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Zinc deficiency-induced phytosiderophore release by the Triticaceae is not consistently expressed in solution culture

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Abstract. The effects of zinc (Zn) and iron (Fe) deficiencies on phytosiderophore (PS) exudation by three barley (*Hordeum vulgare* L.) cultivars differing in Zn efficiency were assessed using chelator-buffered nutrient solutions. A similar study was carried out with four wheat (*Triticum aestivum* L. and *T. durum* Desf.) cultivars, including the Zn-efficient Aroona and Zn-inefficient Durati. Despite severe Zn deficiency, none of the barley or wheat cultivars studied exhibited significantly elevated PS release rates, although there was significantly enhanced PS exudation under Fe deficiency. Aroona and Durati wheats were grown in a further study of the effects of phosphate (P) supply on PS release, using 100 μM KH_2PO_4 as high P, or solid hydroxyapatite as a supply of low-release P. Phytosiderophore exudation was not increased due to P treatment under control or Zn-deficient conditions, but was increased by Fe deficiency. Accumulation of P in shoots of Zn- and Fe-deficient plants was seen in both P treatments, somewhat more so under the KH_2PO_4 treatment. Zinc-efficient wheats and grasses have been previously shown to exude more PS under Zn deficiency than Zn-inefficient genotypes. We did not observe Zn-deficiency-induced PS release and were unable to replicate the results of previous researchers. The tendency for Zn deficiency to induce PS release seems to be method dependent, and we suggest that all reported instances may be explained by an induced physiological deficiency of Fe.

Key words: *Hordeum* (Zn, Fe deficiency) – Iron deficiency – Phytosiderophore – *Triticum* (Zn, Fe deficiency) – Zinc deficiency – Zinc efficiency

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Introduction

Phytosiderophore (PS) is used as a collective term for a family of mugineic acids which have the same biosynthetic pathway from L-methionine to 2′deoxymugineic acid, with subsequent steps dependent on plant species and cultivar (Mori and Nishizawa 1987; Ma et al. 1995). The structure of phytosiderophores, collected as exudate from Fe-deficient barley (*Hordeum vulgare* L.) roots, was first described by Takemoto et al. (1978). It has been clearly established that mugineic acids, singly, or as mixtures, are exuded from the root tips of Fe-deficient graminaceous plants on a photosensitive cycle, and that the chelation of Fe by these exudates is an Fe-acquisition strategy. The PS-Fe(III) complex is actively transported across the plasma membrane without Fe(III) reduction (Römheld and Marschner 1986). It is assumed, although not proven, that Fe efficiency is directly correlated with the rate of release of PS exudation into the rhizosphere (Römheld and Marschner 1986).

It has been postulated that PSs may play a more general role as 'metallophores' and may thus also contribute to the Zn, Mn and Cu nutrition of the Graminae (Welch 1995). Uptake of the PS-Zn complex by maize (*Zea mays* L.) has been observed (von Wirén et al. 1996). Gries et al. (1998) observed Cu-deficiency-induced PS release from the grass *Hordelymus europaeus* (L.) Harz. Increased exudation of PS by Zn-deficient wheat (*Triticum aestivum* L. cv. Ares) and barley (*Hordeum vulgare* L. cv. Europa) were reported by Zhang et al. (1989). Zinc-efficient wheats and grasses have been shown to exude more PS under Zn deficiency than Zn-inefficient genotypes (Cakmak et al. 1996a,b), suggesting a causal linkage. Cakmak et al. (1996a) have theorized that PS exudation under Zn deficiency is an adaptive trait to poor trace-metal availability in calcareous soils.

Conflicting evidence has been presented, suggesting that PS release is a specific response to Fe deficiency alone. Gries et al. (1995) showed that significant PS release from barley (*Hordeum vulgare* L. cv. CM72) was only induced by Fe deficiency, not Zn, Cu or Mn deficiencies. It was subsequently suggested by Cakmak

et al. (1996b) that the barley cultivar used may have been Zn-inefficient and thus incapable of responding to Zn deficiency by increased PS release. However, Walter et al. (1994) halted Zn-deficiency-induced PS exudation from wheat (*Triticum aestivum* L. cv. Aroona) roots by applying Fe-citrate foliarly. They suggested that Zn deficiency induces Fe deficiency by an inactivation of Fe or an impairment of Fe transport, causing exudation of PS. Inactivation of Fe may be caused by the hyperaccumulation of P, an effect frequently seen in Zn-deficient plants grown in solution culture where P is superoptimally supplied (Loneragan and Webb 1993).

Here, we have investigated the Zn-deficiency-induced PS exudation in two barley cultivars known to differ in Zn efficiency, using chelator-buffered nutrient solutions similar to those of Gries et al. (1995). We then re-examined the comparative effects of Zn and Fe deficiency on PS release in three bread wheat cultivars (including Aroona), and the durum wheat cv. Durati. Zinc-efficient Aroona and Zn-inefficient Durati were used by Cakmak et al. (1996a) and Walter et al. (1994) in order to compare PS release under Zn-deficient conditions. Using chelator-buffered nutrient solutions and two methods for providing P, we found no significant increase in PS release from Zn-deficient roots of either species. Possible reasons for the disparity between our results and previously published results are discussed.

Materials and methods

Three barley genotypes were assessed for PS production under Zn-deficient and Fe-deficient conditions in chelator-buffered solution culture. Two barley (*Hordeum vulgare* L.) cultivars differing in Zn efficiency were used; WI-2597 (Zn-efficient) and Galleon (Zn-inefficient) (Graham et al. 1993). For comparison, we also used the cultivar CM-72, previously studied by Gries et al. (1995). Three bread wheats (*Triticum aestivum* L., cv. Aroona, Excalibur and Gatcher) and a durum wheat (*T. durum* Desf., cv. Durati) were similarly assessed. Aroona and Excalibur are Zn-efficient and Durati and Gatcher are Zn-inefficient wheats (Graham et al. 1993).

Plant growth. Seed was surface-sterilized and germinated in nutrient-solution-soaked germination papers (with all trace metals except Fe and Zn), and placed in environmentally controlled growth chambers for 5–7 d. Seedlings were transferred into mesh-bottomed cups (three seedlings per cup), with Teflon beads covering the base of the cups to block light from the photosensitive solution medium. Sets of six cups were fitted into black plastic lids set upon of pots containing 3.0 L of nutrient solution. Thus subsamples of the plants could be removed for root-exudate collection or vegetative harvest, with minimal disturbance to the remaining plants. Seedlings were grown for up to 20 d from transfer to nutrient solutions (at D0) under environmentally controlled conditions, with daylight (photoperiod) of 14 h, night 10 h. All pot treatments were replicated thrice. Temperature was maintained at 22 °C day/17 °C night, relative humidity at 65%, with a peak photon fluence rate of 500 μmol m^{-2} s^{-1} for at least 8 h.

Solution culture. The basal solution culture contained 2 mM $Ca(NO_3)_2$, 1 mM KNO_3, 0.5 mM $MgSO_4$, 0.01 mM H_3BO_3, 0.5 mM NaOH, 8.0 μM $ZnCl_2$, 0.6 μM $MnCl_2$, 1.0 μM $CuCl_2$, 0.1 μM $NiCl_2$ and 0.1 μM $NaMoO_4$. In control solutions, fixed free metal activities of $(Fe^{3+}) = 10^{-16.5}$, $(Mn^{2+}) = 10^{-7.7}$, $(Cu^{2+}) = 10^{-13.2}$, $(Zn^{2+}) = 10^{-9.8}$ and $(Ni^{2+}) = 10^{-14.1}$ were maintained by addition of HEDTA at a concentration equal to the sum of the trace metal concentrations, plus a 25-μM excess. Chemical speciation of the nutrient solutions were calculated by GEOCHEM-PC (Parker et al. 1995). In treatments where Fe- and Zn-deficient conditions were imposed, Fe-HEDTA and Zn-HEDTA were added to the basal solution at 2.4 μM and 0.2 μM respectively, giving $(Fe^{3+}) = 10^{-18.0}$ and $(Zn^{2+}) = 10^{-11.4}$. Solution pH was maintained at pH 6.0 with 1.0 mM 2-(4-morpholino)-ethanesulfonate (Mes), and by daily testing and adjustment with 1 M HCl. Solutions were replaced on D5, D9, and every third day thereafter. Double-deionized (DDI) water was used for all solutions. In half of the treatments in the final experiment, P was conventionally supplied as 100 μM KH_2PO_4. In all other treatments, P was supplied to the plants by the use of solid hydroxyapatite (HAP) suspension (Sigma) in dialysis tubing pouches, as described in detail by Parker (1993). The HAP suspension was repeatedly washed in DDI water and finally resuspended in $Ca(NO_3)_2$ and Mes buffer to a final concentration of 2.5% (w/v) HAP. The suspension was dispensed in 20-ml aliquots into pouches of 15-cm lengths cut from Spectrapor #1 dialysis tubing (MWCO 1300). The pouches were secured at each end with clamps (Spectrapor) and, once completed, were rinsed at least twice in DDI water to remove any splashes of HAP on the outer surface. Two pouches were suspended in the solution of each pot, and were replaced with each solution culture change, the dialysis tubing being susceptible to deterioration over longer periods.

Plant harvests and analysis. Vegetative harvests were carried out after the initial root-exudate collection, at the mid-point of the experiment and after the final root-exudate collection. At each harvest, two cups of plants from each pot were divided into shoots and roots, blotted on absorbent paper and dried overnight at 65 °C. Dry weights were made separately for each cup, but tissue from each pot was bulked for elemental analysis at each harvest. Acid digests of shoots and roots were assayed for Fe, Zn, Mn and Cu by atomic absorption spectrophotometry, and for P by continuous-flow colorimetry using a Technicon Autoanalyzer II. The sequential harvests of plants during the experiment meant that the root mass in solution was approximately constant. The average dry weight of roots per pot throughout the experiment was roughly 0.20 g for wheat and 0.30 g for barley. Single-factor analysis of variance (ANOVA) was carried out on dry-weight yields and elemental analysis.

Phytosiderophore collection and assay. Root-exudate collections were started on the day of the initial vegetative harvest and generally every second day thereafter. Two hours after the beginning of the photoperiod, one cup was removed from each pot and the roots rinsed in DDI water to wash off surface nutrient solution and chelate. The cup was then placed over a collection vessel containing 100 ml DDI water, immersing the roots entirely. The collection vessels were placed in the growth chamber and left for 3 h to collect peak PS release. After replacing the plants in their respective nutrient solutions, 10% Micropur (Roth, Karlsruhe, Germany), an antimicrobial agent, was added to collections to prevent microbial degradation of the root exudate. A 10-mL sample of each collection was immediately assayed for Fe-chelating (PS) agents, using the ferrous-ferrozine colorimetric method described by Gries et al. (1995). To calculate PS release rates based on root dry weight, root weights were linearly interpolated between the harvest dates. Treatment effects on PS release were statistically analyzed by single-factor ANOVA. In the final experiment, simultaneous assays were carried out using a Cu-mobilization assay, described by Walter et al. (1994). A positive, but imperfect correlation between the two assays was found ($R = 0.7$). Both of these assays are indirect, but in trials we found the method of Gries et al. (1995) to be more reproducible. Indirect assays cannot distinguish PS from other Fe-chelating substances, and are not as precise as direct HPLC analysis. However, for our purposes, the indirect assay was more practical, HPLC methods requiring lengthy sample preparation. Ion chromatography analysis of bulk root exudate collections did not detect citrate, which confounds the ferrous-ferrozine assay.

Results

Barley. Barley plants were grown for 17 d, and plants were harvested at D6, D12 and D17, with root exudate collections begun on D6 and every second day thereafter. Phytosiderophore exudation by Zn-deficient plants was not significantly different from that of the control plants, whereas the Fe-deficient plants consistently released at least 20 μmol PS/g root in the 3-h collection period (Fig. 1). Clear differences were seen in plant growth response to Fe and to Zn deficiencies in the distribution of dry weight (Table 1). At the final harvest, the −Fe and −Zn treatments had reduced total relative yield by 50% and 75% respectively. Root weight was enhanced under −Zn treatments, and depressed under −Fe. Levels of Fe and Zn in shoots and roots showed severe deficiency where there was correspondingly low Fe and Zn in the solution culture. Increased Fe and Mn concentrations were seen in shoots of Zn-deficient plants, and increased Zn, Mn and Cu in Fe-deficient shoots. Increased uptake of other divalent cations is often seen in plants suffering from trace-metal deficiencies (Norvell and Welch 1993; Parker 1997). No symptoms of P toxicity were apparent in any plants during the experiment, but shoot P was elevated under by −Fe and especially by the −Zn treatment (to >1% dry weight). The barleys CM-72 and WI-2597 had stronger vegetative growth than Galleon under control conditions. However, there were no major differences between the genotypes in the amount of phytosiderophore produced (per root mass), under any treatment. Differences in Zn efficiency between the barleys were not clear in these solution culture experiments, but field trials in South Australia have shown that WI-2597 is significantly more Zn-efficient than Galleon (Graham et al. 1993).

Wheat. Wheat plants were grown for 17 d and harvested at D11, D13 and D17, with root exudate collections every second day from D9. Despite seemingly adequate Zn and Fe nutrition, an anomalously high PS release (approx. 20 μmol PS/g root) was initially seen in control treatments, decreasing to less than 10 μmol PS/g root by D15 (Fig. 2). Phytosiderophore release from Zn-deficient plants was not significantly different from the control for any of the cultivars, although the two Zn-efficient wheat cultivars, Aroona and Excalibur, appeared to show slightly higher PS exudation than the

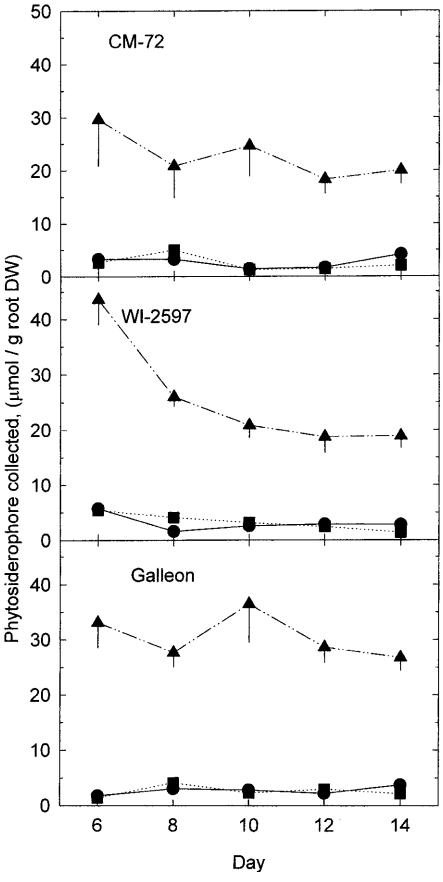

Fig. 1. Phytosiderophore assayed in root exudate collections made over 8 d from three barley cultivars, differing in Zn efficiency: CM-72, WI-2597 (Zn-efficient) and Galleon (Zn-inefficient). Root-exudate collections were assayed for PS using the method outlined in the text. Plants were grown in chelator-buffered solution culture under control (●), Zn-deficient (■), and Fe-deficient (▲), conditions. Values are the means of three replicates, and error bars indicate SE, unless obscured by symbols

Table 1. Shoot and root dry weights (DW), and shoot concentrations of P, Zn, Fe, Mn and Cu, of the final harvests of barley plants grown for 16 d in chelator-buffered solution culture, under control, Zn-deficient and Fe-deficient conditions. The three cultivars of barley differ in Zn efficiency: CM-72, WI-2597 (Zn-efficient) and Galleon (Zn-inefficient). Values are the mean ± SE of three replicates

Cultivar		Shoot DW (g)	Root DW (g)	Concentration (μmol/g DW)				
				P	Zn	Fe	Mn	Cu
CM-72	Control	1.39 ± 0.07	0.474 ± 0.06	69 ± 5	0.36 ± 0.02	0.82 ± 0.01	0.70 ± 0.07	0.09 ± 0.01
	−Zn	0.25 ± 0.02	0.234 ± 0.02	323 ± 36	0.12 ± 0.00	1.72 ± 0.28	1.48 ± 0.28	0.07 ± 0.00
	−Fe	0.48 ± 0.06	0.275 ± 0.02	197 ± 27	1.69 ± 0.10	0.52 ± 0.12	1.99 ± 0.24	0.33 ± 0.05
WI-2597	Control	1.40 ± 0.04	0.434 ± 0.02	83 ± 6	0.42 ± 0.02	1.06 ± 0.34	1.11 ± 0.08	0.13 ± 0.02
	−Zn	0.17 ± 0.01	0.172 ± 0.01	630 ± 41	0.08 ± 0.01	1.85 ± 0.26	2.91 ± 0.21	0.13 ± 0.01
	−Fe	0.60 ± 0.04	0.290 ± 0.02	155 ± 21	1.93 ± 0.02	0.44 ± 0.03	2.67 ± 0.09	0.51 ± 0.01
Galleon	Control	0.67 ± 0.18	0.303 ± 0.04	124 ± 5	0.42 ± 0.04	0.81 ± 0.11	0.85 ± 0.17	0.12 ± 0.03
	−Zn	0.14 ± 0.01	0.140 ± 0.01	478 ± 34	0.09 ± 0.01	2.08 ± 0.38	1.92 ± 0.14	0.21 ± 0.01
	−Fe	0.43 ± 0.07	0.234 ± 0.03	275 ± 75	1.88 ± 0.15	0.47 ± 0.09	2.64 ± 0.12	0.49 ± 0.04

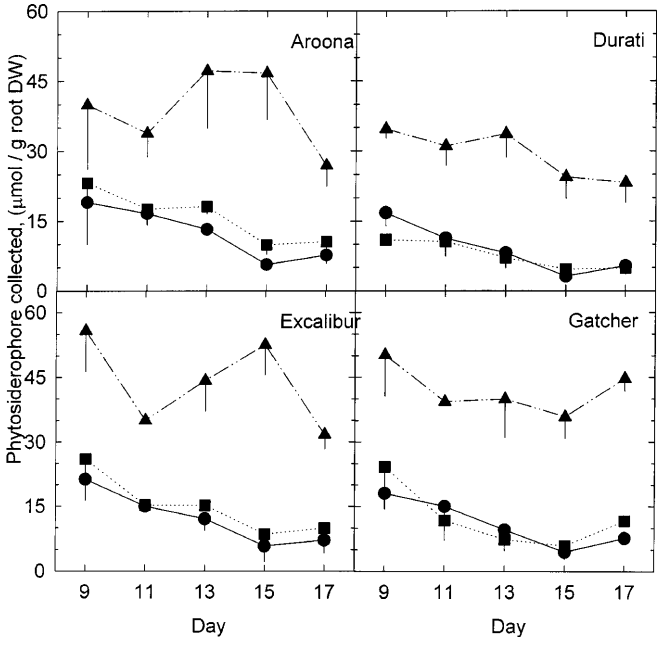

Fig. 2. Phytosiderophore assayed in root exudate collections made over 8 d from four wheat cultivars, differing in Zn efficiency: Aroona, Excalibur (Zn-efficient), Durati and Gatcher (Zn-inefficient). Root-exudate collections were assayed for PS using the method outlined in the text. Plants were grown in chelator-buffered solution culture under control (●), Zn-deficient (■), and Fe-deficient (▲), conditions. Values are the means of three replicates, and error bars indicate SE, unless obscured by symbols

control plants. The PS release from Fe-deficient plants was consistently elevated compared to the control, between 28 and 56 μmol PS/g root.

We could determine no reason for the high basal levels of PS release in this experiment. The peak light level in our experiments was considerably higher than that of other researchers (500 compared to 300 μmol photons m^{-2} s^{-1}) and increased phytosiderophore exudation has been correlated with increased light intensity conditions (Cakmak et al. 1998). However, this increased exudation was not consistently seen and could not be truly attributed to light conditions. Root-exudate collections from this experiment were frozen and later analyzed by ion chromatography for possible Fe-binding organic acids such as citrate, which might have confounded the assay, but no such other Fe-chelates were detected (data not shown).

The dry-weight distribution for wheat was similar to the results for barley: an increased root:shoot ratio under −Zn and a decreased ratio under −Fe treatments (Table 2). The −Fe and −Zn treatments reduced total yield by 70% and 80% respectively, compared to control plants. Increased levels of Mn, Fe and Cu were seen in shoots of Zn-deficient plants, while Fe-deficiency increased Zn, Cu and Mn concentrations. Under Zn-deficient conditions, there was clearly more P accumulated in the shoots of the Zn-efficient plants than the less-efficient genotypes, especially Durati. This may indicate a differential accumulation of P associated with Zn efficiency. Excalibur is known to suffer from severe P toxicity symptoms when grown in Zn-deficient solution culture (Rengel and Graham 1995).

Wheat: P-supply effects. In a third experiment, Aroona and Durati wheats were grown for 20 d and harvested at D6, D12 and D20, with root exudate collections regularly after D10. Zinc-deficient conditions did not increase PS release above the control levels. In Durati, grown with conventionally supplied P, PS release from −Zn treatment plants was consistently lower than from control plants (Fig. 3). Phosphate supplied as HAP slightly but significantly increased PS release from Aroona, but not Durati. Phytosiderophore release in all treatments was consistently lower than in the previous wheat experiment. Plants supplied conventionally with KH_2PO_4 accumulated up to 50% more P in shoots than HAP-supplied plants (Table 3), with Zn-deficient plants showing some P-toxicity symptoms. There was no significant difference between the cultivars

Table 2. Shoot and root dry weights (DW), and shoot concentrations of P, Zn, Fe, Mn and Cu, of the final harvests of wheat plants grown for 17 d in chelator-buffered solution culture, under control, Zn-deficient and Fe-deficient conditions. The four cultivars of wheat differ in Zn: Aroona, Excalibur (Zn-efficient), Durati and Gatcher (Zn-inefficient). Values are the mean ± SE of three replicates

Cultivar		Shoot DW (g)	Root DW (g)	Concentration (μmol/g DW)				
				P	Zn	Fe	Mn	Cu
Aroona	Control	0.90 ± 0.07	0.250 ± 0.03	166 ± 23	0.41 ± 0.04	1.06 ± 0.16	0.95 ± 0.17	0.12 ± 0.02
	−Zn	0.09 ± 0.00	0.118 ± 0.01	662 ± 10	0.16 ± 0.00	3.11 ± 0.33	5.53 ± 0.13	0.51 ± 0.05
	−Fe	0.29 ± 0.04	0.134 ± 0.01	262 ± 17	1.05 ± 0.04	0.54 ± 0.06	1.80 ± 0.17	0.29 ± 0.05
Excalibur	Control	1.09 ± 0.09	0.310 ± 0.02	122 ± 30	0.37 ± 0.03	1.03 ± 0.07	0.92 ± 0.18	0.10 ± 0.01
	−Zn	0.17 ± 0.01	0.211 ± 0.01	867 ± 67	0.14 ± 0.01	2.25 ± 0.10	4.69 ± 0.09	0.41 ± 0.02
	−Fe	0.24 ± 0.07	0.111 ± 0.02	317 ± 52	1.01 ± 0.15	0.49 ± 0.09	1.88 ± 0.19	0.34 ± 0.04
Durati	Control	1.02 ± 0.05	0.296 ± 0.02	151 ± 6	0.45 ± 0.05	1.13 ± 0.21	1.27 ± 0.05	0.13 ± 0.01
	−Zn	0.14 ± 0.02	0.145 ± 0.01	555 ± 79	0.12 ± 0.01	2.06 ± 0.17	4.37 ± 0.14	0.23 ± 0.00
	−Fe	0.38 ± 0.09	0.152 ± 0.03	335 ± 63	0.91 ± 0.09	0.63 ± 0.14	2.37 ± 0.40	0.28 ± 0.03
Gatcher	Control	0.91 ± 0.06	0.265 ± 0.03	136 ± 21	0.33 ± 0.03	0.91 ± 0.17	0.94 ± 0.18	0.10 ± 0.02
	−Zn	0.12 ± 0.01	0.135 ± 0.01	664 ± 45	0.13 ± 0.00	2.08 ± 0.28	3.79 ± 0.44	0.29 ± 0.04
	−Fe	0.17 ± 0.01	0.094 ± 0.00	375 ± 6	0.99 ± 0.03	0.58 ± 0.09	1.89 ± 0.23	0.32 ± 0.01

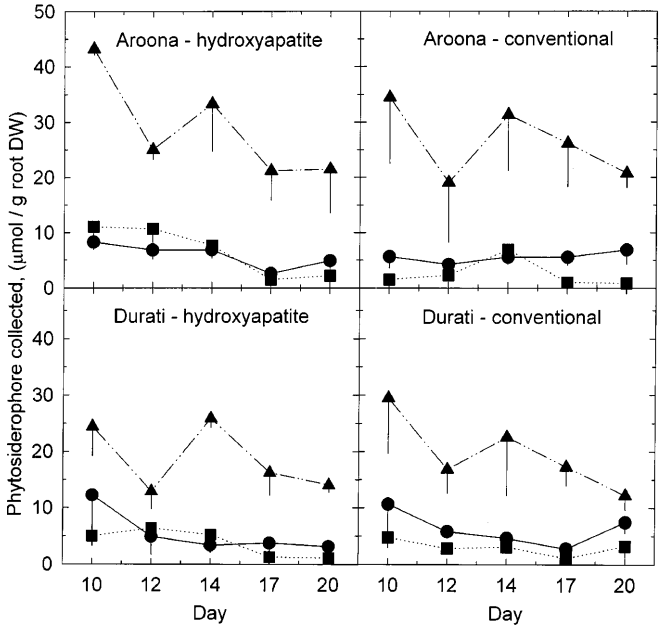

Fig. 3. Phytosiderophore assayed in root exudate collections made over 8 d from two wheat cultivars, Aroona (Zn-efficient) and Durati (Zn-inefficient), grown with different forms of P-supply: solid hydroxyapatite in dialysis-tubing sacs, or conventional P, supplied in solution as 100 µM $KHPO_4$. Plants were grown in solution culture under control (●), Zn-deficient (■), and Fe-deficient (▲), conditions. Root-exudate collections were assayed for phytosiderophore using the method outlined in the text. Values are the means of three replicates, and error bars indicate SE, unless obscured by symbols

in accumulation of P in shoots. At the final harvest, −Fe and −Zn treatments had reduced shoot growth by 50% and 70% respectively, compared to control plants. Iron and Zn levels in plants were similar to those of the previous wheat experiment (data not shown).

Discussion

Our results corroborate those of Gries et al. (1995) showing that Zn deficiency in barley does not induce PS release greater than control levels. We saw no differences in PS release between two barley cultivars known to differ significantly in Zn efficiency. Zhang et al. (1989) reported an increase in PS release from Zn-deficient barley roots, but gave no data for Fe-deficient or control-plant release of PS, making the magnitude of this increase difficult to assess.

In contrast to published results, Zn-efficient wheats (Aroona and Excalibur) grown in −Zn treatments did not release more PS than Zn-inefficient wheats (Durati and Gatcher). Moreover, none of the four genotypes showed significantly elevated PS release rates relative to the control treatments. Phytosiderophore release in both wheat and barley was only significantly increased above control levels by the −Fe treatment.

Our results are at odds with published work, and we sought to explain these differences by examining differences between our methods and those of other researchers. Firstly, we present PS release as a function of root dry weight, while it is frequently presented on a per-plant basis. However, when our results are calculated on a per-plant basis, there is still no significant difference in PS release between control and −Zn treatments. If data from Walter et al. (1994) are recalculated as PS release per gram of root dry weight, there appear to be only minor differences between their data and ours. In all our experiments, the final root masses differed significantly between the control, −Fe and −Zn treatments, with Zn-deficient plants having greater root mass than Fe-deficient plants. We thus preferred to present PS release as a function of root dry weight, which naturally increased during the experiment. Periodic plant harvests during our experiments allowed us to estimate root dry weight for all PS collections.

Secondly, we attempted to maintain root density at a moderate level in 3 L of nutrient solution (see *Materials and methods*) to ensure stable growth conditions. Cakmak et al. (1996a,b) and Walter et al. (1994) grew 30 plants in 600 mL of nutrient solution. The resulting high root density does not lend itself to unequivocal root-exudate responses. Work by Chaboud and Rougier

Table 3. Shoot and root dry weights (DW), and shoot P concentrations of the final harvests of wheat plants grown for 20 d in solution culture under two sources of P-supply, solid hydroxyapatite and conventional KH_2PO_4. Aroona (Zn-efficient), and Durati (Zn-inefficient) wheats were grown under control, Zn-deficient and Fe-deficient conditions. Values are the mean ± SE of three replicates

		Shoot DW (g)	Root DW (g)	P (µmol/g DW)
Hydroxyapatite P				
Aroona	Control	1.22 ± 0.13	0.38 ± 0.04	97 ± 18
	−Zn	0.39 ± 0.07	0.15 ± 0.01	369 ± 68
	−Fe	0.43 ± 0.07	0.21 ± 0.02	163 ± 42
Durati	Control	1.14 ± 0.12	0.28 ± 0.04	108 ± 17
	−Zn	0.30 ± 0.13	0.26 ± 0.03	358 ± 26
	−Fe	0.51 ± 0.15	0.24 ± 0.07	185 ± 46
Conventional P				
Aroona	Control	1.38 ± 0.13	0.39 ± 0.03	212 ± 23
	−Zn	0.39 ± 0.14	0.16 ± 0.02	404 ± 13
	−Fe	0.98 ± 0.22	0.14 ± 0.02	352 ± 13
Durati	Control	1.45 ± 0.09	0.23 ± 0.04	218 ± 23
	−Zn	0.22 ± 0.04	0.18 ± 0.02	389 ± 57
	−Fe	0.38 ± 0.05	0.33 ± 0.05	336 ± 25

(1991) has shown that increasing root mass inside a limited volume of solution usually increases root exudations, perhaps due to increased competition for nutrients.

Obtaining trace-element deficiencies using conventional solution culture can be problematic, and might justify the very high root densities used previously. Parker and Norvell (1999) have discussed in detail the differences between conventional nutrient solutions (such as Hoaglands) and chelator-buffered nutrient solutions. Chelator-buffered nutrient solution can more readily impose severe micronutrient deficiencies than conventional solution culture and the effect of the −Fe and −Zn treatments employed here were severe, especially in the first wheat experiment. However, despite severe Zn-deficiency symptoms, PS release from −Zn treatment plants was not greater than control levels. In contrast, exudation of PS under Fe-deficient conditions increases with the severity of the imposed deficiency, even as relative yield decreases (Gries et al. 1995). In our experiments, PS release under Zn-deficient conditions remained at low levels (certainly compared to Fe-deficient plants), despite severe yield reductions.

We could not duplicate the results obtained by Rengel et al. (1998), who used similar chelator-buffered nutrient solutions to grow the wheat cultivars Warigal and Durati under Zn-deficient conditions, and observed increased PS release by the Zn-efficient Warigal. Rengel et al. (1998) used the high plant density described above, with P supplied conventionally at 100 μM.

Walter et al. (1994) listed several possible reasons for the release of PS by Zn-deficient Graminae, including the possibility of impairment of Fe translocation to the shoot. Most plausible was the suggestion that accumulation of P in Zn-deficient shoots might precipitate Fe-P in the xylem and inactivate Fe in the xylem or shoot. Seminal work (Olsen 1935) showed that Fe and P accumulate in the xylem tissue of maize plants provided with superoptimal PO_4 (1.1 mM). Using scanning electron micrography, Daniels et al. (1973) showed that Fe and P accumulated together in the xylem and metaxylem elements of bean (*Phaseoulus vulgaris* L.) leaves when plants were grown under toxic Cu (8 μM) treatments in a Hoaglands solution culture.

Hyperaccumulation of P in shoots has long been known as a symptom of Zn-deficient plants grown in solution culture – it is an effect not reported for field-grown plants (Loneragan and Webb 1993; Parker 1997). Hyperaccumulation of Fe in shoots of Zn-deficient wheat grown in solution culture has also been observed, up to 5–6 μmol/g shoot dry weight (Walter et al. 1994; Cakmak et al. 1996a). It can be presumed that P was also accumulated in Zn-deficient shoots under the conditions imposed by these researchers, but no data were given. It is unlikely that Fe translocation was impaired under Zn deficiency, since Fe was hyperaccumulated in the *shoots* of the Zn-deficient plants. The precipitation of Fe-P in shoot tissue is a more probable means of reducing the physiological availability of Fe in shoots, thus creating a physiological deficiency and a consequent increase in PS exudation. The occurrence of a physiological Fe-deficiency in the shoots is supported, as Walter et al. (1994) were able to halt PS release in Zn-deficient plants by the foliar application of Fe-citrate.

In our experiments we saw only mild accumulation of Fe in shoots (2.0 μmol/g dry weight), and no significant increase in exudation of PS under Zn deficiency, even when P was supplied at a high rate (100 μM). Accumulation of P in shoots under Zn deficiency was not clearly linked with inactivation of Fe under our growth conditions. We had sought to supply P at levels approximating those of soil solution, using the HAP method. Gries et al. (1995) observed P-deficiency effects on plant growth, using HAP in similar experiments. The accumulation of P by Fe- and Zn-deficient plants in our experiments using the HAP supply was unexpected. Zinc deficiency disrupts the normal regulation of P uptake and transport to the shoot, and the HAP method is an imperfect means of limiting P hyperaccumulation by Zn-deficient plants (Parker 1993).

While PSs are capable of binding transition metal cations other than Fe(III), the binding constants for these other metal ions are generally much weaker (Sugiura et al. 1981; Murakami et al. 1989). Sugiura et al. (1981) estimated the formation constants for PS complexation with Fe(III), Cu(II), Zn(II) and Fe(II) as (log K =) 18.1, 18.3, 10.7 and 8.1, respectively. Nuclear magnetic resonance studies by Iwashita et al. (1983) indicate that there are clear conformational differences between PS-Zn(II) and PS-Co(III) [the latter being an analog for the PS-Fe(III) complex (Mino et al. 1983)]. The PS-Fe(III) complex is taken up at least five times faster than other PS-trace metal complexes (Ma et al. 1993). Ma and Nomoto (1993) showed that high concentrations (50 μM) of the PS-Cu(II) and PS-Zn(II) complexes are necessary to inhibit the uptake of the PS-Fe(III) complex. Thus, even if the general metallophore hypothesis is valid, the stereochemical evidence indicates that the transport system for PS-trace metal uptake is highly specific for PS-Fe uptake.

It is hypothesised that production of PS by members of the Graminae is an evolutionary adaptation to calcareous soils, low in available Fe and other trace metals (Gries and Runge 1992), and that genotypes exhibiting high levels of PS release are Fe-efficient. It has been suggested that increased PS release by Zn-efficient genotypes is similarly an adaptive response to Zn-deficient conditions (Cakmak 1996a,b). However, we have shown that an increased PS release response to Zn-deficient conditions is not consistently expressed in barley or in wheat cultivars known to differ in Zn-efficiency. Erenoglu et al. (1996) also found a poor correlation of PS release with Zn-efficiency in bread wheats, grown under Zn-deficient conditions.

Although it has been shown that PS can mobilize trace metals other than Fe into the soil solution (Treeby et al. 1989), and that the uptake of PS-metal complexes is not restricted to the PS-Fe(III) complex (von Wirén et al. 1996; Rengel et al. 1998), it is not yet clear that the release of PS is a general response to trace-metal deficiencies. There is no evidence that the biochemical synthesis of PS is directly stimulated by deficiency of

trace metals other than Fe. We have discussed possible means by which solution culture conditions may promote PS release by Zn-deficient plants. The evidence for Zn-deficiency-induced PS release is presently confined to solution culture experiments, and awaits confirmatory evidence from soil-grown plants.

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