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Absorption And Translocation Of Chelated Iron By Barley And Rice

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ABSORPTION AND TRANSLOCATION OF

CHELATED IRON BY BARLEY AND RICE

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THESIS

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ABSTRACT

A study was made of iron absorption and translocation in the Mimi variety of barley and the Saturn variety of rice. Various iron chelates were used as sources of iron in nutrient solutions. Radioactive iron was used in some experiments to investigate absorption and translocation of iron in both plant species.

Plants were grown in nutrient solutions with different levels of iron to compare the chlorophyll content, dry weight and iron content in shoots and roots. It was found that iron deficiency decreased chlorophyll content and iron content in tissue and also decreased growth of the shoots and roots. An iron chelate concentration of 10^{-6} M was found adequate to prevent iron deficiency. Higher iron concentrations did not increase growth or chlorophyll contents.

All of the iron chelates used were satisfactory iron sources for barley and rice; however, iron added as HEDTA or EDTA was found to be absorbed and translocated more readily by barley than iron supplied as more stable chelates.

Iron deficient barley plants transported more iron to the shoots when iron was restored than did normal plants. Once the iron requirement of the plant had been satisfied, less iron was absorbed by the plant.

Factors recognized as favoring iron translocation were a lower pH in their growth medium and for rice lower phosphorus levels in the medium. Even relatively high levels

of phosphorus in the nutrient solution did not inhibit translocation of iron in barley.

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INTRODUCTION AND REVIEW OF LITERATURE

Iron is essential for plant nutrition with roles as a constituent of enzymes in photosynthesis, the formation of chlorophyll and transformation of carbohydrates into energy. It is important in respiration and other oxidation systems of plants and is known to play a wide variety of physiological roles within the plant. The requirement for iron in plants is quantitatively intermediate between the macro and micronutrient elements. It has long been known that iron deficiency in green plants results in a reduced concentration of chlorophyll known as chlorosis and reduced plant growth. When chlorosis becomes severe, leaf tissue is devoid of chlorophyll and commonly dies.

Iron deficiency is perhaps the most difficult of all mineral deficiencies to correct. Iron chelates have the property of keeping iron available for plants and are excellent sources of iron for plant growth. Plants can absorb and assimilate iron from iron chelates and correct iron chlorosis. The primary role of the chelating agent is to maintain the iron in a water soluble form and available to the plant root.

Early History

Price (1970) has outlined the early history of iron nutrition. In 1844, Gris demonstrated that iron corrects chlorosis when he applied a solution of iron salts to chlorotic grape leaves and thus restored the green color to the leaves. That iron is an essential element for the growth

of higher plants was established by Sachs in 1860.

Various soil conditions may induce symptoms of iron deficiency. Allyn (1927) and Bennett (1945) have reported that lime-induced chlorosis is caused by the inability of the plant to obtain iron from a calcareous soil and the inactivation of the iron when absorbed by the plant. Ripple (1923) has shown that a high Mn level in water cultures will induce chlorosis and that this condition could be overcome by increasing the iron level of the solution. Willis and Piland (1936) found that copper affected the absorption and utilization of iron in corn and cotton. Linder and Harley (1944) suggested that there is competition between various organic compounds in the plant for the available iron. Schander (1945) related the formation of organic acids, especially citric acid, to the development of iron chlorosis.

The phosphate concentration of the culture solution also influences iron nutrition. Franco and Loomis (1947) found that iron chlorosis developed in plants grown in solutions containing relatively high concentrations of phosphate and suggested that iron is precipitated as iron phosphate in the leaf tissue.

Iron deficiency may result in changes in concentrations of other nutrients in plant tissue. Thorne and Wallace (1944) indicated that chlorotic leaves contain a higher percentage of potassium than calcium. The nitrogenous compounds have been found to be higher in chlorotic than in non-chlorotic plant material by Bennett (1945) and Buslova (1948).

Reuter and Crawford (1946) found the irrigation water applied to grapefruit resulted in development of iron chlorosis in the winter in plantings receiving 2 to 3 acre-inches of water per week in summer and 2 to 3 acre-inches of water every 2 or 3 weeks in winter. Trees receiving 2 to 3 acre-inches of water every 4 to 6 weeks in summer and every 6 to 8 weeks in winter developed normally.

That the transport of iron within the plant might be affected by the pH of the conducting tissue was suggested by Rogers and Shive (1932) when they found iron accumulations occurred in regions where the pH gradient was high. As the pH of a soil or nutrient solution rises above pH 6.5, iron becomes less available due to its precipitation (Bould, 1963).

Several investigations of the intercellular distribution of iron have been reported. Nock and Liebich (1945) reported that 82% of the total iron in spinach leaves is associated with the chloroplasts. They found that five-sixths of the firmly bound iron is absorbed on phosphorus-containing proteins, only traces of iron are in the nucleus and no iron is in the cell wall. Three fractions of the total chloroplastic iron in spinach leaves were differentiated: (1) H_2O-soluble iron, 8%; (2) iron extractable by 0.01 N HCl or 0.001 N KOH, 32%; (3) residual iron, 60%. Whatley, Ordin and Arnon (1951) concluded that chloroplasts represent about one-third of the dry weight in sugar beet leaves and contain 61% of the total iron. Hagen et al. (1952) suggested that there are three

factors governing the chloroplast's ability to absorb iron: (1) mass of the chloroplast, (2) concentration of iron, and (3) iron-binding capacity of the suspension medium. A change in any of these factors may influence the absorption of iron by the chloroplasts. Jacobson (1945) suggested that active iron was localized in the chloroplasts and that non-chloroplast iron could accumulate only when iron was no longer a limiting factor in chlorophyll formation.

Iljin (1951) demonstrated that iron deficiency in plants results in decreased dry weight, salt, and protein nitrogen content and increased content of amino acids and organic acids. Brown and Holmes (1955) observed that leaves of soybeans were susceptible to chlorosis with one source of iron but leaves which developed on the same plant when the plant's source of iron was changed were not chlorotic. Evans (1959) and DeKock et al. (1960) found a positive correlation between the iron content of the nutrient medium and the chlorophyll and heme content of leaves. The activities of enzymes containing heme prosthetic groups, including catalase, peroxidase and cytochrome oxidase were less in iron-deficient than in normal tissues.

In solution and sand culture work it is difficult to keep iron in solution when applied as an inorganic salt because the iron precipitates. In 1951 Jacobson found ferric potassium ethylenediaminetetracetate (EDTA) to maintain a satisfactory supply of available iron in a nutrient solution.

Stewart and Leonard (1952) were able to correct iron chlorosis in citrus trees grown on acidic sandy soil by adding FeEDTA to the soil. This early work has stimulated research on the practical importance of metal chelates for the treatment of iron and micro-nutrient deficiencies in agricultural crops in all parts of the world.

Chelating Agents

Many iron chelates are water-soluble forms of iron which may be used as sources of iron for plants growing in nutrient solution or soil. Brown (1969) states that in order to be useful in agriculture, an iron chelate should have the characteristics of: (1) adequate stability, (2) nontoxic, (3) iron not easily replaced by other metals, and (4) inexpensive.

Chelating agents may function as metal buffers (Stewart and Leonard, 1952):

$$\text{metal ion} + \text{chelating agent} \rightleftharpoons \text{metal chelate}$$

$$K = \frac{(\text{metal chelate})}{(\text{metal ion})(\text{chelating agent})}$$

The K value is commonly referred to as the stability constant of the chelate and will vary depending on the chelating agent, metal and other factors. The chelating agents which have been studied most extensively with regard to iron nutrition are ethylenediaminetetraacetic acid (EDTA), hydroxyethylenediamine di(o-hydroxyphenylacetic acid) (EDDHA), cyclohexane-trans 1,2-diaminotetraacetic acid (CDTA), hydroxyethyl-

ethylenediaminetriacetic acid (HEDTA), diethylenetriamine-pentaacetic acid (DTPA), and an aromatic aminopolycarboxylic acid (APCA). The relative order of decreasing stabilities (K) of these chelates at pH 7 is the following: CDTA > APCA > EDDHA > DTPA > HEDTA > EDTA (Brown, 1956 and 1969). The stability of the metal chelates may change as the pH of the nutrient medium is raised or lowered. Plant species are known to differ greatly in their capacity to absorb iron from a metal chelate and the effectiveness of metal chelates on specific soils is known to differ. In general, as the stability constant increases, iron becomes more resistant to precipitation, but it is also more difficult for roots to absorb the chelated iron.

Brown and Tiffin (1960) suggested that chelating agents may compete with the root for the iron in a nutrient solution and that roots themselves act much like chelating agents in the absorption of iron. Heck and Bailey (1950) first suggested that the metal chelates deliver iron to the roots or absorbing surfaces, but the chelating agent is not absorbed. Later research by Stewart and Leonard (1952) led to the view that both the iron and the chelating agent were absorbed by plants in approximately equivalent quantities; however, more recent studies suggest that normally relatively little of the chelate component is absorbed with the iron (Tiffin and Brown, 1959). Weinstein, Robbins, and Perkins (1954) suggested that iron may be made active through the presence

of a chelating agent within the roots. Other workers observed that iron chelates may be absorbed by the roots of plants and transported into the leaves. It is apparent that the use of organic chelating agents has opened a wide field for research in controlling and explaining the pathways of mineral metablism in plants.

Evidence has been obtained by Tiffin (1966a, b) that iron is translocated in the xylem stream as a chelate of citrate in soybean, sunflower, cucumber and tomato plants. Thus, iron is not precipitated in the xylem under conditions of pH and phosphate concentration that would result in precipitation of the iron ion. Preliminary evidence for a monocot, corn, suggests that citrate may play a similar role in iron translocation in this species as well (Brown and Ambler, 1970).

In the present study, five different iron chelates[1] were used to examine the correlation in the chlorophyll content in barley and rice plants. In some experiments the radio-isotope ^{59}Fe was added to the chelate to provide a sensitive measurement of the absorption and translocation of iron by roots and shoots. The purpose of this investigation was also to study the effect of pH and phosphorus concentration in the nutrient solution on iron uptake and translocation by barley and rice plants.

[1]The chelates investigated include a new experimental chelate 157 NaFe provided by Geigy Agricultural Chemicals, Ardsley, New York. This chelate contains 5.6% iron; however, other characteristics were not available.

MATERIALS AND METHODS

Germination of Barley and Rice Seedlings

Barley (*Hordeum vulgare*, variety Mimi) and rice (*Oryzopsis sativa*, variety Saturn) seeds were soaked in aerated redistilled water for 24 hours. The soaked seeds then were distributed on cheesecloth supported by a plastic frame. The plastic frame was placed in an enamel pan containing a nutrient solution consisting of 0.1 millimole per liter each of KH_2PO_4, $Ca(NO_3)_2$ and $MgSO_4$. The enamel pan was covered with a second enamel pan and the seedlings were grown in the dark at a temperature of about 25 C for 3 and 6 days for barley and rice respectively. The seedlings were allowed to grow in the enamel pan in the greenhouse under fluorescent lights for a few days before being transferred to the polyethylene containers.

Experiments on the Relation of Iron Concentration and Type of Iron Chelate to the Absorption of Iron by Barley and Rice

After germination and early growth in the dark, the seedlings were transferred to the greenhouse and then to the aerated stock solutions in polyethylene containers of an 8 liter capacity. The plants were supported by wrapping cotton about the plant base and inserting the plant in a hole in a 2 cm diameter cork. Three plants were placed in each six hole cork and four corks with plants were inserted into Masonite covers that had been painted with a white epoxy

paint. Twelve plants were supported in each polyethylene container which was wrapped with aluminum foil and placed in a Sherer-Gillett model CEL-37-14 growth chamber (Sherer-Gillett Company, Marshall, Michigan) which provided a 9 hour dark-15 hour light cycle. Ambient temperature varied from the daytime at 25 C to the nighttime at 20 C. The nutrient solutions were varied with respect to their phosphorus content, iron content, and pH. The basic nutrient medium had the following macronutrient composition in millimoles/liter: $Ca(NO_3)_2$, 4.0; KNO_3, 6.0; $MgSO_4$, 2.0; KH_2PO_4, 0.2 or 1.0. The micronutrient composition in micromoles/liter was: KCl, 70.4; $CuSO_4$, 0.32; $MnCl_2$, 0.35; H_3BO_3, 23.0; $ZnSO_4$, 0.76; H_2MoO_4, 0.14. The iron chelate supplied and its concentration was varied in the individual experiments.

In the first experiment barley which had grown 7 days after soaking and rice 14 days after soaking were transferred to the nutrient solution with five levels of iron: 0, 10^{-7}M, 10^{-6}M, 10^{-5}M, and 10^{-4}M. Iron was supplied as FeHEDTA. Harvests were made on the 28th day in barley and 35th day in rice. The fresh material was separated into roots and shoots and rinsed with redistilled water.

Samples for the chlorophyll determination were obtained from the 3rd and 4th leaf of two plants. The fresh leaves were weighed and homogenized in 50 ml of 80% acetone for three minutes using an Omni-Mixer (Ivan Sorvall, Inc.,

Norwalk, Conn.). After homogenizing the sample was extracted with additional 80% acetone, filtered and diluted to a final volume of 100 ml. The absorbence of the resulting solution was measured at 652 mμ with a Bausch and Lomb Spectronic 600 spectrophometer (Bausch and Lomb, Rochester, New York). Chlorophyll concentrations were calculated using the following equation (Bruinsma, 1965):

$$\text{Total chlorophyll (mg/g)} = \frac{27.8 \times D_{652} \times 0.1}{\text{fresh weight (g)}}$$

The remaining separated materials were placed in an oven at 55 C to dry. After the plant materials had dried for two days, they were removed and weighed to determine the yield as dry weight.

The dried plant materials were digested with 10 ml concentrated HNO_3 and 10 ml concentrated $HClO_4$ on an electric hot plate (Maier, 1958). After complete oxidation the samples were taken to dryness, redissolved in 3 N HCl and the volume adjusted to 50 ml with 0.1 N HCl. Iron was then determined colorimetrically using the ortho-phenanthroline method (Johnson and Ulrich, 1959). Ten ml of the 50 ml sample was transferred to a 25 ml volumetric flask, then 1.0 ml of 0.5% o-phenanthroline solution, 1.0 ml of freshly prepared 1% hydroquinone and 5.0 ml of 25% sodium citrate solution was added. The pH was adjusted to 3.5-4.0 with NH_4OH or HCl and the volume brought to 25 ml with redistilled water. The solutions were allowed to stand for one hour; then the

absorbence was determined at 580 mμ with the Bausch and Lomb Spectronic 600 spectrophometer. A standard curve was prepared using standard iron solutions containing 0, 5, 10, 20, 40, 80 and 100 μg of iron to relate optical density to the iron content of the sample.

In the second experiment, barley and rice seedlings were transferred to solutions containing FeEDTA, 157 NaFe, FeHEDTA, FeEDDHA or FeDTPA at a concentration of 10^{-6} M. Growth conditions and plant ages were the same as in the first experiment and the techniques for determining the chlorophyll and iron content and yield were similar to the first experiment.

Radioiron Experiments

The radioisotope ^{59}Fe was used as a tracer in the third and fourth experiments. This radioisotope emits a beta particle with an E_{max} of 1.10 (57%) or 1.29 (43%) Mev and a gamma ray of 0.46 (57%) or 0.27 (43%) Mev and has a half-life of 45 days. Plants were germinated and grown as in earlier experiments. On the 7th day after soaking the seeds for barley or the 14th day for rice, the seedlings were mounted in cork and transferred to the plastic containers which were filled with 8 liters of the nutrient solution previously described. The plants were placed in a growth chamber with the photoperiod and temperature conditions as used in earlier experiments. After six days of growth in this pretreatment nutrient solution, plants were transferred to ^{59}Fe containing solutions under the same growth chamber conditions.

To determine the effect of pretreatment concentration of iron chelate on absorption of ^{59}Fe, barley was grown with 0, 10^{-7} M, 10^{-6} M, and 10^{-5} M FeHEDTA levels in the pretreatment nutrient solution for six days. The plants were then transferred to 940 ml plastic containers containing the usual nutrient solution and ^{59}Fe HEDTA at 10^{-6} M. The labeled chelating agents were prepared by adding ^{59}Fe to the non-radioactive iron chelate to form ^{59}Fe-chelate which was then added to the experimental nutrient solution. In the radioiron experiments 4 to 5 μCi of ^{59}Fe was added to each container. Plants were harvested 24 hours after exposure to the ^{59}Fe-chelate began. The aerial parts and roots were separated and the roots were washed with distilled water. Shoots or roots from individual plants were placed in counting vials and counted with a Nuclear-Chicago Scaler Model 8775 and Well Scintillation Detector DS-202 (Nuclear Chicago Corp., Des Plaines, Illinois) to obtain 10,000 counts or for 1 minute if more than 10,000 counts/minute were detected. The μg values for labeled iron absorbed were calculated from these data based on counts made on aliquots of the ^{59}Fe-chelate stock solutions for each experiment.

Radioiron also was used in experiments with different chelates at 10^{-6} M and with varied phosphorus concentration and pH to determine the effect of these variables on absorption of ^{59}Fe. The same general procedure was followed as previously described for the ^{59}Fe experiment; however,

plants were grown without iron in the pretreatment nutrient solution then transferred to 900 ml plastic containers containing the usual nutrient solution and the ^{59}Fe labeled chelates. In the phosphate experiment KH_2PO_4 was added at 0, 0.1, 0.2, 0.5, 1.0, 2.0, and 4.0 millimoles/liter and pH was maintained at 4.7 to 5.6 in the nutrient solutions. In another experiment pH was adjusted to 3.6, 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0. The pH adjustments were made carefully with KOH and HCl initially and periodically through the 24 hour absorption period. The chelate ^{59}FeEDDHA was used in the phosphate and pH investigations because of its large stability constant and reported stability between pH 3.6 and pH 9.0 (Geigy, 19__).

Statistical Analysis of Results

Data were analyzed by two and three way analysis of variance with replication and regression (Sokal and Rohlf, 1969, Woolf, 1968). The regression equation ($Y = a + bX$) was calculated. The Student-Newman-Keuls Test was also used to show significant differences among treatments.

RESULTS

Effect of Iron Concentration in the Nutrient Solution on Yield, Chlorophyll Content and Total Iron Content in Shoot and Root

In this experiment the effect of various iron concentrations as FeHEDTA on the yield, the chlorophyll content and on total iron content in the shoot and root was examined. In Table 1 for barley and Table 2 for rice, the data show that without iron, growth was restricted and a severe chlorosis developed. Addition of as little as 10^{-7} M iron resulted in appreciable increase in yield, in chlorophyll content and in total iron content of the shoot and root, and chlorosis was no longer acute.

Chlorosis completely disappeared from barley when the iron concentration in the nutrient solution was increased to 10^{-6} M or higher, but yield, chlorophyll content and total iron content in the tissue did not increase within this range. At the highest iron concentration supplied (10^{-4} M) the yield of barley shoots and roots was depressed.

With rice an iron concentration of 10^{-6} M or higher eliminated chlorosis at a phosphate concentration of 0.2×10^{-3} M. At the higher phosphate concentration, 1×10^{-3} M, maximum chlorophyll content was obtained at an iron concentration of 10^{-5} M. The chlorophyll content of the rice tissue was lower at a phosphate concentration of 1×10^{-3} M than at 0.2×10^{-3} M at all concentrations of iron added.

TABLE 1. Chlorophyll content, total iron content and dry weight of barley grown at different concentrations of FeHEDTA and two phosphate concentrations.

Fe concentration	Phosphate concentration	Chlorophyll content	Dry weight[1] Shoot	Root	Total iron content Shoot	Root
M	M	mg/g fresh weight	g	g	µg	µg
0	1×10^{-3}	0.263	1.39	0.725	5.6	1.2
10^{-7}	1×10^{-3}	0.522	3.13	1.72	8.6	5.6
10^{-6}	1×10^{-3}	1.751	7.46	2.87	20.0	12.0
10^{-5}	1×10^{-3}	1.745	7.34	2.75	12.0	16.5
10^{-4}	1×10^{-3}	1.710	4.46	1.729	29.3	64.25
0	0.2×10^{-3}	0.420	1.60	0.94	1.25	5.2
10^{-7}	0.2×10^{-3}	0.902	3.84	2.02	2.50	7.35
10^{-6}	0.2×10^{-3}	1.756	7.03	2.87	26.50	15.8
10^{-5}	0.2×10^{-3}	1.660	6.32	2.57	30.5	35.2
10^{-4}	0.2×10^{-3}	1.932	4.40	1.58	34.40	100.6

[1] The 3rd and 4th leaf were removed from two plants for the chlorophyll determination and the remaining shoot material of all 15 plants was used in the dry weight and total iron determination.

TABLE 2. Chlorophyll content, total iron content and dry weight of rice grown at different concentrations of FeHEDTA and two phosphate concentrations.

Fe concentration	Phosphate concentration	Chlorophyll content	Dry weight[1] Shoot	Dry weight[1] Root	Total iron content Shoot	Total iron content Root
M	M	mg/g fresh weight	g	g	µg	µg
0	1×10^{-3}	0	0.09	0.155	0.6	0.3
10^{-7}	1×10^{-3}	0.162	0.151	0.15	2.0	2.0
10^{-6}	1×10^{-3}	0.376	0.22	0.175	3.2	2.4
10^{-5}	1×10^{-3}	0.818	0.385	0.120	5.8	8.1
10^{-4}	1×10^{-3}	0.625	0.235	0.231	16.5	10.5
0	0.2×10^{-3}	0	0.09	0.198	2.5	1.5
10^{-7}	0.2×10^{-3}	0.613	0.29	0.19	3.4	2.5
10^{-6}	0.2×10^{-3}	1.029	0.50	0.219	4.9	4.8
10^{-5}	0.2×10^{-3}	0.994	0.38	0.13	6.0	10.5
10^{-4}	0.2×10^{-3}	1.038	0.463	0.18	13.0	17.8

[1]The 3rd and 4th leaf were removed from two plants for the chlorophyll determination and the remaining shoot material of all 15 plants was used in the dry weight and total iron determination.

As can be seen from the data in Tables 1 and 2, there is a direct relationship between the amounts of tissue iron and chlorophyll at the lower iron levels but the chlorophyll content does not increase with iron content at higher iron levels.

A comparison of the results from experiments with barley and rice indicated that the growth of barley at a higher phosphate level (1×10^{-3} M) is better than at lower level (0.2×10^{-3} M), but for rice, the opposite seems to be true. In general the nutrient solution used, while satisfactory for barley, was not favorable for rice. The growth and chlorophyll content of rice at both phosphate levels and at all concentrations of iron was considerably less than for barley.

Yield, Chlorophyll Content and Total Iron Content in Tissue Supplied With the Same Concentration (10^{-6} M) of Different Iron Chelates

In the following experiments, five different iron chelates were used in respective culture solutions and the concentration in each case was maintained at 10^{-6} M. Results from two identical experiments done with barley are included in Table 3 and the results for the rice experiment are presented in Table 4. Different chelating agents did not appear to be factors affecting yield, chlorophyll content, or total iron content in tissues examined; however, additional studies permitting a statistical analysis of the data would be required to substantiate this conclusion.

In Table 3, the data for barley clearly show the dry weight of shoots and total iron content were considerably

TABLE 3. Chlorophyll content, total iron content and dry weight of barley grown with different iron chelates at 10^{-6} M.

Chelate	Experiment	Phosphate concentration	Chlorophyll content	Dry weight[1] Shoot	Dry weight[1] Root	Total iron content Shoot	Total iron content Root
		M	mg/g fresh weight	g	g	µg	µg
FeEDTA	A	1×10^{-3}	1.24	4.62	2.11	10.3	6.0
	B	1×10^{-3}	0.86	4.02	1.92	17.5	9.4
157 NaFe	A	1×10^{-3}	1.24	4.82	1.89	14.3	9.3
	B	1×10^{-3}	0.86	5.16	2.26	25.5	12.1
FeHEDTA	A	1×10^{-3}	1.11	5.70	2.09	20.7	7.9
	B	1×10^{-3}	1.05	3.89	1.96	14.5	6.0
FeEDDHA	A	1×10^{-3}	1.26	4.27	1.42	12.8	5.4
	B.	1×10^{-3}	1.57	5.06	2.66	26.5	10.0
FeDTPA	A	1×10^{-3}	0.87	3.10	1.53	11.0	5.3
	B	1×10^{-3}	0.86	3.64	2.05	13.3	7.8
FeEDTA	A	0.2×10^{-3}	1.05	2.19	2.22	6.3	4.9
	B	0.2×10^{-3}	0.85	2.06	2.16	8.0	13.0
157 NaFe	A	0.2×10^{-3}	1.05	2.45	2.29	7.0	7.2
	B	0.2×10^{-3}	0.82	2.55	2.41	10.3	7.4
FeHEDTA	A	0.2×10^{-3}	1.39	2.56	2.44	13.0	7.9
	B	0.2×10^{-3}	1.03	2.36	2.20	10.0	4.8
FeEDDHA	A	0.2×10^{-3}	1.22	1.36	1.10	4.8	3.3
	B	0.2×10^{-3}	1.10	1.55	1.30	14.0	6.5
FeDTPA	A	0.2×10^{-3}	0.99	2.27	2.19	6.8	4.0
	B	0.2×10^{-3}	1.18	2.67	2.53	13.7	7.3

[1]The 3rd and 4th leaf were removed from two plants for the chlorophyll determination and the remaining shoot material of all 15 plants was used in the dry weight and total iron determination.

TABLE 4. Chlorophyll content, total iron content and dry weight of rice grown with different iron chelates at 10^{-6} M.

Chelate	Phosphate concentration	Chlorophyll content	Dry weight		Total iron content	
			Shoot[1]	Root	Shoot	Root
	M	mg/g fresh weight	g	g	µg	µg
FeEDTA	1×10^{-3}	0.52	0.28	0.10	1.5	0.9
157 NaFe	1×10^{-3}	0.74	0.23	0.13	1.5	1.5
FeHEDTA	1×10^{-3}	0.66	0.25	0.12	2.5	3.1
FeEDDHA	1×10^{-3}	0.62	0.19	0.13	2.5	3.2
FeDTPA	1×10^{-3}	0.67	0.23	0.11	2.0	1.5
FeEDTA	0.2×10^{-3}	0.68	0.42	0.16	3.4	4.8
157 NaFe	0.2×10^{-3}	0.89	0.40	0.20	3.2	5.0
FeHEDTA	0.2×10^{-3}	0.76	0.43	0.18	3.4	4.2
FeEDDHA	0.2×10^{-3}	0.47	0.26	0.11	4.0	3.2
FeDTPA	0.2×10^{-3}	0.56	0.45	0.17	2.5	2.9

[1] The 3rd and 4th leaf were removed from two plants for the chlorophyll determination and the remaining shoot material of all 15 plants was used in the dry weight and total iron determination.

higher in seedlings maintained at the higher phosphate level (1×10^{-3} M) than those maintained at the lower level (0.2×10^{-3} M). The dry weights of the roots do not fit this general pattern and the chlorophyll contents do not show differences related to the two phosphate concentrations supplied.

The results of an analogous experiment with rice shown in Table 4 differ sharply, showing that better growth of both the shoots and roots occurred at the lower phosphate level. This generalization encompasses data for dry weight and iron content of both shoots and roots.

Effect of Pretreatment at Different Concentrations of Iron on the Subsequent Absorption and Translocation of Labeled Iron

Experimental plants were grown at four concentrations (0, 10^{-7}, 10^{-6}, and 10^{-5} M FeHEDTA) in the pretreatment. After six days plants were transferred to 940 ml of nutrient solution containing ^{59}FeHEDTA at 10^{-6} M. The data for the absorption of the labeled iron are presented in Tables 5 and 6. The F value in the analysis of variance for plant parts has high significance, indicating that iron retention by the root and iron translocation to the shoot were quite different. In general, more iron is retained in the roots than is translocated to the shoots. The correlation coefficient of iron translocation and iron concentration in the pretreatment shows significance. In other words, different iron concentrations in the pretreatment have affected iron translocation

TABLE 5. The effect of different concentrations of iron in the pretreatment on absorption and translocation of iron by barley supplied with ^{59}FeHEDTA at 10^{-6} M. Iron absorbed from ^{59}FeHEDTA is reported in µg (N = 4).

	Concentration of iron in pretreatment			
Organ	0	10^{-7} M	10^{-6} M	10^{-5} M
Shoot	0.104±0.005a [1]	0.024±0.003b	0.002±0.006b	0.004±0.009b
Root	0.227±0.018a	0.749±0.051b	0.185±0.026a	0.358±0.04c

[1] Standard error and significant differences are indicated by the Student-Newman-Keuls Test. If the letters following the standard error differ for the same organ, then a significant difference exists between these treatments.

TABLE 6. Analysis of variance for iron absorption based upon plant parts and the different concentration of iron in the pre-treatment with barley.

Source of variation	Degree of freedom	Sums of squares	Mean squares	F value
Plant part (A)	1	0.96261	0.96261	387.23218 (D.F.=1, 24)
Iron level (B)	3	0.38177	0.12726	51.19174 (D.F.=3, 24)
Interaction (A x B)	3	0.43957	0.14652	58.94289 (D.F.=3, 24)
Error (R)	24	0.05966	0.00249	
Total	31	1.84361		

$F_{0.01(1, 24)} = 7.82$ $F_{0.01(3, 24)} = 4.72$

into the shoot; the pretreatment without added iron resulted in the most iron being translocated into the shoot.

From the Student-Newman-Keuls Test the results in Table 5 show that significantly more iron was absorbed after a pretreatment of 10^{-7} M; however, plants having the minus iron pretreatment translocated the largest amount of iron to the shoot. While plants receiving the 10^{-5} and 10^{-6} M pretreatments absorbed appreciable quantities of iron, only about 1% of the absorbed iron was translocated to the shoot.

Absorption and Translocation of Labeled Iron from Different Chelates at the Same Concentration

Five different ^{59}Fe labeled chelates were used in these experiments at 10^{-6} M in the nutrient solution during a 24 hour absorption period. The iron absorption and translocation in rice plants did not depend upon the chelating agent supplied as indicated in Tables 7 and 8. Rice plants were supplied with 0.2×10^{-3} M phosphate which had been found most favorable for growth. Roots typically absorbed 0.3 to 0.5 µg of the labeled iron, most of which was retained in root tissues. Translocation seemed to be retarded and only 6-16% of the iron absorbed was translocated into the shoots of the rice plants.

In the case of barley, two levels of phosphate were used as designated in the results in Tables 9 and 10. The chelating agents were found to affect iron translocation and absorption differentially. The content of labeled iron in the root was at least slightly lower than that in the shoot

TABLE 7. The absorption and translocation of iron by rice when supplied with different chelates at 10^{-6} M. Iron absorbed from the labeled chelates is reported in μg (N = 5).

	Chelate				
Organ	FeEDTA	157 NaFe	FeHEDTA	FeEDDHA	FeDTPA
Shoot	0.052±0.001a*	0.039±0.003a	0.044±0.008a	0.041±0.007a	0.052±0.008a
Root	0.356±0.008a	0.404±0.017a	0.347±0.009a	0.524±0.018b	0.271±0.004a

[1]Standard error and significant differences are indicated by the Student-Newman-Keuls Test. If the letters following the standard error differ for the same organ, then a significant difference exists between these treatments.

TABLE 8. Analysis of variance for iron absorption based upon plant parts and different iron chelates with rice.

Source of variation	Degree of freedom	Sums of squares	Mean squares	F value
Plant part (A)	1	1.40381	1.40381	310.71582 (D.F.= 1, 40)
Chelate (B)	4	0.07944	0.01985	4.39556 (D.F.= 4, 40)
Interaction (A x B)	4	0.09549	0.02387	5.28383 (D.F.= 4, 40)
Error (R)	40	0.18072	0.00452	
Total	49	1.75945		

$F_{0.01\ (1,\ 40)} = 7.31$ $F_{0.01\ (4,\ 40)} = 3.83$

TABLE 9. The absorption and translocation of iron by barley supplied with different chelates at 10^{-6} M. Iron absorbed from the labeled chelates is reported in μg (N = 4).

Organ	Phosphate concentration M	Chelate FeEDTA	157 NaFe	FeHEDTA	FeEDDHA	FeDTPA
Shoot	1×10^{-3}	4.362±0.19a[1]	1.911±0.14b	2.514±0.16c	2.601±0.13c	1.837±0.17b
Root	1×10^{-3}	1.712±0.22a	1.512±0.11a	2.454±0.04b	1.426±0.05b	0.953±0.06b
Shoot	0.2×10^{-3}	1.444±0.06a	1.336±0.06a	2.519±0.26b	1.367±0.03a	0.949±0.06a
Root	0.2×10^{-3}	1.027±0.06a	1.297±0.07a	2.444±0.04b	0.602±0.02c	0.514±0.03c

[1]Standard error and significant differences are indicated by the Student-Newman-Keuls Test. If the letters following the standard error differ for the same organ, then a significant difference exists between these treatments.

TABLE 10. Analysis of variance for iron absorption based upon plant parts, phosphate level and different iron chelates with barley.

Source of variation	Degree of freedom	Sums of squares	Mean squares	F value
Plant part (A)	1	8.98268	8.98269	108.946 (D.F. = 1, 60)
Chelate (B)	4	21.14339	5.28585	64.109 (D.F. = 4, 60)
Interaction of A x B	4	5.08742	1.27186	15.425 (D.F. = 4, 60)
Phosphate level (C)	1	12.48906	12.48906	151.474 (D.F. = 1, 60)
Interaction of A x C	1	2.09984	2.09984	25.468 (D.F. = 1, 60)
Interaction of B x C	4	8.17942	2.04485	24.801 (D.F. = 4, 60)
Interaction of A x B x C	4	2.66801	0.66700	8.089 (D.F. = 4, 60)
Error (R)	60	4.94675	0.08245	
Total	79	65.59647		

$F_{0.01}$ (1, 60) = 7.08 $F_{0.01}$ (4, 60) = 3.65

at both phosphate levels for all the different chelate treatments. In Table 9, the data indicate that both absorption and translocation of iron were markedly increased at the higher phosphate concentration except when FeHEDTA was supplied. Phosphorus seems to be necessary for the mechanism functioning in both absorption and translocation of iron.

Effect of Phosphate Concentration on Iron Absorption and Translocation

For these experiments, the nutrient solution was the same as described previously and iron was added as ^{59}FeEDDHA at 10^{-6} M. In the overall series of experiments, phosphate was supplied at concentrations from 0 to 4.0 mM. The results are presented in Tables 11-18. From the analysis of variance (Table 12) it is seen that there are highly significant differences in labeled iron content between barley shoots and roots. In barley, more iron was translocated to the shoot than was retained in the root except at 2 mM, but the inverse is found with rice (Tables 11 and 15). The interactions between plant parts and phosphate levels are also quite significant (Tables 12 and 16) in both barley and rice. Data in Table 15 show that a high concentration of phosphate was especially inhibitory for iron absorption and translocation in rice.

In barley, the effect of phosphate upon the absorption of iron in the root was not significant, except at a level of 2 mM phosphate which appears especially low (Table 11),

TABLE 11. The effect of phosphate concentration on the absorption and translocation of iron by barley from ^{59}FeEDDHA at 10^{-6} M. Iron absorbed from the labeled chelates is reported in µg (N = 5).

	Phosphate concentration (mM)						
Organ	0	0.1	0.2	0.5	1	2	4
Shoot	0.895±0.12a[1]	0.635±0.06b	0.485±0.26c	0.506±0.05c	1.157±0.06d	0.128±0.03e	0.821±0. 7a
Root	0.491±0.02a	0.398±0.02a	0.417±0.04a	0.365±0.02a	0.485±0.03a	0.194±0.04b	0.336±0.02a

[1]Standard error and significant differences are indicated by the Student-Newman-Keuls Test. If the letters following the standard error differ for the same organ, then a significant difference exists between these treatments.

TABLE 12. Analysis of variance for iron absorption based upon plant parts and phosphate levels with barley.

Source of variation	Degree of freedom	Sums of squares	Mean squares	F value
Plant part (A)	1	1.32946	1.32946	68.60017 (D.F. = 1, 56)
Phosphate level (B)	6	2.66263	0.44377	22.89810 (D.F. = 1, 56)
Interaction (A x B)	6	0.96032	0.16005	8.25853 (D.F. = 6, 56)
Error (R)	56	1.08529	0.01938	
Total	69	6.03773		

$F_{0.01}$ (1, 40) = 7.31 $F_{0.01}$ (6, 40) = 3.29

TABLE 13. Analysis of variance for the regression of iron absorption on the phosphate levels with barley roots.

Source of variance	D.F.	Sums of squares	Mean squares	F value
Attributable to regression	1	0.059	0.059	5.557 (D.F. = 1, 33)
Deviation from regression	33	0.353	0.011	
Total	34	0.412		
Intercept = 0.42267			$F_{0.01}$ (1, 30) = 2.84	

TABLE 14. The effect of phosphate concentration on the absorption and translocation of iron by rice from ^{59}FeEDDHA at 10^{-6} M. Iron absorbed from the labeled chelates is reported in µg (N = 5).

	Phosphate concentration (mM)						
Organ	0	0.1	0.2	0.5	1	2	4
Shoot	0.05±0.009a*	0.096±0.021b	0.037±0.012ac	0.014±0.005cd	0.01±0.002d	0.005±0.008d	0.003±0.083d
Root	0.215±0.02a	0.217±0.02a	0.266±0.03b	0.227±0.012a	0.218±0.019a	0.113±0.007c	0.003±0.002d

Standard error and significant differences are indicated by the Student-Newman-Keuls Test. If the letters following the standard error differ for the same organ, then a significant difference exists between these treatments.

TABLE 15. Analysis of variance for iron absorption based upon plant parts and the phosphate levels with rice.

Source of variation	Degree of freedom	Sums of squares	Mean squares	F value
Plant part (A)	1	0.38628	0.38628	347.91821 (D.F. = 1, 56)
Phosphate level (B)	6	0.18275	0.03046	27.43382 (D.F. = 6, 56)
Interaction (A x B)	6	0.09490	0.01582	14.24568 (D.F. = 6, 56)
Error (R)	56	0.6218	0.00111	
Total	69	0.72611		

$F_{0.01\ (1,\ 40)} = 7.31$ $F_{0.01\ (6,\ 40)} = 3.29$

but the effect upon the translocation of iron to the barley shoot was significantly different. Total translocation is highest from a medium of 1 mM phosphate with a minimum occurring at 2 mM; however, iron absorption and translocation was not inhibited at the highest phosphate (4 mM) level. These results indicate that iron absorption and iron translocation in barley may be under different control mechanisms.

Effect of the Hydrogen Ion Concentration on Iron Absorption and Translocation

In this experiment, an attempt was made to minimize pH changes by frequent adjustment of pH in the nutrient solution during the 24 hour period of absorption of ^{59}FeEDDHA at 10^{-6} M. The results for rice (Tables 19-22) emphasized that the hydrogen ion concentration of a nutrient solution exerts an effect both on iron absorption and iron translocation. The Student-Newman-Keuls Test shows in Table 19 that iron translocation at the lower pHs (3.6 to 5) was significantly different from the higher pHs (6 to 9). The absorption of iron by the root is highest in the medium at pH 7 and translocation to the shoot is greatest at pH 3.6. Iron translocation decreased at pH 6 and above, while iron absorption increased from 3.6 to 7. At pH 8 and above absorption of iron was extremely low.

The effect of pH on absorption and translocation of iron by barley was apparent as shown in Tables 21 and 22. In barley, iron absorption from acid, neutral and alkaline

TABLE 16. The effect of pH on the absorption and translocation of iron by rice from ^{59}FeEDDHA at 10^{-6} M. Iron absorbed from the labeled chelate is reported in μg (N = 5).

	pH						
Organ	2.6	4	5	6	7	8	9
Shoot	0.026±0.003a [1]	0.021±0.002a	0.019±0.002a	0.002±0.0005b	0.0003±0.001b	0.0002±0.001b	0.0008±0.002b
Root	0.04±0.003a	0.028±0.003b	0.053±0.002c	0.08±0.008d	0.203±0.02e	0.009±0.0009f	0.014±0.001f

[1]Standard error and significant differences are indicated by the Student-Newman-Keuls Test. If the letters following the standard error differ for the same organ, then a significant difference exists between these treatments.

TABLE 17. Analysis of variance for iron absorption based upon plant parts and the pH for rice.

Source of variation	Degree of freedom	Sums of squares	Mean squares	F value
Plant part (A)	1	0.04550	0.04550	127.77344 (D.F. = 1, 56)
pH level (B)	6	0.06272	0.01045	29.35260 (D.F. = 6, 56)
Interaction (A x B)	6	0.07653	0.01275	35.81546 (D.F. = 6, 56)
Error (R)	56	0.01994	0.00036	
Total	69	0.20470		

$F_{0.01\ (1,\ 40)} = 7.31$ $F_{0.01\ (6,\ 40)} = 3.29$

TABLE 18. The effect of pH on the absorption and translocation of iron by barley from ^{59}FeEDDHA at 10^{-6} M. Iron absorbed from the labeled chelate is reported in µg (N = 5)

	pH						
Organ	3.6	4	5	6	7	8	9
Shoot	0.592±0.08a[1]	0.652±0.13a	0.902±0.06b	0.565±0.09a	0.042±0.006c	0.044±0.006c	0.061±0.02c
Root	0.480±0.07a	0.468±0.03a	0.530±0.07a	0.481±0.08a	0.176±0.003b	0.114±0.01c	0.110±0.006c

[1]Standard error and significant differences are indicated by the Student-Newman-Keuls Test. If the letters following the standard error differ for the same organ, then a significant difference exists between these treatments.

TABLE 19. Analysis of variance for iron absorption based upon plant parts and the pH for barley.

Source of variation	Degree of freedom	Sums of squares	Mean squares	F value
Plant part (A)	1	0.16417	0.16417	16.37466 (D.F. = 1, 56)
pH level (B)	6	4.78352	0.79725	79.51872 (D.F. = 6, 56)
Interaction (A x B)	6	0.61160	0.10193	10.16695 (D.F. = 6, 56)
Error (R)	56	0.56146	0.01003	
Total	69	6.12075		

$F_{0.01}$ (1, 40) = 7.31 $F_{0.01}$ (6, 40) = 3.29

TABLE 20. Analysis of variance for the regression of iron translocation on the pH with rice shoots.

Source of variance	D.F.	Sums of squares	Mean squares	F value
Attributable to regression	1	0.003	0.003	87.715 (D.F.=1,33)
Deviation from regression	33	0.001	0.000	
Total	34	0.005		

Intercept = 0.04216 $F_{0.01}(1, 33) = 2.84$

TABLE 21. Analysis of variance for the regression of iron absorption on pH with rice roots.

Source of variance	D.F.	Sums of squares	Mean squares	F value
Attributable to regression	1	0.000	0.000	0.017
Deviation from regression	33	0.154	0.005	
Total	34	0.154		

Intercept = 0.05639 $F_{0.01\ (1,\ 30)} = 2.84$

TABLE 22. Analysis of variance for the regression of iron absorption on the pH with barley roots.

Source of variance	D.F.	Sums of squares	Mean squares	F value
Attributable to regression	1	0.882	0.882	68.654 (D.F. = 1, 33)
Deviation from regression	33	0.424	0.013	
Total	34	1.306		

Intercept = 0.85169

$F_{0.01\ (1,\ 30)} = 2.84$

TABLE 23. Analysis of variance for the regression of iron translocation on the pH with barley shoots.

Source of variance	D.F.	Sums of squares	Mean squares	F value
Attributable to regression	1	3.095	3.095	65.689 (D.F. = 1, 33)
Deviation from regression	33	1.555	0.047	
Total	34	4.650		

Intercept = 1.39757

$F_{0.01\ (1,\ 30)} = 2.84$

solutions was significantly decreased as pH increased. The statistical analysis (Table 23) shows that more iron was in shoots than in roots when iron was readily absorbed (pH 3.6-6). As the pH of the nutrient solution increased from 3.6 to 5, barley roots and shoots showed increased iron content. A pH of 5.0 results in the maximum absorption and translocation of iron by barley. At pH 7, 8 and 9, iron absorption and translocation greatly decreased.

DISCUSSION

This investigation has shown that the dry weight, iron absorption, and chlorophyll content can be markedly influenced by the iron supply and that the capacity to absorb iron from iron chelates differs considerably between barley and rice. If iron was supplied at a low concentration, a good correlation was obtained between iron content and chlorophyll content in both barley and rice, but no positive correlation could be observed at higher levels of iron in the nutrient solution. Various investigators have presented evidence indicating that, less, the same, or even more iron is present in chlorotic than in green plant tissue, but it was found that under the conditions of this investigation chlorotic shoots contained less iron than green shoots.

Iron is a constituent of the heme group of catalase, cytochrome oxidase, and peroxidase and iron deficiency results in depressed synthesis of iron prophyrin enzymes (Agarwala and Sharma, 1961). Jacobson and Oertli (1956) proposed that lack of iron may inhibit formation of the chloroplasts through inhibition of protein synthesis although a direct effect of iron deficiency on chlorophyll synthesis has also been suggested. Possingham and Brown (1957) explained that growth stops in an iron-deficient medium as a result of an arrest in cell division due to a decrease in the free inorganic iron content of the tissue. The data in Tables 1 and 2 also showed that the dry weight of chlorotic plant

parts is much lower than that of green plant parts. It is inferred that the chlorotic leaves have a low photosynthetic rate due to the low levels of chlorophyll and iron requiring enzymes which are involved in photosynthesis. The absorption system in both barley and rice is exceedingly sensitive to very low concentrations of iron in the external solution; the addition of as little as 10^{-7} M iron remarkably increased chlorophyll content, dry weight and total iron content. The conclusion can be made that iron at a concentration of about 10^{-6} to 10^{-5} M is optimum for both barley and rice; however, an iron concentration of 10^{-4} M definitely depressed the growth of barley.

An attempt was made in the second experiment to determine the effect of different chelating agents on the absorption and translocation of iron. While some variation was observed in chlorophyll contents, iron contents and yield of barley and rice grown for 21 days with different iron chelates, all the chelates used appeared to serve as satisfactory sources of iron for plants growing in solution culture. The pH of the nutrient solution at a phosphate concentration of 1×10^{-3} M was 4.8-4.9 initially and after 21 days of growth of either barley or rice, pH increased to 6.4-6.6. The initial pH of the solution containing a phosphate concentration of 0.2×10^{-3} M was 5.4 to 5.7 and after 21 days of growth the pH increased to 6.9-7.1. The iron chelates of EDTA, HEDTA, DTPA and EDDHA are reported to be stable up to a pH of 8 or

higher. Thus, all of the chelates used with the possible exception of 157 NaFe would be stable in the pH range of the nutrient solutions (Brown, 1969).

The short term experiments with the ^{59}Fe chelates provided a more sensitive measure of the absorption of iron from the different chelates. When barley was grown at a phosphate concentration of 1×10^{-3} M, the following relationships for iron absorption and translocation from the various labeled chelates were observed:

Shoot: EDTA > EDDHA, HEDTA > 157 NaFe, DTPA

Root: HEDTA > EDTA, 157 NaFe, EDDHA, DTPA.

At a phosphate concentration of 0.2×10^{-3} M, the following relationships existed:

Shoot: HEDTA > EDTA, EDDHA, 157 NaFe > DTPA

Root: HEDTA > 157 NaFe, EDTA > EDDHA, DTPA.

The following stability constants have been reported for the iron chelates used: FeEDTA, 25.1; FeHEDTA, 19.6; FeDTPA, 28.6; FeEDDHA, 33; and 157 NaFe, not reported (Geigy, 19__, Brown, 1969). The higher stability constants extend the stability range of the metal chelates to a higher pH range; FeEDDHA is stable in the alkaline range up to at least pH 10. The stability of the iron chelates may change as the pH of the nutrient solution is raised or lowered and the higher the stability constant, the more stable is the complex and the lesser the tendency for the complex to yield Fe ions. In the radioiron experiment with barley, iron was least readily absorbed from DTPA and most readily absorbed from HEDTA and EDTA while EDDHA

and 157 NaFe were intermediate in their ability to supply iron. On the other hand, rice plants absorbed less labeled iron than barley from all the chelates used and also retained most of the labeled iron in the roots. The chelate EDDHA resulted in significantly more iron absorption by the root than the other chelates; however, all chelates resulted in similar amounts of iron translocation to the shoots. The phosphorus requirement of rice is not high and perhaps less than 0.2×10^{-3} M KH_2PO_4 would have proved sufficient and resulted in increased iron translocation.

In the barley study, the total iron content of shoots is higher than that of roots for both phosphate levels and for all the different treatments. Apparently the barley plant has a very effective mechanism for the translocation of iron from the root to the shoot.

The results of the pretreatment experiment with barley reported in Table 5 show that iron is most readily absorbed after a pretreatment of 10^{-7} M FeHEDTA but most translocation occurs after pretreatment without iron. Plants under a preliminary period of iron deficiency developed a slight iron chlorosis and more iron was translocated into the shoots when transferred to the labeled Fe solution. The translocation of iron following either the minus Fe pretreatment or 10^{-7} M FeHEDTA pretreatment is more than that of 10^{-6} M and 10^{-5} M pretreatments. The absorption of labeled iron is significantly lower following pretreatments of 10^{-6} M and 10^{-5} M than after a pretreatment of 10^{-7} M. There were

no apparent relations between the Fe translocated to the shoots and the Fe content of the roots. These results with barley are in contrast to a report by Brown and Bell (1969) that maximum translocation of iron by corn occurs after pretreatment with Fe-containing nutrient solution.

Absorption and translocation of iron by barley and rice grown in nutrient solutions maintained at various phosphate levels was examined. Brown (1956) reported that high levels of phosphorus in the growth medium often have been found to reduce iron absorption and utilization. Aiyar (1946) demonstrated with rice that increasing concentrations of phosphorus caused an increase in the phosphorus concentrations in the root but a decrease in the iron content of the root. Biddulph and Woodbridge (1952) observed that plants grown in media supplying an excess of phosphorus progressively accumulated this element in the leaves, stems and roots. They believed that iron is frequently immobilized by phosphorus within plant tissue. The experiments reported in Tables 2 and 4 indicated that rice grew better at 0.2×10^{-3} M phosphate compared to 1×10^{-3} M. Above a concentration of 0.1×10^{-3} M phosphate iron translocation is markedly decreased (Table 15). Excess phosphorus may be responsible for immobilizing iron and inhibiting iron translocation in the xylem of the rice plant and thus limiting growth. This may explain the low chlorophyll levels in rice provided with either 0.2×10^{-3} M or 1×10^{-3} M phosphate.

A high phosphate concentration in the nutrient solution did not appear to hinder iron absorption or translocation by barley. It is true that barley needed more phosphorus than rice as demonstrated by increased yield at 1×10^{-3} M, compared to 0.2×10^{-3} M. and the higher phosphate concentration did not limit iron absorption in the 21 day absorption study. Barley seems to absorb and translocate iron at phosphate concentrations that would be inhibitory to many other plant species. Phosphorus appears to differentially influence metabolic reactions essential to the absorption and translocation of iron in barley and rice and it is possible to grow barley at a wide range of phosphorus levels without inducing an iron deficiency.

The effect of the hydrogen ion concentration on the absorption and translocation of iron by barley and rice was studied. It was shown that the amount of iron absorbed varied both with the plant species and also with the pH of the nutrient solution. With rice, maximum absorption occurred at pH 7 with minimums at pH 8 and 9; however, translocation decreased greatly at pHs above 5. With barley, total absorption was highest in a medium of pH 5, while both absorption and translocation decreased greatly at pHs above 6. For both species high pH values correspond to low iron content. Several years ago, Thorne and Wallace (1944) indicated that high pH makes iron less available to plants. Klein and Warren (1952) explained that at high pH values iron tends to precipitate

out of solution as ferric hydroxide. Patten and Main (1920) found that iron was precipitated at pH 6.0 and above, thus rendering it unavailable for absorption by the plant. In the present investigation the chelate FeEDDHA should be stable between a pH of 3.6 and 9, and thus pH effects cannot be explained in terms of precipitation of iron in the nutrient solution (Geigy, 19__).

Price (1970) considers the problem of pH and the formation of insoluble ferric hydroxide with a decrease in iron available to plants as pH increases. These results suggest that barley has a mechanism for maintaining Fe in the xylem stream in a soluble state, perhaps as a chelate of citrate. Rice like some plants adapted to acid soils may not form such a chelate in the xylem and thus may depend on a low level of phosphate and low pH to avoid precipitation of iron in the xylem.

SUMMARY

1. The concentration of iron chelate in the growth medium is discussed as the controlling factor affecting the absorption of iron by barley and rice. A concentration of 10^{-6} M was required to prevent iron deficiency symptoms in either barley or rice.

2. It was found that there is a relationship between total iron and chlorophyll content at low iron levels, but the correlation was not apparent at high levels of iron in the culture solution.

3. All the chelates used appeared to serve as satisfactory sources of iron for plants growing in solution culture. The iron chelates of HEDTA and EDTA have lower stability constants and resulted in absorption of more iron by barley than was absorbed from the more stable iron chelates.

4. Most iron was translocated by barley after a pretreatment without iron while iron was absorbed most readily after a pretreatment of 10^{-7} M iron.

5. Iron absorption and translocation by rice was inhibited by a high phosphate concentration in the nutrient solution. The phosphorus requirement of rice is not high and 0.1×10^{-3} M resulted in maximum iron translocation. Barley needed more phosphorus than rice for maximum growth and the higher phosphate concentration did not limit iron absorption or translocation.

6. The hydrogen ion concentration of a nutrient solution exerts an effect both upon the absorption and translocation

of iron. For both barley and rice high pH values correspond to low iron content in the plant tissue. Iron translocation by barley was severely depressed at pH 7 or higher while in rice translocation of iron decreased greatly at a pH of 6 or higher.

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