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Combined effect of iron and zinc on micronutrient levels in wheat (*Triticum aestivum* L.)

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Abstract

A nutrient solution experiment was conducted to investigate the effect of Fe and Zn supply on Fe, Zn, Cu, and Mn concentrations in wheat plants. The experiment used a factorial combination of two Fe levels (0 and 5 mg l^{-1}) and three Zn levels (0, 0.1 and 10 mg l^{-1}). The supply of Fe (5 mg l^{-1}) and Zn (0.1 mg l^{-1}) increased plant dry weight and leaf chlorophyll content compared to the Fe or Zn deficient (0 mg l^{-1}) treatments. However, excess Zn supply (10 mg l^{-1}) reduced plant dry weights and leaf chlorophyll content. Iron supply (5 mg l^{-1}) reduced wheat Zn concentrations by 49%, Cu concentrations by 34%, and Mn by 56% respectively. Zinc supply (10 mg l^{-1}) reduced wheat Fe concentrations by an average of 8%, but had no significant effect on Cu and Mn concentrations. Stepwise regression analyses indicated that Zn, Cu, and Mn concentrations were negatively correlated with root- and leaf-Fe concentrations, but positively correlated with stem-Fe concentrations. Leaf-Mn concentrations were negatively correlated with root-, stem- and leaf-Zn concentrations.

Key words

Fe and Zn effect, Micro-nutrient level, Wheat

Introduction

Iron (Fe), zinc (Zn), copper (Cu), and manganese (Mn) are essential micronutrients for plants and humans (Kaya *et al.*, 1999; Asad and Rafique, 2000; Hao *et al.*, 2007). A deficiency of just one of these nutrients can greatly reduce plant yield and even cause plant death. Micronutrient deficiency, especially Fe and Zn deficiency, is widespread in humans (Graham *et al.*, 1999; Stoltzfus, 2001; FAO, 2002; Liu *et al.*, 2006; Hao *et al.*, 2007). Cereal grains are the most important dietary source of micronutrients in many developing countries. Micronutrient concentrations and bioavailability in cereal grain is generally low. Increasing the micronutrient concentration of cereal grains has been identified as a way of addressing human micronutrient deficiencies (Monthey *et al.*, 1994; Muminjanov *et al.*, 2007; Pahlavan-Rad and Pessarakli, 2009).

A number of attempts have been made to increase micronutrient concentrations in grain. One approach has been to select and/or breed genotypes (cultivars) with greater micronutrient concentrations. However, these attempts have been hampered by limited genotypic variation for grain Zn and Fe concentrations among cultivated wheat cultivars as well as by the complexity of inheritance for grain micronutrient concentrations. Environment also has a significant effect on grain micronutrient concentrations and there is significant interaction between genotype and the environment (Cakmak *et al.*, 2004; Ficco *et al.*, 2008).

Crop management strategies are an important complement to ongoing breeding programs. Studies have shown that Fe, Zn, Cu, and Mn concentrations in rice or wheat grain can be increased by proper irrigation management, N fertilization, and late planting

(Hao *et al.*, 2007; Pearson *et al.*, 2008). A number of studies have reported that the application of micronutrient fertilizers to the soil or crop foliage increased micronutrient concentrations in grain. However, some authors found that foliar sprays resulted in nutritional disorders and imbalances (Ghasemi-Fassaei *et al.*, 2008; Pahlavan-Rad and Pessarakli, 2009).

Interactions among micronutrients affect their uptake, distribution, and utilization in plants (Imtiaz *et al.*, 2003). Many studies have examined these interactive effects, especially between Fe and Zn. For example, Sliman (1990) reported antagonism between Fe and Zn in soybean. Other studies have found that Fe reduced Mn concentrations in Indian mustard (Hamlin *et al.*, 2008) and in soybean leaves (Izaguirre-Mayoral and Sinclair, 2005), but increased Mn concentrations in soybean shoots (Heenan and Campbell, 1983). Other authors reported a negative correlation between Zn and Cu (Pearson *et al.*, 2008; Alloway *et al.*, 2008; Kumar *et al.*, 2009). Murphy *et al.* (2008) found a significant correlation between Cu and Mn in spring wheat.

Most micronutrient research has focused on dicotyledonous crops, such as soybean (Ghasemi-Fasaei *et al.*, 2003) and only on interaction between two micronutrients. Information about the effect of Fe and Zn on monocotyledonous crops, especially wheat, is limited (Ghasemi-Fasaei and Ronaghi, 2008). This experiment investigated interaction between Fe, Zn, Cu, and Mn by measuring the concentration of these micronutrients in wheat plants grown in nutrient solutions containing different amounts of Fe and Zn.

Materials and Methods

The experiment used a 3×2×3 factorial design consisting of three wheat cultivars (Zhengmai 9023, Shaan 512, and Xinong 979), two Fe amounts (0 and 5 mg l^{-1}), and three Zn amounts (0, 0.1 and 10 mg l^{-1}). Iron was supplied as Fe-citrate and Zn was supplied as $ZnSO_4 \cdot 7H_2O$. The 0 and 5 mg Fe l^{-1} treatments will be referred to as the Fe_0 and Fe_5 treatments. The 0, 0.1, and 10 mg Zn l^{-1} treatments will be referred to as the Zn_0, $Zn_{0.1}$, and Zn_{10} treatments. Each treatment was replicated 3 times.

Wheat seeds were soaked in 55°C tap water for 15 min, 3% H_2O_2 for 10 min, and then distilled water for 3 hr. The seeds were germinated for 1 d, and then the seedlings were put in a 4°C refrigerator for a 15 d vernalization period. Afterwards, seedlings were selected for uniformity and transplanted to 2 lit. opaque containers, each covered with a polystyrol-plate with eight holes. One seedling was fixed in each hole and one hole was used as an inlet for the aeration tube. There was one cultivar per pot. The pots were filled with modified Hoagland's solution (Mao, 2004). All chemicals were AR grade. The plants were grown in 1/2 strength Hoagland's solution for 1 week, then the solution was replaced with full strength Hoagland's solution amended with the Fe and Zn treatments. The nutrient solution was aerated continuously. The containers were kept in a growth chamber with day/night temperatures of 25/15°C and a 10 hr photoperiod at 550 μmol $m^{-2}s^{-1}$.

Table - 1: Effect of Fe and Zn on wheat dry weight (g $plant^{-1}$) and leaf chlorophyll content (SPAD value)

Treatment (mg l^{-1})		Dry weight	Leaf chlorophyll
Fe	Zn	(g $plant^{-1}$)	content
0	0	0.71c	29.65b
	0.1	0.65c	29.80b
	10	0.17d	11.15c
5	0	1.97a	36.16a
	0.1	2.11a	35.40a
	10	1.62b	29.18b

The same letter within each column indicates no significant difference at $p<0.05$

Table - 2: Effect of Fe and Zn on Fe concentrations (μg g^{-1}) in wheat tissues

Treatment (mg l^{-1})		Fe concentration (μg g^{-1})		
Fe	Zn	Root	Stem	Leaf
0	0	89^{b}	122^{c}	293^{b}
	0.1	107^{b}	167^{b}	301^{b}
	10	173^{b}	410^{a}	—
5	0	1223^{a}	89^{cd}	306^{ab}
	0.1	1257^{a}	120^{c}	372^{a}
	10	1119^{a}	82^{d}	271^{b}

The same letter within each column indicates no significant difference at $p<0.05$, SPAD=Soil and Plant Analyzer Development

Table - 3: Effect of Fe and Zn on Zn concentrations (μg g^{-1}) in wheat tissue

Treatment (mg l^{-1})		Fe concentration (μg g^{-1})		
Fe	Zn	Root	Stem	Leaf
	0	98c	83b	67^{b}
0	0.1	210c	282^{b}	267^{a}
	10	6679^{a}	2431^{a}	—
5	108^{c}	33^{b}	35^{b}	5 0.1
	119^{c}	111^{b}	56^{b}	10
	3356^{b}	507^{b}	25^{b}	

The same letter within each column indicates no significant difference at $p<0.05$

The chlorophyll content of the second fully expanded leaf was measured in the morning of day 50 using a portable SPAD 502 chlorophyll meter (Minolta Camera Co., Osaka, Japan). Plants were then harvested, separated into roots, stems and leaves, dried at 450°C and weighed. Micronutrient (Fe, Zn, Cu and Mn) concentrations in the plant samples were determined by atomic absorption spectrophotometry (AA320CRT, Shanghai Analytical Instrument Overall Factory, China).

Statistical analyses were performed on all treatments using Excel 2003 and SAS 6.12 software (SAS Institute, Cary, NC, USA). There was no significant difference among the three wheat cultivars, so data in this paper represent the average of all three cultivars. The level of significance (α) was 0.05 ($p \leq 0.05$).

Table - 4: Effect of Fe and Zn on the Cu concentration (µg g^{-1}) in wheat tissue

Treatment (mg l^{-1})		Cu concentration (µg g^{-1})		
Fe	Zn	Root	Stem	Leaf
0	0	87[b]	32[a]	34[a]
	0.1	79 [b]	40[a]	38[a]
	10	128[a]	47[a]	-
5	0	36[c]	10[b]	13[b]
	0.1	36[c]	12[b]	12[b]
	10	40[c]	11[b]	12[b]

The same letter within each column indicates no significant difference at $p<0.05$

Table - 5: Effect of Fe and Zn on the Mn concentration (µg/g) in wheat tissues

Treatment (mg l^{-1})		Cu concentration (µg g^{-1})		
Fe	Zn	Root	Stem	Leaf
0	0	369[b]	136[b]	297[a]
	0.1	314[b]	121[b]	294[a]
	10	518[a]	212[a]	-
5	0	313[b]	73[c]	129[b]
	0.1	306[b]	72[c]	118[b]
	10	183[c]	67[c]	109[b]

The same letter within each column indicates no significant difference at $p<0.05$

Table - 6: Stepwise regression results for Fe, Zn, Cu, and Mn concentrations (mg kg^{-1}) in winter wheat roots, stems and leaves.

Stepwise regression equation	*F* value
$y_{R\text{-}Fe}=1724.9166+3.9491x_{S\text{-}Fe}-18.1584x_{R\text{-}Cu}-4.2976x_{S\text{-}Mn}$	R-Cu<.0001, S-Fe 0.0081, S-Mn 0.1499
$y_{S\text{-}Fe}=-10.9313+0.081x_{S\text{-}Zn}+1.7813x_{S\text{-}Cu}+0.2516x_{R\text{-}Mn}$	S-Zn<.0001, S-Cu 0.0003, R-Mn 0.0333
$y_{L\text{-}Fe}=402.3896-0.0203x_{R\text{-}Zn}-0.0426x_{S\text{-}Zn}-0.7485x_{S\text{-}Mn}$	S-Zn<.0001, S-Mn 0.0342, R-Zn 0.0261
$y_{R\text{-}Zn}=1615.6202+2.0316x_{S\text{-}Zn}-6.4745x_{L\text{-}Mn}$	S-Zn<.0001, L-Mn 0.0698
$y_{S\text{-}Zn}=13.7479+24.3706x_{R\text{-}Cu}-15.8707x_{S\text{-}Cu}-4.3378x_{L\text{-}Mn}$	R-Cu 0.0004, L-Mn<.0001, S-Cu 0.0733
$y_{L\text{-}Zn}=-3.9942+12.9718x_{L\text{-}Cu}-1.0038x_{L\text{-}Mn}$	L-Cu<.0001, L-Mn 0.0694
$y_{R\text{-}Cu}=5.2839+0.8211x_{S\text{-}Cu}+0.366x_{S\text{-}Mn}$	S-Mn<.0001, S-Cu 0.0292
$y_{S\text{-}Cu}=-4.581+0.2648x_{S\text{-}Mn}$	S-Mn<.0001
$y_{L\text{-}Cu}=1.2735+0.1241x_{L\text{-}Mn}$	
$y_{R\text{-}Mn}=147.72+1.6419x_{S\text{-}Mn}$	

R-, S-, L- mean root, stem and leaf

Results and Discussion

Plant dry weights were 2.8 to 9.5 times larger in the Fe_5 treatments compared to the Fe_0 treatments and 1.2 to 4.2 times larger in the Zn_0 and $Zn_{0.1}$ treatments compared to the Zn_{10} treatments (Table 1). Comparison of the F values for the Fe (F=249.8) and Zn (F=12.4) treatments indicated that Fe deficiency (0 mg Fe l^{-1}) had greater effect on wheat growth than excess Zn (10 mg Zn l^{-1}).

Leaf chlorophyll contents were 1.2 to 2.6 times larger in the Fe_5 treatments compared to the Fe_0 treatments (Table 1). Leaf chlorophyll content was significantly ($p<0.05$) correlated with leaf Fe concentration (data not shown). This agrees with Kaya *et al.* (1999) who found a close relationship between Fe application and tomato leaf chlorophyll content. Few studies have examined Fe deficiency in wheat plants, though in general, Fe deficiency can be corrected with Fe fertilizer (Hamlin and Barker, 2008), chelating agents (Nowack *et al*., 2008; Ghasemi-Fasaei and Ronaghi, 2008), or a reduction in the pH of the growth media (Brown and Jones, 2006).

Leaf chlorophyll contents were 1.2 to 2.7 times larger in the Zn_0 and $Zn_{0.1}$ treatments compared to the Zn_{10} treatments (Table 1). This indicated that none of the wheat cultivars in this study were tolerant of excess Zn (10 mg l^{-1}) supply (Walter *et al.*, 2006). Leaf chlorophyll content was lowest in the $Zn_{10}Fe_0$ treatment (Table 1). The addition of Fe to the nutrient solution significantly increased leaf chlorophyll content in the Zn_{10} treatments. These results showed that not only Fe deficiency (0 mg Fe^{-1}), but also excess Zn (10 mg Zn l^{-1}), reduced leaf chlorophyll content in wheat. Our results agree with Fontes and Cox (1998 b) who reported reduced growth and leaf chlorosis in soybean plants grown in culture solutions containing 40 µM Zn. The visual symptoms were similar to those observed in a 0 µM Fe treatment, leading Fontes and Cox (1998a) to conclude that Fe deficiency is one of the factors responsible for reduced growth in plants exposed to Zn toxicity. The mechanism of Zn induced chlorosis is not clear. One possible explanation is that Zn may compete with Fe for an Fe-requiring step in chlorophyll biosynthesis (Kaya *et al.*, 1999). Our results agree with Kaya *et al.* (1999) who found that Fe addition could remedy chlorosis induced by excess Zn.

The effect of Fe supply on wheat tissue Fe concentration varied among plant parts (Table 2). Root-Fe concentrations were 6.5 to 13.7 times larger in the Fe_5 treatments compared to the Fe_0 treatments, whereas stem-Fe concentrations tended to be largest in the Fe_0 treatments. Leaf-Fe concentrations were slightly larger in the Fe_5 treatments compared to the Fe_0 treatments.

The effect of Zn supply on wheat tissue Fe concentration varied depending on Fe supply and plant part. In the absence of Fe (Fe_0 treatments), root-, stem- and leaf-Fe concentrations

increased as Zn supply increased. In the presence of Fe (Fe_5 treatments), root-, stem-, and leaf-Fe concentrations tended to be greater in the $Zn_{0.1}$ treatment compared to the Zn_0 and Zn_{10} treatments, though differences between the treatments were not always significant.

As expected, Zn supply generally increased Zn concentrations in wheat plants (Table 3). Root-Zn concentrations were 38.2 to 31.8 times larger and stem concentrations were 4.6 to 8.6 times larger in the Zn_{10} treatments compared to the $Zn_{0.1}$ treatments. The toxicity limit for Zn depends on plant species, genotype, and growth stage. Toxicity limits generally range between 100 and 500 μg g^{-1} (DW) (Kabata-Pendias, 2001). Our study showed that supply of 10 mg Zn l^{-1} increased Zn concentrations in roots and stems to toxic levels. In contrast, Fe supply generally reduced Zn concentrations in wheat plants (Table 3). Specifically, root-Zn concentrations in the $Fe_5Zn_{0.1}$ and Fe_5Zn_{10} treatments were about half as much as in the $Fe_0Zn_{0.1}$ and Fe_0Zn_{10} treatments. Stem- and leaf-Zn concentrations were one-fifth to two-fifths as much in the Fe_5 treatments as in the Fe_0 treatments. It is interesting to note that Zn supply seemed to have little effect on leaf-Zn concentrations in the Fe_5 treatment.

Antagonism between Fe and Zn is well known. Previous studies have shown that Zn interfered with Fe uptake and translocation, whereas Fe interfered with Zn translocation, but only when Zn concentrations were high (Alloway, 2008). There are three possible mechanisms for this antagonism. First, there could be competition between Zn^{2+} and Fe^{2+} during uptake (Kabata-Pendias, 2001). Second, there could be interference in the chelation process during Fe uptake and translocation (Kabata-Pendias, 2001). Third, there could be competitive inhibition between Zn and Fe during unloading in the xylem (Alloway, 2008).

Iron supply significantly reduced wheat Cu concentrations (Table 4). Root-Cu concentrations were 2.2 to 3.2 times smaller, stem-Cu concentrations were 3.2 to 4.3 times smaller and leaf-Cu concentrations were about one-fourth to one-half as much in the Fe_5 treatments compared to the Fe_0 treatments. In the Fe deficient (Fe_0) treatments, Cu concentrations generally increased as Zn supply increased. In contrast, Zn supply had no significant effect on wheat Cu concentrations in the Fe_5 treatments.

Iron supply generally reduced wheat Mn concentrations, especially in stems and leaves (Table 5). In the Fe deficient (Fe_0) treatments, wheat Mn concentrations increased as Zn supply increased. In contrast, Mn concentrations decreased as Zn supply increased in the Fe_5 treatments.

Results from stepwise regression analysis of Fe, Zn, Cu, and Mn concentrations in wheat tissues are presented in Table 6. Root- and leaf-Fe concentrations were negatively correlated with Zn, Cu, and Mn, whereas stem-Fe concentrations were positively correlated with Zn, Cu and Mn. Root-, stem- and leaf-Zn concentrations were positively correlated with root- and stem-Cu concentrations, but negatively correlated with leaf-Mn concentrations. Root- and stem-Cu concentrations were positively correlated with stem-Mn concentrations.

Interaction between Fe, Zn, Cu and Mn varied among plant parts and micronutrients. A number of studies have reported antagonism between Fe other cationic micronutrients, especially Mn (Izaguirre-Mayoral and Sinclair, 2005; Ghasemi-Fasaei and Ronaghi, 2008). However, most of these studies focused on soybean plants and little was known about micronutrient interactions in wheat. Stepwise analysis in our study indicated the Cu, but not Zn, was significantly correlated with Fe concentrations in wheat. In comparison, both Cu and Mn were significantly correlated with wheat Zn concentrations. This result is consistent with Murphy *et al.* (2008) who found a strong correlation between Cu and Mn, Cu and Zn and Zn and Mn in wheat plants. Balint *et al.* (2007) mapped the quantitative trait loci (QTLs) of wheat and predicted that if a genotype had a high shoot Cu concentration then high concentrations of Fe, Mn and Zn could be also expected. This phenomenon could be useful for breeding wheat with increased micronutrient concentrations.

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