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Foliar Zn Spraying Simultaneously Improved Concentrations and Bioavailability of Zn and Fe in Maize Grains Irrespective of Foliar Sucrose Supply

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Abstract: Zinc (Zn) deficiency is a global nutritional problem that is reduced through agronomic biofortification. In the current study, the effects of foliar spraying of exogenous $ZnSO_4 \cdot 7H_2O$ (0.2% in Quzhou and 0.3% in Licheng, *w/v*) and/or sucrose (10.0%, *w/v*) on maize (*Zea mays* L.) agronomic traits; concentrations of Zn, iron (Fe), calcium (Ca), total phosphorus (P), phytic acid (PA) P, carbon (C), and nitrogen (N); C/N ratios; and Zn and Fe bioavailability (as evaluated by molar ratios of PA/Zn, PA × Ca/Zn, PA/Fe and PA × Ca/Fe) in maize grains were studied under field conditions for two years at two experimental locations. The results confirmed that there were no significant differences in maize agronomic traits following the various foliar treatments. Compared with the control treatment of foliar spraying with deionized water, foliar applications of Zn alone or combined with sucrose significantly increased maize grain Zn concentrations by 29.2–58.3% in Quzhou (from 18.4–19.9 to 25.2–29.6 mg/kg) and by 39.8–47.8% in Licheng (from 24.9 to 34.8–36.8 mg/kg), as well as its bioavailability. No significant differences were found between the foliar spraying of deionized water and sucrose, and between Zn-only and "sucrose + Zn" at each N application rate and across different N application rates and experimental sites. Similar results were observed for maize grain Fe concentrations and bioavailability, but the Fe concentration increased to a smaller extent than Zn. Foliar Zn spraying alone or with sucrose increased maize grain Fe concentrations by 4.7–28.4% in Quzhou (from 13.4–17.1 to 15.2–18.5 mg/kg) and by 15.4–25.0% in Licheng (from 24.0 to 27.7–30.0 mg/kg). Iron concentrations were significantly and positively correlated with Zn at each N application rate and across different N application rates and experimental locations, indicating that foliar Zn spraying facilitated the transport of endogenous Fe to maize grains. Therefore, foliar Zn spraying increased the Zn concentration and bioavailability in maize grains irrespective of foliar sucrose supply while also improving Fe concentrations and bioavailability to some extent. This is a promising agricultural practice for simultaneous Zn and Fe biofortification in maize grains, i.e., "killing two birds with one stone".

Keywords: micronutrients; biofortification; cereals; carbohydrates; foliar application; remobilization

1. Introduction

Maize (*Zea mays* L.), one of the world's paramount cereal crops, along with wheat and rice. It is very popular due to its diverse functionality as a food source for both humans and animals [1,2]. Zinc is an essential micronutrient for the survival of plants, animals, and humans [3]. However, maize grains do not inherently contain enough Zn to meet daily human requirements, particularly when grown in Zn-deficient soils [4]. Since the 1990s, crop Zn deficiency has been very common worldwide, with 33% of the world's total cultivated area containing Zn-deficient soil [5]. In China, more than 40% (60 million hectares) of the soil is Zn-deficient [6,7]. In recent years, an increased crop yield, imbalanced fertilization, large-scale irrigation practices, and changes in climatic and soil conditions (e.g., increases in atmospheric CO_2 and soil available phosphorus) have exacerbated deficiencies of micronutrients, especially Zn and Fe, in the soil–plant systems in many countries, thereby threatening human nutrition [8–16]. It has been reported that nearly one-third of the world's population does not have sufficient Zn intake [17], and 1.9% of the total global burden of disease is caused by Zn deficiency [18]. Limited food diversity and insufficient dietary Zn intake affects about 100 million people in China, mostly children under five and pregnant women living in rural areas [19]. It is therefore of great interest to improve Zn nutrition in maize grains through integrated soil–crop system management to improve food security and provide human health benefits [20,21].

Zn deficiency leads to white mosaic disease in maize. In previous studies, Zn application to Zn-deficient soils was shown to correct the visible symptoms of this condition, significantly increase the maize grain yield by more than 20% [22], and enhance the maize grain Zn concentration [23] by up to 40% [24]. In addition, foliar Zn spraying has been used to improve the quality of edible crop parts as well as nearly double the Zn concentration [25], with a significant and positive linear correlation found between the Zn concentration in wheat grains and the foliar Zn application rates [26]. Compared with the soil application of Zn fertilizer, foliar Zn spraying was found to more effectively enrich maize grains with Zn, resulting in higher grain Zn recovery, especially in Zn-sufficient soils [27]. Iron is also an important microelement that needs to be biofortified to correct Fe deficiency in plants and meet human nutritional requirements [17,28]. According to a recent study by Niyigaba et al. (2019) [29], separate application of Zn and Fe fertilizers increased their concentration in wheat grains more than when they were applied as a combined foliar spray. Most studies of soil and/or foliar Zn application have focused on Zn concentrations in edible parts; however, comprehensive studies of these strategies on Fe and other nutritional quality-related traits are currently lacking. In particular, scientists are interested in determining whether agronomic biofortification of Zn is benign for Fe in cereals [30].

Both the carbohydrate status within the plant and any exogenous sucrose supply influence the transport of Zn into developing grains. The depletion of carbohydrate reserves within cultured wheat ears (through maintaining them in darkness prior to labeling) was shown to reduce the transport of radioisotope ^{65}Zn into grains, possibly due to a decrease in the mass flow of carbohydrates within the phloem [31]. Because of the limitation of the grain sink capacity, when supplied at high rates, exogenous sucrose may accumulate in the peduncle and chaff, resulting in stomatal closure, the abatement of transportation by the xylem, and finally decreased micronutrient (including Zn) accumulation in wheat grains [32]. Several investigators found that the wheat grain Zn concentrations were significantly reduced by increasing the sucrose supply to detached ears, due to a dilution effect resulting from the increase in grain weights [33,34]. Our latest study showed that a synergistic foliar spray of "Zn + sucrose" was more effective for the biofortification of Zn than Zn alone in wheat grains/ears grown under real field conditions [3,35]. Most of the studies mentioned investigated the effects of the carbohydrate status within the plant or exogenous sucrose supply on Zn accumulation in wheat grains under controlled environments including detached ear cultures and field experiments; however, it is less known whether the exogenous sucrose supply (with/without Zn) affects Zn and even Fe accumulation in maize under various environmental conditions. In particular, field experiments are lacking.

The bioavailability of a given element (e.g., Zn and Fe) is related not only to its total nutrient concentration but also to the concentrations of anti-nutritional compounds such as phytic acid (PA) and phenolic compounds [4,36]. Maize grains are rich in phytic acid, which binds tightly with Zn or Fe to form spherical crystals with an insoluble protein structure [37]. A deficiency of phytase in humans and animals has been shown to reduce the bioavailability of Zn and Fe in the digestive tract [36,38]. The molar ratio of PA to Zn or Fe has been widely used as an indicator of the bioavailability of Zn or Fe in the human diet [39–41]. Under normal circumstances, the critical molar ratio of PA/Zn that causes Zn absorption and utilization inhibition is 15–20; a molar ratio of 5–15 represents about 30–35% Zn availability, and higher than 15 represents about 15% Zn availability [42]. The critical value of the molar ratio of PA/Fe is 10 [43]. Calcium ions (Ca^{2+}) could enhance the binding ability of PA with Zn^{2+} to form a PA–Ca–Zn complex; therefore, the molar ratio of PA × Ca/Zn has also been suggested to predict the bioavailability of Zn, and a molar ratio higher than the critical value of 200 is not conducive to Zn absorption and utilization [44]. Therefore, in the present study, the molar ratios of PA/Zn, PA/Fe, PA × Ca/Zn, and PA × Ca/Fe were used as predictors of the potential bioavailability of Fe and especially Zn in maize grains treated by different foliar sprays under field conditions.

The objectives of this research were (1) to quantify the effects of foliar applications of sucrose only, Zn only, and a mixed solution of both on maize grain yields and other agronomic traits; (2) to quantify their effects on the nutritional qualities of Zn and Fe including their concentrations and bioavailability for humans in maize grains; (3) to quantify their effects on C, N, total and phytate P, and Ca concentrations, and on the ratios of C/N and phytate P/total P in maize grains; and (4) to elucidate relationships between maize grain Zn and Fe concentrations across different foliar treatments and experimental locations. The results from these experiments will be useful for providing guidance on agronomic practices aimed at improving the Zn and Fe nutritional qualities of maize grains in the field, thereby providing health benefits to humans.

2. Materials and Methods

2.1. Study Site

The field experiments were conducted at two experimental sites/years in China, Quzhou in Hebei province (in 2010) and Licheng in Shandong province (in 2016), separated by a distance of around 200 km. The detailed climatic conditions in these two cities during the experiments were reported previously by Zhang et al. (2013) [45] and Xia et al. (2019) [46], respectively. Quzhou and Licheng have a similar and typical warm, sub-humid, continental monsoon climate, with rain and heat coinciding in the same season, leading to dry and cold winters and rainy and hot summers. The annual cumulative temperatures above 10 °C are 4000–5000 °C. The annual frost-free period is 175–220 days. Annual precipitation is 500–700 mm, with about 70% of rainfall occurring during the corn growing season. Detailed site locations (geographic coordinates) and soil basal properties are presented in Table 1.

Table 1. Detailed site locations (geographic coordinates) and soil basal properties in 0–20 cm soil layers before maize sowing.

Year	Experimental Site	Geographic Coordinates	Soil Type	pH (2.5:1 Water:Soil Ratio)	Organic Matter (g/kg)	Total Nitrogen (g/kg)	Alkaline Hydrolysis Nitrogen (mg/kg)	Olsen P (mg/kg)	Exchangeable K (mg/kg)	DTPA-Extractable Zn (mg/kg)
2010	Quzhou	115°0′ E, 36°54′ N	Calcareous alluvial soil	8.3	14.5	0.92	-	14.9	130.5	2.8
2016	Licheng	117°04′ E, 36°42′ N	Calcareous alluvial soil	8.0	15.7	-	90.5	20.2	142.6	1.5

2.2. Experimental Design and Crop Management

For the Quzhou experiment, summer maize (*Zea mays* L., cv. Zhengdan 958) was planted in split-plots (with nitrogen fertilizer in the main plots and foliar spraying treatments in the sub-plots) with three replicates. The main plot treatments included zero N supplement and 105 kg N/ha as urea based on in-season root-zone N management strategies [2,45]. The sub-plot treatments consisted of four foliar treatments (T1–T4), as shown in Table 2. Before tasseling, nine plants were tagged for spraying with each foliar treatment. For the Licheng experiment, summer maize (cv. Zhengdan 958) was grown in fields with uniform fertility and a N application rate of 225 kg/ha as urea. Foliar treatments are shown in Table 2. Each spraying treatment was performed on three replicates, and five plants were tagged before tasseling for each replicate.

Table 2. Foliar spraying treatments.

Treatments		Solution Composition
T1	Control	Deionized water
T2	Sucrose	10.0% (*w*/*v*)
T3	$ZnSO_4 \cdot 7H_2O$ (Zn)	0.2% in Quzhou and 0.3% in Licheng (*w*/*v*)
T4	Zn + Sucrose	A combination of T2 and T3

The foliar treatments were repeated four times at seven- (in Licheng) or 7–10- (in Quzhou) day intervals after tasseling. It was almost at the flowering stage (BBCH 61–65) when the maize plants were sprayed with solutions for the first time and at the dough or physiological maturity stage (BBCH 85–87) the last time. All solutions contained 0.01% (*v*/*v*) TWEEN 20 as a surfactant, and 50 (in Quzhou) or 100 (in Licheng) mL were applied to the maize leaves of each plant each time using a 1.5-L manual pneumatic hand-held sprayer with a rod (DEEPBANG, Guangdong, China).

The two experiments were conducted in a winter wheat–summer maize rotation system. Detailed information about fertilizer applications and crop husbandry was previously described by Zhang et al. (2013) [45] and Xue et al. (2014) [2] for the experiment in Quzhou and by Xia et al. (2019) [46] for the experiment in Licheng. Weeds were well controlled; no obvious water, disease, or pest stress was observed during the maize growing season, and no fungicide or pesticide was used.

2.3. Plant Sampling and Nutrient Analysis

At maturity, all sprayed plants were harvested manually to investigate agronomic traits. After being rapidly washed with deionized water, grain samples were oven-dried at 60–65 °C for 72 h and then ground with a stainless-steel grinder (RT-02B, Rong Tsong, Taizhong, Taiwan). Ground samples were digested with HNO_3–H_2O_2 in a microwave accelerated reaction system (CEM, Matthews, NC, USA). The concentrations of nutrients (Zn, Fe, P, and Ca) in the digested solutions were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES, OPTIMA 3300 DV, PerkinElmer, Waltham, MA, USA). A reference sample IPE556 (Wageningen University, Wageningen, The Netherlands) in Quzhou or IPE568 (Wageningen University, The Netherlands) in Licheng was included in each batch of digestions to ensure analytical quality. Analysis of IPE556 gave values of 46.7 mg Zn/kg and 36.2 mg Fe/kg, which are in good agreement with the certified values of 46–54 mg Zn/kg and 36–46 mg Fe/kg, respectively. Analysis of IPE568 gave values of 58.26 mg Zn/kg, 40.28 mg Fe/kg, 3.23 g P/kg, and 0.464 g Ca/kg, which are also in good agreement with the certified values of 51.34–60.46 mg Zn/kg, 38.05–54.15 mg Fe/kg, 3.09–3.49 g P/kg, and 0.307–0.501 g Ca/kg, respectively. The phytate P concentration was analyzed according to the method of Haug and Lantzsch (1983) [47]. Phytate P was converted to PA by dividing by 0.282. The C and N concentrations and ratios of C/N were determined using Multi N/C 3100 (Analytik Jena AG, Thuringia, Germany). All nutrient concentrations in maize grains were expressed on a dry weight basis.

2.4. Statistical Analysis

Data from the single-factor completely randomized design and split-plot design experiments were subjected to an analysis of variance (ANOVA) using SAS software (SAS 8.0, SAS Institute, Cary, NC, USA), and mean values were compared by Fisher's protected least significant difference (LSD) test at the 5% level. SPSS Statistics 17.0 (SPSS Incorporation, Chicago, IL, USA) software was used for calculating Pearson correlation coefficients.

3. Results

3.1. Maize Agronomic Traits and Grain Zn and Fe Accumulation in Quzhou

Split-plot ANOVAs revealed significant impacts of N application rates on maize yield traits, grain Zn contents, and both Fe grain concentrations and contents, as well as significant effects of foliar treatments on grain Zn concentrations and Zn and Fe contents (Table 3). Irrespective of foliar treatments, N supply increased the maize grain dry weight, the total dry weight including grain and straw, the hundred grain dry weight, the grain Zn content, and both the Fe grain concentration and content compared to when no N fertilizer was applied. Across the two N application rates, compared with foliar spraying of deionized water (T1) and sucrose (T2), foliar Zn spraying alone (T3) or in combination with sucrose (T4) generally resulted in significant increases in grain Zn concentrations from the initial 18.4–19.9 mg/kg to 25.2–29.6 mg/kg by 29.2–58.3%, and in grain Zn contents from 1.2–2.1 mg/plant to 1.7–3.2 mg/plant by 30.8–83.3%. No significant differences were observed between foliar spraying of deionized water and sucrose, and between Zn-only and "Sucrose + Zn" treatments. Similarly, T3 and T4 also enhanced Fe concentrations from 13.4–17.1 (in treatments T1 and T2) to 15.2–18.5 mg/kg (4.7–28.4%) and Fe contents from 0.9–1.8 to 1.0–2.0 mg/plant (0–44.4%). There were significant differences between T3 and both T1 and T2 (Table 3).

Table 3. Effects of different foliar treatments and N application rates on maize grain dry weights (GDW), total dry weights (TDW) including grain and straw, hundred grain dry weights (HGDW) and grain Zn concentrations and contents in the field experiment at Quzhou.

Parameters		Treatments	N application Rates (kg/ha)			ANOVA	
			0	105	Mean		
GDW (g per ear)	T1	Deionized water	67.6 ± 1.7 a	104.9 ± 16.4 a	86.2 ± 7.3 A	*N*	0.0052
	T2	Sucrose	66.8 ± 19.3 a	104.6 ± 13.6 a	85.7 ± 16.3 A	*T*	0.4983
	T3	$ZnSO_4 \cdot 7H_2O$ (Zn)	75.6 ± 19.0 a	114.6 ± 25.2 a	95.1 ± 22.1 A	*N* × *T*	0.9898
	T4	Sucrose + Zn	65.9 ± 10.0 a	99.7 ± 10.9 a	82.8 ± 10.5 A		
		Mean	69.0 ± 11.3 B	106.0 ± 8.9 A	87.5 ± 9.9		
TDW (g per plant)	T1	Deionized water	136.5 ± 12.0 a	224.3 ± 26.6 a	180.4 ± 9.0 A	*N*	<0.0001
	T2	Sucrose	134.3 ± 30.2 a	206.0 ± 12.2 a	170.2 ± 20.9 A	*T*	0.4732
	T3	$ZnSO_4 \cdot 7H_2O$ (Zn)	143.4 ± 33.9 a	221.7 ± 29.5 a	182.5 ± 30.7 A	*N* × *T*	0.8051
	T4	Sucrose + Zn	134.1 ± 13.1 a	201.5 ± 10.0 a	167.8 ± 11.3 A		
		Mean	137.1 ± 20.7 B	213.4 ± 8.8 A	175.2 ± 14.4		
HGDW (g)	T1	Deionized water	28.3 ± 2.9 a	32.5 ± 2.5 a	30.4 ± 0.5 A	*N*	0.0001
	T2	Sucrose	28.1 ± 1.3 a	30.7 ± 1.1 a	29.4 ± 0.6 A	*T*	0.2814
	T3	$ZnSO_4 \cdot 7H_2O$ (Zn)	27.3 ± 1.5 a	31.4 ± 1.4 a	29.4 ± 0.9 A	*N* × *T*	0.6057
	T4	Sucrose + Zn	27.5 ± 2.2 a	31.0 ± 1.4 a	29.2 ± 0.7 A		
		Mean	27.8 ± 1.9 B	31.4 ± 1.3 A	29.6 ± 0.5		
Grain Zn concentration (mg/kg)	T1	Deionized water	18.4 ± 1.1 b	19.9 ± 1.0 b	19.1 ± 0.5 B	*N*	0.4941
	T2	Sucrose	19.5 ± 2.8 b	18.7 ± 2.7 b	19.1 ± 2.4 B	*T*	<0.0001
	T3	$ZnSO_4 \cdot 7H_2O$ (Zn)	28.6 ± 1.0 a	28.0 ± 1.1 a	28.3 ± 0.3 A	*N* × *T*	0.2453
	T4	Sucrose + Zn	25.2 ± 1.4 ab	29.6 ± 5.6 a	27.4 ± 2.4 A		
		Mean	22.9 ± 0.6 A	24.1 ± 2.2 A	23.5 ± 1.0		

Table 3. *Cont.*

Parameters		Treatments	N application Rates (kg/ha) 0	105	Mean	ANOVA	
Grain Zn content (mg per plant)	T1	Deionized water	1.2 ± 0.1 b	2.1 ± 0.3 b	1.7 ± 0.1 B	*N*	0.0357
	T2	Sucrose	1.3 ± 0.2 b	1.9 ± 0.2 b	1.6 ± 0.1 B	*T*	0.0008
	T3	$ZnSO_4 \cdot 7H_2O$ (Zn)	2.2 ± 0.6 a	3.2 ± 0.6 a	2.7 ± 0.6 A	*N* × *T*	0.5642
	T4	Sucrose + Zn	1.7 ± 0.3 ab	2.9 ± 0.6 a	2.3 ± 0.3 A		
		Mean	1.6 ± 0.3 B	2.5 ± 0.2 A	2.1 ± 0.2		
Grain Fe concentration (mg/kg)	T1	Deionized water	13.4 ± 1.4 a	17.1 ± 0.9 ab	15.2 ± 1.2 B	*N*	0.0091
	T2	Sucrose	14.4 ± 1.4 a	15.4 ± 2.0 b	14.9 ± 0.3 B	*T*	0.0587
	T3	$ZnSO_4 \cdot 7H_2O$ (Zn)	17.2 ± 3.3 a	17.9 ± 1.6 ab	17.6 ± 2.1 A	*N* × *T*	0.1656
	T4	Sucrose + Zn	15.2 ± 1.6 a	18.5 ± 1.2 a	16.8 ± 0.6 AB		
		Mean	15.0 ± 1.8 B	17.2 ± 0.9 A	16.1 ± 0.8		
Grain Fe content (mg per plant)	T1	Deionized water	0.9 ± 0.1 a	1.8 ± 0.2 ab	1.3 ± 0.1 B	*N*	0.0325
	T2	Sucrose	1.0 ± 0.4 a	1.6 ± 0.1 b	1.3 ± 0.2 B	*T*	0.0470
	T3	$ZnSO_4 \cdot 7H_2O$ (Zn)	1.3 ± 0.6 a	2.0 ± 0.4 a	1.7 ± 0.5 A	*N* × *T*	0.7565
	T4	Sucrose + Zn	1.0 ± 0.3 a	1.8 ± 0.1 ab	1.4 ± 0.2 AB		
		Mean	1.1 ± 0.3 B	1.8 ± 0.1 A	1.4 ± 0.2		

Values are means ± standard deviation ($n = 3$). Values in the same column followed by different lowercase letters are significantly different among various foliar treatments according to Fisher's Protected LSD test ($p < 0.05$). Values followed by different capital letters are significantly different between different *N* application rates (horizontal comparison) or among various foliar treatments (vertical comparison) according to Fisher's Protected LSD test ($p < 0.05$).

3.2. Maize Agronomic Traits in Licheng

Compared with the control treatment, there were some numerical differences (an increase or decrease to some extent) in maize grain dry weights, hundred grain dry weights, and other ear traits following foliar spraying of sucrose and/or Zn (Table 4). However, as expected, non-significant differences among various foliar treatments were observed in the statistical analysis.

Table 4. Maize agronomic traits among various foliar spraying treatments in the field experiment at Licheng.

Treatments		Grain Dry Weight (g/ear)	Hundred Grain Weight (g)	Number of Kernels per Ear	Ear Diameter (mm)	Ear Length (cm)	Bald Tip Length (cm)	Bald Tip Length/Ear Length (%)
T1	Deionized water	142.8 ± 15.7 a	37.8 ± 2.2 a	485.6 ± 119.5 a	50.9 ± 3.1 a	20.8 ± 3.5 a	1.5 ± 0.4 a	7.2 ± 2.2 a
T2	Sucrose	139.6 ± 10.4 a	38.6 ± 1.7 a	518.4 ± 103.4 a	48.5 ± 5.2 a	21.9 ± 1.5 a	2.2 ± 1.1 a	10.0 ± 2.0 a
T3	$ZnSO_4 \cdot 7H_2O$ (Zn)	143.6 ± 13.2 a	36.1 ± 1.3 a	523.4 ± 96.6 a	46.0 ± 4.8 a	21.3 ± 1.3 a	2.2 ± 0.4 a	10.3 ± 4.9 a
T4	Sucrose + Zn	150.9 ± 20.1 a	37.9 ± 1.3 a	500.0 ± 120.7 a	50.0 ± 2.7 a	20.0 ± 1.2 a	1.6 ± 1.1 a	8.0 ± 5.5 a

Values are means ± standard deviation ($n = 3$). Values in the same column followed by same lowercase letters are not significantly different among various foliar treatments according to Fisher's Protected LSD test ($p < 0.05$).

3.3. Maize Grain Zn and Fe Concentrations, Contents, and Bioavailability in Licheng

Except for significant reductions in the molar ratios of PA × Ca/Zn and PA × Ca/Fe, foliar spraying of sucrose-only (T2) did not significantly affect maize grain Zn and Fe accumulation (concentrations and contents) or molar ratios of PA/Zn and PA/Fe as compared with the foliar spraying of deionized water (Table 5). In comparison with the control (T1), foliar Zn-only spraying (T3) significantly increased the grain Zn concentration from 24.9 to 36.8 mg/kg (47.8%) and the Zn yield from 3.6 to 5.3 mg/plant (47.2%). In contrast, it significantly reduced the molar ratio of PA/Zn from 34.9 to 22.1 (36.7%) and PA × Ca/Zn from 70.1 to 35.8 (48.9%). Similarly, T3 significantly enhanced the grain Fe concentration by 25.0% and the content by 26.5%, and significantly reduced the molar ratio of PA/Fe by 24.5% and PA × Ca/Fe by 39.8%. Similar results were found in the Zn + sucrose treatment (T4). Except for the molar ratios of PA × Ca/Zn and PA × Ca/Fe, the grain Fe concentration in T4 and the molar ratio of PA/Fe in T3, both foliar Zn-biofortified treatments including T3 and T4 had significantly higher grain Zn and Fe accumulation and bioavailability than T2. No significant differences were found between Zn-only and Zn + sucrose (Table 5) foliar treatments.

Table 5. Effects of different foliar treatments on Zn and Fe concentrations, contents and molar ratios of PA/Zn, PA × Ca/Zn, PA/Fe, and PA × Ca/Fe in maize grains in the field experiment at Licheng.

	Treatments	Zn Concentrations (mg/kg)	Zn Contents (mg/plant)	PA/Zn	PA × Ca/Zn	Fe Concentrations (mg/kg)	Fe Contents (mg/plant)	PA/Fe	PA × Ca/Fe
T1	Deionized water	24.9 ± 0.8 b	3.6 ± 0.1 b	34.9 ± 3.6 a	70.1 ± 3.5 a	24.0 ± 2.8 b	3.4 ± 0.3 b	31.4 ± 4.3 a	63.0 ± 5.2 a
T2	Sucrose	23.8 ± 0.6 b	3.3 ± 0.1 b	32.6 ± 2.8 a	37.9 ± 1.9 b	23.1 ± 0.5 b	3.2 ± 0.1 b	28.9 ± 2.8 ab	33.6 ± 2.1 b
T3	$ZnSO_4·7H_2O$ (Zn)	36.8 ± 2.0 a	5.3 ± 0.3 a	22.1 ± 2.0 b	35.8 ± 7.6 b	30.0 ± 3.9 a	4.3 ± 0.5 a	23.7 ± 4.1 bc	37.9 ± 7.1 b
T4	Sucrose + Zn	34.8 ± 0.8 a	5.3 ± 0.2 a	18.9 ± 2.4 b	36.7 ± 11.5 b	27.7 ± 1.2 ab	4.2 ± 0.2 a	20.4 ± 2.3c	39.5 ± 10.8 b

Values are means ± standard deviation ($n = 3$). Values in the same column followed by different lowercase letters are significantly different among various foliar treatments according to Fisher's Protected LSD test ($p < 0.05$).

3.4. Concentrations of Carbon, Nitrogen, Total and Phytate Phosphorus and Calcium, and Ratios of C/N and Phytate P/Total P in Maize Grains in Licheng

Compared with the control, foliar spraying of sucrose, Zn, or sucrose + Zn had no significant effects on carbon concentration or on the ratio of phytate P/total P, whereas the total and phytate P concentrations were significantly reduced by the sucrose + Zn treatment. Also, the Ca concentration was reduced in the sucrose-only treatment (Table 6). Sucrose alone or in combination with Zn significantly decreased the nitrogen concentration, but significantly increased the ratio of C/N as compared to the control and the Zn-only treatment.

Table 6. Effects of different foliar treatments on the concentrations of carbon, nitrogen, phytate P, total P, and Ca, and ratios of C/N and phytate P/total P in maize grains in the field experiment at Licheng.

	Treatments	Carbon Concentration (%)	Nitrogen Concentration (%)	C/N	Phytate-P Concentration (g/kg)	Total P Concentration (g/kg)	Phytate P/Total P (%)	Ca Concentration (mg/kg)
T1	Deionized water	42.8 ± 0.5 a	1.78 ± 0.02 a	24.07 ± 0.06 b	2.49 ± 0.18 a	3.24 ± 0.08 a	76.8 ± 7.5 a	80.8 ± 6.1 a
T2	Sucrose	42.7 ± 0.6 a	1.69 ± 0.03 b	25.27 ± 0.27 a	2.22 ± 0.25 ab	3.01 ± 0.10 ab	73.8 ± 6.8 a	46.6 ± 1.7 b
T3	$ZnSO_4·7H_2O$ (Zn)	42.8 ± 0.1 a	1.76 ± 0.03 a	24.36 ± 0.40 b	2.33 ± 0.17 ab	3.07 ± 0.25 ab	76.2 ± 7.2 a	65.4 ± 17.0 ab
T4	Sucrose + Zn	42.7 ± 0.3 a	1.70 ± 0.01 b	25.11 ± 0.12 a	1.88 ± 0.27 b	2.79 ± 0.07 b	67.4 ± 8.2 a	77.5 ± 19.0 a

Values are means ± standard deviation ($n = 3$). Values in the same column followed by different lowercase letters are significantly different among various foliar treatments according to Fisher's Protected LSD test ($p < 0.05$).

3.5. Relationships between Zn and Fe Concentrations in Maize Grains

There were significant and positive linear correlations between Zn and Fe concentrations in maize grains when no N or 105 kg/ha N was applied, and across both N supply levels in the field experiment at Quzhou (Figure 1). Maize grain Fe concentrations were also significantly and positively correlated with Zn concentrations in the field experiment at Licheng and across both experimental sites (Figure 1).

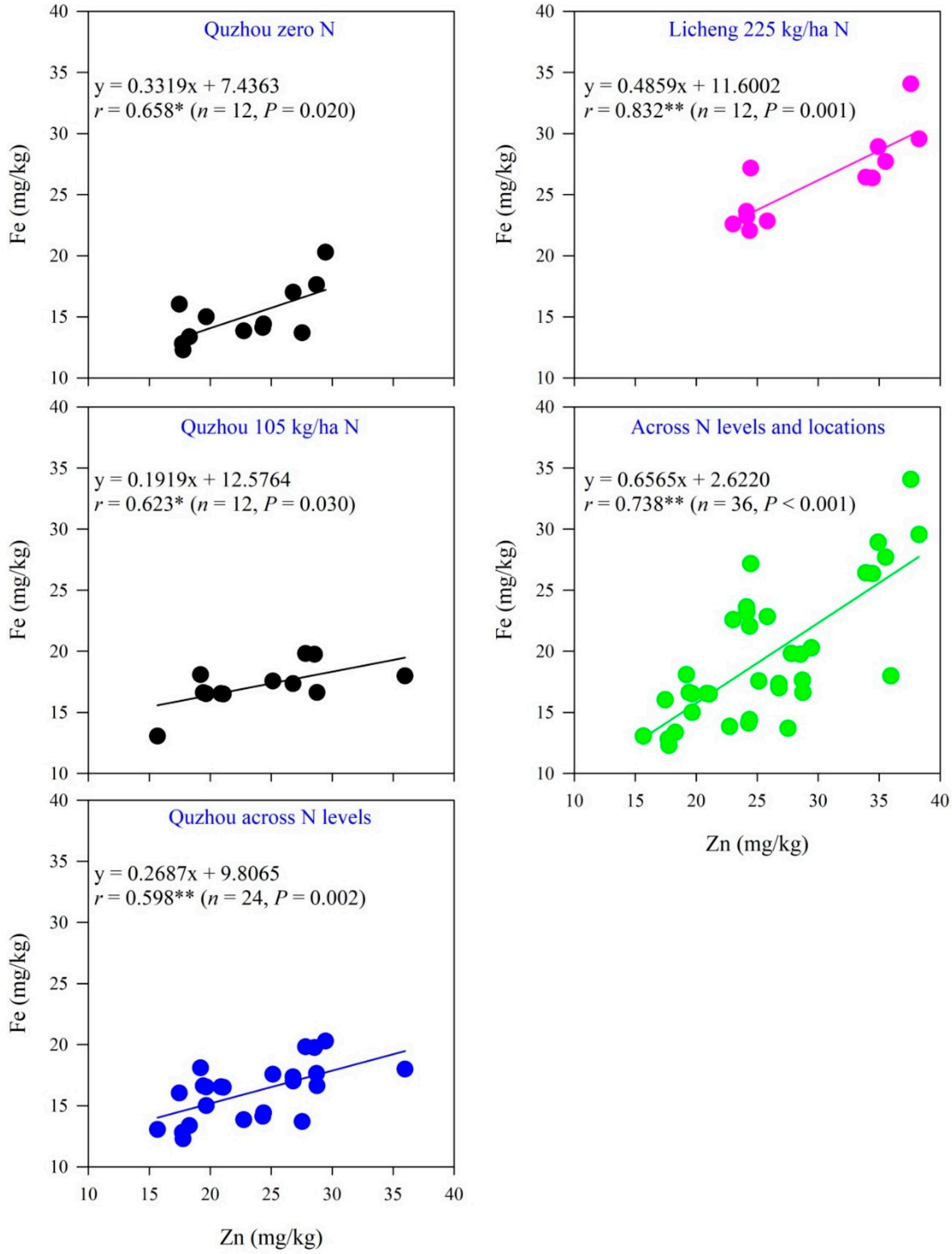

Figure 1. Relationships between Zn and Fe concentrations in maize grains across all foliar spraying treatments at different nitrogen (N) application rates and different experimental locations. * indicates significance at $p \leq 0.05$; **, significance at $p \leq 0.01$.

4. Discussion

In a study by Mohsin et al. (2014) [48], the combined application of Zn as seed priming and foliar spray significantly improved the performance of maize hybrids, including the plant height, cob length, cob diameter, 1000 grain weight, biological yield, grain yield, and harvest index. Interestingly, increased Zn concentrations in maize kernels were also positively correlated with the grain yield, 1000 grain weight, cob length, and cob diameter [48]. Zinc fertilization has also been reported to have additional benefits like promoting growth at early stages and improving tolerance/resistance to abiotic/biotic stresses [36,49]. In the current study, foliar Zn and/or sucrose applications did not affect the yield or other agronomic traits of maize in Quzhou and Licheng (Tables 3 and 4), suggesting that the dry-matter accumulation in maize grains is less dependent on exogenous foliar Zn and/or carbohydrate supply, at least under the conditions used in this study. Similar results were previously reported for maize and wheat under pot or field conditions [3,26,27,35,38,50,51]. For Zn, this finding may be attributed to the high Zn concentration in soil and the suitable soil conditions, and thus the good Zn nutritional status of plants. It could also be due to the very late application of Zn, which failed to promote crop growth during the seedling stage [27]. As an exception, the exogenous foliar spraying of Zn effectively supplemented the demand of wheat plants for Zn, which reduced drought-induced oxidative cell damage due to an improved antioxidative defense ability [52], thus increasing the grain yield under drought conditions, even in soil with a high DTPA–Zn concentration [53]. It has been observed that increasing sucrose concentrations within the optimal level substantially enhance grain yields and single grain weights of wheat and rice under detached-ear culture conditions [33,34,54]. Sasaki et al. (2005) [54] speculated that an increased supply of sucrose during the grain-filling period might improve the activity of enzymes involved in starch synthesis and lead to an increase in single grain weights. Hence, the effects of foliar applications of Zn and/or sucrose on maize yield situations warrant further research under various environmental conditions.

Consistent with other studies, the foliar supply of Zn alone significantly increased maize grain Zn accumulation [27] as well as the estimated Zn bioavailability in this study (Tables 3 and 5). It has been reported that Zn concentrations in cereal grains are significantly and positively correlated with those in leaves, suggesting the importance of the source strength of physiologically available Zn within vegetative tissues, which can be effectively translocated or remobilized to the grain sink after flowering [50,55–57]. Hence, the foliar applied Zn would undoubtedly penetrate across the cuticle into maize leaves and contribute to an increase in grain Zn accumulation, as presented in the current study. Various studies have confirmed that eating Zn-biofortified maize helps individuals to meet their Zn requirements [58]. The current marked increase in the maize grain Zn concentration (9.2 and 11.9 mg/kg on average in Quzhou and Licheng, respectively) caused by Zn-only spraying would have a measurable impact, improving the human dietary intake of Zn to alleviate malnutrition [50,59]. Therefore, foliar Zn spraying should be adopted as an effective way to biofortify maize with Zn. However, to overcome Zn malnutrition, a Zn concentration of more than 37 mg/kg in the whole grain [60] and an increase of 30 mg/kg in the endosperm (www.harvestplus.org) of maize (because the major proportion of the maize grain is comprised of endosperm) are recommended. In terms of Zn bioavailability, the critical molar ratio of PA/Zn is 15–20; a value of 5–15 represents about 30–35% Zn availability, and ≥15 represents about 15% Zn availability [42]. The effectiveness of foliar Zn spraying on grain Zn concentrations is related to the spray timing, location, rates, type of fertilizers (e.g., nano-particles), maize genotypes, and environmental conditions [60]. Based on the low maize grain Zn concentrations (<37 mg/kg) and high PA/Zn values (> 15) achieved by foliar spraying of 0.2% or 0.3% $ZnSO_4{\cdot}7H_2O$ (*w*/*v*) in our present study (Tables 3 and 5), there is still a need to further enhance Zn absorption by maize, its translocation to grains, and its bioavailability, possibly by appropriately increasing the spraying frequency and Zn concentration and spraying with other beneficial fertilizers (e.g., urea) or stimulators [51]. Gomez-Coronado et al. (2016) [61] found that the foliar spraying of 0.5% $ZnSO_4{\cdot}7H_2O$ (*w*/*v*) on wheat increased grain Zn concentrations effectively with a better bioavailability. It is worth pointing out that the spraying doses of 50 or 100 mL for each maize plant were only applied under our experimental conditions, not for the actual practice. For actual large-scale practices, drone spraying with a higher Zn concentration solution containing some high efficient additives is possible to save a lot of doses, which need to be further studied.

In 1953, it was reported that the addition of sucrose to urea sprays reduced the injury of maize leaves, perhaps by reducing the rate of urea absorption and increasing the rate of urea translocation within the plant [62]. In our previous studies, foliar application of sucrose + Zn was associated with greater improvements in the concentration, content, and bioavailability of Zn in wheat (a C3 plant) than the spraying of Zn only [3,35]. As mentioned by Zhao et al. (2014) [51], the relatively higher effectiveness of sucrose + Zn may be attributed to (1) the longer drying time of the spraying solution; (2) enhanced leaf cuticle penetration; and (3) enhanced translocation of Zn from the absorption site to the grain sink. However, in the current study, this was shown to be totally ineffective for C4 maize (Tables 3 and 5). Although treatments of foliar applications of Zn alone and combined with sucrose increased maize grain Zn concentrations, contents, and bioavailability, no significant differences were found between these two treatments regardless of the N application rate and experimental locations. It was found that ^{14}C-sucrose was not transported within the grain in the same way as ^{65}Zn [63]. The spraying of only sucrose had no impact on either grain Zn accumulation or bioavailability (as estimated by the molar ratio of PA/Zn) of maize (Tables 3 and 5). In addition, foliar sucrose supply (with or without Zn) did not affect the maize grain C concentration significantly; rather, the significantly lower N concentrations led to significantly higher ratios of C/N (Table 6). However, higher C/N ratios were not associated with higher Zn accumulation and bioavailability in maize grains (Table 3, Table 5, and Table 6). Therefore, unlike wheat, foliar sucrose spraying played no role in lowering the deliquescent relative humidity of spray solutions [64] or facilitating swelling of the cuticle by the absorption of substantial amounts of water to form "water-filled pores" [65] to enhance the penetration of hydrophilic solutes across the maize cuticle [64] and ultimately increase the rate/amount of foliar Zn absorption. Simultaneously, its effect on the remobilization and translocation of Zn from maize leaves to grains might be negligible. Whether the sprayed sucrose, accompanying Zn or not, entered leaf cells and was translocated from leaves to grains needs to be further investigated. In a study by Myers et al. (2014) [12], in comparison with values under ambient CO_2 conditions, the decline of grain Zn concentrations at elevated CO_2 levels was notable in C_3 crops, but the effect was less pronounced in C_4 crops, which is consistent with the differences in their physiological processes. A high concentration of CO_2 in C_4 crops internally resulted in photosynthesis being CO_2-saturated even under ambient CO_2 conditions, leading to no stimulation of photosynthetic carbon assimilation at elevated CO_2 levels under mesic growing conditions [66]. Similarly, the more prominent impact of foliar spraying "sucrose + Zn" than the foliar spraying Zn alone on improving Zn accumulation and bioavailability in wheat but not in maize grains in our study may also be consistent with the physiology of different types of crops. Maize (as a C_4 plant) has a higher photosynthetic capacity to produce more carboxylates than C_3 wheat; hence, maize grain mineral accumulation is less dependent on an exogenous foliar sucrose supply.

The relationship between Zn and Fe concentrations in major cereal crops (maize, rice, and wheat) is still uncertain, mostly antagonistic [67], seldom synergistic [68–70], and sometimes indifferent [71]. If biofortification of Zn in cereals leads to a loss of Fe, this is unacceptable, because the malnutrition caused by Fe deficiency is no less severe than that of Zn [30]. Saha et al. (2015) [30] reported that Zn application through soil (as basal) in combination with two foliar sprays (at maximum tillering (6–8 leaves) and flowering/silking stages) yielded Zn-dense but Fe-starved grains (including the ultimate processed food products) and straws/stalks of most cultivars (~90%) of maize, rice, and wheat. A significant positive linear correlation was found between the grain Fe and Zn concentrations in maize (but not wheat) in different soils and foliar Zn treatments in the Loess Plateau, China [27]. However, these studies did not clearly distinguish between the effects of soil Zn application and foliar Zn spraying. Soil and foliar applications have been reported to have differential effects on Zn concentrations of maize kernels [60]. Our study revealed that effects of various foliar treatments on maize grain Fe accumulation and bioavailability were similar to those on Zn. Foliar Zn spraying simultaneously improved the concentrations and bioavailability of Zn and Fe in maize grains irrespective of the foliar sucrose supply, but Fe increased to a lesser extent than Zn (Tables 3 and 5). Significant and positive linear correlations between Zn and Fe concentrations in maize grains were observed at each N concentration and across both N supply levels in the field experiments at both Quzhou and Licheng as well as across these two different experimental locations (Figure 1). In contrast to Saha et al. (2015) [30], this indicates that Zn enrichment by foliar spraying of Zn has a benign effect on Fe in maize grains. This result suggests that this process can successfully achieve simultaneous Zn and Fe biofortification. In case of foliar Zn applications, genetic mechanisms regulating Zn absorption by maize leaves and translocation to grains are still unidentified [60]. It has also been reported that the foliar application of Zn not only increases the transport of shoot Fe to grains but also reduces cadmium toxicity in maize and other cereals [69]. This synergetic effect of Zn and Fe may be attributed to pleiotropic effects or linkage among the genes governing Zn and Fe accumulation in kernels. There are large numbers of genes involving the transport of various metals, some of them encoding proteins that are capable of transporting multiple metals, and the QTLs for these traits are also co-localized in the same chromosomal regions [72].

5. Conclusions

Foliar spraying of Zn and sucrose alone or in combination did not impact the agronomic traits of maize, including yield, indicating that dry-matter accumulation in grains is less dependent on exogenous foliar Zn and/or carbohydrate supply, at least under the conditions used in this study. The foliar applied Zn (with or without sucrose) undoubtedly penetrated the cuticle into maize leaves and contributed to an increase in grain Zn accumulation and bioavailability. The spraying of sucrose alone had no impact on either grain Zn accumulation or bioavailability. The combination of zinc and sucrose did not lead to a further significant increase in maize grain Zn accumulation and bioavailability compared with Zn-only spraying. In addition, the significantly lower N concentrations caused by the spraying of sucrose (with or without Zn) led to significantly higher ratios of C/N in maize grains, rather than higher Zn accumulation and bioavailability. Therefore, unlike the previously reported effects on wheat, foliar spraying of exogenous sucrose did not promote the absorption of Zn by maize leaves and its transport to grains. This may be consistent with the physiology of different types of crops. C_4 maize has a higher photosynthetic capacity to produce more carboxylates than C_3 wheat, leading to maize grain mineral accumulation that is less dependent on an exogenous foliar sucrose supply. Similar results were obtained for maize grain Fe accumulation and bioavailability, but the Fe concentration increased less than that of Zn. The Fe concentration was significantly and positively correlated with Zn, indicating that foliar Zn spraying facilitated the transport of endogenous Fe to maize grains. Therefore, foliar spraying of Zn achieved biofortification of both Zn and Fe in maize grains simultaneously, i.e., "killing two birds with one stone."

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