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Obligatory Reduction of Ferric Chelates in Iron Uptake by Soybeans

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ABSTRACT

The contrasting Fe^{2+} and Fe^{3+} chelating properties of the synthetic chelators ethylenediaminedi(*o*-hydroxyphenylacetate) (EDDHA) and 4,7-di(4-phenylsulfonate)-1,10-phenanthroline (bathophenanthrolinedisulfonate) (BPDS) were used to determine the valence form of Fe absorbed by soybean roots supplied with Fe^{3+}-chelates. EDDHA binds Fe^{3+} strongly, but Fe^{2+} weakly; BPDS binds Fe^{2+} strongly but Fe^{3+} weakly. Addition of an excess of BPDS to nutrient solutions containing Fe^{3+}-chelates inhibited soybean Fe uptake-translocation by 99+%; $[Fe(II)(BPDS)_3]^{4-}$ accumulated in the nutrient solution. The addition of EDDHA caused little or no inhibition. These results were observed with topped and intact soybeans. Thus, separation and absorption of Fe from Fe^{3+}-chelates appear to require reduction of Fe^{3+}-chelate to Fe^{2+}-chelate at the root, with Fe^{2+} being the principal form of Fe absorbed by soybean.

The mechanism by which a root utilizes Fe from highly stable Fe^{3+}-chelates has remained elusive since the introduction of these materials to plant nutrition. Two hypotheses were advanced: (*a*) Fe and chelating agent (chelator) are separated before Fe uptake, or (*b*) the Fe chelate enters the plant intact and is separated as the Fe is utilized. The first hypothesis is supported by the work of Tiffin *et al.* (32) and Tiffin and Brown (31), who found the chelator to be excluded during short term experiments in which several plant species under Fe-stress accumulated large amounts of Fe. They consistently observed a slow entry of chelator into both green and chlorotic (Fe-stressed) plants (31). Early data from other workers supported the alternative hypothesis (18), but more recent reports support the conclusion that Fe and chelator can be separated at the root, especially in the case of Fe-deficient dicotyledonous plants (15, 19).

Brown *et al.* (6, 9) suggested that the separation of Fe from chelator before entry of Fe involved reduction of the Fe^{3+}-chelate to Fe^{2+}-chelate. Tests with four chelators revealed that the ability of an Fe-efficient plant to tolerate an excess of chelator was not related to the stability constant of the Fe^{3+}-chelate (9). Since not all of the Fe^{2+} stability constants were available, conclusions about reduction of the Fe^{3+}-chelates were not possible.

More recent information about the separation of Fe from Fe^{3+}-chelates poses a severe test for any theory that does not consider the valence form of Fe that is exchanged. Bates *et al.* (3) demonstrated that exchange of Fe^{3+} from Fe-EDTA to transferrin was a slow first-order process with a half-time of 3.5 days at pH 7.5. The fact that Fe^{2+}-chelates of the EDTA family of chelators have both a much faster exchangeability and much lower stability than the corresponding Fe^{3+}-chelates led us to search for evidence of reductive separation of Fe from Fe^{3+}-chelates by plants.

We tested this hypothesis by use of the Fe^{2+} color reagent BPDS,[2] which forms a soluble, nonautoxidizable Fe^{2+}-chelate of high stability and low exchangeability (14). We found that this chelator prevented uptake of Fe from Fe^{3+}-chelates and concurrently, $FeBPDS_3$ accumulated in the nutrient solution. These results demonstrate that separation and absorption of Fe from Fe^{3+}-chelate requires reduction of Fe^{3+}-chelate to Fe^{2+}-chelate at the root before uptake of Fe^{2+} by the plant.

MATERIALS AND METHODS

Nutrient Solutions. Two nutrient solutions were used. The first was a modified one-fifth Steinberg solution (29) with varied Fe treatments (one-fifth Steinberg). The second nutrient solution (30) had one-fifth the macronutrients and one-half the micronutrients of the first and contained, in micromoles per liter: 250 Ca, 50 Mg, 200 K, 850 N, 30 S, 10 P, 3.0 B, 1.2 Mn, 0.25 Zn, 0.10 Cu, 0.05 Mo, and 35 Cl with varied Fe treatments (1/25 Steinberg).

Plant Growth and Culture Techniques. Hawkeye soybean, *Glycine max* (L.) Merrill, seeds were germinated for 3 days, then transferred to a one-fifth Steinberg solution containing 1 μM FeEDDHA and grown under partial shading. At 7 days seedlings were bound in groups of five or eight plants and four groups were transferred to 8 liters of fresh nutrient solution in each treatment. The plants were grown in a light chamber with 8-hr dark and 16-hr light periods (1500 ft-c) at 23 ± 2 C.

Two methods of solution culture were used. In one (single-change method), the growth solution was one-fifth Steinberg

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[2] Abbreviations: EDDHA: ethylenediaminedi(*o*-hydroxyphenylacetate); CDTA: *trans*-1,2-diaminocyclohexanetetraacetic acid; DTPA: diethylenetriaminepentaacetic acid; BPDS: 4,7-di(4-phenylsulfonate)-1,10-phenanthroline, bathophenanthrolinedisulfonate; $FeBPDS_3$: $[Fe(II)(BPDS)_3]^{4-}$; Fe^{3+}BPDS: $[(BPDS)_2Fe(III)OFe(III)BPDS)_2]^{4-}$; TPTZ: 2,4,6-tris(2-pyridyl)-1,3,5-triazine; Fe^{3+} chel: (any Fe^{3+}-chelate); pFe^{2+}: $-\log[Fe^{2+}]$; pFe^{3+}: $-\log[Fe^{3+}]$.

with varied Fe. The single change of nutrients and Fe had to last until the plants were used for isotope absorption experiments 11 days later; plants supplied 1 μM FeDTPA at day 7 became chlorotic on day 17. In the other culture method (2-day change), nutrient solutions were replaced every 2 days (30). The growth solution was $\frac{1}{25}$ Steinberg with 1 μM FeEDDHA. At day 15 varied Fe treatments were imposed (conditioning nutrient). The plants were used in isotope absorption experiments after 8 days of conditioning. When conditioned at low Fe levels these 23-day-old plants were not chlorotic. During growth and conditioning periods, the roots of each group of plants were separated to prevent any breakage of roots in the transfer to isotope absorption nutrients.

Isotope Absorption Techniques. Experiment 1 involved a topped-plant exudate-collection technique (1, 29). Exudate was collected for 20 hr. Experiments 2 and 3 involved intact plants. In both experiments the plants were transferred from the conditioning nutrient at midday to 1 liter of absorption nutrient after the roots were briefly rinsed with deionized water. The culture vessels were 1-liter beakers wrapped with black plastic and covered with black Plexiglas plant supports to exclude light.

^{59}Fe-labeled Fe^{3+}-chelates with other treatments as described for the individual experiments (legends of Tables I, II, and III) were supplied to the plants in vigorously aerated nutrient solutions. Aliquots of absorption nutrient solution were removed at intervals for ^{59}Fe and absorbance measurements. In experiment 3 sufficient water was added to each vessel 10 min before each sampling to replace water lost by evapotranspiration and sampling. Appropriate control solutions were prepared and sampled with the experimental treatments.

At the end of the absorption experiments the tops were cut off at the cotyledonary node, and the roots were rinsed for 15 sec in flowing deionized water. Root fresh weights were obtained after adhering water had dripped off the roots.

Preparation of $^{59}Fe^{3+}$-Chelates. Quantities of chelators (102% of total Fe in solution for DTPA or EDTA, on a molar basis) were dissolved in enough NaOH to bring the solution to pH 7. The selected amount of $^{59}FeCl_3$ in 0.5 N HCl was added and the pH was raised to 7 with NaOH. Carrier $Fe(NO_3)_3 \cdot 9H_2O$ was added and, after stirring, the pH of the solution was adjusted to 6.0. This solution was then diluted to the desired volume. An aliquot (5 ml/liter) of the $^{59}Fe^{3+}$-chelate was pipetted into each experimental absorption solution about 2 hr before the experiment was to begin; any competing chelators were pipetted into the solution 1 hr later. Aerated solution of Fe chelates of the EDTA family of chelators are quantitatively Fe^{3+}-chelates.

Isotope Measurement. ^{59}Fe was assayed using a gamma scintillation spectrometer. Count rates were corrected for decay and, where necessary, for sample volume. The amount of ^{59}Fe (μmoles) was calculated from specific radioactivity relationships. Selected portions of wilted plants were conveniently prepared for ^{59}Fe assay by wrapping in a square of 3-mil plastic film; this wrapped plant material was stuffed tightly into the bottom of a 25 $\times$ 150 mm counting tube.

"Reductant" Measurement. Compounds accumulating in the conditioning nutrient solution which were capable of reducing inorganic Fe^{3+} (reductants) were measured by a modification of the method of Ambler *et al.* (1). To a 20-ml aliquot of the nutrient solution, the following were added with mixing after each addition: 1.00 ml of 4 mM Fe^{3+} in 2 N HCl, 2.00 ml of 2.50 mM TPTZ, 1.00 ml of 4 M sodium acetate. The absorbance of these solutions at 592 nm was measured after 24 hr in the dark. Appropriate water or nutrient solution blanks were treated as samples.

In vitro reduction of FeDTPA by reductant was measured as follows: to 1.00 ml of 2.50 mM FeDTPA + 2.50 mM excess DTPA the following additions were made with stirring: 2 ml of 2 N acetate buffer, pH 4.6; 2 ml of 3.75 mM BPDS; 10 ml H_2O; reductant; H_2O to 25 ml. The absorbance at 535 nm was measured at 30 min, 12 hr, and 24 hr.

Measurement of Fe^{3+}-Chelate Reduction. Reduction of Fe^{3+}-chelates was measured spectrophotometrically with the ferrous color reagent BPDS. The molar absorptivity of $FeBPDS_3$ is 2.2×10^4 at 535 nm (14). $FeBPDS_3$ produced at the root by reduction of the Fe^{3+}-chelates accumulated in the nutrient solution; 5-ml aliquots were withdrawn with time for absorbance measurement. A control assay solution with no plants added was also sampled with time (blank or control value). The reduction experiments were performed in the darkened beakers described above for routine isotope absorption experiments.

RESULTS

The experiments were designed to determine the effect of the Fe^{2+} trapping reagent, BPDS, and other chelators on Fe uptake by Hawkeye soybeans. Chlorotic (Fe-stressed) plants were generally used because they absorb and translocate much more Fe than Fe-sufficient plants during short term experiments.

Experiment 1. The effect of several concentrations of BPDS, EDTA, DTPA, CDTA, and EDDHA on soybean uptake-translocation of Fe from ^{59}FeDTPA was determined. ^{59}Fe in the stem exudate of topped plants was measured. The treatments and results are shown in Table I.

BPDS was the most effective inhibitor of Fe uptake-translocation, followed by EDTA > DTPA > CDTA $\gg$ EDDHA. BPDS inhibited ^{59}Fe movement to the exudate by 99.7% even at the lowest level of competitor used in this experiment. $FeBPDS_3$ accumulated in the nutrient solution. BPDS was 10 to 100 times more inhibitory than EDTA, DTPA, or CDTA. EDDHA, the chelator with the highest Fe^{3+} stability constant, only slightly inhibited or actually promoted Fe uptake-translo-

Table I. *Effect of Chelator Excess on Fe Uptake-Translocation by Topped Soybeans (Experiment 1)*

The plants were grown by the single-change method with 1 μM FeDTPA. The topped root systems of eight 18-day-old chlorotic plants were transferred to 1 liter of one-fifth Steinberg solution containing 5 μM ^{59}FeDTPA (10 μc/l) with excess chelators as shown. Exudate was collected for 20 hr. BPDS forms a 3:1 chelate with Fe; the other chelators bind Fe 1:1.

Competing Chelator		^{59}Fe in Exudate	
compound	μM	*nmoles*	*% of control*
None		268.4[1]	100
BPDS	15	0.71	0.26
BPDS	48	0.36[1]	0.13
BPDS	150	0.12[1]	0.04
EDTA	16	9.55	3.56
EDTA	50	5.12	1.91
DTPA	16	32.8	12.22
DTPA	50	2.06	0.77
CDTA	16	29.4	10.95
CDTA	50	5.43	2.02
EDDHA	5	197.3	73.5
EDDHA	16	295.7[1]	110.5
EDDHA	50	97.8[1]	36.4

[1] Means of duplicate treatments; other values are for unreplicated treatments.

Table II. *Effect of BPDS on Fe Uptake-Translocation by Intact Soybeans under Different Degrees of Fe-Stress (Experiment 2)*

The plants were grown by the single-change method with pretreatments as shown. Each group of eight intact plants, 18- or 20-days-old, was transferred to 1 liter of one-fifth Steinberg solution containing 5 μM ^{59}FeDTPA (10 μc/1) ± BPDS. The absorption period was 20 hr. The standard errors of the means were calculated for the contents of isotope in the tops.

	Pretreatment		Treatment	Isotope in Tops	
	Fe	DTPA	BPDS		
	μM			*nmoles/plant* ± SE	*% of control*
18-Day-old plants	1	1	0	503.0 ± 68.3	100
	1	1	48	0.84 ± 0.10	0.17
	20	20	0	37.3 ± 9.92	100
	20	20	48	1.01 ± 0.14	2.71
	20	200	0	423.8 ± 69.3	100
	20	200	48	0.61 ± 0.07	0.14
20-Day-old plants	1	1	0	414.5 ± 62.9	100
	1	1	48	1.41 ± 0.31	0.34
	20	20	0	95.3 ± 14.8	100
	20	20	48	1.51 ± 0.16	1.59
	20	200	0	386.8 ± 69.1	100
	20	200	48	0.88 ± 0.09	0.23

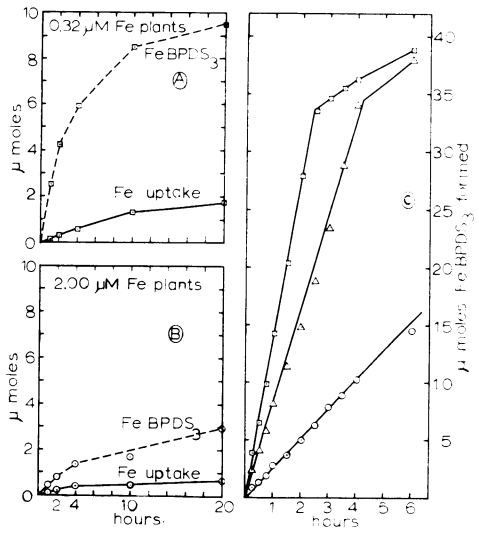

FIG. 1. Time course of reduction of Fe^{3+}-chelates and Fe uptake by soybeans (Experiment 3). Bundles of five plants were conditioned for different Fe-stress: ⊙: 2 μM Fe; ⊡: 0.32 μM Fe; △: 0.10 μM Fe. A and B: Plants supplied 1 liter of 10 μM ^{59}FeEDTA ± 96 μM BPDS: $FeBPDS_3$ accumulating in the nutrient solution (- - -); Fe absorbed in the absence of BPDS (——). C: Plants supplied 40 μM Fe^{3+} + 160 μM BPDS; reduction measured by accumulation of $FeBPDS_3$.

cation, whereas the chelator with the highest Fe^{2+} stability constant, BPDS, was a severe inhibitor. It is clear that in these trials with topped plants, the competition of excess chelators for Fe is for Fe^{2+}.

Experiment 2. Intact rather than topped plants were used in this experiment. The plants were under different degrees of Fe-stress from limited Fe supply or excess chelator (pretreatment, Table II). Only the plants on the low Fe pretreatment were chlorotic.

Plants under severe Fe-stress from either low Fe or high DTPA pretreatment absorbed and translocated more Fe than the Fe-sufficient plants (Table II). The Fe-stressed plants, in the absence of BPDS, absorbed 98% of the total ^{59}Fe (5 μmoles) in the absorption nutrient and transported 60 to 80% of it to the tops during the 20-hr absorption period. Fe uptake-translocation was inhibited about 99.8% by the BPDS treatment. About 1 nmole of ^{59}Fe reached the plant tops from each of the BPDS treatments regardless of the Fe-stress on plants.

Thus, a similar result was observed for intact and topped plants: uptake of Fe from FeDTPA is prevented by trapping Fe^{2+} generated at the root, indicating a reductive separation of Fe from Fe^{3+}-chelate before uptake of Fe^{2+} by the plant. Further, the plants adapted to the excess chelator in the conditioning nutrient solution by increasing their ability to reduce and absorb Fe.

Experiment 3A. This experiment was designed to examine the relationship between reduction of Fe^{3+}-chelate and uptake of Fe. The formation of the red $FeBPDS_3$ was used as a measure of the reduction of FeEDTA at the root. Data for pretreatments 0.10 and 0.32 μM Fe were similar, thus, only data for the 0.32 μM Fe pretreatment are shown graphically. Plants under moderate Fe-stress (pretreatment 0.32 μM Fe, Fig. 1A) reduced more Fe^{3+} than the plants conditioned with 2.0 μM Fe (Fig. 1B). ^{59}Fe transported to the tops was higher in plants with greater Fe-stress (Table III).

Experiment 3B. This experiment was exactly like experiment 3A except Fe^{3+}BPDS replaced the ^{59}FeEDTA. The measurement of reduction of FeEDTA by trapping Fe^{2+} as $FeBPDS_3$ necessarily underestimates the rate of reduction because EDTA catalyzes oxidation of Fe^{2+} (21). Use of Fe^{3+}-BPDS as the source of Fe^{3+} precludes the oxidative back reaction. The results are shown in Figure 1C. The experiment was terminated at 6 hr because the Fe-stressed plants had reduced nearly all the Fe^{3+}BPDS in the solution. Note that when Fe^{3+}BPDS was not in short supply, its reduction was linear with time.

The initial rate of reduction of FeEDTA and Fe^{3+}BPDS and the rate of Fe uptake by these plants conditioned for different Fe-stress are shown in columns 4 and 5 of Table III. These results show a 4- to 8-fold higher rate of reduction when autoxidation of the Fe^{2+} product was precluded by measurement of Fe^{3+}BPDS reduction.

Reductants. An accumulation of materials capable of reducing inorganic Fe^{3+} (reductants) in the nutrient solutions of Fe-stressed plants has been observed (1, 11). Reductant in the conditioning nutrient solutions was measured daily and the mean rate of accumulation for the last 2 days before harvest is

Table III. *Rate of Reduction of Fe^{3+}-Chelates and Fe Uptake by Soybean Plants Conditioned at Three Levels of Fe-Stress (Experiment 3)*

The plants were grown by the 2-day-change method and conditioned for 8 days at three levels of FeEDDHA: 0.10, 0.32 and 2 μM. Groups of five plants were transferred to 1 liter of 1/25 Steinberg solution with either 10 μM ^{59}FeEDTA (10 μc/1) (columns 2 and 3), 10 μM ^{59}FeEDTA + 96 μM BPDS (column 4), or 40 μM Fe^{3+} + 160 μM BPDS (Fe^{3+} BPDS) (column 5).

FeEDDHA in Conditioning Nutrient	Fe Uptake by Control Plants	Fe in Tops of Control Plants	Reduction of FeEDTA	Reduction of Fe^{3+} BPDS	Reductant in Conditioning Nutrient
μM	*μmoles/20 hr*				
2	0.66	0.14	6.0	51	0.09
0.32	1.75	0.90	45.0	273	0.69
0.10	1.35	0.83	37.0	163	0.92

shown in Table III (column 6). The compounds reduce inorganic Fe^{3+} rapidly, the reaction being nearly complete within 5 min at pH 1. However, reduction of FeDTPA is very slow: the natural reductants reduce only 5% as much FeDTPA as inorganic Fe^{3+} during 20 hr of incubation. Thus, only 5% of the reductant values measured with inorganic Fe^{3+} are reported in Table III (column 6) to permit a fair comparison between ^{59}Fe uptake rate (column 2), Fe^{3+} chelate reduction rate (column 4 and 5), and reductant (column 6). The results show that it is unlikely that the reductants accumulating in the nutrient solution could account for the rate of reduction of Fe^{3+}-chelates by intact soybean roots (column 6 $\ll$ column 4 or 5).

DISCUSSION

Fe-chelates have been recognized for 20 years as an available source of Fe for plants. But few attempts have been made to determine how plants obtain Fe from the Fe^{3+}-chelates or why plant species differ in this ability. It was clearly shown that plants were able to separate Fe from Fe^{3+}-chelates (31, 32), but the mechanism of the separation was not considered.

The contrasting chelators, BPDS and EDDHA, used in this study show obligatory reduction of Fe^{3+}-chelates at the root. The Fe^{2+} produced by the root was trapped by BPDS and remained in the nutrient solution as $FeBPDS_3$. Intact $FeBPDS_3$ does not appear to enter plant roots, probably because of its size and high negative charge. The extreme inhibition of Fe uptake-translocation by BPDS (99.8%) while control treatments allowed 98% removal of ^{59}Fe from the nutrient solution and 80% transport of nutrient ^{59}Fe to the plant tops during a 20-hr period clearly substantiates the obligatory Fe^{3+}-chelate reduction and Fe^{2+} uptake by soybeans. The higher rate of reduction of Fe^{3+}-chelates by Fe-stressed plants indicates that Fe^{3+}-chelate reduction is an integral part of the Fe-stress response (7). The obligatory reduction described here has also been demonstrated for seven other varieties of soybean, five other species in four families, and *Chlorella sorokiniana* by direct measurement of BPDS inhibition of Fe uptake-translocation from Fe^{3+}-chelates (unpublished).

Theoretical Considerations. In considering the mechanism of Fe uptake from Fe^{3+}-chelates, one must include at least these influences on Fe availability: (*a*) the effect of H^+ concentration and valence state of the Fe on stability of Fe-chelates, and (*b*) the effect of H^+ concentration and valence state of the Fe on the exchange of Fe from Fe-chelates. H^+ affects the activity of free Fe in a solution of Fe-chelate. The relationship pFe^{3+} *versus* pH is shown for several chelators in Figure 2A. The curves were calculated for a 1% excess of chelator over Fe according to Chaberek and Martell (12). Fe^{3+}BPDS is shown by a dashed line because it forms a dimer that is not well characterized (33). However, it is clear that Fe^{3+}BPDS has a lower effective stability at pH 4.6 than FeEDDHA because Fe^{3+} exchanges from BPDS to EDDHA nearly quantitatively (unpublished).

H^+ concentration controls the effective stability of an Fe-chelate based on the pK of the individual ionizing ligand groups of the multidentate chelator. The stability constant (pH independent) is calculated for the most ionized form of the chelator. The pK values of the ligand functional groups vary for the chelators shown in Figure 2A; thus, EDDHA with the two phenolic ligands (with high pK values) forms metal chelates whose effective stabilities are much more sensitive to increased H^+ concentration than chelates of EDTA.

Curves for pFe^{2+} *versus* pH are shown for two chelators and BPDS in Figure 2B. A value of 9.3 was used for the stability constant of Fe^{2+}EDDHA. This is a maximum value selected from a plot of stability constant *versus* atomic number for the divalent cations Mn, Fe, Co, Ni, Cu, and Zn according to Irving and Williams (17). Most chelators fit this plot regardless of their Fe^{2+}/Fe^{3+} stability constant ratio. The Fe^{2+} stability constant for EDDHA has not been reported. Other constants used in these calculations were obtained from a compilation of constants (28) and the literature (4, 27). The pFe^{2+} in $FeBPDS_3$ solutions depends on the Fe and BPDS concentrations and the cube of the excess BPDS concentration because it is a tris chelate (12). In contrast, only the chelator-Fe ratio has any appreciable effect on the pFe for 1:1 EDTA type chelates until the chelate approaches 1% dissociation. The curves in Figure 2, A and B, illustrate how different BPDS and EDDHA are in terms of their competition for Fe^{2+} and Fe^{3+}. Since BPDS severely inhibits while EDDHA barely inhibits Fe uptake-translocation for Fe^{3+}-chelates (even when Fe^{3+}-chelate remains available to the plant in the experimental solution), it is obvious that the inhibitory action of excess chelator results from competition for Fe^{2+} formed at the root by a reductive process.

This conclusion helps explain the effects of excess chelators in studies by Brown *et al.* (8–10). For each plant species,

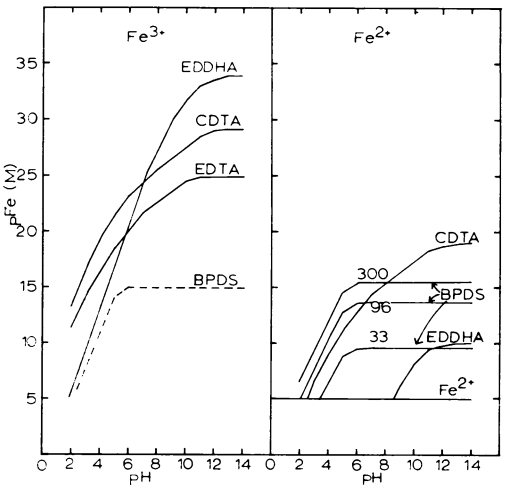

FIG. 2. Calculated pFe *versus* pH according to Chaberek and Martell (13). A: pFe^{3+} for solutions containing 10 μM Fe^{3+} + 10.1 μM EDDHA, CDTA, EDTA or 60 μM BPDS; B: pFe^{2+} for solutions containing 10 μM Fe^{2+} + 100 μM EDDHA, 100 μM CDTA or 33, 96, or 300 μM BPDS.

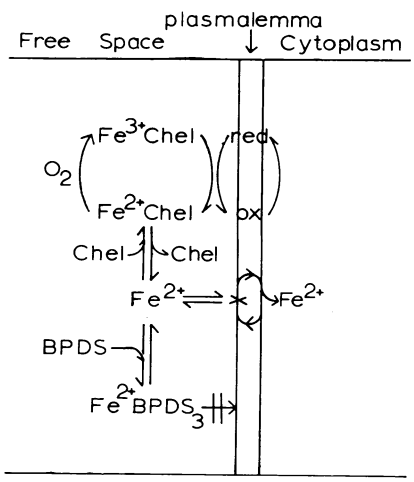

FIG. 3. Model for Fe uptake by plants.

EDDHA (weakest Fe^{2+} competitor) was least toxic and CDTA (strongest Fe^{2+} competitor) was most toxic. The rate of reduction, the ability of the plant to compete for Fe^{2+}, and the extent of the Fe-stress response could explain differences they observed among species.

The exchange of Fe from Fe^{3+}- and Fe^{2+}-chelates has been generally unappreciated in studies of Fe uptake from chelates, although equilibria have been considered (22, 23). Recently, Bates *et al.* (3) described the exchange of Fe from nitrilotriacetate, citrate, or EDTA to transferrin. Exchange from FeEDTA was first order and slow. The effect of pH on exchangeability of Fe^{2+}- and Fe^{3+}-chelates has not been studied as extensively as it has been for many divalent cations (26), but it is clear that ferroin-type Fe^{2+}-chelates exchange slowly while EDTA-type Fe^{2+}-chelates exchange very rapidly. Fe^{3+}-chelates of the EDTA-type exchange relatively slowly, and the rate of exchange is much slower as pH increases. Our preliminary study of exchange of Fe^{3+} from FeEDDHA to CDTA at varied pH shows half-times of exchange of 20 min at pH 3, 16 days at pH 5, 1 year (extrapolated) at pH 6, and too slow to measure at pH 7 and 8. Thus, exchange of Fe^{3+} from FeEDDHA is kinetically rather than thermodynamically controlled at nutrient solution and soil pH. The uptake of Fe from FeEDDHA at pH 6 or above therefore implies use of a mechanism for Fe release from the chelate other than simple exchange.

Most higher plants grow well on Fe supplied as FeEDDHA at pH 6 or above (22). Loneragen *et al.* (24) grew many plant species with a circulating nutrient solution containing 3 μM FeEDDHA regulated at pH 5.7. Further, the alga *Chlamydomonas mundana* grows well in the presence of chelator excess at pH 6 and above (25). Hutner's medium contains a 10-fold excess of EDTA over Fe (16). Fe-inefficient mutants of *E. coli* that fit our model of Fe^{3+}-chelate reduction have recently been observed (13). The experimental demonstration of a reductive-uptake mechanism in soybean, as well as the theoretical arguments based on valence state, stability, and exchangeability indicate to us that most lower and higher plants use a reductive mechanism in Fe uptake.

Model for Plant Fe Uptake. The scheme shown in Figure 3 summarizes our information on the mechanism of Fe uptake. Fe^{3+}chel is reduced at the root to Fe^{2+}chel. The Fe^{2+}chel can be oxidized (21), or can dissociate to free Fe^{2+} and chel. The chel diffuses into the nutrient solution and accumulates as Fe is removed from Fe^{3+}chel; chel can enter the root separately from Fe^{2+} entry, or chel can compete for Fe^{2+}. The free Fe^{2+} pool is in equilibrium with chel, any other competing Fe^{2+} chelator (as BPDS), and the root Fe^{2+} uptake system. We include the carrier system (X at outside of plasmalemma) because the root competes for Fe^{2+} much like a chelator, though no physical evidence has been obtained. Kannan (20) has indicated that a Fe^{2+} carrier exists in rice roots. $FeBPDS_3$ does not enter the root, but Fe^{2+} is available by dissociation and exchange in the absence of excess competing BPDS.

Translocation of Fe from the root involves Fe citrate; Fe^{3+} citrate is the only Fe-chelate normally found in stem exudate (30). An examination of Fe transport and citrate release to the stem exudate has recently shown citrate release can be Fe-dependent; one of the experiments demonstrated obligatory reduction of Fe^{3+}-chelates for Fe uptake by tomato (5).

The source of electrons for the reduction of Fe^{3+}-chelates is not clear. Large amounts of reducing compounds accumulate in the nutrient solution of Fe-stressed soybean. However, these compounds reduce Fe^{3+}-chelates very slowly. Other species do not release as much of these compounds to the nutrient solutions when under Fe-stress, even though they display a 200-fold Fe-stress response. We show a reducing system at the plasmalemma (red designates the reduced electron carrier; ox, the oxidized), since a cytochrome or flavin on the cell membrane might transfer electrons from inside the cell and thus reduce Fe^{3+}-chelates. No physical evidence supports this hypothesis. The reduction observed with soybean roots apparently occurs outside the epidermal or cortical cells because the product ($FeBPDS_3$) accumulates in the nutrient solution.

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