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Differential Response of Two Soybean Genotypes to Zinc Induced Iron Deficiency Chlorosis

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Abstract. A greenhouse experiment using nutrient solutions was conducted to study the influence of zinc on the growth and composition of an Fe-efficient (Hawkeye) and an Fe-inefficient (PI-54619-5-1) soybean genotypes at various levels of Fe. Three levels of zinc (0.1, 1.0 and 5.0 mg/l) in combination with three levels of Fe (0.1, 1.0 and 5.0 mg/l) were used.

In general, increased zinc levels resulted in a growth reduction in both genotypes with the Fe-inefficient plants being more sensitive to Zn level. Increased Zn resulted in a decrease in Fe uptake and transport from the roots to the plant tops. The reduction in Fe transport may be greater than the reduction in uptake and resulted in an accumulation of Fe in the roots. The Fe-efficient genotype had a higher Fe content than the Fe-inefficient at corresponding treatment levels. Increasing Fe level offset the adverse effect of Zn on the growth and Fe uptake in both genotypes.

Introduction

Heavy metals may cause various degrees of Fe deficiency to develop in plants, depending on the plant's ability to respond to Fe-stress. Watanabe *et al.* [1] reported that corn grown in nutrient solutions experienced a depression in growth as Zn was increased from 0.75 to 2.25 μM when Fe was held constant at 2 ppm. Iron deficiency symptoms existed at all levels of P in that experiment and disappeared only when the Fe concentration was doubled to 4 ppm. However, the concentration of Fe in the plants and Fe uptake were not depressed by Zn. In contrast, Rosell and Ulrich [2] reported that increasing the Zn supply to sugarbeets from 0 to 12 ppm resulted in a reduction in Fe concentration in beet leaves from 900 to 90 ppm. Brown and Jones [3] studied nutrient interactions in grain sorghum and noted that as Zn was supplied to correct a soil deficiency, Fe concentrations in the plant tissue tended to decline significantly. Iron application to the soil, however, had no significant effect on Zn concentrations in the plants.

There is no doubt that nutritional interactions between Fe and Zn do exist in plants, but the explanation for this interaction is not completely understood. The main objective of this work was to study the effect of Zn levels on growth and Fe nutrition of soybeans at different levels of Fe in nutrient solutions.

Materials and Methods

Plant culture

Seeds of an Fe-efficient genotype Hawkeye (HA) and an Fe-inefficient genotype (PI-54619-5-1 (PI) of soyabeans *Glycine max* (L.) Merr. were obtained from the USDA Regional Soyabean Laboratory, Urbana, Illinois. The seeds were sterilized prior to planting by treatment with 75% ethanol (v/v) for three minutes followed by extensive rinsing with distilled water. The seeds were then inoculated with *Rhizobium japonicum* in the form of a commercial preparation (Agway, Inc. Syracuse, New York). The inoculated seeds were germinated on a pre-washed sand and seven days after germination, four seedlings were transplanted into 20.5 cm diameter pots containing perlite as a supporting media. Basic nutrient solution (1/2 strength Hoagland # 2) was applied to all treatments daily for three days after transplanting to ensure adequate growth of the seedlings. Thereafter, each treatment received its own solution daily. Three levels of Zn as $ZnSO_4$, $7H_2O$ (0.1, 1.0 and 5.0 mg/l) in combination with three levels of Fe as FeEDTA (0.1, 1.0 and 5.0 mg/l) in the Hoagland No. 2 were used as treatment solutions.

Analytical procedure

Fourty days after planting, plants were harvested and tops and roots were separated. Root samples were rinsed twice with deionized water to remove surface contaminants. Plant material was dried in a forced air oven at 75°C for 48 hr. Dry weights were recorded and the dried materials were ground in a stainless steel Wiley mill using a 30 mesh screen. Samples were digested in a mixture of nitric and perchloric acids, phosphorus determined by the vanado-molybdate method [4] and iron and zinc determined by atomic absorption spectrophotometry. The data obtained were statistically analysed [5].

Results and Discussion

Plant growth response

Zinc toxicity symptoms ranging from slight to severe developed on leaves of the Fe-inefficient genotype seven days after treatment with Zn levels ranging from 0.1 to

5.0 mg/l at low and medium Fe levels (0.1 and 1.0 mg/l). Toxicity symptoms did not develop at the highest Fe level (5.0 mg/l). A slight intervenal chlorosis developed on new leaves of the Fe-efficient genotype at the highest Zn and lowest Fe level three weeks after treatments started.

By harvest (40-day-old plants), the symptoms consisted of chlorosis and spotty nechrosis of the primary leaves; with severe interveinal chlorosis, rugosity and nechrosis of the trifoliates of the PI plants grown at the higher Zn levels and low and medium Fe levels. The Fe-efficient genotype HA developed almost the same symptoms on plants grown at the highest Zn and lowest Fe levels. The symptoms observed were very similar to those described by White *et al.* [6].

Dry matter production

The HA genotype produced more dry matter than the PI genotype (Table 1). Increasing Fe level gave an increase in dry matter production of both genotypes with a greater increase with the PI than with the HA genotype. Top dry weight of both genotypes increased with increasing Zn level from 0.1 to 1.0 mg/l and then decreased in going from 1.0 to 5.0 mg/l. The root dry weight increased significantly with increased Zn level up to 5.0 mg/l. The lowest dry matter production was found for plants having the most severe visual symptoms. These results are in agreement with those obtained by Brown [7] and Clarck [8].

Table 1. Dry matter yield and of soybean plants at different levels of iron and zinc

Treatment		Tops		Roots	
Fe	Zn	HA	PI	HA	PI
μg/ml		g. dry wt./plant			
0.1	0.1	3.52*[d]	2.13[g]	0.48[f]	0.42[f]
	1.0	3.52[d]	2.16[g]	0.56[d]	0.48[e]
	5.0	2.93[e]	1.86[h]	0.65[b]	0.50[de]
1.0	0.1	3.54[d]	3.16[e]	0.53[e]	0.52[d]
	1.0	3.78[bc]	3.32[d]	0.61[c]	0.55[c]
	5.0	3.58[d]	2.28[f]	0.70[a]	0.55[c]
5.0	0.1	3.70[c]	3.72[b]	0.54[de]	0.54[c]
	1.0	4.11[a]	3.90[a]	0.63[bc]	0.59[b]
	5.0	3.84[b]	3.48[c]	0.71[a]	0.61[a]

* Values are means of four replicates. Values within a column followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's Multiple Range Test. Underscored values are not significantly different at $P = 0.05$.

Phosphorus content

The P content of the plants is given in Table 2. The highest P contents were obtained at the lowest Fe and highest Zn levels. The PI genotype had higher P in the plant tops than the HA genotype at low Fe levels, and lower P than HA at high Fe levels. These results agree quite well with the visual symptoms, in that chlorotic plants accumulated more P than the non-chlorotic ones. The lowest P content of the tops was associated with the highest dry matter production.

Table 2. Phosphorus content of soybean plants at different levels of iron and zinc

Treatment		Tops		Roots	
Fe	Zn	HA	PI	HA	PI
μg/ml		mg. P/g dry wt.			
0.1	0.1	4.82*[e]	6.97[a]	10.07[d]	7.62[d]
	1.0	5.08[d]	5.75[c]	12.27[ab]	9.10[bc]
	5.0	5.75[b]	6.32[b]	12.40[a]	9.27[bc]
1.0	0.1	4.80[e]	5.45[c]	9.97[d]	7.57[d]
	1.0	5.10[d]	4.82[d]	11.92[bc]	8.90[c]
	5.0	6.20[a]	6.55[b]	12.25[ab]	11.65[a]
5.0	0.1	5.23[cd]	4.65[de]	9.87[d]	7.62[d]
	1.0	5.10[d]	4.40[e]	11.72[c]	9.42[b]
	5.0	5.30[c]	4.87[d]	11.72[c]	9.40[b]

* Values are means of four replicates. Values within a column followed by the same letter are not significantly different at P = 0.05 according to Duncan's Multiple Range Test. Underscored values are not significantly different at P = 0.05.

Phosphorus content of the roots did not follow exactly the same trend as the tops. However, the highest P contents were found at the lowest Fe and the highest Zn treatments.

These results are in accordance with the observations of [9] that Fe-inefficient sorghum plants accumulated more P in the tops than Fe-efficient plants at low levels of Fe. With the Fe-inefficient sorghum, P dominated the system because the Fe-stress-response mechanism was not present to counteract the interference of P on Fe uptake.

Iron content

The iron content data are given in Table 3. From these data it is clear that plant Fe contents are sensitive to both Fe and Zn treatments. In both genotypes, Fe content increased at any level of Zn with increasing Fe in the nutrient solution. However, the Fe content of the plant tops tended to increase and then decrease with increasing Zn level. For plant roots, increased Zn levels in the nutrient solutions had a tendency to give reduced Fe contents in roots of both genotypes. These results are in agreement with those obtained by Ambler *et al.* [10]. They found that Zn suppressed the formation of reductants in the roots and consequently reduced Fe uptake by soybean roots. The decrease occurring in Fe content of both HA and PI soyabean plants with 5.0 mg/l Zn were offset by increasing Fe level in the nutrient solution.

Table 3. Iron content of soybean plants at different levels of iron and zinc

Treatment		Tops		Roots	
Fe	Zn	HA	PI	HA	PI
μg/ml		μg Fe/g dry wt.			
0.1	0.1	85.18*c	59.79f	187.50d	106.25d
	1.0	92.41d	59.42f	186.25d	98.75de
	5.0	70.73f	47.39g	158.75f	96.25e
1.0	0.1	95.34c	73.97d	196.25c	143.75b
	1.0	96.03c	81.70c	195.00c	137.50b
	5.0	86.76e	63.81e	166.25e	128.75c
5.0	0.1	99.71b	95.61b	298.75a	236.25a
	1.0	105.43a	99.28a	296.25a	236.25a
	5.0	105.99a	94.06b	290.00b	230.00a

* Values are means of four replicates. Values within a column followed by the same letter are not significantly different at P = 0.05 according to Duncan's Multiple Range Test. Underscored values are not significantly different at P = 0.05.

The Fe content data agrees with the visual symptoms observed and dry matter production (Table 1) in that the lowest dry matter production was for those plants having the lowest Fe content and showing Fe-deficiency chlorosis. The relationship between Fe content and top dry weight is highly and positively correlated ($r = 0.97$). This data indicates an increasing trend in dry matter production up to an Fe content in the tops of 105 μg/g (Fig. 1).

Total Fe uptake (Table 4) shows greater differences between the two genotypes than does Fe content. Because of the greater dry matter yield and Fe content of the HA plants at lower Fe levels, total Fe uptake was more than two fold greater than that of the PI plants. However, at the highest Fe levels, total uptake by the PI plants was closer to that of the HA.

The amount of dry matter produced per unit of Fe taken up (Table 5) decreases as Fe level in the nutrient solution increases. Such an effect would be expected as the Fe content increases above the minimum amount required for the plant. The

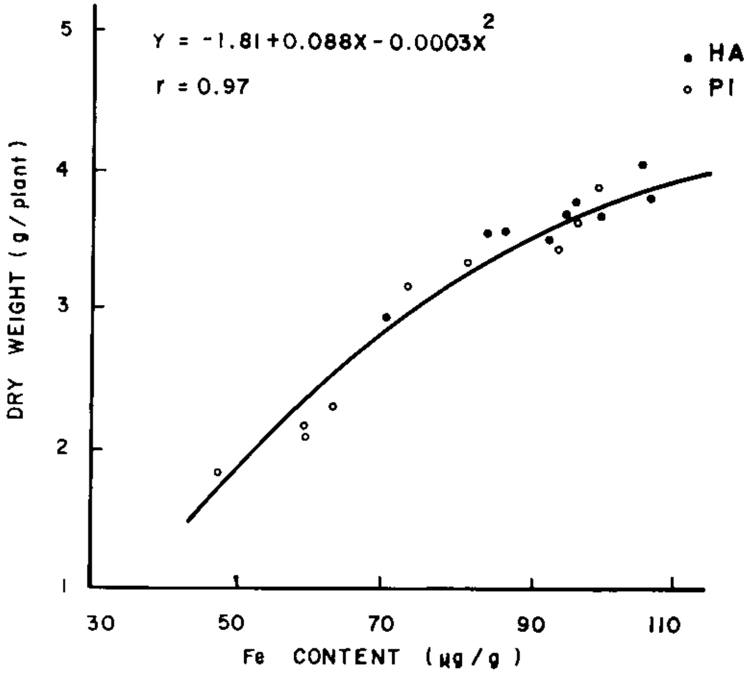

Fig. 1. Relationship between iron content and dry weight of soybean plant top

Table 4. Total iron uptake of soybean plants at different levels of iron and zinc

Treatment		Fe uptake					
		Tops		Roots		Total	
Fe	Zn	HA	PI	HA	PI	HA	PI
— μg/ml —		— μFc/plant —					
0.1	0.1	299.74[f]*	127.66[g]	89.52[f]	44.64[d]	389.26[f]	172.30[g]
	1.0	325.03[e]	129.35[g]	104.79[e]	47.67[d]	429.82[de]	176.02[g]
	5.0	207.61[g]	88.17[h]	102.40[e]	48.15[d]	310.01[g]	136.32[h]
1.0	0.1	338.23[d]	234.31[e]	104.51[e]	74.36[c]	442.74[d]	308.67[e]
	1.0	363.24[c]	271.83[d]	119.95[d]	75.96[c]	483.19[c]	347.79[d]
	5.0	311.01[f]	145.85[f]	115.96[d]	71.44[c]	426.97[e]	271.28[f]
5.0	0.1	369.20[c]	356.15[b]	160.54[c]	126.96[b]	529.73[b]	483.11[b]
	1.0	433.25[a]	387.16[a]	186.67[b]	138.75[a]	619.92[a]	525.91[a]
	5.0	407.21[b]	328.02[c]	205.16[a]	140.31[a]	612.37[a]	468.33[c]

* Values are means of four replicates. Values within a column followed by the same letter are not significantly different at P = 0.05 according to Duncan's Multiple Range Test. Underscored values are not significantly different at P = 0.05.

increase in dry matter production per unit of Fe with an increase in Zn level may indicate that Zn is not interfering with Fe utilization but rather with uptake and translocation. Increasing Zn level from 0.1 to 1.0 mg/l seems to have synergistic effect on Fe accumulation in the plant tops and on total plant uptake, while at the highest level of Zn (5.0 mg/l), the amount of Fe in the tops and the total plant uptake was reduced indicating an antagonistic effect of Zn on Fe.

Table 5. Dry matter per unit of Fe and Top Fe/Total Fe of soybeans.

Treatment		Dry matter/Unit Fe				Fe in Tops/Total Fe	
		Tops		Roots			
Fe	Zn	HA	PI	HA	PI	HA	PI
μg/ml		mg/μg Fe					
0.1	0.1	11.74	16.72	5.36	9.41	0.77	0.79
	1.0	10.82	16.83	11.75	10.07	0.75	0.73
	5.0	14.14	21.10	6.35	10.38	0.67	0.64
1.0	0.1	10.49	13.52	5.36	6.99	0.76	0.76
	1.0	10.41	12.24	5.09	7.24	0.75	0.78
	5.0	11.52	15.67	6.04	7.70	0.73	0.67
5.0	0.1	10.03	10.46	3.36	4.25	0.70	0.74
	1.0	9.49	10.07	3.37	4.25	0.70	0.74
	5.0	9.43	10.63	3.46	4.35	0.66	0.70

The fraction of Fe translocated to the tops decreased with increasing Zn level at all Fe levels. This effect would seem to indicate that Zn may interfere with Fe translocation. This effect is also indicated by an increase in uptake by the roots with an increase in Zn level. At the highest Fe level, there may still be an effect of Zn on Fe translocation but there is enough Fe uptake to provide an adequate supply to the plant tops.

Zinc content

Zinc content tended to increase with increasing Zn level in the nutrient solution at any given Fe level. Increasing Fe supply up to 5.0 mg/l had no significant effect on the Zn content of tops and roots at the 0.1 mg/l Zn treatment in both genotypes. However, at 1.0 and 5.0 mg/l zn in the nutrient solution, Zn content was significantly reduced as the Fe level was raised from 0.1 to 5.0 mg/l (Table 6).

The effect of Fe level on Zn content is in marked contrast to the effect of Zn on Fe content (Table 3). Zinc seems to interfere with Fe uptake and translocation at the lowest Fe and highest Zn levels, thereby creating an Fe deficiency. Iron, however, interferes with Zn uptake only at the highest Zn levels, thereby preventing a toxicity.

Table 6. Zinc content of soybean plants at different levels of iron and zinc

Treatment		Tops		Roots	
Fe	Zn	HA	PI	HA	PI
μg/ml		μg Zn/g dry wt.			
0.1	0.1	39.19^{*g}	37.48^{f}	33.25^{f}	26.75^{e}
	1.0	103.57^{d}	78.50^{d}	130.75^{d}	82.50^{c}
	5.0	329.02^{a}	150.89^{b}	631.00^{a}	567.75^{a}
1.0	0.1	37.77^{g}	35.24^{f}	30.75^{f}	26.25^{e}
	1.0	68.86^{e}	66.31^{e}	72.00^{e}	70.75^{d}
	5.0	306.23^{b}	189.06^{a}	626.00^{b}	567.25^{a}
5.0	0.1	38.25^{g}	36.36^{f}	31.00^{f}	25.00^{e}
	1.0	64.42^{f}	66.22^{e}	71.25^{e}	67.50^{d}
	5.0	128.85^{c}	130.28^{c}	355.00^{c}	353.75^{b}

* Values are means of four replicates. Values within a column followed by the same letter are not significantly different at P = 0.05 according to Duncan's Multiple Range Test. Underscored values are not significantly different at P = 0.05.

These results are in agreement with those obtained by Reddy *et al.* [11]. They reported that the depression of Zn uptake and translocation to shoots of soybean plants was of higher magnitude at higher levels of Fe and Mn in the nutrient media. They concluded that interactions between Zn and Fe as well as Zn and Mn occur during uptake and translocation processes. On the other hand, Lee *et al.* [12] reportd that competition for root absorption sites existed between Zn and Fe. Zinc appeared to interfere with Fe uptake, whereas Fe did not interfere with Zn uptake. In this study, it appears that Fe interfered with Zn uptake only at high plant contents of Zn.

In summary, plants subjected to excessive Zn in the nutrient solution can suffer Fe deficiency chlorosis not only from low levels of Fe, but also from an inability to utilize available Fe present. Additional Fe added to the growth media can overcome the adverse effects of Zn. In order to prevent Fe deficiency chlorosis not only the absolute amount of Fe in the growing media must be considered, but also the ratio of Fe to Zn.

The Fe-inefficient genotype, PI, is more sensitive to high amounts of Zn in the nutrient solution than the Fe-efficient genotype HA. Although foliar analysis is quite effective in diagnosing Zn or Fe deficiencies, foliar Fe content appears to be a poor indicator of Fe availability in the growing media because of the Zn-Fe interactions.

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اختلاف درجة استجابة صنفين من نبات فول الصويا لنقص الحديد الناتج عن زيادة الزنك في وسط النمو

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ملخص البحث. أجريت التجربة في محلول مغذي تحت ظروف محكمة في الصوبة الزجاجية لدراسة تأثير مستويات مختلفة من الزنك (١,٠، ١,٠، ٥,٠ مجم/لتر) مع مستويات مختلفة من الحديد (١,٠، ١,٠، ٥,٠ مجم/لتر) على النمو وامتصاص الحديد لصنفين من فول الصويا PI - 54619-5-1 (PI), Haw-keye (HA).

وقد أوضحت الدراسة أن زيادة تركيز عنصر الزنك في وسط النمو أدى إلى اختزال نمو النبات في كلا الصنفين مع ظهور أعراض الإصفرار على النباتات خاصة في التركيز المنخفض من الحديد، ويرجع ذلك أساسًا إلى أن زيادة الزنك تؤدي إلى نقص امتصاص الحديد وانتقاله من المجموع الجذري إلى المجموع الخضري، وربما يكون قلة انتقال الحديد أكثر من معدل الامتصاص مما أدى إلى تراكم الحديد في الجذور. وأظهر الصنف HA كفاءةً أكثر في امتصاص الحديد من الصنف PI عند أي مستوى من الحديد والزنك في المحلول المغذي، وقد أدت زيادة تركيز الحديد في وسط النمو إلى التغلب على التأثير المعاكس لعنصر الزنك على امتصاص الحديد ونمو النباتات.