



Research article

Genotype-dependent biofortification of winter wheat grain with Iron, Zinc, and Selenium via drone-assisted foliar application

Chao Jin¹, Yining Wang¹, Fangfang Liu¹, Zifu Ni¹, Min Zhang^{1,*} and Chengwei Li^{1,2,*}

¹ School of Biological Engineering, Henan University of Technology, 450001 Zhengzhou, China

² State Key Laboratory of Cotton Biobreeding and Integrated Utilization, School of Agriculture and Biomanufacturing, Zhengzhou University, Zhengzhou, 450001, China

* **Correspondence:** Email: zhangmin01@haut.edu.cn (M.Z.), lcw@haut.edu.cn (C.L.); Tel: +8618623713183.

Abstract: Micronutrient deficiency in staple crops is a global health challenge. Simultaneous biofortification with multiple elements (Fe, Zn, Se) provides a critical physiological solution; however, its field efficacy via innovative delivery across diverse genotypes remains underexplored. To evaluate this, a field experiment in Xinxiang, Henan Province, China during the 2024–2025 growing season assessed unmanned aerial vehicle (UAV)-mediated foliar applications. Eight winter wheat varieties received an Fe-Zn-Se cocktail at three application rates (T1–T3) during the heading and filling stages. Results showed highly genotype- and rate-dependent concentrations ($P < 0.01$), with maximum absolute gains reaching 38.31 mg/kg for Fe (in ZM895), 30.12 mg/kg for Zn (in JZ439), and 0.44 mg/kg for Se (in JZ439). While Fe concentration was primarily genotype driven, Zn and Se levels were highly sensitive to application rates, peaking at T3. Furthermore, this application synergistically increased Cu and Ca without disrupting Mn, Mg, or P homeostasis. Ultimately, successful UAV biofortification requires coupling optimal application rates with responsive genotypes. Varieties LD1, ZM895, and JZ439 demonstrated exceptional multi-element enrichment capacity and are highly recommended for large-scale agricultural programs.

Keywords: wheat; unmanned aerial vehicle (UAV); foliar application; biofortification; micronutrients; mineral nutrients; genotype

1. Introduction

Wheat is one of the most important food crops in providing calories, proteins, and mineral nutrition to global human populations [1]. Over the past 50 years, the Green Revolution has doubled global wheat production. These agricultural advances sustained the growing world population and curtailed the fraction of undernourished individuals globally [2]. However, the increase in calorie availability was not matched with synchronized enhancement in micronutrient intake in many parts of world, especially in the less affluent fraction of the human population, resulting in various functional disorders and diseases [3]. There is an impetus, therefore, to improve the nutritional quality of wheat grains, at least with respect to micronutrients, such as iron (Fe), zinc (Zn), and selenium (Se).

Iron is involved in various biochemical processes, such as oxygen transport and enzymatic reactions [4,5]. Iron deficiency anemia affects up to 1.6 billion people globally, with the highest burden in sub-Saharan Africa and South Asia [6]. Zinc deficiency can also lead to numerous abnormalities in human metabolism and hormonal disorders [7]. According to research statistics, nearly one-third of the global population is deficient in Zn and more than 10% of the people with zinc deficiency have less than 50% of the recommended daily Zn intake in their diet [7,8]. As another vital micronutrient, selenium is particularly known for its protective role in human immune, endocrine, reproductive systems, and brain function [9,10]. Selenium supplementation supports antioxidant defense, immune function, and thyroid hormone metabolism [11,12].

In many resource-poor developing countries, achieving dietary diversity to provide adequate micronutrient consumption is generally difficult for most people. The provision of required vitamins and micronutrients through staple or energy-rich food is therefore considered a cost-effective strategy to address micronutrient malnutrition and other nutrition-associated health issues. Agronomic biofortification, complementing traditional breeding and genetic enhancements, employs a fertilizer-based approach involving the application of micronutrients to soil and/or foliage, either individually or in various combinations. A number of studies have been conducted to examine the variability for grain Fe, Zn, and Se concentrations following foliar spraying of micronutrient fertilizer in wheat varieties and the landraces [13–16], most of which have focused on application of a single or rarely two micronutrients. However, achieving simultaneous biofortification of Fe, Zn, and Se remains a major physiological challenge. Specifically, the co-application of multiple divalent cations (e.g., Fe and Zn) often triggers antagonistic interactions and competitive inhibition during foliar uptake and vascular translocation [15]. Consequently, the physiological dynamics and practical feasibility of co-accumulating these essential elements via a unified multi-nutrient spray remain critically unresolved [14]. In addition, traditional crop fortification is usually conducted by manual foliar spraying, which is labor intensive, tedious, and lacks the precision and consistency that are required for comparative analysis. The recent advent of unmanned aerial vehicles (UAVs) has provided a potentially useful alternative approach of biofortification of Fe, Zn, and Se in wheat, the effectiveness of which remains to be investigated.

UAVs are increasingly employed for precision foliar application due to their operational flexibility, high efficiency, and cost-effectiveness [16]. However, while UAVs hold great potential for micronutrient biofortification, the physiological impacts of multi-element co-delivery remain largely unexplored. Therefore, we hypothesized that simultaneous foliar application of a cocktail mix of Fe, Zn, and Se via UAV would successfully increase these micronutrient concentrations in wheat grains in a genotype-dependent manner, without inducing severe ionic antagonism. The objective of this study was to test this hypothesis by investigating genotypic variations and plant responses across different

winter wheat varieties and spraying application rates. Our study successfully achieved this objective by identifying highly-responsive wheat varieties and optimal application rate parameters, thereby laying a solid foundation for the broad application of multi-element UAV biofortification in precision agriculture.

2. Materials and methods

2.1. Plant materials

Selected winter wheat (*Triticum aestivum* L.) varieties, including BN4199, BNAK58, XM26, ZM895, ZM379, BN207, LD1, and JZ439, were cultivated under specified field conditions.

2.2. Field experiments

Field experiments were conducted at the Xinxiang experimental station (35.2°N, 113.8°E), Xinxiang, Henan Province, China, during the 2024–2025 winter wheat growing season. The soil at the experimental site is predominantly a clay-loam. Soil samples collected from the 0–30 cm depth exhibited the following physicochemical properties: A pH of 8.0 (measured in a 1:2.5 soil-to-water suspension), organic matter content of 12.2 g/kg, alkaline hydrolysis nitrogen (N) of 65.1 mg/kg, available potassium (K) of 112.7 mg/kg, and Olsen P of 19.1 mg/kg. Prior to sowing, a commercial compound fertilizer was applied evenly to the field at a rate of 975 kg/ha as a basal application to secure optimal crop growth.

The field experiment was arranged in a randomized complete block design (RCBD) with a split-plot arrangement and three replicates (blocks) to account for potential spatial field heterogeneity. The total experimental area was approximately 1 hm². To accommodate the continuous flight path of the UAV, the main plots were assigned to the four foliar application treatments: (1) Water (CK); (2) 1.5% FeSO₄ · 7H₂O + 1.5% ZnSO₄ · 7H₂O + 0.02% Na₂SeO₃ (T1); (3) 3% FeSO₄ · 7H₂O + 3% ZnSO₄ · 7H₂O + 0.05% Na₂SeO₃ (T2); and (4) 5% FeSO₄ · 7H₂O + 5% ZnSO₄ · 7H₂O + 0.1% Na₂SeO₃ (T3). Meanwhile, the sub-plots consisted of the eight winter wheat varieties fully randomized within each main plot.

To optimally match the 5 m effective spray width of the UAV and effectively prevent spray drift between treatments, each sub-plot covered a strictly defined area of 80 m² (16 m × 5 m), with appropriate buffer zones and mechanical walkways established between adjacent plots. For yield and nutrient analysis, five representative sites, each measuring 1 m², were randomly chosen and harvested manually from each sub-plot.

2.3. UAV flight parameters and foliar application strategy

A DJI T20 hexacopter UAV (DJI Technology Co., Ltd., Shenzhen, China) was employed for the foliar application of micronutrient mixtures. To ensure uniform coverage and optimal deposition, the flight altitude was maintained at 2 m above the wheat canopy, with a constant flight speed of 4 m/s and an effective spray width of 5 m. Based on standard operational guidelines calibrated specifically for wheat canopy penetration, the spray volume was precisely set at 27 L/ha. The centrifugal nozzles were configured to produce a median droplet diameter (VMD) of approximately 90 µm, ensuring optimal spray distribution, adequate canopy coverage, and sufficient droplet density without the need for supplementary in-field deposition testing. The successful and uniform biofortification of target

elements in the harvested grains further validated the efficacy of this standardized spray quality. The applications were strategically conducted at two key phenological stages: Heading and grain-filling. To maximize leaf nutrient absorption, minimize rapid evaporative loss, and prevent droplet drift, UAV spraying operations were strictly constrained to specific meteorological thresholds. The applications were performed on clear evenings (after 18:00) when the ambient temperature ranged from 15 °C to 25 °C, and the wind speed was stably below 2.0 m/s. Meteorological conditions were continuously monitored to ensure that these thresholds were met and no rainfall occurred 24 h before or after the application.

2.4. Sample preparation and nutrient analysis

In each experiment, five sites, each measuring 1 m², were randomly chosen and harvested separately. Following harvest, the grains from each sample were promptly washed using deionized water and subsequently dried in an oven at 60 °C until a constant weight was reached. The grains were then ground to powder prior to being dissolved in the mixture of HNO₃ and H₂O₂ (1:1, v:v). The concentrations of the target elements (Fe, Zn, and Se) and other mineral nutrients (Ca, Mg, P, Mn, and Cu) were determined using inductively coupled plasma mass spectrometry (ICP-MS, Wuhan Yihe Analytical Company, Wuhan, China) according to the national standard method. Quality assurance (QA) and quality control (QC) were strictly implemented using blank corrections, calibration curves, and certified reference materials. Multi-point calibration curves were constructed for Fe, Zn, and Se, with the standard acceptance criterion of correlation coefficients (R^2) being ≥ 0.995 . Based on the standard instrument protocol and a 2.0 g sample weight, the limits of detection (LOD) were 0.3, 0.2, and 0.003 mg/kg for Fe, Zn, and Se, respectively, while the limits of quantification (LOQ) were 1.0, 0.5, and 0.01 mg/kg. To validate the accuracy of elemental quantification at trace concentrations (e.g., Se), an agricultural reference material was analyzed in parallel as a quality control check, yielding an acceptable elemental recovery rate ranging from 90% to 110%, thereby assuring the reliability and reproducibility of the nutrient data.

2.5. Statistical analysis

To evaluate the biofortification efficacy, absolute gain and relative enrichment efficiency (REE) were calculated using the following formulas:

$$\text{absolute gain} = \text{concentration (treatment)} - \text{concentration (CK)}$$

$$\text{REE (\%)} = [(\text{concentration (treatment)} - \text{concentration (CK)}) / \text{concentration (CK)}] \times 100$$

where concentration (treatment) represents the micronutrient concentration in the treated groups, and concentration (CK) represents the corresponding value in the control group.

Data were subjected to two-way analysis of variance (ANOVA) to evaluate the main effects of wheat variety, application rate, and their interactions. Prior to the ANOVA, the assumptions for parametric tests were statistically verified: The normal distribution of the data was evaluated using the Kolmogorov-Smirnov (K-S) test, and the homogeneity of variances was confirmed by Levene's test. For each variety, one-way ANOVA followed by the least significant difference (LSD) test was performed to compare mean values among different treatment groups at a significance level of $P < 0.05$. Different lowercase letters were used to indicate significant differences. The aforementioned variance analyses were conducted using IBM SPSS Statistics 22.0. Furthermore, hierarchical cluster analysis

was conducted and visualized using GraphPad Prism 8 to evaluate the similarities among wheat varieties. Before clustering, the raw data were standardized using Z-scores to eliminate the dimensional influence of variables with different magnitudes. The clustering was automatically executed by the software using the average linkage method, with Euclidean distance acting as the similarity metric.

Additionally, an exploratory correlation analysis was conducted to investigate the potential synergistic or antagonistic relationships among the REE of Zn, Se, and Fe. Pearson correlation coefficients (r) were calculated and visualized as a heatmap using Graphpad Prism 8. Statistical significance for the correlations was determined at $P < 0.05$.

3. Results

3.1. Varietal and rate-dependent responses of grain Fe, Zn, and Se

A two-way ANOVA was performed, considering wheat variety (genotype) and foliar spray application rate as the primary factors. The contribution of each factor to the variation was further quantified using the partial eta squared (η^2) (Table 1). For Fe concentration, the genetic factor played a dominant role, with the order of influence being: variety ($\eta^2 = 0.963$) > variety $\times$ application rate interaction ($\eta^2 = 0.906$) > application rate ($\eta^2 = 0.817$). In contrast, the spraying application rate was the primary driver for Zn and Se. Specifically, the application rate exerted the greatest effect on Zn concentration ($\eta^2 = 0.891$) compared to variety ($\eta^2 = 0.841$). A similar but more extreme trend was observed for Se, where the application rate contribution ($\eta^2 = 0.950$) far exceeded that of genotype ($\eta^2 = 0.867$).

Table 1. Two-way ANOVA of the effects of wheat variety, foliar spray application rate, and their interaction on grain Fe, Zn, and Se concentrations.

Source of variation	Element	DF	Mean square	F-value	P-value	Partial η^2
Variety (V)	Fe	7	966.99	120.259	0.000	0.963
	Zn	7	212.982	24.134	0.000	0.841
	Se	7	0.03	29.739	0.000	0.867
Application rate (R)	Fe	3	383.857	47.738	0.000	0.817
	Zn	3	766.921	86.903	0.000	0.891
	Se	3	0.203	204.35	0.000	0.95
V $\times$ R	Fe	21	118.601	14.75	0.000	0.906
	Zn	21	26.289	2.979	0.003	0.662
	Se	21	0.012	12.14	0.000	0.888
Error	Fe	32	8.041	-	-	-
	Zn	32	8.825	-	-	-
	Se	32	0.001	-	-	-

*Note: DF: Degree of freedom.

3.2. Genotype-specific biofortification efficiency under foliar micro-fertilizer

To further elucidate the differential responses of wheat varieties to the UAV-based micronutrient cocktail, the concentration profiles of grain Fe, Zn, and Se were analyzed. Results indicated that foliar treatments induced distinct genotypic sensitivities among the tested varieties (Figure 1).

3.2.1. Iron (Fe) enrichment patterns

The efficacy of Fe biofortification was highly variety-dependent. For the majority of the varieties (i.e., LD1, JZ439, BN207, ZM895, XM26, and BNAK58) grain Fe concentration significantly increased under at least one application rate (Figure 1(A)). Among these responsive genotypes, ZM895 and LD1 achieved peak Fe concentrations of 76.59 mg/kg (under T2) and 65.35 mg/kg (under T3), respectively, representing substantial improvements over their respective controls. Notably, XM26 responded most efficiently to the lowest application rate (T1), where it reached a peak value of 68.71 mg/kg. In stark contrast, ZM379 and BN4199 exhibited remarkable stability in grain Fe concentration, with no significant variation observed across all treatment levels ($P > 0.05$). This lack of response highlights a potential physiological bottleneck in certain genotypes concerning Fe uptake or translocation following foliar application.

3.2.2. Zinc (Zn) enrichment patterns

Unlike Fe, grain Zn concentration showed a more universal upward trend across the varieties, although the magnitude of this increase varied (Figure 1(B)). For most varieties, grain Zn concentration followed a linear enrichment pattern, increasing proportionally with the spraying concentration and reaching significant elevations under the T3 treatment (with the exception of BN207). Interestingly, BN207 and ZM379 displayed a “peak-and-decline” pattern, in which Zn concentration increased at lower application rates but stabilized or slightly decreased as the rate reached T3. Similarly, for BNAK58, significant Zn enhancement was exclusively achieved under the T2 application rate. These diverse response profiles suggest a variety-specific threshold for Zn absorption and storage in wheat grains.

3.2.3. Selenium (Se) enrichment patterns

Grain Se concentration was the most sensitive to the foliar treatment among the three target elements. All tested wheat varieties achieved significant Se enrichment under the T3 treatment (Figure 1(C)). The most dramatic response was observed in ZM379, which reached a concentration of 0.54 mg/kg under T3, an 8.65-fold increase compared to its control. Regarding genotypic variation, while BN207 and BNAK58 reached a concentration plateau at higher application rates, the other six varieties demonstrated a consistent, dose-dependent increase in Se. The robust response of Se across all genotypes, including those that were less responsive to Fe (e.g., BN4199), further reinforces the ANOVA finding that application rate, rather than genotype, is the primary driver of grain Se enrichment.

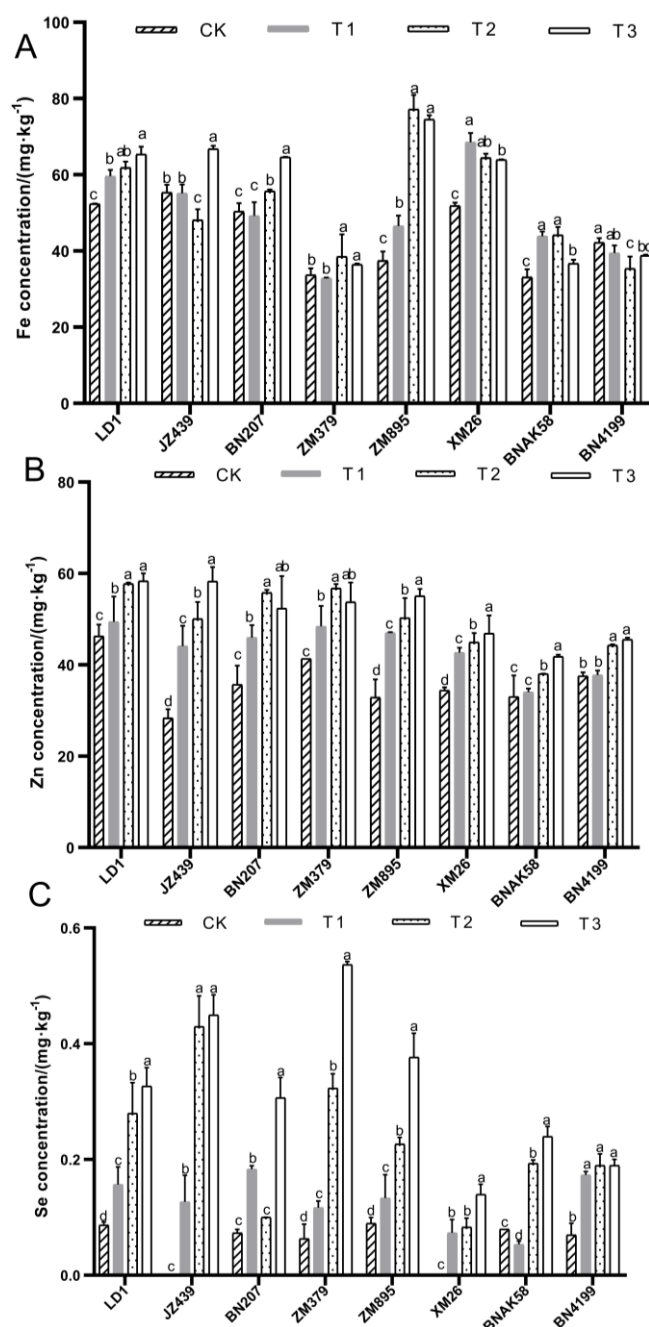


Figure 1. Concentrations of target micronutrients in wheat grains under varying application rates of the foliar cocktail. (A) Iron (Fe) concentration; (B) Zinc (Zn) concentration; and (C) Selenium (Se) concentration across different wheat varieties.

3.2.4. Absolute gain and relative enrichment efficiency (REE) analysis

To quantify biofortification potential while avoiding mathematical distortions from near-zero baselines, both absolute gain (mg/kg) and REE were evaluated (Table 2). For Fe, enrichment varied widely. ZM895 showed the highest absolute gain ($38.31 \text{ mg}/\text{kg}$; 101.5% REE at T2), followed by XM26 ($16.44 \text{ mg}/\text{kg}$; 31.7% REE at T1), while most varieties exhibited modest 10%–25% REE increases. Notably, despite exhibiting mathematically negative gains in specific cases (e.g., BN4199 across treatments and JZ439 at T1/T2), statistical analysis revealed no significant difference from the

control ($P > 0.05$). Rather than indicating an inhibitory effect, this suggests the foliar treatments did not alter the inherent Fe homeostasis in these genotypes.

Table 2. Absolute gain and REE of grain Fe, Zn, and Se in eight wheat varieties under different spraying treatments compared to the control.

Variety	Element	T1 (%)		T2 (%)		T3 (%)	
		Absolute gain (mg/kg)	REE (%)	Absolute gain (mg/kg)	REE (%)	Absolute gain (mg/kg)	REE (%)
LD1	Fe	7.29	13.9	9.53	18.2	13.02	24.9
	Zn	3.29	7.2	11.22	24.5	12	26.1
	Se	0.07	82.4	0.19	229.4	0.24	282.4
JZ439	Fe	-0.19	-0.4	-6.48	-11.9	12.23	22.3
	Zn	16.02	51.6	21.96	79.2	30.12	108.6
	Se	0.14	—	0.43	—	0.44	—
BN207	Fe	-1.05	-2.3	4.77	10.1	13.57	26.8
	Zn	10.01	27.6	19.94	55	15.6	51
	Se	0.11	152.3	0.03	36.4	0.23	318.2
ZM379	Fe	-0.1	0.1	5.5	20.1	3.46	10.5
	Zn	7.04	17	15.36	37	12.26	29.7
	Se	0.06	88.5	0.26	—	0.47	—
ZM895	Fe	8.26	16	38.31	101.5	36.46	93.4
	Zn	13.8	41.9	17.4	52.5	22.16	67.5
	Se	0.06	62.2	0.14	151.7	0.29	323
XM26	Fe	16.44	31.7	12.3	24	11.92	23.2
	Zn	8.35	23.3	11.18	33.7	12.58	36.5
	Se	0.08	—	0.08	—	0.14	—
BNAK58	Fe	10.65	33.3	10.81	33.8	3.92	13
	Zn	0.78	1	4.59	13.7	8.41	24.7
	Se	-0.03	-37.5	0.11	141.7	0.15	191.7
BN4199	Fe	-2.76	-7.5	-6.77	-15.4	-3.62	-8.5
	Zn	0.23	1.1	6.75	17.8	8	21.4
	Se	0.11	150	0.11	171.4	0.12	171.4

*Note: Absolute gain was calculated as the difference between the specific treatment group and the control (CK) average—indicates that REE (%) was intentionally excluded to prevent mathematical distortion due to non-detectable or near-zero baseline values in the CK group, as relying on absolute gain provides a more biologically accurate assessment for these extreme responses.

Conversely, Zn enrichment was robust and consistent. JZ439 exhibited the strongest response, gaining 16.02 mg/kg (51.6% REE) at T1 and peaking at 30.12 mg/kg (108.6% REE) at T3. BN207 and ZM895 also achieved substantial Zn enrichments (19.94 and 17.40 mg/kg at T2, respectively) with >50% REE, reflecting a relatively linear response to the foliar cocktail.

Regarding Se, absolute gains revealed a distinct “hyper-response” pattern. ZM379 and JZ439 displayed extreme sensitivity, netting 0.47 and 0.44 mg/kg respectively under T3. Varieties with quantifiable baseline Se similarly showed profound gains, notably LD1 (0.24 mg/kg gain; 282.4%

REE at T3). Overall, robust Se enrichment occurred consistently across most varieties at higher application rates. This underscores that UAV-based foliar application is an exceptionally efficient, genotype-independent pathway for Se biofortification, whereas Fe and Zn enhancements face stronger genotypic “bottlenecks”.

3.2.5. Correlation analysis of grain Fe, Zn, and Se absolute gain

To further investigate the internal relationships between the concentration of different microelements following foliar application, and to avoid statistical artifacts caused by extreme baselines in relative percentage calculations, a Pearson correlation analysis was performed on the absolute gain (mg/kg) of Zn, Se, and Fe in wheat grains ($n = 24$, Figure 2). The results revealed a moderate-to-strong positive correlation between the absolute concentration of Zn and Se ($r = 0.64$, $P < 0.01$), suggesting a significant synergistic effect in the transport and accumulation of Zinc and Selenium under UAV-based foliar spraying. In contrast, the absolute gain of Fe showed weaker correlations with the other two elements, with correlation coefficients of $r = 0.32$ for Zn and a mere $r = 0.04$ for Se. These rigorous statistical findings based on absolute mass gain indicate that the enrichment mechanism of Fe in grains is relatively independent and is less influenced by the synchronized application rate variations of the Zn and Se treatments.

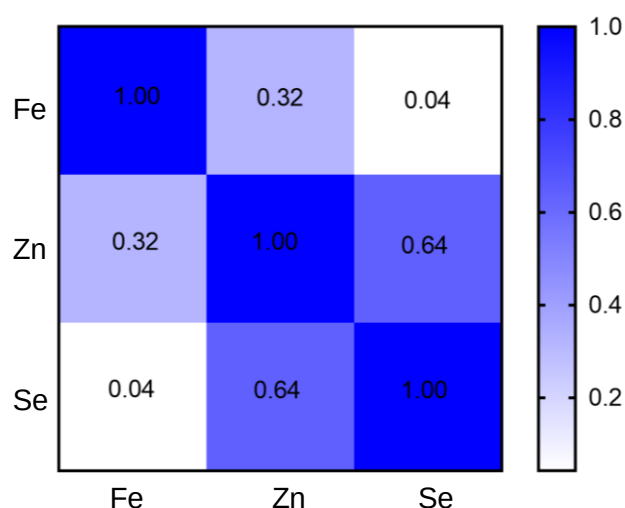


Figure 2. Heatmap of Pearson correlation analysis for the absolute gain of grain Zn, Se, and Fe following foliar application. The color intensity in the heatmap represents the magnitude of the Pearson correlation coefficient (r), as indicated by the color scale on the right. Darker blue shades with values closer to 1.0 denote a stronger positive correlation. The dark blue squares on the diagonal represent the self-correlation of each parameter ($r = 1.0$). Zn, Se, and Fe denote the absolute gain (mg/kg) of the respective elements.

3.3. Impact of micronutrient cocktail on mineral homeostasis

To ensure that the biofortification of Fe, Zn, and Se does not disrupt the nutritional balance of wheat grains, the concentrations of five other essential minerals (i.e., copper [Cu], manganese [Mn], calcium [Ca], magnesium [Mg], and phosphorus [P]) were monitored (Table 3). The results indicated that the UAV-mediated micronutrient cocktail effectively enriched the target elements while

maintaining or even improving the homeostasis of other mineral nutrients. A notable synergistic effect was observed particularly for Cu and Ca concentration. For instance, in LD1, BN207, and BNAK58, grain Cu concentration increased significantly ($P < 0.05$). However, this pattern was not universal; in genotypes such as JZ439, Cu concentrations fluctuated or even slightly decreased under certain treatments (e.g., decreasing from 5.82 mg/kg in CK to 5.16 mg/kg at T2), indicating an un-patterned, genotype-specific response to the foliar cocktail.

Table 3. Effects of varying spraying treatments on the concentrations of numerous mineral elements in the grains of different wheat varieties.

Sample name		Mineral element concentration (mg/kg)				
		Cu	Mn	Ca	Mg	P
LD1	CK	3.78 ± 0.13 ^c	23.34 ± 2.07 ^b	534.28 ± 9.23 ^b	1082.65 ± 9.91 ^{ab}	5271.77 ± 143.40 ^a
	T1	6.80 ± 0.12 ^a	37.74 ± 2.05 ^a	642.11 ± 24.54 ^a	1024.51 ± 82.16 ^b	4468.20 ± 87.95 ^b
	T2	6.37 ± 0.15 ^b	42.21 ± 2.78 ^a	627.45 ± 7.65 ^a	1238.12 ± 108.05 ^a	4899.67 ± 59.32 ^{ab}
	T3	6.29 ± 0.15 ^b	39.23 ± 1.69 ^a	730.29 ± 56.50 ^a	1312.23 ± 132.90 ^a	5452.93 ± 240.32 ^a
JZ439	CK	5.82 ± 0.01 ^b	31.59 ± 0.80 ^a	341.52 ± 8.44 ^b	1006.34 ± 3.65 ^b	4560.82 ± 6.27 ^a
	T1	6.54 ± 0.13 ^a	31.54 ± 0.00 ^a	418.83 ± 9.11 ^a	1122.81 ± 29.08 ^{ab}	3844.34 ± 127.70 ^b
	T2	5.16 ± 0.33 ^c	24.82 ± 3.86 ^b	431.53 ± 16.63 ^a	1127.23 ± 36.00 ^{ab}	3789.97 ± 110.15 ^b
	T3	5.66 ± 0.20 ^{bc}	28.79 ± 0.72 ^{ab}	445.55 ± 9.60 ^a	1163.22 ± 83.18 ^a	4323.06 ± 472.26 ^a
BN207	CK	2.77 ± 0.05 ^c	20.07 ± 3.48 ^a	415.02 ± 2.87 ^b	1080.63 ± 44.89 ^a	4487.85 ± 11.23 ^a
	T1	5.76 ± 0.04 ^b	18.16 ± 2.87 ^a	449.07 ± 8.68 ^b	1036.24 ± 74.22 ^a	4405.19 ± 472.10 ^a
	T2	5.85 ± 0.03 ^b	21.09 ± 4.55 ^a	447.64 ± 13.51 ^b	1098.38 ± 51.44 ^a	4413.73 ± 83.89 ^a
	T3	6.21 ± 0.06 ^a	21.80 ± 3.16 ^a	558.25 ± 12.42 ^a	1110.37 ± 92.34 ^a	4236.72 ± 59.40 ^a
ZM379	CK	3.78 ± 0.14 ^b	22.65 ± 1.47 ^a	385.12 ± 13.37 ^b	965.60 ± 101.35 ^a	3499.46 ± 181.24 ^b
	T1	4.50 ± 0.02 ^a	23.80 ± 2.54 ^a	373.21 ± 8.78 ^b	988.19 ± 33.42 ^a	3669.22 ± 239.80 ^{ab}
	T2	4.34 ± 0.13 ^a	20.29 ± 0.10 ^a	406.82 ± 45.51 ^{ab}	1008.34 ± 62.96 ^a	3475.22 ± 202.05 ^b
	T3	4.84 ± 0.35 ^a	17.55 ± 2.31 ^b	437.81 ± 7.60 ^a	1031.00 ± 54.04 ^a	4259.40 ± 199.34 ^a
ZM895	CK	3.70 ± 0.10 ^b	25.64 ± 2.16 ^a	332.00 ± 21.00 ^b	970.48 ± 5.10 ^b	4370.93 ± 343.10 ^a
	T1	3.96 ± 0.18 ^{ab}	19.37 ± 1.96 ^b	397.16 ± 19.83 ^a	929.91 ± 11.08 ^b	3872.91 ± 47.93 ^a
	T2	3.88 ± 0.03 ^{ab}	18.46 ± 2.73 ^b	407.72 ± 30.22 ^a	864.67 ± 106.38 ^c	3931.77 ± 421.13 ^a
	T3	4.21 ± 0.17 ^a	18.32 ± 2.03 ^b	445.58 ± 52.88 ^a	1073.56 ± 127.12 ^a	3904.06 ± 143.51 ^a
XM26	CK	3.46 ± 0.05 ^b	21.72 ± 0.03 ^a	403.24 ± 31.23 ^a	1276.20 ± 232.0 ^a	3954.39 ± 121.24 ^b
	T1	4.58 ± 0.04 ^a	17.57 ± 1.71 ^a	440.57 ± 28.14 ^a	1359.93 ± 152.3 ^a	4668.22 ± 121.58 ^a
	T2	3.56 ± 0.25 ^b	19.39 ± 1.73 ^a	373.68 ± 51.71 ^a	1303.22 ± 167.0 ^a	4416.08 ± 209.43 ^{ab}
	T3	3.91 ± 0.05 ^b	19.59 ± 1.53 ^a	411.20 ± 18.06 ^a	1336.83 ± 186.2 ^a	4778.22 ± 78.02 ^a
BNAK58	CK	1.67 ± 0.11 ^b	19.10 ± 0.89 ^a	274.38 ± 32.53 ^b	1086.81 ± 112.45 ^a	4735.57 ± 405.47 ^a
	T1	2.36 ± 0.08 ^a	18.01 ± 0.13 ^a	516.64 ± 40.63 ^a	1045.65 ± 94.40 ^a	4291.93 ± 14.31 ^a
	T2	2.53 ± 0.05 ^a	18.73 ± 0.12 ^a	537.39 ± 81.37 ^a	1149.35 ± 35.40 ^a	4329.87 ± 52.51 ^a
	T3	2.45 ± 0.10 ^a	18.11 ± 0.21 ^a	453.91 ± 76.76 ^a	1116.26 ± 30.22 ^a	4544.46 ± 255.85 ^a
BN4199	CK	2.49 ± 0.07 ^b	19.07 ± 0.41 ^a	446.30 ± 4.21 ^c	995.14 ± 91.01 ^a	3645.68 ± 222.75 ^b
	T1	2.85 ± 0.14 ^a	18.19 ± 0.11 ^b	630.33 ± 43.61 ^a	1022.10 ± 91.33 ^a	4104.83 ± 483.10 ^{ab}
	T2	2.84 ± 0.06 ^a	19.42 ± 0.12 ^a	500.76 ± 18.57 ^b	966.41 ± 92.42 ^a	3775.25 ± 260.16 ^b
	T3	2.76 ± 0.20 ^a	17.50 ± 0.07 ^c	551.74 ± 11.85 ^b	925.54 ± 34.79 ^a	4196.30 ± 92.40 ^a

In contrast, the macro-minerals P and Mg remained remarkably stable across most genotypes and treatments, with no significant adverse changes observed. This stability serves as a key indicator that the spray application rates used in this study (up to 5%) did not interfere with primary mineral metabolism. Nevertheless, for manganese (Mn), a distinct bidirectional trend was observed. For instance, in BN4199, Mn concentration significantly decreased under foliar treatments, whereas in LD1, it showed a significant increase. Similar variations in Mn concentration among distinct genotypes following exogenous micronutrient applications have been previously documented [14,17].

From a dietary perspective, it is crucial to note that despite these genotype-specific fluctuations or localized competitive inhibitions, the absolute trace element concentrations of Cu (3–6 mg/kg) and Mn (17–42 mg/kg) remained well within nutritionally adequate and safe limits across all treatments. This confirms that the targeted hyper-concentration of Fe, Zn, and Se was successfully achieved without compromising the fundamental nutritional integrity or inducing secondary trace element deficiencies in the wheat grain.

4. Discussion

Foliar spraying of micro-fertilizer represents an advance in agrotechnology with improved efficacy, which also enables more accurate investigations on the uptake efficiency of micro-nutrients in crops. In this study we investigated the genotypic variation in the uptakes of Fe, Zn, and Se in several cereal grain crops, including JZ439, LD1, and a number of representative winter wheat varieties, in response to foliar application of UAV spraying of the cocktail mix of these micro-nutrients in variable application rates. We have demonstrated that substantial increase in the concentrations of Fe, Zn, and Se in the grains harvested from the cereal plants that were subjected to UAV foliar application of the micro-nutrients in a genotypic- and fertilizer application rate-dependent manner. These macro-level findings indicate that genotypic selection is the most critical prerequisite for Fe biofortification. Exogenous supply levels (spraying application rates) are the decisive drivers for Zn and Se enrichment. The significant variety $\times$ application rate interactions across all three elements imply that different wheat genotypes possess distinct sensitivity and efficiency in responding to UAV-mediated micronutrient delivery, necessitating a variety-specific approach for optimal biofortification. We have also observed that the concentrations of some other mineral elements, such as Cu and Ca, were also significantly elevated as the result of foliar application of Fe, Zn, and Se, which is intriguing and warrants further investigation in future studies.

4.1. Foliar spraying of multi-nutrient cocktail as an effective strategy for biofortification

Soil fertilization or manual foliar spraying of single micronutrient has been commonly used to improve the concentration of crop trace elements [18–20]. The application of UAV technology has hitherto focused on spraying pesticides and herbicides [21,22], and there are few reports if any on the use of UAV precision fertilization for nutrient enhancement to reduce the stress of soil metal pollution on plants. In this study, the operational feasibility of UAV foliar spraying of cocktail solutions that entail variable application rates of Fe, Zn, and Se was evaluated to achieve micro-nutrient fortification. Although this study did not include a manual application control (e.g., backpack or tractor spraying) to directly compare deposition or absorption efficiency, our results demonstrate that UAV-based application represents a viable and operationally feasible approach for large-scale micronutrient

biofortification in modern agricultural production. It is conceivable that the spraying efficiency is affected by flight speed, flight height, spraying application rate, and spray volume. In the present study we have found that the effectiveness of the Fe, Zn, and Se fortification through UAV foliar spraying varies between genotypes, and there is an interaction between genotype and mineral concentration, reinforcing the importance of testing the effectiveness of biofortification by foliar application in a species or genotype dependent manner, rather than assuming they would all behave the same.

4.2. Effects of spraying mixed micro-fertilizer on other mineral elements

With the increase in spraying application rate, Fe, Zn, and Se enrichment or concentration in cereal grains were increased significantly, but the magnitude of increase varied considerably. The response of some other mineral elements, such as Cu, Mn, Ca, Mg, and P, to the spraying of Fe, Zn, and Se varied greatly, without a consistent pattern of enhancement in response to the increase in the application rate of the micronutrient cocktail. It is not known how the foliar application of Fe, Zn, and Se mixed micro-fertilizer affected the concentration of other mineral elements in cereal grains. Nevertheless, the results of this study manifested the phenotypic flexibility and a complex relationship between individual micro-elements in their metabolism and concentration in cereal grains, and the lack of a consistent effect on the concentration of many other mineral elements in cereal grains as the result of increase in Fe, Zn, and Se reflects the co-existence of both ion antagonistic and synergistic effects [23,24]. From a physiological perspective, divalent cations, such as Fe^{2+} , Zn^{2+} , Cu^{2+} , and Mn^{2+} , share similar chemical properties and frequently compete for the same non-selective cation channels and specialized membrane transporters during foliar absorption and subsequent phloem loading. Notably, members of the ZIP (ZRT-, IRT-like protein) family are well-documented to possess broad substrate specificity for multiple transition metals. Consequently, an influx of exogenous Fe and Zn from the foliar spray can competitively bind to these ZIP proteins and other transporters, potentially inhibiting the uptake and phloem translocation of endogenous specific trace elements like Mn or Cu, which explains the significant decrease in Mn concentration observed in specific sensitive genotypes like BN4199 in our results [25,26]. This intensified competition at the transporter level, combined with the complementary roles of other transport proteins [27], and the physiological characteristics of cereal species per se. As there are only few similar research results in this area, the effect of applying exogenous Fe, Zn, and Se on the uptake and concentration of multiple mineral elements in the grains and their underlying mechanisms warrant further investigation.

4.3. The effects of genotype and the application rate of micro-nutrients can be optimized for maximizing fortification

Given the prominent genotypic effects on the mineral concentration in cereal grains as revealed in this study, cereal types and wheat genotypes suitable for nutrient fortification need to be taken into consideration in order to achieve the goal of maximizing the efficiency of micro-nutrients biofortification. There are two aspects that need to be considered in selecting a suitable genotype, which include the background concentration of mineral elements that have already present in the grains, and the amplitude of elevation of mineral elements following foliar spraying. As an essential element, Fe fortification has become a hotspot of research in recent years [25]. In the present study, genotype appeared to be the biggest influencing factor for the grain concentration of the fortified micronutrient.

Except for ZM379 and BN4199, all the cereals assessed in this study showed significantly improved Fe concentration in the grains of the plants that were sprayed with a cocktail of Fe, Zn, and Se at various application rates on the leaves by using UAV.

Studies have shown that Zn fertilizer application in soil and on leaf surface can both effectively increase the concentration of Zn in wheat grains and achieve the target value of biofortification [28–30]. In this study, the concentration of Zn in wheat grains was increased in proportion with the increase in spraying application rate, except for BN207 and ZM379, and the spraying application rate exerted the greatest effect on Zn concentration in wheat grains. Medium application rate T2 and high application rate T3 treatments achieved substantial and consistent absolute gains in grain Zn concentration (e.g., up to 30.12 mg/kg in JZ439), successfully realizing the targeted nutritional enhancement. It is therefore that Zn concentration in the micronutrient fertilizer should be the primary consideration for Zn biofortification in cereals.

Previous studies have shown that Se concentration in wheat grains is positively correlated with application rate of Se fertilizer [31,32]. In the present study, Se concentration in winter wheat grains was elevated along with the increase in fertilizer application rate, except for BN207 and BNAK58. The Se concentration in wheat grains was most affected by the treatment application rate. Both medium (T2) and high (T3) application rate treatments consistently prompted robust absolute gains in Se concentration across most tested varieties, displaying a distinct hyper-response pattern (netting up to 0.47 mg/kg in ZM379 at T3), thereby effectively maximizing Se biofortification. Therefore, to a large extent, the application rate of leaf micronutrient fertilizer determines the effectiveness of Se biofortification in cereal grains.

Overall, our findings in this study demonstrate that foliar spraying by using UAVs is a viable approach for micronutrient fortification. Genotype, fertilizer application rate, and their interactions determine its effectiveness as measured by the micronutrient concentration in the grains.

4.4. Comprehensive evaluation and varieties recommendation

To evaluate the overall biofortification efficiency and provide practical recommendations for UAV-based agricultural practices, the nutritional fortification standards were defined as grain Fe > 55 mg/kg, Zn > 45 mg/kg, and Se > 0.1 mg/kg. These thresholds were rationally established in accordance with the agricultural industry standard of the People's Republic of China for biofortified edible agricultural products (NY/T 4174-2022) [33]. Based on these thresholds, a hierarchical cluster analysis was performed on all treatment groups (Figure 3), categorizing the outcomes into three distinct groups.

The first group, designated as category I (high-efficiency enrichment), consists of treatments where all three elements (Fe, Zn, and Se) successfully reached the fortification standards. This category primarily includes highly responsive varieties, specifically LD1, ZM895, and JZ439 (under T3 treatment). These samples achieved the most significant synergistic enrichment, making them the most suitable candidates for producing high-density biofortified flour. Category II represents a moderate enrichment level, characterized by treatments where only Fe and Zn met the fortification standards, while the response in Se concentration was relatively insufficient. This group includes treatments, such as BN207 T2, XM26 T2, and XM26 T3. Finally, category III comprises treatments where only Zn and Se met the standards, whereas Fe concentration remained substantially below the target. Even at specific spray application rates, varieties in this cluster (e.g., JZ439 T2 and ZM379 T3) failed to show a balanced improvement, confirming the existence of physiological thresholds for certain elements in

specific genotypes.

Based on this comprehensive evaluation and the strict fortification criteria, this study identifies LD1, ZM895, and JZ439 as the most amenable varieties for simultaneous multi-micronutrient fortification via UAV spraying. To maximize nutritional value, the T3 application rate (5%) cocktail is recommended as the optimal treatment for these specific varieties.

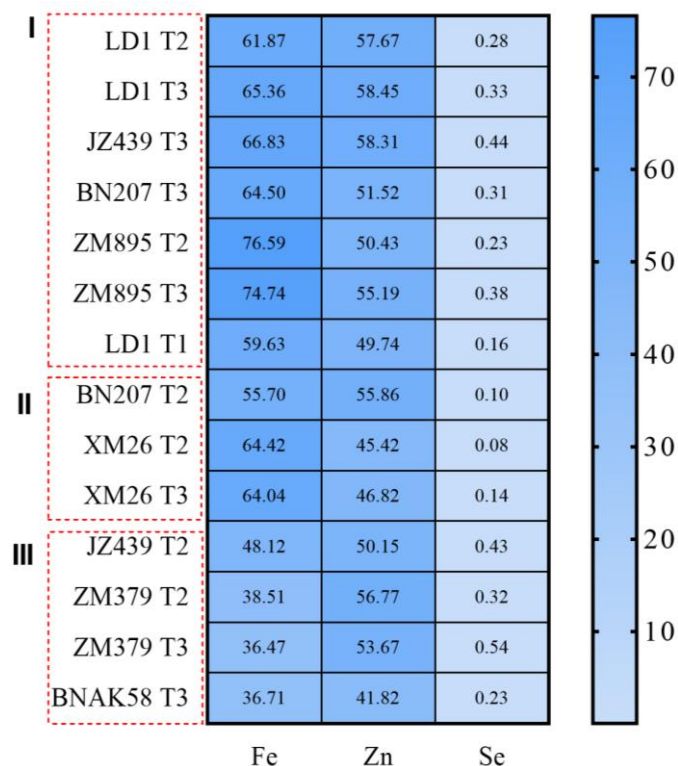


Figure 3. Cluster analysis of trace elements concentration in wheat grains.

4.5. Study limitations

While our findings demonstrate the viability of UAV-based micronutrient fortification, it is important to formally acknowledge that the current results derive from a single edaphoclimatic environment and a single growing season. Inter-annual climate variations, particularly fluctuations in relative humidity and ambient temperature, can significantly affect leaf cuticle permeability, droplet drying time, and ultimately the foliar absorption efficiency of the micro-fertilizers. Given these environmental dynamics, the single-environment design represents a limitation of the present study. Consequently, although the T3 application rate cocktail exhibited optimal enrichment in our trial, such specific dose recommendations require rigorous multi-seasonal and multi-location validation before broad, large-scale commercial application. Furthermore, as pointed out from an agricultural engineering perspective, this study primarily focused on agronomic and physiological responses (e.g., final elemental concentration in grains). Critical spray quality parameters specific to UAV applications, such as droplet deposition, leaf retention rate, and vertical distribution uniformity within the canopy, were not empirically measured. Therefore, future interdisciplinary studies combining agronomic biofortification with precise agricultural engineering evaluations, as well as multi-year climate field tests, are necessary to fully elucidate the specific advantages and stability of UAV delivery systems.

across diverse agro-ecological zones.

5. Conclusions

In conclusion, this study confirms the underlying hypothesis that UAV-based foliar application of a compound Fe/Zn/Se fertilizer is a feasible approach for improving the mineral nutritional quality of wheat grains, and that this efficacy is highly genotype-dependent. Based strictly on the tested experimental data, grain Fe, Zn, and Se concentrations were significantly governed by the interaction between wheat genotype and fertilizer application rate. Reaffirming the determining role of the genotype, it played a predominant role in grain Fe concentration, whereas Zn and Se enrichment were more strongly driven by the application rate. Under the specific conditions tested in this single-season study, the T3 application rate generally showed the strongest effect on target micronutrient concentration. Consequently, LD1, ZM895, and JZ439 were identified as the most promising cultivars for simultaneous Fe, Zn, and Se enrichment via UAV-assisted biofortification under these tested conditions. In addition, the foliar application increased Cu and Ca concentrations while maintaining relatively stable levels of Mn, Mg, and P. These findings provide targeted agronomic evidence for precision-oriented biofortification. Future multi-environment and multi-seasonal trials are essentially required to evaluate the broad environmental stability and economic feasibility of these applications before large-scale commercial implementations.

Author Contributions

Min Zhang planned and designed the research; Chao Jin, Fangfang Liu and Yining Wang performed the research and analyzed data; Chengwei Li conceived and supervised research; Chao Jin and Min Zhang wrote the paper. Zifu Ni revised the paper. All authors have read and approved the final version of the manuscript for publication.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare that there is no interest conflict.

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