Improving Efficiency of Iron Uptake by Soybeans

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Introduction

In order to conveniently study the distribution, chemical form, and bioavailability of iron in plant foods, plants are intrinsically labeled with isotopes of the mineral. Usually this involves growing the plants hydroponically and introducing the label via the nutrient solution.

Iron is the most difficult nutrient to keep in solution in hydroponic systems. The solubility of iron is highly pH dependent. It is more soluble in acid solutions and precipitates with phosphates in alkaline solutions (7). Chelating agents combine with micronutrients such as iron to form soluble complexes or chelates. By increasing the solubility of iron, these chelates play an important role in transporting iron to the plant roots (5). Certain chelating agents are more effective than others. EDDHA (ethylenediamine di (o-hydroxyphenylacetate)) has been shown to be effective over a wide pH range (pH 4-9) (3) and promote iron uptake-translocation in iron-stressed soybeans (1). DTPA (diethylenetriaminepentaacetate) is effective over the pH range 4-7.8 (3).

Soybeans typically absorb $<10\%$ of available iron. Accumulation of iron by soybeans is less efficient than other trace minerals such as zinc. In an effort to discover optimal conditions for intrinsically labelling soybeans with ^{59}Fe, this study investigated the efficiency of incorporation of a single does of $^{59}FeCl_3$ into hydroponically grown soybeans under three different conditions: 1) nutrient solution containing DTPA, 2) nutrient solution containing EDDHA, and 3) root iron stripped with ferrozine prior to dosing.

Materials and Methods

Soybeans seeds [Glycine Max. (L) Merr. 'Century'] were germinated in vermiculite. After two weeks seedlings were transferred to 2 liter plastic pots containing a modified Hoagland-Arnon nutrient solution (4), measured pH 6.4. Iron was added as FeDTPA, sodium ferric diethylenetriaminepentaacetate (Sequestrene, Ciba Geigy Corp., Greensboro, N.C.). The nutrient solution was aerated continuously and replenished daily. There were six plants per pot and twelve plants per treatment. Plants were grown outdoors to the flowering stage at which time a single dose of 0.15 μCi of $^{59}FeCl_3$ was added to each pot. After two weeks of exposure to the $^{59}FeCl_3$, the roots were removed and discarded. The six plants from each pot were weighed together and assayed for ^{59}Fe in a whole body gamma counter.

Three treatments were initiated at flowering. Group I plants remained in the modified Hoagland-Arnon nutrient solution (Table I) throughout the entire study. This has been the usual procedure for our lab (7). The chelating agent was DTPA. Four days prior to receiving the $^{59}FeCl_3$ dose, group II plants were transferred to Chaney's nutrient solution (Table 1) which contained an excess of EDDHA and had a pH of 7.2. The plants remained in Chaney's nutrient solution for the duration of the study. Group III plant roots were placed in a solution of 250mM sodium dithionite, a reducing agent, and 1.5mM ferrozine, a strong Fe^{2+} chelator, one day prior to dosing to strip iron from the roots. Nitrogen gas was bubbled through the solution to keep it oxygen-free, thus preventing reoxidation of Fe^{2+}. After this treatment, the plants were transferred to Chaney's nutrient solution for the remainder of the study.

TABLE 1. Concentration of Individual Elements in Nutrient Solutions

Element	Modified Hoagland-Arnon Nutrient Solutions[1]	Chaney's Nutrient Solution[2]
	mM	mM
N	15	15
K	8.6	1.12
Ca	4	5
P	1	0.02
S	2	2
Mg	2	5
	μM	μM
Mn	9	2
B	46	10
Zn	0.77	1
Cu	0.32	0.4
Co	—	0.2
Mo	0.5	0.2
Fe	45	4
DPTA	45	
EDDHA		40
pH	6.4	7.2

[1]Ref. 4
[2]Ref. 2

Results and Discussion

Accumulation of ^{59}Fe by soybeans in the three treatment groups is shown in Table II. Plants in group I, exposed to DTPA, incorporated 4.6% of the ^{59}Fe dose into plant shoots. Plants in group II, exposed to a molar excess of EDDHA, incorporated 10.3% of the dose. This is more than twice as much as for group I. Stripping iron from the roots of the plants in Group III increased the uptake of ^{59}Fe to 13.0% of the dose when EDDHA was the chelating agent. The stripping process caused the plants to wilt and growth was stunted when compared to plants in groups I and II.

Until recently, the best model for iron uptake by soybean plants was proposed by Chaney et al (1) who found that iron must be in the reduced form (Fe^{2+}) to be

TABLE 2. Efficiency of ^{59}Fe Accumulation by Soybean Plants

Treatment	cpm ^{59}Fe	Total weight of plants (g)	% ^{59}Fe dose in plant shoot
Group I DTPA	12,112 ± 575	681	4.6 ± 0.2^a
Group II EDDHA	27,175 ± 4465	735	10.3 ± 1.7^b
Group III EDDHA, stripped roots	34,402 ± 11,744	459	13.0 ± 4.4^c

Different Superscripts denote significant difference at $P \leq 0.05$

absorbed. It has been known for several years that soybean roots are capable of reducing Fe^{3+}-chelates in their immediate vicinity. This reducing ability is greatly enhanced in iron deficiency.

According to Chaney et al (1), the Fe^{3+}-chelate is reduced to the Fe^{2+}-chelate at the root. The Fe^{2+}-chelate can be oxidized or can dissociate to free Fe^{2+} and free chelating agent. The free Fe^{2+} can be absorbed by the root, complex with the chelating agent, or complex with any competing chelating substance in the nutrient solution.

Evidence is accumulating that the reduction of Fe^{3+}-chelate is an enzymatic process that takes place at the plasmalemma of the epidermal cells on the root surface (6). This enzyme would be embedded in the plasmalemma and capable of transporting electrons across the membrane. Recently Sijmons and Bienfait (6) determined that cytosolic NADPH is the electron donor for extracellular Fe^{3+} reduction in iron deficient bean roots. They found that the supply of reduced pyridine nucleotides in lateral roots of iron deficient beans was greatly enhanced, and that the level of cytosolic NADPH was strongly lowered when iron deficient roots were exposed to extracellular iron salts. This indicates that electrons are transported from the cytosolic NADPH to the Fe^{3+} outside the cell via a transmembrance electron carrier (Figure 1). Sijmons and Bienfait concluded that one of the functions of trans-plasma transport systems in plant roots is the reduction of extracellular Fe^{3+}-chelates which is a necessary step in the uptake of iron by the roots.

There was an excess of EDDHA in the nutrient solution used in groups II and III. This excess chelating agent bound both the radioactive and non-radioactive forms of iron so that there was an equilibrium between the $^{59}Fe^{3+}$-chelate and Fe^{3+}-chelate complexes. Therefore, all iron-chelate in the nutrient solution was equally available

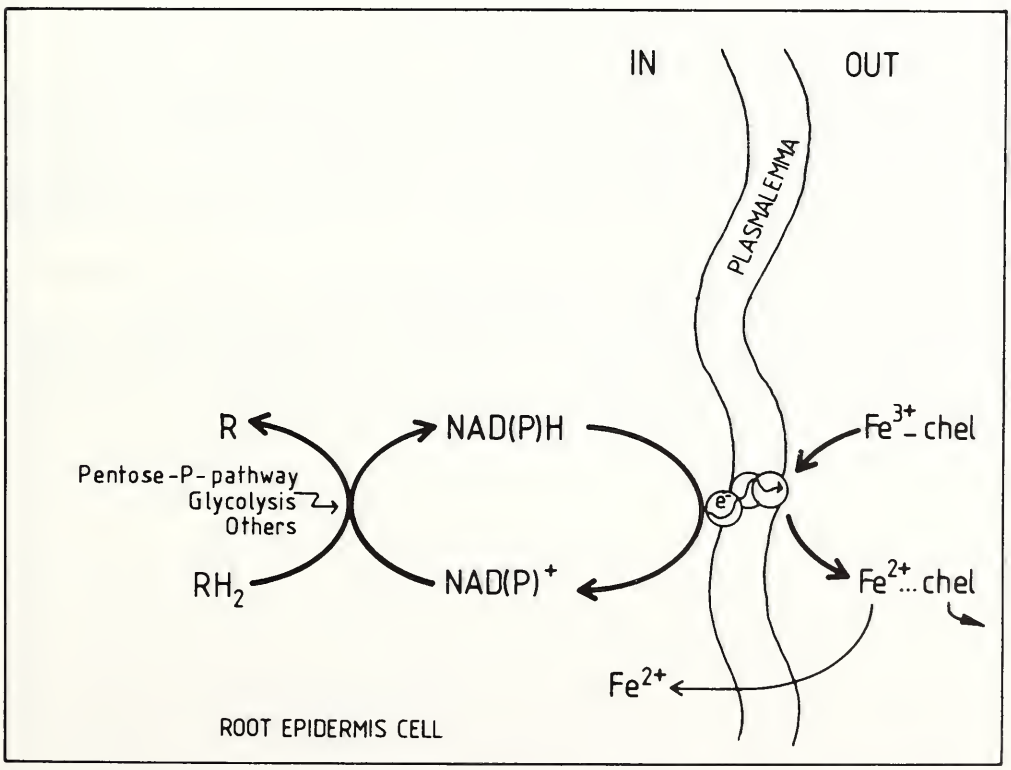

FIGURE 1. Diagram for Fe^{3+} reduction mechanism by root epidermis cells adapted from Sijmons and Bienfait (6).

for absorption. EDDHA is a more efficient chelating agent than DTPA because it binds Fe^{3+} very tightly which keeps it from precipitating out of solution and makes it more available for absorption. EDDHA has a greater affinity for Fe^{3+} and a lower affinity for Fe^{2+} than does DTPA. When the Fe^{3+}-chelate is reduced to Fe^{2+}-chelate at the root, EDDHA releases the iron very readily. Thus EDDHA delivers iron to the roots more efficiently than DTPA. Also, soybeans have been shown to adapt to excess chelating agent in nutrient solutions by increasing their ability to reduce and absorb iron (1). EDDHA was present in excess but DTPA was not. A third reason for increased efficiency of accumulation of Fe^{3+} by group II plants over group I plants could be the lower iron concentration of the nutrient solution.

The further increase in ^{59}Fe uptake by the plants in group III was due to the removal of non-radioactive iron from the immediate vicinity of the roots. There was less competition for absorption between the non-radioactive iron and the ^{59}Fe than in group II. Therefore, more of the ^{59}Fe was available for absorption. There was an increase in the uptake of ^{59}Fe, but not necessarily total iron. The drawback to this increased ^{59}Fe uptake is that the procedure used to strip iron from the roots caused the plants to wilt and their growth to be stunted.

The data presented here indicate that nutrient solutions containing EDDHA result in more efficient uptake of an iron label than those containing DTPA. Prior stripping of iron from roots is not recommended.

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