Article

Not peer-reviewed version

Appropriate Soil Fertilization or Drone-based Foliar Zn Spraying Can Simultaneously Improve Yield and Micronutrient (Particularly for Zn) Nutritional Quality of Wheat Grains

Xue Gao , Qiang Zhao , Nuo Yuan , Xiaojing Li , Bin Zhang , Yinghua Zhu , Lingan Kong , Zhaohui Wang * , Haiyong Xia *

Posted Date: 12 August 2024

doi: 10.20944/preprints202408.0768.v1

Keywords: zinc; micronutrient; macronutrient; agronomic biofortification; compound fertilizer; drone technology; foliar spraying

Article

Appropriate Soil Fertilization or Drone-based Foliar Zn Spraying Can Simultaneously Improve Yield and Micronutrient (Particularly for Zn) Nutritional Quality of Wheat Grains

Xue Gao [1], Qiang Zhao [2,3], Nuo Yuan [3,4], Xiaojing Li [3], Bin Zhang [3,5], Yinghua Zhu [2], Lingan Kong [3,5], Zhaohui Wang [6,7,*] and Haiyong Xia [2,3,4,5,6,*]

[1] Institute of Agricultural Resources and Environment, State Key Laboratory of Barley and Yak Germplasm Resources and Genetic Improvement, Xizang Academy of Agricultural and Animal Husbandry Sciences, Lhasa 850002, China
[2] School of Agronomy, Anhui Agricultural University, Hefei 230036, China
[3] Crop Research Institute, National Engineering Research Center of Wheat and Maize/Shandong Technology Innovation Center of Wheat, Shandong Academy of Agricultural Sciences, Jinan 250100, China
[4] School of Agriculture, Ludong University, Yantai 264025, China
[5] State Key Laboratory of Nutrient Use and Management, Shandong Academy of Agricultural Sciences, Jinan 250100, China
[6] State Key Laboratory for Crop Stress Resistance and High-Efficiency Production, Northwest A&F University, Yangling 712100, China
[7] College of Natural Resources and Environment, Northwest A&F University, Yangling 712100, China
* Correspondence: haiyongxia@cau.edu.cn; w-zhaohui@263.net (Z.W.)

Abstract: To better understand effects of agronomic practices on yield-nutrition relationships in wheat (*Triticum aestivum* L.) grains for Zn biofortification while improving yields simultaneously, effects of different soil fertilization and different drone-based foliar spraying treatments were investigated. The mixture of Zn or increasing N but simultaneously reducing P in compound fertilizers were all practical to increase grain yields and Zn concentrations in calcareous soils tested. However, the overall effects are limited, with a maximal yield increase by only 7.0% and a maximal increase of the grain Zn density from 19.4 to 27.0 mg/kg, which is far below the target biofortification value of 40-50 mg/kg. Unfortunately, there was a negative side effect, which decreased Fe and Mn concentrations and the Fe bioavailability. Notably, grain yields and Zn concentrations were significantly increased by drone-based foliar Zn sprayings from 7.5 to 8.6~8.8 t/ha by 12.0-17.3% and from 33.5 to 41.9~43.6 mg/kg by 25.1~30.1%, respectively, indicating a double-win in yield and nutrition. A spraying of $ZnSO_4{\cdot}7H_2O$ increased grain Zn concentrations and accumulation more so than ZnO, indicating the importance of chemical Zn forms in determining the effectiveness of foliar spraying. Moreover, foliar Zn sprayings simultaneously increased grain concentrations and accumulation of Fe, Mn and Cu, demonstrating multiple benefits. There were positive correlations between Zn and Fe, Mn or Cu, indicating synergistic but not antagonistic interactions. Compared to micronutrients, concentrations of grain macro- and medium nutrients (N, P, K, Ca, Mg) were less affected. Thus, a double-win in grain yields and micronutrient (particulaly for Zn) nutrition could be effectively achieved through appropriate soil fertilization and foliar Zn spraying. These results provide a better understanding of the yield-nutrition relationship among wheat grain yields, Zn and other nutrient elements for a better integrated manipulation to achieve a win-win situation in yield and nutrition.

Keywords: zinc; micronutrient; macronutrient; agronomic biofortification; compound fertilizer; drone technology; foliar spraying

1. Introduction

Wheat (*Triticum aestivum* L.), as one of the world's important food crops, accounts for about one third of human daily food demand [1–3]. Facing the challenge of continuous population growth, undoubtedly, much more wheat production is required to satisfy the increasing demand [4,5]. To date, the Guinness World Record for the highest wheat grain yield is 17.95 t/ha [6]. However, large yield gaps, i.e., the difference between the practical farmers' yields achieved in large areas and the potential highest yields that can be achieved by using the best soil-crop management practices with the best adapted cultivar, exist in in many regions of the world [7]. For example, the average wheat grain yield of China in 2023 is only 5.83 t/ha [8]. Zinc (Zn) is an essential micronutrient for wheat growth and human health [9]. However, wheat grains generally contain low Zn, which can't meet the daily human nutrition requirement [10]. Chen et al. [11] investigated wheat grain Zn concentrations in seven major wheat production provinces of China and found an average of only 23.3 mg/kg, which is far lower than the recommended biofortification target value of 40-50 mg/kg [12]. Therefore, while wheat grain yield needs to increase, it must simultaneously increase its Zn concentration to achieve both food and nutrition security.

Unfortunately, negative yield-nutrition trade-offs in wheat grains were frequently reported. High-yielding wheat cultivars generally had low gluten/protein concentrations [13], and wheat lines with low phytate, an antinutritional compound that reduces grain Zn bioavailability, were always along with low grain yields [14]. With the variety release years increasing from 1838 to 2012 in the UK, concentrations of Zn, iron (Fe) and protein in wheat grains were all unintentionally decreased, indicating modern wheat varieties with higher grain yields than old ones have lower nutritional quality in minerals [15,16]. Thus, the "Green Revolution" was actually constrained by the yield-nutrition "dilution effect" [17–19], and achieving high yield and high nutritional quality simultaneously was an unavoidable dilemma and challenge for wheat breeders [20,21]. Actually, the "dilution effect" was reported to be mainly due to wheat breeding but seldom due to agronomic management practices, which were rarely considered in the studies above-mentioned [22]. Whether agronomic practices, e.g., fertilization, can effectively overcome the yield-nutrition dilution effect or not is an emerging important question worthy to be answered [23,24].

Increasing Zn supply via soil fertilization and/or foliar spraying could effectively correct or prevent the symptom occurrence of Zn deficiency, ensure sufficient Zn uptake by wheat, and improve grain yields and Zn concentrations, particularly in calcareous soils with high pH, and low soil organic matter and moisture [10]. Notably, many previous studies have shown that foliar Zn spraying is much more effective and much more economically efficient (low dose) than soil Zn application in increasing Zn concentration and bioavailability in the wheat grain/flour [23]. In the HarvestZinc international study involving 7 countries (China, India, Kazakhstan, Mexico, Pakistan, Turkey, and Zambia), 23 experimental sites, 10 different wheat varieties, and 3 years, the grain Zn concentration was increased by an average of 83.5% achieved by foliar Zn spraying, and by only 12.0% by soil Zn application [25]. Numerous studies have shown that increasing nitrogen (N) supply moderately (not excessively) increases wheat grain yields as well as improves grain Zn and N concentrations, i.e., the "N-Zn synergism", but the input of phosphorus (P) fertilizer generally reduces crop grain Zn concentrations, being termed the "P-Zn" antagonism [12,23,26].

Therefore, the optimized N and P fertilizers in combination with appropriate soil and foliar Zn applications may act as a double-win strategy to simultaneously achieve high wheat grain yields and high grain Zn concentrations/bioavailability [23]. However, most previous researches have focused on the dose effects of a single element (N, P, or Zn), but less on their chemical forms and the integration or interactions among these elements, and most studies on foliar Zn spraying were conducted using small watering cans or knapsack sprayers with high labor costs and low efficiency, resulting in a clear separation between the experimental work and the farmers' practices [12]. There is a lack of research on compound, slow/controlled release, and organic/microbial fertilizers, and water and fertilizer integration technology adopted by farmers, and a lack of research on the development of new types of high-efficient foliar Zn fertilizers and foliar spraying techniques using modern agricultural drones.

In addition to Zn, other micronutrient elements, such as Fe, manganese (Mn) and copper (Cu), and macronutrients including N, P, potassium (K), calcium (Ca) and magnesium (Mg), are also essential nutrient elements determining wheat grain yields and nutritional quality and human dietary health [22,27–30]. Most studies on wheat grain Zn biofortification focus on only a few of these

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 August 2024 doi:10.20944/preprints202408.0768.v1

elements (no more than 3 in most situations), but less on the whole suite of these mineral nutrients in wheat grains. Consequently, there is a lack of systematic understanding on the effects of different crop management practices on changes of these micro- and macro- nutrients in wheat grains, their cross-talks among each other and relationships with grain yields.

In this study, we changed the component ratio of N, P_2O_5, K_2O and micronutrient in compound fertilizers through enlarging N or micronutrient and shrinking P in the soil fertilization experiment, sprayed foliar solutions with different chemical forms of Zn or without Zn using an agricultural drone, and investigated their effects on (1) wheat grain yields, yield components and other agronomic traits including the plant height (PH), spike length (SL), spike number (SN) per 666.7 m^2, kernel number per spike (KNPS), thousand kernel weight (TKW), grain yield (Y), and harvest index (HI); (2) grain micronutrient accumulation including Zn, Fe, Mn and Cu; (3) changes of grain macronutrients (N, P, K, Ca, Mg); and (4) relationships among the agronomic and nutritional traits across different soil fertilization or foliar spraying treatments. The bioavailability of Zn and Fe, estimated by molar ratios of phytic acid (PA)/Zn, PA/Fe, PA × Ca/Zn and PA × Ca/Fe in wheat grains in the soil fertilization experiment was also investigated. These findings would provide a better understanding of the yield-nutrition relationship among agronomic practices, wheat yields and grain nutritional quality to achieve food security in quantity and quality, and biofortification of wheat grains with micronutrient (especially for Zn) to alleviate malnutrition.

2. Materials and Methods

2.1. Study Site

Field experiments were conducted at two sites/years during the winter wheat growing season from October to June in a winter wheat-summer maize rotation system. The soil fertilization treatments were conducted at Jiyang Experimental Station, Shandong Academy of Agricultual Sciences, Jinan, China, during 2020-2021. The foliar spraying treatments were conducted at Liuyuan planting base, Maifeng Wheat Planting Professional Cooperative in Dongming County, Heze, China, during 2022-2023. The two sites are located in west of Shandong Province of China, the area has a typical continental and warm-temperate monsoon climate, with cold and dry spring and winter, and a hot and rainy summer. The annual mean temperature is 12.0-14.0 °C and the annual frost-free period is 195-220 days. The annual precipitation is 500-700 mm, with 70% rainfall occurring during June-September. Detailed site information including geographic coordinates and soil basal properties in 0-20 cm soil layers of the two experimental sites before wheat sowing are presented in Table 1.

Table 1. Detailed site information including geographic coordinates and soil basal properties in 0-20 cm soil layers before wheat sowing.

Experimental year	Site	Geographic coordinates	Soil type	pH (2.5:1 Water : soil ratio)	Organic matter (g/kg)	Total nitrogen (g/kg)	Olsen-P (mg/kg)	Exchangeable K (mg/kg)	DTPA-extractable Zn (mg/kg)
2020-2021	Jiyang	116°58'E, 36°58'N	Calcareous alluvial soil	8.1	12.7	0.94	23.1	103.5	1.7
2022-2023	Liuyuan	115°07'E, 35°02'N	Calcareous alluvial soil	8.3	13.5	0.82	15.3	98.7	1.2

2.2. Experimental Design and Crop Management

For the soil fertilization experiment, the single-factor randomized block design was applied with 5 treatments and 4 replicates. Corresponding to 5 treatments, five different compound fertilizers, (NPK 15-15-15 as a control, 15-15-15+Micronutrient, 17-17-17, 26-10-15 and 30-10-11, Stanley Agricultural Group Co., Ltd., Linshu, China), were evenly distributed and incorporated into the upper 20 cm of the soil prior to wheat planting, respectively, with a quantity of 750 kg·ha^{-1}. The planting area of each treatment was 400 m^2 (10 m × 40 m).

For the foliar spraying experiment, the single-factor randomized block design was applied with 3 treatments (Table 2) and 3 replicates. These three treatments included: (1) spraying of deionized water as a control (CK); (2) spraying of a mixed solution with deionized water and ZnO (2.0%, *w*/*v*); and (3) spraying of a mixed solution with deionized water and $ZnSO_4 \cdot 7H_2O$ (2.0%, *w*/*v*), labeled Zn. All solutions contained 0.01% (*v*/*v*) TWEEN 20 as a surfactant and each time a dosage of 10 L/ha was sprayed to the area of 660 m^2 (44 m × 15 m) for each treatment using an agricultural drone (DJI AGRAS T40, DJI Agriculture, Shenzhen, China). All foliar spraying treatments were conducted three times. The spraying occurred for the first time 5 days after wheat flowering on May 8, and was repeated on May 17 and May 23, respectively, at 6-9 day intervals (Table 2).

Table 2. Treatments of drone-based foliar spraying.

Treatment	Number of sprays/ spraying date	Deionized water (L/ha)	$ZnSO_4 \cdot 7H_2O$ (kg/ha)	ZnO (kg/ha)
CK [1]		150 L/ha	0	0
ZnO	First/2023-05-08	150 L/ha	0	3 kg/ha
Zn		150 L/ha	3 kg/ha	0
CK		150 L/ha	0	0
ZnO	Second/2023-05-17	150 L/ha	0	3 kg/ha
Zn		150 L/ha	3 kg/ha	0
CK		150 L/ha	0	0
ZnO	Third/2023-05-23	150 L/ha	0	3 kg/ha
Zn		150 L/ha	3 kg/ha	0

[1] CK: spraying of deionized water; ZnO: spraying of a mixed solution with deionized water and ZnO; Zn: spraying of a mixed solution with deionized water and $ZnSO_4 \cdot 7H_2O$.

In addition, for each treatment of the two experiments above-mentioned, a 112.5 kg of N ha^{-1} (supplied as urea) was top-dressed with irrigation/rainfall during the regreening-jointing stage. Winter wheat (*Triticum aestivum* L.) variety "Jimai 22" was sown around 20 October in autumn and harvested around 10 June in the following year. All plots were adequately irrigated and no obvious biotic (weeds, pests, disease, etc.) and abiotic (drought, cold damage, etc.) stress was observed during the wheat growing season. To control aphids, omethoate (2-dimethoxyphosphinoylthio-N-methylacetamide) was sprayed at the booting stage, and no fungicide was applied.

2.3. Plant Sampling and Nutrient Analysis

At maturity, a 1-m^2 area of spikes in each plot of the two experiments was manually harvested to determine the wheat grain yield and yield components (SN: spike number, KNPS: kernel number per spike, and TKW: thousand kernel weight). In addition, 10 random plants of each plot were used to determine the average above-ground plant height (PH) and spike length (SL).

After determination of the grain yield and yield components, grain samples were rapidly washed with deionized water, oven-dried at 65 °C for 72 h until constant weight, and then ground with a stainless steel grinder. Sub-samples after ground were digested with HNO_3-H_2O_2 in a microwave accelerated reaction system (CEM Corp., Matthews, North Carolina, USA). The concentrations of Zn, Fe, Mn, Cu, P, K, Ca and Mg in the digested solutions were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES, Avio™ 200, PerkinElmer, Waltham, Massachusetts, USA). A certified reference grain material (IPE556, Wageningen University) and two blanks were included in each digestion batch to ensure analytical quality. The grain N concentration was determined by the H_2SO_4-H_2O_2 digestion-micro-Kjeldahl method. Phytate-P concentration was determined by the method of Haug and Lantzsch [31], and converted to PA by dividing by 0.282. The molar ratios of PA/Zn, PA × Ca/Zn, PA/Fe and PA × Ca/Fe were calculated to predict the bioavailability of Zn and Fe in wheat grains.

2.4. Statistical Analysis

Data from the single-factor randomized block design experiments were subjected to one-way analysis of variance (ANOVA) using The SAS System for Windows V8 (SAS Institute Inc., Cary, North Carolina, USA) and the mean value was compared according to Fisher's protected least significant difference (LSD, $p \leq 0.05$). Pearson's correlation analysis and principal component analysis (PCA) were performed by OriginPro 2021 (OriginLab Corp., Northampton, Massachusetts, USA).

3. Results

3.1. Grain Yields, Yield Components and Other Agronomic Traits

Soil fertilization had non-significant impacts on plant height, spike number and thousand kernel weight (Table 3). Among different soil fertilization treatments, the spike lengths varied from 6.5 to 7.3 cm, and were in the order of "30-10-11" > "26-10-15" > "17-17-17" > "15-15-15+Micronutrient" > "NPK (15-15-15)". The treatment of 15-15-15+Micronutrient had the lowest kernel number per spike, which was significantly lower than 17-17-17, which had the maximum value. The grain yield was significantly increased from 7.1 t/ha in the control treatment of 15-15-15 to 7.4~7.6 t/ha in other soil fertilization treatments, with increases of 4.2~7.0%. Compared to the control of 15-15-15, only the treatment of 26-10-15 decreased the harvest index significantly (Table 3).

Table 3. Grain yields, yield components and other agronomic traits of wheat as affected by different soil fertilization treatments and different drone-based foliar spraying treatments.

Experiment	Treatment	Plant height (cm)	Spike length (cm)	Spike number (10000/666.7 m²)	KNPS	TKW (g)	Grain yield (t/ha)	HI (%)
Soil Fertilization	N-P_2O_5-K_2O (15-15-15)	72.4a [1]	6.5c	34.4a	34.5ab	48.2a	7.1b	63.2a
	15-15-15+Micronutrient	74.5a	6.7bc	36.5a	32.3b	48.2a	7.5a	61.1ab
	17-17-17	72.3a	6.9abc	39.2a	36.5a	47.3a	7.6a	62.0ab
	26-10-15	75.5a	7.1ab	35.7a	33.4ab	48.2a	7.5a	59.8b
	30-10-11	75.6a	7.3a	36.8a	36.2ab	46.7a	7.4a	61.5ab
Foliar Spraying	CK	77.7a	8.3a	39.8a	36.8a	43.9b	7.5b	59.4a
	ZnO	84.5a	8.4a	41.4a	37.5a	45.7a	8.6a	61.5a
	Zn	78.7a	8.1a	41.8a	39.9a	46.2a	8.8a	61.8a

[1] Values are means of four replicates for the soil fertilization experiment, in which the nutrient component ratio of the compound fertilizer varies for different treatments, and values are means of three replicates for the foliar spraying experiment. CK: spraying of deionized water; ZnO: spraying of a mixture of deionized water and ZnO; Zn: spraying of a mixture of deionized water and $ZnSO_4·7H_2O$. KNPS: kernel number per spike; TKW: thousand kernel weight; HI: harvest index. Values in each experiment followed by different lowercase letters in the same column are significantly different among treatments at $p \leq 0.05$.

Among all agronomic traits, significant effects of foliar spraying treatments were observed on the thousand kernel weight and grain yield. Foliar spraying of Zn-containing fertilizers significantly

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 August 2024 doi:10.20944/preprints202408.0768.v1

increased the thousand kernel weight from 43.9 g in the control to 45.7~46.2 g by 4.1~5.2%, and significantly increased the grain yield from 7.5 to 8.6-8.8 t/ha by 14.7~17.3% (Table 3).

3.2. Grain Nutrient Concentrations and Acquisition

For the soil fertilization experiment, the grain Zn concentration increased from 19,9 mg/kg in the control treatment of 15-15-15 to 23.5 mg/kg by 18.1% in 15-15-15+Micronutrient, to 22.5 mg/kg by 13.1% in 26-10-15, and to 27.0 mg/kg by 35.7% in 30-10-11 (Table 4). There were significant differences between treatments of 15-15-15 or 17-17-17 and 30-10-11. Similar trends occurred in grain Zn acquisition, and grain Cu concentrations and acquisition. Compared to the control, the grain Fe concentrations were significantly reduced by treatments of 26-10-15 and 30-10-11, and the grain Mn concentration was significantly reduced by 26-10-15. However, no significant differences in grain Fe and Mn acquisition were found between the control and other treatments (Table 4).

Table 4. Grain nutrient concentration and acquisition of wheat as affected by different soil fertilization treatments.

Parameter	Treatment	Zn	Fe	Mn	Cu	N	P	K	Ca	Mg
Concentration		mg/kg				g/kg				
	$N-P_2O_5-K_2O$ (15-15-15)	19.9b [1]	26.4a	26.5a	2.4ab	19.2a	3.1a	4.5a	0.33a	1.39ab
	15-15-15+ Micronutrient	23.5ab	26.0ab	25.2ab	2.6ab	18.6a	3.0a	4.7a	0.34a	1.41ab
	17-17-17	19.4b	25.0ab	26.1ab	2.3b	17.0a	3.0a	4.6a	0.34a	1.37b
	26-10-15	22.5ab	22.2c	23.9b	2.4ab	18.6a	3.0a	4.6a	0.32a	1.38b
	30-10-11	27.0a	24.2b	25.7ab	2.8a	19.9a	3.2a	4.8a	0.33a	1.45a
Acquisition		g/ha				kg/ha				
	$N-P_2O_5-K_2O$ (15-15-15)	141.2b	187.7ab	188.1a	17.4b	136.9a	12.6b	32.2a	2.3a	9.9a
	15-15-15+ Micronutrient	176.2ab	195.0a	189.0a	19.3ab	139.7a	14.6ab	35.0a	2.5a	10.6a
	17-17-17	144.5b	189.1ab	196.6a	17.4b	128.1a	14.1ab	34.5a	2.6a	10.3a
	26-10-15	168.9ab	167.6b	180.1a	17.8ab	140.4a	15.2a	34.3a	2.4a	10.4a
	30-10-11	197.6a	178.3ab	188.5a	20.8a	145.9a	14.7ab	35.2a	2.5a	10.7a

[1] Values are means of four replicates. Values followed by different lowercase letters in the same column are significantly different among treatments at $p \leq 0.05$.

Compared to grain micronutrients, the concentrations and acquisition of grain macronutrients (N, P, K) and medium nutrients (Ca, Mg) were less affected by different compound fertilizers. Significant differences were observed between the treatments of 17-17-17 or 26-10-15 and 30-10-11 only in grain Mg concentrations, and between 15-15-15 and 26-10-15 only in grain P acquisition (Table 4).

For the foliar spraying experiment, concentrations and acquisition of grain Zn, Fe, Mn, and Cu in treatments of foliar spraying with Zn (ZnO or $ZnSO_4 \cdot 7H_2O$) were all significantly higher than those in the control treatment (Table 5). The grain Zn, Fe, Mn, and Cu concentrations were significantly increased from 33.5 mg/kg to 41.9~43.6 mg/kg by 25.1~30.1%, from 25.9 mg/kg to 28.3~29.0 mg/kg by 9.3~12.0%, from 13.3 mg/kg to 14.4~15.9 mg/kg by 8.3~19.5%, and from 4.5 mg/kg to 4.8~5.4 mg/kg by 6.7~20.0%, respectively, with highest values observed in the treatment of $ZnSO_4·7H_2O$ (except for Mn). Moreover, there were significant differences in grain Zn, Mn and Cu concentrations between the foliar spraying treatments of ZnO and $ZnSO_4·7H_2O$. Correspondingly, the grain Zn, Fe, Mn, and Cu acquisition were significantly increased by 42.9~51.9%, by 24.7~30.7%, by 26.8~36.7%, and by 22.6~40.4%, respectively, with maximum values noticed in the treatment of $ZnSO_4·7H_2O$ (except for Mn). Significant differences in grain Fe, Mn and Cu acquisition were found between the foliar spraying treatments of ZnO and $ZnSO_4·7H_2O$ (Table 5).

Table 5. Grain nutrient concentration and acquisition of wheat as affected by different drone-based foliar spraying treatments.

Parameter	Treatment	Zn	Fe	Mn	Cu	P	K	Ca	Mg
Concentration		mg/kg				g/kg			
	CK	33.5c [1]	25.9b	13.3c	4.5c	3.5a	4.3a	0.392ab	1.5a
	ZnO	41.9b	28.3a	15.9a	4.8b	3.6a	4.0a	0.389b	1.5a
	Zn	43.6a	29.0a	14.4b	5.4a	3.7a	4.2a	0.430a	1.5a
Acquisition		g/ha				kg/ha			
	CK	252.5b	195.0c	100.0c	33.7c	26.5b	32.1c	3.0c	11.0b
	ZnO	360.8a	243.2b	136.7a	41.3b	31.0ab	34.7ab	3.3b	12.8a
	Zn	383.5a	254.9a	126.8b	47.3a	32.5a	36.9a	3.8a	13.0a

[1] Values are means of three replicates. CK: spraying of deionized water; ZnO: spraying of a mixture of deionized water and ZnO; Zn: spraying of a mixture of deionized water and $ZnSO_4·7H_2O$. Values followed by different lowercase letters in the same column are significantly different among treatments at $p \leq 0.05$.

Compared with the control, both foliar spraying treatments of ZnO and $ZnSO_4·7H_2O$ did not lead to significant changes in grain P, K, Ca, and Mg concentrations, but were associated with significantly higher grain P, K, Ca and Mg acquisition in most situations, with highest values observed in the treatment of $ZnSO_4·7H_2O$ (Table 5).

3.3. Concentrations of Phytic Acid and Phytate-P, Phytate-P/P Ratios, and Molar Ratios of PA/Zn, PA × Ca/Zn, PA/Fe and PA × Ca/Fe in Wheat Grains

Variations in compound fertilizers had non-significant impacts on concentrations of phytic acid and phytate-P, and ratios of phytate-P/P in wheat grains (Table 6). Molar ratios of PA/Zn and PA × Ca/Zn decreased from 40.2~46.5 in treatments of N-P_2O_5-K_2O (15-15-15) and 17-17-17 to 33.5~38.4 in other treatments, and from 327.1~394.7 to 278.5~307.6, respectively. Both treatments of 15-15-15+Micronutrient and 30-10-11 had significantly lower molar ratios of PA/Zn and PA × Ca/Zn than 17-17-17. In addition, the molar ratio of PA × Ca/Zn in the treatment of 26-10-15 was also significantly lower than that of 17-17-17 (Table 6). Compared with the control, molar ratios of PA/Fe and PA × Ca/Fe were all significantly increased by the treatment of 26-10-15, and the molar ratio of PA/Fe was also significantly increased by 30-10-11. There were no significant differences in molar ratios of PA/Fe and PA × Ca/Fe between treatments of 15-15-15 and 15-15-15+Micronutrient or among treatments of 17-17-17, 26-10-15 and 30-10-11 (Table 6).

Table 6. Concentrations of phytic acid (PA) and phytate-P, ratios of phytate-P/P, and molar ratios of PA/Zn, PA × Ca/Zn, PA/Fe and PA × Ca/Fe in wheat grains as affected by different soil fertilization treatments.

Treatment	PA (g/kg)	Phytate-P (g/kg)	Phytate-P/P	PA/Zn	PA × Ca/Zn	PA/Fe	PA × Ca/Fe
N-P_2O_5-K_2O (15-15-15)	8.1a [1]	2.3a	0.74a	40.2ab	327.1ab	26.0c	212.5b
15-15-15+Micronutrient	8.1a	2.3a	0.75a	34.3b	290.3b	26.6bc	225.9ab
17-17-17	8.9a	2.5a	0.82a	46.5a	394.7a	30.1abc	257.5ab
26-10-15	8.8a	2.5a	0.82a	38.4ab	307.6b	33.4a	268.6a
30-10-11	8.8a	2.5a	0.79a	33.5b	278.5b	31.0ab	259.2ab

[1] Values are means of four replicates. Values followed by different lowercase letters in the same column are significantly different among treatments at $p \leq 0.05$.

3.4. Principle Component Analysis (PCA) of Various Parameters of Wheat as Affected by Soil Fertilization and Foliar Spraying

The principle component analysis revealed the data distribution in the soil fertilization experiment (Figure 1a) and in the foliar spraying experiment (Figure 1b). It showed a better visualization of the relationships and great variation present among all the investigated parameters and among different treatments performed on wheat (Figure 1). Two principal components (PCs) accounted for 46.3% (PC1-24.6%, PC2-21.7%) of the total variance of all data in the soil fertilization experiment (Figure 1a), and accounted for 67.1% (PC1-47.7%, PC2-19.4%) of the total variance of all data in the foliar spraying experiment (Figure 1b). There were clear separations in data distribution areas among five soil fertilization treatments (Figure 1a), and especially among three foliar spraying treatments (Figure 1b).

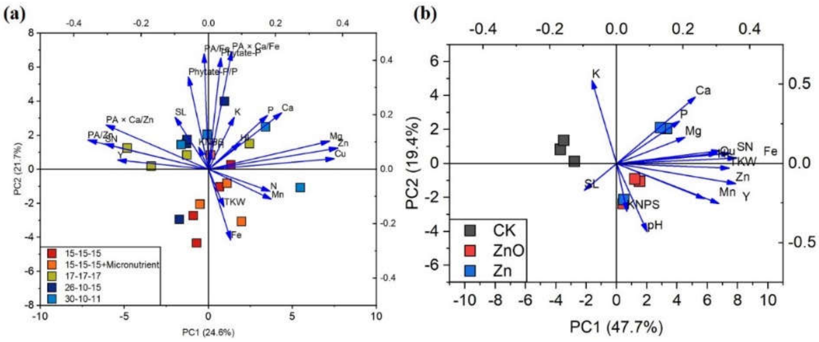

Figure 1. Principle component analysis (PCA) of the effects of different soil fertilization treatments (a) and foliar spraying treatments (b) on various investigated parameters of wheat plants. 15-15-15, 17-17-17, 26-10-15, and 30-10-11 are ratios of N-P_2O_5-K_2O in compound fertilizers (a). In panel (b), CK: spraying of deionized water; ZnO: spraying of a mixed solution with deionized water and ZnO; Zn: spraying of a mixed solution with deionized water and $ZnSO_4 \cdot 7H_2O$. The abbreviations of various parameters investigated are as follows: yield (Y), plant height (PH), spike length (SL), spike number per 666.7 m^2 (SN), kernel number per spike (KNPS), thousand kernel weight (TKW), HI (harvest index), concentrations of Zn, Fe, Mn, Cu, N, P, K, Ca, Mg and phytate-P, ratios of phytate-P/P, and molar ratios of phytic acid (PA)/Zn, PA × Ca/Zn, PA/Fe and PA × Ca/Fe in wheat grains.

3.5. Relationships among Grain Yield Traits and Nutritional Quality-Related Parameters

Across all data in the soil fertilization experiment, SN was positively correlated with grain yield, but negatively correlated with TKW (Figure 2a). There were significantly positive correlations between PH and SL, and between KNPS and HI. Both the grain yield and SN were negatively

 doi:10.20944/preprints202408.0768.v1

correlated with grain Cu concentration. In addition, the grain yield and SN were negatively correlated with grain Mn concentration and grain Zn concentration, respectively. Significantly positive correlations were observed between PH and grain K concentration, between SN and molar ratios of PA/Zn or PA × Ca/Zn in wheat grains, and between HI and grain Ca concentration (Figure 2a).

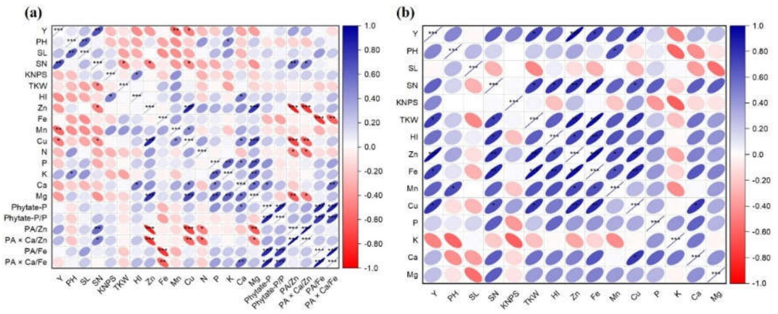

Figure 2. Corrplot representing correlations among measured grain yields, yield components, other agronomic traits, and grain nutritional parameters of wheat crop across different soil fertilization treatments (a) and across different foliar spraying treatments (b). Positive correlations are displayed in blue and negative correlations in red. The color legend on the right-hand side of corrplot shows correlation coefficients and the corresponding colors. The intensity of the color is proportional to the correlation coefficient, and the ellipse size indicates the range of scattered experimental data points. "*", "**" and "***" indicate significant correlations at $p \leq 0.05$, 0.01 and 0.001, respectively. The abbreviations are as follows: yield (Y), plant height (PH), spike length (SL), spike number per 666.7 m^2 (SN), kernel number per spike (KNPS), thousand kernel weight (TKW), harvest index (HI), concentrations of Zn, Fe, Mn, Cu, N, P, K, Ca, Mg and phytate-P, ratios of phytate-P/P, and molar ratios of phytic acid (PA)/Zn, PA × Ca/Zn, PA/Fe and PA × Ca/Fe in wheat grains.

Grain concentrations of Zn, Mn, Ca, and Mg were all positively correlated with Cu, and Zn was positively correlated with Mg. Both Zn and Cu were negatively correlated with molar ratios of PA/Zn and PA × Ca/Zn, and Fe was negatively correlated with molar ratios of PA/Fe and PA × Ca/Fe (Figure 2a).

For N and Mg, they were all negatively correlated with molar ratios of PA/Zn and PA × Ca/Zn. For P, K, Ca and Mg, there were positive correlations among grain concentrations of each other (with an exception of K and Ca). In addition, Ca was positively correlated with phytate-P and the molar ratio of PA × Ca/Fe. There were significantly positive correlations among grain phytate-P concentrations, phytate-P/P ratios, and molar ratios of PA/Fe and PA × Ca/Fe (Figure 2a).

Considering all date in the foliar spraying experiment, all significant correlations observed between two different parameters were positive, there were no significantly negative correlations (Figure 2b). Among parameters of the grain yield, SN, TKW, grain concentrations of Zn, Fe and Cu, any two of them (with an exception of the grain yield and SN) were all positively correlated with each other. The HI was positively correlated with SN and grain concentrations of Zn and Fe. The grain Mn concentration was positively correlated with PH and grain concentrations of Zn and Fe. There was a significantly positive correlation between grain concentrations of Cu and Ca (Figure 2b).

4. Discussion

4.1. Appropriate Soil Fertilization can Simultaneously Increase the Wheat Grain Yield while Improving Grain Zn Nutritional Quality, but the Overall Effect is Limited

Previous studies reported Zn concentrations in wheat grains increased with moderate Zn or N fertilizers [13,32] but decreased with P fertilizer [33–36]. And both the phenomena of "N-Zn synergism" and "P-Zn antagonism" and underlying mechanisms have been well established and also summarized in several latest review papers published [12,23,37,38]. In accordance, our study showed that the mixture of Zn, or increasing N but simultaneously reducing P and K in compound fertilizer were all practical to increase the grain yield of wheat and grain Zn density in the calcareous soil tested (Tables 3-4).

 doi:10.20944/preprints202408.0768.v1

Regarding the addition of Zn into compound fertilizers, previous researches also indicated the biofortification effect of Zn in compound fertilizers was not interfered by other component elements [39]. Liu et al. [40] found that Zn application to soil at 50 kg $ZnSO_4 \cdot 7H_2O$/ha increased the wheat grain yield by 8.8%, which was due to the improvement of thousand kernel weight and tillers number. In the current study, compared to the control, an increase of 5.6% of wheat grain yield was achieved by addition of micronutrient into the compound fertilizer, possibly due to the slightly increase of spike number (Table 3).

However, here the overall effects of Zn addition or changing the composition ratio of N-P_2O_5-K_2O in compound fertilizers are limited, with a maximal increase of the wheat yield by only 7.0%, and a maximal increase of the grain Zn concentration from 19.4 to 27.0 mg/kg, which is far below the target value of 40-50 mg/kg for biofortification. Moreover, there may be a negative side effect, which decreased grain Fe and Mn concentrations, and Fe bioavailability (Table 6). The limited effect of soil fertilization may be attributed to the relatively low soil DTPA-Zn (only 1.7 mg/kg prior to sowing) of the experimental site and insufficient Zn addition into the compound fertilizer. Liu et al. [40] showed that when the DTPA-Zn in the typical calcareous soil of North China Plain exceeded 4.09 mg/kg after Zn fertilizer application, a 45 mg/kg of wheat grain Zn concentration can be achieved to be within the range of the target biofortification value of 40-50 mg/kg.

4.2. Foliar Zn Spraying using Agricultural Drones is a High-efficient Approach to Biofortify Wheat Grains with Zn while Enhancing Grain Yields, thus Showing a Great Potential to be Adopted in Large Areas by Farmers

Most studies showing significant effects of foliar Zn spraying in increasing wheat grain Zn concentrations/bioavailability to the target biofortification value were conducted using small watering cans in pot/plot experiment or using knapsack sprayers in a lager field area [10,13,23,41]. However, primarily due to the low work efficiency and high labor costs, such foliar spraying methods aiming to Zn biofortification are not conductive to be adopted by farmers, thus inevitably hinders the wide promotion of foliar Zn spraying in large areas. Our study innovated a high-efficient way to conduct the foliar Zn spraying on wheat plants, i.e., using an agricultural drone (e.g., DJI AGRAS T40, DJI Agriculture, Shenzhen, China) in combination with the suitable Zn spraying solution concentration, which greatly saves labor, improves the work efficiency and achieves the target biofortification value of grain Zn simultaneously.

Promisingly, the wheat grain yield was increased from 7.5 to 8.6~8.8 t/ha by 12.0-17.3%, meanwhile, the grain Zn concentration was increased from 33.5 to 41.9~43.6 mg/kg by 25.1~30.1%, indicating a double-win in yield and nutrition (Tables 3 and 5). The yield increase caused by foliar Zn spraying has been observed under drought, even in a high soil DTPA-Zn condition [42]. The exogenous foliar Zn supply may supplement the Zn demand of wheat plants due to the drought-induced insufficient Zn uptake from soil and simultaneously reduce the drought-induced oxidative cell damage by improving the antioxidative defense ability [43]. Our study demonstrated that the yield increase was mainly due to the increased thousand kernel weight, and there was a significantly positive correlation between the wheat grain yield and TKW (Table 3; Figure 2b).

In agreement with other studies [41,44–46] and our previous study [13], foliar Zn spraying significantly increased wheat grain Zn concentrations as well as total Zn accumulation (Table 5), indicating the critical role of the pool of available Zn within and on leaves in determining grain Zn deposition, especially when the Zn availability in soils (mostly calcareous or drought conditions, with low organic matter contents and moisture) is restricted during the wheat grain-filling period [47]. The foliar-sprayed Zn could penetrate into leaves, be transported/remobilized from leaves to wheat grains via phloem, where the mobility of Zn is high due to concentrated chelating solutes, such as organic acids, peptides, etc. [48–56].

When using small watering cans in pot/plot experiments or using knapsack sprayers in larger field areas, the suitable concentrations of foliar Zn spraying generally fall in the range of 0.1~0.5% $ZnSO_4 \cdot 7H_2O$ (w/v) in water, with a dose of normally 500~1000 L/ha each time (at least two or three times), and excessive concentrations would cause obvious foliar damage and a yield penalty

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 August 2024 doi:10.20944/preprints202408.0768.v1

[13,41,44,45]. However, in our current study, a drone-based spraying of 3 kg $ZnSO_4 \cdot 7H_2O$/ha, i.e., 2.0% $ZnSO_4 \cdot 7H_2O$ (w/v) in water, which is four times of the recommended maximal concentration of 0.5% as known from the traditional foliar Zn spraying method, with a dose of 150 L/ha each time (three times in total), did not lead to a yield penalty but simultaneously improved wheat grain yields and increased grain Zn concentrations to the target biofortification value of 40~50 mg/kg. In addition to the obvious characteristics of high concentrated Zn spraying solution and low dose of water used, the drone-based spraying is high-efficient and both labor- and time-saving. Here, the work efficiency using the current model of drone (DJI AGRAS T40, DJI Agriculture, Shenzhen, China) can reach 40 ha per day. Therefore, compared with the traditional foliar Zn spraying method, the present drone-based technology showed obvious advantages and progress, updated our understanding on manipulation of foliar Zn spraying, and would undoubtedly have great potential to be adopted in large areas by farmers in the very near future.

4.3. A Double-win in Wheat Grain Yield and Micronutrient Nutrition rather than a "Dilution Effect" on Zn due to Yield Increase could be Effectively Achieved through Appropriate Soil Fertilization and Foliar Zn Spraying

The breeder's dilemma-yield or nutrition arises from the negative relationship between crop yield and its nutritional quality [20,21,57]. A "dilution effect" between wheat grain yields and grain micronutrient concentrations (especially for Zn) has been observed during breeding [15,16], and such correlation could be precisely describe by a negative linear relationship [22]. However, in the present study, appropriate soil fertilization by the addition of Zn or changing the component ratio of N-P_2O_5-K_2O in compound fertilizers, and especially foliar Zn spraying, increased wheat grain yields and simultaneously elevated grain Zn concentrations, achieving a double-win in yield and Zn nutrition.

Moreover, foliar Zn spraying simultaneously increased concentrations of Fe, Mn and Cu in wheat grains (Table 5), demonstrating multiple benefits. There were positive correlations between concentrations of Zn and Fe, Mn or Cu in wheat grains (Figure 2b), indicating synergistic but not antagonistic interactions. Rakshit et al. [58] reported the concentrations of Zn and Fe in wheat grains were both elevated by foliar Fe fertilization. Our previous study on maize also showed that foliar spraying of Zn alone increased grain Zn and Fe concentrations simultaneously, i.e., "killing two birds with one stone" [59]. The underlying mechanisms regulating these positive crosstalks need a further in-depth investigation.

There were significantly positive correlations between wheat grain yields and concentrations of Zn, Fe or Cu (Figure 2b), indicating a double-win in wheat grain yields and micronutrient nutrition. Thus, our study clearly proved that the yield increase and nutrition improvement could be realized simultaneously by appropriate agronomic management practices (e.g., fertilization), suggesting a critical role of agronomic management practices on balancing the seesaw of yield-nutrition. The current study may shed light on closing the divide between more nutritious wheat grains and higher grain yields to maximize the benefits to food security and human health [57].

4.4. Integrated Practical Strategies for Simultaneously Improving Yield and Micronutrient (Particularly for Zn) Nutritional Quality of Wheat Grains

To maximize grain Zn/micronutrient nutritional quality while maintaining high wheat grain yields, a comprehensive approach integrating soil and foliar fertilization is needed [10,22,60]. For example, compared to soil or foliar Zn application alone, a combined application resulted in an increase of 109% and 47% in the grain Zn concentration, respectively [61]. The current results indicated that soil fertilization alone is limited to increase wheat grain yields and especially for grain Zn concentrations (Tables 3-4). For instance, the maximal value of grain Zn concentrations due to soil fertilization was only 27.0 mg/kg, far below the target biofortification value of 40-50 mg/kg. On the other hand, there is a possibility to make grain Zn concentrations increase from the current levels of 41.9~43.6 mg/kg further to approach or exceed 45~50 mg/kg, if incorporating the appropriate soil fertilization (the addition of Zn and/or changing the component ratio of N-P_2O_5-K_2O in compound fertilizers) into the foliar Zn spraying experiment. Here, we strongly suggest the integration of

Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 12 August 2024 doi:10.20944/preprints202408.0768.v1

appropriate soil fertilization (the addition of Zn and/or moderately increasing N but decreasing P in compound fertilizers of N-P_2O_5-K_2O) with foliar Zn spraying in farming management systems to maximize the yield-nutrition benefits (Figure 3).

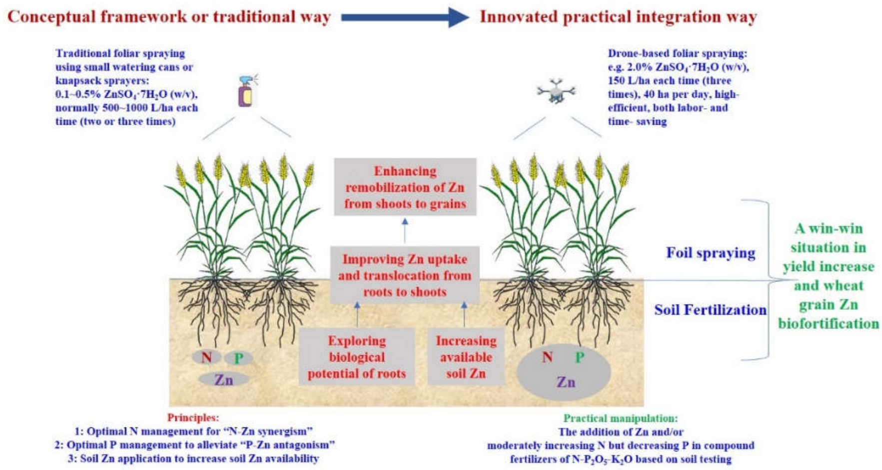

Figure 3. A schematic diagram showing integrative strategies for simultaneously achieving yield increase and wheat grain Zn biofortification.

These combined measures are in accordance with the conceptual framework previously proposed by plant nutritionists for integrative strategies to attain wheat yield goal and to harvest more grain Zn [11,12,23]. In the conceptual framework for wheat grain Zn biofortification, at least three aspects should be considered and gracefully managed: (i) a moderate N supply to soil, to fully explore the potential of “N-Zn synergism”; (2) a slimming P supply to soil, to effectively alleviate the “P-Zn antagonism”; and (3) an adequate Zn supply to soil for essential root uptake during the whole growth period of wheat, and a sufficient physiologically available Zn pool in leaves by foliar Zn spraying during the wheat grain-filling stage after anthesis (Figure 3). As farmers seldom apply Zn or P alone, the development of simple and practical integration technologies, e.g., the addition of Zn and/or tailoring the component ratio of N-P_2O_5-K_2O in compound fertilizers based on soil testing should be easy-done and embraced by farmers, and especially the drone-based technology for foliar spraying with Zn aiming at Zn biofortification would be particularly welcomed due to its high effectiveness in terms of both labor- and time-saving.

Remarkably, our study showed a spraying of $ZnSO_4·7H_2O$ increased the grain Zn concentration and accumulation more so than ZnO (Table 5), indicating the importance of chemical forms of Zn in determining the effectiveness of foliar Zn spraying. The ZnO nanoparticles have been proved to be more effective than $ZnSO_4·7H_2O$ and/or ZnO for Zn biofortification of wheat [46,62–65]. In addition, the spraying timing, frequency, and amounts all influenced the effectiveness of foliar Zn applications [12,65]. However, the previous studies are mostly conducted using the spraying devices of small cans or knapsack sprayers, there is a lack of information on using drones [65].

Our findings underscore the potential of using drones to boost wheat grain yields and Zn enrichment. Foliar spraying of a mixture of Zn and pesticide could reduce the cost without apparent compatibility issues [66–68]. Plant hormones (auxin, cytokinin, abscisic acid, ethylene) may influence grain Zn accumulation of cereal crops [30,69–71]. To fully explore the potential and finally establish the drone-based precision and high-efficient integrated fertilization technology for wheat grain Zn biofortification, the spraying timing, frequency, chemical forms (e.g. nanoparticles), and amounts, in combination with the latest drone product (new model) and other chemical agents (pesticides, plant hormones, etc.), should be optimized and integrated further. Researches have demonstrated that foliar spraying of both $ZnSO_4$ and $FeSO_4$ simultaneously improved grain Zn and Fe concentrations [72], and a cocktail micronutrient solution was effective to biofortify wheat grains simultaneously with Zn, selenium, iodine, and partly with Fe while ensuring the yield productivity [73]. Therefore, it is promising to upgrade the drone-based technology from biofortification of only Zn to co-biofortification of multiple micronutrient elements, achieving a multi-win for a better human health.

5. Conclusions

Our study proved that a double-win in wheat grain yield and micronutrient nutrition rather than a "dilution effect" on Zn due to yield increase could be effectively achieved through appropriate fertilization management practices. Appropriate soil fertilization by moderately enlarging N or micronutrients and shrinking P in compound fertilizers can simultaneously increase the wheat grain yield while improving grain Zn nutritional quality in calcareous soils, but the overall effect is limited. Foliar Zn spraying using agricultural drones is a more high-efficient approach to biofortify wheat grains with Zn while enhancing grain yields than soil fertilization, thus showing a great potential to be adopted in large areas by farmers. Here, we strongly recommend the integration of appropriate soil fertilization (the addition of Zn and/or tailoring the component ratios of N-P_2O_5-K_2O in compound fertilizers based on soil testing) with the drone-based foliar Zn spraying in farming management systems to maximize the yield-nutrition benefits. Our current findings underscore the potential of using drones to boost wheat grain yields and Zn enrichment. In the future, it is promising to upgrade the drone-based technology from biofortification of only Zn to co-biofortification of multiple micronutrient elements, achieving a multi-win for a better human health.

Author Contributions: Conceptualization, X.G., B.Z., Y.Z., Z.W. and H.X; formal analysis, X.G., Q.Z., N.Y. and H.X; investigation, Q.Z., N.Y. and X.L.; writing, X.G., L.K., Z.W. and H.X. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Shandong Provincial Natural Science Foundation (ZR2021MC059), the Open Project Program of State Key Laboratory for Crop Stress Resistance and High-Efficiency Production (SKLCSRHPKF13), the Xizang Key R&D Program (XZ202001ZY0042N), the State Key Laboratory of Nutrient Use and Management, and the National Key R&D Program of China (2023YFD1900600).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The data are contained within the article.

Acknowledgments: Dr. Lei Ma from Institute of Agricultural Resources and Environment, Shandong Academy of Agricultural Sciences, Jinan, China, is gratefully acknowledged for technical assistance in correlation matrix plot and principle component analysis. We also thank the editor and anonymous reviewers for their valuable comments and suggestions.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Cakmak, I. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant Soil* **2008**, *302(1-2)*, 1-17.
2. Poursarebani, N.; Nussbaumer, T.; Šimková, H.; Šafář, J.; Witsenboer, H.; van Oeveren, J.; Doležel, J.; Mayer, K.F.X.; Stein, N.; Schnurbusch, T. Whole-genome profiling and shotgun sequencing delivers an anchored, gene-decorated, physical map assembly of bread wheat chromosome 6A. *Plant J*. **2014**, *79*, 334-347.
3. Erenstein, O.; Jaleta, M.; Abdul Mottaleb, K.; Sonder, K., Donovan, J.; Braun, H.-J. Global trends in wheat production, consumption and trade. In *Wheat Improvement*: *Food Security in a Changing Climate*; Reynolds, M.P., Braun, H.-J., Eds.; Springer International Publishing, 2022; pp. 47-66.
4. Godfray, H.C.J.; Beddington, J.R.; Crute, I.R.; Haddad, L.; Lawrence, D., Muir, J.F.; Pretty, J.; Robinson, S.; Thomas, S.M.; Toulmin, C. Food security: the challenge of feeding 9 billion people. *Science* **2010**, *327*, 812-818.
5. Martre, P.; Dueri, S.; Guarin, J.R.; Ewert, F.; Webber, H.; Calderini, D.; Molero, G.; Reynolds, M.; Miralles, D.; Garcia, G.; Brown, H.; George, M.; Craigie, R.; Cohan, J.-P.; Deswarte, J.-C.; Slafer, G.; Giunta, F.; Cammarano, D.; Ferrise, R., Gaiser, T.; Gao, Y.; Hochman, Z.; Hoogenboom, G.; Hunt, L.A.; Kersebaum, K.C.; Nendel, C.; Padovan, G.; Ruane, A.C.; Srivastava, A.K.; Stella, T.; Supit, I.; Thorburn, P.; Wang, E.; Wolf, J.; Zhao, C.; Zhao, Z.; Asseng, S. Global needs for nitrogen fertilizer to improve wheat yield under climate change. *Nat. Plants* **2024**, *10*, 1081-1090.

6. Guinness World Records. Available online: https://www.guinnessworldrecords.com/world-records/highest-wheat-yield/ (accessed on 31 July 2024).
7. van Ittersum, M.K.; Cassman, K.G.; Grassini, P.; Wolf, J.; Tittonell, P.; Hochman, Z. Yield gap analysis with local to global relevance-a review. *Field Crop. Res.* **2013**, *143*, 4-17.
8. National Bureau of Statistics. Available online: https://www.stats.gov.cn/sj/zxfb/202307/t20230715_1941239.html (accessed on 31 July 2024).
9. Natasha, N.; Shahid, M.; Bibi, I.; Iqbal, J.; Khalid, S.; Murtaza, B.; Bakhat, H.F.; Farooq, A.B.U.; Amjad, M.; Hammad, H.M.; Niazi, N.K.; Arshad, M. Zinc in soil-plant-human system: a data-analysis review. *Sci. Total Environ.* **2022**, *808*, 152024.
10. Cakmak, I.; Kutman, U.B. Agronomic biofortification of cereals with zinc: a review. *Eur. J. Soil Sci.* **2018**, *69(1)*, 172-180.
11. Chen, X.P.; Zhang, Y.Q.; Tong, Y.P.; Xue, Y.F.; Liu, D.Y.; Zhang, W.; Deng, Y.; Meng, Q.F.; Yue, S.C.; Yan, P.; Cui, Z.L.; Shi, X.J.; Guo, S.W.; Sun, Y.X.; Ye, Y.L.; Wang, Z.H.; Jia, L.L.; Ma, W.Q.; He, M.R.; Zhang, X.Y.; Kou, C.L.; Li, Y.T.; Tan, D.S.; Cakmak, I.; Zhang, F.S.; Zou, C.Q. Harvesting more grain zinc of wheat for human health. *Sci. Rep.* **2017**, *7*, 7016.
12. Xia, H.; Wang, L.; Qiao, Y.; Kong, W.; Xue, Y.; Wang, Z.; Kong, L.; Xue, Y.; Sizmur, T. Elucidating the source-sink relationships of zinc biofortification in wheat grains: a review. *Food Energy Secur.* **2020**, *9(4)*, e243.
13. Xia, H.; Xue, Yanf.; Liu, D.; Kong, W.; Xue, Yanh.; Tang, Y.; Li, J.; Li, D.; Mei, P. Rational application of fertilizer nitrogen to soil in combination with foliar Zn spraying improved Zn nutritional quality of wheat grains. *Front. Plant Sci.* **2018**, *9*, 677.
14. Guttieri, M.J.; Peterson, K.M.; Souza, E.J. Agronomic performance of low phytic acid wheat. *Crop Sci.* **2006**, *46(6)*, 2623-2629.
15. Shewry, P.R.; Pellny, T.K.; Lovegrove, A. Is modern wheat bad for health? *Nat. Plants.* **2016**, *2(7)*, 1-3.
16. Shewry, P.R.; Hassall, K.L.; Grausgruber, H.; Andersson, A.A.M.; Lampi, A.-M.; Piironen, V.; Rakszegi, M.; Ward, J.L.; Lovegrove, A. Do modern types of wheat have lower quality for human health? *Nutr. Bull.* **2020**, *45*, 362-373.
17. Garvin, D.F.; Welch, R.M.; Finley, J.W. Historical shifts in the seed mineral micronutrient concentration of US hard red winter wheat germplasm. *J. Sci. Food Agric.* **2006**, *86(13)*, 2213-2220.
18. Zhao, F.J.; Su, Y.H.; Dunham, S.J.; Rakszegi, M.; Bedo, Z.; McGrath, S.P. Variation in mineral micronutrient concentrations in grain of wheat lines of diverse origin. *J. Cereal Sci.* **2009**, *49(2)*, 290-295.
19. Velu, G.; Ortiz-Monasterio, I.; Cakmak, I.; Hao, Y.; Singh, R.P. Biofortification strategies to increase grain zinc and iron concentrations in wheat. *J. Cereal Sci.* **2014**, *59(3)*, 365-372.
20. Morris, C.E.; Sands, D.C. The breeder's dilemma—yield or nutrition? *Nat. Biotechnol.* **2006**, *24*, 1078-1080.
21. Gashu, D.; Nalivata, P.C.; Amede, T.; Ander, E.L.; Bailey, E.H.; Botoman, L.; Chagumaira, C.; Gameda, S.; Haefele, S.M.; Hailu, K.; Joy, E.J.M.; Kalimbira, A.A.; Kumssa, D.B.; Lark, R.M.; Ligowe, I.S.; McGrath, S.P.; Milne, A.E.; Mossa, A.W.; Munthali, M.; Towett, E.K.; Walsh, M.G.; Wilson, L.; Young, S.D.; Broadley, M.R. The nutritional quality of cereals varies geospatially in Ethiopia and Malawi. *Nature* **2021**, *594*, 71–76.
22. Xia, H.Y.; Li, X.J.; Qiao, Y.T.; Xue, Y.H.; Yan, W.; Ma, L.; Zhao, Q.Y.; Kong, L.A.; Xue, Y.F.; Cui, Z.L.; van der Werf, W. Dissecting the relationship between yield and mineral nutriome of wheat grains in double cropping as affected by preceding crops and nitrogen application. *Field Crop. Res.* **2023**, *293*, 108845.
23. Zhang, W.; Xue, Y.-F.; Chen, X.-P.; Zhang, F.-S.; Zou, C.-Q. Zinc nutrition for high productivity and human health in intensive production of wheat. *Adv. Agron.* **2020**, *163*, 179–217.
24. Zhao, Q.; Cao, W.; Chen, X.; Stomph, T.J.; Zou, C. Global analysis of nitrogen fertilization effects on grain zinc and iron of major cereal crops. *Glob. Food Secur.* **2022**, *33*, 100631.
25. Zou, C.Q.; Zhang, Y.Q.; Rashid, A.; Ram, H.; Savasli, E.; Arisoy, R.Z.; Ortiz-Monasterio, I.; Simunji, S.; Wang, Z.H.; Sohu, V.; Hassan, M.; Kaya, Y.; Onder, O.; Lungu, O.; Yaqub Mujahid, M.; Joshi, A.K.; Zelenskiy, Y.; Zhang, F.S.; Cakmak, I. Biofortification of wheat with zinc through zinc fertilization in seven countries. *Plant Soil.* **2012**, *361(1-2)*, 119-130.
26. Yu, B.; Zhou, C.; Wang, Z.; Bucher, M.; Schaaf, G.; Sawers, R.J.H.; Chen, X.; Hochholdinger, F.; Zou, C.; Yu, P. Maize zinc uptake is influenced by arbuscular mycorrhizal symbiosis under various soil phosphorus availabilities. *New Phytol.* **2024**, *243(5)*, 1936-1950.
27. Yan, X.; Wu, P.; Ling, H.; Xu, G.; Xu, F.; Zhang, Q. Plant nutriomics in China: an overview. *Ann. Bot.* **2006**, *98*, 473-482.
28. Marschner, P. *Marschner's Mineral Nutrition of Higher Plants*; Academic Press, Elsevier: San Diego, CA, USA, 2012.
29. Posma, J.M.; Garcia-Perez, I.; Frost, G.; Aljuraiban, G.S.; Chan, Q.; Van Horn, L.; Daviglus, M.; Stamler, J.; Holmes, E.; Elliott, P.; Nicholson, J.K. Nutriome–metabolome relationships provide insights into dietary intake and metabolism. *Nat. Food* **2020**, *1*, 426-436.
30. Wang, L.; Xia, H.; Li, X.; Qiao, Y.; Xue, Yanh.; Jiang, X.; Yan, W.; Liu, Y.; Xue, Yanf.; Kong, L. Source-sink manipulation affects accumulation of zinc and other nutrient elements in wheat grains. *Plants* **2021**, *10*, 1032.

31. Haug, W.; Lantzsch, H.J. Sensitive method for the rapid determination of phytate in cereals and cereal products. *J. Sci. Food Agric.* **1983**, *34(12)*, 1423-1426.
32. Xue, Y.-F.; Yue, S.-C.; Zhang, Y.-Q.; Cui, Z.-L.; Chen, X.-P.; Yang, F.-C.; Cakmak, I.; McGrath, S.P.; Zhang, F.S.; Zou, C.-Q. Grain and shoot zinc accumulation in winter wheat affected by nitrogen management. *Plant Soil* **2012**, *361(1–2)*, 153-163.
33. Zhang, W.; Liu, D.Y.; Li, C.; Cui, Z.L.; Chen, X.P.; Russell, Y.; Zou, C.Q. Zinc accumulation and remobilization in winter wheat as affected by phosphorus application. *Field Crop. Res.* **2015**, *184*, 155-161.
34. Zhang, W.; Liu, D.Y.; Liu, Y.M.; Cui, Z.L.; Chen, X.P.; Zou, C.Q. Zinc uptake and accumulation in winter wheat relative to changes in root morphology and mycorrhizal colonization following varying phosphorus application on calcareous soil. *Field Crop. Res.* **2016**, *197*, 74-82.
35. Zhang, W.; Liu, D.Y.; Liu, Y.M.; Chen, X.P.; Zou, C.Q. Overuse of phosphorus fertilizer reduces the grain and flour protein contents and zinc bioavailability of winter wheat (*Triticum aestivum* L.). *J. Agric. Food Chem.* **2017**, *65*, 1473-1482.
36. Adnan, M. Integrated effect of phosphorous and zinc on wheat quality and soil properties. *Adv. Environ. Biol.* **2016**, *10*, 40-45.
37. Bingham, F.T.; Garber, M.J. Solubility and availability of micronutrients in relation to phosphorus fertilization. *Soil Sci. Soc. Am. J.* **1960**, *24*, 209-213.
38. Zhang, W.; Zhang, W.; Wang, X.; Liu, D.; Zou, C.; Chen, X. Quantitative evaluation of the grain zinc in cereal crops caused by phosphorus fertilization. A meta-analysis. *Agron. Sustain. Dev.* **2021**, *41*, 6.
39. Montalvo, D.; Degryse, F.; da Silva, R.C.; Baird, R.; McLaughlin, M.J. Agronomic effectiveness of zinc sources as micronutrient fertilizer. *Adv. Agron.* **2016**, *139*, 215-267.
40. Liu, D.Y.; Zhang, W.; Pang, L.L.; Zhang, Y.Q.; Wang, X.Z.; Liu, Y.M.; Chen, X.P.; Zhang, F.S.; Zou, C.Q. Effects of zinc application rate and zinc distribution relative to root distribution on grain yield and grain Zn concentration in wheat. *Plant Soil* **2017**, *411*, 167-178.
41. Zhang, Y.Q.; Sun, Y.X.; Ye, Y.L.; Karim, M.R.; Xue, Y.F.; Yan, P.; Meang, Q.F.; Cui, Z.L.; Cakmak, I.; Zhang, F.S. Zinc biofortification of wheat through fertilizer applications in different locations of China. *Field Crop. Res.* **2012**, *125*, 1-7.
42. Karim, M.R.; Zhang, Y.Q.; Zhao, R.R.; Chen, X.P.; Zhang, F.S.; Zou, C.Q. Alleviation of drought stress in winter wheat by late foliar application of zinc, boron, and manganese. *J. Plant Nutr. Soil Sci.* **2012**, *175*, 142-151.
43. Cakmak, I. Role of zinc in protecting plant cells from reactive oxygen species. *New Phytol.* **2000**, *146*, 185-205.
44. Cakmak, I.; Kalayci, M.; Kaya, Y.; Torun, A.A.; Aydin, N.; Wang, Y.; Arisoy, Z.; Erdem, H.; Yazici, A.; Gokmen, O.; Ozturk, L.; Horst, W.J. Biofortification and localization of zinc in wheat grain. *J. Agric. Food Chem.* **2010**, *58*, 9092-9102.
45. Zhang, M. Investigations of the Effects of Zn biofortification in Different Wheat Genotypes. PhD Thesis, Yangzhou University, Yangzhou, China, 2017.
46. Zhang, T.; Sun, H.; Lv, Z.; Cui, L.; Mao, H.; Kopittke, P.M. Using synchrotron-based approaches to examine the foliar application of $ZnSO_4$ and ZnO nanoparticles for field-grown winter wheat. *J. Agric. Food Chem.* **2018**, *66(11)*, 2572-2579.
47. Kutman, U.; Kutman, B.; Ceylan, Y.; Ova, E.; Cakmak, I. Contributions of root uptake and remobilization to grain zinc accumulation in wheat depending on post-anthesis zinc availability and nitrogen nutrition. *Plant Soil* **2012**, *361(1-2)*, 177-187.
48. Haslett, B.S.; Reid, R.J.; Rengel, Z. Zinc mobility in wheat: uptake and distribution of zinc applied to leaves or roots. *Ann. Bot.* **2001**, *87(3)*, 379-386.
49. Uauy, C., Distelfeld, A., Fahima, T., Blechl, A., Dubcovsky, J. A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science* **2006**, *314(5803)*, 1298-1301.
50. Waters, B.M.; Uauy, C.; Dubcovsky, J.; Grusak, M.A. Wheat (*Triticum aestivum*) NAM proteins regulate the translocation of iron, zinc, and nitrogen compounds from vegetative tissues to grain. *J. Exp. Bot.* **2009**, *60(15)*, 4263-4274.
51. Erenoglu, B.; Nikolic, M.; Römheld, V.; Cakmak, I. Uptake and transport of foliar applied zinc (^{65}Zn) in bread and durum wheat cultivars differing in zinc efficiency. *Plant Soil*, **2002**, *241(2)*, 251-257.
52. Erenoglu, E.B.; Kutman, U.B.; Ceylan, Y.; Yildiz, B.; Cakmak, I. Improved nitrogen nutrition enhances root uptake, root-to-shoot translocation and remobilization of zinc (^{65}Zn) in wheat. *New Phytol.* **2011**, *189(2)*, 438-448.
53. Palmer, L.J.; Dias, D.A.; Boughton, B.; Roessner, U.; Graham, R.D.; Stangoulis, J.C.R. Metabolite profiling of wheat (*Triticum aestivum* L.) phloem exudate. *Plant Methods*, **2014**, *10*, 27.
54. Pearce, S., Tabbita, F., Cantu, D., Buffalo, V., Avni, R., Vazquez-Gross, H.; Zhao, R.; Conley, C.J.; Distelfeld, A.; Dubcovksy, J. Regulation of Zn and Fe transporters by the *GPC1* gene during early wheat monocarpic senescence. *BMC Plant Biol.* **2014**, *14*, 368.

 doi:10.20944/preprints202408.0768.v1

55. Gupta, N.; Ram, H.; Kumar, B. Mechanism of zinc absorption in plants: uptake, transport, translocation and accumulation. *Rev. Environ. Sci. Biotechnol.* **2016**, *15*, 89-109.
56. Gupta, O.P.; Pandey, V.; Saini, R.; Narwal, S.; Malik, V.K.; Khandale, T.; Ram, S.; Singh, G.P. Identifying transcripts associated with efficient transport and accumulation of Fe and Zn in hexaploid wheat (*T. aestivum* L.). *J. Biotechnol.* **2020**, *316*, 46-55.
57. Diepenbrock, C.H.; Gore, M.A. Closing the divide between human nutrition and plant breeding. *Crop Sci.* **2015**, *55*, 1437-1448.
58. Rakshit, R.; Patra, A.K.; Purakayastha, T.J.; Singh, R.D.; Pathak, H.; Dhar, S. Super-optimal NPK along with foliar iron application influences bioavailability of iron and zinc of wheat. *Proc. Nat. Acad. Sci., India Sect. B: Biol. Sci.* **2016**, *86*, 159-164.
59. Xia, H.Y.; Kong, W.; Wang, L.; Xue, Y.; Liu, W.; Zhang, C.; Yang, S.; Li, C. Foliar Zn spraying simultaneously improved concentrations and bioavailability of Zn and Fe in maize grains irrespective of foliar sucrose supply. *Agronomy* **2019**, *9(7)*, 386.
60. Stangoulis, J.C.R.; Knez, M. Biofortification of major crop plants with iron and zinc - achievements and future directions. *Plant Soil* **2022**, *474*, 57-76.
61. Cakmak, I., Pfeiffer, W.H., McClafferty, B. Biofortification of durum wheat with zinc and iron. *Cereal Chem.* **2010**, *87*, 10-20.
62. Sun, H.; Du, W.; Peng, Q.; Lv, Z.; Mao, H.; Kopittke, P.M. Development of ZnO nanoparticles as an efficient Zn fertilizer: Using synchrotron-based techniques and laser ablation to examine elemental distribution in wheat grain. *J. Agric. Food Chem.* **2020**, *68(18)*, 5068-5075.
63. Lv, Z.; Zhong, M.; Zhou, Q.; Li, Z.; Sun, H.; Bai, J.; Liu, J.; Mao, H. Nutrient strengthening of winter wheat by foliar ZnO and Fe_3O_4 NPS: Food safety, quality, elemental distribution and effects on soil bacteria. *Sci. Total Environ.* **2023**, *893*, 164866.
64. Mazhar, Z.; Akhtar, J.; Alhodaib, A.; Naz, T.; Zafar, M.I.; Iqbal, M.M.; Fatima, H.; Naz, I. Efficacy of ZnO nanoparticles in Zn fortification and partitioning of wheat and rice grains under salt stress. *Sci. Rep.* **2023**, *13*, 2022.
65. Sánchez-Palacios, J.T.; Henry, D.; Penrose, B.; Bell, R. Formulation of zinc foliar sprays for wheat grain biofortification: a review of current applications and future perspective. *Front. Plant. Sci.* **2023**, *14*, 1247600.
66. Ortiz-Monasterio, I.; Cardenas, M.E.; Cakmak, I. Zinc Biofortification in Wheat through Foliar Fertilization Combined with Pesticides. The 4th International Zinc Symposium: Improving Crop Production and Human Health, Sao Paulo, Brazil, 2015.
67. Wang, X.-Z.; Liu, D.-Y.; Zhang, W.; Wang, C.-J.; Cakmak, I.; Zou, C.-Q. An effective strategy to improve grain zinc concentration of winter wheat, aphids prevention and farmers' income. *Field Crop. Res.* **2015**, *184*, 74-79.
68. Wang, Y.-H.; Zou, C.-Q.; Mirza, Z.; Li, H.; Zhang, Z.-Z.; Li, D.-P.; Xu, C.-L.; Zhou, X.-B.; Shi, X.-J.; Xie, D.-T.; He, X.-H.; Zhang, Y.-Q. Cost of agronomic biofortification of wheat with zinc in China. *Agron. Sustain. Dev.* **2016**, *36(3)*, 44.
69. Gao, S.P.; Xiao, Y.H.; Xu, F.; Gao, X.K.; Cao, S.Y.; Zhang, F.X.; Wang, G.D.; Sanders, D.; Chu, C.C. Cytokinin-dependent regulatory module underlies the maintenance of zinc nutrition in rice. *New Phytol.* **2019**, *224*, 202-215.
70. Chen, L.; Zhao, J.; Song, J.; Jameson, P.E. Cytokinin dehydrogenase: a genetic target for yield improvement in wheat. *Plant Biotechnol. J.* **2020**, *18(3)*, 614-630.
71. Wang, Z.; Wang, Y.; Du, Q.; Yan, P.; Yu, B.; Li, W.-X.; Zou, C.-Q. The auxin signaling pathway contributes to phosphorus-mediated zinc homeostasis in maize. *BMC Plant Biol.* **2023**, *23(1)*, 20-32.
72. Zhang, Y.Q.; Shi, R.L.; Karim, R.M.; Zhang, F.S.; Zou, C.Q. Iron and zinc concentrations in grain and flour of winter wheat as affected by foliar application. *J. Agric. Food Chem.* **2010**, *58*, 12268-12274.
73. Zou, C.Q.; Du, Y.F.; Rashid, A.; Ram, H.; Savasli, E.; Pieterse, P.J.; Ortiz-Monasterio, I.; Yazici, A.; Kaur, C.; Mahmood, K.; Singh, S.; Le Roux, M.R.; Kuang, W.; Onder, O.; Kalayci, M.; Cakmak, I. Simultaneous biofortification of wheat with zinc, iodine, selenium, and iron through foliar treatment of a micronutrient cocktail in six countries. *J. Agric. Food Chem.* **2019**, *67*, 8096-8106.