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DISCLAIMER

IRON ABSORPTION FROM DIFFERENT SOURCES IN SOIL BY TWO DIFFERENT SOYBEAN VARIETIES (IRON EFFICIENT AND IRON INEFFICIENT)

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Abstract

Two soybean varieties (*Glycine max* [L.] Merr. Bragg) were grown in a glasshouse with and without a chelating agent in calcareous Hacienda loam soil which had been equilibrated prior to the test with 0, 1, 2.5, 5, 10, 25, or 50 ppm Fe from $FeSO_4$ each labeled with ^{59}Fe. Fe, ^{59}Fe, and specific activities of Fe (cpm/μg Fe) were all higher for the Hawkeye than for the PI54619-5-1 soybean. Only with the chelating agent were substantial quantities of Fe from $FeSO_4$ in the plants. In another test the two soybean varieties were grown in a glasshouse in noncalcareous Yolo loam soil which had been equilibrated with essentially carrier-free ^{59}Fe. The specific activity of Fe was again higher in Hawkeye than in PI54619-5-1. The amount of Fe extracted from the soil by different extracting agents after cropping was not always related to the specific activity of the extract. Specific activities of Fe in the soybeans grown without the chelating agent were nearly like that of the 1/10 *N*

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HCl extract, and those in the soybeans grown with the chelating agent were nearly like that of the DTPA (diethylene triamine pentaacetic acid) (chelating agent) extract. A reducing agent extracted as much Fe as did the chelating agent, but the specific activity of the extracted Fe was much lower with the reducing agent.

Introduction

Iron-efficient plants, like Hawkeye soybeans, absorb more Fe than Fe-inefficient plants, like PI54619-5-1 soybeans, from both soil and from synthetic chelating agent sources (1, 2). The addition of carrier-free ^{59}Fe to calcareous soil in which the Fe-efficient rough lemon and Fe-inefficient trifoliate orange were grown resulted in greater contents of the ^{59}Fe in the former than in the latter even more so than the differences in total Fe (6, 7). There was a great difference even in the presence of synthetic chelating agents. A possible conclusion from the previous studies was that there are different sources of Fe in the soil and these are differentially available to different species or varieties (3, 4). The addition of carrier-free ^{59}Fe to soil does not label all or even much of the Fe which is available to plants. Chelating agents themselves may cause a separation of soil Fe into more than one available source. This study was directed at determining if ^{59}Fe and EDDHA (ethylenediamine (di[o-hydroxyphenylacetic acid]) would help ascertain if there is more than one major source of Fe in soil available to plants and how efficient and inefficient plants differ in their ability to absorb any different sources of Fe that may exist in soil.

Materials and Methods

Calcareous Hacienda loam soil (7) was equilibrated with ^{59}Fe (10^6 cpm/500 g) containing sufficient $FeSO_4$ to give either 0, 1, 2.5, 5, 10, 25, or 50 ppm Fe on the dry weight basis in the soil. Each soil received the same amount of ^{59}Fe, but differing amounts of total Fe. PI54619-5-1 (Glycine max (L.) Merr. Bragg) soybeans or Hawkeye soybean seedlings were planted into the soil in 500-g quantities with eight replicates. EDDHA (sodium salt) without Fe was supplied to half the pots and at a rate equivalent to 2.5 ppm Fe per dry weight of soil if Fe had been added. The plants were grown in a glasshouse for 12 days. At this time leaves and stems were separated, washed in 0.1 N HCl and in distilled-deionized water, then dried, weighed, and prepared for analysis by emission spectrography (8). ^{59}Fe was counted in the samples with a scintillation-well counter.

High specific activity ^{59}Fe (about 40 μc/600 g soil) was uniformly mixed (sprayed in solution on thinly spread-out soil which was later thoroughly mixed) and equilibrated for 3 weeks with noncalcareous Yolo loam soil and Hawkeye and PI54619-5-1 soybeans were grown 12 days as explained above. Half of the plants received the chelating agent EDDHA at a rate equivalent to 5 ppm Fe per dry weight of soil, but no Fe was applied (1:1 chelate assumed). All plants received 100 ppm N per dry weight of soil as NH_4NO_3 at the start of the test. The plants were prepared as above. After the plants were removed from the soil, extracts were made of 10-g quantities of it with 20 ml of DTPA (diethylene triamine pentaacetic acid) (5), 1/10 M HCl, 1 M HCl, and 10^{-3} M hydroquinone. The latter is a reducing agent and its use was predicated on the observation that ability of efficient species to accumulate Fe is directly related to the reducing capacity of the root (2). Iron in these extracts was determined colorimetrically with o-phenanthroline. All results for ^{59}Fe were calculated to the date of starting of the experiment to correct for differences due to half-life of ^{59}Fe.

Results and Discussion

All the PI54619-5-1 plants in the Hacienda loam were yellow from Fe chlorosis. This occurred even though leaves of those with EDDHA contained generally more

Fe than Hawkeye soybeans without EDDHA (Table 1). The latter were green as were all Hawkeye plants. Application of labeled Fe to the soil resulted in relatively little uptake of it by either plant variety unless EDDHA was also added. More of the labeled Fe was taken up by Hawkeye plants than PI54619-5-1 plants with or without the chelating agent (Table 1).

An important point is that the ^{59}Fe-labeled Fe was essentially not the Fe available to plants without the chelating agent. The chelating agent made the labeled Fe more available than it otherwise was. The addition of $FeSO_4$ at 50 ppm per dry weight of soil had little effect on the uptake of Fe without chelating agent; some of it was taken up, but it did not seem to add to the total uptake. With the chelating agent, increasing quantities of the added ^{59}Fe from $FeSO_4$ were found in the plants. As this quantity increased, there was a decreased amount of Fe in the plants from other sources.

It is not possible to determine with the chelate treatments if all the Fe available to the plants were chelated or if some were available without being chelated. The near constant specific activity of Fe in the plants with chelates would indicate that the chelating agents chelated close to an equal amount of Fe in all treatments and that the Fe chelated from the added $FeSO_4$ source largely displaced other Fe which would be chelated. Two sources of Fe at least then were being utilized, but both would be subject to chelation before absorption by the plants.

Further confirmation of the hypothesis that different sources of Fe are available to plants was obtained in the second experiment. The plants grown in noncalcareous Yolo loam (Table 2) contained more Fe than those in calcareous Hacienda loam (Table 1). The specific activity of ^{59}Fe in plants was slightly higher for the Hawkeye than for the PI54619-5-1 variety with or without the chelating agent applied to the soil (with the calcareous soil (Table 1) it was much higher for Hawkeye than for PI54619-5-1 soybeans). The chelating agent approximately doubled the specific activities of ^{59}Fe in the leaves without changing the total Fe content of leaves. This indicates that the chelating agent was extracting and making available a source of Fe that otherwise was not very available to the plants.

The data from the extracting agents also indicated that the agents extracted somewhat different sources of Fe from the soil (Table 3). For example, the reducing agent extracted more Fe than did the chelating agent, but the specific activity of the ^{59}Fe with the reducing agent was only one-eighth that with the DTPA. The specific activity of the 1/10 M HCl extract was most like that of both soybean varieties grown while that with the DTPA extract was most like the soybeans grown with the EDDHA. These results circumstantially provide evidence that different sources of Fe which are differentially available to different plants do exist in soil.

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Table 1. Iron and ^{59}Fe contents of leaves of two varieties of soybeans with and without chelating agents.

Fe added to soil ppm	0 EDDHA Yield mg/plant	0 EDDHA cpm Fe^{59}/µg Fe	0 EDDHA Fe ppm	0 EDDHA Fe* ppm	0 EDDHA Fe-Fe* ppm	EDDHA Yield	EDDHA cpm Fe^{59}/µg Fe	EDDHA Fe ppm	EDDHA Fe* ppm	EDDHA Fe-Fe* ppm
PI54619-5-1 soybeans										
0	190	0.0	52	0.00	52	171	62.4	61	0.00	61
1	198	0.0	56	0.00	56	185	70.8	73	0.73	72
2.5	224	0.0	53	0.00	53	180	65.6	62	1.88	60
5	161	0.0	43	0.00	43	168	45.0	66	2.41	64
10	143	6.9	40	0.32	40	180	54.8	61	6.69	54
25	194	1.5	49	0.24	49	202	42.0	78	16.5	62
50	198	1.0	29	0.60	29	216	44.9	74	29.8	44
Hawkeye soybeans										
0	262	7.8	60	0.00	60	247	76.9	75	0.00	75
1	283	14.5	50	0.11	50	230	66.1	93	1.15	92
2.5	324	8.3	59	0.25	59	244	70.1	81	3.01	78
5	302	11.0	50	0.44	54	234	52.5	67	3.30	64
10	212	9.4	53	1.07	52	228	80.6	71	12.4	59
25	348	2.6	43	1.70	41	206	50.4	82	19.4	63
50	322	3.8	63	2.79	60	253	48.7	78	38.3	40
LSD .05	63	4.0	8	0.21	8	63	15.2	14	3.0	7
LSD .01	83	5.2	12	0.27	12	83	20.0	NS	4.2	9

Fe in plants from added FeSO$_4$ as determined from specific activity relationships. Fe-Fe represents the nonlabeled Fe in the leaves. All data on dry weight basis.

Table 2. Fe and specific activity of it in trifoliate leaves of the soybean plants grown in Yolo soil (12 replicates). All data are on dry weight basis.

Soybean and treatment	Fe in trifoliate leaves	Specific activity
	ppm	cpm $^{59}Fe/\mu g$ Fe
PI54619-5-1 control	106	8.1
PI54619-5-1 + EDDHA	100	18.7
Hawkeye control	118	10.6
Hawkeye + EDDHA	118	20.5
LSD .05	8	1.1
LSD .01	11	1.4

Table 3. Fe extracted from the Yolo loam soil by different agents and specific activity of the Fe. (12 replicates) All data are on dry weight basis.

Extractant	Fe in extract	Specific activity of Fe in extract
	ppm	cpm $^{59}Fe/\mu g$ Fe
DTPA	14.4	30.1
1/10 N HCl	1.6	13.0
1 N HCl	33.0	81.7
10^{-3} M hydroquinone	18.8	3.6
LSD .05	2.2	4.1
LSD .01	2.9	5.3