children and adolescents. Iron and zinc are essential for immune system function, wound healing, infection prevention, and antioxidant activity (Sangeetha et al 2022). (Gupta et al 2015) state that although iron deficit results in severe cognitive issues and maternal death, zinc deficiency affects growth and immunity and increases maternal mortality. In developing nations, where diets are frequently cereal-based and deficient in minerals and vitamins, these deficits are particularly common among women and children (Ghimire et al 2019, Gupta et al 2015, Ramzan et al 2020). In Nepal, anemia among women of reproductive age increased from 35% in 2011 to 41% in 2016 (Ghimire et al 2019), and malnutrition rates remain high, with 6.1% of the population undernourished and 36% of children under five stunted (Grebmer et al 2020).

According to (Stangoulis and Knez 2022), high-yielding wheat cultivars have increased yields by up to 175% over the course of 120 years. Iron (Fe) and zinc (Zn) contents have, however, decreased by 11–25% as a result. This decrease is caused by modern wheat's larger grain sizes, changed bran to endosperm ratios, deteriorating soil quality, and climate change variety (Murphy et al 2008, CIMMYT 2020). Intensive use of chemical fertilizers, particularly those high in phosphorus, also hinders plants' ability to absorb zinc and iron (Cakmak 2008). Soil limitations, a rainy environment, and inadequate irrigation further reduce Nepal's supply of these minerals.

Crops are enhanced with micronutrients by biofortification, increasing their nutrient content. This can be accomplished in wheat by increasing the levels of zinc and iron in the seeds by agronomic and genomic methods

(Gupta et al 2015, Ngozi 2013). Higher yield under deficiency, disease resistance, decreased seed rate, enhanced nutrition, greater seed viability, and increased resistance to abiotic stress are some advantages (Cakmak 2008). The Nepal Agriculture Research Council (NARC) developed five biofortified wheat varieties in 2020 that had better yield and disease resistance in addition to having 20–40% more zinc and iron than commercial varieties (CIMMYT 2020).

Food and mineral fortification is frequently used in developed nations to improve wheat nutrition, but it is expensive and unavailable in developing nations like Nepal (Cakmak 2008, CIMMYT 2020, Tewari et al 2017). Grain micronutrient content and cereal yield are improved by agronomic techniques like zinc and iron fertilization, which provide an affordable and practical solution to zinc and iron (Cakmak 2008, Velu et al 2013). By increasing crop micronutrient value, agronomic biofortification could aid in the fight against hidden hunger in nations like Nepal (CIMMYT 2020, Gupta et al 2015, Ngozi 2013). According to Yilmaz et al. (1987), applying zinc fertilizers and foliar sprays can boost grain zinc concentration by three to four times and improve productivity. (Firdous et al 2018) found that combining soil and foliar Zn application also improves yield and grain nutritional quality. Considering the above facts, present study was carried out to examine the effect of zinc and iron levels and their interaction on yield, its attributes, and zinc and iron content in biofortified and non-biofortified wheat varieties.

MATERIALS AND METHODS

Experiment site

The research was conducted at the Agronomy farm of Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal, from November 2021 to April 2022, covering one wheat growing season. The site, located at 184.86 meters altitude in Bagmati province (27°40'53.2"N, 84°20'54.7"E), has been under a rice-wheat cropping system for the past five years. The soil was sandy loam, slightly acidic, with medium levels of total nitrogen (0.16%) and organic matter (3.13%), and low levels of available phosphorus (25.95 kg ha⁻¹), potassium (96.32 kg ha⁻¹), and zinc (0.80 mg ha⁻¹), while iron content was high (24 mg ha⁻¹). The average maximum temperature ranged from 23.5°C in February to 34.1°C in April, and the minimum temperature ranged from 11.6°C in December to 24.02°C in April. Total rainfall during the cropping period was 132.7 mm, with the highest rainfall of 71.8 mm in April. Average relative humidity was 78.81%, ranging from 65.5% to 95%.

Plant materials

The study used two bread wheat varieties: Zinc Gahun 2, a biofortified variety with high grain zinc and iron content, and Vijay, a non-biofortified variety popular in plain regions of Nepal. Seeds of both varieties were obtained from the National Wheat Research Program (NWRP), Bhairahawa, Rupendehi. Sowing was done manually on November 29, 2021, with a seed rate of 120 kg ha⁻¹ and a row spacing of 25 cm, using continuous seeding in rows. The individual plot size was 4×3 m with an area of 12 m².

Experimental details

The experiment was conducted using a strip-split plot design with a total of twelve treatment combinations. The vertical plots (strips) included two wheat varieties (Zinc Gahun 2 and Vijay), the horizontal plots had two iron levels (0 and 10 kg Fe ha⁻¹ soil application), and the subplots had three zinc levels (0, 10, and 20 kg Zn ha⁻¹ soil application), with three replications.

Cultural practices

After rice harvest, the field was deep-plowed with a cultivator and cross-harrowed twice, 15 days before planting. Stubbles and dried weeds were manually removed, and final harrowing was done a day before sowing to level the field. The recommended fertilizer dose (120:50:50 N, P₂O₅, K₂O) was applied using urea, DAP, and muriate of potash. Phosphorus and potassium were applied as a basal dose, while nitrogen was split into two applications: 50% at basal and 50% at the Crown Root Initiation (CRI) stage. Zinc and iron were also applied as basal doses using zinc sulphate and iron sulphate, which contained 21 % Zn and 19 % Fe, respectively. One irrigation was provided at grain filling stage, 105 days (March 13, 2022) after sowing for a better grain set. The crop was manually harvested on April 2, 2022, dried for two days, and threshed with a thresher.

Data collection

Measurement of growth and yield parameters

During physiological maturity stage, effective tillers were counted from one entire row of 3 m from middle of each plot and converted to per square meter. Plant height and spike length were measured using a scale. The number of grains per spike was manually counted from twenty randomly selected spikes from each plot and averaged. At physiological maturity, 8.25 m² net area of each plot was harvested to measure biological yield.

Grain yield was recorded after threshing on a plot basis and expressed in kg ha⁻¹ at 12% moisture content, and thousand-grain weight was measured using an electronic balance. Harvest index was calculated by dividing grain yield by biological yield, both adjusted to 0% moisture content, and expressed as percentage. Sterility was assessed by counting the number of grains in the F1 and F2 florets of ten central spikelets and averaging the values. Then sterility percentage was estimated using the formula which is known as Thai method.

$$\text{Sterility \%} = \frac{20 - \text{No. of grains per (F1+F2) florets of 10 central spikelets}}{20} \times 100\%$$

Measurements of Zinc and Iron content in wheat grain

Zinc (Zn) and iron (Fe) concentrations were determined using an atomic absorption spectrophotometer, as proposed by (Steponeniene et al 2003). After the grain samples were dried and milled, 5 grams were put into a sterile digesting vessel. To aid with digestion, strong nitric and hydrochloric acids were mixed together. The absorbance of the diluted sample was used to measure the amounts of Zn and Fe, and the results were represented in mg kg⁻¹ or ppm. The analysis was conducted at the Agriculture Technology Center (ATC), Lalitpur, Nepal.

Statistical analysis

The field and lab data were organized in MS Excel and analyzed using Analysis of Variance (ANOVA) in R-studio. ANOVA tested the significance of each parameter, and significant results were further analyzed using DMRT for mean comparison at the 5% and 1% levels (Gomez and Gomez 1983).

RESULTS

Yield attributing characters

Wheat varieties were found significantly different for the traits: number of grains per spike, effective tillers per square meter, thousand grain weight, and sterility (Table 1).

Table 1. Yield attributes of wheat as influenced by the varieties, iron and zinc levels at Rampur, Chitwan, Nepal 2021-2022

| Treatments | Effective tillers m ² | Number of grains spike ⁻¹ | Thousand-grain weight (g) | Sterility % |
|---|----------------------------------|--------------------------------------|---------------------------|--------------------|
| Vertical factor: Varieties | | | | |
| Zinc Gahun 2 | 355.72 ^a | 54.76 ^a | 36.87 ^b | 4.33 ^b |
| Vijay | 332.42 ^b | 33.77 ^b | 50.71 ^a | 7.88 ^a |
| SEm (±) | 2.12 | 0.53 | 0.78 | 0.19 |
| LSD (=0.05) | 12.89 | 3.20 | 4.75 | 1.20 |
| CV (%) | 2.61 | 5.05 | 7.57 | 13.79 |
| Horizontal factor: Iron levels (kg ha ⁻¹) | | | | |
| 0 | 350.89 | 43.80 | 44.36 | 5.59 |
| 10 | 337.55 | 44.73 | 43.22 | 6.62 |
| SEm (±) | 4.79 | 0.48 | 0.98 | 0.23 |
| LSD (=0.05) | NS | NS | NS | NS |
| CV (%) | 5.90 | 4.61 | 9.55 | 16.00 |
| Sub plot factor: Zinc levels (kg ha ⁻¹) | | | | |
| 0 | 330.08 | 42.16 ^b | 42.73 | 6.43 ^{ab} |
| 10 | 350.72 | 43.96 ^{ab} | 43.85 | 6.70 ^a |
| 20 | 351.41 | 46.68 ^a | 44.81 | 5.18 ^b |
| SEm (±) | 10.57 | 0.94 | 1.31 | 0.42 |
| LSD (=0.05) | NS | 2.83 | NS | 1.26 |
| CV (%) | 10.63 | 7.40 | 10.40 | 23.97 |
| Grand mean | 344.07 | 44.26 | 43.79 | 6.10 |

Treatments mean followed by the common letter (s) within a column are non-significantly different with each other based on DMART at 5% level of significance. CV: Coefficient of variation, LSD: Least significant difference

Zinc Gahun 2 had significantly higher grains per spike (54.75), and effective tillers per square meter (355.72) compared to Vijay, while Vijay had a higher thousand grain weight (50.71 g) than Zinc Gahun 2 (Table 1). Soil-applied Zn significantly ($p < 0.05$) improved grain numbers per spike though the effects of Zn and Fe on effective tillers per square meter and thousand grain weight were non-significant (Table 1). Applying 20 kg Zn ha⁻¹ resulted in significantly more grains per spike (46.68) as compared to control. Additionally, Zn application at 20 kg ha⁻¹ also reduced sterility by 19.44% compared to the control, with significant variation between varieties. There was no significant effect of the iron treatment used for this experiment on the studied yield attributes.

There was a significant interaction between iron and zinc levels on the number of ET m⁻² (Table 2). The highest number of ET m⁻² was observed with 20 kg Zn ha⁻¹ without iron fertilization, which was statistically similar to 10 kg Zn ha⁻¹ with iron (360.55) and 10 kg Zn ha⁻¹ without iron (340.88). The lowest number of ET m⁻² (328.72) occurred in the control plot with no zinc and iron.

Table 2. Number of effective tillers per meter square of wheat as influenced by the interaction between iron level and zinc level at Rampur, Chitwan, 2021-2022

| Zinc level (kg ha ⁻¹) | Number of effective tillers m ⁻² | |
|-----------------------------------|---|----------------------|
| | Iron level (kg ha ⁻¹) | |
| | 0 | 10 |
| 0 | 328.72 ^b | 331.44 ^b |
| 10 | 340.88 ^{ab} | 360.55 ^{ab} |
| 20 | 382.16 ^a | 320.66 ^b |
| SEm (±) | 14.94 | |
| LSD (=0.05) | 44.80 | |
| CV (%) | 10.63 | |

Treatments mean followed by the common letter (s) are non-significantly different with each other based on DMRT at 5% level of significance. CV: Coefficient of variation, LSD: Least significant difference

Grain yield, straw yield and harvest index

Wheat grain yield was significantly affected by varieties, Fe levels, and Zn levels, but their interaction had no significant impact (Table 3). Zinc Gahun 2 produced a higher grain yield (4821.75 kg ha⁻¹) than Vijay (4271.92 kg ha⁻¹). Applying 10 kg Fe ha⁻¹ increased yield (4764.18 kg ha⁻¹) by 10.04% compared to control (4329.49 kg ha⁻¹).

Table 3. Grain yield, straw yield and harvest index of wheat as influenced by the varieties, iron and zinc levels at Rampur, Chitwan, Nepal 2021-2022

| Treatments | Grain yield (kg ha ⁻¹) | Straw yield (kg ha ⁻¹) | Harvest index |
|---|------------------------------------|------------------------------------|---------------|
| Vertical factor: Varieties | | | |
| Zinc Gahun 2 | 4821.75 ^a | 7299.17 | 0.42 |
| Vijay | 4271.92 ^b | 6846.98 | 0.40 |
| SEm (±) | 82.56 | 89.61 | 0.01 |
| LSD (=0.05) | 501.98 | NS | NS |
| CV (%) | 7.70 | 5.37 | 6.06 |
| Horizontal factor: Iron levels (kg ha ⁻¹) | | | |
| 0 | 4329.49 ^b | 7434.58 | 0.39 |
| 10 | 4764.18 ^a | 6711.57 | 0.43 |
| SEm (±) | 59.90 | 335.04 | 0.01 |
| LSD (=0.05) | 364.20 | NS | NS |
| CV (%) | 5.58 | 21.29 | 11.03 |
| Sub plot factor: Zinc levels (kg ha ⁻¹) | | | |
| 0 | 4265.63 ^b | 7004.95 | 0.40 |
| 10 | 4678.06 ^a | 7054.39 | 0.41 |
| 20 | 4696.81 ^a | 7159.87 | 0.42 |
| SEm (±) | 103.58 | 267.70 | 0.01 |
| Grand mean | 4546.83 | 7073.07 | 0.41 |
| LSD (=0.05) | 310.54 | NS | NS |
| CV (%) | 7.89 | 13.11 | 8.21 |

Treatments mean followed by the common letter (s) within a column are non-significantly different with each other based on DMART at 5% level of significance. CV: Coefficient of variation, LSD: Least significant difference

Zinc application also significantly boosted grain yield, with 10 kg ha⁻¹ (4678.06 kg ha⁻¹) and 20 kg ha⁻¹ (4696.81 kg ha⁻¹) showing statistically similar results. The control yielded the lowest (4265.63 kg ha⁻¹), with 20 kg Zn ha⁻¹ resulted in a 10.11 % increment in grain yield as compared with the control plot. Straw yield and harvest index were not significantly affected by any factor (Table 3).

Nutrient content

Zinc and Iron content in wheat grain

Grain zinc content in wheat was influenced by varieties and zinc levels, but not by iron levels (Table 4). Zinc Gahun 2 had significantly higher grain zinc content (40.47 mg kg⁻¹) compared to Vijay (31.72 mg kg⁻¹). The highest zinc content (39.60 mg kg⁻¹) was observed with 20 kg Zn ha⁻¹, followed by 10 kg Zn ha⁻¹ (36.79 mg kg⁻¹). The non-Zn application plot had the lowest zinc content (31.89 mg kg⁻¹).

Iron content in wheat grain was influenced by varieties, zinc levels, and iron levels (Table 4). The average iron content was 35.95 mg kg⁻¹. Zinc Gahun 2 had significantly higher iron content (39.29 mg kg⁻¹) than Vijay (32.61 mg kg⁻¹). Soil-applied iron significantly increased grain iron content yielding the highest (39.30 mg kg⁻¹), 20.55% higher than the control. Zinc application reduced iron content, with 20 kg Zn ha⁻¹ resulting in the lowest iron content (34.06 mg kg⁻¹), followed by 10 kg Zn ha⁻¹ (36.05 mg kg⁻¹). The grain iron content was significantly higher in the control (0 kg Zn ha⁻¹), 10.80% more than with 20 kg Zn ha⁻¹ application.

Table 4. Grain zinc and iron content of wheat as influenced by the varieties, iron and zinc levels at Rampur, Chitwan, Nepal 2021-2022

| Treatments | Grain content (mg kg ⁻¹) | |
|---|--------------------------------------|---------------------|
| | Zn | Fe |
| Vertical factor: Varieties | | |
| Zinc Gahun 2 | 40.47 ^a | 39.29 ^a |
| Vijay | 31.72 ^b | 32.61 ^b |
| SEm (±) | 1.34 | 0.82 |
| LSD (=0.05) | 8.19 | 5.04 |
| CV (%) | 15.81 | 9.79 |
| Horizontal factor: Iron levels (kg ha ⁻¹) | | |
| 0 | 36.99 | 32.60 ^b |
| 10 | 35.20 | 39.30 ^a |
| SEm (±) | 1.32 | 0.18 |
| LSD (=0.05) | NS | 1.10 |
| CV (%) | 15.56 | 2.15 |
| Sub plot factor: Zinc levels (kg ha ⁻¹) | | |
| 0 | 31.89 ^c | 37.74 ^a |
| 10 | 36.79 ^b | 36.05 ^{ab} |
| 20 | 39.60 ^a | 34.06 ^b |
| SEm (±) | 0.84 | 0.69 |
| LSD (=0.05) | 2.52 | 2.087 |
| CV (%) | 8.07 | 6.70 |
| Grand mean | 36.10 | 35.95 |

Treatments mean followed by the common letter (s) within a column are non-significantly different with each other based on DMART at 5% level of significance. CV: Coefficient of variation, LSD: Least significant difference

Grain Zn content due to interaction between Fe and Zn level

A significant interaction was found between Fe and Zn application levels on grain zinc content (Table 5).

Table 5. Grain zinc content of wheat as influenced by the interaction between iron level and zinc level at Rampur, Chitwan, 2021-2022

| Zinc level (kg ha ⁻¹) | Grain zinc content (mg kg ⁻¹) | |
|-----------------------------------|---|---------------------|
| | Iron level (kg ha ⁻¹) | |
| | 0 | 10 |
| 0 | 30.88 ^d | 32.90 ^{cd} |
| 10 | 35.96 ^{bc} | 37.62 ^b |
| 20 | 44.13 ^a | 35.08 ^{bc} |
| SEm (±) | 1.19 | |
| LSD (=0.05) | 3.56 | |
| CV (%) | 8.07 | |

Treatments mean followed by the common letter (s) are non-significantly different with each other based on DMRT at 5% level of significance. CV: Coefficient of variation, LSD: Least significant difference

The highest zinc content (44.13 mg kg⁻¹) was observed with 20 kg Zn ha⁻¹ without iron application. Higher Zn application without Fe increased grain zinc content, while 10 and 20 kg Zn ha⁻¹ combined with 10 kg Fe showing similar, but higher than the 0 kg Zn application.

Grain Fe content due to interaction between Fe level and varieties

A significant interaction was found between Fe levels and wheat varieties on grain iron content (Table 6). Zinc Gahun 2 had significantly higher iron content (42.71 mg kg⁻¹) without zinc application. The iron content in Vijay was similar across all zinc levels. The lowest iron content (31.63 mg kg⁻¹) was observed in Vijay with 20 kg Zn ha⁻¹ application.

Table 6. Grain iron content of wheat as influenced by the interaction between varieties and zinc levels at Rampur, Chitwan, 2021-2022

| Zinc level (kg ha ⁻¹) | Grain iron content (mg kg ⁻¹) | |
|-----------------------------------|---|--------------------|
| | Varieties | |
| | Zinc Gahun 2 | Vijay |
| 0 | 42.71 ^a | 32.77 ^c |
| 10 | 38.68 ^b | 33.42 ^c |
| 20 | 36.49 ^b | 31.63 ^c |
| SEm (±) | 0.98 | |
| LSD (=0.05) | 2.95 | |
| CV (%) | 6.70 | |

Treatments mean followed by the common letter (s) are non-significantly different with each other based on DMRT at 5% level of significance. CV: Coefficient of variation, LSD: Least significant difference

Grain Fe content due to interaction between Fe level and Zn level

There was significant interaction between Fe and Zn application levels on grain iron content (Table 7). The highest iron content (42.91 mg kg⁻¹) was observed with 10 kg Fe ha⁻¹ without zinc application. When 20 kg Zn ha⁻¹ was applied without Fe, the grain iron content significantly decreased (32.36 mg kg⁻¹). Higher Fe application without zinc increased grain iron content, while increased zinc levels reduced it.

Table 7. Grain iron content of wheat as influenced by the interaction of both iron and zinc levels at Rampur, Chitwan, 2021-2022

| Zinc level (kg ha ⁻¹) | Grain iron content (mg kg ⁻¹) | |
|-----------------------------------|---|--------------------|
| | Iron level (kg ha ⁻¹) | |
| | 0 | 10 |
| 0 | 32.57 ^d | 42.91 ^a |
| 10 | 32.86 ^{cd} | 39.24 ^b |
| 20 | 32.36 ^d | 35.76 ^c |
| SEm (±) | 0.98 | |
| LSD (=0.05) | 2.95 | |
| CV (%) | 6.70 | |

Treatments mean followed by the common letter (s) are non-significantly different with each other based on DMRT at 5% level of significance. CV: Coefficient of variation, LSD: Least significant difference

DISCUSSION

Yield attributing characters

The Zinc Gahun 2 variety had significantly higher ET m⁻², grains per spike, and lower sterility compared to Vijay, which had the highest thousand grain weight. These yield differences were due to their genetic makeup. Zinc Gahun 2 has higher grain number and denser spikes contributed to lower sterility, as reported by (Adhikary et al 2009). Vijay's increased thousand grain weight resulted from larger, heavier grains, but fewer grains each spike, similar with studies of (Suleiman et al 2014, Acharya et al 2017). According to (Hassan et al 2019) the increase in grains per spike can be ascribed to improved photosynthetic translocation with Zn treatment boosting physiological functioning. Additionally, (Hafeez et al 2021) discovered that applying Zn and Fe at 10 and 12 kg ha⁻¹ greatly increased the number of grains per spike. Because Zn-treated plots had better anther and pollen development than untreated ones, the positive effect of zinc on grain number was associated with decreased sterility. In wheat lacking zinc, (Yilmaz et al 1987) observed poor anther and pollen formation. These findings also accord with (Firdous et al 2018), who observed that Zn application reduced sterility and increased grains per spike.

Grain yield

Zinc Gahun 2 had significantly higher grain yield in this study primarily due to better yield attributes, such as grains per spike, effective tillers, thousand grain weight, and lower sterility. Yield variation among the varieties may reflect genetic differences, in line with previous studies by (Yasseen and Hussain 2021, Acharya et al 2017 and Hafeez et al 2021) and the yield potential reported in the MoALD released varietal list (MoALD 2023). The increase in grain yield in Zn-treated plots was due to a higher number of grains per spike and reduced sterility (Table 2). (Yilmaz et al 1987) stressed Zn's significance in anther and pollen development, crucial for seed set. In addition to supporting metabolic processes like the metabolism of carbohydrates, lipids, and nucleic acids

(Marschner 1955, Tripathi et al 2015), zinc also plays a role in photosynthesis, respiration, hormone production, enzyme activation, and protein synthesis, all of which increase yield (Jalal et al 2020, Tripathi et al 2015). Because iron promotes the synthesis of proteins and nucleic acids, carbohydrates, and chlorophyll, it also greatly boosted grain yield (Fageria et al 2002). Findings by (Das et al 2020 and Keram et al 2013) on the beneficial effects of Zn and Fe on yield corroborated with (Abbas et al 2009) conclusion that Fe fertilization increased grain yield by improving growth and yield.

Grain Zn and Fe content

The wheat variety Zinc Gahun 2 had significantly higher grain Zn content than Vijay (Table 4), primarily due to its genetic makeup. According to the findings of (Hafeez et al 2021), Zinc Gahun 2 was more effective at accumulating Zn in grains. According to (Andersso et al 2017), fortified varieties such as Zinc Gahun 2 are specifically designed to accumulate high levels of zinc. According to (Hussain et al 2018), because of their bigger grain size, cultivars with higher TGW and more endosperm, like Vijay, typically have lower Zn concentration. In line with (Hafeez et al 2021), who discovered that soil-applied Zn improves grain Zn levels, Zn application at various doses considerably enhanced grain Zn content compared to control plots (Table 4). Higher Zn absorption and improved mobilization to grains are the causes of this (Hussain et al 2016). (Hegelund et al 2012) demonstrated that remobilization during grain filling is the primary source of zinc in wheat and barley grains. Similarly, (Jalal et al 2020) and (Yaseen and Hussain 2021) discovered that Zn fertilization improves Zn uptake and redistribution from leaves to grains, increasing grain Zn content. Research generally supports that Zn supplementation, either soil, foliar, or seed application, promotes Zn accumulation in grains (Cakmak 2010, Jalal et al 2020, Laik et al 2019, Yaseen and Hussain 2021).

Zinc Gahun 2 had a much greater grain Fe concentration than Vijay (Table 4), which is consistent with the findings of (Hafeez et al 2021), who discovered that biofortified cultivars accumulate more Fe than non-biofortified cultivars. In a similar vein, adding 10 kg Fe ha⁻¹ considerably raised the grain Fe content (39.30 mg kg⁻¹), which was 20.55% higher than the control. These findings are comparable to those of (Hafeez et al 2021), who discovered that grain Fe content was considerably increased by applying 12 kg ha⁻¹ of Fe. Conversely, grain Fe content decreased when Zn doses were increased from 0 to 20 kg Zn ha⁻¹. According to (Jalal et al 2020), who similarly noted a decrease in grain Fe content with higher Zn levels, the highest Fe content (37.74 mg kg⁻¹) was found at 0 kg Zn ha⁻¹ and the lowest (34.06 mg kg⁻¹) at 20 kg Zn ha⁻¹. Zn's impact on assimilate accumulation in grains may be the cause of this, as elevated Zn levels might hinder Fe uptake by obstructing its movement within the phloem of the plant.

Interaction between Zn and Fe on grain Zn and Fe content

The interaction between iron and zinc levels significantly affected grain Zn and Fe content (Table 5 and Table 7). This is consistent with (Jalal et al 2020), who observed that increasing Zn levels led to a uniform decrease in grain Fe content. (Fageria et al 2002) and (Prasad et al 2016) reported that the negative interaction between Zn and Fe in soil interferes with Zn absorption and its translocation from roots to shoots. (Yaseen and Hussain 2021) and (Ramzan et al 2020) also noted this antagonistic effect. The Fe-Zn interaction is positive at low nutrient concentrations but negative at higher levels (Prasad et al 2016). (Pahlavan-Rad and Pessarakli 2009) also discovered that the Zn-Fe interaction had a major impact on Zn concentration.

The evaluation of the effects of zinc and iron application on grain yield and micronutrient concentration in both conventional and biofortified wheat varieties is this study's primary strength. It illustrates how agronomic biofortification can successfully raise nutritional quality and productivity while addressing micronutrient deficiencies in Nepal. However, the results are limited in their wider applicability because they are based on a single site and season. Additionally, the study only looked at soil applications and restricted rates, underscoring the need for more multi-location, multi-season studies that use a variety of application techniques and evaluate long-term effects.

CONCLUSION

Soil application of Zn and Fe increased the Zn and Fe content in the grain by 24.17 and 20.55 % respectively. Zinc Gahun 2 contained 27.58 and 20.48 % more grain Zn and Fe content, respectively as compared with Vijay. Biofortification also increased grain yield and pollen fertility of wheat varieties. Therefore, agronomic biofortification through soil application of Zn and Fe based fertilizers could be suggested to wheat growers for increased grain yield, yield attributes, grain Zn and Fe in both biofortified and non-biofortified wheat varieties. But farmers should also be cautious about the corresponding antagonizing effects of Zn and Fe fertilization on their grain accumulation to harvest the maximum grain yield with optimum grain Zn and Fe concentrations.

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AUTHORS' CONTRIBUTION

The main author Uttam Poudel carried out the experiment, collected data, prepared ANOVA and manuscript, and Shrawan K. Sah guided and helped in the entire process of the experimentation to the writing of the manuscript and reviewed final draft of manuscript.

CONFLICT OF INTEREST

The authors have no any conflict of interest to disclose.

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