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Effect of Zinc on Iron-Stress-Response Mechanism of Two Soybean Genotypes

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Abstract. Zinc-toxicity enhanced the Fe-stress-response mechanism in Hawkeye (Fe-efficient) but not in PI-54619-5-1 (Fe-inefficient) soybean plant. Hawkeye plants were more effective in reducing the pH of the culture solutions and in taking up Fe than the PI plants. The drop in solution pH seems to occur cyclically in response to Fe insufficiency induced by high zinc levels. The severity and nature of stress symptoms induced by high levels of zinc depends on the plant cultivar.

Introduction

Heavy metals may cause various degrees of Fe deficiency to develop in plants. Rosell and Ulrich [1] reported that increasing the Zn supply to sugarbeets from 0 to 12 ppm in the nutrient solution resulted in a reduction in Fe concentration in beet leaves from 900 to 90 ppm. Zinc toxicity in crop plants also causes chlorosis of the leaves which can be alleviated by increased supply of Fe; in this case, however, the zinc-induced chlorosis is not correlated with a decreased transport of Fe to the levels [2]. It is suggested that chlorosis may arise from Fe/Zn competition for an Fe-requiring step in the biosynthesis of chlorophyll.

Plant species and cultivars differ in their ability to utilize inorganic Fe and Fe-chelates for Fe nutrition. Marschner *et al.* [3] reported that the so called Fe-efficient plant species are able to lower the pH of the nutrient medium and to increase the reducing capacity of the root surface under Fe stress conditions, upon which Fe availability and absorption increase rapidly.

The objective of the study reported here was to determine if the Fe-stress-response mechanism (lowering the pH) is enhanced more in Fe-efficient than in Fe-inefficient cultivars when they were grown at various levels of Zn in solution cultures, thereby affecting the absorption and transport of Fe to the plant tops.

Materials and Methods

Soybean seeds *Glycine max* (L.) Merr. of Fe-efficient Hawkeye (HA) and Fe-inefficient PI-54619-5-1 (PI) genotypes were sterilized prior to planting by a treatment with 75% Ethanol (V/V) for three minutes followed by extensive rinsing with distilled water. The seeds were inoculated with *Rhizobium japonicum* in the form of commercial preparation (Agway, Inc. Syracuse, N.Y.). The inoculated seeds were germinated on prewashed sand. Seven days after, three seedlings were transplanted into 3.8 L plastic pot containing half strength Hoagland solution No.1 (N as NO_3^- only). Fourteen days after planting and thereafter, the solution was changed to the treatment solutions. Three levels of Zn (0.1, 0.5 and 1.0 mg L^{-1}) were used with Hoagland solution No. 1 to prepare the solution Treatment. The treatment solutions were adjusted to pH = 6.0 with KOH. The pH of the culture solution was measured daily without any pH adjustment after that. Plants were grown in open glasshouse on a 16 hr/8 hr day/night regime. Illumination was provided by daylight, supplemented with incandescent lamps. Eight days after treatment, plants were harvested and tops and roots 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. Iron and Zn determinations were made by atomic absorption spectrophotometry. The data were statistically analysed [4].

Results and Discussion

A – General observations

Plants of the Fe-efficient genotype (HA) showed no symptoms during the experimental period, except a slight chlorosis observed on those plants treated with the highest Zn level (1.0 mg L^{-1}). In contrast, plants of the Fe-inefficient genotype (PI) developed chlorosis after about five days of treatment except for those plants grown at the lowest Zn level (0.1 mg L^{-1}).

At harvest, there were pronounced differences between HA and PI plants at the medium and the high Zn treatments. Hawkeye plants grew very well whereas PI plants were severely chlorotic.

B – pH changes

The soybean genotypes responded differently to Zn levels in affecting the pH of the growing media. Hawkeye plants decreased the pH of solution culture when grown at high Zn levels, while the pH of the PI growing media increased in the eight days period (Fig. 1). The HA plants apparently released enought H^+ ions from their roots when they were under the stress of high Zn level. The pH of their root zone was lowered which favors Fe solubility and uptake. The reduction in pH was most pronounced at the highest concentration of Zn in solution and seems to occur cyclically, probably in response to Fe deficiency stress. The Fe-inefficient genotype PI plants were not able to reduce the solution pH even at high Zn level. These plants developed visual symptoms which were probably due to an Fe insufficiency.

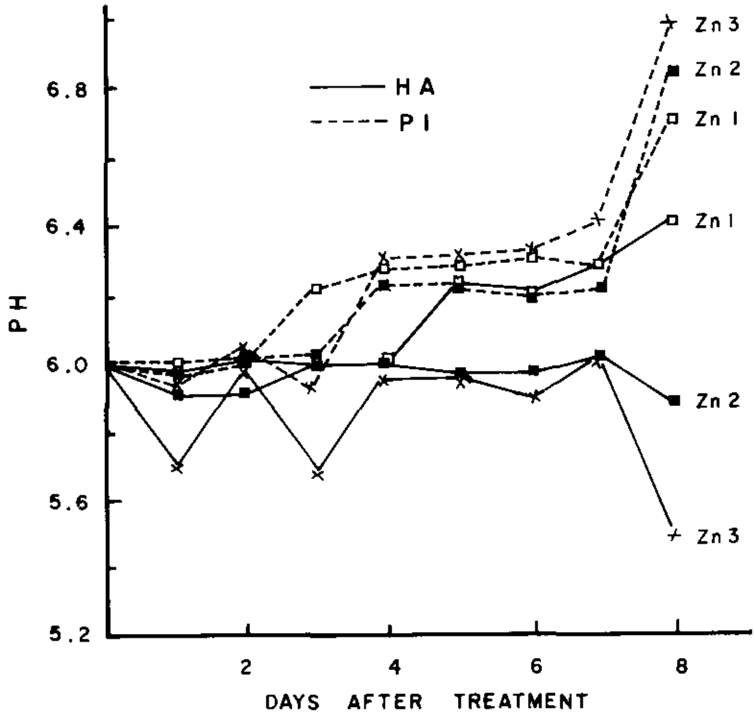

Fig. 1. pH of solution cultures as affected by soybean genotypes and zinc level

As a result of NO_3^- absorption, OH^- ions are excreted by plant roots which may result in an increase in solution pH and a decrease in Fe solubility and availability for plants. Both genotypes, however, were able to take up and translocate adequate amounts of Fe at the lowest Zn level in solution culture without suffering Fe deficiency. These results are in agreement with those reported by Ambler *et al.* [5]. They concluded that Fe-stress-response in plants is an adaptive response to Fe deficiency, which causes metabolic changes that favor Fe uptake and utilization. The release of H^+ ions from roots of Fe-efficient plants is probably one of the factors involved in the Fe-stress-response [6].

C – Dry matter

At the lowest Zn level (0.1 mg L^{-1}), both HA and PI soybean genotypes produced the same amount of dry matter (Table 1). Dry matter production decreased with increasing Zn level for both genotypes, but the reduction was greater in the PI. Initially, the HA plants did not show Fe deficiency symptoms, but at later stage of growth the plants at the highest Zn level showed some Fe deficiency chlorosis.

Table 1. Dry matter yield and phosphorus, iron, and zinc content of soybean plants at different zinc levels in solution culture.

Zn	Dry matter		P		Fe		Zn	
level	HA	PI	HA	PI	HA	PI	HA	PI
μg/ml	g/plant		%		μg/g			
				Tops				
0.1	0.76 a	0.76 a	0.89 a	0.71 a	98.33 a	81.67 a	80.00 c	63.33 c
0.5	0.67 b	0.61 b	0.83 b	0.68 a	81.67 b	63.33 b	113.33 b	101.67 b
1.0	0.65 b	0.55 c	0.81 b	0.61 b	63.33 c	41.67 c	151.67 a	121.67 a
				Roots				
0.1	0.20 a	0.18 a	1.08 a	1.04 b	1000.00 b	986.70 c	100.00 c	58.33 c
0.5	0.19 a	0.15 b	1.16 a	1.12 ab	1000.00 b	1210.00 b	168.33 b	161.67 b
1.0	0.16 b	0.12 c	1.26 a	1.17 a	1416.70 a	1433.30 a	530.00 a	303.33 a

* Values are means of three replicates. Means for a given plant part within a genotype followd 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 difference observed in response between HA and PI plants suggests that HA plants were able to take up enough Fe to withstand the adverse effect of increasing Zn on Fe uptake and translocation. The Zn effect observed in this study is in agreement with the observations of Lingle *et al.* [7]. They reported that Zn may be acting as a metabolic poison to some key essential to Fe absorption.

D – Iron content

Increasing levels of Zn in the growing medium reduced significantly Fe content of both HA and PI soybean plant tops. At any given level, HA plant tops contained significantly higher amounts of Fe than those of the PI. This may explain why the PI soybean plants developed Fe deficiency chlorosis and produced less dry matter than those of HA soybean plants. The greatest top dry matter production was obtained when Fe content of plant tops was the highest (Table 1). There was a highly positive correlation between top dry matter production and Fe content of plant tops (r = 0.89, Fig. 2).

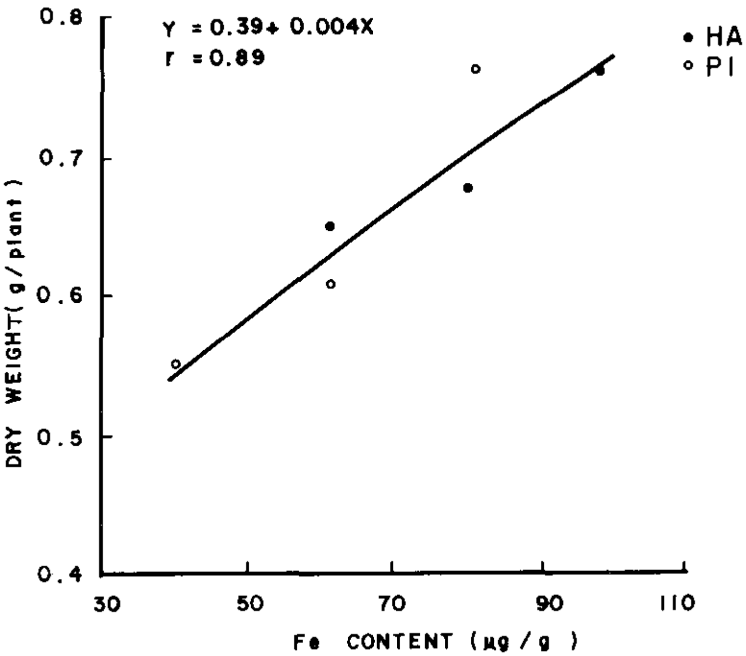

Fig. 2. Relationship between iron content and dry weight of soybean plant top

Increasing Zn level in solution culture increased Fe retention in the roots and affected its translocation to the plant tops. Hawkeye plants were able to overcome this inability effect of Zn and translocated more Fe to the plant than did PI plants (Table 2).

Similar results were obtained by Ambler *et al.* [8]. They concluded that Zn interferes with the translocation of Fe by inhibiting the reducing capacity of the root or accentuates other reactions detrimental to Fe transport. Cumbus *et al.* [9] reported that Zn may act as a competitive cation for Fe in active metabolic transport particularly at the loading site into the stem of watercress plants.

The amount of dry matter produced per unit Fe taken up increased with increasing Zn level in solution culture from 0.1 to 1.0 mg L^{-1}, which may indicate that the adverse effect of Zn on Fe translocation was much greater than on dry matter production (Table 2).

Table 2. Total iron uptake, Dry matter/Unit Fe, and Top Fe/Total Fe of HA and PI soybean genotypes at different levels of zinc in solution culture.

Zn	Fe uptake						Dry matter/Unit Fe				Top Fe/Total Fe	
	Top		Root		Total		Top		Root			
Level	HA	PI	HA	PI	HA	PI	HA	PI	HA	PI	HA	PI
μg/ml	μg Fe/plant						mg/μg Fe					
0.1	74.43a*	62.12a	196.67b	177.67a	271.10a	239.78a	10.17	12.24	1.00	1.01	0.27	0.26
0.5	54.72b	38.65b	186.67b	185.60a	241.38b	224.25a	12.22	15.79	1.00	0.83	0.22	0.17
1.0	41.37c	22.95c	231.33a	176.83a	272.70a	199.78b	15.79	23.99	0.70	0.70	0.15	0.12

* Values are means of three 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.

E – Zn content

Increasing Zn level in the nutrient solution resulted in increased Zn content in tops and roots of both soybean genotypes. The Zn content of HA plants was greater than that of PI plants at any Zn level (Table 1). With increasing Zn level, the total Zn uptake increased in tops and roots of both HA and PI genotypes. This increase in total Zn uptake is due to a greater increase inZn content than the decrease in dry matter with increasing Zn level. The fraction of total Zn translocated to plant tops decreased with increasing Zn level in the nutrient solution. This may indicate that the accumulation of Zn in plant tops is less than the amount accumulated by roots (Table 3).

Increasing Zn content and total uptake with increasing Zn level tended to result in decreased Fe content and translocation to the plant top. The reduction in dry matter production that occured may also be due to the presence of high amount of Zn that could have been toxic to the soybean plants. Another possibility is that Zn may have interfered with Fe metabolism resulting in the visual symptoms that were observed at harvest.

The increased Zn/Fe ratio in the plant tops in response to increasing Zn level suggests the presence of excess Zn and/or deficiency of Fe within the plant where Zn may compete and/or interfere with Fe in some metabolic function. These results are in agreement with those of Agarwala *et al.* [10] and Olsen and Brown [11].

In conclusion, it is clear from the data obtained that the Fe-inefficient genotype PI was more susceptible to high levels of Zn than the Fe-efficient genotype HA.

Table 3. Total zinc uptake, dry matter production/Unit Zn, and Top Zn/Total Zn of HA and PI soybean plants grown at different zinc levels in solution culture.

Zn level	Zn uptake		Dry matter/Unit Zn		Top Zn/Total Zn	
	HA	PI	HA	PI	HA	PI
μg/ml	—— μg/plant ——		—— mg/μg Zn ——			
			Tops			
0.1	60.80*	48.13	12.50	15.79	0.75	0.82
0.5	75.93	62.02	8.82	9.83	0.70	0.72
1.0	98.59	66.92	6.69	8.21	0.54	0.65
			Roots			
0.1	20.00	10.50	10.00	17.14		
0.5	31.98	24.25	5.94	6.18		
1.0	84.80	36.40.	1.89	3.30		

* Values are means of three replicates.

Hawkeye plants were able to tolerate higher levels of Zn without suffering Fe insufficiency which may suggest some genetic differences between the two genotypes enabling HA plants to tolerate higher Zn levels. The greater Fe-efficiency of HA compared with PI soybean plants was related to the development of a low pH in the root zone. This drop in solution pH seems to occur cyclically in response to Fe stress induced by high Zn level and resulted in greater efficiency in the uptake and translocation of Fe to the plant tops.

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References

[1] Rosell, R.A. and Ulrich, A. "Critical Zinc Concentrations and Leaf Minerals of Sugarbeet Plants." *Soil Sci.*, **97** (1964), 152–167.

[2] Rosen, J.A., Pike, C.S. and Golden, M.L. "Zinc, Iron, and Chlorophyll Metabolism in Zinc-Toxic Corn." *Plant Physiol.*, **59** (1977), 1085–1087.

[3] Marschner, H., Kalish, A. and Romheld, V. Mechanism of Iron Uptake in Different Plant Species. In: Whermann, J. (ed.) *7th Int. Coll. Plant Anal.* Fertilizer Problems, Hannover, 273–281 (1974).

[4] Steel, R.G.D. and Torrie, J.H. *Principles and Procedures of Statistics*. 2nd ed. N.Y.: McGraw-Hill Book Company, Inc., 1980.

[5] Ambler, J.E., Brown, J.C. and Gauch, H.G. "Sites of Iron Reduction in Soybean Plants." *Agron.J.*, **63** (1971), 95–97.

[6] Brown, J.C. and Jones, W.E. "pH Changes Associated with Iron-stress Response." *Physiol. Plant.*, **30** (1974), 148–152.

[7] Lingle, J.C., Tiffin, L.O. and Brown, J.C. "Iron Uptake-translocation of Soybean as Influenced by Other Cations." *Plant Physiol.*, **38** (1963), 71–76.

[8] Ambler, J.E., Brown, J.C. and Gauch, H.G. "Effect of Zinc on Translocation of Iron in Soybean Plants." *Plant Physiol.*, **46** (1970), 320–323.
[9] Cumbus, I.P., Hornsey, D.J. and Robinson, L.W. "The Influence of Phosphorus, Zinc, and Manganese on Absorption and Translocation of Iron in Watercress." *Plant Soil*, **48** (1977), 651–660.
[10] Agarwala, S.C., Bisht, S.S. and Sharma, C.P. "Relative Effectiveness of Certain Heavy Metals in Producing Toxicity and Symptoms of Iron Deficiency in Barley." *Can. J. Bot.*, *55* (1977), 1299–1307.
[11] Olsen, R.A. and Brown, J.C. "Light Induced Reduction of Fe as Related to Causes of Chlorosis in Cotton." *J. Plant Nutr.*, **3** No. 5 (1981), 767–787.

تأثير الزنك على ميكانيكية استجابة صنفين من فول الصويا للإجهاد الناتج عن نقص عنصر الحديد

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ملخص البحث. أوضحت النتائج المتحصل عليها أن زيادة تركيز عنصر الزنك في المحلول المغذى لدرجة السمية أدت إلى تشجيع استجابة النبات للإجهاد الناتج عن نقص الحديد في الصنف Haw Keye (HA) بينما لم تكن هناك أية استجابة في الصنف (PI) 1 - 5 - 54619 - PI وكانت نباتات الصنف HA أكثر كفاءة في خفض pH المحلول المغذى وامتصاص الحديد بالمقارنة بنباتات الصنف PI ويبدو أن الانخفاض في الـ pH يحدث في صورة دورية أو متعاقبة نتيجة عدم كفاية الحديد في وسط النمو والناتج عن وجود تركيزات عالية من الزنك. لذلك يمكن القول إن شدة وطبيعة الإجهاد ودرجة الاستجابة الناتجة عند المستويات المرتفعة لعنصر الزنك تعتمد على صنف النبات.