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Role of the Root Apoplasm for Iron Acquisition by Wheat Plants[1]

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ABSTRACT

The role of the root apoplasm for iron acquisition was studied in wheat (*Triticum aestivum* L. cv Ares) grown in nutrient solution under controlled environmental conditions. To obtain different levels of Fe in the root apoplasm, plants were supplied in the dark for 5 hours (preloading period) with various ^{59}Fe-labeled Fe compounds [Fe(III) hydroxide; microbial siderophores: Fe rhodotorulic acid (FeRDA) and ferrioxamin (FeDesferal[3]), and synthetic Fe chelate (FeEDDHA)], each at a concentration of 5 micromolar. Large pools of apoplasmic Fe were formed after supplying Fe(III) hydroxide or FeRDA, but no such pools were observed after supplying FeDesferal or FeEDDHA. Depending on plant Fe nutritional status (preculture ± 0.1 millimolar FeEDTA), apoplasmic Fe was used to different extent for translocation to the shoot. Under Fe deficiency, a much greater fraction of the apoplasmic Fe was utilized than in Fe-sufficient plants, as a result of the different rates of phytosiderophore release. Because of the diurnal rhythm in release of phytosiderophores in Fe-deficient plants, the utilization of the apoplasmic Fe for translocation into the shoot started 2 hours after onset of the light period and was dependent on the concentration of Fe in the apoplasm, which followed the order: Fe(III) hydroxide ≫ FeRDA ≫ FeDesferal = FeEDDHA. From these results, it can be concluded that in soil-grown plants the apoplasmic Fe pool loaded by various indigenous Fe compounds such as siderophores in the soil solution can be an important Fe source in graminaceous species, particularly during periods of limited Fe supply from the soil.

In higher plants, two distinct mechanisms exist for Fe acquisition under conditions of limited Fe supply in the growth medium (10). The first mechanism has been referred to as strategy I and is found in all dicots and monocots with the exception of graminaceous species (13). This strategy is characterized by an inducible plasma membrane-bound reductase, enhancement of hydrogen ion release, and release of both "reductants" and chelating root exudates. The second mechanism has been referred to as strategy II and is confined to graminaceous species (10). This strategy is characterized by enhanced release of phytosiderophores that form chelates with Fe(III), and a highly specific uptake system for Fe(III) phytosiderophores without reduction to Fe(II) at the plasma membrane.

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In plants utilizing strategy I, such as bean and *Chlorophytum* under Fe deficiency and associated rhizosphere acidification, considerable amounts of apoplasmic Fe in the roots can be mobilized and translocated to the shoots (3). In soybean, genotypical differences in resistance to Fe deficiency-induced chlorosis are correlated with differences in accumulation of Fe in the root apoplasm and utilization of this pool under Fe deficiency (8). In contrast, in maize (strategy II) the apoplasmic pool of Fe seems to contribute only little to Fe transport to the shoots, even under Fe deficiency (3). In view of the distinct diurnal rhythm in root release of phytosiderophores under conditions of Fe deficiency (10, 19) and the corresponding differences in mobilization of zinc in the root apoplasm (20), mobilization of apoplasmic Fe may also strongly depend on the period of the day at which studies are made. The objective of the present work was to study the relationships between amounts of Fe in the root apoplasm and time course of mobilization of this pool for shoot transport in Fe-deficient and Fe-sufficient wheat plants. Different amounts of Fe in the root apoplasm were obtained by preloading of roots during the dark period with Fe(III) from different sources.

MATERIALS AND METHODS

Wheat (*Triticum aestivum* L., cv Ares) grains were germinated in quartz sand moistened with saturated $CaSO_4$ solution. After 4 d, the roots were gently washed several times with distilled water and the seedlings transferred to a continuously aerated nutrient solution (pH 6.2) of the following composition (mM): K_2SO_4 0.7; $MgSO_4$ 0.5; $Ca(NO_3)_2$ 2.0; KCl 0.1; KH_2PO_4 0.1; H_3BO_3 1×10^{-3}; $MnSO_4$ 1×10^{-3}; $CuSO_4$ 2.5×10^{-4}; $(NH_4)_6Mo_{27}O_{24}$ 1×10^{-5}; $ZnSO_4$ 1×10^{-3}; and for the control (Fe-sufficient) plants, 0.1 mM FeEDTA. After 10 d of growth, the plants were transferred to a nutrient solution without Fe and Zn to achieve roots with low apoplasmic Fe and Zn. Low apoplasmic Zn concentrations were envisaged to prevent substantial Zn mobilization in the apoplasm by phytosiderophores released under Fe deficiency. Plants were kept in this solution for another 2 d. During this period, the control (Fe-sufficient) plants received foliar sprays with solutions of 0.3% $ZnSO_4$ and 0.3% Fe-citrate. Plants not supplied with Fe (Fe-deficient plants) received only 0.3% $ZnSO_4$ and showed slight chlorosis after 12 d preculture in nutrient solution. Plants were grown in a growth chamber with light/dark regimens of 16/8 h, a light intensity of 220 μmol m^{-2} s^{-1} (fluorescent tubes, Sylvania, cool white FR 96T12), a temperature of 25 ± 1°C, and a RH of 65 to 75%.

For loading the root apoplasm with Fe, both Fe-deficient and Fe-sufficient plants were transferred during the dark period (6 h before onset of the light period) to continuously aerated nutrient solution with ^{59}Fe-labeled Fe compounds at a concentration of 5×10^{-6} M with a specific activity of 528 GBq mol^{-1} Fe. Zinc, Mn, and Cu were withheld from the nutrient solution during loading to keep the concentration low of competing heavy metal cations for exchange sites in the apoplasm. After loading for 5 h, the roots were washed with 10^{-4} M $CaSO_4$ solution (pH 6.2) for 10 min. Thereafter, some of the plants were harvested for determination of the amounts of ^{59}Fe in the apoplasm (apoplasmic Fe), roots, and shoots at zero time. The remaining plants were transferred to a nutrient solution without Zn, Fe, Mn, and Cu supply (pH 6.2). For mobilization and uptake of the apoplasmic Fe pool, these plants were harvested after 0, 3, 5, 9, 13, and 18 h. The roots were rinsed for 10 min with 1×10^{-3} M $CaSO_4$ solution and apoplasmic (extracellular) Fe was removed and determined by using a reductant (sodium dithionite, 7.5 mM) and a chelator for Fe(II) (2,2-bipyridyl, 1.5 mM) under a continuous flow of N_2 for 5 min (3). Plants were separated into roots and shoots, and freeze-dried. After dry weight determination and grinding, subsamples were used for Chl determination and for dry ashing at 500°C for 5 h. Chl concentrations were determined in the shoot dry matter according to Arnon (1), and in the ash solution radioactive ^{59}Fe was determined by scintillation counting and micronutrients with a Beckman Spectraspan type M VI directly coupled plasma-emission spectrometer. After the preculture without Fe supply (4 d germination + 12 d in nutrient solution), the Fe-deficient plants (16-d-old) showed symptoms of mild chlorosis, indicating a high release rate of phytosiderophores from the roots (10).

For collection of root exudates (phytosiderophores), plant roots were briefly rinsed with distilled water and then transferred to 500 mL double distilled water for 4 h between 2 and 6 h after onset of the light period. Distilled water was used instead of a Ca solution to avoid precipitation of Ca salts during subsequent concentration of the solution. Comparisons between distilled water and $CaSO_4$ solutions showed no significant differences in release of phytosiderophores by roots, presumably due to the release of Ca^{2+} from the apoplasm into the distilled water. After filtration, the solution containing root exudates was evaporated under vacuum in a water-bath (50°C) to a volume of 20 mL. The amount of Fe(III) mobilized by the root exudates was taken as a parameter to estimate the amount of phytosiderophores released by the root. Freshly precipitated Fe(III) hydroxide was shaken with the root exudates at 25°C for 2 h. After filtration, the mobilized Fe was photometrically determined at 562 nm as Fe(II) ferrozine complex after addition of a reductant (hydroxylamine hydrochloride) and ferrozine (3-[2-pyridyl]-5,6-bis[4-phenylsulfonic acid]-1,2,4-triazine) (18). As revealed by HPLC (11), more than 90% of the Fe(III) mobilization by the root exudates of Fe-deficient plants could be attributed to the phytosiderophore, 2′-deoxy-mugineic acid.

The data presented in tables and figures are the means (± SD) of four replicates.

RESULTS

The root and shoot dry weight and Chl concentrations of the Fe-deficient plants were significantly lower than those of the Fe-sufficient plants (Table I). The shoot concentration of Fe of the Fe-deficient plants was very low but the concentrations of manganese and particularly zinc and copper were significantly higher than in the Fe-sufficient plants. The rate of root release of phytosiderophores was about 35 times higher in the Fe-deficient as compared with the Fe-sufficient plants (Table I).

After the 5 h loading period in the dark, the concentrations of apoplasmic Fe and root Fe differed widely between the various ^{59}Fe sources, whereas the Fe nutritional status had only a marginal effect (Table II). After loading with ^{59}Fe hydroxide, the concentrations of ^{59}Fe in the root apoplasm and in the roots to a lesser extent (after removal of apoplasmic ^{59}Fe) were several times higher than after loading with FeRDA.[3] In contrast to these two Fe sources, the concentrations of ^{59}Fe in both root apoplasm and roots were very low after loading with the chelated ^{59}Fe sources FeDesferal and FeEDDHA. The differences in ^{59}Fe concentrations in the apoplasm and roots between FeRDA and the other two chelates (FeDesferal and FeEDDHA) are presumably caused by their different stabilities.

During the ^{59}Fe loading period in the dark, only very small amounts of ^{59}Fe were translocated to the shoots, regardless of the Fe nutritional status of the plants and the ^{59}Fe source supplied (Fig. 1). In the subsequent light period, the translocation rate of ^{59}Fe to the shoots remained very low in plants supplied with the stable Fe chelates FeDesferal and FeEDDHA, which is in accordance with the small root apo-

[3] Abbreviations: FeRDA, Fe rhodotorulic acid; FeDesferal, ferrioxamine B methanesulfonate; FeEDDHA, synthetic Fe chelate.

Table I. *Effect of Iron Nutritional Status on Dry Weight, Concentrations of Chl and Micronutrients in the Shoots, and Release Rate of Phytosiderophores in Wheat (Plant Age 16 d)*

Fe Preculture	Dry Weight		Chl	Micronutrients				Release of Phytosiderophores
	Roots	Shoot		Fe	Zn	Mn	Cu	
± 100 μM FeEDTA	mg plant^{-1}		mg g^{-1} dry wt	mg kg^{-1} shoot dry wt				μmol Fe eq g^{-1} root dry wt 4 h^{-1}
+Fe	24	57	11.3	263	89	142	8	0.2
−Fe	16[a]	41[a]	6.9[a]	29[a]	167[a]	218[a]	16[a]	6.9[a]

[a] Significantly different (P = 0.05) from +Fe preculture.

Table II. *Concentrations of Apoplasmic (Extracellular) and Root ^{59}Fe in Fe-Sufficient (+Fe) and Fe-Deficient (−Fe) Wheat Plants after Supply with 5 μM of ^{59}Fe-labeled Fe Sources (Preloading) in Nutrient Solution for 5 h, in the Dark (Plant Age 16 d, pH 6.2)*

Root ^{59}Fe Was Determined after Removal of the Extracellular ^{59}Fe.

^{59}Fe Sources	Apoplasmic ^{59}Fe Preculture		Root ^{59}Fe Concentration Preculture	
	+Fe	−Fe	+Fe	−Fe
	μmol ^{59}Fe g^{-1} root dry wt			
Fe hydroxide	4600 ± 875	5000 ± 950	426 ± 113	407 ± 39
FeRDA	295 ± 75	412 ± 150	167 ± 67	284 ± 38
FeDesferal	2 ± 0.2	8 ± 0.5	8 ± 3	9 ± 1
FeEDDHA	5 ± 0.9	2 ± 0.4	8 ± 2	12 ± 2

plasmic ^{59}Fe pool (Table II). There was only a tendency for the Fe-deficient plants to translocate somewhat more ^{59}Fe into the shoots than was the case in the Fe-sufficient plants (Fig. 1B). In contrast, in plants in which the root apoplasmic ^{59}Fe pool was loaded with FeRDA and particularly with Fe hydroxide, ^{59}Fe translocation into the shoots during the subsequent light period was much greater (Fig. 1A), particularly in the Fe-deficient plants. These differences between Fe sources in ^{59}Fe translocation correspond well with the amount of ^{59}Fe in the root apoplasm and in roots after the loading period (Table II). Despite the distinct effect of the Fe nutritional status on ^{59}Fe translocation into the shoots of plants pretreated with FeRDA or Fe hydroxide, the relatively large amounts of ^{59}Fe in the roots were not significantly decreased (Table II).

DISCUSSION

After substantial loading of the root apoplasmic pool with Fe (Table II), mobilization of Fe from this source and translocation into the shoots was related to the rate of phytosiderophore release by the roots (Table I). Accordingly, after onset of the light period, translocation increased more in Fe-deficient than in Fe-sufficient plants (Fig. 1). This confirms results on relationships between utilization of Fe from Fe(III) hydroxide by various graminaceous species and their corresponding differences in release of phytosiderophores (16). A large proportion of Fe associated with roots is likely to be adsorbed to fixed negative charges in the root apoplasm (4) or precipitated as Fe(III) oxide-hydrates at the rhizoplane. In our experiments, after the 5 h loading period with Fe hydroxide more than 90% of the Fe associated with the roots was in the apoplasmic pool (Table II). The Fe in this pool can be mobilized by phytosiderophores and subsequently taken up by the specific uptake system for Fe(III) phytosiderophores (16).

The failure of Fe-deficient maize to utilize apoplasmic Fe (3) may reflect either a much lower phytosiderophore release in this plant species (16, 17) or a rapid microbial degradation of phytosiderophores in nutrient solution culture (14, 17), or both these factors.

Enhanced utilization of FeEDDHA in Fe-deficient compared with Fe-sufficient barley plants has been found by Clarkson and Sanderson (5), but these results could not be confirmed (16). In the present experiments with wheat plants pretreated with FeEDDHA, the uptake and translocation of Fe was also very small and independent of Fe nutritional status (Fig. 1). Presumably, in the experiments of Clarkson and Sanderson (5) the chelation of Fe(III) was incomplete and some inorganic Fe(III) was accumulated in the root apoplasmic pool, as indicated by the spot-like location of labeled Fe at the rhizoplane. Thus, the enhanced utilization of FeEDDHA in Fe-deficient barley plants probably reflects mobilization of apoplasmic inorganic Fe(III) by phytosiderophores.

Phytosiderophores are released at much higher rates in Fe-deficient plants (Table I) and follow a distinct diurnal rhythm with a maximum at about 4 to 6 h after onset of the light period (10, 19). Accordingly, the translocation of Fe to the shoots increased steeply in Fe-deficient plants after onset of the light period (Fig. 1). However, the translocation of Fe continued thereafter more or less continuously. This discrepancy between timing of phytosiderophore release and Fe translocation is probably the result of the experimental conditions. The released phytosiderophores mobilize Fe in the root apoplasm by formation of Fe(III) phytosiderophores. In nutrient solution culture, part of these Fe(III) phytosiderophores may diffuse into the external solution and be taken up subsequently. In contrast to the diurnal rhythm in phytosiderophore release, the uptake of the Fe(III) phytosiderophores by the highly specific system in roots of graminaceous species is not affected by the period of day (V. Römheld, unpublished). Additionally, the continuous translocation of Fe to the shoots during the light period (Fig. 1) could be derived from a pool formed within the roots after onset of the light

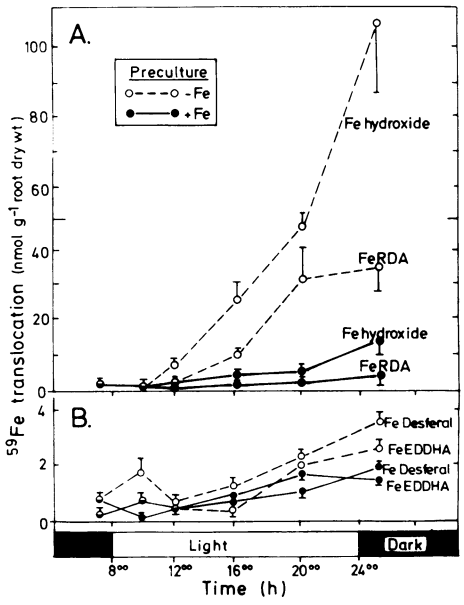

Figure 1. Time course of ^{59}Fe translocation from the root apoplasmic ^{59}Fe pool in Fe-deficient and Fe-sufficient wheat plants. Root apoplasmic pool was loaded during the dark period for 5 h with ^{59}Fe by supplying 5 μM ^{59}Fe from different sources: Fe hydroxide, FeRDA (A) or FeDesferal, FeEDDHA (B). After ^{59}Fe loading, the plants were transferred to nutrient solutions without Fe for up to 19 h.

period. The chemical nature of this Fe pool in roots is not known. It is also not clear whether Fe(III) phytosiderophores are involved in Fe translocation from roots to the shoot (9, 11). Experimental evidence is lacking so far on a role of nicotianamine in long distance transport of Fe from roots to the shoot.

The poor utilization of Fe from microbial siderophores such as FeDesferal (Table II, Fig. 1) confirm earlier results with Fe-deficient graminaceous species (16, 17). Like Fe-EDDHA, FeDesferal is also a complex with high stability (log K_s = 30.6) and, thus, the contribution of Fe from this source both for loading of the apoplasmic pool (Table II), and also for the subsequent mobilization of Fe by phytosiderophores and transport to the shoot, is small. Like graminaceous species (strategy II), in plant species with strategy I microbial siderophores with high stability are also poor sources of Fe, because the Fe(III) in these compounds is not readily reduced by the plasma membrane-bound reductase (2, 15). However, microbial siderophores are important for solubilization of Fe(III) in soils and thus for Fe transport in the soil solution to plant roots (12). Utilization of Fe from microbial siderophores in long-term experiments with plants grown both in nutrient solution (6) and in soil (7), therefore, may be mainly of indirect nature. Microbial siderophores may maintain a continuous and thus a substantial supply of soluble Fe to the root surface. Depending on their chelate stability, they may make an important contribution to the apoplasmic Fe pool and thus the source of Fe readily mobilized by phytosiderophores in graminaceous species.

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