



Wheat and Maize Grown on Two Contrasting Zinc-deficient Calcareous Soils Respond Differently to Soil and Foliar Application of Zinc

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Abstract

Assessing the effects of soil and foliar application of Zn on plant growth and P and Zn uptake by wheat (*Triticum durum* L.) and maize (*Zea mays* L.) grown on calcareous soils. Plants were pot-grown in fertile soil (Vertisol) and a less fertile one (Inceptisol). Six Zn treatments were compared: control (no Zn application), soil Zn application (3 mg Zn kg⁻¹), and foliar Zn application (~1.0 mg Zn plant⁻¹) to plants at different phenological stages, with one treatment involving splitting the total amount of Zn supplied between two stages. Soil treatment and early foliar spraying reduced P uptake and translocation to edible parts in wheat and maize plants. Soil application increased the Zn content of wheat grains and maize ears relative to the foliar application. Soil application and foliar spraying at stem elongation or flowering in wheat led to the greatest increase in grain Zn content (up to 69%) and use efficiency, and also to the lowest grain P/Zn ratio (especially in the Vertisol). All Zn treatments increased maize ear biomass relative to the control plants in both the Vertisol (138–227%) and the Inceptisol (27–41%). Splitting the application of Zn to wheat leaves was inefficient but maximized maize biomass production in both soils. Wheat and maize responded differently in terms of crop production and quality to soil and foliar Zn treatments, the response being soil-dependent. Fertilization strategies for cereals on calcareous soils should consider the P and Zn interaction (soil and plant levels).

Keywords Zn availability · Zn biofortification · P–Zn interaction · Cereals · Foliar spraying · Soil application · Zinc use efficiency

1 Introduction

Hidden hunger or malnutrition due to micronutrient deficiencies affects more than two billion people in the world (Bailey et al. 2015). Although multiple efforts and remarkable progress toward plant breeding for crop biofortification

have been made in recent years, releasing new but traditionally bred varieties is a time-consuming process, often taking 8–20 years (Van Der Straeten et al. 2020). Using micronutrient fertilizers has proved useful to biofortify agricultural crops in the short and medium terms. However, properly using them requires a solid knowledge of the target plant and soil system. In the last few years, a new paradigm for developing fertilizers and sustainable fertilization strategies has emerged in agriculture (Bindraban et al. 2015). The new products and strategies revolve around a better understanding of plant physiology, soil processes, and plant–soil interactions as the means to reduce the use of fertilizers and increase the efficiency of the nutrients they contain (Bindraban et al. 2020; Macintosh et al. 2019).

Zinc (Zn) is an essential micronutrient for plant and human health (Cakmak and Kutman 2018; Read et al. 2019), and its Zn deficiency is a fairly common occurrence in humans and in livestock (Alloway 2009; Cakmak 2008; Jurowski et al. 2014). Thus, it plays a key role in plant

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growth and strongly influences crop yield and quality (Cakmak and Kutman 2018). The main origin of Zn deficiency in humans is a diet based on plants grown on soils with limited Zn availability. Zn deficiency is a major agricultural problem because cereals are staple foods for developing economies, and roughly one-half of all arable land cropped with cereals in the world is Zn-deficient (Graham and Welch 1997), especially in rural areas. It is even worse in rural areas of these countries.

The availability of Zn in soil depends on physical and chemical properties (Alloway 2009) such as mineral composition (CaCO₃, iron oxides), texture, pH, moisture, and organic matter, among others. Also, the availability of Zn in soil and its uptake by plants is influenced by soil phosphorus (P) content and P fertilization (Zhang et al. 2015; Sánchez-Rodríguez et al. 2017; Liu et al. 2020a, b). Phosphorus and Zn availability in calcareous soils, which account for approximately 30% of all arable land in the world (Chen and Barak 1982), are somewhat limited. As a result, cereals find it difficult to absorb these two nutrients, which results in impaired growth, and in reduced crop yields (Akhtar et al. 2019), and quality (especially with P fertilizers applied in the absence of Zn) (Bindraban et al. 2020; Sánchez-Rodríguez et al. 2021b). Moreover, the effects of soil and foliar Zn application on P and Zn accumulation in plants remain a matter of debate (Akhtar et al. 2019; Bindraban et al. 2020; He et al. 2021).

Zinc fertilizers enhance Zn uptake by plants. The effectiveness of such fertilizers, which are usually applied to soil or sprayed to plant aerial parts, depends on some soil properties and on the particular crop. Thus, the complex dynamics of Zn upon application to the soil reduces its efficiency. Specifically, an alkaline pH strongly reduces the solubility of Zn (Marschner 1993; Vonwiren et al. 1993) and causes it to coprecipitate with other elements such as P or Ca and be adsorbed onto the surface of CaCO₃, Fe oxides, or clays (Alloway 2009; Marschner 2011). For these reasons, only high enough Zn rates (> 10 kg ha⁻¹) are expected to have a favorable effect on soil Zn availability and crop yield (Liu et al. 2019; Sánchez-Rodríguez et al. 2021a). On the other hand, foliar Zn application, which supplies smaller amounts of Zn than soil application, avoids the complex dynamics of this element in soil and is more effective toward crop biofortification (Cakmak 2008; Zia et al. 2020). However, as explained by Rehman et al. (2021), certain physiological parameters such as leaf penetration and later translocation of Zn play a crucial role when the nutrient is applied to leaves. In conclusion, plants (cereals included) differ in their sensitivity to Zn deficiency and response to Zn fertilization (Cakmak and Kutman 2018), so both factors should be considered in addition to soil properties when nutrient use efficiency is to be increased by the use of sustainable agricultural strategies.

Although Zn fertilizers have been shown to influence P dynamics (Raliya and Tarafdar 2013; Raliya et al. 2016), their effects on P partitioning in different plant species and tissues are unclear (Bindraban et al. 2020). In this work, we followed a mechanistic approach to improve existing knowledge on Zn deficiency and P–Zn interactions in plant–soil systems. For this purpose, two of the most cultivated cereals in the world (viz., wheat and maize, which differ in their sensitivity to Zn deficiency; Marschner 2011) were grown on two Mediterranean calcareous soils (a Vertisol and an Inceptisol) with contrasting Zn and P availability. Experiments involved six different Zn treatments, namely: control (no Zn application), an initial application to the soil, and four foliar treatments at different phenological stages including one in which Zn was split between two stages. Our hypotheses were (a) that the effects of the treatments would be more apparent in plants grown on the less limiting calcareous soil (Vertisol) and depend on the particular plant species (wheat or maize); (b) that applying Zn to the soil would reduce plant P uptake; (c) that Zn fertilization would alter P and Zn translocation and partitioning within plants; and (d) that the most effective Zn application strategy in terms of plant growth and crop biofortification, P and Zn translocation and partitioning, Zn availability in edible plant parts and Zn use efficiency would be species dependent.

2 Material and Methods

2.1 Soil Sampling and Properties

Samples were collected from the topsoil layer (0–25 cm) of two different Spanish agricultural soils located in Fernán Núñez (37°42′13″ N; 4°42′43″ W), province of Córdoba, and Argamasilla del Alba (39°04′23″ N; 3°04′35″ W) in Ciudad Real. Samples were air dried for 1 week, after which rock fragments and roots were removed by sieving through 1 cm of mesh. Then, about 500 g of each sample was sieved to 2 mm and used for analysis. The soils were a fertile Vertisol (VER; Haploxerert, cropped with a wheat/sunflower rotation) and a less productive Inceptisol (INC; Calcixerpt, from a vineyard) (Soil Survey Staff 2014). Their properties are summarized in Table 1. The clay content as determined with the pipette method (Gee and Bauder 1986) was 330 g kg⁻¹ for VER and 100 g kg⁻¹ for INC. Both soils had a low content in organic carbon (OC) as determined by rapid dichromate oxidation (Walkley and Black 1934) and an alkaline pH (in a 1:2.5 soil:water suspension owing to their substantial content in CaCO₃ as determined according to van Wesemael (1951) (calcium carbonate equivalent, especially high in INC; Table 1). Available P as extracted according to Olsen et al. (1954) and determined with the Molybdate Blue method (Murphy and Riley 1962) was higher in VER

Table 1 Properties of the soils (*VER*, Vertisol; *INC*, Inceptisol). Mean value of two duplicates are shown for clay content and calcium carbonate equivalent (CCE) and mean value of three duplicates and SE are shown for *OC*, organic carbon; *EC*_{1:5}, electrical conductivity

Soil	Clay	OC	EC _{1:5}	pH _{1:2.5}	CCE	P _{Olsen}	Zn _{DTPA}	Fe _{DTPA}	Mn _{DTPA}	Cu _{DTPA}	P _{Olsen} /Zn _{DTPA}
	g kg ⁻¹	g kg ⁻¹	dS m ⁻¹		g kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹	
VER	330	8.8±0.1	0.11±0.01	7.8±0.1	297	10.3±0.5	0.26±0.08	4.65±0.11	4.59±0.11	3.72±0.06	40
INC	100	8.4±0.1	0.10±0.01	7.9±0.0	560	18.7±0.8	0.17±0.01	1.34±0.06	5.06±0.04	0.23±0.07	110

than in *INC*. Finally, available Zn as extracted with DTPA (Zn_{DTPA}; Lindsay and Norvell 1978) and determined by atomic absorption spectrophotometry was low in both soils and below the critical threshold (0.5 mg kg⁻¹; Lindsay and Norvell 1978) (Table 1). Available Fe, Mn, and Cu were determined following the same methodology as explained for Zn_{DTPA}.

2.2 Plant Cultivation

After drying and sieving, the soil samples were homogenized and used to fill 72 cylindrical PVC pots per soil. Each pot, which was 5 cm in diameter and 15 cm tall and furnished with a cellulose acetate filter wedged into a hole in the bottom to allow drainage, was filled with 250 g of soil and used to sow three seeds of durum wheat (*Triticum durum* L. cv. Calero) or maize (*Zea mays* L. cv. ES Zoom). Before sowing, seeds were pregerminated for 72 h in sterilized filter paper moistened with deionized water. Subsequently, wheat and maize were thinned to one plant per pot 19 days after sowing (DAS). Pots were watered daily with deionized water to keep moisture at ca. 70–85% of the soil water holding capacity. Also, each pot was supplied with 10–20 mL per week, depending on the specific growth stage, of a Zn-free Hoagland solution [Ca(NO₃)₂·4H₂O (5 mM), KNO₃ (5 mM), KH₂PO₄ (1 mM), MgSO₄ (2 mM), Fe (EDDHA) (20 mM), KCl (0.05 μM), H₃BO₃ (25 μM), MnSO₄·H₂O (2 μM), CuSO₄·5H₂O (0.5 μM), and Na₂MoO₄ (3 μM)] to ensure

ity in the extract 1:5 soil:water; pH_{1:2.5}, soil pH in the extract 1:2.5 soil:water; P_{Olsen}, P extracted with 0.5 M NaHCO₃; DTPA, diethylenetriaminepentaacetic acid-extractable Zn, Fe, Mn, and Cu (Zn_{DTPA}, Fe_{DTPA}, Mn_{DTPA}, and Cu_{DTPA}, respectively)

that no deficiency other than that of Zn would occur. Experiments were conducted in a growth chamber under controlled conditions (photoperiod 14 h day⁻¹, 23.5/16 °C day/night, 70% relative humidity, and radiation of 325 μmol m⁻² s⁻¹). Then, the plants were harvested 105 DAS (wheat) or 85 DAS (maize).

2.3 Zinc Treatments and Experimental Design

Table 2 depicts the different Zn treatments. Control plants (C) were fertilized with no Zn. For the soil application treatment (S), an amount of a Zn solution containing 9 mg of Zn and prepared by dissolving 40 mg of ZnSO₄·7H₂O (22.5% Zn) in 1 L of deionized water was homogeneously sprayed over an amount of 3 kg of each soil (equivalent to 3 or 7.5 kg Zn ha⁻¹). These Zn-treated soil samples were air dried for a week and used to fill 12 pots. For the foliar treatments, a fresh ZnSO₄·7H₂O solution containing 1 mg Zn mL⁻¹ (treatments F1 to F3) or 0.5 mg Zn mL⁻¹ (treatment F4) and 0.5% Tween 80 @ (v/v) was prepared. Then, an airbrush was used to spray 5.4 or 7.2 mL of the solution to the aerial part of the corresponding six wheat or maize plants, respectively. Before the foliar treatment, which was applied outside the growth chamber, the surface of the treated pots was covered with aluminum foil to prevent the soil from receiving any Zn. The four foliar treatments were applied to each plant species at different growth stages according to the BBCH scale (Witzenberger and Lancashire 2001). Thus, F1 was

Table 2 Zinc treatments applied to wheat and maize plants. DAS, days after sowing

Treatment	Wheat			Maize		
	Zn fertilization	Application time (DAS)	Growth stage (BBCH)	Zn fertilization	Application time (DAS)	Growth stage (BBCH)
Control (C)	0			0		
Soil (S)	3 mg Zn kg ⁻¹	–7		3 mg Zn kg ⁻¹	–8	
Foliar 1 (F1)	0.9 mg Zn plant ⁻¹	19	23 (three tillers)	1.2 mg Zn plant ⁻¹	19	13 (three leaves)
Foliar 2 (F2)	0.9 mg Zn plant ⁻¹	45	39 (flag leaf)	1.2 mg Zn plant ⁻¹	44	30 (one node)
Foliar 3 (F3)	0.9 mg Zn plant ⁻¹	56	60 (flowering)	1.2 mg Zn plant ⁻¹	57	33 (three nodes)
Foliar 4 (F4)	0.45 mg Zn plant ⁻¹ +0.45 mg Zn plant ⁻¹	½ 45 ½ 56	½ 39 (flag leaf) ½ 60 (flowering)	0.6 mg Zn plant ⁻¹ +0.6 mg Zn plant ⁻¹	½ 44 ½ 57	½ 30 (one node) ½ 33 (three nodes)

Growth stage according to the BBCH-scale: 10–19, leaf development; 20–29, tillering; 30–39, stem extension; 60–69, flowering

applied at tillering in wheat (19 DAS) and leaf development in maize (19 DAS), whereas F2 was applied at the end of stem extension in wheat (45 DAS) and the beginning of that stage in maize (one node observed, 44 DAS), and F3 at flowering in wheat (56 DAS) and a later stem extension stage in maize (three nodes observed, 57 DAS). The total amount of Zn supplied by F4 was split over two different applications corresponding to the growth stages of F2 and F3 in each crop. Therefore, the amount of Zn applied to each plant with the four foliar treatments was 0.9 mg for wheat and 1.2 mg for maize (Table 2). The foliar treatments were applied at earlier growth stages in maize than in wheat for practical reasons, namely: the complexity of foliar applications and the potential damage to maize plants once they have reached stem elongation under field conditions. The Zn rates used were based on previous research work (Cakmak and Kutman 2018; Sánchez-Rodríguez et al. 2021a).

The effects of the crop (wheat and maize), soil (VER and INC), and Zn treatment were assessed by establishing six experimental units (one pot with one plant) per crop per soil per Zn combination. The pots (144 in all) were distributed according to a completely randomized design in the growth chamber.

2.4 Plant Analyses, Phosphorus, and Zinc Partitioning

Plant height and leaf chlorophyll index (SPAD) were measured at 64 DAS in wheat and 79 DAS in maize. A SPAD-502 Portable Chlorophyll Meter (Konica Minolta Co., Osaka, Japan) was used to measure leaf chlorophyll index (as given in so-called SPAD units) in the last two totally expanded leaves (6–8 measurements per leaf) in each plant. At harvest (105 DAS in wheat and 85 DAS in maize), the plants were cut 1 cm above the soil surface to avoid contamination and washed with tap water followed by deionized water (twice for 2 min) to remove unabsorbed Zn. Then, they were dried at 65 °C in an oven for at least 72 h and weighed for dry matter. Plant parts (leaf, stem, chaff, and grain in wheat; leaf, stem–bottom part including the first three nodes, stem–upper part including the remainder of the stem, tassel, and ear in maize) were separated and weighed. Also, wheat grains were counted. Grain and ear weight were used as proxies for yield in wheat and maize, respectively.

Plant parts were separately ground in a mill and digested with a mixture of acids, using 3 mL of 65% HNO₃ and 1 mL of 60% HClO₄ for 0.2 g of sample (Zasoski and Burau 1977). The P and Zn contents of the different plant parts were determined by using the Molybdate Blue method of Murphy and Riley (1962) and atomic absorption spectrophotometry, respectively. The ensuing data were multiplied by the dry weight of each plant part, and P and Zn partitioning were

calculated by division into the total content of each nutrient in the plant.

2.5 Phosphorus to Zinc Ratio and Zinc Use Efficiency

The P/Zn ratio in each plant part was calculated as an indicator of Zn availability for human or animal consumption (Cakmak 2008), especially in wheat grain and maize ear. Zinc use efficiency (ZnUE) was calculated as follows:

$$\text{Grain or Plant ZnUE (\%)} = 100 \times \frac{\text{Zn content (S, F1, F2, F3, or F4)} - \text{Zn content (C)}}{\text{Zn applied}} \quad (1)$$

where Zn content (S-soil treatment, F1, F2, F3, or F4) and Zn content (C-control treatment) are the grain and plant Zn content at harvest (grain and whole plant, respectively) depending on the particular treatment, and Zn applied is the amount of Zn used in each treatment.

The agronomic efficiency (AE) of Zn application was calculated by dividing grain weight (wheat) or ear weight (maize) into the amount of Zn supplied by each treatment (Ladha et al. 2005).

2.6 Statistical Analysis

The experimental results were separately subjected to factorial analysis of variance (ANOVA) with two factors (soil and Zn treatment) per crop. When the soil × Zn treatment interaction was significant ($P < 0.05$) and for grain (wheat) and ear (maize) P and Zn concentrations, a one-way ANOVA was used to assess the effect of Zn treatments on each individual soil. When differences in the ANOVA were significant ($P < 0.05$), the least significance difference (LSD) test was used to identify differences between soils and treatments. Levene's test was used to check for variance homoscedasticity and logarithmic or square root transformations were applied when needed. All statistical computations were done with the software Statistix v. 10.0 (Analytical Software, Tallahassee, FL, USA).

3 Results

3.1 Plant Growth and Biomass

The only significant differences in plant height or SPAD were those between soils, the values for the plants grown on VER exceeding those for the plants on INC (Table 3). In maize, there was a significant interaction between soil and Zn treatment in SPAD ($P = 0.0002$; Table 3). This interaction is illustrated in Figs. S1A and S1B: relative to unfertilized plants (control), the treatments increased

Table 3 Factorial ANOVA (soil and treatment) for plant height and leaf chlorophyll index (SPAD values) for wheat and maize plants (64 and 79 days after sowing, respectively), and dry weight of the different plant parts at harvest (105 and 85 days after sowing, respectively).

The values shown are means ($n=36$, soil; $n=12$, treatment). Different letters denote significant differences between soils or Zn treatments as per LSD post hoc test ($P<0.05$). Soils: VER, Vertisol; INC, Inceptisol

Wheat		Plant height (cm)	SPAD	Leaf (g)	Stem (g)	Grain number	Chaff (g)	Grain (g)	Plant (g)
Soil	VER	46.9 a	51.8 a	0.29	0.47 a	21.3 a	0.39 a	0.84 a	1.89 a
	INC	43.2 b	25.3 b	0.27	0.35 b	5.9 b	0.19 b	0.26 b	0.89 b
	<i>P</i> -value	0.002	0.000	0.242	0.000	0.000	0.000	0.000	0.000
Treatment	Control	43.0	35.5	0.24 b	0.35	14.6	0.27 b	0.52	1.43 a
	Soil	43.8	40.5	0.29 ab	0.41	13.0	0.28 b	0.54	1.49 a
	Foliar 1	44.4	37.9	0.33 a	0.43	12.3	0.26 b	0.57	1.08 b
	Foliar 2	47.0	41.9	0.27 b	0.43	15.5	0.38 a	0.61	1.57 a
	Foliar 3	47.0	35.4	0.30 ab	0.42	14.4	0.26 b	0.57	1.42 a
	Foliar 4	45.1	40.2	0.25 b	0.42	11.9	0.29 b	0.50	1.35 a
	<i>P</i> -value	0.184	0.091	0.039	0.305	0.630	0.049	0.7020	0.011
Interaction	<i>P</i> -value	0.707	0.238	0.325	0.887	0.623	0.255	0.816	0.082
Maize		Plant height (cm)	SPAD	Leaf (g)	Stem-bottom (g)	Stem-upper (g)	Tassel (g)	Ear (g)	Plant (g)
Soil	VER	78.2 a	27.7	1.44 a	1.88 a	1.71 a	0.07 a	0.92 a	6.01
	INC	69.3 b	23.0	1.10 b	1.31 b	0.92 b	0.04 b	0.14 b	3.42
	<i>P</i> -value	0.000	0.000	0.00	0.000	0.000	0.010	0.000	0.000
Treatment	Control	72.7	23.3	1.33	1.27 b	1.21	0.02 b	0.24	4.08
	Soil	74.7	25.4	1.11	1.54 a	1.32	0.07 a	0.62	4.68
	Foliar 1	73.6	27.0	1.13	1.62 a	1.14	0.08 a	0.44	4.41
	Foliar 2	75.5	25.3	1.30	1.70 a	1.41	0.06 ab	0.57	5.03
	Foliar 3	71.9	26.1	1.22	1.71 a	1.33	0.04 ab	0.59	4.91
	Foliar 4	74.2	25.2	1.25	1.73 a	1.46	0.04 a	0.70	5.19
	<i>P</i> -value	0.156	0.084	0.027	0.004	0.018	0.039	0.000	0.000
Interaction	<i>P</i> -value	0.181	0.000	0.028	0.421	0.005	0.252	0.002	0.021

SPAD in wheat grown on INC—not significantly with soil treatment; however, some treatments (F2, F3 and, especially, F4) had the opposite effect on the plants grown on VER.

Overall, both cereals produced more biomass on VER than on INC (Table 3). Although dry weight in the different plant parts of wheat was only slightly affected by Zn treatment (especially leaf and chaff), F1 resulted in the smallest plant dry weight (mainly in INC; see Fig. S2). In maize, the control plants had the smallest dry weights of stem bottom and tassel (Table 3). Significant interactions between the two factors were observed in leaf, stem upper part (Figs. S1C, S1D, S1D, and S1F), and ear and maize plant dry weights. Whichever the application method, Zn significantly increased ear production in maize grown on VER (Fig. 1A) and also plant dry weight in maize grown on INC (Fig. 1D). However, only F4, followed by F2 and F3, significantly increased plant dry weight relative to control plants in maize grown on VER (Fig. 1C), and no differences in ear production were observed in those grown on INC (Fig. 1D).

3.2 Phosphorus and Zinc Concentrations in Wheat Grain and Maize Ear

Grain P concentrations in wheat were significantly reduced by soil and F2 treatments in VER and INC, respectively (Fig. 2A and B). Also, grain Zn concentrations at harvest were significantly increased in soil, F3 and F2 treated wheat plants (in this sequence) relative to control plants in both soils (Table S1). Based on the results of the one-way ANOVA, however, the differences were only significant in wheat plants grown on VER (Fig. 2C and D). In maize, the treatments caused no substantial changes in ear P or Zn concentrations relative to control plants (Fig. S3). Table S1 shows the results of the factorial ANOVA.

3.3 Phosphorus and Zinc Contents

The P and Zn contents of grains, ears, and total plants were higher in wheat and maize grown on VER than they were in the plants grown on INC (Table S2, Figs. 3 and S4). There were many significant interactions between plant P and Zn contents (see the results of the factorial ANOVA

Fig. 1 Ear and plant dry weight at harvest (85 days after sowing) in maize plants as a function of soil (A and C, VER-Vertisol; B and D, INC-Inceptisol) and treatment: C, no Zn was added; S, Zn was applied at 3 mg kg^{-1} to the soil before sowing; F1, Zn was sprayed at $1.2 \text{ mg plant}^{-1}$ to leaves at leaf development; F2, same as F1 but at the stem extension—one node stage; F3, same as F1 but at stem extension—three nodes stage; F4, Zn was sprayed at a total rate of $1.2 \text{ mg plant}^{-1}$ to leaves at both the stem extension—one node and the three-node stage (one-half each time). The values shown are means together with their standard errors (SE, $n=6$). Different letters denote significant differences between Zn treatments as per an LSD post hoc test ($P < 0.05$)

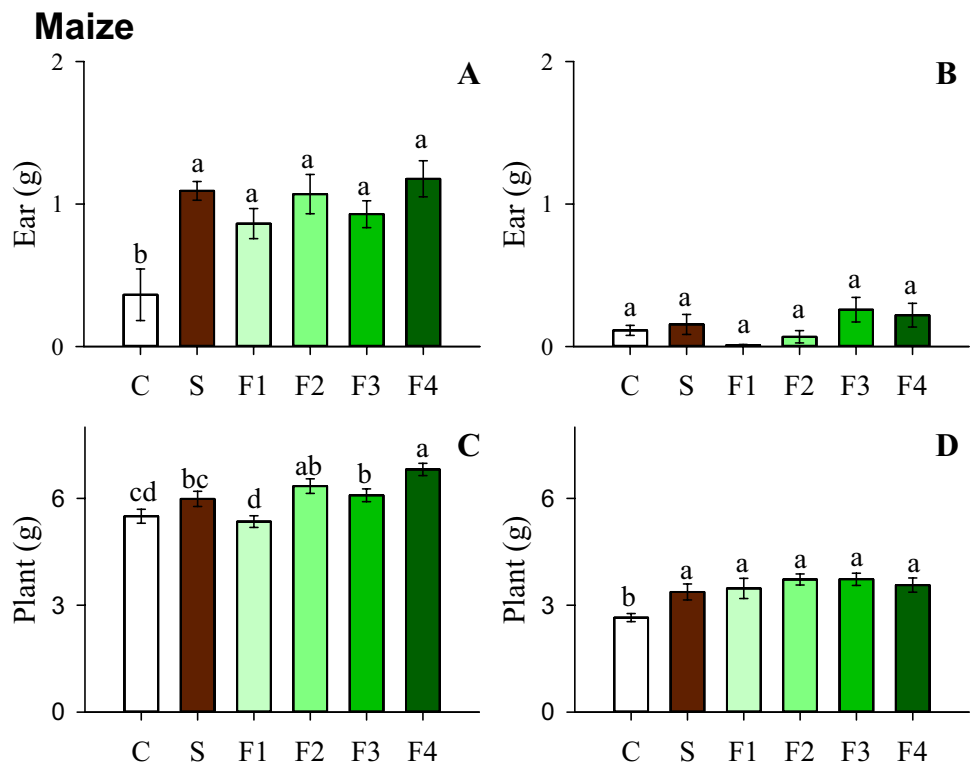
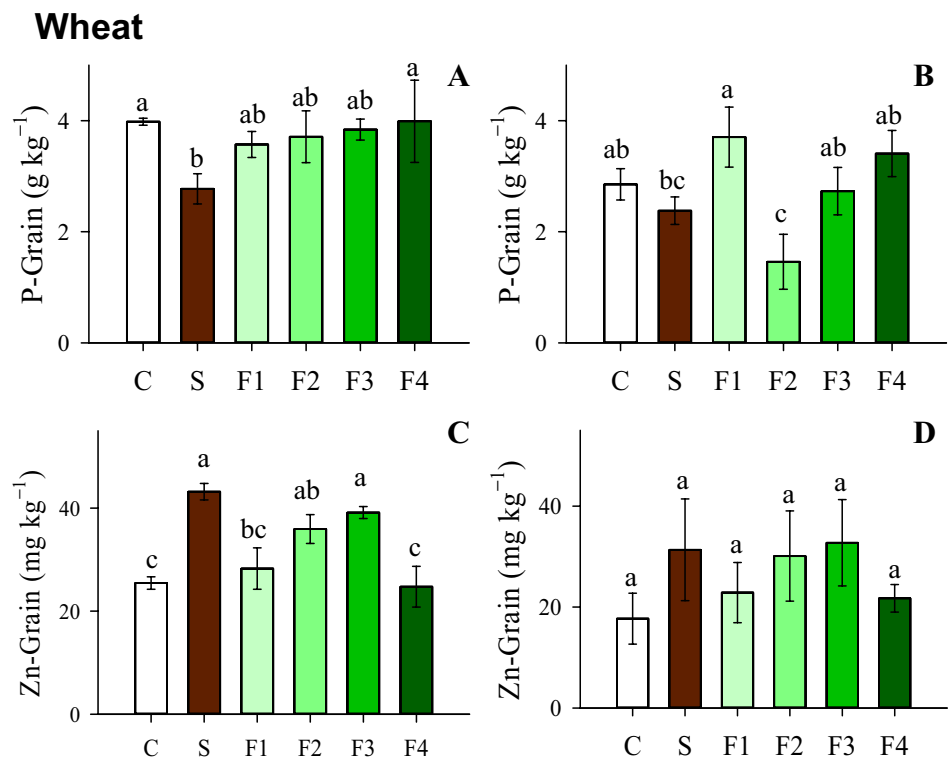


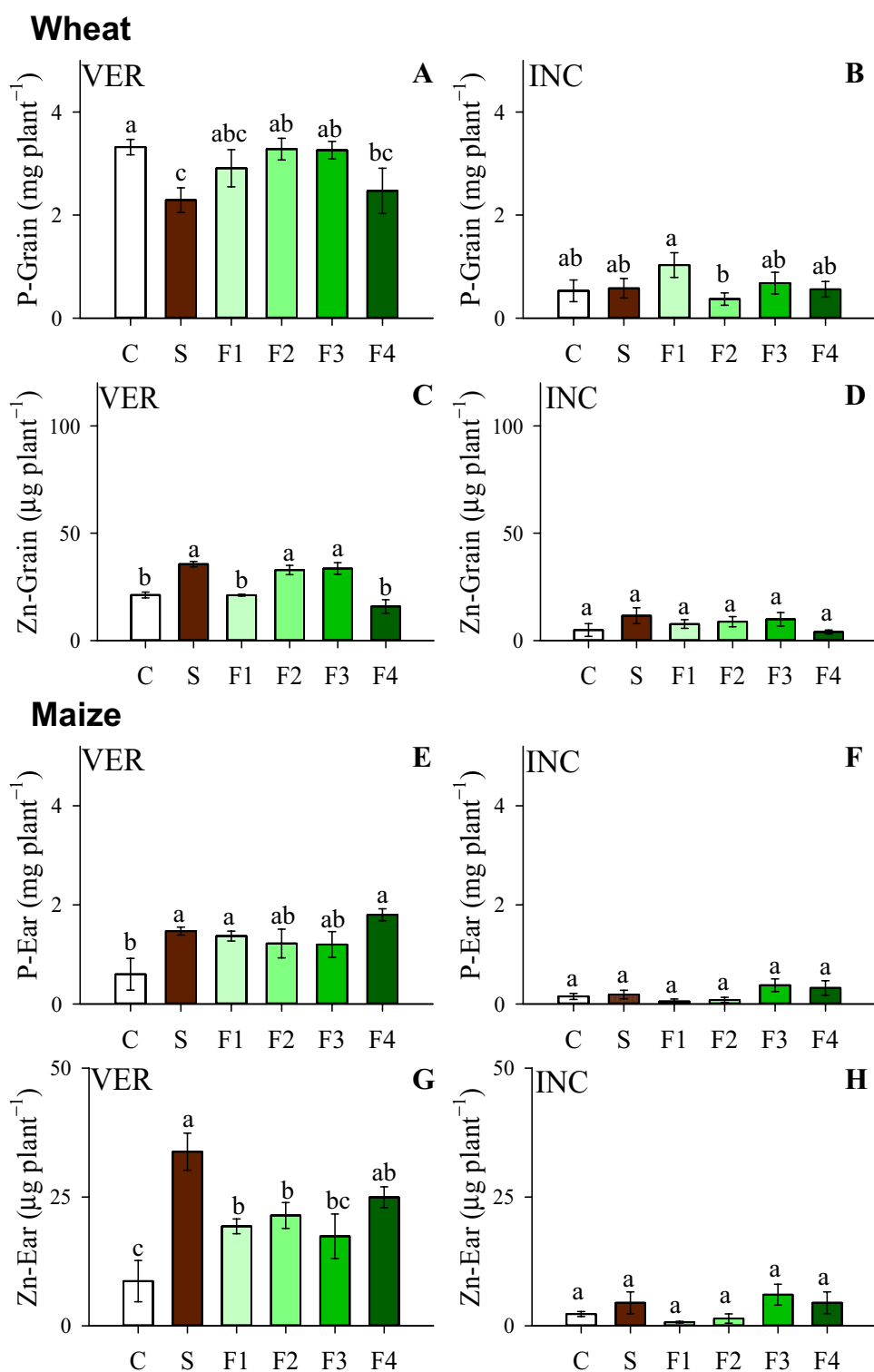
Fig. 2 Grain P and Zn concentrations at harvest (105 days after sowing) in wheat plants as a function of soil (A and C, VER-Vertisol; B and D, INC-Inceptisol) and treatment: C, no Zn was added; S, Zn was applied at 3 mg kg^{-1} to the soil before sowing; F1, Zn was sprayed $0.9 \text{ mg Zn plant}^{-1}$ to leaves at tillering; F2, same as F1 but at the stem extension; F3, same as F1 but at flowering; F4, Zn was sprayed at a total rate of $0.9 \text{ mg Zn plant}^{-1}$ to leaves at both the stem extension and flowering (one-half each time). The values shown are means together with their standard errors (SE, $n=6$). Different letters denote significant differences between Zn treatments as per an LSD post hoc test ($P < 0.05$)



in Table S2). This led us to examine the previous variables separately in each soil and crop (see Fig. 3 for grains and ears and Fig. S4 for plants). All treatments reduced grain P

content (significantly with soil and F4 treatments), and soil, F2 and F3 treatments significantly increased grain Zn content, relative to control wheat plants grown on VER (Fig. 3A

Fig. 3 Grain and ear P and Zn contents at harvest in wheat (A–D) and maize (E–H) plants (105 and 85 days after sowing, respectively) as a function of soil (VER–Vertisol, INC–Inceptisol) and treatment: C, no Zn was added; S, Zn was applied at 3 mg Zn kg^{-1} to the soil before sowing; F1, Zn was sprayed at 0.9 , and $1.2 \text{ mg Zn plant}^{-1}$ to leaves at tillering in wheat and leaf development in maize; F2, same as F1 but at the stem extension–one node stage in maize; F3, same as F1 but at flowering in wheat and stem extension–three nodes stage in maize; F4, Zn was sprayed at a total rate of $0.9 \text{ mg Zn plant}^{-1}$ in wheat and $1.2 \text{ mg Zn plant}^{-1}$ to leaves at both the stem extension and flowering (one-half each time) and at both the stem extension–one node and the three-node stages, respectively (one-half each time). Mean value and SE are shown ($n=6$). Different letters indicate significant differences between Zn treatments according to the LSD post hoc test ($P < 0.05$)



and B). In maize, Zn application increased ear P and Zn contents (P significantly with soil, F1 and F4 treatments; and Zn with soil, F1, F2, and F4 treatments) relative to control plants (Fig. 3E and G). The results in INC were inconsistent because of its limited production (Fig. 3B, D, F, and H). Total plant P content was reduced by soil treatment in wheat

on VER and in maize on both soils, and also by F1 and F2 treatments in maize on both soils, relative to unfertilized plants (control; Figs S4A, S4B, S4E, and S4F). In addition, total plant Zn content was significantly increased by all Zn treatments in wheat and maize grown on VER, and also by F2, F3, and F4 treatments in both plant species grown on

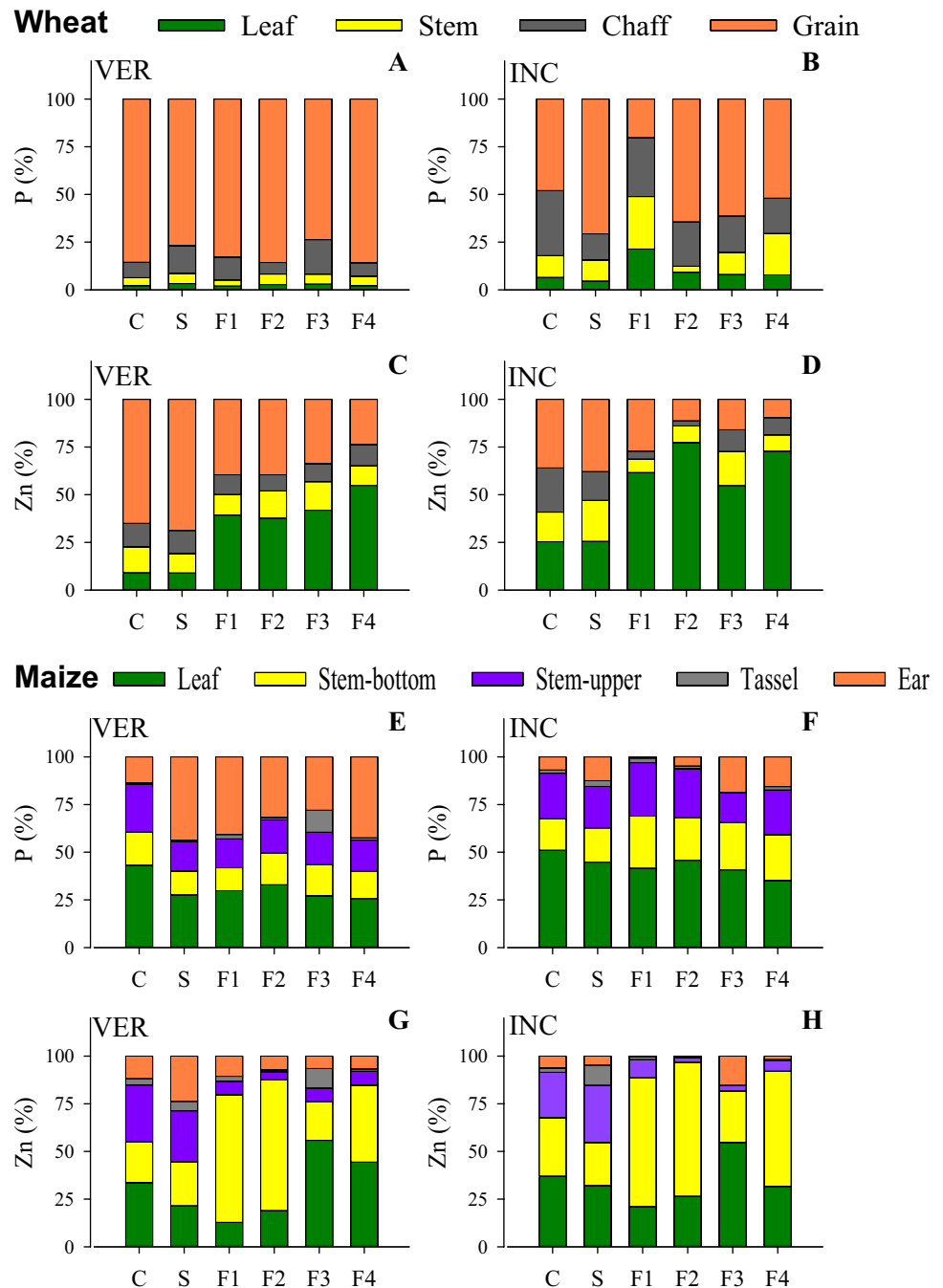
INC, relative to control plants (Figs. S4C, S4D, S4G, and S4H).

3.4 Phosphorus and Zinc Partitioning

Figure 4 shows the P and Zn distribution among plant parts. In general, the P and Zn contents of grains (wheat) and ears (maize) were higher in the plants grown on VER than in those grown on INC. In wheat, P was more markedly allocated to grain (> 75% in VER and 50–70% in INC); by

exception, F1 plants grown on INC accumulated the least amounts of P in grains (Fig. 4A and B). In maize, however, more P was allocated to leaves, followed by ears (Zn-treated plants grown on VER) and/or stems (plants grown on INC) (Fig. 4E and F). Zn distributed similarly in wheat grown on both soils; also, control and soil treatments led to Zn accumulating preferentially in grain (up to 70% in VER and 35–40% in INC) than in other plant parts (around 25–45% in VER and 15–30% in INC; Fig. 4C and D). Zn distribution in maize was more homogeneous. Thus, soil and F3

Fig. 4 P and Zn partitioning at harvest in wheat (A–D) and maize (E–H) plants (105 and 85 days after sowing, respectively) as a function of soil (VER–Vertisol, INC–Inceptisol) and treatment: C, no Zn was added; S, Zn was applied at 3 mg Zn kg^{-1} to the soil before sowing; F1, Zn was sprayed 0.9 , and $1.2 \text{ mg Zn plant}^{-1}$ to leaves at tillering in wheat and leaf development in maize; F2, same as F1 but at the stem extension in wheat and stem extension–one node stage in maize; F3, same as F1 but at flowering in wheat and stem extension–three nodes stage in maize; F4, Zn was sprayed at a total rate of $0.9 \text{ mg Zn plant}^{-1}$ in wheat and $1.2 \text{ mg Zn plant}^{-1}$ to leaves at both the stem extension and flowering (one-half each time) and at both the stem extension–one node and the three-node stages, respectively (one-half each time). Mean value is shown ($n=6$)



treatments resulted in more Zn being allocated to ears in plants grown on VER and INC, respectively. On the other hand, F1, F2, and F4 treatments minimized the Zn contents of ears in maize grown on INC (Fig. 4G and H). Also, foliar treatments led to more Zn accumulating in leaves (wheat) or stems (maize) than in grains or ears.

3.5 Zinc Availability and Use Efficiency

Applying Zn to soil or plant leaves decreased the P/Zn ratio in all plant parts except maize ear (Table 4). Thus, the grain P/Zn ratio in wheat was significantly reduced by soil, F2, and F3 treatments, though not by F1 or F4, relative to control on either soil (Fig. 5A and B). Treatments soil, F2, and F3 led to the highest grain ZnUE values in wheat on VER (Fig. 5C) but resulted in no significant differences in plants grown on INC. In wheat, plant ZnUE and AE were greater in VER than they were in INC, the former parameter peaking with F2 and F3, followed by F4, and then soil treatment and F1 (Table 4). In maize, plant ZnUE was significantly

higher with F2, F3, and F4 than it was with soil treatment and F1. Also, applying Zn to the soil significantly increased ear ZnUE (Fig. 5E) and AE (Fig. 5G) in maize grown on VER relative to the foliar treatments—no differences in this respect were observed in INC, however (Fig. 5F and H).

4 Discussion

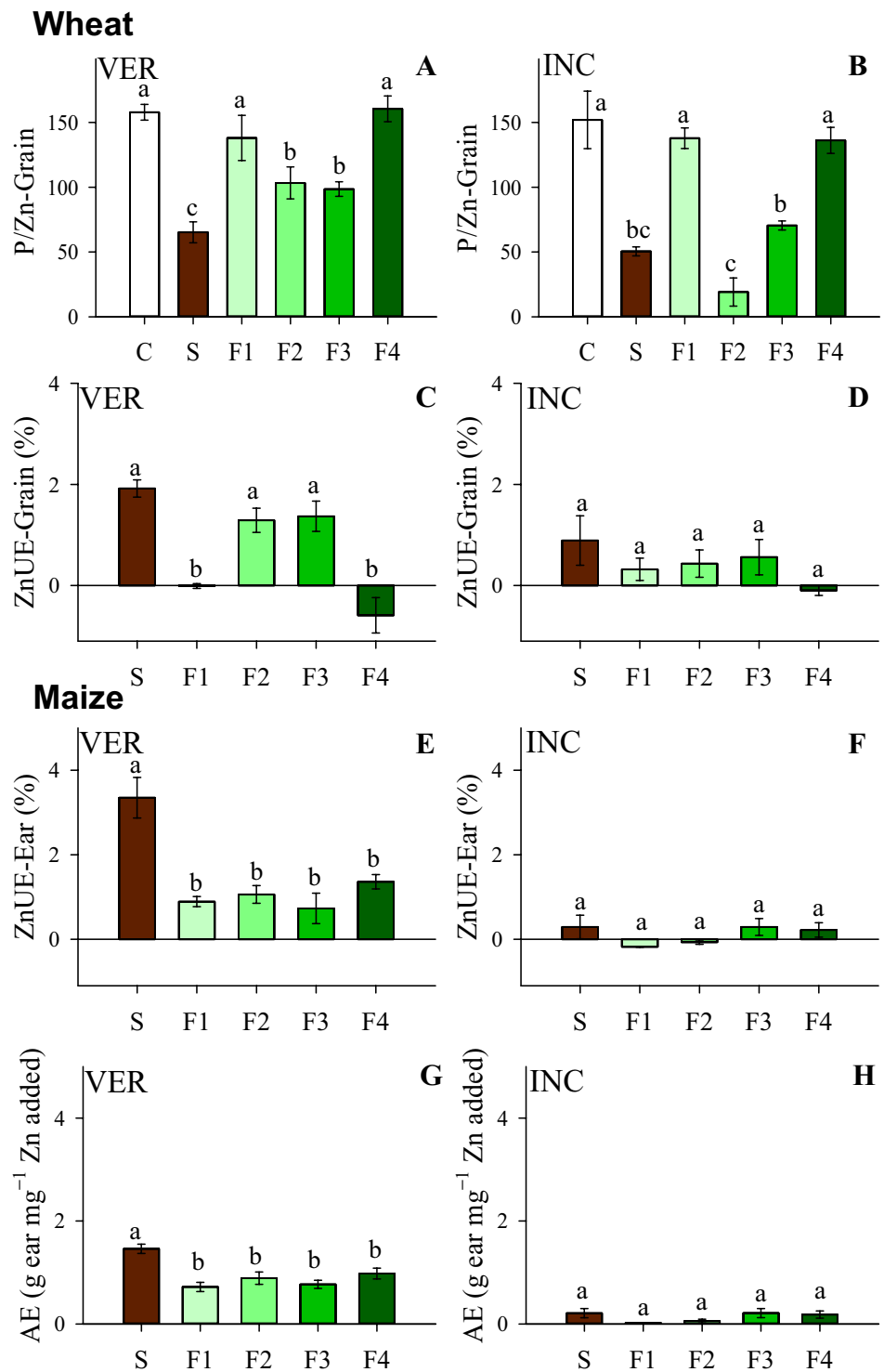
The factor soil played a key role in plant growth and the effects of the different Zn treatments on wheat and maize plants. The two calcareous soils examined had a low content in organic carbon and limited availability of nutrients such as Zn owing to the presence of substantial amounts of CaCO₃ (Alloway 2009). The Inceptisol (INC) had a stronger limiting effect on plant growth (height and biomass) stronger than that of the Vertisol (VER) for both crops probably as the result of its higher content in CaCO₃ (> 500 vs. 297 g kg⁻¹) and lighter texture. Also, the poorer Zn availability in INC must have boosted differences between plants grown on it

Table 4 Factorial ANOVA (soil and treatment) for P/Zn ratio, Zn use efficiency (ZnUE, %, grain/ear, and plant), and agronomic efficiency (AE, g of grain/ear per mg of Zn applied to the soil/plant) in wheat and maize plants at harvest (105 and 85 days after sowing, respectively). The values shown are means ($n=36$, soil; $n=12$, treatment).

Factor	Wheat	P/Zn-leaf	P/Zn-stem	P/Zn-chaff	P/Zn-grain	ZnUE-grain (%)	ZnUE-plant (%)	AE-grain (g/mg Zn)	
Soil	VER	9.68	24.8	64.4	120.6	0.79	4.53 a	0.96 a	
	INC	7.56	27.5	60.6	95.85	0.42	3.59 b	0.32 b	
	<i>P</i> -value	0.189	0.321	0.623	0.000	0.050	0.029	0.000	
Treatment	Control	28.7 a	43.8	99.6 a	155.2	na	na	na	
	Soil	11.2 b	23.6	33.2 cd	59.3	1.40	2.07 c	0.72	
	Foliar 1	5.90 bc	40.8	82.6 ab	138.0	0.15	1.99 c	0.63	
	Foliar 2	1.58 c	15.1	75.6 ab	65.0	0.86	6.47 a	0.67	
	Foliar 3	2.29 c	8.61	26.1 d	85.8	0.96	6.17 a	0.64	
	Foliar 4	2.10 c	24.2	57.0 bc	149.4	0.35	3.59 b	0.55	
	<i>P</i> -value	0.000	0.000	0.000	0.000	0.000	0.000	0.397	
Interaction	<i>P</i> -value	0.978	0.041	0.087	0.008	0.027	0.148	0.558	
Factor	Maize	P/Zn-leaf	P/Zn-stem (bottom)	P/Zn-stem (upper)	P/Zn-tassel	P/Zn-ear	ZnUE-ear (%)	ZnUE-plant (%)	AE-ear (g/mg Zn)
Soil	VER	32.4	15.3	36.0 b	11.6	64.6	1.48	16.60	0.96
	INC	27.5	11.1	43.6 a	19.4	56.7	0.11	15.04	0.14
	<i>P</i> -value	0.084	0.022	0.022	0.000	0.220	0.000	0.295	0.000
Treatment	Control	78.3 a	40.8	57.9 a	21.3	65.4	na	na	na
	Soil	27.2 c	13.4	13.2 c	4.92	45.0	1.82	7.89 b	0.83
	Foliar 1	43.0 b	6.49	48.6 a	21.9	68.6	0.35	7.07 b	0.36
	Foliar 2	18.0 d	2.22	52.1 a	14.2	60.8	0.50	21.59 a	0.47
	Foliar 3	5.74 d	13.1	37.2 b	16.0	68.4	0.52	19.48 a	0.50
	Foliar 4	7.50 d	3.47	29.1 b	13.1	69.0	0.79	23.09 a	0.58
	<i>P</i> -value	0.000	0.000	0.000	0.000	0.287	0.000	0.000	0.000
Interaction	<i>P</i> -value	0.630	0.006	0.126	0.000	0.946	0.000	0.113	0.001

Different letters denote significant differences between soils or Zn treatments as per LSD post hoc test ($P<0.05$). Soils: VER, Vertisol; INC, Inceptisol. na, non-available because this value is used to calculate ZnUE and AE for the rest of Zn treatments

Fig. 5 P to Zn ratio (A, B) and zinc use efficiency in grain (ZnUE-Grain, C, D) at harvest in wheat plants (105 days after sowing), and ZnUE-Ear (E, F) and agronomic efficiency (AE, G, H) at harvest in maize plants (85 DAS) as a function of soil (VER-Vertisol, INC-Inceptisol) and treatment: C, no Zn was added; S, Zn was applied at 3 mg Zn kg⁻¹ to the soil before sowing; F1, Zn was sprayed 0.9 and 1.2 mg Zn plant⁻¹ to leaves at tillering in wheat and leaf development in maize; F2, same as F1 but at the stem extension in wheat and stem extension–one node stage in maize; F3, same as F1 but at flowering in wheat and stem extension–three nodes stage in maize; F4, Zn was sprayed at a total rate of 0.9 mg Zn plant⁻¹ in wheat and 1.2 mg Zn plant⁻¹ to leaves at both the stem extension and flowering (one-half each time) and at both the stem extension–one node and the three-node stages, respectively (one-half each time). Mean value and SE are shown (n=6). Different letters indicate significant differences between Zn treatments according to the LSD post hoc test (P < 0.05)



and on VER. According to Sacristán et al. (2019), the soil Zn phytoavailability decreases with increasing P_{Olsen}/Zn_{DTPA} . Such ratio was higher in INC than in VER (110 vs. 40), which explains the poor performance of the former relative to the latter soil.

The results observed in this study confirm our first hypothesis, namely: that the effects of the Zn treatments

would be species dependent and more apparent in plants grown on the less limiting calcareous soil (VER). Because Zn is involved in chlorophyll production, its deficiency impairs plant growth and crop yield (Cakmak et al. 1999). In fact, Zn application to leaf in treatments F2, F3, and F4 decreased the leaf chlorophyll content of maize 79 DAS in plants grown on VER but increased it with all

foliar treatments in plants on INC. This may have resulted from a dilution effect in maize on VER—which exhibited increased plant biomass production with *F2* and *F4*—that did not occur in INC. Chlorophyll production was increased in plants grown on INC as a result of foliar treatments; by contrast, a sizeable portion of Zn applied to the soil (treatment S) was rendered not phytoavailable by reactions of the metal in the calcareous environment (Alloway 2009) (e.g., adsorption onto CaCO_3 and Fe oxide surfaces or precipitation with P). In line with that, Zn fertilization enhanced plant growth and increased potential yield (ear) in maize but not in wheat, those effects being more marked in the plants grown on INC and VER, respectively. The increase in maize plant dry weight with *F2*, *F3*, and *F4* (11–24% regarding control plants) in VER was concomitant with the peak observed in plant ZnUE. Ear production in maize grown on VER (but not on INC) was also increased between 138 (*F1*) and 227% (*F4*) for all Zn treatments, including soil treatment, in comparison with control plants. Zn fertilization of maize increases yield and/or biomass production more frequently than it results in biofortification (Manzeke et al. 2014; Imran and Rehim 2017; Liu et al. 2020a, b; Sánchez-Rodríguez et al. 2021b), which agrees with our results. Although durum wheat and maize are sensitive to Zn deficiency, the latter species seemingly require higher soil Zn contents to grow well (Singh et al. 1987; Martens and Westermann 2018). Liu et al. (2019) previously found applying Zn at rates above 20 kg ha^{-1} to soil to significantly increase wheat growth and yield, but, consistent with our results, Sánchez-Rodríguez et al. (2017) observed no effect of Zn fertilization in pot experiments and nor did Zou et al. (2012) and Zou et al. (2019) in field experiments. Recently, Sánchez-Rodríguez et al. (2021a) reported significantly increased wheat yields in one out of eleven field experiments after a single event of soil Zn fertilization of calcareous soils similar to those examined here. Also, wheat yield can be expected to rise by the effect of Zn fertilization in soils with an extremely low Zn availability ($< 0.25 \text{ mg Zn kg}^{-1}$; Cakmak et al. 1996).

Interestingly, splitting the Zn dose between two different phenological stages (treatment *F4*) did not have a similar effect on wheat and maize. It resulted in the highest ear and maize plant dry weight in VER, the effect on dry weight being even stronger than that of *F3*. However, *F4* had no effect on wheat growth or yield, as observed by Sánchez-Rodríguez et al. (2021a) in field experiments on similar calcareous soils. The greater size (and, especially, foliar area) of maize relative to wheat may have prevented a toxic effect of Zn on early sprayed maize plants (*F1*) and facilitated Zn absorption and translocation to ears when applied at two phenological stages (*F4*).

Additionally, spraying Zn to the aerial part at the tillering stage (treatment *F1*) decreased wheat yield by 25% relative to the control plants. These plants (treatment *F1*) exhibited

a decreased weight at harvest as the likely result of a toxic effect of Zn (Broadley et al. 2007) (small plants at an early phenological stage receiving substantial amounts of the metal). These results highlight the importance of choosing the appropriate Zn fertilization method for each crop.

Furthermore, Zn application also influenced crop quality and plant zinc content. Grain/ear Zn content and ratio P/Zn were affected by Zn treatment, mainly in wheat plants, as only slight effects were seen in maize. The grain Zn concentrations of wheat plants not fertilized with Zn (control) were 25.5 mg kg^{-1} in VER and 17.7 mg kg^{-1} in INC [too low for human nutrition (Cakmak et al. 2010; Cakmak and Kutman 2018)]; also, the difference between the two soils can be ascribed to the fact that available Zn was higher in VER than it was in INC. Although grain Zn concentrations were increased by application of Zn to the soil treatment and the aerial part of wheat at later phenological stages (*F2* and *F3*) in both soils, the differences between treatments were significant only in VER. Plants were grown on this soil produced grain whose Zn concentrations were above or near the recommended values for biofortified wheat [$> 37.0 \text{ mg Zn kg}^{-1}$, Cakmak et al. (2010); i.e., soil Zn treatment, $43.2 \text{ mg Zn kg}^{-1}$; foliarly sprayed with Zn at stem extension, $35.9 \text{ mg Zn kg}^{-1}$, or flowering, $39.1 \text{ mg Zn kg}^{-1}$]. These treatments increased this grain quality variable by 41% (*F2*) to 69.5% (soil treatment) relative to the control plants. Treatments soil, *F2*, and *F3* increased not only grain Zn concentration in plants grown on VER but also grain Zn availability in wheat grown on either soil, by the effect of the treatments significantly decreasing the grain P/Zn ratio concomitantly with the highest grain ZnUE value in VER. By contrast, Zn foliar spraying at tillering and splitting applied Zn between stem extension and flowering were both ineffective to increase grain Zn content (28.3 and $24.7 \text{ mg Zn kg}^{-1}$, respectively, in VER) and to reducing the P/Zn ratio in grain. Again, a reduced foliar area (*F1*, small wheat plants) and application of an identical amount of Zn at two different phenological stages in wheat (*F4*) should have resulted in increased Zn losses relative to the other foliar treatments. No effects on Zn concentration or P/Zn ratio in maize ear were observed irrespective of Zn treatment. This result can be ascribed to a dilution effect (Cakmak et al. 1999; Sánchez-Rodríguez et al. 2017) increasing ear (VER) or biomass production (INC) relative to the control plants. The differential response of the two cereals to Zn fertilization (viz., crop biofortification in wheat, and plant growth and increased potential yield in maize) is consistent with previous reports (Cakmak and Kutman 2018; Rehman et al. 2021). Indeed, maize is a C4 plant with greater Zn requirements than wheat—a C3 plant and, hence producing less biomass. Also, previous experiments involving ^{70}Zn and fluorescence microscopy showed wheat to be superior to maize in terms of leaf penetration of Zn, Zn uptake, and translocation of Zn sprayed to leaves

(Rehman et al. 2021). Therefore, the fact that the response to Zn fertilization was soil- and plant-dependent confirms our first hypothesis.

The second hypothesis was that applying Zn to the soil would reduce plant P uptake. This was supported by our results in wheat (decrease in plant P content in VER resulting from the application of Zn to the soil or spraying it at two different phenological stages and in plants grown on INC and subjected to foliar Zn spraying at stem extension) and maize (P contents were reduced in both soils when Zn was applied to the soil or at early growth stages, F2). We would like to remark that the adverse effect on wheat P uptake as a result of splitting the Zn dose has never been observed previously under similar conditions. In addition, P translocation to grain in wheat was also impaired by soil Zn application in VER, and by foliar Zn spraying at stem extension (F2) in INC. This could have occurred because P and Zn in soil react to form insoluble compounds that are coadsorbed onto mineral constituents (Agbenin 1998; Diaz-Barrientos et al. 1990; Pérez-Novo et al. 2011), thereby reducing uptake and translocation to aerial plant parts (Marschner 2011). Also, a negative interaction between P and Zn within the plant could explain the negative effect of Zn application on total P content at harvest, and the decreased proportion of P allocated to grains (P partitioning) in F1 and/or F2 treated plants grown on INC. This was less apparent in VER and with foliar Zn application at late phenological stages (both crops and soils, F2 and F3 in wheat, and F3 and F4 in maize) and suggests that these treatments minimized the effects on P nutrition in the plants. Also, Sánchez-Rodríguez et al. (2021b) previously observed an imbalance in P and Zn absorption and translocation in maize plants grown on calcareous soils receiving only one of the nutrients. The imbalance detracted from maize growth, ear production, translocation of the two nutrients to grains, and grain quality (Zn availability included). This is consistent with the results of He et al. (2021) for alfalfa grown on calcareous soil, and it partially supports our third hypothesis (Zn fertilization would alter P translocation).

Our results support our third and fourth hypotheses, i.e. that Zn fertilization would alter P and Zn translocation, the effectiveness of the Zn application strategy in terms of plant growth and crop biofortification, P and Zn translocation and partitioning, Zn availability in edible plant parts, and Zn use efficiency is species-dependent. Based on the results shown here, foliar Zn treatments at a late phenological stage—if applied near flowering—are to be preferred for wheat, and so is Zn application to the soil for maize. Some related aspects, however, are worth of note. Thus, late foliar applications of Zn resulted in the highest use efficiency for this nutrient; in fact, as reflected in Zn contents, more Zn was absorbed by the plants and higher plant ZnUE and, generally, lower P/Zn ratios, were obtained as a result. Consistent with previous

results of Rehman et al. (2021), most Zn applied to aerial plant parts accumulated in leaves and stems (> 50 and $\geq 75\%$ with the foliar treatments on wheat and maize, respectively), thereby facilitating Zn translocation to grain during the reproductive stages (Cakmak 2008; Sánchez-Rodríguez et al. 2021a). However, the control plants, and those on soil supplied with Zn, accumulated higher amounts of the absorbed Zn in grain (see Zn partitioning), the latter result being a consequence of increased ZnUE values in grain ear and also of increased AE values—the effects being more marked in wheat than in maize and in VER than in INC. The adverse effect of soil Zn application on P uptake in wheat and maize observed here is not a common occurrence in field experiments except for other cereals such as rice (Ding et al. 2021). Ueda et al. (2021) suggested that Zn modulates P uptake in plants under Zn deficient conditions, but this interaction is complex. In addition, there are multiple studies on the cross-talks between macro and micronutrients, including P and Zn, because this knowledge is vital to improving the efficiency in agroecosystems (reviewed in Fan et al. 2021).

Finally, our results warrant discussion in terms of spatial and soil limitations in pot experiments. Although the rate of Zn applied to soil was 3 mg kg^{-1} , equivalent to a single application of $7.5 \text{ kg Zn ha}^{-1}$, its effects on soil P availability and plant P and Zn uptake were probably maximal because of Zn being uniformly sprayed to the soil. Under field conditions, Zn concentrations after soil fertilization will decrease with increasing depth and similar effects on crops can be expected following several applications of Zn and/or tillage to mix the top 15–30 cm of soil. Also, plant roots can explore other soil horizons if P availability is reduced by Zn concentration resulting in the precipitation of Zn phosphates.

5 Conclusion

The effects of Zn fertilization in plant growth, yield, and quality depended on the Zn treatment, plant species (wheat or maize), and soil fertility. Soil treatment and foliar Zn spraying at stem elongation or flowering are the recommended treatments for wheat biofortification as they led to the highest grain Zn concentrations, lowest P/Zn ratio in the grain—and hence increased Zn availability for human and animal diets—and, most important in agronomic and environmental terms, very high Zn use efficiencies. On the other hand, early foliar treatments could result in Zn toxicity to wheat and reduce P translocation to grains. Splitting Zn application to leaves between two different phenological stages is seemingly an effective strategy for maize; in fact, together with soil application and/or late foliar Zn spraying, it maximized potential yield and plant biomass. However, foliar Zn treatments have some disadvantages such as increased costs (especially when doses are split between two

or more stages), technical complexity, and potential mechanical damage to crops at stem extension or a later stage under field conditions. For these reasons, building up available Zn by application to soil appears to be the most realistic strategy for maize. Sustainable strategies for wheat and maize cultivation on calcareous soils should specifically consider plant species, soil fertility, and the potential effect of Zn treatments on P–Zn interactions in soil and within plants (viz., accumulation and translocation of these nutrients to edible parts).

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Availability of Data and Material The data are available under request.

Code Availability Not applicable.

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors declare no competing interests.

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