Phosphorus Tolerance and Sensitivity of Soybeans as Related to Uptake and Translocation [1, 2]

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Introduction

The differing responses of 'Lincoln' and 'Chief' soybean varieties to high levels of phosphorus in nutrient solution culture were described by Howell (3). Chief, now called a tolerant variety, responded favorably to phosphorus concentrations as high as 3.62 mM, whereas Lincoln, a sensitive variety, was adversely affected by concentrations of 1.61 mM or higher. Total phosphorus in mature Chief plants increased with increasing phosphorus in the nutrient solution throughout the entire range studied, while in Lincoln plants it increased only up to nutrient solution concentrations of 0.72 mM and then decreased greatly because of reduced growth caused by phosphorus toxicity. Symptoms of phosphorus injury appear on the cotyledons and leaves of sensitive plants within 3 days to a week after start of treatment, depending on experimental conditions and the age of the plants.

The objectives of the present study were to (1) compare uptake and translocation of phosphorus by sensitive and tolerant varieties; (2) relate sensitivity to phosphorus content of the tissues; and (3) determine the relative importance of roots and tops in the response to high phosphorus.

Materials and Methods

Soybean seeds (*Glycine max* (L.) Merr.) of the varieties Lincoln and Chief were germinated in sand flats in the greenhouse. By the sixth day the seedlings were usually 1.5 to 3 inches high, and the unifoliate leaves were expanding. At this stage, seedlings were transplanted to nutrient solution containing the concentration of phosphate under study in a growth room. Aluminum foil-wrapped 1-liter beakers with loosely-fitted plastic lids were used as containers. Ten or fewer seedlings, suspended by their cotyledons through holes in the lid, were placed in each beaker. The room was operated at 27° and with constant light from pink and blue fluorescent lamps (ca 1500 ft-c). Relative humidity was about 45 %.

In experiments with grafted plants, seedlings at the transplanting stage were grafted about 1 cm below the cotyledons. The scion was removed by making a V-shaped cut in the hypocotyl. The wedge of the scion was then set in the V-cut of the stock of another plant and secured by wrapping a small piece of pressure-sensitive vinyl-coated tape around the graft. Time between making a cut and joining the scion to a new stock was 1 to 5 minutes. No wax or other grafting aid was used. The grafted plants were grown 1 week in the shade in a growth chamber in the greenhouse at 21 to 24° and 95 to 100 % relative humidity and were then transplanted to beakers of nutrient solution in the growth room or to quartz gravel in the greenhouse. The tape was removed at the time of transplanting. A short length of greenhouse wire tie was wrapped around each grafted plant in the growth room to support it in the plastic lid. In the greenhouse, plants were flooded periodically with nutrient solution.

The nutrient solution was the same as previously described (4) and at the low phosphorus level (.32 mM) contained major salts in the following millimolar concentrations: KCl, 0.60; KH_2PO_4, pH 5.5, 0.32; $(NH_4)_2SO_4$, 0.34; $Ca(NO_3)_2$, 1.22; $MgSO_4$, 2.06.

Iron concentration was 53.7 μM as Fe DTPA. Micronutrient concentrations were Cu 0.16, Zn 0.15, B 5.55, and Mn 2.00 μM. Additional phosphorus when required was supplied as NaH_2PO_4, pH 5.5.

The plants were harvested on the third or the fourth day unless otherwise indicated. Freshly harvested plants were divided into root, shoot, and cotyledons and dried at 80° overnight. Phosphorus was determined by counting P^{32} content of dried plant parts, or by the method of Fiske and SubbaRow (2) on ash solutions prepared as follows: dried plants were charred in 6 N $Mg(NO_3)_2$ and ashed 22 hours at 470°; the ash was taken up in 6 N HCl and made to volume. Radioactivity was measured in a gas flow proportional counter. The amount of phosphorus absorbed was calculated from the specific activity of the nutrient solution. The P^{32} method was more suitable for determining absorbed phosphorus because of the large ratio of total to absorbed phosphorus.

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Table I. *Phosphorus Absorption by 10 Lincoln and Chief Soybean Plants on Low and High Phosphorus*

Each value is average uptake per beaker (10 plants). Phosphorus concentrations of nutrient solutions were 0.32 mM (low) or 3.2 mM (high). Absorbed phosphorus was determined by P^{32} content. In 3 experiments of either 3 or 4 days duration, 41 of 60 Lincoln plants on high P developed symptoms of phosphorus toxicity. These symptoms consist of a reddish brown marginal discoloration and sponginess of the cotyledons; in severe cases the affected area may include the entire cotyledon; as the plants get older the symptoms appear as reddening of the veins and interveinal area followed by necrosis of young expanded leaves.

	Root		Shoot		Cotyledons		Total	
	Low P	High P	Low P	High P	Low P	High P	Low P	High P
				(mg P per 10 plants)				
Lincoln	2.9 ± 0.2	5.4 ± 0.5	3.3 ± 0.3	5.9 ± 0.6	0.8 ± 0.04	2.0 ± 0.2	7.0	13.3
Ratio H:L		1.86		1.79		2.50		1.90
Chief	1.8 ± 0.2	3.1 ± 0.3	2.2 ± 0.3	3.4 ± 0.4	0.5 ± 0.02	1.1 ± 0.03	4.5	7.6
Ratio H:L		1.72		1.55		2.20		1.69

Table II. *Phosphorus Absorption by Lincoln and Chief Soybeans During Development of Phosphorus-Toxicity Symptoms*

P^{32} was applied for 6 hours on the day indicated. Plants were harvested at the end of the 6-hour period. On day 1, P^{32} was given during the first 6 hours after transplanting. Each item is average for 10 plants. Nine of the Lincoln plants on high phosphorus had toxicity symptoms by the end of the experiment.

Variety and Day	Root		Shoot		Cotyledons		Total	
	Low P	High P	Low P	High P	Low P	High P	Low P	High P
				(μg P per plant)				
Lincoln:								
1	15 ± 0.5	27 ± 0.1	3 ± 0.1	10 ± 0.5	1 ± 0.1	2 ± 0.3	19	39
2	36 ± 1.0	63 ± 2.1	11 ± 0.3	22 ± 1.0	6 ± 0.3	13 ± 0.4	53	98
3	35 ± 1.1	68 ± 2.4	13 ± 0.8	34 ± 0.9	4 ± 0.3	10 ± 0.6	52	112
4	39 ± 0.8	69 ± 2.3	17 ± 0.9	36 ± 1.9	3 ± 0.3	5 ± 0.2	59	110
5	25 ± 0.7	82 ± 2.6	17 ± 0.8	44 ± 2.0	2 ± 0.1	8 ± 0.5	44	134
Chief:								
1	15 ± 0.5	22 ± 0.4	5 ± 0 2	7 ± 0.1	1 ± 0.1	2 ± 0.1	21	31
2	31 ± 1.1	46 ± 1.1	13 ± 0.4	19 ± 0 9	7 ± 0.3	11 ± 0.4	51	76
3	28 ± 0.7	38 ± 0.8	14 ± 0.5	18 ± 0.5	4 ± 0.2	2 ± 0.3	46	58
4	24 ± 0.3	41 ± 0.8	13 ± 0.4	16 ± 0.4	1 ± 0.1	2 ± 0.1	38	59
5	25 ± 0.7	56 ± 2.1	14 ± 0.6	27 ± 2.0	1 ± 0.1	3 ± 0.2	40	86

Results

Phosphorus uptake by Lincoln and Chief seedlings during the first 3- to 4-day period after transplanting is shown in table I. This is the period during which initial symptoms of toxicity appear on sensitive plants. In the 3 experiments summarized in table I, cotyledon symptoms appeared on 41 of 60 Lincoln plants on high phosphorus. Lincoln plants took up more phosphorus than Chief on both phosphorus levels and had higher ratios of uptake on high P to that on low P. Chief absorbed only slightly more on high P than Lincoln did on low. These results were confirmed with another pair of varieties, Chippewa (sensitive) and Adams (tolerant).

Phosphorus absorption by Lincoln and Chief plants during 6-hour periods on each of the first 5 days after start of low or high P treatment is shown in table II. Absorption by both varieties is low during the first 6 hours after transplanting, but the difference in variety response to phosphorus level is immediately observed. On low P, uptake by the 2 varieties was similar throughout the 5 days of the experiment. On high P, increased absorption by Lincoln was quickly apparent, and the variety difference increased throughout the period except possibly on the fifth day. In this experiment plants of both varieties absorbed about 2.5 times as much phosphorus on the second day as on the first. Phosphorus uptake by Lincoln on high P continued to increase throughout the period while that by Chief was less consistent.

Lincoln seedling plants grow more rapidly than Chief so the increase in phosphorus absorption by Lincoln during culture on high P as shown in table II might be a reflection of the increasing growth of the plant. However, Lincoln on high P solutions also absorbed more phosphorus per unit fresh weight than Chief (table III). The highest absorption rate by both varieties on both phosphorus levels occurred on the second day, indicating recovery from a slightly phosphorus-starved condition or from the shock of transplanting.

Toxicity symptoms appear occasionally on Chief plants, especially if they are exposed to very high

Table III. *Phosphorus Absorption per Unit Weight by Lincoln and Chief Soybeans During Toxicity Development*

Variety and Day	Root		Shoot		Cotyledons	
	Low P	High P	Low P	High P	Low P	High P
	(μg P per g fr wt)					
Lincoln:						
1	61	120	14	40	1	5
2	124	199	34	63	11	21
3	95	183	27	78	7	17
4	90	151	30	55	5	8
5	44	165	22	59	3	13
Chief:						
1	65	95	20	30	2	4
2	105	172	42	65	14	22
3	85	122	36	41	8	3
4	63	110	24	29	2	4
5	75	146	23	44	2	6

Table IV. *Phosphorus Content of and Development of Toxicity Symptoms by Lincoln and Chief Soybeans on Normal and Very High Phosphorus Levels*

After 4 days of absorption, phosphorus content was determined on the ash by method of Fiske and SubbaRow (2).

Variety and Phosphorus Concentration	Content of				Symptoms per 10 plants
	Root	Shoot	Cotyledons	Total	
(mM) Lincoln:	(mg P per 10 plants)				
0.32	4.9	9.9	3.6	18.4	0
3.2	7.4	13.7	4.9	26.0	8
8.0	9.4	16.0	6.4	31.8	9
16.0	9.1	20.6	6.7	36.4	10
24.0	9.2	18.7	7.0	34.9	10
32.0	11.8	20.7	7.8	40.3	10
Chief:					
0.32	3.2	6.7	3.2	13.1	0
3.2	4.5	9.2	3.2	16.9	1
8.0	4.9	10.2	3.3	18.4	0
16.0	5.0	11.3	4.5	20.8	6
24.0	6.7	12.1	4.5	23.3	9
32.0	5.9	12.5	7.0	25.4	10

Table V. *Phosphorus Toxicity Ratings of Grafted Soybean Plants Grown on High-Phosphorus Solution in the Greenhouse*

These data are the averages for 36 plants: 1, normal; 5, very sensitive.

Genotype of scion	Genotype of stock	Toxicity rating
Lincoln	Chief	1.1
Lincoln	Lincoln	5.0
Chief	Chief	1.0
Chief	Lincoln	4.9

phosphorus concentrations (4). It was therefore of interest to compare the phosphorus contents of Lincoln and Chief plants over a range of phosphorus concentrations sufficient to cause toxicity symptoms on both varieties. Phosphorus levels from 0.32 to 32 mM were used. To compensate for the increase in potassium and sodium added along with the phosphate, control beakers were included with appropriate levels of KCl and NaCl. The additional KCl and NaCl caused no change in phosphorus content of plants. Lincoln plants developed toxicity symptoms as usual on 3.2 mM phosphorus, but Chief plants rarely developed symptoms on levels below 16 mM (table IV). On this treatment phosphorus content of Chief cotyledons averaged 0.45 mg per plant. The minimum nutrient solution phosphorus concentration for development of toxicity symptoms on Lincoln plants is about 1.6 mM (3). The phosphorus content of Lincoln cotyledons on solution of this concentration, as interpolated from the data of table IV, would be about 0.45 mg per plant. Phosphorus percentage (dry weight basis) in cotyledons of both varieties was about 1.2 when symptoms appeared.

The varietal difference in response to phosphorus is determined primarily in the roots. When Lincoln shoots were grafted onto Chief roots and vice versa, the sensitivity response of the top was determined by the genotype of the root (table V). Chief tops grown on Lincoln roots were virtually as sensitive as Lincoln tops. Similarly, Lincoln tops grown on Chief roots showed no significant sensitivity.

Table VI. *Phosphorus Accumulation by Grafted Soybean Plants in the Growth Room*

The absorption period was 3 days. Data are the averages for 2 composite ash samples of 6 plants each. Accumulated phosphorus was determined by P^{32} content.

Plant part	Chief on Lincoln		Chief on Chief		Lincoln on Chief		Lincoln on Lincoln	
	Low P	High P	Low P	High P	Low P	High P	Low P	High P
	(mg P per plant)							
Root	0.17	0.35	0.10	0.17	0.12	0.25	0.17	0.35
Shoot	0.17	0.30	0.12	0.17	0.15	0.22	0.15	0.27
Cotyledons	0.07	0.17	0.03	0.08	0.08	0.17	0.10	0.22

Phosphorus uptake by grafted plants during 3 days in the growth room also reflected the genotype of the root (table VI). Lincoln roots accumulated more phosphorus than Chief roots on both low and high P and regardless of the genotype of the attached shoot. The shoots of both varieties accumulated more phosphorus on Lincoln roots than on Chief. The cotyledons, which were above the graft and therefore of the same genotype as the shoot, accumulated less phosphorus than roots and shoots, but also seemed to reflect the root genotype.

Discussion

The reaction of a plant as tolerant or sensitive to high phosphorus seems to be determined largely by the amount of phosphorus in the plant. Chief plants, which ordinarily tolerate high solution levels of phosphorus, developed phosphorus-toxicity symptoms when the phosphorus content of the cotyledons reached about 0.45 mg per plant, or 1.2% (dry wt). This is about the level that causes cotyledon symptoms on Lincoln. However, a phosphorus concentration of about 16 mM was required in the nutrient solution to produce this internal level in Chief while only about 1.6 mM gave a similar effect in Lincoln.

The critical interaction of variety and external phosphorus supply appears to occur in the roots or at the root-nutrient medium junction. When grafted plants were grown in a high-phosphorus medium, both Chief and Lincoln tops on Lincoln roots developed severe toxicity symptoms and contained comparable amounts of phosphorus. When Chief roots were used, tops of neither variety developed toxicity symptoms nor did they contain as much phosphorus as on Lincoln roots.

Metabolic differences related to the phosphorylation system may also contribute to the difference in varietal response (Howell, manuscript in preparation), but it would appear that any such metabolic mechanism would have to exert its controlling action in the roots or in the active uptake of phosphorus by the roots.

The greater responsiveness of sensitive varieties to phosphorus concentration at low and intermediate levels may have favored selection of these varieties over competing lines in experimental plant breeding programs. It has been noted that increasing the phosphorus supply within normal ranges stimulates uptake by the sensitive variety Lincoln, more than by the tolerant variety Chief and that increased phosphorus stimulates vegetative growth of Lincoln more than that of Chief during the first few days of treatment. Conceivably the greater responsiveness of sensitive varieties is an advantage under field conditions. It may be significant that nearly all soybean varieties currently recommended in the northern half of the United States are sensitive to high phosphorus (4) although most of these varieties are derived from crosses involving Lincoln and a tolerant parent which produce equal numbers of sensitive and tolerant lines (1).

Summary

Phosphorus nutrition of soybean varieties tolerant (Chief) and sensitive (Lincoln) to high phosphorus was studied during the period of toxicity symptom development. Increasing the phosphorus supply stimulated P uptake by the sensitive variety more than by the tolerant, and the varietal difference was evident during the first 6 hours of high-phosphorus treatment. Characteristic symptoms of toxicity occurred when phosphorus content of the cotyledons reached 0.45 mg per plant (1.2% dry wt). The sensitive variety attained this level on an external concentration of about 1.6 mM; the tolerant variety required 16 mM. When tops of the tolerant variety were grafted to roots of the sensitive variety, the tolerant variety developed phosphorus toxicity symptoms on a nutrient solution phosphorus concentration of 1.6 mM. Conversely, the tops of the sensitive variety accumulated less phosphorus and did not develop toxicity symptoms when grafted to roots of the tolerant variety. The critical genotypic difference in phosphorus nutrition thus appears to reside in the roots. The tolerance of the tolerant variety is due primarily to reduced phosphorus accumulation.

Literature Cited

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