Iron Uptake-Transport of Soybeans as Influenced by Other Cations

John C. Lingle\(^2\), Lee O. Tiffin, \& John C. Brown

Mineral Nutrition Laboratory for Pioneering Research, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland

Introduction

Of the many factors affecting the absorption of iron by plants, the interference of other metal ions appears quite important. Hewitt (8, 9), Nicholas (14, 15), and Millikan (12, 13) found nickel, cobalt, zinc, and copper to induce iron deficiencies in a number of annual crops. Nicholas found high concentrations of these interfering metals reduced the iron content of the leaves of affected plants. More recently, Brown and Tiffin found that heavy applications of zinc salts applied to a zinc-deficient soil induced iron deficiency in corn after lighter applications corrected zinc deficiency of corn and certain other crops (7). Reuther and Smith (16) showed that continued applications of copper salts and copper fungicides induced iron deficiencies in Florida citrus crops. Brown et al. (4) felt that a high copper-manganese to iron ratio caused iron chlorosis to develop in milo in certain calcareous western soils. Milo grew normally in similar soils where this ratio was lower.

High concentrations of Ca in the soil have been thought to be a major causative factor of Fe chlorosis (1, 20) but the effect of this ion in soils is difficult to separate from the effect of soil pH on Fe availability.

Recent reviews (1, 2, 20) of the research on Fe nutrition of plants point out that even though the interference of some metals with Fe absorption and metabolism are well known, little is known of the mechanism of these phenomena. The work of Hopkins et al. (10) and Somers and Shive (17) indicated that Mn appeared to oxidize ferrous Fe to the more insoluble ferric form. Millikan (12) found that Mo would to some extent overcome the toxic effects of heavy metals on flax, but did not suggest how it did this.

Brown et al. (3, 6) have found that roots of chlorotic Hawkeye soybean (Glycine max) develop the capacity to reduce and absorb ferrous Fe more rapidly than roots of green plants. Furthermore, Tiffin (18) has determined that most of the Fe is translocated to the top in the form of Fe malate or malonate. It was thought that an interfering ion (Mn, Cu, Zn, Ca) may take the place of Fe in the malic or malonic acid complexes and that this may be one mechanism whereby other metals could interfere with the translocation of Fe. The purpose of these experiments was to determine whether or not various cations interfered with rapid uptake of Fe by chlorotic soybean plants and, if so, to study the mechanism whereby this interference was accomplished.

Materials & Methods

Hawkeye soybean seeds (Glycine max. L.) were germinated and the plants grown in a light chamber in minus Fe nutrient solution according to the method outlined by Brown and Tiffin (5). The plants were grown in bundles of ten. The nutrient solution contained 55 ppm N (33 ppm NO\textsubscript{3}-N and 2 ppm NH\textsubscript{4}-N), 0.8 ppm P, 0.6 ppm S, 50 ppm Ca, 6 ppm Mg, 42 ppm K, 0.2 ppm Mn, 0.08 ppm Zn, 0.02 ppm Cu, 0.12 ppm B, and 0.02 ppm Mo. The chemically pure salts used included Ca(NO\textsubscript{3})\textsubscript{2} \cdot 4H\textsubscript{2}O, Mg (NO\textsubscript{3})\textsubscript{2} \cdot 6H\textsubscript{2}O, NH\textsubscript{4}NO\textsubscript{3}, (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, K\textsubscript{2}HPO\textsubscript{4}, K\textsubscript{2}SO\textsubscript{4}, MnCl\textsubscript{2} \cdot 2H\textsubscript{2}O, ZnSO\textsubscript{4} \cdot 7H\textsubscript{2}O, CuSO\textsubscript{4} \cdot 5H\textsubscript{2}O, H\textsubscript{3}BO\textsubscript{3}, and Na\textsubscript{2}MoO\textsubscript{4} \cdot 2H\textsubscript{2}O. When grown in this manner, the plants initially showed chlorosis on the 14th day of growth. They were very chlorotic by the 17th day and the third trifoliate leaf had just unfolded when the absorption experiments were usually initiated.

The uptake-transport of Fe was studied in both decapitated and intact plants. The concentration of Fe\textsuperscript{59} was determined in the stem exudate of decapitated plants after collecting it for 20 hours in small culture tubes bathed in ice water. For intact plants the absorption period was 10 hours. The Fe\textsuperscript{59} concentration in both tops and roots was determined by ashing a portion of the dried plant material in steel planchets for 4 hours at 550\textdegree C. The resultant ash was wet down with water, redried under infra-red lamps, and the activity counted in a gas flow counter. Radioassay of the nutrient solutions at the beginning and end of each experiment provided additional estimates of the Fe absorbed.

The basal absorption solution used for testing the interfering ions was half the concentration of that used for growing the plants prior to these tests with the following exceptions: When micronutrient ca-

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\(^2\) Participated in this work while on Postdoctoral Research Fellowship with Mineral Nutrition Laboratory. Present Address: Department of Vegetable Crops, University of California, Davis.
tions Mn, Zn, or Cu were the interfering cations the respective ion was omitted from the basal absorption solution to provide a control level; when macronutrient cations Ca, K + Rb, or Mg were the interfering cations, the concentration contained in the basal absorption solution provided a control level; when macronutrient cations Ca, K + Rb, or Mg were the interfering cations, the concentration contained in the basal absorption solution provided a control level of the respective ion. Iron, labeled with Fe⁵⁹, (specific activity ~ 180 μc/mg Fe) was supplied as ferric ethylenediamine di(o-hydroxyphenylacetate) (FeEDDHA) at 2 × 10⁻⁶ M or 1 × 10⁻⁵ M. Interfering ions were added as solutions of equimolar concentrations of Cl⁻ and SO₄²⁻ salts at concentrations ranging from 6.25 × 10⁻⁴ to 1.25 × 10⁻² M Ca, 1.36 × 10⁻⁴ to 1.9 × 10⁻² M Mg, 5.5 × 10⁻⁴ to 1.1 × 10⁻² M K + Rb, 0 to 6.4 × 10⁻⁵ M Mn, 0 to 5 × 10⁻⁵ M Zn, or 0 to 5 × 10⁻⁶ M Cu. Each liter of absorption nutrient was adjusted to pH 6.5 with NaOH or HCl prior to the start of the absorption period.

In some experiments, Mn⁵⁴ or Zn⁶⁵ were used to label the interfering Mn or Zn ions at the specific activity of 18 μc Mn⁵⁴/mg Mn and 15 μc Zn⁶⁵/mg Zn. This made it possible generally to follow the movement of these interfering ions in the plant and specifically locate them if they appeared as Mn or Zn malate or malonate in the stem exudate.

In order to confirm the presence of Fe containing compounds in the stem exudate as well as to

Fig. 1. The concentration of Fe in the stem exudate of chlorotic Hawkeye soybean plants as influenced by increasing concentrations of Mn, Zn, Cu, Ca, Mg, and K plus Rb in the nutrient solution. Absorption period 20 hours. Fe in the nutrient solution was 2 × 10⁻⁶ M FeEDDHA.
characterize the Mn and Zn compounds in the xylem stream, paper electrophoretic separation of 10 μl portions of the exudate from plants absorbing the respective isotopes was made as outlined by Tiffin and Brown (18). After drying, the papers were exposed to X-ray film for various periods of time depending on the radioactivity of the various exudates.

**Results & Discussion**

The effect of the various metals on Fe uptake-transport in the soybean is summarized in figures 1 and 2. All metals interfered with the uptake-transport of Fe at high concentrations in the nutrient solution, but the level at which this interference became effective depended upon the ion species involved. Figure 1A shows that Mn became effective as an interfering ion when the concentration of this element exceeded $2 \times 10^{-6} \text{M}$ or at concentrations approximately equimolar with the Fe. At lower concentrations Mn stimulated the absorption of Fe. Experiments using higher Fe concentrations in the nu-

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**Fig. 2.** The uptake of Fe (calculated on the basis of the amount of Fe$^{59}$ leaving the nutrient solution in 20 hr) and the exudate production by decapitated chlorotic Hawkeye soybean plants as influenced by increasing concentrations of Mn, Zn, Cu, Ca, Mg, or K plus Rb in the nutrient solution. Fe was added as $2 \times 10^{-6} \text{M FeEDDHA}$. Absorption period 20 hours.
trient solution showed this general equimolar relationship with Mn still to be true. Figure 1C shows that the interference of Cu with Fe uptake-transport took place over the range 1 \( \times 10^{-7} \) to 5 \( \times 10^{-6} \) m. At lower concentrations Cu stimulated Fe absorption. In contrast, Zn interfered strongly over the entire range of concentrations studied (fig 1B). An additional experiment was run in duplicate to check the interference at the lower concentrations of Zn. This experiment showed that Zn reduced the uptake-transport of Fe by 15% when it was present in the nutrient solution at levels as low as 5 \( \times 10^{-5} \) m (0.0032 ppm).

Figures 1D, E, and F show that Ca, K + Rb, and Mg must be present in much higher concentrations in the substrate to interfere with Fe uptake-transport to any great degree. Calcium concentrations had to exceed 5 \( \times 10^{-3} \) m before the concentration of Fe in the exudate was lower than the control. Potassium plus rubidium concentration did not interfere until their concentration exceeded 1 \( \times 10^{-3} \) m. Magnesium interfered when its concentration was higher than 2.5 \( \times 10^{-3} \) m. All of the interfering cations except Zn enhanced the Fe content of the exudate when supplied at low concentrations. Increases of 20 to 50% in the Fe content of the exudate over the control treatments were observed. This effect was observed in the case of Cu at concentrations as low as 5 \( \times 10^{-8} \) m in the nutrient solution, and the effect was still observed at 5 \( \times 10^{-7} \) m.

Viets (19) found that when multivalent ions were added to nutrient solutions at low concentrations they enhanced the absorption of monovalent ions. This has often been referred to as the Viets effect. In this study it was found that mono- as well as polyvalent ions can enhance the uptake of Fe when added at low concentrations. These ions must be exerting some common effect, the nature of which can only be explained by future research.

The question immediately arises, "Is the concentration of Fe in the stem exudate a true reflection of absorption by the roots?" Comparison of graphs in figure 2 with those in figure 1 will show a close agreement of the amount of Fe leaving the nutrient solution with the concentrations of Fe found in the exudate. The volume of exudate produced in 20 hours as influenced by the concentration of different cations in the absorption solution is also shown in figure 2.

It is apparent from these data that all elements do not depress Fe uptake-transport and exudate production in like manner. Of all the metals studied, Zn exerted the strongest effect on both Fe uptake and exudate production. Increases in the Ca or Mg concentration depressed exudate production to a greater extent than they depressed Fe concentration in that exudate. Kramer (11) in his discussion on active vs. passive water uptake by plants attaches considerable importance to stem exudation as being that portion of the water that is actively absorbed.

If this is true, then these data would indicate that increased concentrations of metallic ions in the substrate can act as metabolic inhibitors.

The small quantity of Zn required to reduce absorption-transportation of Fe and exudate production indicates that Zn specifically may be acting as a metabolic poison to some key reaction essential to Fe absorption. The action of Zn is much more severe than that of the other cations.

Mn\(^{54}\) and Zn\(^{65}\) Studies. These experiments were identical to previous Mn and Zn interference studies except that Mn\(^{54}\) and Zn\(^{65}\) were used instead of Fe\(^{59}\). Fe\(^{59}\) was used to label the Fe in the control (or minus Mn or Zn) treatments.

Table I shows that more Mn was absorbed from the nutrient solution than Fe, but that less Mn was found in the stem exudate than Fe. Radioactivity of the respective isotopes in the exudate was a maximum of 1,500 cps/ml for Mn\(^{54}\) but exceeded 20,000 cps/ml for Fe\(^{59}\). Table I also shows much the same comparative relationship for Zn\(^{65}\) and Fe\(^{59}\).

Table I

<table>
<thead>
<tr>
<th>Isotope &amp; conc in nutrient solution</th>
<th>Uptake of isotope* %</th>
<th>Uptake transported** Ec</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Fe(^{59})</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Mn(^{54})</td>
<td>74</td>
</tr>
<tr>
<td>1.5</td>
<td>Zn(^{65})</td>
<td>99</td>
</tr>
</tbody>
</table>

* Absorbed from absorption nutrient solution.
** Found in exudate.

Figure 3 shows radioautograms of electrophoretic separations of nutrient solutions of Zn and Mn and of exudate from plants receiving Fe\(^{59}\), Mn\(^{54}\), or Zn\(^{65}\) in the absorption nutrient solution. The Fe\(^{59}\) is located principally in the same spot as shown previously to be Fe malate (18). What little Mn\(^{54}\) appeared in the exudate moved toward the cathode in a discrete spot with the same mobility as the Mn\(^{54}\) added to the nutrient solution. This indicates that it was present in the exudate as the cation. Zn\(^{65}\) in the exudate also moved toward the cathode but appeared in three spots. One spot remained at the origin, one migrated to the same position as inorganic Zn\(^{65}\) in the nutrient solution, and one half-way between the other two. The Zn\(^{65}\) in the latter spot may be complexed in some organic compound. Neither Mn or Zn appeared to interfere by substitution for Fe in the malate complex.

From the foregoing data, one would immediately wonder if intact plants would react the same as desiccated plants. Since Zn was the strongest interfering ion, experiments were conducted to study the influence of Zn on the Fe uptake by intact plants. From previous experiments (table I) it was noted...
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Fig. 3. Radioautogram of paper electrophoretic separations of Fe\(^{65}\), Mn\(^{65}\), and Zn\(^{65}\) compounds in nutrient solutions and stem exudates of decapitated chlorotic Hawkeye soybean plants. Path 1, nutrient solution containing Fe\(^{65}\)EDDHA. Path 2, exudate from plants absorbing Fe\(^{65}\)EDDHA. Path 3, nutrient solution containing Zn\(^{65}\)SO\(_4\). Path 4, exudate from plants absorbing Zn\(^{65}\)SO\(_4\). Path 5, nutrient solution containing Mn\(^{64}\)Cl\(_2\). Path 6, exudate from plants absorbing Mn\(^{64}\)Cl\(_2\). Path 1 exposed 60 hours, path 2 exposed 24 hours, path 3 exposed 72 hours, path 4 exposed 45 days, path 5 exposed 72 hours, path 6 exposed 30 days. The shadow on path 6 was too weak to develop a satisfactory picture, and is outlined.

that even though Zn was readily absorbed by the roots, little was translocated to the tops in the xylem stream. Identical experiments with intact plants were, therefore, conducted, one using Fe\(^{65}\) and the other using Zn\(^{65}\) (table II). As shown previously in figure 2B, zinc depressed the uptake of Fe by the roots and even more strongly depressed its translocation to the tops. Data in table II are in agreement with data in figure 2B which show that Zn is readily absorbed by the roots but very little is translocated to the tops of the plants. These experiments were also conducted at higher levels of Fe (1 \(\times\) \(10^{-5}\) M) in the nutrient solutions. The effect of Zn at these higher Fe levels were very similar to those in table II, except that the reduction of the Fe content of the roots and tops by Zn was less drastic than at the lower Fe level.

The interference with Fe uptake by other cations appears to be dependent on both the cation and the crop species involved. Millikan (13) noted that Cu was the strongest interfering ion in his work with flax. Of several species tested, Brown and Tiffin (7) found that only corn developed Fe chlorosis with heavy Zn fertilization. Nicholas (14) noted differences in the Fe chlorotic symptoms of the tomato depending on whether Co, Ni, or Zn was the interfering ion, and that the tomato seemed most sensitive to Ni.

Though varying concentrations of micro- and macronutrient cations were not used to induce Fe deficiencies in the growth period, it is quite evident that such ions strongly interfere with the rapid absorption and translocation of Fe by Fe-deficient plants. It was pointed out previously that Fe-deficient Hawkeye soybean plants develop a greater capacity to reduce and absorb ferric Fe than green plants (6). In another experiment (unpublished) Zn strongly depressed the absorption of Fe but did not affect the reduction of ferric to ferrous Fe. It would appear likely that two separate reactions are involved in the absorption and the translocation of Fe in Hawkeye soybeans.

It seems probable that these cations are affecting some metabolic reaction or reactions induced in the Hawkeye soybean plant by a limited Fe supply and it may differ from the heavy metal toxicities reported by Hewitt (9), Nicholas (14), and Millikan (12, 13). This would then seem to indicate that many heavy metals can act as interfering ions but the relative effectiveness of each element will depend largely on the crop in question or the growth medium used.

Summary

Iron-deficient (chlorotic) Hawkeye soybean plants (Glycine max L.) develop a greater capacity to absorb Fe than iron-sufficient plants. The effect of the interfering ions Mn, Zn, Cu, Ca, Mg, and K + Rb on the uptake-transport of Fe by chlorotic plants was determined by measuring the disappearance of Fe\(^{65}\) from the nutrient solution, the concentration of Fe\(^{65}\) in the stem exudate of decapitated plants, and the distribution of Fe in intact plants.

With increasing concentrations of the interfering cations (except Zn) in the nutrient solution, Fe concentration in the stem exudate first increased and then decreased to very low levels. Instead of enhancing the absorption of Fe, small concentrations of Zn in the substrate depressed it over the entire range tested. Zn was the strongest interfering ion studied in decapitated plants and also interfered with the uptake-transport of Fe by intact plants. Zn not only reduced the uptake of Fe by the roots but, to a greater extent, interfered with the translocation of Fe to the tops.

Table II

Fe\(^{65}\) & Zn\(^{65}\) Concentrations in Chlorotic Hawkeye Soybean Plants as Influenced by Zinc Concentration in Nutrient Solution*

<table>
<thead>
<tr>
<th>ZnSO(_4) in nutrient solution</th>
<th>Isotope labeled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe(^{65})</td>
</tr>
<tr>
<td></td>
<td>cps/g dry wt</td>
</tr>
<tr>
<td>(M \times 10^{-6})</td>
<td>Roots</td>
</tr>
<tr>
<td>0</td>
<td>9,890</td>
</tr>
<tr>
<td>0.5</td>
<td>8,260</td>
</tr>
<tr>
<td>1.0</td>
<td>6,535</td>
</tr>
<tr>
<td>5.0</td>
<td>1,820</td>
</tr>
</tbody>
</table>

* Fe in nutrient solution was 2 \(\times\) \(10^{-6}\) M FeEDDHA. Absorption period, 10 hours.
With the exception of Ca and Mg, increasing concentrations of the interfering cations enhanced and then depressed the volume of stem exudate produced. Calcium and Mg decreased exudate production long before the Fe concentration in the exudate was depressed.

Radioautograms of electrophoretically separated components of stem exudate revealed that Mn\(^{14}\) and Zn\(^{65}\) did not substitute for Fe in the malate complex. Instead, these two isotopes move toward the cathode, either as free ions or organic compounds of similar mobility.

**Literature Cited**