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Roots of Iron-Efficient Maize also Absorb Phytosiderophore-Chelated Zinc[1]

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To investigate the recognition of Zn-phytosiderophores by the putative Fe-phytosiderophore transporter in maize (*Zea mays* L.) roots, short-term uptake of ^{65}Zn-labeled phytosiderophores was compared in the Fe-efficient maize cultivar Alice and the maize mutant ys1 carrying a defect in Fe-phytosiderophore uptake. In ys1, uptake and translocation rates of Zn from Zn-phytosiderophores were one-half of those in Alice, but no genotypical difference was found in Zn uptake and translocation from other Zn-binding forms. In ys1 and in tendency also in Alice, Zn uptake decreased with increasing stability constant of the chelate in the order: $ZnSO_4$ ≥ Zn-desferrioxamine > Zn-phytosiderophores > Zn-EDTA. Adding a 500-fold excess of free phytosiderophores over Zn to the uptake solution depressed Zn uptake in ys1 almost completely. In uptake studies with double-labeled ^{65}Zn-^{14}C-phytosiderophores, ys1 absorbed the phytosiderophore at similar rates when supplied as a Zn-chelate or the free ligand. By contrast, in Alice ^{14}C-phytosiderophore uptake from the Zn-chelate was 2.8-fold higher than from the free ligand, suggesting that Alice absorbed the complete Zn-phytosiderophore complex via the putative plasma membrane transporter for Fe-phytosiderophores. We propose two pathways for the uptake of Zn from Zn-phytosiderophores in grasses, one via the transport of the free Zn cation and the other via the uptake of nondissociated Zn-phytosiderophores.

In calcareous soils, in which Fe and Zn deficiencies in plants are widespread, high soil pH decreases the solubility of both metals to very low levels, mainly because of the formation of inorganic Fe complexes and an increased Zn adsorption by soil constituents (Moraghan and Mascagni, 1991). Since both metals possess a relatively high affinity for organic chelators, the presence of synthetic or naturally occurring chelators can enhance considerably the metal concentrations in the soil solution (Norvell, 1991). As a consequence of these similarities in physicochemical behavior, Fe and Zn availabilities are often affected in the same manner by plant-induced processes at the soil-root interface (Marschner et al., 1986; Dinkelaker et al., 1989). For example, phytosiderophores released by Fe-deficient graminaceous plant species efficiently chelate and mobilize both micronutrient metals (Treeby et al., 1989). Furthermore, the release of phytosiderophores is also induced under Zn deficiency (Zhang et al., 1989; Cakmak et al., 1994; Walter et al., 1994). Although the induction of phytosiderophore release and the mobilization by phytosiderophores are similar for Fe and Zn, distinct differences are found in their uptake by roots. Grasses can absorb Fe via a nondissociated Fe(III)-phytosiderophore complex (Römheld and Marschner, 1986), whereas Zn is believed to enter plant roots only as a nonchelated divalent cation (Halvorson and Lindsay, 1977), which has been confirmed by numerous observations showing that Zn uptake is highly dependent on its free ion activity (Bell et al., 1991; Norvell and Welch, 1993). The beneficial effect of chelators in Zn acquisition, therefore, has been restricted to Zn mobilization, whereas Zn uptake is actually inhibited by high chelator concentrations (Laurie et al., 1991; Zhang et al., 1991).

Short-term uptake studies with double-labeled Fe-phytosiderophores provided evidence for the existence of an uptake system for nondissociated Fe(III)-phytosiderophores at the root plasma membrane of graminaceous plant species (Marschner et al., 1989). This putative Fe-phytosiderophore transporter is inducible under Fe deficiency (Römheld, 1987), is sensitive to chilling and proton uncouplers (Mori, 1994), has a high affinity for Fe-phytosiderophores ($K_m \approx 10$ μM; von Wirén et al., 1995), and does not transport microbial or synthetic Fe-chelates (Römheld and Marschner, 1986; Bar-Ness et al., 1992). These results lead to the concept of a specific recognition and transport of Fe-phytosiderophores across the root plasma membrane in grasses (Römheld and Marschner, 1986). Uptake rates of other metal-phytosiderophores were lower than those of Fe-phytosiderophores and were regarded as a consequence either of chelate splitting prior to metal uptake (Marschner et al., 1989; Zhang et al., 1991) or of irreversible metal binding to the transporter when a subsequent inhibition of Fe-phytosiderophore uptake was observed (Ma and Nomoto, 1993; Ma et al., 1993). Doubts remained, however, about the metal specificity in Fe-phytosiderophore uptake (Kochian, 1993) and whether the ligand concomitantly enters the root when bound to metals other than Fe.

The objective of the present study was to address the question of the specificity of the metal component in Fe-phytosiderophore transport. We have focused on the up-

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Abbreviations: DMA, 2′-deoxymugineic acid; *e*HMA, *epi*-hydroxymugineic acid.

take of Zn-phytosiderophores for two reasons: first, Zn-phytosiderophores have similar structural conformations as Fe-phytosiderophores (Iwashita et al., 1983), providing a theoretical chance for their recognition by the transport protein; and second, the uptake of Zn-phytosiderophores could be of ecological significance because Fe and Zn deficiencies in plants often occur under the same environmental conditions. To determine the contribution of the Fe-phytosiderophore transporter in Zn uptake, we used the maize (*Zea mays* L.) mutant ys1 as a reference genotype, since it has been shown to carry a defect in Fe-phytosiderophore uptake (von Wirén et al., 1994). Short-term uptake studies with ^{65}Zn single- and double-labeled phytosiderophores were conducted with the mutant and the wild-type maize cv Alice, both precultured under Fe deficiency to stimulate phytosiderophore-transport activity (von Wirén et al., 1995).

MATERIALS AND METHODS

Plant Preculture and Production of Phytosiderophores

Seeds of maize (*Zea mays* L. cv Alice and mutant yellow-stripe 1, ys1) were placed between filter paper sheets moistened with saturated $CaSO_4$ solution and germinated at 25°C in the dark. After 3 d, seedlings were transferred to a continuously aerated nutrient solution composed of 2.0 mM $Ca(NO_3)_2$, 0.7 mM K_2SO_4, 0.5 mM $MgSO_4$, 0.1 mM KH_2PO_4, 0.1 mM KCl, and micronutrients: 10 μM H_3BO_3, 0.5 μM $MnSO_4$, 0.5 μM $ZnSO_4$, 0.2 μM $CuSO_4$, and 0.01 μM $(NH_4)_6Mo_7O_{24}$. Plants were cultured either without Fe supply (−Fe treatment) or supplied with 10^{-4} M Fe(III)-EDTA (+Fe treatment). From d 9 onward, nutrient solutions were changed daily and the pH was adjusted to 5.5. Alice-Fe plants were cultivated without Fe supply from the beginning, whereas ys1-Fe plants were precultured for 9 d with 10^{-5} M Fe(III)-EDTA because a lower amount of Fe is stored in their seeds. From d 13 onward, micronutrients were omitted from the nutrient solution to avoid interactions of apoplasmic metal ions with radioactively labeled Zn-phytosiderophores in the uptake solution. All plants were cultivated for 15 d in a growth chamber at 60% RH, with a light intensity of 250 μmol m^{-2} s^{-1} at plant height and a day-night temperature regime of 16 h/25°C and 8 h/22°C, respectively. The light was provided by fluorescent tubes (Sylvania, cool-white FR96 T12).

For collecting large amounts of phytosiderophores, wheat (*Triticum aestivum* L. cv Ares) or barley (*Hordeum vulgare* L. cv Taiga) plants were grown in nutrient solution as described above without supplying Fe until Fe-deficiency chlorosis appeared. Nutrient solutions were then replaced by distilled water every morning 2 h after the onset of the light period, and root exudates were collected over a 4-h period. Crude root exudates were treated with Micropur (Roth, Karlsruhe, Germany) to prevent microbial degradation of phytosiderophores, filtered through coarse filter paper, and concentrated by evaporation under vacuum at 50°C. Thereafter, phytosiderophores were partially purified by gradient elution through a Dowex 50 WX 8 cation-exchange column (Serva, Heidelberg, Germany) using 0.5 to 2.0 M HCl as eluant. In the purified samples, phytosiderophores were quantified by HPLC (Mori et al., 1987) and identified exclusively as DMA in wheat or as *e*HMA (>95%), mugineic acid, and DMA (<5%) in barley. ^{14}C-labeled phytosiderophores were obtained from barley plants by the same procedure, except that during a period of 2 weeks Fe-deficient barley plants were placed in a plexiglass box and exposed to 4.4 MBq of $^{14}CO_2$, produced by the reaction of $Ba^{14}CO_3$ with 50% lactic acid. Root exudates were collected the following morning and purified as above. An equivalent amount of DMA or ^{14}C-labeled phytosiderophores was mixed with $^{65}ZnSO_4$ (specific activity 2.2 TBq mol^{-1} Zn), and aliquots were taken for direct scintillation counting or spotted onto paper discs for ashing in a carbon oxidizer (TRI-CARB B306, Canberra-Packard, Dreieich, Germany) prior to scintillation counting.

Uptake Experiments with Labeled Compounds

For short-term uptake experiments, Alice and ys1 plants were transferred to fresh micronutrient-free nutrient solution and supplied with 1 or 10 μM ^{65}Zn-labeled DMA or ^{14}C-phytosiderophores (>95% *e*HMA). As previously shown, the use of different phytosiderophore species does not influence genotypical differences in Fe(III)-phytosiderophore uptake (Römheld and Marschner, 1990; von Wirén et al., 1994). Complexes of Zn with phytosiderophores, desferrioxamine, and EDTA were prepared by mixing the ligand and $ZnSO_4$ in equimolar concentrations. Uptake rates of ^{59}Fe-labeled DMA were determined as described previously (von Wirén et al., 1995). For the first experiments (Tables I and II), uptake periods of 1 and 2 h were chosen to avoid a significant depletion of the radioisotopes in the uptake solution and, at the same time, to measure radioisotope translocation to the shoots, which usually requires uptake periods of >40 min (Pearson and Rengel, 1995). In subsequent experiments (Fig. 1; Table III), radioisotope accumulation was determined in roots after 30 min. All experiments started 7 h after the onset of the light period and were conducted under the same environmental conditions as described above. The uptake experiments were terminated by transferring the roots to 1 mM $CaSO_4$ for 10 min and subsequently to 1 mM Na_2EDTA for 15 min to exchange and chelate apoplasmic root Zn. Shoots were immediately separated from roots, and the remaining radioactivity in the uptake solution was measured. Shoots and roots were dried at 60°C, weighed, and in the case of ^{14}C uptake ashed in a carbon oxidizer, from which the ash was recovered. ^{65}Zn- and ^{59}Fe-labeled samples were ashed at 550°C, digested in 3 N HNO_3, resuspended in 1.5 N HCl, and finally assayed for radioisotope activity by liquid scintillation spectrophotometry.

Data from at least three replicates of samples from the same experimental conditions were tested by analysis of variance, and when analysis of variance generated a significant *F* value ($P \leq 0.05$), treatment means were compared by the Scheffé test. Data are presented as mean values ± SE.

RESULTS

In Alice plants adequately supplied with Fe, rates of Zn uptake from ^{65}Zn-labeled phytosiderophores were similar to Fe uptake rates from the corresponding Fe-phytosiderophores (Table I). Fe-deficiency treatment, which generated moderately chlorotic leaves in both genotypes, enhanced root uptake and shoot translocation of Fe in Alice more than 2-fold, whereas Zn uptake and translocation were less enhanced. By contrast, ys1 plants absorbed and translocated much more Zn than Fe from the phytosiderophore complex with no response to the Fe-deficiency treatment. In both genotypes, Zn uptake from phytosiderophores was considerably lower than from $ZnSO_4$, and Zn translocation rates followed the same trend.

Zn uptake was determined in the presence of the following ligands differing in their stability constant for Zn: desferrioxamine, $10^{6.2}$ (Richardson et al., 1989); phytosiderophores, $10^{10.7}$ (Sugiura et al., 1981); and EDTA, $10^{13.4}$ (Richardson et al., 1989). In ys1 and in tendency also in Alice, Zn uptake and translocation rates from different binding forms decreased in the following order: $ZnSO_4 \geq$ Zn-desferrioxamine > Zn-*e*HMA > Zn-EDTA (Table II). There was an inverse relationship between the Zn accumulation after 1 h and the affinity of the ligand for Zn. A striking difference between genotypes was observed in the uptake of Zn from Zn-phytosiderophores, in which uptake rates of ys1 were one-half of those in Alice.

In all experiments, apoplasmic root Zn accumulated during the uptake experiment was desorbed and accounted for less than 2% of plant Zn uptake (data not shown). Furthermore, shoot translocation rates followed the same trend as root uptake rates (Tables I and II), although SDS were relatively high. These observations suggested that root concentrations of Zn reliably reflected intracellular accumulation of Zn in the roots, allowing us to measure only root uptake rates in the following experiments and to shorten the uptake period to 30 min. As a consequence, the radioactive tracer in the uptake solution was depleted by less than 5% (uptake period of 30 min) instead of 10 to 18% (uptake period of 1 and 2 h, respectively).

Table I. *Root uptake and shoot translocation of Zn and Fe in the Fe-efficient maize cv Alice and the maize mutant ys1 supplied with 10^{-6} M ^{65}Zn-labeled $ZnSO_4$ or ^{65}Zn- and ^{59}Fe-labeled e*HMA

The plants were 14 d old and precultured with or without a supply of 10^{-4} M Fe(III)EDTA. Experimental conditions: nutrient solution pH 6.0, uptake period 2h; $n = 4$, means ± SE.

Cultivar and Preculture	$^{65}ZnSO_4$	^{65}Zn-*e*HMA	^{59}Fe-*e*HMA
	μmol Zn g^{-1} root dry wt h^{-1}		*μmol Fe g^{-1} root dry wt h^{-1}*
Root uptake			
Alice + Fe	1.08 ± 0.14	0.57 ± 0.04	0.80 ± 0.08
Alice − Fe	1.27 ± 0.11	0.79 ± 0.05	1.82 ± 0.04
ys1 + Fe	1.06 ± 0.06	0.32 ± 0.09	0.02 ± <0.01
ys1 − Fe	0.96 ± 0.12	0.32 ± 0.06	0.03 ± <0.01
Shoot translocation			
Alice + Fe	0.30 ± 0.09	0.13 ± 0.02	0.29 ± 0.02
Alice − Fe	0.13 ± 0.02	0.10 ± 0.02	0.56 ± 0.09
ys1 + Fe	0.27 ± 0.04	0.06 ± 0.02	<0.01
ys1 − Fe	0.13 ± 0.04	0.05 ± 0.01	<0.01

Adding an excess of free phytosiderophores (DMA) to ^{65}Zn-labeled DMA led to a decrease of Zn uptake rates in both genotypes (Fig. 1). In ys1, however, a 500-fold excess of DMA over Zn decreased Zn uptake to 10% of its initial level, whereas in Alice Zn uptake decreased only to 50% of its initial level. In both genotypes, the excess of free ligand reduced Zn uptake by a similar amount, namely about 0.6 to 0.7 μmol Zn g^{-1} root dry weight h^{-1}. This DMA-repressible amount was attributed to the uptake of nonchelated Zn^{2+}.

To determine whether Zn-phytosiderophores are absorbed with or without chelate splitting, the uptake of double-labeled Zn-phytosiderophores was investigated. Free ^{14}C-labeled phytosiderophores (*e*HMA), which served as a control treatment, were absorbed at similar rates in both genotypes (Table III). In ys1 this background level of ^{14}C-phytosiderophore uptake did not change when [^{14}C]*e*-HMA was supplied as a Zn-chelate, indicating that there was no net uptake of the nondissociated Zn-*e*HMA complex. In contrast, in Alice uptake rates of the phytosiderophore chelator were strongly enhanced when supplied as a Zn-chelate. Compared to ys1, accumulation of Zn was 2.5-fold higher in Alice and confirmed the difference in Zn uptake between the two genotypes (Tables I and II), also at a 10-fold higher concentration (10 μM; Table III). Furthermore, the difference in the Zn uptake rate between Alice and ys1 (2.56 μmol Zn g^{-1} h^{-1}) closely matched their difference in ^{14}C uptake (2.41 μmol *e*HMA g^{-1} h^{-1}), suggesting an uptake path for nondissociated Zn-phytosiderophores in Alice, which is absent in ys1 (Table III).

DISCUSSION

The present study demonstrates that plant roots can absorb Zn not only as a divalent cation (Halvorson and Lindsay, 1977) but also in a chelated form, namely as Zn-phytosiderophores. On the basis of our experimental data we suggest that the uptake of Zn-phytosiderophores is mediated by the putative transport protein(s) for Fe-phytosiderophores in the root plasma membrane of graminaceous plant species. Primary evidence for such a contribution of the Fe-phytosiderophore transporter was obtained when uptake rates of ^{65}Zn from ^{65}Zn-labeled chelates were compared between the wild-type maize cv Alice and the maize mutant ys1, which carries a defect in Fe-phytosiderophore uptake (von Wirén et al., 1994, 1995). Except with phytosiderophores, Zn uptake rates from either ionic or chelated binding forms did not differ considerably between genotypes (Table II). This indicates that general root parameters, such as surface area, did not cause the genotypical difference in Zn-phytosiderophore uptake. Furthermore, Zn uptake rates from Zn-phytosiderophores by Alice were twice as high as by ys1 and seemed to be slightly induced under Fe-deficiency stress (Tables I and II), pointing to a role of the uptake system for Fe-phytosiderophores in the uptake of Zn-phytosiderophores.

In both genotypes, rates of Zn uptake decreased with the increasing stability constant of the ligand for Zn (Table II). This implied that it was free Zn and not the bound metal that determined the uptake rate and that the observed rates

Table II. *Root uptake and shoot translocation of Zn in the Fe-efficient maize cv Alice and the maize mutant ys1 supplied with 10^{-6} M ^{65}Zn-labeled $ZnSO_4$, Zn-desferrioxamine, Zn-eHMA, or Zn-EDTA*

The plants were 14 d old and precultured with or without a supply of 10^{-4} M Fe(III)-EDTA. Experimental conditions: nutrient solution pH 6.0, uptake period 1 h; $n = 4$; means ± SE.

Cultivar and Preculture	$^{65}ZnSO_4$	^{65}Zn-Desferrioxamine	^{65}Zn-eHMA	^{65}Zn-EDTA
		μmol Zn g^{-1} root dry wt h^{-1}		
Root uptake				
Alice + Fe	1.14 ± 0.09	0.80 ± 0.12	0.77 ± 0.05	0.13 ± 0.07
Alice − Fe	1.21 ± 0.12	1.48 ± 0.06	0.89 ± 0.06	0.11 ± 0.01
ys1 + Fe	0.85 ± 0.12	0.89 ± 0.07	0.36 ± 0.03	0.11 ± 0.01
ys1 − Fe	1.03 ± 0.10	0.99 ± 0.11	0.34 ± 0.03	0.07 ± 0.02
Shoot translocation				
Alice + Fe	0.24 ± 0.03	0.09 ± 0.03	0.17 ± 0.01	0.02 ± <0.01
Alice − Fe	0.20 ± 0.04	0.28 ± 0.05	0.14 ± 0.01	0.02 ± <0.01
ys1 + Fe	0.17 ± 0.02	0.14 ± 0.02	0.09 ± 0.02	0.02 ± <0.01
ys1 − Fe	0.23 ± 0.04	0.26 ± 0.06	0.09 ± 0.02	0.01 ± <0.01

of Zn uptake resulted from the uptake of Zn^{2+} after chelate splitting (Laurie et al., 1991). It is interesting that similar results have been reported for the uptake of Zn by human fibroblast cells, in which Zn uptake also decreases with increasing chelate stability (Ackland and McArdle, 1990). In mammalian cells Zn^{2+} is the preferred form for Zn intake (Failla and Cousins, 1978; Ackland and McArdle, 1990) and substrate for the recently cloned Zn transporter ZnT-1 (Palmiter and Findley, 1995). Similarly, Zn uptake by plant roots strongly depends on the activity of free Zn^{2+} (Lindsay, 1972; Bell et al., 1991; Norvell and Welch, 1993), and kinetic parameters for time- and concentration-dependent root uptake of Zn^{2+} (Mullins and Sommers, 1986; Santa Maria and Cogliatti, 1988) indicate a transporter-mediated uptake process. The presence of ligands thus modifies the free Zn concentration and hence the substrate concentration for the transporter, which finally modulates the uptake rate. So far, no evidence has been found for a metabolically active uptake of chelated Zn species by plant roots (Halvorson and Lindsay, 1977; Laurie et al., 1991).

Repressing the activity of free Zn^{2+} by stabilizing the formation of the Zn-phytosiderophore complex with the addition of a 500-fold excess of DMA over Zn decreased uptake rates of Zn by a similar absolute amount in both genotypes (Fig. 1), indicating that this repression was due to the absence of uptake of initially nonchelated Zn. The remaining uptake rate of Zn still exceeded 600 nmol Zn g^{-1} root dry weight h^{-1} in Alice but approached 0 in ys1. Since only Alice carries an efficient uptake system for Fe-phytosiderophores (von Wirén et al., 1995), we suggest that at 500 μM excess DMA the remaining Zn uptake rate in roots of Alice almost completely reflects Zn-phytosiderophore uptake via the putative Fe-phytosiderophore transporter. However, the possibility cannot be excluded that Zn-phytosiderophores were recognized and transported by a separate transport protein. In this case, the mutation in ys1 would affect both the Fe- and Zn-phytosiderophore transporters at the same time, e.g. through a common transcription factor or another protein required for uptake. To date, this possibility remains a matter of speculation and requires further investigation at the molecular level.

Our experiments clearly showed that there were two components contributing to the uptake of Zn in Fe-efficient maize, one of which was repressible by chelation and the other of which was dependent on an intact transport system for Fe-phytosiderophores (Fig. 2). We assume

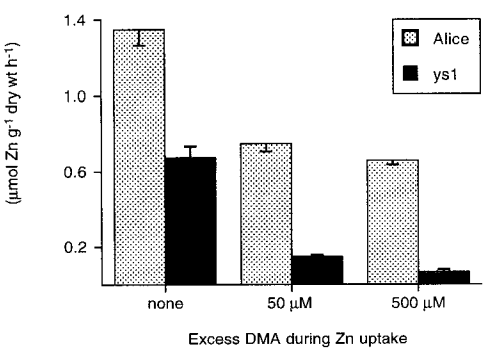

Figure 1. Root uptake of Zn in the Fe-efficient maize cv Alice and the maize mutant ys1 supplied with 10^{-6} M ^{65}Zn-labeled DMA with and without excess free DMA. The plants were 15 d old and precultured in Fe-deficient nutrient solution, and leaf chlorosis was similar in both genotypes. Experimental conditions: nutrient solution pH 6.0, uptake period 30 min, $n = 6$. Results are means ± SE.

Table III. *Root uptake of ^{14}C-labeled eHMA and ^{65}Zn-labeled Zn in the Fe-efficient maize cv Alice and the maize mutant ys1 supplied with 10^{-5} M ^{14}C-labeled eHMA or double-labeled ^{65}Zn-[^{14}C]eHMA*

The plants were 15 d old, precultured in Fe-deficient nutrient solution, and had a similar degree of leaf chlorosis in both genotypes. Experimental conditions: nutrient solution pH 6.0, uptake period 30 min, purity of [^{14}C]eHMA > 95%; $n = 6$; means ± SE.

Cultivar and Preculture	[^{14}C]eHMA	^{65}Zn-[^{14}C]eHMA
	μmol eHMA g^{-1} root dry wt h^{-1}	
Alice − Fe	1.04 ± 0.13	2.92 ± 0.15
ys1 − Fe	0.80 ± 0.07	0.51 ± 0.12
	μmol Zn- g^{-1} root dry wt h^{-1}	
Alice − Fe	0	4.25 ± 0.43
ys1 − Fe	0	1.69 ± 0.10

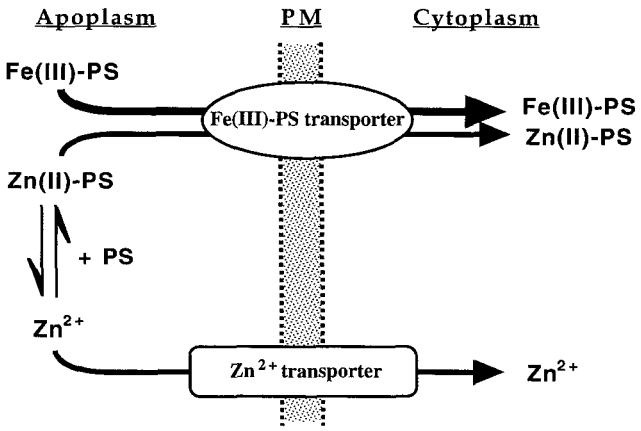

Figure 2. Proposed pathways for the uptake of Zn from Zn-phytosiderophores by root cells of graminaceous plant species. PM, Plasma membrane; PS, phytosiderophores.

that in the absence of excess phytosiderophores Zn-phytosiderophores are in equilibrium with the free cation species and its ligand, allowing the uptake of free Zn^{2+} via a putative Zn^{2+} transporter. With increasing concentrations of free phytosiderophores, the chelated Zn species predominates and is transported across the root plasma membrane only via a functional transport system for Fe-phytosiderophores. This model is in agreement with the results obtained by Ma and Nomoto (1993), who found that a 5-fold excess of Zn-phytosiderophores over ^{59}Fe-labeled phytosiderophores repressed Fe uptake rates by about 50%. Such repression indicates a competition between Fe- and Zn-phytosiderophores for the same transport sites. Our model does not support the conclusion of Ma et al. (1993), who interpreted 5-fold higher uptake rates of Fe from ^{59}Fe-labeled phytosiderophores compared to the Zn and Cu uptake rates from their corresponding phytosiderophores as a specific recognition and transport of Fe-phytosiderophores by the phytosiderophore uptake system. Likewise, our model also demands a reinterpretation of the data obtained by Marschner et al. (1989) and Zhang et al. (1991), who explained the beneficial effect of phytosiderophores on Zn uptake only by an enhanced mobilization of Zn from exchange sites in the soil or root apoplasm, excluding the possibility of Zn-phytosiderophore uptake. In this respect, we agree with the speculation of Kochian (1993) of the possibility for an uptake of Fe- and Zn-phytosiderophores via the same transport protein.

Our model is supported by the comparison of root uptake of free ^{14}C-labeled phytosiderophores with their ^{65}Zn-double-labeled complexes. In the wild-type maize cv Alice supplied with double-labeled ^{65}Zn-^{14}C-phytosiderophores, the root uptake of [^{14}C]*e*HMA increased 2.8-fold over the uptake of the free ligand (Table III). Thus, at least 64% of [^{14}C]*e*HMA uptake in Alice was related to the concomitant uptake of Zn. Because the unfolded structure of the free ligand (Sugiura and Nomoto, 1984) is probably not recognized by the Fe-phytosiderophore uptake system, the absorption of the ligand in the absence of Zn is thought to result from nonspecific uptake, e.g. via root lesions, and/or from the chelation and subsequent absorption of nonlabeled Zn (or other micronutrients) in the root apoplasm formed during plant preculture.

The role of the putative Fe-phytosiderophore transporter in the uptake of Zn-phytosiderophores was elucidated by using the maize mutant ys1 as a reference genotype and monitoring the level of nonspecific and inefficient phytosiderophore uptake. In contrast to Alice, ys1 plants absorbed [^{14}C]*e*HMA from the free phytosiderophore ligand or its Zn chelate at similar rates (Table III), indicating that both the Zn-phytosiderophore complex and its free ligand entered the roots of ys1 via the same pathway. Moreover, Zn uptake in ys1 corresponded to that in Alice after the amount of phytosiderophore-bound Zn uptake was subtracted from total Zn uptake in Alice. Both genotypes took up similar amounts of nonchelated Zn (Tables I and II; supported by Fig. 1), but only Alice additionally took up considerable amounts of chelated Zn, whereas the maize mutant ys1 failed to take up nondissociated Zn-phytosiderophores. We therefore suggest that the ability of plants to take up phytosiderophore-chelated Zn depends on an intact transport system for Fe-phytosiderophores in the root plasma membrane and, thus, is expected to be confined to Fe-efficient strategy II plants.

A direct comparison of ^{59}Fe and ^{65}Zn uptake rates from their labeled phytosiderophores requires considering the proportion of free metal uptake that was close to 0 for Fe (as represented by the values for ys1, Table I) but amounted to about 50% for Zn under our experimental conditions. Uptake rates of phytosiderophore-chelated Fe are, therefore, at least 2- to 10-fold higher than those for Zn (Table I; Marschner et al., 1989; Ma et al., 1993). This observation emphasizes a preference of the phytosiderophore transporter for the Fe-chelate. In our opinion, the strong dependence of Fe acquisition on the phytosiderophore system still justifies the term "phytosiderophore" instead of "phytochelate or phytometallophore," as suggested elsewhere (Crowley et al., 1991; Welch, 1995). The present results, however, demand a restriction of the term "specific" recognition and uptake of Fe-phytosiderophores by the transport protein (Römheld and Marschner, 1986; Marschner et al., 1989; Ma et al., 1993) to the ligand but not to the chelated metal.

Our findings also bear general implications for Zn nutrition of plants. In high pH soils, chelators can markedly increase Zn mobilization from the solid phase and thus enhance the concentration of total Zn in the soil solution as well as subsequent plant uptake (Dinkelaker et al., 1989). In low pH soils or nutrient solutions, in which Zn availability either is not at all or is less restricted, chelators generally repress uptake rates of Zn by plant roots (Halvorson and Lindsay, 1977; Bell et al., 1991; Norvell, 1991). This mode of action also seems to be true for phytosiderophores (Treeby et al., 1989; Zhang et al., 1991; Fig. 1), except that chelate splitting is not a prerequisite for the uptake of Zn from Zn-phytosiderophores (Table III). Higher uptake rates of free Zn compared to its chelated species (Table I) imply that free Zn^{2+} remains the preferential form for Zn uptake even in the presence of Zn-phytosiderophores. The puta-

tive Zn^{2+} transporter probably has a higher transport capacity for its substrate than the phytosiderophore transporter for Zn-phytosiderophores. Nevertheless, the uptake of phytosiderophore-chelated Zn will become predominant with decreasing free Zn and increasing phytosiderophore levels as in calcareous soils, where free Zn^{2+} concentrations may decrease below 0.01 μM (Bruemmer et al., 1986). As a response to low Zn availability, graminaceous plants can induce the release of phytosiderophores (Zhang et al., 1989; Cakmak et al., 1994; Walter et al., 1994), which may accumulate in the rhizosphere to concentrations of up to 1 μM (Shi et al., 1988). A 100-fold excess of phytosiderophores over Zn considerably represses the efficiency of uptake of free Zn, whereas under the same conditions uptake rates of phytosiderophore-chelated Zn might be 5 to 10 times higher than that of free Zn (Fig. 1). Zn-phytosiderophore uptake could be of similar importance in the case of Fe deficiency, in which, otherwise, Zn-deficiency in plants might be induced by the high phytosiderophore concentrations in the rhizosphere. With regard to the coincidence of Fe and Zn deficiencies in calcareous soils (Moraghan and Mascagni, 1991), we argue that the direct uptake of Zn-phytosiderophores via the phytosiderophore transporter might be an ecological advantage for graminaceous plants to cover their demand for Zn.

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