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Evidence for a Specific Uptake System for Iron Phytosiderophores in Roots of Grasses[1]

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VOLKER RÖMHELD* AND HORST MARSCHNER
Institut für Pflanzenernährung, Universität Hohenheim, Postfach 70 05 62, 7000 Stuttgart 70, Federal Republic of Germany

ABSTRACT

Roots of grasses in response to iron deficiency markedly increase the release of chelating substances ('phytosiderophores') which are highly effective in solubilization of sparingly soluble inorganic Fe^{III} compounds by formation of Fe^{III}phytosiderophores. In barley (*Hordeum vulgare* L.), the rate of iron uptake from Fe^{III}phytosiderophores is 100 to 1000 times faster than the rate from synthetic Fe chelates (*e.g.* Fe ethylenediaminetetraacetate) or microbial Fe siderophores (*e.g.* ferrichrome). Reduction of Fe^{III} is not involved in the preferential iron uptake from Fe^{III}phytosiderophores by barley. This is indicated by experiments with varied pH, addition of bicarbonate or of a strong chelator for Fe^{II} (*e.g.* bathophenanthrolinedisulfonate). The results indicate the existence of a specific uptake system for Fe^{III}phytosiderophores in roots of barley and all other graminaceous species. In contrast to grasses, cucumber plants (*Cucumis sativus* L.) take up iron from Fe^{III}phytosiderophores at rates similar to those from synthetic Fe chelates. Furthermore, under Fe deficiency in cucumber, increased rates of uptake of Fe^{III}phytosiderophores are based on the same mechanism as for synthetic Fe chelates, namely enhanced Fe^{III} reduction and chelate splitting. Two strategies are evident from the experiments for the acquisition of iron by plants under iron deficiency. Strategy I (in most nongraminaceous species) is characterized by an inducible plasma membrane-bound reductase and enhancement of H^+ release. Strategy II (in grasses) is characterized by enhanced release of phytosiderophores and by a highly specific uptake system for Fe^{III}phytosiderophores. Strategy II seems to have several ecological advantages over Strategy I such as solubilization of sparingly soluble inorganic Fe^{III} compounds in the rhizosphere, and less inhibition by high pH. The principal differences in the two strategies have to be taken into account in screening methods for resistance to 'lime chlorosis'.

Studies on root responses to iron deficiency during the last three decades were mainly focused on dicotyledonous species. Root responses of most dicotyledonous and in some monocotyledonous species to iron deficiency are characterized by an increase in the activity of a NADPH dependent reductase (2, 20, 25) and of an ATPase-driven proton efflux pump (23). Both reactions may enhance the solubilization of sparingly soluble inorganic Fe^{III} in the rhizosphere to some degree but they distinctly increase the rate of splitting and reduction of Fe^{III} from synthetic Fe^{III}chelates at the plasma membrane of root epidermal cells (5, 20). Plant species with this response to iron deficiency may be described as Fe efficient. Recently, this adaptation has been defined as strategy I (21).

These responses of roots to iron deficiency are absent in grasses. Based on this, grasses have been classified as Fe inefficient (16, 22). However, roots of grasses (barley) have been demonstrated (13) to have an ability to solubilize and take up iron from sparingly soluble inorganic Fe^{III}. That grasses respond to iron deficiency by enhanced release of chelating substances for iron has been meanwhile well established (27, 28). These substances have been characterized chemically as nonproteinogenic amino acids such as mugineic and avenic acid (26). In analogy to the iron deficiency-induced release of siderophores by many microorganisms (10, 15, 29) these chelating substances released by roots were termed phytosiderophores (28). Thus, grasses differ from dicots (strategy I) principally in the response of their roots to iron deficiency and have adapted to substrates with low iron solubility by a different mechanism (strategy II; 21).

Roots of grasses such as rice also have a much greater capability to take up iron from Fe^{III}phytosiderophores compared to iron from synthetic Fe^{III}chelates (14). This may indicate a further peculiarity of the roots from grasses, namely the presence of efficient uptake systems for Fe^{III}phytosiderophores.

The objective of this work was to study, in more detail, the mechanisms of Fe^{III}phytosiderophore uptake by roots of grasses differing in their iron nutritional status. Comparisons between the uptake rates of iron from phytosiderophores, synthetic chelates and microbial siderophores were emphasized particularly. Furthermore, in the uptake studies cucumber plants (strategy I) were included for further evaluation of the principle differences in the strategies I and II.

MATERIALS AND METHODS

Preculture of Plants. Barley (*Hordeum vulgare* L., cv Europa) and cucumber (*Cucumis sativus* L., cv Chinesische Schlange) were precultured under controlled climatic conditions (day/night 16/8 h; light intensity, 40 W/m^2, temperature 25/23°C; and RH, 70–80%) in nutrient solutions with 100 μM FeEDTA (control) or without iron supply (Fe-deficient plants). The nutrient solution was continuously aerated and had the following composition: 2.00 mM $(Ca(NO_3)_2$, 0.75 mM K_2SO_4, 0.65 mM $MgSO_4$, 0.5 mM KH_2PO_4, 1 μM H_3BO_3, 0.1 μM $MnSO_4$, 0.05 μM $ZnSO_4$, 0.05 μM $CuSO_4$, 0.005 μM $(NH_4)_6Mo_7O_{24}$.

On a shoot dry weight basis the Chl content of the Fe-sufficient (control, supplied with 0.1 M FeEDTA) plants and the Fe-deficient plants was 11.5 mg/g and 5.5 mg/g in barley and 12.2 mg/g and 7.8 mg/g in cucumber, respectively. For the studies on solubilization and uptake of iron, 10 d old cucumber and 15 d old barley plants were used. In one of the experiments the mechanism of iron uptake was compared in a range of plant species: Corn (*Zea mays* L., cv Garbo), rye (*Secale cereale* L., cv Merkator), sorghum (*Sorghum bicolor* Moench, cv SPV 393), wheat (*Triticum aestivum* L., cv Okupt), peanut (*Arachis hypogaea* L., cv Böhm), potato (*Solanum tuberosum* L., cv DTO 2), sunflower (*Helianthus annuus* L., cv Sorex), tomato (*Lycopersi-*

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con esculentum Mill., cv Hellfrucht).

Solubilization and Uptake of ^{59}Fe. For these studies, iron was supplied either as $^{59}Fe^{III}$chelate or as $^{59}Fe^{III}$hydroxide. The ^{59}Fe chelate (0.03–3.0 μM; 1–5 TBq/mol Fe) was added to the nutrient solution in 250 ml flasks (15 barley and 10 cucumber plants, respectively, per flask). The ^{59}Fe hydroxide (2–10 TBq/mol Fe) was not mixed with nutrient solution but supplied separately in dialysis tubes (Servapor ϕ 16 mm, Serva Feinbiochemica GmbH, Heidelberg, FRG) at a level of 12.5 μmol Fe/tube. The dialysis tubes were inserted into the flasks with the 250 ml nutrient solution. The suspension of ^{59}Fe hydroxide in the dialysis tubes was continuously aerated. ^{59}Fe labeled Fe hydroxide and Fe chelates were prepared as described elsewhere (1, 20).

To compare the rate of ^{59}Fe uptake from Fe^{III}phytosiderophores by Fe-deficient barley or cucumber plants with Fe-sufficient (control) plants a mixed culture was chosen. ^{59}Fe hydroxide was supplied in a dialysis tube (see above) and Fe-deficient barley plants (as donor for phytosiderophores) and either barley plants (Fe-sufficient) or cucumber plants (Fe-sufficient or Fe-deficient) were transferred to the same nutrient solution for the uptake study. Samples were taken several times from this nutrient solution for the determination of the concentration of ^{59}Fe solubilized by the phytosiderophores. At the end of the uptake study (24 h), the extracellular Fe of the roots was measured according to Bienfait et al. (3). Thereafter, the roots and shoots were separated, dried at 100°C and ashed at 500°C. ^{59}Fe was determined by scintillation counting.

Each experiment was repeated twice with 4 to 6 replicates each. The standard deviation from the mean never exceeded 15% of the values shown.

RESULTS

Uptake of Fe by Cucumber and Barley Plants, Supplied Either as Fe^{III}Chelate or Fe^{III}Hydroxide. Raising the concentration of $^{59}Fe^{III}$EDDHA[2] resulted in increased rates of ^{59}Fe uptake in both species (Table I). However, cucumber and barley plants differed in the rates of ^{59}Fe uptake from ^{59}FeEDDHA in two ways: the rate of ^{59}Fe uptake was much faster in the Fe-sufficient cucumber plants (+Fe). In cucumber, but not in barley, the Fe-deficient plants (−Fe) responded by a large increase (a factor of 100–200) in rate of ^{59}Fe uptake from $^{59}Fe^{III}$EDDHA.

Table I. *Rates of Fe Uptake by Fe-Sufficient (+Fe) and Fe-Deficient (−Fe) Cucumber and Barley Plants Supplied with Either $^{59}Fe^{III}$EDDHA (A) or $^{59}Fe^{III}$Hydroxide in Dialysis Tubes (B)*

^{59}Fe was supplied in nutrient solution (pH 6.5–7.0) for 24 h. Plants were precultured with 0.1 mM FeEDTA (+Fe) or without Fe (−Fe). Values are means of six replicates.

Treatment	Fe Uptake Rates			
	Cucumber preculture		Barley preculture	
	+Fe	−Fe	+Fe	−Fe
	nmol Fe/g root dry wt·24 h			
(A) $^{59}Fe^{III}$EDDHA				
3×10^{-8} M	5.4	487.2	0.5	0.6
3×10^{-7} M	31.8	4840.4	1.3	2.4
3×10^{-6} M	185.2	35858.3	7.9	12.8
(B) $^{59}Fe^{III}$hydroxide in dialysis tubes (12.5 μmol Fe/tube)	9.2	24.1	1.1	791.8

[2] Abbreviations: FeDFOB, ferrioxamine; FeDTPA, ferric diethylenetriamine pentaacetate; FeEDDHA, ferric ethylenediaminedi(*o*-hydroxyphenylacetate); FeHEDTA, ferric *N*-(2-hydroxyethyl)ethylendiaminetriacetate; BPDS, bathophenanthrolinedisulfonate; CCCP, carbonyl cyanide *m*-chlorophenyldrazone; DCCD, *N,N'*dicyclohexyl carbodiimide.

Table II. *Time Course of Solubilization of Inorganic Iron from ^{59}Fe Labeled Fe^{III}Hydroxide by Root Exudates of Intact Barley and Cucumber Plants*

The $^{59}Fe^{III}$hydroxide was supplied in dialysis tubes in nutrient solution (pH 6.5–7.5). Preculture of the plants with 0.1 mM FeEDTA (+Fe) or without Fe (−Fe). (A) *in vitro* studies (without plants). (B) *In vivo* studies with the plants in nutrient solution.

Fe Preculture (+/−Fe)	Concentration of Solubilized Iron in Nutrient Solution at h of ^{59}Fe Supply:					
	0.5	2	4	6	12	24
	nM					
(A) Without plants						
+ Root exudates from Fe-sufficient barley plants[a]	<10	<10	<10	<10	<10	<10
+ Root exudates from Fe-deficient barley plants[a]	<10	35	100	190	360	555
+ KEDDHA (3×10^{-6} M)	<10	15	65	225	435	615
(B) With plants						
Barley (15/pot)						
+Fe	<10	<10	<10	<10	<10	<10
−Fe	20	30	50	70	20	10
Cucumber (10/pot)						
+Fe	<10	<10	<10	<10	<10	<10
−Fe	<10	10	10	10	<10	<10

[a] Root exudates of 15 barley plants, collected during the first 6 h of the light period. Root exudates were added to a nutrient solution (without plants) after sterile filtration.

As expected from the low solubility of Fe^{III}hydroxide (Table II), rate of ^{59}Fe uptake from $^{59}Fe^{III}$hydroxide was low in the Fe-sufficient plants of both species (Table I). The rate of ^{59}Fe uptake by the Fe-deficient plants increased only slightly in cucumber but more than 700-fold in barley. Thus, cucumber requires the supply of soluble Fe (Fe chelates) for the enhancement of rates of Fe uptake in the deficient plants, but a supply of sparingly soluble inorganic Fe^{III} is needed for barley. About two-thirds of the ^{59}Fe taken up from the $^{59}Fe^{III}$hydroxide by the Fe-deficient barley plants had been translocated into the shoot within 24 h. Roots of Fe-deficient barley plants release root exudates which act as strong chelators of iron supplied as Fe^{III}hydroxide (Table II). The root exudates were obviously identical with the amino acids classified as phytosiderophores as shown by separation techniques with cation and anion exchange resins (28). The chelating properties of these plant-born chelators are comparable with the synthetic chelator EDDHA as shown by a similar time course of iron solubilization (Table IIA).

The release of phytosiderophores by the roots of Fe-deficient barley plants was demonstrated by the higher concentrations of soluble ^{59}Fe in the nutrient solution (Table IIB). During the first 6 h, the ^{59}Fe concentration in the nutrient solution of the Fe-deficient barley plants increased constantly; thereafter the ^{59}Fe concentration declined again. The lower ^{59}Fe concentrations in the presence of Fe-deficient barley plants (Table IIB) compared to the *in vitro* experiments with root exudates only (Table IIA) may be due to the following: microbial decomposition of the root exudates, absorption of the solubilized ^{59}Fe by the roots, decrease in rates of root release of phytosiderophores due to diurnal fluctuations, or to recovery from Fe deficiency.

The concentration of soluble ^{59}Fe in the nutrient solutions

with Fe-deficient cucumber plants was only slightly increased in the time period between 2 and 6 h after onset of the experiment (Table IIB).

Fe^{III} solubilized by phytosiderophores is taken up by Fe-deficient barley plants at a rate of 10^2 to 10^3 times higher than Fe from synthetic Fe^{III}chelates such as Fe^{III}EDDHA as shown in Table I. These comparatively low uptake rates are not only confined to various synthetic Fe^{III}chelates, but also hold true for Fe^{III}siderophores such as ferrichrome; *i.e.* Fe^{III}chelates of microbial origin (Table III). The relatively high uptake rates of Fe from Fe^{III}caffeate, Fe^{III}rhodotorulic acid and Fe^{III}HEDTA are obviously causally related to their lower stability and precipitation of Fe^{III}hydroxide at the root surface. This is indicated by the high pool of extracellular Fe at the end of the uptake period (Table III). Extracellular Fe, *i.e.* precipitation of Fe^{III}hydroxide at the root surface and in the apparent free space of the cortex, may act as readily accessible source of Fe for the phytosiderophores released by the barley roots under iron deficiency.

An unexpected result was that the high rates of Fe uptake from phytosiderophores were not only confined to chlorotic (Fe-deficient) barley. They also occured at only slightly lower rates in the green (Fe-sufficient) barley plants, when Fe^{III}phytosiderophores became available for uptake by Fe-sufficient plants in mixed culture with Fe-deficient plants (Table III). This indicates that the important step for regulation of Fe uptake in barley is the rate of release of phytosiderophores and not the uptake rate of Fe phytosiderophores.

Involvement of an Fe^{III} Reduction Step in Fe Uptake. Barley and cucumber plants not only differ in the solubilization of sparingly soluble inorganic iron and the utilization of synthetic Fe^{III}chelates (Table I) but also in their mechanism of iron uptake, particularly the necessity of a reduction step prior to the uptake by root cells (Table IV). The role of a reduction step for iron uptake can be demonstrated by the inhibiting effect of BPDS, a strong chelator for Fe^{2+}.

In cucumber plants, the rates of ^{59}Fe uptake from both synthetic chelates and phytosiderophores increase rapidly under Fe deficiency (Table IV). However, as indicated by the severe decrease in ^{59}Fe uptake by addition of BPDS, reduction of Fe^{III} to Fe^{II} is an obligatory step for the enhancement of iron uptake under iron deficiency. This is irrespective of whether or not Fe^{III} is supplied as a synthetic (FeEDDHA) or as a plant-made (phytosiderophore) chelate. Fe-deficient barley plants fail to increase the rate of ^{59}Fe uptake from $^{59}Fe^{III}$EDDHA. However, the rates of ^{59}Fe uptake from $^{59}Fe^{III}$phytosiderophores compared to the synthetic chelate are more than 700 times higher and are not affected by the addition of BPDS. Thus, reduction of Fe^{III} to Fe^{II} is not involved in uptake of Fe^{III}phytosiderophores in barley. This is true for both Fe-deficient and Fe-sufficient barley plants.

Effect of the pH on the Uptake of Fe Supplied as Fe Chelates. The rate of ^{59}Fe uptake by Fe-deficient cucumber plants rapidly declines with increase in pH from 5.0 to 8.8 (Table V). The decline is independent of whether iron is supplied as synthetic Fe^{III}chelates or Fe^{III}phytosiderophores. Such a pH effect on iron uptake in cucumber is to be expected from the well documented inhibitory action of high pH on the Fe^{III}reductase at the root surface.

The rate of ^{59}Fe uptake by barley from Fe^{III}phytosiderophores is much less inhibited by high pH in the Fe-deficient plants (Table VI). The inhibition is confined to the pH range up to 7.5 and does not exceed about 50% of the values obtained at pH 5.0. This is so even in the presence of relatively high concentrations of bicarbonate. Compared to the Fe-deficient barley plants, not only is the level of ^{59}Fe uptake by the Fe-sufficient plants much lower but also the pH effect is greater. The solubilization and uptake of iron in the Fe-sufficient plants is presumably mediated by unspecific root exudates whose release and/or effectiveness are more pH dependent. The results of Tables V and VI are further indications of the differences between cucumber and barley plants in their mechanisms of solubilization and uptake of iron under iron deficiency.

Diversity of the Phytosiderophore System for the Uptake of Iron (Strategy II) in Higher Plants. Table VII shows that only the graminaceous species possess the ability to utilize Fe^{III}hydroxide as a source for high rates of iron uptake under iron deficiency. In the grass species tested, the capability for uptake of iron supplied as Fe^{III}hydroxide decreases in the following order: barley > wheat > rye ≫ corn ≫ sorghum.

All dicotyledoneous species tested had limited ability to enhance the utilization of Fe^{III}hydroxide under iron deficiency

Table III. *Rate of ^{59}Fe Uptake and Concentration of Extracellular ^{59}Fe in Fe-Sufficient (+Fe) and Fe-Deficient (−Fe) Barley Plants Supplied with $^{59}Fe^{III}$Hydroxide (in Dialysis Tubes) or Various Forms of $^{59}Fe^{III}$Chelates in the Nutrient Solution (pH 6.5–7.5) for 24 h*

Values are means of six replicates.

^{59}Fe Sources	^{59}Fe Uptake Rate Preculture		Extracellular Fe Preculture	
	+Fe	−Fe	+Fe	−Fe
	nmol ^{59}Fe/g root dry wt·24 h		*nmol ^{59}Fe/g root dry wt*	
Fe hydroxide (1×10^{-8}–1×10^{-7} M Fe phytosiderophores)[a]	410.2	505.3	4.3	4.3
Fe siderophores (3×10^{-7} M)				
FeDFOB	3.1	12.9	5.8	6.3
Ferrichrome	3.4	8.7	4.4	3.6
Fe rhodotorulic acid[b]	19.6	86.9	68.0	36.8
Synthetic chelates (3×10^{-7} M)				
FeEDDHA	1.3	2.4	2.3	0.6
FeEDTA	1.6	6.1	4.4	1.5
FeDTPA	6.4	4.5	5.2	1.3
FeHEDTA	12.0	28.3	17.3	9.1
Fe (caffeate)$_3$[b]	44.8	213.5	201.3	50.0

[a] Mixed culture of Fe-deficient and Fe-sufficient barley plants supplied with ^{59}Fe hydroxide in dialysis tubes. The Fe^{III}phytosiderophores were formed by root exudates of the Fe-deficient plants with the Fe hydroxide.

[b] Substantial precipitation on inorganic ^{59}Fe on the root surfaces during the uptake experiments.

Table IV. *Effect of the Ferrous Chelator BPDS Added to the Nutrient Solution on the Uptake Rate of ^{59}Fe in Fe-Sufficient and Fe-Deficient Cucumber and Barley Plants Supplied with Either $^{59}Fe^{III}$EDDHA (3×10^{-8} M) or $^{59}Fe^{III}$Phytosiderophores (1×10^{-8}–7×10^{-8} M)*

^{59}Fe was supplied in nutrient solution (pH 6.5–7.0) for 24 h. Preculture of the plants with 0.1 mM FeEDTA (+Fe) or without Fe (−Fe).

Treatment	^{59}Fe Uptake Rate			
	Cucumber preculture		Barley preculture	
	+Fe	−Fe	+Fe	−Fe
	nmol ^{59}Fe/g root dry wt·24 h			
(A) ^{59}FeEDDHA				
−BPDS	5.4	487	0.5	0.6
+BPDS, 50 μM	2.3	99	0.5	0.5
(B) ^{59}Fe phytosiderophores[a]				
−BPDS	6.5	387	381	498
+BPDS, 50 μM	2.9	66	372	510

[a] Supply of $^{59}Fe^{III}$phytosiderophores was achieved in mixed culture with Fe-deficient barley plants and supply of $^{59}Fe^{III}$hydroxide in a dialysis tube. The concentration of $^{59}Fe^{III}$phytosiderophores in the nutrient solution was measured continuously during the uptake experiment.

Table V. *Effect of the pH and of Bicarbonate on Uptake of Fe in Fe-Deficient Cucumber Plants Supplied with either $^{59}Fe^{III}$EDDHA (10^{-7} M) of $^{59}Fe^{III}$Phytosiderophores (1×10^{-8}–7×10^{-8} M)*

^{59}Fe was supplied in nutrient solution for 24 h to roots. pH was adjusted by addition of NaOH, $CaCO_3$, or 10 mM $KHCO_3$. Values are means of six replicates.

pH of Nutrient Solution	^{59}Fe Uptake Rates	
	^{59}FeEDDHA	^{59}Fe phytosiderophore[a]
	nmol ^{59}Fe/g root dry wt·24 h	
5.0	575.5 (100.0%)	414.0 (100.0%)
6.5	345.3 (60.0%)	263.8 (63.7%)
7.5 (+$CaCO_3$)	281.9 (48.9%)	125.5 (30.3%)
8.0	105.1 (18.3%)	48.4 (11.7%)
8.8 (+$KHCO_3$)	30.3 (3.5%)	21.2 (5.1%)

[a] Mixed culture with Fe-deficient barley plants and supply of $^{59}Fe^{III}$hydroxide in dialysis tubes.

Table VI. *Effect of the pH and of Bicarbonate in the Nutrient Solution on the Rates of Fe Uptake in Fe-Sufficient (+Fe) and Fe-Deficient (−Fe) Barley Plants*

^{59}Fe was supplied as $^{59}Fe^{III}$hydroxide in dialysis tubes in nutrient solution for 24 h. The pH was adjusted by addition of NaOH, $CaCO_3$, or 10 mM $KHCO_3$. Values are means of six replicates.

pH of Nutrient Solution	^{59}Fe Uptake Rates Preculture	
	+Fe	−Fe
	nmol ^{59}Fe/g root dry wt·24 h	
5.0	67.9 (100.0%)	741.1 (100.0%)
6.5	21.1 (32.0%)	681.9 (92.0%)
7.5 (+$CaCO_3$)	2.0 (2.9%)	393.2 (53.1%)
8.0	1.6 (2.4%)	359.8 (48.5%)
8.8 (+$KHCO_3$)	2.0 (2.9%)	373.4 (50.4%)

(Table VII). The Fe-deficient dicots increased the uptake of ^{59}Fe uptake from this source of iron only by a factor of about 3 to 4 compared to a factor of between 7 and 73 in the grasses. This demonstrates that the phytosiderophore system (strategy II), *i.e.* the ability of solubilization of sparingly soluble inorganic Fe^{III} compounds and the subsequent uptake of Fe^{III}, is confined to

Table VII. *Chlorophyll Content and Rates of Fe Uptake by various Plant Species as Affected by the Fe Nutritional Status*

Plants were precultured with 0.1 mM FeEDTA (+Fe), or without Fe (−Fe). For the uptake studies, Fe was supplied as $^{59}Fe^{III}$hydroxide in dialysis tubes to roots of intact plants in nutrient solution (pH 6.5–7.0) for 24 h.

Plant Species	Chl Content Preculture		^{59}Fe Uptake Rates Preculture	
	+Fe	−Fe	+Fe	−Fe
	mg/g dry wt		*nmol/g root dry wt·24 h*	
Strategy II				
Barley	12.2	7.8	9.3	567.8
Wheat	12.1	5.2	6.3	458.1
Rye	12.4	6.4	26.2	358.1
Corn	11.9	6.1	5.2	183.0
Sorghum	11.7	6.9	5.3	37.3
Strategy I				
Cucumber	11.5	5.5	8.5	30.2
Peanut	11.7	5.5	2.5	9.6
Potato	10.9	6.2	2.8	12.1
Sunflower	9.2	4.8	3.9	12.1
Tomato	11.2	5.1	4.1	14.7

the grass species. Enhancement of Fe uptake by the tested dicots under iron deficiency relies on the supply of soluble Fe^{III} compounds (chelates) and reduction to Fe^{II} at the uptake sites (strategy I).

DISCUSSION

The present paper provides further evidence for principle genotypical differences in higher plants in the mechanisms of solubilization and uptake of iron in response to iron deficiency. The principle differences between strategy I and strategy II are summarized schematically in Figure 1. These two strategies are distributed in a systematic order in the plant kingdom (21).

Strategy I is typical for dicots and nongraminaceous monocots, and is understood fairly well (21). In plant species with strategy I, supplied with chelated Fe^{III} the transport of iron across the plasma membrane of root cells is preceded by Fe^{III} reduction and chelate splitting (5, 20). Fe^{III} reduction under iron deficiency is enhanced by a dramatic increase in the activity of a plasma membrane-bound 'reductase' (2). As a rule, the rate of H^+ extrusion is increased also in the Fe-deficient roots (18). Enhancement of the reductase activity and of H^+ extrusion is confined to the apical root zones (21, 23) and leads to a severalfold increase in the rate of iron uptake by the apical root zones. At suboptimal Fe supply, these events may occur rhythmically (19). Occasionally, an enhanced release of phenolics can be observed also from roots of Fe-deficient plant species with strategy I (16, 20).

Our knowledge on strategy II is limited. Up to now strategy II has been found only in graminaceous species, and Fe deficiency-induced increases in reductase activity and H^+ extrusion generally are absent (18, 21). Accordingly, the ability is lacking in grasses under Fe deficiency for enhanced utilization of Fe^{III}chelates such as FeEDDHA or FeEDTA (Tables I and III). However, barley and other grasses under iron deficiency can markedly increase the utilization of sparingly soluble Fe^{III}hydroxide (Tables I and VII). Nonproteinogenic amino acids (phytosiderophores) such as mugineic or avenic acid are responsible for this. These phytosiderophores are released by the roots of grasses under iron deficiency and form stable chelates with Fe^{3+} (26, 28).

Phytosiderophores are released in accord with a diurnal

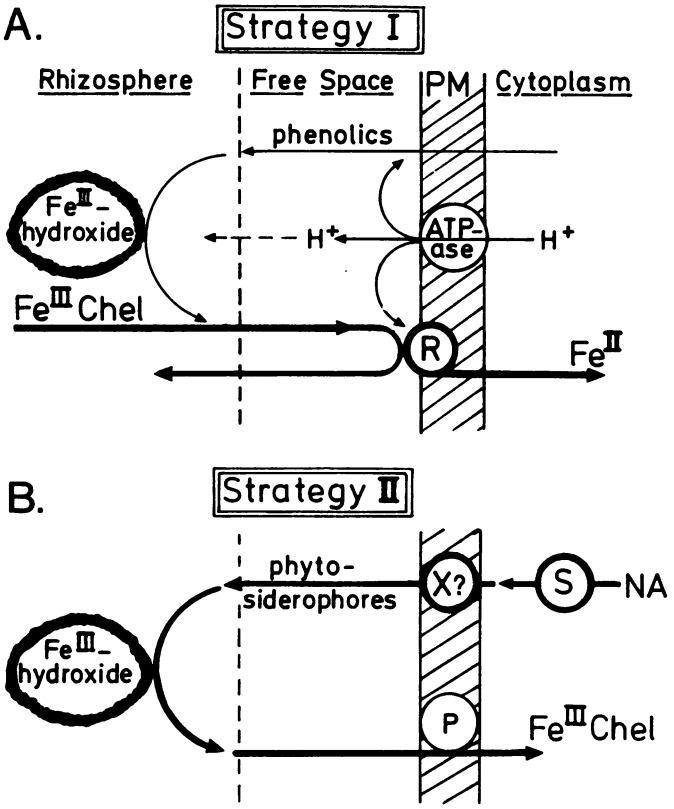

FIG. 1. Model of strategy I and II in higher plants for solubilization and uptake of iron. A, Strategy I: inducible reductase Ⓡ and ATP-ase. B, Strategy II: inducible synthesis of phytosiderophores Ⓢ from the precursor nicotianamine (NA), extrusion of phytosiderophores Ⓧ, and a specific transport system for ferrated phytosiderophores Ⓟ.

rhythm with a maximum a few hours after onset of the light period (28). The fluctuations in the concentration of soluble iron in the nutrient solution (Table II) may in part reflect this rhythmical release. However, feedback regulation mechanisms resulting from the iron nutritional status of the roots may also be involved in these rhythmic events, in analogy to strategy I (19).

The enhanced utilization of FeEDDHA in Fe-deficient barley (7) is in apparent contradiction to the results shown in Table I. However, it became evident that higher rates of utilization of iron from chelates by Fe-deficient barley plants are closely related to higher levels of extracellular inorganic Fe^{III} in comparison of Fe^{III}chelates of differing stability (Table III). The inorganic Fe^{III} originates from the decay of Fe^{III}chelates and may act as a readily available source of iron for chelation by the root-released phytosiderophores. This explanation agrees with reports of high utilization of FeHEDTA or Fe^{III}caffeate by rice and barley (11, 14, 26). Thus, the reports on faster rates of iron uptake from chelated iron by Fe-deficient grass species are probably misinterpretations. According to the data in Table III, the assumption of Sugiura and Nomoto (26) that FeHEDTA as a structural analog of ferrated mugineic acid is taken up by the same phytosiderophore system also is not justified. An additional source of error that has to be considered is the incomplete chelation of inorganic Fe^{III} during the preparation of synthetic chelates such as ^{59}FeEDDHA (7).

Fe^{III}phytosiderophores are readily and preferentially taken up by grasses (Tables I, III, and VII). This agrees with results of Mino *et al.* (14) and Takagi *et al.* (28) on rice supplied with Fe^{III}phytosiderophores. The preferential uptake of Fe^{III}phytosiderophores by barley—and also presumably by other grasses—does not require an Fe^{III} reduction step at the plasma membrane as indicated by the ineffectiveness of BPDS (Table IV). In addition, the iron uptake by Fe-deficient barley plants is only moderately inhibited by high pH or high bicarbonate concentrations (Table VI). These factors are well known for their strong inhibitory effects on the plasma membrane-bound reductase and thus on Fe^{III} reduction and Fe uptake in Fe-deficient species with strategy I (Table V; 20). Thus, Fe^{III} reduction as an obligatory step in Fe uptake is typical for Strategy I but not for strategy II. This is true for strategy I even when Fe^{III}phytosiderophores are supplied for cucumber as shown in Table IV.

The uptake system in grasses is rather selective for Fe^{III}phytosiderophores. Microbial siderophores with high stability are comparably poor sources for iron uptake by barley (Table III) and rice (14, 26). Microbial siderophores in soils may be an important factor involved in iron solubility (17). However, a particular importance of these siderophores for the iron nutrition of grasses as it has been recently been suggested (8, 17) must be questioned. The microbial Fe^{III}siderophores are poor sources of iron, also for plant species with strategy I since the ferric iron in these compounds is not easily reducible by the plasma membrane-bound reductase (2, 20). One may speculate that the transport of Fe^{III}phytosiderophores as a whole molecule across the plasma membrane of grasses also is mediated by a specific transport (receptor) protein in analogy to the transport mechanism of siderophores in microorganisms (15, 29). This speculation seems to be justified according to preliminary results with the use of double labeled Fe phytosiderophores. However, Fe uptake by microorganisms and grasses differ. In microorganisms, the receptor proteins are highly selective for the various siderophores and differ even between strains of fungi (29). The transport system in grasses is not very selective for the various Fe phytosiderophores. Fe phytosiderophores of barley (mainly ferrated mugineic acid) are readily taken up by rice (14) and sorghum (V Römheld, unpublished data). Another difference is that the synthesis and/or activity of the siderophore receptor proteins in microorganisms are closely related to the iron nutritional status (10, 15). However, in grasses, at least in barley (Table III), and sorghum (V Römheld, unpublished data), the uptake of Fe phytosiderophores is similar in chlorotic (Fe-deficient) and FeEDTA precultured green (Fe-sufficient) plants. Thus, the principal step of regulation of iron uptake (Fig. 1) seems to be the rate of synthesis Ⓢ from the possible precursor nicotionamine (9) and/or extrusion of phytosiderophores Ⓧ but not the rate of uptake Ⓟ. In such a regulatory system the involvement of a shuttle transport mechanism, as it exists for siderophores in microorganisms (recycling of siderophores; 10) is not very likely. In addition, phytosiderophores are fairly readily decomposed by microorganisms. Only about 10% of the phytosiderophores released by the roots were taken up as Fe equivalents based on a rough calculation, in the experiments presented here.

The common precursor for phytosiderophores is probably nicotianamine (9). Nicotianamine is neither released from the roots under Fe deficiency (24) nor is the corresponding Fe chelate (Fe nicotianamine) preferentially taken up by roots (14). According to terminology for siderophores (10, 15), the classification of nicotianamine as a phytosiderophore (4) is not appropriate.

Clarkson and Sanderson (7) presented evidence for a preferential and enhanced uptake of iron in the apical root zones of Fe-deficient barley plants. Unfortunately, their methods did not differentiate between whether the faster rates of iron uptake in the apical zones are the result of enhanced release of phytosiderophores or the results of uptake of the Fe phytosiderophores. With seminal roots of maize, a similar uptake rate in the different root zones was observed when the various root zones were exposed to the same circulating nutrient solution (12). However, the level of iron uptake was several times higher with a supply of inorganic Fe^{III} than with FeEDTA. This would indicate that

the apical root zones are the preferential sites for production and release of phytosiderophores, but that the uptake rate of Fe^{III}phytosiderophores is similar along the whole root. If this assumption can be verified by further experiments, strategy II also would differ from strategy I since all of the individual components of the iron deficiency response mechanism, including the enhanced rate of iron uptake, are confined to the apical root zones (21).

The ability of chlorotic plants to increase the rate of iron uptake from Fe^{III}hydroxide as source of iron differs among various species of grasses (Table VII). These differences are mainly the result of corresponding differences in the release of phytosiderophores (21, 27). Graminaceous species, such as rice and sorghum, which release only small amounts of phytosiderophores, are, however, very efficient in the uptake of ferrated mugineic acid (14; V Römheld, unpublished data). Thus, only the rate of release of phytosiderophores correlates positively with the resistance of the various grass species to iron deficiency on calcareous soils ('lime chlorosis'; 21). Selection and breeding programs for resistance to lime chlorosis in grasses therefore have to be focused on high rates of release of phytosiderophores rather than on high uptake rates of ferrated phytosiderophores. Furthermore the role of pH (addition of $CaCO_3$ and bicarbonate) is less important in screening programs for grasses than in corresponding programs for species with strategy I.

From the ecological point of view, strategy II seems to have some advantages for adaptation to calcareous soils over strategy I. The advantages are the lower sensitivity to high pH and high bicarbonate concentrations (Table VI) and the ability to solubilize sparingly soluble inorganic Fe^{III} in the rhizosphere by release of phytosiderophores. In addition, high concentrations of Ca^{2+} and Mg^{2+} which are common on calcareous soils have no inhibitory effect on the solubilization of Fe^{III} by phytosiderophores (14). In contrast, strategy I is severely inhibited by high pH and high bicarbonate and relies on the supply of chelated iron to the uptake sites at the plasma membrane. In agreement with this, grasses on wet calcareous soils are usually less sensitive to lime chlorosis than dicots (6, 22). On the other hand, grasses are more sensitive than dicots to phosphate induced chlorosis, particularly in nutrient solution culture (1, 6, 21). The reasons for these differences in the interference with phosphate between Strategy I and II is not known.

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