EFFECTS OF MANGANESE INDUCED CHLOROSIS ON THE ABSORPTION AND TRANSLOCATION OF ZINC BY SOYBEAN PLANTS

by

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Iron chlorosis of soybean plants has been studied by many investigators. It has been found that root cells of iron starved plants (non-chlorotic) accumulate a great amount of Fe 59 when grown in the presence of iron[5,12]. As the soybean plant becomes more chlorotic it has a greater capacity to absorb iron[1]. There was a greatly increased rate of transport and accumulation of iron in the leaves of the chlorotic plants[2,19]. The condition that takes place in soybean roots grown under deficient iron concentrations and the mechanism whereby the iron is absorbed and accumulated more readily is still not known. Have chlorotic plants the same ability to absorb more of other metal irons? The solution of this problem may help the elucidation of the mechanism. The present work was undertaken to study the effect of chlorosis on Zn absorption and its mechanism.

Iron chlorosis was reproduced by treating the plants with a high level of manganese in the nutrient solution[23]. Shive *et al*[15,16,17] and Twyman[20,21] have reported that a high concentration of manganese had a depressive effect on the absorption of iron from nutrient solutions and upon maintenance of high level of water-soluble iron in plant tissues, symptoms of iron chlorosis was induced. Weinstein and Robbins[24], on the basis of biochemical research, concluded that a high nutrient level of manganese in the presence of low iron induces a true iron deficiency.

The use of radioactive Zn provided a very sensitive means of both quantitative and qualitative determinations of Zn as compared to the Polarographic determination[11] or Dithizone method[7].

MATERIALS AND METHODS

Soybean plants (Glycine max Merr.) were used in these experiments. The seeds were germinated and grown in sand in the greenhouse for two weeks, After which they were transplanted to 1 gallon crocks containing an aerated half-strength nutrient solution described below. They were again transplanted one day later to full-strength nutrient solutions. For comparison of chlorosis resulting from true iron deficiency, seeds were germinated in petri-dishes using deionized water. They were then treated as described above. Nutrient solutions were made with deionized water and C. P. grade salts. Constant aeration was provided throughout the experiment and the

nutrient solution was changed every week. The composition of the nutrient solution used is given as follows.

KH_2PO_4	0.001 M	$(H_3BO_3)B$	0.1 p.p.m.
$Ca(NO_3)2 \cdot 4H_2O$	0.0045 M	$(CuSO_4 \cdot 5H_2O)$ Cu	0.01 p.p.m.
K_2SO_4	0.0015 M	$(H_2MoO_4 \cdot H_2O)$ Mo	0.01 p.p.m.
$MgSO_4 \cdot 7H_2O$	0.002 M	Fe (Fe EDTANa_2)	1.267 p.p.m.
HNO	0.0013 M	$(MnSO_4)$ Mn	0.25 p.p.m.
(ZnCl) Zn	0.1 p.p.m.		

Nutrient solution for high manganese level was the same as the solution for other treatment except that the concentration of Mn was 10.00 p.p.m., and the nutrient solution for minus Fe was the one in which no Fe-Na_2 EDTA was added.

In about four weeks, the young leaves of the high Mn treated plants showed chlorotic symptoms. The absorption period of Zn 65 was 48 hours. At the end of the inductive period (4 weeks), the cotyledons were removed and the plants were transferred to 1-gallon crocks containing the appropriate nutrient solution and 48.32 micro-curies of Zn 65 per 3.5 liters of nutrient solution. After 48 hours, the plants were then harvested in duplicate, the roots were washed with $ZnCl_2$ solution and distilled water.

For exudate collecting[8], the plants were cut off below the cotyledons and short lengths of rubber were snugly fitted to the stumps. Exudate was collected from these cups at four hour intervals for one day.

For preparing gross autoradiograms, the whole plants were pressed and dried in the oven at 50–60°C for 24 hours. They were then mounted on paper. Radioautograms were made with Kodak "blue brand X-ray film". The specimen plants were placed directly on 8×10 inch film sheets. They were placed in a specially made exposing box and exposed for 9 days. The films were then developed. The exposure, development and reproduction of the films were maintained uniformly throughout so that semi-quantitative comparisons between autoradiograms may be made.

Cation and anion exchange resins were used in both column and batch tests. Amberlite IR-120 was prepared in the sodium form by treatment with sodium chloride and hydrochloric acid; amberlite IR-410 in the acetate form was prepared by treatment with sodium acetate and acetic acid. After passing exudate through the resin column, they were washed with distilled water and then eluted with appropriate eluent. Fractions of 3 ml of eluvante were collected and tested for radioactivity. In batch work with resins, exudate samples were placed in contact with the resin in a 50-ml beaker for 2 hours under refrigeration with occasional stirring. The supernatant was then decanted and tested for radioactivity.

Paper chromatograms were prepared with Whatman No. 3 mm paper. The solvent systems used for the separations of amino acids was n-butanol-acetic-water (4:1:1).

Amino acid spots were detected by 0.1% ninhydrin in 95 alcohol[6]. Autoradiograms prepared as described above.

Measurement of radioactivity; Zn 65 in its decay, emits both positrons and r-rays as shown by the following reactions[14]:

$$_{30}Zn^{65} \longrightarrow {}_{29}Cu^{65} + {}_{+1}e^{0}$$

Emission of positrons, as shown in the above equation, is of secondary importance to the electron capture; shown by equation below:

$$_{30}Zn^{65} - {}_{1}e^{0} \longrightarrow {}_{29}Cu^{65}$$

The positrons emitted have relatively low radiation energies. Thus in the measurements of Zn only hard penetration r-rays were counted and no correction was necessary for self-absorption of particles within the sample, counting rates lower than 10,000 cpm the coincidence correction was neglected.

For measurements of radioactivity, a well type scintillation counter was used. A count rate of 6130 cpm is approximately equivalent to 0.28 micro-grams of Zn.

RESULTS AND DISCUSSION

1. The first experiment was designed to compare the relative level of newly absorbed Zn and its distribution in intact plants that had different precultural histories. The Mn treated plants showed a characteristic chlorosis as described by Weinstein & Robbins[24]. The minus Fe plants showed no signs of chlorosis as compared to that of normal plants. The results from whole plants were shown by autoradiograms. Both high Mn-induced chlorotic and Fe-deficient plants accumulated greater amounts of Zn in the leaves and stems.

Greater amounts of Zn 65 were also found in the exudates of chlorotic soybean plants than in the normal green plants (Table 1) and this situation was further confirmed by the autoradiograms.

Table 1. Radioactivity found in exudates of chlorotic and normal green plants

Treatment	Assay (cpm/0.3 ml of exudate)
High Mn (chlorotic)	639.4±11
Normal (green)	579.0±11

2. The effect of chlorosis on the absorption of Fe and Zn is of interest. Investigations on iron compounds in the exudates showed that iron in the exudate existed in the form of Fe-malate and malonate[13,18]. It was also found that some type of organic compounds or compounds within the plant seems to control Fe absorption and translocation[3,4]. Ulrick[22], working with excised barley roots, concluded that when excess cations were absorbed, organic acids were formed as a response to the tendency toward an increase in pH of the root sap. All this sug-

gests the possibility that increase in organic compound in the plants may be responsible for the greater transport and accumualtion of Fe and Zn in chlorotic plants.

Zinc phosphate has a pKsp of 47.9[9]. This indicates that its solubility is less than that of iron phosphate (pKsp=35)[13]. Organic acids and certain amino acids are naturally occurring chelating agents that form water soluble metal complexes. An increase of these compound in the root sap can increase the solubility of metal compounds and thus may facilitate its mobility and translocation. As a test for this hypothesis, the following experiments were undertaken to see if zinc in the exudate were combined with chelates.

Exudate obtained has a pH of 6.6–6.8. Zinc content was gradually decreased with time after cutting. One ml exudate contained about 9.5 milli-micro-gram of Zn. Equal amounts of exudates from chlorotic plants were applied to both anion and cation exchange resin columns. As shown in Table 2, all activities were absorbed by the cation exchanger (Amberlite IR-120). Elution with 4 NHCl recovered all the activities absorbed.

Table 2. Activities in washing distilled water passed through cation & anion exchange resins

No. Portion	Amberlite IR-410	Amberlite IR-120
1	95± 8.4	0.4±7.3
2	788.8±11.6	11.0±7.8
3	1,172.8±11.8	7.4±7.7
4	387.9±10	6.7±7.7
5	234.8±9.2	0.4±7.3
6	159.7±8.8	6.9±7.7
7	103.0±8.5	5.6±7.7
8	28.2±8.0	4.3±7.4

* To both columns 3 ml of exudates, each have approximately 59400 cpm, were applied.

* Corrections are made for background & activities present in distilled water which has an activity of about 15.3±7.7 cpm/3 ml distilled water (10).

* Percent recovery from amberlite IR-410 was about 90%.

To eliminate the possibility of a filtering action by resin bed. a batch test was also carried out. Table 3 shows that all the Zn again was removed from the exudate.

The existence in the xylem exudate of a Zn-anionic complex seems to have been eliminated by ion exchange studies. However, possibility still existed that some amino acids with larger stability constants of Zn complex may chelate Zn in the xylem exudate.

Table 3. Activity of exudate Zn in the supernatant fluids of cation & anion exchange resins

Resins	Assays (cpm)
Amberlite IR-410	1180±11.8
Amberlite IR-120	28±8

* The same as Table 2

Paper chromatographic experiments showed that exudate Zn from chlorotic plants are located principally in some spots which are ninhydrin positive. It may be noted that chlorotic plants were not supplied with EDTA Na_2 Fe as the normal green plants, in which Fe was supplied as EDTA Na_2 Fe. Zinc was found to be mostly combined with this synthetic chelating compound. This may be explained by the larger stability constant of Zn-EDTA complex.

The greater accumulation of zinc in the chlorotic plants may be contributed by two factors. The first is a greater rate of absorption of the roots. Brown *et al*[2] have found that roots of chlorotic Hawkeye soybean develop the capacity to reduce and absorb ferric Fe more rapidly than roots of green plants. In the case of Zn absorption, the mechanism is not known. The second contribution is the greater rate of translocation. An increase in the exudate of malate or malonate may increase the transport of Fe. However zinc, in the present work, has been found to be bound to some ninhydrin positive compounds possibly amino acids. Whether or not the root sap or exudate of chlorotic plants have a greater amino acid content is unknown and awaits further study.

It is suggested that a metal complex present in xylem exudate may not be present in the xylem stream of a transpiring plant and may be in the transport form of iron under those conditions. Sap collected from transpiring plants appears to be a more satisfactory subject to be studied.

SUMMARY

1. The chlorotic soybean plants accumulated a greater amount of zinc than the healthy green plants. The same phenomenon has been found in the absorption of iron.
2. This paralellism found between the effects of chlorosis upon the accumulation of zinc and upon the accumulation of iron arose the speculation, whether or not it was the response of an increase of some naturally occurring chelating compounds.
3. Zinc in the exudated was not found to be bound as an anion.
4. Paper chromatography demonstrated that zinc was bound to some ninhydrin positive substances, which require further identification.

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