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SHORT COMMUNICATION

Phosphorylated compounds in soybeans [*Glycine max* (L.) Merr.] as affected by phosphorus levels in solution*

Summary

Phosphorylated compounds in four varieties of soybeans (Lincoln and Clark, sensitive; Chief and L9, tolerant) were studied in relation to plant sensitivity to high phosphorus supply as indicated by reduced dry matter yield. Total P in all tissues indicated that sensitive varieties took up P from solution more readily than tolerant ones, especially at low P levels. Tolerant varieties had a greater protein synthesis rate at high solution P concentration, while sensitive varieties reached a maximum rate at a lower P concentration.

Introduction

Although P sensitivity in plants has been investigated by several workers [2 3 4 6 7 11], the relationship of P-containing compounds to P response has not been adequately studied. Phosphorylated compounds themselves are unique in role and ubiquitous in plant metabolism. Previous experiments [5 7 8] showed that P uptake and content of various P fractions were related to the P sensitivity and tolerance of some soybean varieties to high P supply.

The objectives of the present study were: first, to investigate the effects of P supply on phosphorylated compounds in different tissues of sensitive and tolerant soybean varieties, and second, to attempt to explain the results in terms of a mechanism for P sensitivity.

Materials and methods

Four varieties of soybean,** Lincoln and Clark (sensitive) and Chief and L9 (tolerant) were selected because of their distinctive differences in sensitivity and tolerance to high P treatment. Phosphorus content of weight-selected soybean seeds showed no difference among varieties.

Plants were grown in a controlled environment chamber using a split-

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** Seed was obtained from the U.S. Regional Soybean Laboratory, ARS, USDA, Urbana, Illinois. L9 is a composite of three F_3 lines from Clark[6] × Chief selected for P-tolerance and developed by Dr. R. L. Bernard.

medium technique with continuous aeration of the solution*. Hoagland's minus-P nutrient solution of one-tenth strength was used. Duplicate samples of four plants were grown for each of five P treatments (0 to 4.0 mg P/liter, applied as $NaH_2PO_4.H_2O$). New solutions were provided every other day to maintain the P concentration as constant as possible. Plants were harvested 28 days after germination; separated into roots, stems, trifoliolate leaves, and terminal buds; frozen with liquid nitrogen; and lyophilized.

The general scheme for extracting phosphorylated compounds was presented previously [8]. Inorganic phosphate was determined by the ascorbic acid method [9]. Content of RNA–P and DNA–P were measured by UV absorption at 260 and 268 nm, respectively, after standardizing against P and sugar content procedures.

Results and discussion

Plants usually respond with increased growth to increasing level of P supply, but when sensitive to high P, exhibit reduced growth. The results in Table 1 show that with increasing P concentration up to 3.0 mg P per liter in solution, dry weights increased significantly for all four varieties of soybeans grown. The sensitive varieties, Lincoln and Clark, decreased at 4.0 mg P per liter, while the tolerant varieties, Chief and L9, continued to gain. Previous data [8] had shown Chief to increase in weight even up to 18 mg P per liter in solution. Reduction in growth for the sensitive varieties was not accompanied by toxicity symptoms, such as severe stunting, browning of leaves, chlorosis, or leaf drop.

TABLE 1

Yield of plant tops (mg dry weight*) for four soybean varieties grown at various P concentrations

P concentration mg/l	Lincoln	Clark	Chief	L9
0.0	360	430	210	450
1.0	530	640	470	760
2.0	640	790	570	810
3.0	670	870	630	830
4.0	520	780	690	990
LSD (.05)	95	80	60	90

* Mean of four plants.

* For experimental details see: Lee, K. W. 1968. Phosphorus fractions and growth of the soybean plant as affected by phosphorus supply. Ph.D. Thesis, University of Minnesota, St. Paul.

Total P content in the youngest trifoliolate leaves, as shown in Table 2, indicated that sensitive varieties took up P from solution more readily than tolerant ones, especially at low P levels. Similar stimulation in P uptake by sensitive varieties was also shown by Foote and Howell [5]. The varietal difference in total P was also noticeable in other tissues, especially in the roots (data not shown), whose P status controls the general P metabolism of the whole plant [10].

TABLE 2

Total P, acid-soluble organic-P, and phosphatide-P fractions (μg/mg dry wt) in trifoliolate leaves*

P concentration mg/l	P content**					
	PT	*Ps*	*Pt*	*PT*	*Ps*	*Pt*
	Lincoln			Chief		
0.0	2.58	0.06	0.21	2.70	0.12	0.26
1.0	4.12	0.14	0.53	3.45	0.12	0.29
2.0	5.01	0.58	0.63	4.80	0.47	0.46
3.0	6.50	0.77	1.16	5.94	0.73	0.61
4.0	6.78	0.84	1.03	6.51	0.87	0.70
	Clark			L9		
0.0	2.41	0.20	0.44	2.50	0.18	0.42
1.0	3.59	0.24	0.69	3.16	0.20	0.60
2.0	4.89	0.47	1.06	4.23	0.34	0.98
3.0	5.72	0.77	1.10	4.80	0.45	1.00
4.0	6.17	0.97	1.06	5.29	0.81	1.04

* Youngest trifoliolate leaf, next to terminal bud.
** P-fraction: *PT*, total P; *Ps*, acid-soluble organic-P; and *Pt*, phosphatide-P. Mean of two composite samples of four plants.

The acid-soluble organic-P fraction (*Ps*), phosphate esters and nucleotides, increased with increasing P in solution without apparent varietal differences. The content of phosphatide-P (*Pt*) was greater in Lincoln than in Chief, whereas no difference was found between Clark and L9 (Table 2). The increase in the amount of *Pt* reflects possibly an increase in the amount of intracellular membranes and indicates cell enlargement. Therefore, it is suggested that Lincoln showed an ability to grow rapidly with quick response to P supply in the immature leaves.

The inorganic-P fraction (*Pi*) was affected by external P supply more than any other fraction in both sensitive and tolerant varieties (Table 3). Barr and Ulrich [1] reported similar results with lima beans. The percentage of *Pi* in total P generally increased with increasing P concentration, particularly in the roots. Among leaves of different growth stages the percentage of *Pi* in total P was greatest in the terminal bud. Since orthophosphate P has an im-

TABLE 3

Inorganic-P content (μg/mg dry wt) in roots, stems, and leaves* of P-sensitive and -tolerant soybeans

P concn.	P content**							
mg/l	root	stem	T3	TB	root	stem	T3	TB
	Lincoln				Chief			
0.0	0.22	0.31	0.60	0.86	0.25	0.22	0.47	0.78
1.0	0.62	0.46	1.05	1.58	0.48	0.30	0.72	1.95
2.0	1.35	0.58	1.10	1.96	0.92	0.42	1.26	2.25
3.0	2.29	1.45	1.76	2.52	1.37	0.84	1.70	2.78
4.0	3.71	2.20	2.07	2.66	2.21	1.60	2.00	2.80
	Clark				L9			
0.0	0.67	—	0.23	0.16	0.60	—	0.24	0.19
1.0	—	—	0.43	0.41	—	—	0.34	0.29
2.0	4.59	—	0.65	0.73	3.47	—	0.65	0.73
3.0	—	—	1.10	1.14	—	—	1.05	1.17
4.0	7.90	—	1.40	1.37	6.06	—	1.19	1.25

* Separate leaf parts: youngest trifoliolate leaf (T3), next to the terminal bud (TB).
** Mean of two composite samples of four plants.

portant direct effect upon glycolytic systems, the increase in *Pi* in high P solutions may indicate that the reduction in growth by the Lincoln and Clark varieties could not be ascribed only to changes in the *Pi* fraction.

Increasing P in solution resulted in increases in RNA–P and DNA–P in the trifoliolate leaves of all four varieties (Table 4). There were no apparent differences in RNA and DNA contents between sensitive and tolerant varieties. Both fractions of nucleic acids were much lower in stems and roots than in leaves (data not shown). This was possibly due to the cells of stems and roots, being more mature (lower RNA) and fewer in number per unit weight (lower DNA).

If DNA is assumed to be constant for all somatic cells, the ratio of RNA–P to DNA–P represents the RNA content per cell and thus may be considered to be an index of protein synthesis capacity per cell. In Table 4, the sensitive varieties show a slight decrease in the RNA–P/DNA–P ratio for the youngest trifoliolate leaves at high P supply, even though other tissues did not show any consistent differences between sensitive and tolerant varieties.

When RNA–P content was expressed as a ratio with total nitrogen content, Lincoln and Clark showed a decrease at high P in solution in the youngest trifoliolate leaves as well as in roots. Chief and L9, however, showed a slight increase in RNA–P/N. These results may indicate that tolerant varieties had an increasing rate of protein synthesis at high P in solution, while sensitive varieties reached a maximum at a lower P concentration.

TABLE 4

RNA–P, DNA–P (μg/mg dry wt) and ratio of RNA–P/N (μg/mg) in trifoliolate leaves*

P concn. mg/l	RNA–P	DNA–P	RNA–P/N	RNA–P	DNA–P	RNA–P/N
		Lincoln			Chief	
0.0	1.29	0.42	35	1.44	0.41	33
1.0	1.87	0.53	61	1.88	0.44	53
2.0	2.08	0.50	68	2.12	0.49	62
3.0	2.32	0.48	60	2.39	0.51	70
4.0	2.31	0.53	57	2.42	0.52	73
		Clark			L9	
0.0	1.20	0.34	39	1.27	0.39	37
1.0	1.84	0.39	50	1.64	0.38	53
2.0	2.21	0.50	64	1.82	0.44	64
3.0	2.27	0.48	74	1.86	0.44	65
4.0	2.24	0.50	70	1.90	0.45	66

* Youngest trifoliolate leaf, next to terminal bud. Mean of two composite samples of four plants.

It has been shown that total P, inorganic-P and RNA–P were the main P fractions in which sensitive and tolerant varieties differed. Sensitive varieties took up P more readily than tolerant ones; RNA–P decreased at high P supply primarily in leaves of sensitive varieties.

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