DIFFERENTIAL ABSORPTION OF METAL CHELATE COMPONENTS BY PLANT ROOTS [1]

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The use of synthetic metal chelators in bionutrition has become well known since the initial work of Schatz and Hutner (8) and Hutner, et al (3). Early investigators (1, 3, 8, 12,13) considered the chelating agent as a carrier which delivered metals to absorbing surfaces, but was not itself absorbed. This view was soon modified, however, when the uptake of the chelating agent or a decomposition product was demonstrated (2, 4, 6, 11) by use of C^{14}-tagged chelates. The increased concentrations of iron in plant materials (2, 4, 6, 11, 14), in addition to the presence of C^{14}, suggested the probability that metal chelate components were absorbed together. By use of N^{15}-tagged chelate, Wallace and co-workers (15) give data from citrus which indicate an equivalent uptake of iron and chelating agent. In later work (16), however, they reported cases in which high ratios of iron to chelating agent indicated that the uptake of these components may not be equivalent. The same lack of equivalence was shown by Krauss and Specht (4) in studies with green algae. Although some evidence may be adduced in favor of equivalent uptake, it is possible that considerable exchange may also take place at the root surface.

This paper reports the differences in the quantities of iron and of chelating agent absorbed from an iron chelate by plant roots. The results indicate an absorption mechanism which involves some absorption of the chelating agent, but a much greater absorption of iron which was released from the chelate at the root surface.

MATERIALS AND METHODS

The plants were: Eldorado No. 882 zinnia (*Zinnia elegans* Jacq.), PI-54619-5-1 soybean (*Glycine max* (L.) Merr.), and Greystripe sunflower No. F-4688-20 (*Helianthus annuus* L.). The seed sources were: zinnia, F. W. Bolgiano & Co., Washington, D.C.; soybean, the Plant Industry Station, Beltsville, Md.; and sunflower, Northrup King & Co., Berkeley, Calif.

The chelating agent was ethylenediamine di(*o*-hydroxyphenylacetic acid)—an aminopolycarboxylic acid. This chelating agent is a phenolic analog of EDTA (5). It has been called EDDHA, APCA, EHPG, and Chel-138. In this paper the term EDDHA will be used. When combined with ferric iron this compound gives a deep red color. The absorption spectra of FeEDDHA at six concentrations are shown in figure 1. The optical density plotted against concentration follows Beer's Law up to 10 ppm Fe.

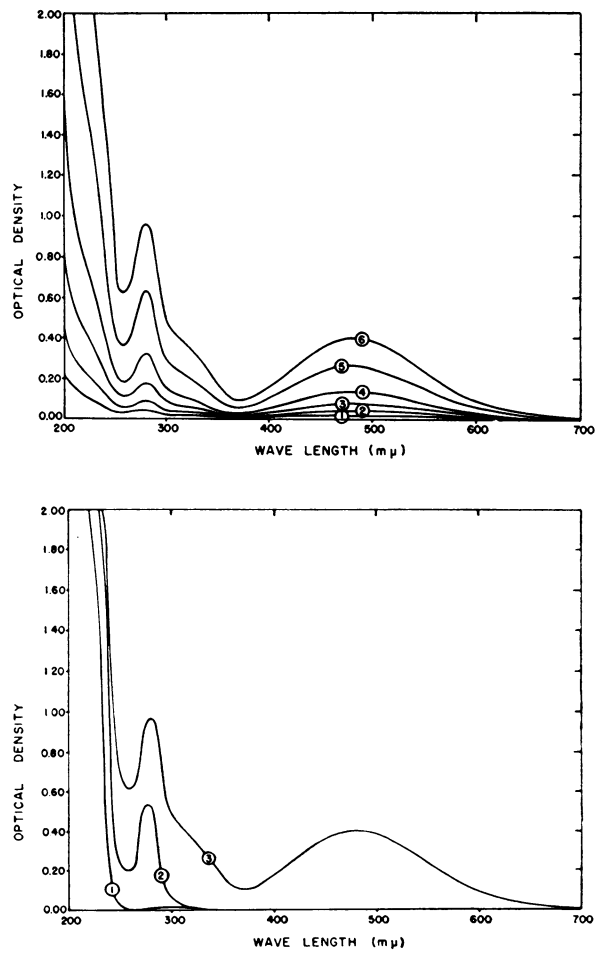

FIG. 1. Absorption spectra of FeEDDHA solutions. (Curves 1-6 correspond respectively to concentrations of 2, 4, 8, 16, 32, and 48 mg FeEDDHA per liter.)

FIG. 2. Absorption spectra of nutrient solutions. (Curves correspond to solutions as follows: 1. Nutrient solution alone, 2. Nutrient solution plus 50 mg EDDHA per liter, and 3. Nutrient solution plus 55 mg FeEDDHA per liter.)

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The base nutrient medium was an iron-free modified Steinberg solution (10) adjusted to pH 6.8. A liter of the nutrient medium contained 150 mg $Ca(NO_3)_2 \cdot 4H_2O$; 35 mg $Mg(NO_3)_2 \cdot 6H_2O$; 9 mg NH_4NO_3; 12 mg K_2SO_4; 30 mg KNO_3; 5 mg K_2HPO_4; 1.3 mg $(NH_4)_2HPO_4$; 0.234 mg $MnCl_2 \cdot 4H_2O$; 0.204 mg H_3BO_3; 0.088 mg $ZnSO_4 \cdot 7H_2O$; 0.02 mg $CuSO_4 \cdot 5H_2O$, and 0.012 mg $Na_2MoO_4 \cdot 2H_2O$. This solution showed little absorbance at 280 mμ and no absorbance at 480 mμ when measured in a Beckman DU spectrophotometer (see curve 1, fig 2).

The nutrient plus EDDHA absorbs strongly at 280 mμ, but not at 480 mμ (see curve 2, fig 2). The Na and K chelates of EDDHA were found to absorb at 280 mμ. The nutrient medium plus FeEDDHA and other metal chelates of this acid absorb strongly at 280 mμ, but only FeEDDHA gives a specific but weaker absorbance at 480 mμ (see curve 3, fig 2). Due to the interfering absorbance of various compounds at 280 mμ, 480 mμ was the most satisfactory wavelength for the determination of FeEDDHA. Preliminary investigations disclosed that colored exudates could be obtained from stems of certain plants when their roots were in FeEDDHA. Later experiments with these exudates indicated an absence of interfering absorption by other compounds at 480 mμ. Thus the determination of FeEDDHA in both nutrient and exudate was possible.

Seeds of the test plants were germinated between layers of moist muslin on stainless steel wire frames placed in Pyrex trays. The muslin was kept moist by extending it over the edges of the wire frames into water maintained at one-fourth inch below the seeds. The seeds and trays were covered with aluminum foil and kept in darkness at 75° F for 3 days. Seedlings with roots one-half inch long were transferred to aerated nutrient solution and grown in a constant temperature room at 75° F. Illumination was provided for 16 hours each day by banks of fluorescent and incandescent lamps at an intensity of 1,500 ft-c. After the soybeans and sunflowers had grown for 3 days and the zinnias for 10 days, selected seedlings were bound in groups and transferred to individual jars of nutrient medium for further growth periods (12 additional days for soybeans and sunflowers and 20 days for zinnias). The number of seedlings in each group was as follows: four zinnia; ten soybean, and ten sunflower. In one experiment 30 sunflower plants were used.

For collection of exudate the roots of the plants were washed and the groups were placed individually in 1 liter beakers of aerated nutrient containing varied treatments of EDDHA and FeEDDHA. The beakers were covered with plastic in order to hold the plant stems in place and to prevent excessive evaporation of the solution. The stems were cut off 10 cm above the roots, bent over, and the cut ends were pushed through a hole in the plastic covering of a 50 ml beaker from which exudate samples could be pipetted for analysis (fig 3). Calibrated centrifuge tubes were used for collecting fractions at time intervals. Collecting assemblies were covered with bell jars and kept in darkness at 75° F.

Three methods were used for analyses of exudate and nutrient medium. The optical density of FeEDDHA was measured by a Beckman DU spectrophotometer. Total Fe was determined on ashed material by the colorimetric *o*-phenanthroline method (7). The iron chelating capacity of exudate or nutrient was determined as follows: 2.9 μg $Fe^{55, 59}$ with a specific activity of 937 cps/μg was added to each ml of exudate or nutrient. After adding radioiron the samples were brought to pH 6, heated to 90° C, then set aside to equilibrate for 12 hours. Samples were then brought to pH 8 and heated to 90° C to precipitate non-chelated iron, after which they were filtered through No. 42 Whatman paper into tin planchets, brought slowly to dryness, and counted in a proportional counter. For the nutrient and exudate controls no more than 1.5 and 0.5 %, respectively, of the added activity passed through the filter paper. By this method it was possible to identify very small quantities of iron which were taken as an index of the chelating capacity of nutrients or exudates.

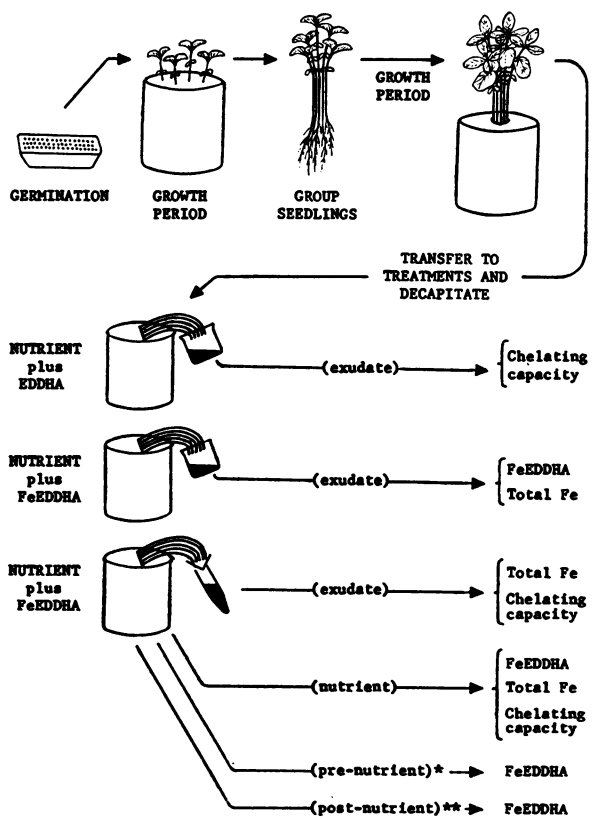

FIG. 3. Schematic representation of the procedure employed in treating and sampling plants and media to study the absorption of metal chelate components by plant roots.

TABLE I

EFFECT OF EDDHA IN NUTRIENT SOLUTION ON IRON-CHELATING CAPACITY OF EXUDATE FROM DECAPITATED ZINNIA, SUNFLOWER, AND SOYBEAN PLANTS

NUTRIENT SOLUTION TREATMENTS	CHELATING CAPACITY OF PLANT EXUDATES (IN TERMS OF $Fe^{55, 59}$ CHELATED)		
EDDHA PPM	ZINNIA PPM	SUNFLOWER PPM	SOYBEAN PPM
0	0.003	0.016	0.012
50	0.011	0.032	0.077
200	0.038	0.081	0.097
400	0.091	0.198	0.316
600	0.422	0.329	...

For determining chelating capacity of exudate, an aliquot was collected from groups of zinnia plants and groups of sunflower plants with roots in nutrient medium which contained 0, 50, 200, 400, and 600 mg EDDHA per liter. The same nutrient series was used in an experiment with soybeans except that the 600 ppm level was eliminated. Each of these experiments was repeated several times.

To determine in plant exudates the ratio of total iron to chelated iron, exudate was collected from additional groups of zinnia, soybean, and sunflower plants with roots in base nutrient containing 0, 55, 220, 440, and 660 mg FeEDDHA per liter.

In an experiment designed to give the time course of absorption, a base nutrient containing 55 mg FeEDDHA per liter was prepared and a 10 ml sample, hereafter designated "pre-nutrient," was set aside for later analysis. Thirty sunflower plants were then placed in 1 liter of the nutrient medium. After the pre-nutrient sample at zero time, a 5 ml sample of exudate and of nutrient was taken at 5, 10.5, 16.5, 23, 31.5, and 42.5 hours. Changes in FeEDDHA, total iron, and iron-chelating capacity of the nutrient solutions, and the concurrent changes in total iron and chelating capacity of the exudates were determined.

A final experiment was performed to determine whether the sunflower plants in the time-course experiment had absorbed iron and left EDDHA in the nutrient. The pre-nutrient sample taken at zero time and a sample of the nutrient after it had contained plants for 42.5 hours (hereafter designated "post-nutrient") were used for this purpose. The absorbance of these samples was first determined, after which excess Fe^{+++} was added to both the pre-nutrient and post-nutrient. Absorbance readings were then taken at time intervals to determine the relative rates and extent of the chelation of Fe^{+++} as FeEDDHA.

RESULTS

The effect of EDDHA in the iron-free nutrient medium on the iron chelating capacity of plant exudates is given in table I. For the exudates of all three plant species there is an upward trend in iron chelating capacity which corresponds to the increased levels of EDDHA in the nutrient medium. Adding radioiron to the exudates caused the appearance of a pale red color, indicating the presence of $Fe^{55, 59}$ EDDHA. The color reaction indicates that the chelate in the exudate was iron free previous to iron addition.

Data from experiments in which FeEDDHA was added to the nutrient medium (table II) show that plant exudates increase both in FeEDDHA and in total iron as the nutrient concentrations of FeEDDHA are increased. The iron concentrations (as FeEDDHA) in the exudates are very low when compared to total iron. This difference suggests the separation of significant quantities of iron from FeEDDHA at the root surface with iron-free EDDHA remaining in the nutrient.

Table III shows a decrease of FeEDDHA and total iron in the nutrient medium as affected by root absorption of decapitated sunflower plants. The loss of Fe (as FeEDDHA) was from 5.4 to 3.6 ppm, or 1.8 ppm. Iron chelating properties of the nutrient increased, however, showing at the end of the experiment that nutrient samples retained (against pH 8

TABLE II

CHELATED FE (FEEDDHA) AND TOTAL FE CONCENTRATIONS IN EXUDATE FROM DECAPITATED ZINNIA, SUNFLOWER, AND SOYBEAN PLANTS AS AFFECTED BY INCREASED LEVELS OF FEEDDHA IN NUTRIENT SOLUTION

NUTRIENT SOLUTION TREATMENTS	CHELATED FE AND TOTAL FE IN PLANT EXUDATES					
	ZINNIA		SUNFLOWER		SOYBEAN	
FEEDDHA PPM	FE (FEEDDHA) PPM*	FE (TOTAL) PPM	FE (FEEDDHA) PPM*	FE (TOTAL) PPM	FE (FEEDDHA) PPM*	FE (TOTAL) PPM
0	0.00	0.1	0.00	0.3	0.00	0.1
55	0.05	0.6	0.07	1.4	0.12	1.5
220	0.05	2.3	0.07	1.2	0.31	2.8
440	0.34	4.6	0.21	3.9	0.51	3.6
660	0.31	5.3	0.36	5.0	0.78	4.6

* Values have been adjusted to compensate for exudate optical density values: 0.0000 for zinnia; 0.0088 for sunflower; and 0.410 for soybean, obtained on the zero FeEDDHA nutrient treatment.

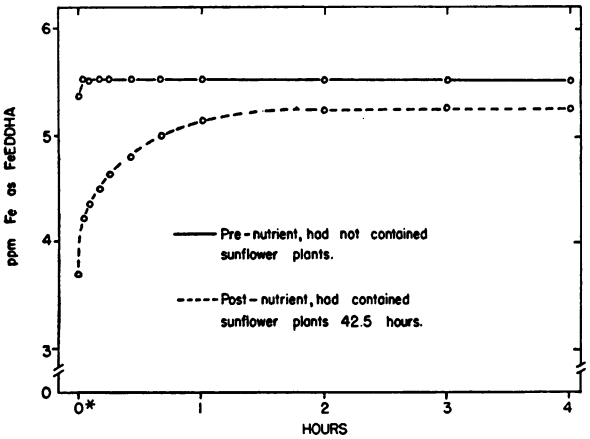

FIG. 4. Rate and extent of chelation of Fe, measured as FeEDDHA at 480 mμ, by a nutrient solution before and after containing decapitated sunflower plants.

and filtering) approximately 0.3 ppm $Fe^{55, 59}$. The exudate shows first a sharp rise, then a decline in total iron. Radioiron levels retained by exudate were low, indicating a low iron chelating capacity of the exudate. The progressive reduction of FeEDDHA in the nutrient medium (table III) might suggest the equivalent loss of both components of the chelate; but the concomitant increase in iron chelating capacity shows that iron-free EDDHA may have been left in the nutrient medium. Similar results were obtained in three confirmatory experiments.

To resolve this question, Fe was added to aliquots of the medium sampled at the beginning and at the end of 42.5 hours growth of sunflower plants. Figure 4 shows the restoration of the FeEDDHA complex after the addition of excess Fe to both solutions. This demonstrates the presence of considerable iron-free chelating agent in the medium.

DISCUSSION

The quantities of radioiron chelated by plant exudates were very low (table I) indicating the presence of only small amounts of chelating agent. However, there was even some radioiron held by the exudate from plants in zero EDDHA treatments. These values suggest the presence of natural chelators which are capable of holding iron soluble against high pH, heat, and filtering. The quantities of Fe held by natural chelators were deducted from the remaining exudate values for the respective plant exudates in order to determine radioiron chelated as FeEDDHA.

If calculations are made on a combining weight basis (55 g Fe to 360 g EDDHA = 1 : 6.5), the overall average for the 11 observations in table I indicates that 0.3 % of the EDDHA concentration in the nutrient medium can be found as iron chelate in the plant exudate. Broken down to individual species these observations range from 0.10 % to 0.45 %, with an average of 0.2 % for zinnia; 0.20 % to 0.33 %, with an average of 0.25 % for sunflower; and 0.27 % to 0.84 %, with an average of 0.53 % for soybean.

EDDHA entered roots from an iron deficient medium. It is therefore capable of entering roots in an iron-free form. This fact reinforces the possibility that this agent may enter roots as the chelate of various metals.

Small quantities of FeEDDHA were found in plant exudates. The iron chelate in the exudate suggests, but does not prove, that Fe penetrated the roots in this form. The quantities of chelated Fe are low when compared to total Fe. Calculations from table II show that on the average only 1 out of every 12 Fe atoms in the total plant exudate was in the form FeEDDHA. Expressed as a percentage of total Fe, the chelated Fe was 5.9 % for zinnia, 5.8 % for sunflower, and 12.5 % for soybean.

Large quantities of Fe were made available to the root by the metal chelate. In some cases the Fe concentration in the exudate was much higher than the corresponding concentration of Fe in the nutrient. Data from table III show that concentrations of Fe in the exudate range to nearly 20 times the Fe levels in the nutrient. On the average, the concentration of total Fe in the exudate is eight times greater than total Fe in the nutrient. The approximate total loss

TABLE III

CHANGES IN FE (FEEDDHA), TOTAL FE, AND CHELATING CAPACITY* OF NUTRIENT SOLUTION CONTAINING SUNFLOWER PLANTS AND CONCURRENT CHANGES IN TOTAL FE AND CHELATING CAPACITY OF PLANT EXUDATE

NUTRIENT AND EXUDATE SAMPLED AT: HRS	NUTRIENT SOLUTION			EXUDATE	
	FE (FEEDDHA) PPM	FE (TOTAL) PPM	FE CHELATING CAPACITY* PPM	FE (TOTAL) PPM	FE CHELATING CAPACITY* PPM
0	5.4	5.5	0.043	...	...
5	...	5.4	0.177	3	0.008
10.5	4.5	4.5	0.227	25	0.004
16.5	...	3.5	0.282	69	0.006
23.0	3.6	3.2	0.289	54	0.005
31.5	...	3.3	0.315	24	0.003
42.5	3.6	3.2	0.302	6	0.012

* In terms of $Fe^{55, 59}$ chelated

of Fe from the nutrient was 2 mg/l. One half of this amount was found in the 30 ml of exudate.

Comparison of Fe (FeEDDHA) in pre- and post-nutrient in figure 4 shows that pre-nutrient contained 5.4 ppm of chelated Fe before plants were placed in the nutrient. After 42.5 hours the sunflower plants had reduced this to 3.6 ppm of chelated Fe. This represents a loss of 1.8 mg of Fe from the liter of nutrient. If this quantity of iron had been accompanied by equivalent chelating agent, the nutrient should have lost 11 mg EDDHA. This was not the case. One hour after adding excess Fe^{+++} to the samples the complexing of iron free EDDHA remaining in the post-nutrient was nearly complete. Optical density readings at 3 and 4 hours were identical, showing equilibrium. The difference at equilibrium between chelated Fe values for the pre- and post-nutrient is 0.25 ppm Fe. On a combining weight basis (1:6.5), 1.6 ppm EDDHA would be required to chelate 0.25 ppm Fe. The quantity, 1.6 mg EDDHA, represents the loss of chelating agent from 1 liter of nutrient medium in 42.5 hours. This is a seventh the expected EDDHA loss if EDDHA had been absorbed with Fe in equivalent quantities.

The valence state and the form of Fe entering the root is unknown. If EDDHA entered in this case as FeEDDHA, then, on the average, the ratio of non-chelated Fe atoms entering sunflower roots to those entering as FeEDDHA would be approximately 6:1.

Experiments both with animals and plants are in general agreement with these results. Seeberg, et al (9) found that iron from iron chelates was absorbed from the gastrointestinal tract of anemic rats and utilized for hemoglobin regeneration at the same rate as iron from ferrous sulfate. Applied intravenously the iron was not readily available to the body. It would appear that iron is released from the chelate in rats by some mechanism in the gastrointestinal tract before being absorbed; in the blood stream the metal ions remain attached to the chelate. Krauss and Specht (4), studying the absorption of C^{14}-labeled FeEDTA by Scenedesmus cells, found that EDTA or some breakdown product was absorbed. However, their calculations showed insufficient EDTA present to account for the high iron levels. In a series of experiments, only enough EDTA to account for 1/50 to 1/15 of the iron was found in the Scenedesmus cells.

Wallace, et al (16) added $Fe^{59}N^{15}$ EDTA to nutrient cultures growing bush beans and found that the isotopes were fairly well distributed in most plant parts. Ratios of Fe^{59}/N^{15}EDTA indicated, however, that the uptake, translocation, and accumulation of iron and EDTA by plants may not have been equivalent. The ratio for the entire leaf was 1.41, for the stem 0.98, and for the entire plant 2.79. A very noticeable difference in the amounts of iron and chelating agent was observed in root analyses, where the ratio of Fe/EDTA was 6.13. This fact suggests that Fe was separated from the iron chelate at the root surface. A further study (16) with soybeans in sand culture containing FeEDDHA also revealed large amounts of Fe in the roots.

One of the most emphasized facts in literature on chelates has been the increase of iron in plant parts following iron chelate application. Although the generally accepted view has been that both components are absorbed together, it seems reasonable to assume that at least some of the many reported instances of high Fe levels in plants may be due to the separation of Fe from the chelate by the roots.

The uptake of non-equivalent quantities of chelate components by a few plants suggests the need for more extensive investigations among various plant families in order to determine how widespread this phenomenon may be. To expect all types of plants to respond alike in their interactions with factors relating to iron supply in a nutrient medium is not possible. Therefore, some plants may be expected to manifest only threshold responses to chelated Fe, whereas others may utilize chelated Fe very readily. And though conclusive demonstrations are not available at the present time, it may be that some plants will be found to absorb readily both components in equivalent quantities.

From these experiments it is concluded that zinnia, sunflower, and soybean plants do not absorb Fe and EDDHA in equivalent quantities, but that Fe is released to the roots, with most of the EDDHA remaining in the nutrient medium.

Summary

To study the absorption of metal chelate components, zinnia, sunflower, and soybean plants were grown in media containing ethylenediamine di(*o*-hydroxyphenylacetic acid), EDDHA, or the ferric chelate of this acid, FeEDDHA. Plants were decapitated and the exudates were collected for analyses of total iron, chelated iron, and chelating capacity.

Exudates from plants with roots in EDDHA contained small amounts of EDDHA. The average concentration was 0.3 % of that in the nutrient medium.

Exudate from plants with roots in FeEDDHA contained small amounts of FeEDDHA but relatively large amounts of total Fe. The average ratio of chelated Fe to total Fe was 1:12.

A time course experiment with sunflowers and FeEDDHA showed the progressive loss of Fe from the nutrient solution and the accumulation of Fe in the exudate. The FeEDDHA chelate delivered Fe to the root. As a result, the average total Fe in the exudate was eight times the average concentration of total Fe in the medium. With the loss of Fe from the nutrient, there was a seven fold increase in the iron chelating capacity of the nutrient. Analyses showed that the increase in chelating capacity was due to an increase in iron free EDDHA concomitant with Fe uptake by the roots. It is concluded, therefore, that these plant species selectively absorb Fe, the EDDHA remaining, for the most part, in the nutrient medium.

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