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ORIGINAL ARTICLE/SHORT PAPER

Iron deficiency causes zinc excess in *Zea mays*

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Abstract

Iron deficiency stress causes a severe reduction in plant growth. Although Fe deficiency causes an imbalance in divalent heavy metal nutrients, the mechanisms underlying the growth reduction caused by this imbalance remain unclear. We investigated Zn uptake and accumulation in maize under Fe-deficient conditions. Under Fe-deficient conditions, Zn uptake was 15-fold higher and Zn accumulation was 16-fold higher than that under normal nutrient conditions. The Zn content of maize leaves under Fe-deficient conditions was >0.4 mg g^{-1} dry weight, which was higher than the content of plants grown in a nutrient solution containing 50 μM $ZnCl_2$. Plant growth under conditions of both Fe and Zn deficiency was significantly higher than that under only Fe-deficient conditions. Moreover, Fe deficiency increased the thiol content of the plant. These results indicate that Fe deficiency causes excess uptake and accumulation of Zn, and that the stress resulting from the Zn overload accelerates growth reduction in maize.

Key words: iron deficiency, maize, thiol content, xylem sap, zinc excess.

INTRODUCTION

Heavy metals, such as Fe, Cu, Mn and Zn, function as micronutrients and are indispensable for plant growth (Haydon and Cobbett 2007; Marschner 1995). Deficiencies of these heavy metals cause severe growth reduction (Marschner 1995; Mori 1999; Broadley *et al.* 2007). Although essential, these heavy metals are toxic to plants if present in excess (Broadley 2007).

The uptake of these heavy metals has been studied at the physiological and molecular levels (Marschner 1995). Several types of transporters have been identified on the plasma membrane of root cells (Haydon and Cobbett 2007). Studies on the substrate specificity of these transporters have revealed that the uptake of Fe, Cu, Mn and Zn is competitive and antagonistic (Cohen *et al.* 1988; Korshunova *et al.* 1999; Roberts *et al.* 2004; Schaaf *et al.* 2004). These studies have indicated that if some heavy metals are present in excess, they can cause a deficiency in another heavy metal. The impairment of plant growth results from the combined effects of the excess and deficiency of these heavy metals.

Iron-deficient soil, such as calcareous soil, covers approximately one-third of all cultivated soil. Iron deficiency is a problem throughout the world and leads to a severe reduction in crop yield (Mori 1999). Studies have reported that Fe deficiency accelerates the excess uptake and accumulation of Mn and Zn (Alams *et al.* 2001; Kobayashi *et al.* 2003; Mozafar 1997). However, the physiological effects of the uptake of heavy metals in excess and their accumulation as a result of Fe deficiency remain to be clarified.

Among these heavy metals, Zn competes with Fe for the transporters HvIRT1 and HvIRT 2 (Pedas *et al.* 2008) and ZmYS1 (Murata *et al.* 2006). Therefore, Zn uptake is expected to be affected the most under Fe deficiency in graminaceous plants. In the present study, we evaluated the toxic effect of excess Zn uptake and accumulation as a result of Fe deficiency in maize.

MATERIALS AND METHODS

Plant materials and growth conditions

Experimental plants were cultivated in a glasshouse that was thermoregulated at 24 ± 2°C under natural light

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conditions. Maize seeds (*Zea mays* cv. Honey bantam) were soaked in deionized water overnight, placed on filter paper moistened with deionized water and kept in a vertical position for 3 days. The seedlings were then transferred to plastic containers filled with a nutrient solution containing 2 mmol L^{-1} $Ca(NO_3)_2$, 0.7 mmol L^{-1} K_2SO_4, 0.5 mmol L^{-1} $MgSO_4$, 0.1 mmol L^{-1} KCl, 0.1 mmol L^{-1} KH_2PO_4, 0.1 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA)-Fe, 0.01 mmol L^{-1} H_3BO_4, 0.5 μmol L^{-1} $MnSO_4$, 0.5 μmol L^{-1} $ZnSO_4$, 0.2 μmol L^{-1} $CuSO_4$ and 0.01 μmol L^{-1} $(NH_4)_6Mo_7O_{24}$. The pH of the nutrient solution was adjusted to 5.0 with H_2SO_4. The seedlings were allowed to grow in the nutrient solution for 7 days. The seedlings were then transferred to plastic containers filled with one of the following solutions: a complete nutrient solution (+Fe+Zn), a nutrient solution without EDTA-Fe (–Fe+Zn), a nutrient solution without $ZnSO_4$ (+Fe–Zn), a solution without either EDTA-Fe or $ZnSO_4$ (–Fe–Zn) or a nutrient solution with 50 μmol L^{-1} $ZnCl_2$ (50 μmol L^{-1} $ZnCl_2$). The 50 μmol L^{-1} $ZnCl_2$ solution was prepared by adding 50 μmol L^{-1} $ZnCl_2$ to the complete nutrient solution. The seedlings were grown for 21 days under each of the abovementioned nutrient conditions.

Determination of the Fe and Zn contents

The Fe and Zn contents of the maize leaves were measured according to the method of Kanai *et al.* (2007). The dried maize shoots were digested with HNO_3 : $HClO_4$ (4:1) at 100–180°C for 4 h. The Fe and Zn contents of the digested products were analyzed using an atomic absorption spectrophotometer (AA-680; Shimadzu, Kyoto, Japan).

Xylem sap collection

Maize plants were cut 1 cm above the roots and the xylem sap was collected (for 1 h) using a micropipette; this method is a slight modification of the method of Mitani and Ma (2005). Xylem sap in each treatment was collected from five plants and then transferred into a single tube.

Determination of the soluble thiol content

The content of reduced, soluble thiol compounds was measured using the method of Wawrzynski *et al.* (2006). Approximately 100 mg of leaf tissue was homogenized in liquid N_2 and 500 μL of ice-cold 0.1 mol L^{-1} HCl was added to the samples. After vigorous mixing, the samples were centrifuged (10 min, 20,000 *g*, 4°C) and the supernatants were collected for analysis. The supernatants were added in aliquots of 200 μL to 800 μL of buffer (775 μL of 0.5 mol L^{-1} K_2HPO_4 and 25 μL of 10 mmol L^{-1} 5,5′-dithiobis-2-nitrobenzoic acid [DTNB]) and the reactions were allowed to continue for 5 min.

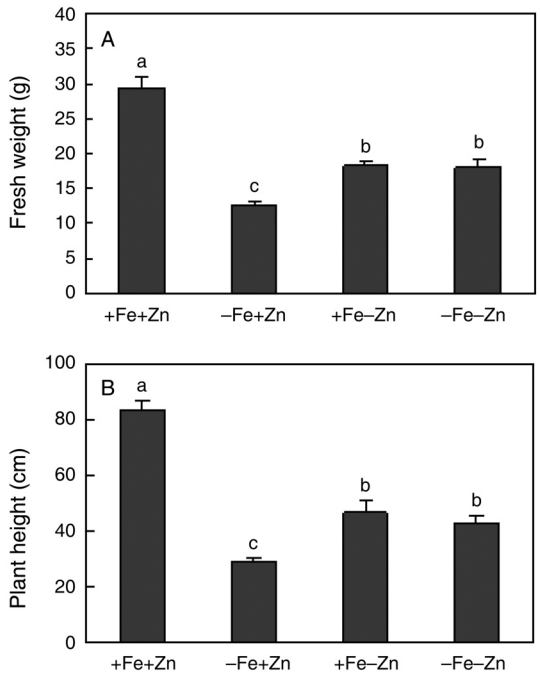

Figure 1 (A) Fresh weight and (B) plant height of each maize plant grown under +Fe+Zn, –Fe+Zn, +Fe–Zn and –Fe–Zn conditions. Plants were grown in the following nutrient solutions for 21 days: with Fe and Zn (+Fe+Zn), without Fe (–Fe+Zn), without Zn (+Fe–Zn) and without either Fe or Zn (–Fe–Zn). The fresh weight and plant height values are mean ± standard error ($n = 4$). Significant differences between the mean values are indicated by different letters (Student's *t*-test; $P < 0.05$).

The absorbance of the samples was measured at 412 nm and corrected against the absorbance of a sample without DTNB. The content of soluble thiols was expressed as nanomoles of thiol per gram fresh weight (FW).

RESULTS

Plant growth and Fe and Zn contents in maize grown under Fe deficiency

The FW and plant height of the maize plants and the contents of Fe and Zn in the leaves were measured. The FW and plant height of plants grown under the –Fe+Zn condition were less than half the FW and plant height of the plants grown under the +Fe+Zn condition (Fig. 1A,B). However, the FW and plant height of the plants grown under the –Fe–Zn condition were greater than the FW and plant height of the plants grown under the –Fe+Zn condition and approximately equal to those of the plants grown under the +Fe–Zn condition (Fig. 1A,B). The removal of Mn or Cu under the –Fe+Zn condition did not improve the FW or plant height of the plants (data not shown).

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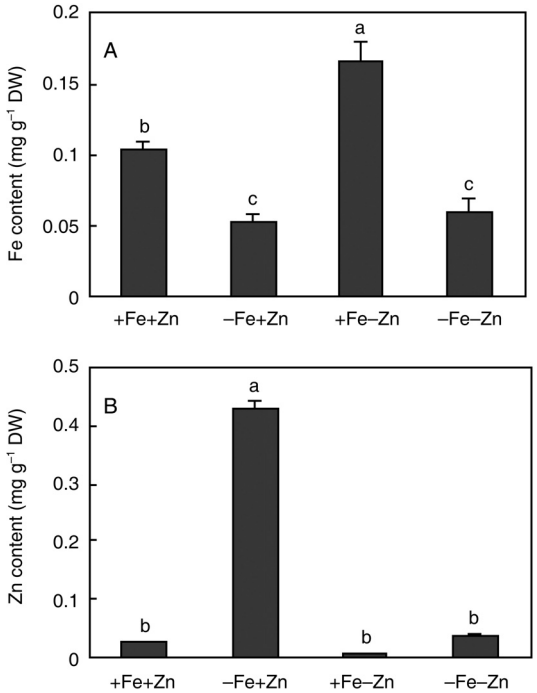

Figure 2 (A) Iron and (B) Zn contents in maize plants grown under +Fe+Zn, –Fe+Zn, +Fe–Zn and –Fe–Zn conditions. Plants were grown in the following nutrient solutions for 21 days: with both Fe and Zn (+Fe+Zn), without Fe (–Fe+Zn), without Zn (+Fe–Zn) and without either Fe or Zn (–Fe–Zn). The values are mean ± standard error ($n = 3$). Significant differences between the mean values are indicated by different letters (Student's *t*-test; $P < 0.05$). DW, dry weight.

The Fe content of the plants grown under the –Fe+Zn condition was approximately half that of plants grown under the +Fe+Zn condition and equal to that of the plants grown under the –Fe–Zn condition (Fig. 2A). The Zn content of the plants grown under the –Fe+Zn condition was 16-fold that of the plants grown under the +Fe+Zn condition, whereas the Zn content of the plants grown under the +Fe–Zn condition was approximately half that of the plants grown under the +Fe+Zn condition (Fig. 2B).

These results suggest that the growth inhibition that occurs under the –Fe+Zn condition can be attributed not only to the low Fe content, but also to the high Zn content in the plant.

Change in the thiol content of the maize leaves

To confirm whether maize plants under the –Fe+Zn condition respond to the stress caused by excess Zn, we investigated the thiol content in the leaves of the maize plants. Thiol compounds act as antioxidants and heavy metal chelators and their contents increase under heavy metal stress (Sharma and Dietz 2006). We measured the thiol content of the leaves of maize plants grown under the +Fe+Zn, –Fe+Zn, –Fe+Zn and –Fe–Zn conditions.

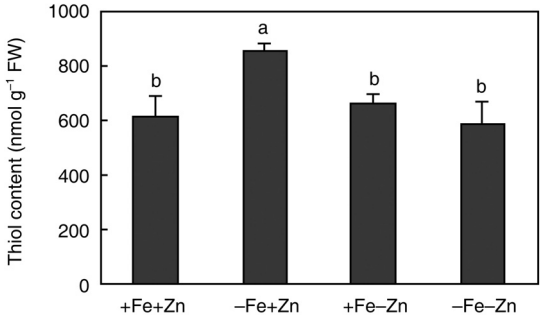

Figure 3 Thiol content of maize leaves grown under +Fe+Zn, –Fe+Zn, +Fe–Zn and –Fe–Zn conditions. Plants were grown in the following nutrient solutions for 21 days: with both Fe and Zn (+Fe+Zn), without Fe (–Fe+Zn), without Zn (+Fe–Zn) and without either Fe or Zn (–Fe–Zn). The values of thiol content are represented as mean ± standard error ($n = 3$). Significant differences between the mean values are indicated by different letters (Student's *t*-test; $P < 0.05$). FW, fresh weight.

The thiol content of the leaves of plants grown under the –Fe+Zn condition was 1.5-fold that of plants grown under the +Fe+Zn condition, and was the highest among the plants grown in the four different types of nutrient solutions (Fig. 3). The thiol content of the leaves of plants grown under the +Fe–Zn condition was equal to that of the plants grown under the +Fe+Zn and –Fe–Zn conditions (Fig. 3). This result indicated that Zn accumulation in the leaves induced the synthesis of thiol compounds.

Excess uptake and accumulation of Zn in maize plants grown under Fe-deficient conditions

Plant growth was inhibited under the –Fe+Zn condition (Fig. 1A,B) and the plants accumulated large amounts of Zn (Fig. 2B). The thiol content of the maize plants increased under the –Fe+Zn condition (Fig. 3). To compare the Zn concentrations in the xylem sap and leaves under the –Fe+Zn condition with those under the excess Zn condition, we measured the Fe and Zn concentrations in the xylem sap and the leaves of the plants grown under the +Fe+Zn and –Fe+Zn conditions, and in the nutrient solution containing 50 μmol L^{-1} $ZnCl_2$. The Zn concentration in the xylem sap of the plants grown under the –Fe+Zn condition was 15-fold that of the plants grown under the +Fe+Zn condition and 1.2-fold that of the plants grown under the 50 μmol L^{-1} $ZnCl_2$ condition (Fig. 4A), although the Zn concentration in the nutrient solution used under the –Fe+Zn condition was 0.5 μmol L^{-1}. The Zn content of the leaves of the plants grown under the –Fe+Zn condition was 16-fold that of the plants grown under the +Fe+Zn condition (Fig. 4B) and approximately 80% of that of

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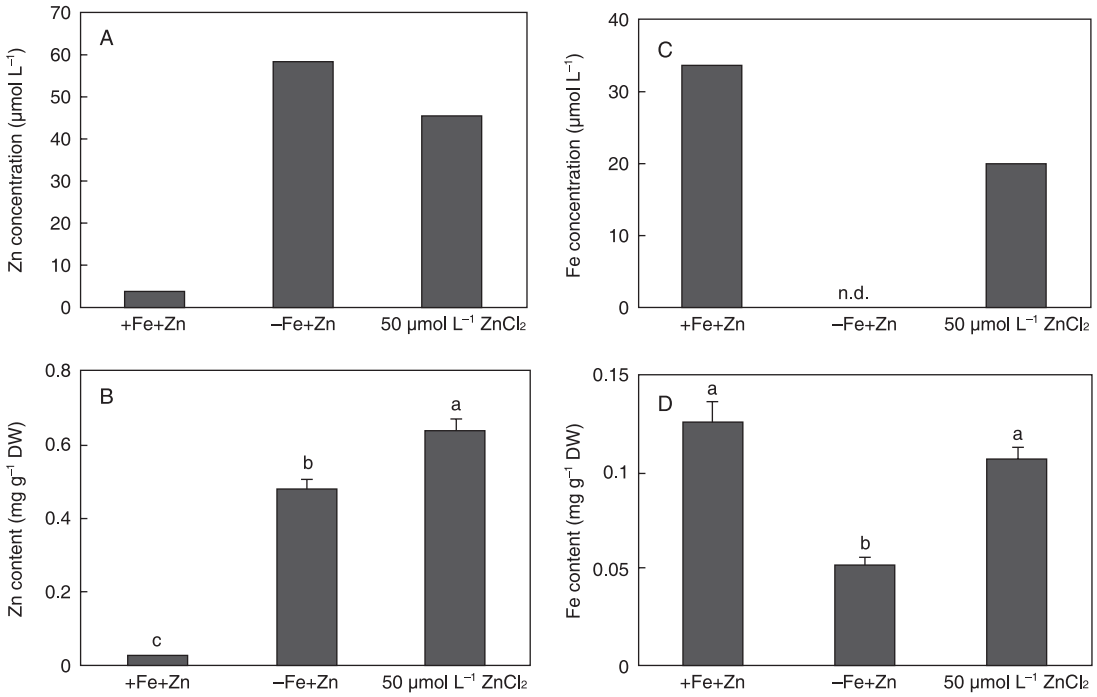

Figure 4 (A) Zinc content of the xylem sap collected from the roots of maize plants grown under the +Fe+Zn condition or –Fe+Zn condition or in the 50 μmol L^{-1} $ZnCl_2$ nutrient solution. (B) Zinc content of the maize leaves of plants grown under the +Fe+Zn condition or –Fe+Zn condition or in the 50 μmol L^{-1} $ZnCl_2$ nutrient solution. (C) Iron content in the xylem sap collected from the roots of maize plants grown under the +Fe+Zn condition or –Fe+Zn condition or in the 50 μM $ZnCl_2$ nutrient solution. The Fe content in the plants grown under the –Fe+Zn condition was below the detection limit. (D) Iron content in the leaves of maize plants grown under the +Fe+Zn condition or –Fe+Zn condition or in the 50 μmol L^{-1} $ZnCl_2$ nutrient solution. Plants were grown under the +Fe+Zn or –Fe+Zn conditions or in the 50 μmol L^{-1} $ZnCl_2$ nutrient solution for 21 days. (B,D) Values are the mean ± standard error ($n = 4$). Significant differences between the mean values are indicated by different letters (Student's *t*-test; $P < 0.05$). (A,C) The xylem sap in each treatment was collected from five plants and then transferred into a single tube. DW, dry weight.

the plants grown in the nutrient solution with 50 μmol L^{-1} $ZnCl_2$ (Fig. 4B). In the xylem sap of the plants grown in the 50 μmol L^{-1} $ZnCl_2$ nutrient solution, the Fe concentration was approximately 70% of that of the plants grown under the +Fe+Zn condition (Fig. 4C); however, no significant difference was observed between the Fe contents of the leaves of plants grown under the +Fe+Zn condition and those grown in the 50 μmol L^{-1} $ZnCl_2$ nutrient solution (Fig. 4D). These results indicated that the levels of Zn uptake and accumulation in maize plants grown under the –Fe+Zn condition were approximately the same as those in the plants grown in the 50 μmol L^{-1} $ZnCl_2$ nutrient solution. The Zn content of the leaves of the plants grown under the –Fe+Zn and 50 μmol L^{-1} $ZnCl_2$ condition was over 0.4 mg g^{-1} dry weight, which is the level of Zn that is toxic for plants (Broadley *et al.* 2007).

DISCUSSION

Some graminaceous plants are known to secrete mugineic acids (MAs), which belong to a family of phytosiderophores. Mugineic acids solubilize inorganic Fe by chelation and the resulting Fe–MA complex is absorbed at the root surface (Mori 1999). However, it has been reported that MAs also chelate heavy metals other than Fe (Murata *et al.* 2006; Schaaf *et al.* 2004). In our study, the Zn contents of the maize plants grown under the –Fe+Zn condition was 16-fold the contents of plants grown under the +Fe+Zn condition (Fig. 2), and the Zn concentration in the xylem sap of the plants grown under the –Fe+Zn condition was 15-fold greater (Fig. 4). These results indicate that Zn uptake increased under the –Fe+Zn condition. In graminaceous plants, heavy metal–MA complexes are taken up through YS1 in maize (Curie *et al.* 2001), and the substrate specificities of YS differ among plant species (Harada *et al.* 2007; Murata *et al.* 2006). However, HvYS1 from barley has selective specificity for Fe–MA, whereas ZmYS1 from maize has a broad specificity for heavy metal–MA complexes (Murata *et al.* 2006). Iron deficiency caused a fourfold increase in the Zn concentrations in the xylem sap of barley (Alams *et al.* 2001), but caused a 15-fold increase (Fig. 4a) in maize. The broad specificity of

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ZmYS1 might cause the excess Zn uptake by maize plants grown under the Fe-deficient condition.

In contrast, dicotyledonous plants, such as *Nicotiana tabacum* and *Arabidopsis thaliana*, do not secrete MAs. In *Arabidopsis*, YSL1 and YSL 3 contribute only slightly to the uptake of Fe and Zn in roots, but they contribute substantially to the establishment of metal ion homeostasis and the loading of metal ions into seeds (Waters *et al*. 2006). However, Fe deficiency causes excess Zn uptake in tobacco and *Arabidopsis* (Kobayashi *et al*. 2003; Thomine *et al*. 2003). AtIRT1 and AtIRT 2 transport not only Fe, but also Zn (Henriques *et al*. 2002; Vert *et al*. 2001). Moreover, HvIRT1 transports both Fe and Zn (Pedas *et al*. 2008). These reports suggest that IRT also contributes to excess Zn uptake under Fe deficiency and that both monocotyledonous and dicotyledonous plants suffer from excess Zn uptake under Fe deficiency.

Excess Zn accumulation is toxic for plants and severely affects plant growth (Broadley *et al*. 2007). The Zn concentrations in the xylem sap and leaves of the maize plants grown under the –Fe+Zn condition was almost equal to the concentrations in the plants grown in the nutrient solution with 50 μmol L^{-1} $ZnCl_2$ (Figs. 4A,B), which has a Zn concentration that is 100-fold higher than that of the normal nutrient solution. These results indicate that the maize plants grown under the –Fe+Zn condition suffered from stress on account of excess Zn; moreover, the degree of Zn stress might be nearly equal to that under the 50 μmol L^{-1} $ZnCl_2$ condition, in which the Zn concentration is toxic (Broadley *et al*. 2007). Souza and Rauser (2003) indicated that the growth of maize in a nutrient solution containing 50 μmol L^{-1} $ZnCl_2$ was less than that in the normal nutrient solution, and the thiol content was also higher. The thiol content of the plants grown under the –Fe+Zn condition was 1.5-fold that of the plants grown under the +Fe+Zn condition (Fig. 3). This result indicates that the –Fe+Zn condition strongly induces the synthesis of thiol compounds and that maize plants grown under the –Fe+Zn condition suffer from physiological stress as a result of excess Zn.

In the present study, we evaluated Zn uptake and accumulation in Fe-deficient maize and found that growth inhibition under Fe-deficient conditions is significantly associated with the uptake and accumulation of excess Zn. Improving the tolerance of plants to excess Zn might facilitate the recovery of their growth under Fe deficiency.

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