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Iron-stress Response in Mixed and Monocultures of Soybean Cultivars

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ABSTRACT

Hawkeye (Fe-efficient) and PI-54619-5-1 (Fe-inefficient) soybeans (*Glycine max* [L.] Merr.) were grown in mixed and monoculture nutrient solutions to evaluate an inhibitory effect of PI-54619-5-1 on the uptake of Fe by Hawkeye. The ability of Hawkeye to take up Fe (Fe-stress response) was dependent on the degree of Fe stress (Fe deficiency) and was not the result of an inhibitory substance released by PI-54619-5-1 in mixed culture (Hawkeye + PI-54619-5-1).

The degree of Fe stress in Hawkeye was dependent on the amount of Fe taken up by the plant. Hawkeye took up more Fe and developed less Fe stress in mixed than in monoculture because in mixed culture PI-54619-5-1 did not utilize Fe as efficiently as Hawkeye which allowed more Fe to be available per HA plant. Thus, Fe-stress response, rather than any inhibitory substances produced by PI-54619-5-1 plants, controls the uptake of Fe in Hawkeye soybean.

Wallace *et al.* (10) grew Hawkeye and PI-54619-5-1 soybeans in mixed and monoculture in nutrient solution containing $^{59}Fe^{3+}$-labeled calcareous soil. Because the uptake of Fe by HA[1] plants was less in mixed than in monoculture, they postulated that roots of PI plants may excrete a substance that interferes with Fe accumulation in HA plants. The inhibitory "substance" was never identified.

The conclusion of Wallace *et al.* (10) was recently supported by Elmstrom and Howard (7) who observed a differential ^{59}Fe accumulation in the primary leaves of HA and PI plants grown in mixed and monoculture. They indicated that the inhibitory factor appears to be associated with the plant rather than the solution, and the effect is retained by HA plants even after their removal from the mixed culture.

The objective in this study was to develop an alternate interpretation to that of Wallace *et al.* (10) and Elmstrom and Howard (7) based on differential "Fe-stress response" of PI and HA soybean. Our interpretation notes that Fe-deficient HA plants absorb more Fe than Fe-sufficient HA plants or Fe-sufficient or Fe-deficient PI plants (2). In other words, Fe uptake rates depend on the degree of Fe-deficiency (Fe-stress) imposed and the ability of the plant to make a regulatory biochemical response to Fe-deficiency. Hawkeye and PI soybeans differ in their response to an Fe-stress; HA develops more response than PI.

[1] Abbreviations: FeEDDHA: Fe-ethylenediamine di(*o*-hydroxyphenylacetate); FeDTPA: Fe-diethylenetriaminepentaacetate; BPDS: 4,7-di(4-phenylsulfonate)-1,10-phenanthroline, bathophenanthrolinedisulfonate; HA: Hawkeye; PI: PI-54619-5-1.

MATERIALS AND METHODS

Nutrient Solutions. A modified 0.1 Steinberg solution (9), was used to repeat, in part, experimental work reported by Elmstrom and Howard (7) on 16 soybean (*Glycine max* [L.] Merr.) plants grown in mixed or monoculture. The nutrient solution contained in μM: $Ca(NO_3)_2\cdot 4H_2O$, 630; $Mg(NO_3)_2\cdot 6H_2O$, 140; K_2HPO_4, 220; NH_4NO_3, 110; KNO_3, 30; $(NH_4)_2SO_4$, 70; $MnCl_2\cdot 4H_2O$, 1.18; $ZnSO_4\cdot 7H_2O$, 0.31; $CuSO_4\cdot 5H_2O$, 0.08; H_3BO_3, 3.29; and $NaMoO_4\cdot 2H_2O$, 0.5. Another nutrient solution, 0.2 Steinberg solution, was used that contained twice the salt concentration of the first nutrient solution except that P, in the second solution, was 70 μM instead of 220 μM.

Growth of Plants. HA and PI seeds were germinated between layers of moist muslin on stainless steel wire frames over deionized water in Pyrex trays. Selected 3-day-old seedlings were transferred to plastic rings with holes for the seedlings. The plastic rings were supported on a 10-liter Pyrex battery jar containing a modified 0.1 Steinberg solution minus Fe. The plants were either 7 or 10 days old (indicated in each experiment) when they were bunched in groups of four plants and grown in mixed or monoculture in a light chamber under 8-hr dark and 16-hr light period (1500 ft-c) at 23 ± 2 C.

Assays. Radioiron was used in nutrient solutions as ^{59}FeEDDHA with a specific radioactivity of 1200 or 6000 cpm/μg Fe. ^{59}Fe was assayed with a γ-scintillation spectrometer. Total Fe of plant parts was determined by *o*-phenanthroline method (8). The term, top leaves, in the text means the youngest two trifoliolate leaves.

Reduction of Fe^{3+} DTPA by Roots. Reduction of Fe^{3+} DTPA by the roots of soybean plants was measured according to Chaney *et al.* (6) using the ferrous color reagent, BPDS. The nutrient solution, initial pH 6.0, contained 10 μM Fe^{3+} DTPA and 50 μM BPDS. Ferrous iron at the root was trapped by BPDS, forming the highly stable ferrous chelate (Fe^{2+} $[BPDS]_3$) in the nutrient solution. Measurements were made periodically on 5-ml aliquots.

RESULTS

Experiment 1, 16-Plant Culture. Ten-day-old HA and PI soybean plants (four of a cultivar/bundle, four bundles/8 liters of solution) were grown for 7 days in mixed or monocultures: 16 HA; 12 HA + 4 PI; 4 HA + 12 PI; and 16 PI plants. The plants were grown in 0.1 Steinberg solution containing 1.1, 5.6, or 11.1 μM ^{59}FeEDDHA. The initial pH of the solutions was adjusted to 6.8 with 0.1 N NaOH, and the pH of the solutions was determined daily.

HA plants had little or no effect on the uptake of Fe into the top leaves of PI plants (Table I). However, the tops of HA plants contained more ^{59}Fe in mixed than in monoculture; uptake by HA was greatest where four HA and 12 PI plants were grown together (Table I). In these cultures, PI plants took up less Fe per plant than HA plants, thus leaving more nutrient Fe for the HA plants. The PI plants were chlorotic on 1.1 μM FeEDDHA but green on 5.6 and 11.1 μM FeEDDHA. HA plants were green on all levels of FeEDDHA but the tops contained less Fe when grown on the 1.1 than on the 5.6 or 11.1 μM FeEDDHA treatments.

Iron in the primary leaves was not as good index of Fe uptake as iron in the top two leaves. ^{59}Fe and total Fe concentrations were about the same in top leaves, but they were considerably different in primary leaves. Both in mono and mixed HA-PI cultures, HA contained more Fe than PI soybeans (Table I).

Table I. *Effect of Mixed or Monoculture on the Concentration of Fe in the Top Two Leaves and the Primary Leaves of Sixteen 17-day-old Soybean Plants following a 7-day Absorption Period*

Upper number represents ^{59}Fe, and lower number represents total Fe for each treatment. Specific radioactivity of ^{59}Fe was 1200 cpm/μg Fe.

Culture ^{59}FeEDDHA	Number of Each Cultivar in Mixed and Monoculture					
	HA			PI		
	HA 16	HA + PI 12 4	HA + PI 4 12	HA + PI 12 4	HA + PI 4 12	PI 16
μM	μg ^{59}Fe or total Fe/g dry wt					
Top two leaves						
1.1	63	68	73	38	34	35
	63	66	78	41[1]	39[1]	41[1]
5.6	66	70	87	61	62	58
	70	72	89	64	63	62
11.1	85	93	101	76	80	78
	90	96	103	76	81	79
Primary leaves						
1.1	111	119	102	38	21	34
	131	130	139	96	97	85
5.6	177	191	195	86	62	65
	201	223	232	117	113	124
11.1	185	207	242	95	93	91
	238	249	301	126	139	135

[1] Chlorotic.

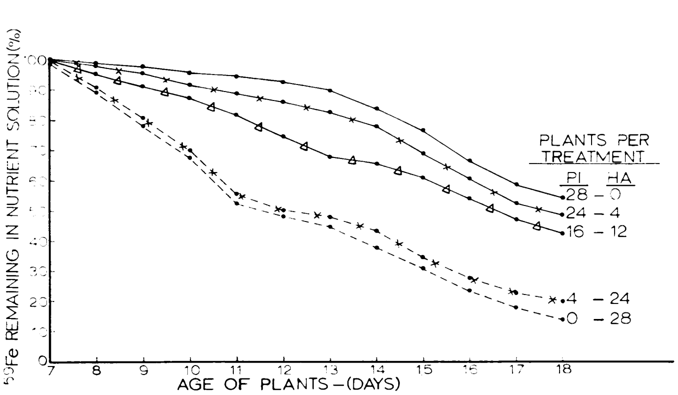

FIG. 1. ^{59}Fe remaining in nutrient solution as affected by mixed and monoculture of PI and HA soybean (nutrient ^{59}FeEDDHA was 1.0 μM).

Table II. *Effect of Mixed or Monoculture on the Concentration of Fe in the Top Two Leaves of Twenty-eight 18-Day-Old Soybean Plants Following an 11-Day Absorption Period*

Upper number represents ^{59}Fe, and lower number represents total Fe foı each treatment. Specific radioactivity of ^{59}Fe was 1200 cpm/μg Fe.

Culture ^{59}FeEDDHA	Number of Each Cultivar in Mixed and Monoculture							
	HA				PI			
	HA 28	HA + PI 24 4	HA + PI 12 16	HA + PI 4 24	HA + PI 24 4	HA + PI 12 16	HA + PI 4 24	PI 28
μM	μg ^{59}Fe or total Fe/g dry wt							
Top two leaves								
0.5	42	44	50	67	35	34	33	34
	43[1]	45[1]	52	68	38[1]	37[1]	37[1]	35[1]
1.0	47	47	59	75	36	35	34	35
	48[1]	49[1]	62	75	40[1]	40[1]	38[1]	37[1]
1.5	53	58	65	80	43	43	42	43
	54	58	68	81	46[1]	44[1]	44[1]	43[1]

[1] Chlorotic.

Experiment 2, 28-Plant Culture. The levels of Fe used in experiment 1 were the same as those used by Elmstrom and Howard (7). In experiment 2, the number of plants was increased from 16 to 28 in order to increase the Fe stress produced in the monoculture.

Seven-day-old HA and PI soybean plants were grouped (four of a cultivar/bundle; seven bundles/8 liters of solution) and grown for 11 days in mixed or monocultures: 28 HA; 24 HA + 4 PI; 12 HA + 16 PI; 4 HA + 24 PI; and 28 PI. The plants were grown in 0.2 Steinberg solution containing 0.5, 1.0, or 1.5 μM FeEDDHA. Nutrient FeEDDHA was either unlabeled or labeled with ^{59}Fe on day 7 or day 18. Preliminary work established 70 μM phosphorus to be sufficient for normal growth and development during the 11-day absorption period. The initial pH of solutions was adjusted to 6.8 with 0.1 N NaOH and then measured daily.

The removal of nutrient ^{59}Fe (1.0 μM on day 7), from mixed and monocultures, was followed from day 7 to day 18 (Fig. 1). On day 18, HA plants had removed approximately 85% of the ^{59}Fe, but PI plants had removed only about 45% of the ^{59}Fe from the monocultures. ^{59}Fe uptake decreased with increasing proportion of PI plants in the mixed plant cultures.

PI and HA soybeans, grown on 0.5, 1.0, and 1.5 μc ^{59}FeEDDHA, had developed different degrees of Fe chlorosis by day 18. All PI plants were chlorotic and HA plants were mildly chlorotic on the 0.5 and 1.0 μM ^{59}FeEDDHA treatments. On the 1.5 μM ^{59}FeEDDHA treatment, top leaves of HA were green (81 μg Fe/g dry weight), whereas PI top leaves were chlorotic (43 μg Fe/g dry weight) (Table II). The Fe concentration in plants did not differ appreciably in mixed or monculture (Table II), whereas Fe concentration was greater in HA in mixed than in monoculture. Hawkeye plants from cultures containing 12 HA plants or fewer contained more Fe than those from cultures containing more than 12 HA plants.

PI and HA soybeans, grown on 0.5, 1.0, and 1.5 μM FeEDDHA, at day 18 had developed different degrees of Fe deficiency (Fe-stress). These plants from mixed and monoculture were transferred to 1 liter of nutrient solution containing 10 μM FeEDDHA labeled with 10 μc ^{59}Fe. Plants were harvested after an absorption period of 6 hr, and tops were analyzed for total Fe and ^{59}Fe. Less than 0.1 μg $^{59}Fe/g$ dry weight was accumulated in the tops of PI plants regardless of treatment. In contrast, ^{59}Fe uptake was greater in HA plants grown in mono- or near monoculture (Table III).

Other PI and HA plants, Fe-stressed for 11 days in mixed or monoculture on 1.0 μM FeEDDHA, were tested separately (four of a cultivar/bundle) for capacity to reduced Fe^{3+} DTPA at the root. The reduction of Fe^{3+} DTPA at the root of mixed *versus* monoculture Fe-stressed plants was measured by trapping ferrous iron with BPDS (Table IV). HA soybeans had much greater reduction than PI soybeans, and HA from monoculture had greater reduction than HA from mixed cultures (Table IV). Both reduction of Fe^{3+} and release of H^{+} ions (Table V) by the root are associated with increased uptake of Fe. Thus, HA plants responded more to an Fe stress than did PI plants under the conditions of the experiments. This response was dependent on the proportion of HA plants in the culture, being greatest in HA monoculture. Reduction of Fe^{3+} DTPA by Fe-stressed HA plants was up to 100-fold greater than by PI plants.

DISCUSSION

Plant species and cultivars differ in their ability to absorb and translocate Fe. The uptake of Fe appears to result from metabolic changes that occur in Fe-deficient (Fe-stress) plants that favor Fe uptake (1). Increased Fe uptake by a plant caused by Fe stress is termed "Fe-stress response" of the plants. HA soybeans show greater Fe-stress response than PI plants (4).

The experimental work of Elmstrom and Howard (7), in which they observed a differential ^{59}Fe accumulation by primary leaves of HA and PI plants grown in mixed or in monocultures, was repeated. Our ^{59}Fe data were in general agreement with theirs for primary leaves. However, we found the Fe status of the plant top (youngest two trifoliolate leaves) to be a better index of Fe stress in soybean than the primary leaves. For example, when Fe-deficiency symptoms developed in tops of some HA and PI plants, the primary leaves of each cultivar were green on all treatments.

Table III. *Effect of Fe Stress on the Accumulation of ^{59}Fe in the Top Two Leaves of 18-Day-Old HA and PI Soybean during a 6-Hr Absorption Period*

$^{59}FEDDHA$ was 10 μM. Specific radioactivity of ^{59}Fe was 6000 cpm/μg Fe. Upper number represents ^{59}Fe and lower number represents total Fe for each treatment.

Preculture FeEDDHA	Number of Each Cultivar in Mixed or Monoculture							
	HA				PI[1]			
	HA 28	HA + PI 24 4	HA + PI 12 16	HA + PI 4 24	HA + PI 24 4	HA + PI 12 16	HA + PI 4 24	PI 28
μM	μg ^{59}Fe or total Fe/g dry wt							
Top Two leaves								
0.5[2]	15	11	7	3				
	58	59	60	71	38	37	37	35
1.0	13	10	6	2				
	60	63	65	78	40	40	38	36
1.5	11	4	2	1				
	66	68	74	80	45	44	44	43

[1] PI ^{59}Fe data omitted because <0.1 μg $^{59}Fe/g$ dry wt was accumulated in plant tops regardless of treatment.

[2] Greatest Fe stress.

Table IV. *Effect of Fe-Stress Response on the Reduction of Fe^{3+} DTPA with Time by Roots of HA and PI Soybeans*

Plants were Fe-stressed from day 7 to day 18 on 1.0 μM FeEDDHA. Each cultivar bundle, 18 days old, was then transferred to 1 liter of modified 0.2 Steinberg solution (P = 70 μM) containing 10 μM Fe^{3+}DTPA and 50 μM BPDS.

Time	Number of Each Cultivar in Mixed or Monoculture					
	HA			PI		
	HA 28	HA + PI 24 4	HA + PI 4 24	HA + PI 24 4	HA + PI 4 24	PI 28
hr	μM Fe^{2+} *BPDS formed in nutrient solution/4 plants*					
0.5	1.0	0.6	0.3	0.01	0.01	0.01
1.0	2.0	1.1	0.7	0.03	0.04	0.03
1.5	3.2	1.7	1.2	0.07	0.07	0.06
2	4.6	2.3	1.5	0.13	0.15	0.13
3	6.3	2.8	1.9	0.26	0.27	0.26
4	7.0	3.7	2.4	0.30	0.31	0.30
5	7.9	4.8	2.8	0.34	0.33	0.33
6	8.5	5.7	3.3	0.37	0.36	0.37

Table V. *Effect of PI and HA Plants in Mixed or Monoculture on pH Change of Nutrient Culture with Time*

Nutrient FeEDDHA was 1.0 μM.

Number of Each Cultivar in Mixed andMonoculture		Age of Plants (days)						
HA	PI	7	9	10	14	15	16	18
		pH						
0	28	6.8	6.1	5.3	4.6	4.5	4.9	6.4
4	24	6.8	6.1	5.2	4.5	4.4	4.8	6.4
12	16	6.8	6.1	4.9	4.3	4.2	4.4	6.5
24	4	6.8	6.0	4.5	4.0	4.0	4.5	6.4
28	0	6.8	5.9	4.4	4.0	4.0	4.5	6.3

Elmstrom and Howard's work (7) may be interpreted in terms of regulatory Fe-stress response rather than by an inhibitory factor associated with the plants.

PI Plants. Iron-inefficient PI plants had a low Fe-stress response and took up about the same quantity of Fe irrespective of the number of HA plants.

HA Plants. Fe-efficient HA plants had a high Fe-stress response and accumulated Fe according to the nutrient Fe concentration and the number of HA plants present in the culture. The Fe concentration in HA plants was less in plants from mono than mixed culture. Thus, HA plants from monoculture would be under greater Fe stress than those from mixed culture and would develop more Fe-stress response than plants from mixed cultures.

In this study, the regulatory "Fe-stress response" was affected by growing PI and HA soybeans in mixed *versus* monoculture, and forms the basis for suggesting an alternate interpretation of work by Wallace *et al.* (10), who suggested that PI roots excreted an inhibitory substance. When PI and HA soybeans were grown in mixed cultures, PI soybean did not utilize the Fe in the nutrient solution as efficiently as HA soybean, thereby allowing more Fe for each HA plant. This led to HA plants developing less Fe-stress response. HA plants grown in monoculture contained less Fe and developed a greater Fe-stress response than when grown in mixed culture (HA + PI). This was evidenced also by a greater reduction of Fe^{3+} DTPA and H^+ ion released by HA plants from monoculture.

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