Soil and foliar zinc biofortification of broccolini: effects on plant growth and mineral accumulation

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Abstract

Millions of people have Zn-deficient diets and Zn-biofortified crops could prevent such deficiency. The aim of this study was to evaluate the use of agronomic Zn biofortification of broccolini, a new hybrid crop variety derived from a cross between kalian cabbage and broccoli. Plants were grown in pots using a Zn deficient soil. Four fertiliser treatments were tested: (1) control; (2) soil application of 5 mg $ZnSO_4.7H_2O$ kg^{-1} soil; (3) foliar application at the early flowering stage of 0.5% (w/v) $ZnSO_4.7H_2O$; (4) combined soil and foliar treatments. Florets were harvested in four sequential harvests. There was a decrease in both growth and leaf composition of Zn, Ca, Fe and Mg. Soil Zn application increased floret production. There were increases in the Zn concentration stem+leaves and florets of 12- and 2.5-fold in foliar and soil+foliar treatments, respectively. PA:Zn molar ratios decreased under both foliar and soil+foliar treatments. Boiling reduced Zn concentration by 40%, along with a decrease of other mineral nutrients. A soil+foliar treatment can increase both plant growth and Zn concentration in broccolini, and boiled 100 g portion of biofortified florets fertilized at rates in this study would deliver ~49 mg Zn, a 46% increase than in the non-biofortified broccolini.

Keywords: Zinc, Brassica, Bioavailability, Nutrient uptake, Phytate

Introduction

Zinc (Zn) deficiency affects about 17% of the world's population and is one of the most common micronutrient deficiencies (WHO 2016). It has been estimated that up to 0.5 million children under five years of age die from causes related to Zn deficiency each year (Krebs et al. 2014). Although Zn deficiency is more common in Low and Middle Income countries, it is also found in High Income countries such as Spain. For example, Sanchez et al. (2009) found that 56% of the Spanish population had intakes less than 10 mg day^{-1}, with 15 mg day^{-1} being the Recommended Dietary Intake (RDI; FAO/WHO, 2000). Dietary Zn deficiency has often been attributed to agricultural production on soils with little phytoavailable Zn (Alloway 2008) which can lead to reductions in the Zn concentrations in their edible parts and also poor yield (Cakmak et al. 2010; Gomez-Coronado et al. 2016). In Zn-deficient soils, agronomic biofortification has been shown as a potentially effective way to increase Zn concentration in major crop types including cereals (Cakmak et al. 2010; Gomez-Coronado et al. 2016) and legumes (Rafique et al. 2015; Poblaciones and Rengel 2017). Zinc sulphate is the most widely used fertilizer demonstrating an effective increase in production when applied to the soil and increasing Zn accumulation when applied as a foliar spray (Cakmak et al. 2010; Hussain et al. 2012; Gomez-Coronado et al. 2016).

Brassica crops are an excellent dietary source of mineral and trace elements, vitamin and other organic compounds, including Zn (Moreno et al. 2006; Broadley et al., 2008; 2010; Francisco et al. 2017). In part due to their perceived health benefits, the consumption and production of Brassica crops has increased considerably in Spain. For example, broccoli consumption was 1.8 kg per capita per year and with a production >40,000 ha (MAPA 2018). Despite its high nutritional value, broccoli is not fully accepted due to its specific aroma and taste. For this reason, seed breeders are trying to develop varieties with milder flavours. One of them is the hybrid between kalian (*Brassica oleracea*, also known as Chinese kale or Chinese broccoli) and broccoli (*Brassica oleracea* var. *italica* L.) (Martinez-Hernandez et al. 2013a). It is commercially known as Bimi®, Tenderstem®, Vellaverde® or Broccolini®. The main physiological difference with broccoli, cauliflower or cabbage that the harvest is staggered and not just one at the end of the growth cycle. In countries such as Spain, where Brassica crops have experienced one of the largest increases in area in recent years, the cultivation of this hybrid could be economically valuable, since its price in the market is much higher.

Brassica crops are generally rich in Zn, ranged widely between them: values between 21 to 66 mg Zn kg^{-1} were found in broccoli (Kaluzewicz et al. 2016; Slosar et al. 2017), and around 70 mg Zn kg^{-1} in broccolini (Martinez-Hernandez et al. 2013a). Furthermore, the phytic acid (PA) concentration, which is one of the most important antinutrients, is relatively low in Brassica crops, as Ogbede et al. (2015) found in cabbage (2.2-3.0 g kg^{-1}). Phytic acid can inhibit intestinal Zn absorption because it forms stable complexes with minerals including Ca, Fe, Mg and Zn (Walter et al. 2002; Wang et al. 2009).

There is limited information on agronomic Zn biofortification of Brassica crops in the literature. Slosar et al. (2017) found increases of 8-18% with foliar Zn application. White et al. (2018) explored the potential limits to Zn biofortification in cabbage and broccoli before yield penalties occurred and identified a wide range of critical shoot Zn concentrations of between 74 and 1666 mg Zn kg^{-1}. The aim of this study was to determine the effect of soil and foliar agronomic Zn biofortification on the yield and Zn concentration of a broccolini hybrid, including effects on PA:Zn molar ratios and the retention of Zn after cooking.

Materials and methods

Experimental design and crop management

Plants were grown between 31st October and 27th February 2018 in a naturally-lit greenhouse at School of Agronomy Engineering, Extremadura University, Badajoz, Spain (38°89′ N, 6°97′ W; 186 m above sea level). During the experiment, the greenhouse temperature was 18 ± 6 ºC during the day and 13 ± 3 ºC during the night, with a relative humidity between 62% (midday) to 82% (midnight).

A Zn-deficient sandy soil was collected from the area of Tierra de Barros region in Western Spain (38°88’ N, 7°04′ W). The soil was air-dried and sieved to <5 mm. Four subsamples of the sieved soil were analysed for various physico-chemical properties. The soil had pH of 6.5 ± 0.1 (mean ± standard error) determining with a calibrated pH meter (10 g soil: 25 mL deionised H_2O), organic carbon 2.8 ± 0.1 g kg^{-1} (Walkley-Black method), nitrate nitrogen 1.3 ± 0.1 mg kg^{-1}, ammonium nitrogen 2.7 ± 0.2 mg kg^{-1} (extracted with 1 M potassium chloride for 1 h at 25 ºC and measured on a Lachat Flow Injection Analyzer), available phosphorus 15 ± 0.4 mg kg^{-1} and potassium <15 ± 0.5 mg kg^{-1} (Colwell method). Plant-available Zn was 0.35 ± 0.03 mg kg^{-1} by extraction with DTPA (diethylenetriamine pentaacetic acid) (Lindsay and Norwell 1978), and the extracted Zn was determined by inductively-coupled plasma mass spectrometry (ICP-MS), as described for stem+leaves and florets samples below. A Brassica Laboratory Reference Material (LRM) and blanks were included in each batch of samples. All the results were reported on a dry weight basis.

Seeds of broccolini cv. Broccolini Rapini were sown in a seedbed containing commercial substrate after being surface-sterilised by soaking in 80% v/v ethanol for 60 s and washing thoroughly with deionised water. Four weeks after sowing, plants were transplanted to 30-cm-high and 30-cm-diameter free-draining pots containing 8.5 kg soil. To ensure Zn was the only nutrient limiting the growth, the following basal nutrients (in mg pot^{-1}) were added, followed by a thorough mixing: 767 KH_2PO_4; 1189 K_2SO_4; 341 $MgSO_4.7H_2O$; 809 NH_4NO_3; 1278 $CaCl_2.2H_2O$; 85 $MnSO_4$-H_2O; 17 $CuSO_4.5H_2O$; 4.3 $CoSO_4.7H_2O$; 1.7 $Na_2MoO_4.2H_2O$, 6.0 H_3BO_3. Soil Zn treatments (see below) consisted of spraying Zn sulphate solution to the soil surface. After the application of basal nutrients and Zn, the soil in each pot was thoroughly mixed. Extra application of 809 mg per pot NH_4NO_3 was applied after every three weeks to avoid N deficiencies. During plant growth, plants were watered with deionised water every two days to maintain 60% of the water holding capacity. There were no incidences of pests or diseases during the experiment.

The experiment was arranged in completely randomized block design with four Zn treatments and four replicates. The Zn treatment consisted of: no Zn application (control); soil application of 5 mg $ZnSO_4.7H_2O$ kg^{-1} (soil); foliar application at the early beginning of flowering of 15 mL pot^{-1} of distilled water spray with 0.5% (w/v) $ZnSO_4.7H_2O$ (foliar); and the combination of the soil and foliar applications (soil+foliar). Foliar Zn treatments were applied in the late afternoon; spraying continued being all the leaves are covered.

Plant material analysis

Florets were harvested sequentially after the first florets had matured (at the end of January, eight weeks after sowing) and once more each week for a total of four harvests. At each harvest, the number of florets, average floret height, weight, and total floret weight was determined. Florets were washed with running deionised water over a mesh and rinsing with deionised water three times, and then lyophilized at -58 ºC. Samples were split so that nutrient composition (Zn, Ca, Fe, Mg, phytic acid and their respective molar ratios) could be analysed in both raw and boiled florets (boiled for 5 min in 400 mL of deionised water in Pyrex flasks).

At the end of the plant growth, the whole plant (stem+leaves) was harvested just above the soil surface and washed with running deionised water over a mesh and rinsing with deionised water three times. Plant height and weight of stem+leaves were measured and total number of florets, their average and total weight were also calculated. Stem+leaves were dried at 60°C for 72 hours in an oven until constant weight, and weighed.

Total Zn, Ca, Fe and Mg concentrations were determined in plants (stem+leaves), florets and boiled florets (Thomas/Alcock, method ref). Accurately weighed powdered samples (each approx. 20 mg DM) were digested using a microwave system (Anton Paar Gmbh, Graz, Austria) using a mix of 2 mL 70% Trace Analysis Grade (TAG) HNO_3, 1 mL Milli-Q water (18.2 MΩ cm; Fisher Scientific UK Ltd, Loughborough, UK), and 1 mL H_2O_2. Two operational blanks and two samples of certified reference material (CRM: tomato leaf SRM 1573a NIST, Gaithersburg, MD, USA) were included approximately in each digestion run. Following digestion, each tube was made up to a final volume of 15 mL by adding 11 mL Milli-Q water, then transferred to a 25 mL universal tube (Sarstedt Ltd., Nümbrecht, Germany) and stored at room temperature. Samples were further diluted 1:5 with Milli-Q water into 13 ml tubes (Sarstedt Ltd.) prior to analysis by ICP-MS (Thermo Fisher Scientific iCAPQ, Thermo Fisher Scientific, Bremen, Germany). The Zn-specific recovery from CRMs was 95% compared with certified CRM values. Nitrogen content was determined separately in stem+leaves, florets and boiled florets by using Kjeldahl method using a Kjeltec system.

To estimate the bioavailability of Zn, Ca, Fe and Mg, phytic acid (PA) was determined in the whole shoot (stem+leaves), and in raw and cooked florets using a PA-total phosphorus assay kit (Megazyme, County Wicklow, Ireland). Duplicate samples of a certified reference material provided by the kit (oat flour) were included in every 20 samples. Phytic acid to Zn, Ca, Fe and Mg molar ratios were estimated using a 65% grain P conversion ratio and subsequently dividing by the respective Zn, Ca, Fe and Mg concentrations.

Statistical analysis

Soil Zn-DTPA and whole shoot (stem+leaves) determinations were subjected to one-way ANOVA based on Zn treatment (control, soil, foliar and soil+foliar). Average floret height and weight, number of florets and total floret weight in each harvest, as well as Ca, Fe, Mg, Zn, and PA concentration and molar ratios in raw and cooked florets were subjected to two-way ANOVA based on Zn treatment, harvest (week 8, week 9, week 10 and week 11 after sowing) and their interaction. To test for significant differences, treatment means were compared using Fisher's protected least significant difference (LSD) test at $P<0.05$. The hypotheses of normality and homoscedasticity were determined by Kolmogorov-Smirnov and Levene's tests, respectively. All analyses were performed using Statistix v. 8.10 for Windows (Analytical Software, Tallahassee, FL, USA).

Results

Zinc application significantly increased DTPA-extractable soil Zn from 0.39 mg kg^{-1} to 1.35 and 1.28 mg kg^{-1} from control, to soil and soil+foliar treatment, respectively (Table 1).

Broccolini plant growth and nutrient composition

Zinc application significantly affected shoot weight, Zn concentration and PA:Zn molar ratio (Table 1). Plant weight (stem+leaves) was significantly higher in both soil and soil+foliar treatments. Mean plant height was 44.6 ± 3.3 cm (mean ± SE), with 6.1 ± 0.7 florets of 0.323 ± 0.04 g DM from a total biomass of 1.85 ± 0.26 g D.M. (Table 1). Zinc concentration in shoots (stem+leaves), increased significantly when foliar Zn was applied, in both foliar and soil+foliar treatments. Zinc concentrations were 12.8- and 6.1-fold greater than control and soil Zn treatments, respectively with 9 and 19 mg Zn kg^{-1}, respectively (Table 1). Zinc bioavailability expressed as the PA:Zn molar ratio, was significantly lower when Zn was applied, especially in the foliar and soil+foliar treatments (Table 1). The mean concentrations of other nutrients was not significantly influenced by Zn applications, and were 22.1 ± 0.7 g Ca kg^{-1}, 33.7 ± 15.2 mg Fe kg^{-1}, 11.5 ± 1.8 g N kg^{-1} and 2.9 ± 0.7 g Mg kg^{-1}. The mean PA concentration in the stem and leaves was 1.8 ± 0.1 mg kg^{-1}, resulting in PA to Ca, Fe and Mg molar ratios of 0.005 ± 0.001, 0.48 ± 0.1 and 0.023 ± 0.005, respectively (Table 1).

Broccolini floret growth

Floret height was significantly affected by Zn application, with the soil+foliar application resulting in the tallest florets (Table 2). The number of florets, their average weight and total floret weight were affected by harvest. While the number of florets was almost constant until the last harvest, with ~5 florets per harvest, the number of florets was significantly greater in the final harvest, with 8.9 florets. Floret weight decreased in the sequence Harvest 1 > Harvest 2 = 3 > Harvest 4, from 0.51 g at the first harvest to 0.20 g at the final harvest. The interaction effect of Zn treatment*harvest was only statistically significant for total floret weight (Table 2). Total floret weight in the first harvest was up to 1.7-times greater in the soil and soil+foliar treatments than in the control treatment (Table 2).

Raw broccolini floret nutrient composition

Zinc application significantly influenced the raw broccolini floret composition of the studied nutrients (except N). Soil+foliar Zn application resulted in the largest Zn concentration (96.1 mg Zn kg^{-1}), similarly for Ca (5.8 g Ca kg^{-1}) and Fe (57.4 mg Fe kg^{-1}) concentration. Harvest influenced all the nutrients, in general the earlier harvests had greater nutrient concentrations than later harvests (Figure 1). The interaction of Zn treatment*harvest was statistically significant for raw broccolini floret Ca, Fe and Zn composition (Figure 1). Floret Zn concentration decreased from 153.5 and 166.6 mg Zn kg^{-1} in soil+foliar and foliar in the first harvest to 102.6 and 100.8 mg kg^{-1} in the second harvest. However, the sharpest decline was from harvest two, decreasing up to 62.9 and 67.6 mg kg^{-1} in harvest three, and up to 54.7 and 52.0 mg kg^{-1} in harvest four, which was week 11 after sowing. While in total Ca, soil+foliar stands out in all the harvest with a clearly negative tendency; in Fe, the treatments with higher total Fe with a less marked decrease were foliar and soil+foliar.

Raw broccolini floret PA concentration

Zinc application did not significantly affect the PA concentration of the raw broccolini florets. Altered PA:Zn molar ratios (and those for the other nutrients) in the florets were therefore driven by effects of Zn application on nutrient composition of the florets (Figure 2). The PA concentration of the florets decreased with harvest, but to a lesser extent that the nutrient concentration of the florets, therefore PA:nutrient molar ratios increased (Figure 2).

Boiled broccolini floret

Boiling decreased the Zn concentration of boiled broccolini florets by 45% (Figure 1). There were also reductions in Ca (20%), Fe (8%), Mg (20%), and N (60%) concentration. Processing caused an increase of ~8% in PA concentration, resulting in increases in molar ratios of 16.6% in PA:Ca, 13.7% in PA:Fe, 26.5% in PA:Mg and 43.8% in PA:Zn (Figure 2).

Discussion

Soil application of 5 mg $ZnSO_4.7H_2O$ kg^{-1} was an effective dose, which increased DTPA-Zn concentration up to more than 1.2 mg kg^{-1} (Table 1). This increase was similar to those found by Poblaciones and Rengel (2017) in field peas and by Gomez-Coronado et al. (2016) in wheat. Despite Brassicas having a relatively low sensitivity to Zn deficiency (Alloway 2008), soil application resulted in an increase of ~15% in plant weight. White et al. (2018) did not find increases in shoot dry weight due to the soil Zn application in different Brassicas. Slosar et al. (2016 and 2017) observed a yield increase between 8.2 to 17.5% in broccoli after foliar Zn application, but at higher doses than used in this study.

Despite the low soil DTPA-Zn in the control pots, the nutritional quality of broccolini plant and florets is evident. Floret Zn concentration (39 mg Zn kg^{-1}) in control plants is similar to the target concentration established by the HarvestPlus program for cereals of 38 mg Zn kg^{-1}, although less than the target concentration of 61 mg Zn kg^{-1} for legumes (Huett et al. 1997). Martinez-Hernandez et al. (2013a) reported higher concentrations of Zn and Fe, but similar concentrations of Ca, Mg and N in bimi florets than in this study. Liu et al. (2018) reported lower levels of Zn, Ca and Mg, but similar concentrations of Fe in broccoli than in this study. Obgede et al. (2015) reported higher concentrations of Ca in cabbage, but lower concentrations of Fe, N and Zn than in this study. Therefore, broccolini, is nutritionally valuable as a source of minerals for human nutrition. Given that 90% of the plant production comprises stem+leaves, they are also a valuable potential source of nutrients for animal feed.

Soil application increased Zn concentration ~10 mg kg^{-1} in both stem+leaves and in florets. As expected, foliar application increased Zn concentrations (by ~ 3 times) to a greater extent than soil application, with the increased in stem+leaves (by ~ 12- times) being larger than in florets.

These increases were larger in the two first harvests and decreased in later harvests. The increases were much higher than those found by Slosar et al. (2017) in broccoli or by Gomez-Coronado et al. (2016) in cereals but similar than those found for legumes (Poblaciones and Rengel 2017). Hence, it appears that broccolini may accumulate large amounts of Zn in the whole plant, stem+leaves, and florets, after Zn application. Interestingly, Zn application did not significantly affect the concentration of other nutrients in stem+leaves, but foliar Zn application significantly increased floret Ca and Fe concentrations. These data indicate the potential of agronomic biofortification of broccolini with Zn, without incurring negative consequences for other nutrients.

The Zn concentration of stem+leaves after foliar Zn application remained very high relative to the florets, which indicates the relative low translocation of the Zn to the florets. Furthermore, the decrease in floret Zn concentration (also observed for Ca and N) with harvest potentially reflects a decrease in nutrient mobility over time. To optimise agronomic Zn biofortification for sequentially-harvested crops such as broccolini, it will be important to conduct field experiments where growth is not limited by the size of the pots. In addition, it will be important to understand the interactions between N and Zn which might affect translocation, as has been seen previously for wheat (Ref). It is also critical to ensure that maximising yield will be critical if farmers are to adopt agronomic biofortification programs.

Bioavailability, estimated from PA:Zn molar ratio was greater in stem+leaves, which had PA concentration ~3.8-times less than in florets. Higher PA concentrations than observed in this study were reported by Mohammed and Luka (2013) and Ogbede et al. (2015) in green, red or Chinese cabbage, which had phytate contents of ~3.0 g kg^{-1}. In all the Zn treatments and harvests PA:Zn molar ratio of stem+leaves and florets exceeded the recommended level 15 for adequate bioavailability (Gibson 2007) in only the control pots. Calcium, Fe and Mg bioavailabilities were good, with PA:nutrient molar ratios less than the recommended level of 0.24 for PA:Ca (Morris and Ellis 1998), 10 for PA:Fe (Engle-Stone 2005) and 0.2 for PA:Mg

(Evans and Martin 1988) in all treatments. It will be important to understand the effects of Zn on PA concentration in sequential harvests under field conditions.

Losses of nutrients during boiling were 45% in Zn, together with reductions of 19% for Ca, 8% for Fe, 21% for Mg, and 39% for N in Zn. Phytic acid concentration increase by ~8% after boiling, indicating that bioavailability will be reduced for all the nutrients after boiling. Reductions in the nutritional quality of broccoli have been reported due to thermal degradation and leakage in the cooking fluids (Lee and Kader 2000; Roy et al. 2009). Nevertheless, Schnepf and Driskell (1994) reported no differences in the texture scores and loss of colour for broccoli prepared by boiling (Kala and Prakash 2004). Similar losses of nutrients after boiling were found by Poblaciones and Rengel (2017) in field peas. Processing steps including grilling and vacuum-based cooking treatments may have less impact on nutritional composition and warrants further study (Martinez-Hernandez et al., 2013b).

Conclusion

The Recommended Dietary Allowance (RDA) of minerals for males and females between 19 and 65 years (FAO/WHO 2000) include: 15 mg Zn, 700 mg Ca, 18 mg Fe, and 240 mg Mg. From the optimal treatment in this study (soil+foliar Zn), an intake of 100 g of boiled florets of broccolini would supply 40% of the RDA for Zn, 77% for Ca, 27% for Fe, and 80% for Mg in the first harvest. Whilst boiling decreased the majority of the nutrients in broccolini, the PA:nutrient molar ratios, were sufficiently low to ensure a good bioavailability of Zn, together with Ca, Fe, Mg and Zn in broccolini under agronomic Zn biofortification.

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Conflicts of Interest

The authors declare no conflict of interest

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Table 1. Mean ± standard error in soil DTPA-Zn, shoot (stem+leaves) height and weight, total number of florets and total floret weight, total Ca, Fe, Mg, N, Zn and phytic acid concentrations in whole shoot and their respective molar ratios (PA:Ca, PA:Fe, PA:Mg and PA:Zn molar ratios) as affected by the Zn treatment.

Zn treatment	**Soil DTPA-Zn ($mg\ kg^{-1}$)**	**Shoot height (cm)**	**Shoot weight (g DW)**	**Total number of florets**	**Total florets weight (g DW)**
No-Zn	0.39 ± 0.41b	40.3 ± 4.7 a	18.7 ± 1.8 b	6.0 ± 1.0 a	1.7 ± 0.2 a
Soil	1.35 ± 1.36 a	43.3 ± 3.6 a	21.3 ± 1.9 ab	6.0 ± 1.4 a	1.7 ± 0.2 a
Foliar	0.33 ± 0.58 b	44.7 ± 3.9 a	19.5 ± 0.9 b	5.7 ± 0.8 a	1.9 ± 0.5 a
Soil+Foliar	1.28 ± 0.92a	50.1 ± 6.0 a	22.8 ± 0.8 a	6.8 ± 1.8 a	2.1 ± 0.7 a

Zn treatment	**Shoot total Ca ($g\ kg^{-1}$)**	**Shoot total Fe ($mg\ kg^{-1}$)**	**Shoot total Mg ($g\ kg^{-1}$)**	**Shoot total N ($mg\ kg^{-1}$)**	**Shoot total Zn ($mg\ kg^{-1}$)**
No-Zn	23.5 ± 1.6 a	37.4 ± 4.5 a	2.8 ± 0.7 a	11.3 ± 1.1 a	8.7 ± 1.1 b
Soil	20.7 ± 0.8 a	33.5 ± 2.2 a	2.9 ± 0.7 a	12.3 ± 1.3 a	18.8 ± 3.0 b
Foliar	23.1 ± 2.0 a	34.5 ± 4.5 a	3.1 ± 0.2 a	11.2 ± 1.8 a	120.7 ± 37.8 a
Soil+Foliar	21.3 ± 1.8 a	29.5 ± 1.4 a	2.7 ± 0.2 a	11.3 ± 0.9 a	110.0 ± 8.9 a

Zn treatment	**Shoot PA ($g\ kg^{-1}$)**	**Shoot PA:Ca molar ratio**	**Shoot PA:Fe molar ratio**	**Shoot PA:Mg molar ratio**	**Shoot PA:Zn molar ratio**
No-Zn	1.7 ± 0.1 a	0.004 ± 0.001 a	0.40 ± 0.05 a	0.023 ± 0.002 a	19.5 ± 1.2 a
Soil	1.8 ± 0.1 a	0.006 ± 0.001 a	0.45 ± 0.03 a	0.022 ± 0.002 a	9.6 ± 1.6 b
Foliar	2.0 ± 0.1 a	0.006 ± 0.001 a	0.50 ± 0.07 a	0.023 ± 0.002 a	2.5 ± 0.8 c
Soil+Foliar	1.9 ± 0. 3 a	0.005 ± 0.001 a	0.55 ± 0.07 a	0.027 ± 0.005 a	2.1 ± 0.4 c

Means in a column with different letters were significantly different ($P \leq 0.05$) according to the Fisher's protected LSD test for the Zn treatment.

Table 2. Mean ± standard error in number of florets, average floret height and weight and total floret weight as affected by Zn treatment and number of harvest (weeks after sow).

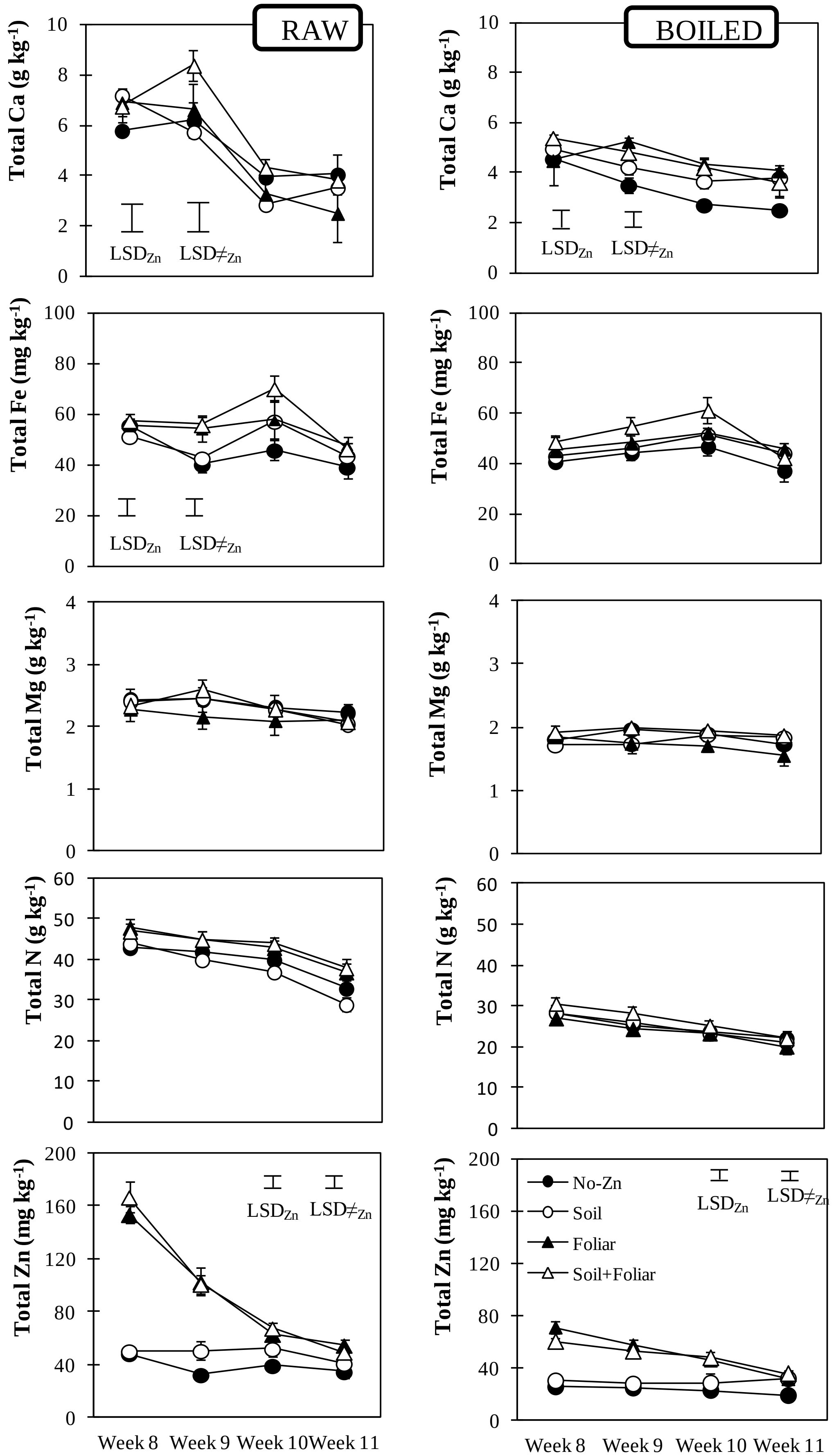
Treatment	Average florets height (cm)	Number of florets	Average florets weight (g DW)	Total florets weight (g DW)
0-Zn				
Week 8	11.3 ± 1.9 a	4.4 ± 0.9 a	0.50 ± 0.04 a	2.22 ± 0.45 b
Week 9	14.6 ± 0.6 a	5.3 ± 0.3 a	0.29 ± 0.03 a	1.43 ± 0.31 c-f
Week 10	11.1 ± 1.6 a	6.0 ± 1.6 a	0.26 ± 0.03 a	1.55 ± 0.43 b-f
Week 11	12.1 ± 0.4 a	8.3 ± 2.2 a	0.20 ± 0.02 a	1.54 ± 0.30 b-f
Soil				
Week 8	11.3 ± 0.8 a	5.8 ± 1.0 a	0.57 ± 0.08 a	3.10 ± 0.29 a
Week 9	13.2 ± 1.4 a	6.0 ± 1.2 a	0.32 ± 0.02 a	1.86 ± 0.21 bcd
Week 10	11.3 ± 0.8 a	3.8 ± 0.9 a	0.32 ± 0.03 a	1.20 ± 0.31 def
Week 11	12.6 ± 1.6 a	7.3 ± 2.4 a	0.20 ± 0.01 a	1.38 ± 0.39 c-f
Foliar				
Week 8	10.9 ± 0.9 a	4.8 ± 0.6 a	0.42 ± 0.03 a	1.95 ± 0.13 bcd
Week 9	12.8 ± 1.4 a	4.5 ± 1.5 a	0.23 ± 0.02 a	1.08 ± 0.42 ef
Week 10	13.6 ± 1.2 a	5.3 ± 0.9 a	0.35 ± 0.04 a	1.80 ± 0.35 b-e
Week 11	11.5 ± 1.0 a	9.5 ± 1.7 a	0.23 ± 0.01 a	1.96 ± 0.09 bcd
Soil+Foliar				
Week 8	14.3 ± 0.8 a	7.3 ± 1.1 a	0.54 ± 0.02 a	3.83 ± 0.46 a
Week 9	13.7 ± 0.8 a	3.0 ± 0.0 a	0.26 ± 0.03 a	0.78 ± 0.08 f
Week 10	13.9 ± 0.6 a	6.3 ± 1.0 a	0.29 ± 0.03 a	1.76 ± 0.16 b-e
Week 11	13.6 ± 1.1 a	10.8 ± 0.9 a	0.20 ± 0.01 a	2.14 ± 0.18 bc

Means in a column with different letters were significantly different ($P \leq 0.05$) according to the Fisher's protected LSD test for the harvest moment.

Figure captions

Figure 1: Total Ca, Fe, Mg, N and Zn concentrations ± standard errors in raw (left) and boiled (right) florets as affected by the Zn treatments in the different harvest (from week 8 to week 11 after sowing). Vertical bars represent LSD ($P \leq 0.05$) for comparison: LSD_{Zn}, same Zn treatment; $LSD_{\neq Zn}$, different Zn treatment.

Figure 2: Phytic acid and PA:Ca, PA:Fe, PA:Mg and PA:Zn molar ratios ± standard errors in raw (left) and boiled (right) florets as affected by the Zn treatments in the different harvest (from week 8 to week 11 after sowing). Vertical bars represent LSD ($P \leq 0.05$) for comparison: LSD_{Zn}, same Zn treatment; $LSD_{\neq Zn}$, different Zn treatment.



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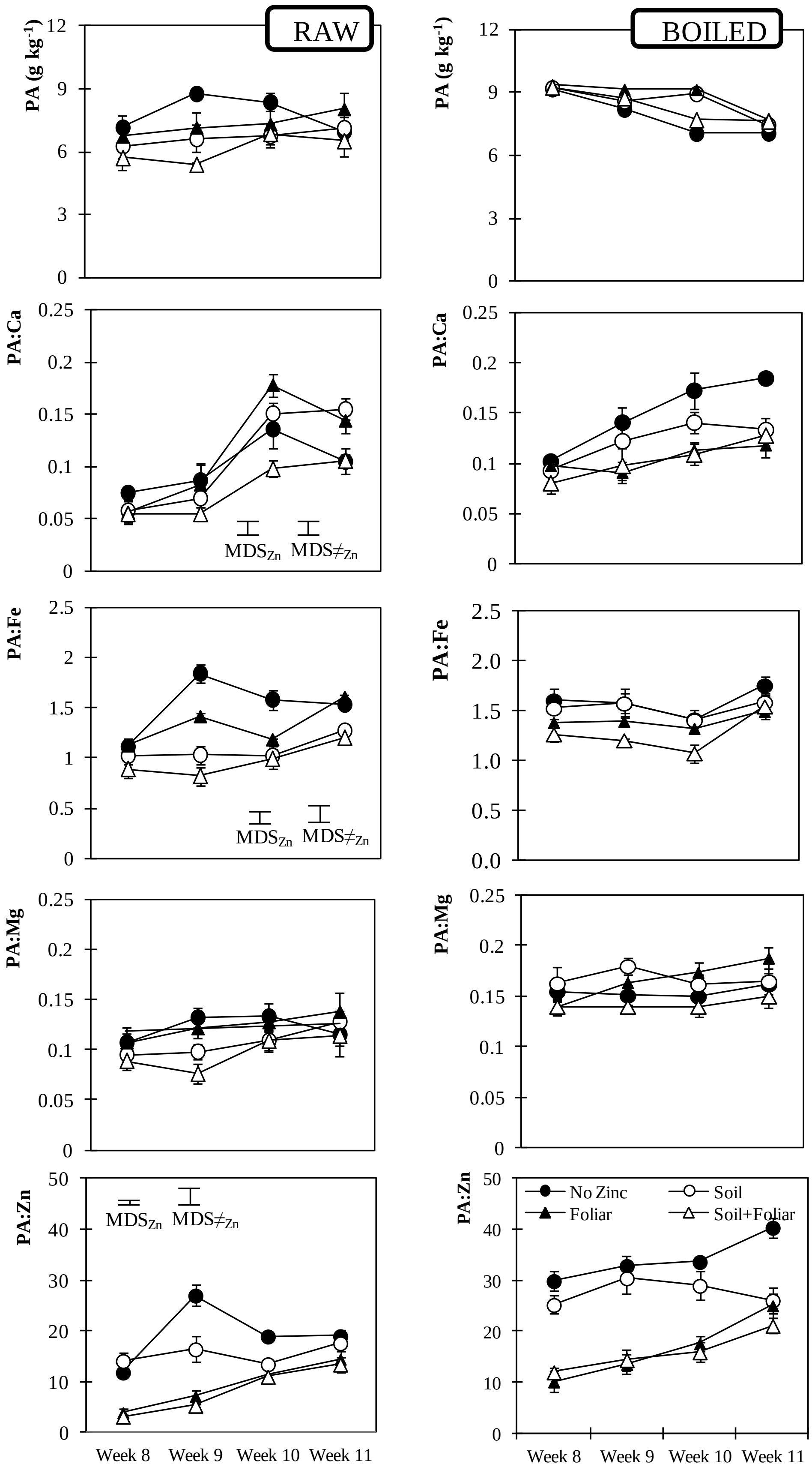
RAW
BOILED
PA (g kg-1)
PA:Ca
PA:Fe
PA:Mg
PA:Zn
MDSZn
MDS≠Zn
No Zinc
Soil
Foliar
Soil+Foliar
Week 8
Week 9
Week 10
Week 11

FIGURE 2