Plant Physiol. (1974) 54, 582–585

Iron Requirement and Iron Uptake from Various Iron Compounds by Different Plant Species

Received for publication December 11, 1973 and in revised form May 16, 1974

RUDOLF A. CHRIST
Ciba-Geigy Ltd., Agrochemicals Division, Basel, Switzerland

ABSTRACT

The Fe requirements of four monocotyledonous plant species (*Avena sativa* L., *Triticum aestivum* L., *Oryza sativa* L., *Zea mays* L.) and of three dicotyledonous species (*Lycopersicum esculentum* Mill., *Cucumis sativus* L., *Glycine maxima* (L.) Merr.) in hydroponic cultures were ascertained. Fe was given as NaFe-EDDHA chelate (Fe ethylenediamine di(O-hydroxyphenylacetate). I found that the monocotyledonous species required a substantially higher Fe concentration in the nutrient solution in order to attain optimum growth than did the dicotyledonous species. Analyses showed that the process of iron uptake was less efficient with the monocotyledonous species. When the results obtained by using chelated Fe were compared with those using ionic Fe, it was shown that the inefficient species were equally inefficient in utilizing Fe^{3+} ions. However, the differences between the efficient and the inefficient species disappeared when Fe^{2+} was used. This confirms the work of others who postulated that Fe^{3+} is reduced before uptake of chelated iron by the root. In addition, it was shown that reduction also takes place when Fe is used in ionic form. The efficiency of Fe uptake seems to depend on the efficiency of the root system of the particular plant species in reducing Fe^{3+}. The removal of Fe from the chelate complex after reduction to Fe^{2+} seems to present no difficulties to the various plant species.

Fe chlorosis is one of the symptoms most frequently found in cultures grown entirely in nutrient solutions. Many attempts have been made to explain the mechanism of iron uptake, but there remains some contradictory evidence. Although many facts have been established with respect to short term Fe uptake and Fe transport as measured with labeled Fe (5, 9–11, 17), particularly in soybeans, little information is available on the actual Fe requirement of various plant species in order to ensure their optimal growth. The experiments described were designed to evaluate the Fe requirement of four monocotyledonous and three dicotyledonous plant species. Since great differences in Fe requirement were found, I attempted to investigate which process in the course of Fe uptake could cause these great differences.

MATERIALS AND METHODS

Plant Material and Growth Conditions. The following monocotyledonous plant species were used: oat (*Avena sativa* L. cv. Flemingskrone), wheat (*Triticum aestivum* L. cv. Probus), rice (*Oryza sativa* L. cv. Ribe), and corn (*Zea mays* L. cv. Orla 234). Dicotyledons used were: tomato (*Lycopersicum esculentum* Mill. cv. Rheinlands Ruhm), cucumber (*Cucumis sativus* L. cv. Chinesische Schlange), and soybeans (*Glycine maxima* (L.) Merr. cv. Hardee).

The seeds were germinated on quartz sand for 5 to 10 days. Then the plantlets were transferred to Plexiglas holders and further developed in nutrient solutions. They were usually kept in all Fe-free nutrient solution until a weak Fe chlorosis developed (10–20 days according to plant species). Then the plants were transferred to the experimental nutrient solution. They were treated there for 14 days (rice for 21 days), and thereafter scores on chlorosis and fresh weight were determined.

The plants were kept in controlled-climate chambers under Hg high-pressure light of 15,000 to 20,000 lux (12 hr day), at a relative humidity of ± 80% and at a temperature of 20 C ± 1.

Nutrient Solutions. The basic nutrient solution was made up according to Hewitt (15): only the Fe content was varied. In order to ensure that the Fe content was according to design, irrespective of the pH of the nutrient solution, the most stable Fe chelate (NaFe-EDDHA[1] or Sequestrene 138 Fe) was used throughout these test series. The Fe concentrations tested ranged from 0.025 to 12.8 μg/ml of Fe, each concentration being double the previous one. Analyses confirmed that the correct amount of Fe remained in solution.

In the course of the investigation I desired to compare chelate-bound Fe with Fe in the ionic form.

A new method (13) permitted a fair comparison between the stable chelate and $FeSO_4$ and $FeCl_3$. Plants were grown alternately in solutions with and without Fe, in order to avoid interference between Fe and other compounds of the nutrient solution (3 days with Fe + $Ca(NO_3)_2$ and 4 days of full nutrient solution, without Fe). In this way the addition of reducing agents together with Fe were also tested without difficulty.

Analyses. Fe analyses were carried out by atomic absorption spectrophotometry. The plant material was extracted beforehand with 6 M HCl.

Scores. In all experiments fresh weights of plant shoots were determined at the end of the experiment. Chlorosis symptoms were scored according to the following scale: 1 = yellow with necrosis; 2 = yellow with some green; 3 = green with some yellow; 4 = normal appearance (completely green). It proved useful to score intermediate symptoms with half scores.

Statistical Analysis. Fresh weight was taken per plant holder (3 to 7 plants according to plant species). Treatments were replicated six times (*i.e.*, with six holders) and an analysis of variance was carried out followed by the Student-Newman-Keuls' test of significance at the $P = 0.05$ level.

[1] Abbreviations: NaFe-EDDHA: Fe chelate of ethylenediamine di(o-hydroxyphenylacetic acid); NaFe-DTPA: Fe chelate of diethylenetriaminepentaacetic acid.

RESULTS

In Figure 1, scores of chlorosis are shown plotted against the Fe concentration in the nutrient solution. In the top part of the figure the effect on monocotyledonous plants is drawn, in the bottom part the effect on the dicotyledonous species. It can be seen that oat and wheat become completely green only when 3.2 μg/ml of Fe were given. Corn needed 6.4 μg/ml and rice as much as 12.8 μg/ml of Fe. These are quite large Fe quantities as compared with those required by tomatoes (0.4 μg/ml Fe), cucumbers, and soybeans (0.8 μg/ml of Fe). The difference between rice and tomato is as high as a factor of 30.

The fresh weights of shoots show a similar dependence on the Fe concentration in the nutrient solution (Fig. 2). Corn and rice reached their optimum only with 12.8 μg/ml of Fe, whereas all the dicotyledonous plants attained their best growth with only 0.2 to 0.4 μg/ml of Fe (factor 60). It is surprising to find such a difference in Fe requirement between the various species.

The question arises whether the monocotyledonous plants need a higher Fe content in the nutrient solution or within the plant than the dicotyledonous species, or whether they are just less efficient in taking up Fe from a particular concentration of Fe in the nutrient solution. Analyses of the plant shoots after these treatments showed that the Fe contents of all the monocotyledonous species when grown in a solution containing 1.6 μg/ml of Fe were appreciably lower than the ones of the dicotyledonous species. At the Fe concentration of 12.8 μg/ml only corn showed a somewhat lower Fe content: oat and wheat were similar to the dicotyledonous species. I concluded that the plant species which require high concentrations in the nutrient solution are less effective in taking up Fe. Various authors reported such differences in efficiency on different soy-

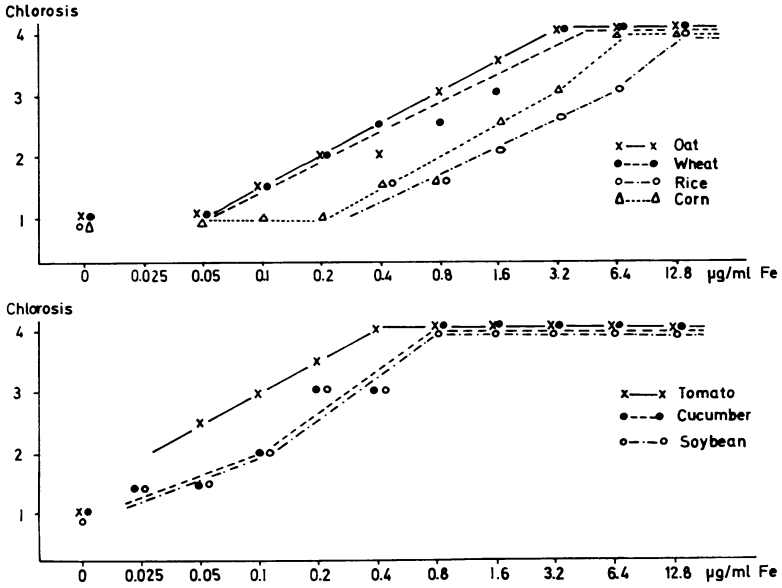

FIG. 1. Chlorosis scores of various plant species plotted against the Fe concentration in the nutrient solution (given as NaFe-ED DHA).

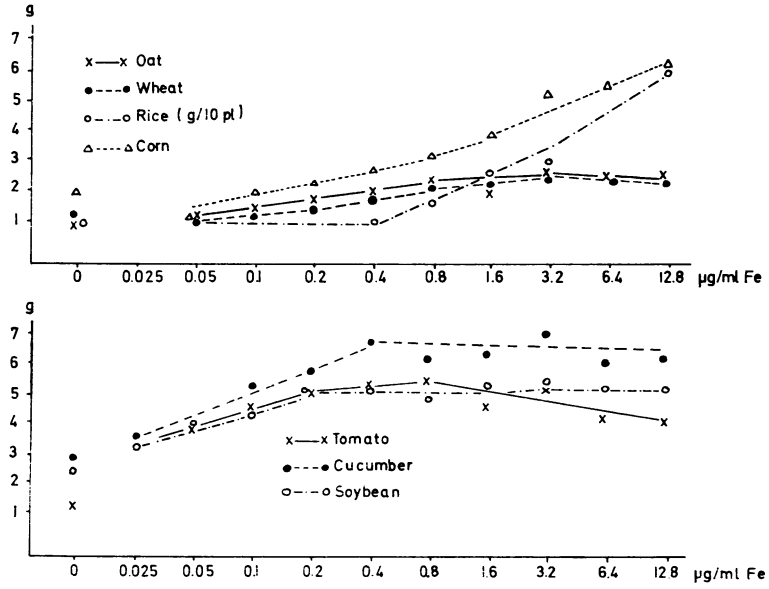

FIG. 2. Fresh weights of plant shoots after two weeks cultivation in nutrient solutions containing various concentrations of Fe as NaFe-EDDHA. Values are g per single plant, except for rice where g per 10 plants are given.

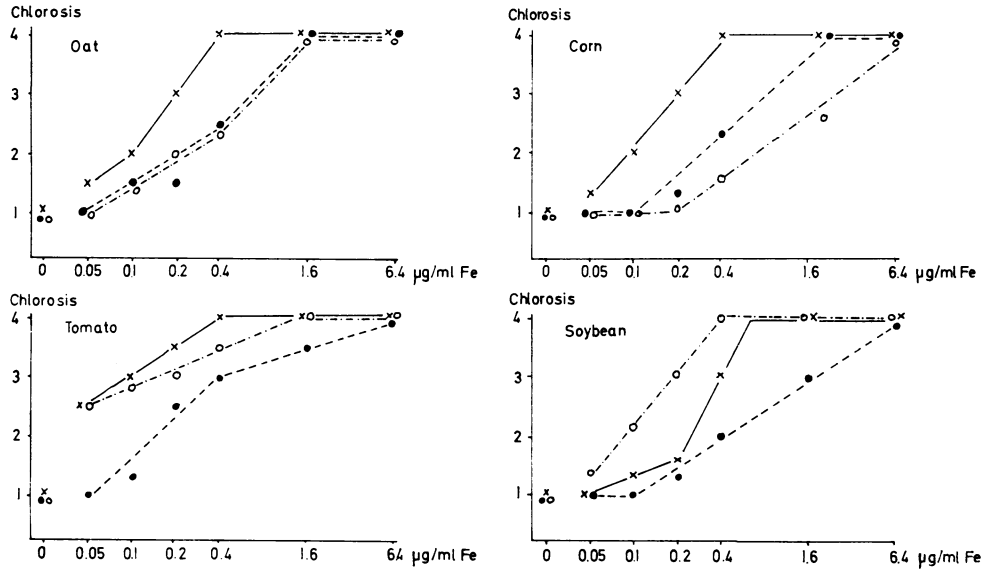

FIG. 3. Chlorosis scores of four plant species after treatment in alternating nutrient solutions with increasing concentrations of three different Fe compounds. ×——×: $FeSO_4$; ○- - -○: $FeCl_3$; ○-·-·○: NaFe-EDDHA.

bean cultivars when Fe uptake was measured with labeled Fe chelates (1, 3, 6, 14). Brown *et al.* (10) found that *Sorghum vulgare* var. Wheatland milo was less efficient in taking up Fe from the chelate NaFe-DTPA than were soybean plants. Figures 1 and 2 prove that other plant species, too, differ in the efficiency of this uptake.

Since Fe was given in its most stable form, as the chelate NaFe-EDDHA, it was of interest to investigate whether this variation in efficiency was mainly a result of differences in the removal of Fe from the very stable chelate, or of the Fe uptake in general. A comparison of Fe chelate with Fe in the ionic form was attempted, using some of the plant species. Corn and oat were chosen as relatively inefficient plant species and soybean and tomato as efficient plants. The method of alternating solutions, as described earlier (13), was used. Such a direct comparison between Fe in the chelated form and Fe in ionic form has never been attempted before because Fe in the ionic form is not stable in a nutrient solution containing a phosphate concentration which supports normal growth. Fe was given as the EDDHA-chelate as $FeSO_4$ and as $FeCl_3$.

Figure 3 shows the effect of the three Fe compounds on chlorosis symptoms with the four chosen test plants. The effect on fresh weight was similar. Again corn, when given NaFe-EDDHA, required a high Fe concentration (6.4 µg/ml of Fe) to achieve a completely green color, whereas soybeans required only 0.2 µg/ml of Fe. Fe^{3+} chloride was usually fairly close to Sequestrene 138 Fe, but $FeSO_4$ was more effective in curing Fe chlorosis than the other two Fe compounds, particularly when used with oat and corn. In fact, all four plant species turned completely green at the 0.4 µg/ml Fe concentration. The differences between monocotyledonous and dicotyledonous plant species were no longer apparent when $FeSO_4$ was used.

Additional experiments proved that the anion (SO_4^{2-} *versus* Cl^-) was of no importance. Therefore the valency of Fe was entirely responsible for the efficiency of Fe uptake.

This conclusion was confirmed in another way. By using the same culture method of alternating nutrient solutions, plants were grown with Fe^{3+} and Fe^{2+} supplied in the ionic form with and without the addition of a reducing agent (10 µg/ml of hydroquinone) together with the Fe supply. Table I shows the results of this experiment in terms of the shoot fresh weights.

The results show that the addition of hydroquinone had no effect when given with Fe^{2+} but it increased the fresh weights significantly ($P = 0.05$) of three out of four test plants when they were supplied with Fe^{3+}. This increase was such that the fresh weights were statistically identical with the ones obtained using Fe^{2+}. Only tomatoes, which were very effective in using Fe^{3+} (Fig. 1), did not show a response to the addition of hydroquinone.

Table I. *Shoot Fresh Weights after 2-week Treatment with Alternating Solutions Containing Fe^{2+} or Fe^{3+} with and without the Addition of Hydroquinone*

	Hydroquinone	Oat	Corn	Soybeans	Tomatoes
		0.2 µg/ml of Fe	0.3 µg/ml of Fe	0.1 µg/ml of Fe	0.1 µg/ml of Fe
	µg/ml	g fresh wt/plant shoot			
$FeCl_2$	0	2.86 a[1]	6.36 a	3.1 a	2.31 a
$FeCl_2$	10	2.84 a	5.21 a b	3.09 a	1.92 a
$FeCl_3$	0	2.23 b	4.36 b	2.15 b	1.78 a
$FeCl_3$	10	2.62 a	6.97 a	2.96 a	1.58 a

[1] Differences between figures in the same column not followed by the same letter are statistically significant ($P = 0.05$).

DISCUSSION

The data show that the Fe requirement of various plant species can differ appreciably. These differences are not due to various quantities of Fe being necessary for optimal growth, but are caused by the plants being more or less efficient in the Fe uptake.

Various papers (2, 4, 6, 7, 12, 16) have postulated that the reduction of Fe^{3+} to Fe^{2+} was essential in the uptake of Fe from Fe chelates and was important for the process of removing the Fe from the chelate complex. But in these earlier publications chelated Fe (NaFe-EDDHA) was always used, and it was not known whether or not the removal of the Fe from the chelate complex was contributing to the difference in efficiency of the various plants.

In this paper I applied a new method which allowed the com-

parison between Fe in the ionic form and Fe chelates. The data show that the reduction of Fe^{3+} to Fe^{2+} also took place when Fe was given in the ionic form and that the reduction to Fe^{2+} was indeed the important process which was carried out more or less effectively by the various species. As soon as Fe^{2+} was supplied in the ionic form the efficiency differences disappeared, *i.e.*, all plant species became fully green at the same low Fe concentration. Fe^{3+} in the ionic form was required in considerably higher concentrations by the inefficient species. I was tempted to speculate that Fe^{3+} ions cannot be admitted through the plasmalemma into the symplast because of the $^{3+}$ charge.

I did not determine how the removal of Fe from other chelate complexes could affect the Fe uptake. Soybean and tomato were more effective in taking up Fe from the EDDHA chelate than from Fe^{3+} in the ionic form (Fig. 3). This is surprising since removal of Fe from a complex should be more difficult than from ionic Fe. There is no explanation for this effect yet. It seems, however, that in the case of the EDDHA chelate, removal does not cause any additional difficulties for the plant. Chaney *et al.* (12) showed that reduction takes place within the Fe complex. Therefore the plant has to remove the Fe^{2+} from the chelate. In most chelates the stability of the Fe^{2+} complex is considerably lower than of the Fe^{3+} complex. This is particularly so with the EDDHA chelate, where the stability of the Fe^{2+} complex is reduced to a low value. Investigations with other chelates will have to show in what way the removal of Fe^{2+} from the complex affects the Fe uptake and whether plant species are more or less effective in this respect too.

No generalization about the relative efficiency of monocotyledonous species and dicotyledonous species can be made from the evidence of the few plants examined. More species will have to be investigated before the general conclusion can be drawn that monocotyledonous species are less efficient in reducing Fe^{3+} than dicotyledonous species.

Acknowledgment—Thanks are due to Mrs. C. Barmettler for carrying out all the practical experiments with extraordinary skill.

LITERATURE CITED

1. Ambler, J. E. and J. C. Brown. 1972. Iron-stress response in mixed and monocultures of soybean cultivars. Plant Physiol. 50: 675–678.
2. Ambler, J. E., J. C. Brown, and H. G. Gauch. 1971. Sites of iron reduction in soybean plants. Agron. J. 63: 95–97.
3. Bhan, K. C., A. Wallace, and E. J. Krohn. 1962. Effect of pH and nitrogen source on the ability of corn and soybean to obtain iron chelated with EDDHA. Agron. J. 54: 119–121.
4. Brown, J. C. 1972. Competition between phosphate and the plant for Fe from Fe^{2+} Ferrozine. Agron. J. 64: 240–43.
5. Brown, J. C. and R. L. Chaney. 1971. Effect of iron on the transport of citrate into the xylem of soybeans and tomatoes. Plant Physiol. 47: 836–840.
6. Brown, J. C., R. S. Holmes, and L. O. Tiffin. 1961. Iron chlorosis in soybeans as related to the genotype of rootstalk: 3. Chlorosis susceptibility and reductive capacity at the root. Soil Sci. 91: 127–132.
7. Brown, J. C. and W. E. Jones. 1962. Absorption of Fe, Mn, Zn, Ca, Rb, and Phosphate ions by soybean roots that differ in their reductive capacity. Soil Sci. 94: 173–179.
8. Brown, J. C., L. O. Tiffin, and R. S. Holmes. 1960. Competition between chelating agents and roots as a factor affecting absorption of iron and other ions by plant species. Plant Physiol. 35: 878–886.
9. Brown, J. C., L. O. Tiffin, A. W. Specht, and J. W. Resnicky. 1961. Iron absorption by roots as affected by plant species and concentration of chelating agent. Agron. J. 53: 81–85.
10. Brown, J. C., L. O. Tiffinn, A. W. Specht, and J. W. Resnicky. 1961. Stability and concentration of metal chelates as factors in iron chlorosis of plants. Agron. J. 53: 85–90.
11. Brown, J. C., C. R. Weber, and B. E. Caldwell. 1967. Efficient and inefficient use of iron by two soybean genotypes and their isolines. Agron. J. 59: 459–462.
12. Chaney, R. L., J. C. Brown, and L. O. Tiffin. 1972. Obligatory reduction of ferric chelates in iron uptake by soybeans. Plant Physiol. 50: 208–213.
13. Christ, R. A. 1974. A method to compare the effect of ionic iron with iron chelates in nutrient solution cultures. Plant Physiol. 54: 579–581.
14. Emlstrom, G. W. and F. D. Howard. 1969. Iron accumulation, root peroxidase activity, and varietal interactions in soybean genotypes that differ in iron nutrition. Plant Physiol. 44: 1108–1114.
15. Hewitt, E. J. 1966. Sand and water culture methods used in the study of plant nutrition. Tech. Commun. No. 22. Communication Bureau of Horticulture and Plantation Crops, East Malling.
16. Hoefner, W. 1972. Aufnahme und Sorption von zwei- und dreiwertigem Eisen durch Sonnenblumenpflanzen (*Helianthus annuus* L.) Z. Pflanzenernährung 131: 130–138.
17. Tiffin, L. O. 1970. Translocation of iron citrate and phosphorus in xylem exudate of soybean. Plant Physiol. 45: 280–283.