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Effect of Phosphorus and Zinc Nutrition on Soybean Seed Phytic Acid and Zinc[1]

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ABSTRACT

The relationships between nutrient P and Zn levels and the phytic acid, P, and Zn concentrations in soybean (*Glycine max* L. Merr. cv 'Williams 79') seed were studied. Phytic acid increased linearly from 4.2 to 19.2 milligrams per gram as nutrient P treatment was varied from 2.0 to 50 milligrams per liter and Zn was held constant at 0.05 milligrams per liter. Leaf P concentration during seed development was found to be closely related to the concentrations of seed P and phytic acid. Leaf and seed Zn concentrations both responded positively to increasing nutrient Zn treatment. The effects of P treatment on plant and seed P and phytic acid were largely independent of the effects of Zn treatment on leaf and seed Zn. Phytic acid to Zn molar ratios ranging from 3.6 to 33.8 were observed.

The effects of nutrient P treatments on the concentrations of phytic acid, seed P, and leaf P were also studied in the P-sensitive (gene *np*) cultivars 'Harosoy' and 'Clark' and their respective P-tolerant (gene *Np*) near-isogenic lines L66-704 and L63-1677. In general, the positive relationships observed among nutrient P, leaf P, seed P, and phytic acid concentrations were similar to those observed in the studies with Williams 79. When fertilized with low or moderate nutrient P (2.5 and 25.0 milligrams P per liter, respectively) no significant differences in any parameter were observed between Harosoy or Clark and their respective P-tolerant isolines. When fertilized with high nutrient P (100 milligrams P per liter), Harosoy seed had a significantly higher concentration of phytic acid (30 milligrams per gram) than did seed of its P-tolerant near-isogenic line L66-704 (24.2 milligrams per gram phytic acid), whereas no significant difference was observed between Clark and its P-tolerant near-isogenic line L63-1677 (22.8 and 21.6 milligrams per gram, respectively). Variation in the phytic acid concentrations in the mature seed of the cultivars and isolines more closely paralleled leaf P concentrations observed during seed development (49 days after flowering), than those observed at the onset of seed development (14 days after flowering). Electrophoresis and ion-exchange chromatography revealed that partially phosphorylated intermediates do not appear when phytic acid accumulation is greatly reduced by limiting the nutrient P or when accumulation is greatly accelerated by excess P.

Phytic acid accounts for 60 to 80% of the total P in soybean seeds (24) and is a source of Pi for the seedlings. However, fertilizer P is lost when phytic acid is removed from the field along with the seeds at harvest. The phytic acid content of legume-based and cereal-based diets is also undesirable from the standpoint of animal nutrition, since binding of Zn to phytic acid is reported to reduce the bioavailability of this essential trace element (26). Diets with phytic acid to Zn molar ratios greater than 20 have been reported to result in lowered Zn retention and reduced growth of rats (16, 20, 26). Previous work has shown that mature seeds of various soybean cultivars contained 14 to 23 mg phytic acid g^{-1}, and the molar ratio of phytic acid to Zn was 26 to 44 (24).

There is considerable interest in lowering the phytic acid content of soy products used for food (10). Reduction of phytic acid level by genetic means has been suggested as a permanent way of improving the Zn bioavailability of soybeans (21), but mature seeds low in phytic acid are not available to establish the validity of this idea. Furthermore, there is no information for any crop concerning the effect of an abnormally low phytic acid level on seed maturation, germination, and seedling vigor.

It is likely that the phytic acid content of mature soybean seeds could be altered for experimental purposes by varying the nutrient P supplied to the plants, because nutrient P level is positively correlated with phytic acid accumulation in mature seeds of several other crop plants (1, 18, 19). Furthermore, a single gene which regulates the uptake of nutrient P has been identified in soybeans (3, 12). Phosphorus-sensitive genotypes accumulate P in the shoots and develop toxicity symptoms when given high nutrient P; P-tolerant genotypes modulate the uptake of P and do not develop toxicity symptoms when nutrient P is high.

Here we report on the extent of variation in seed phytic acid and Zn observed when soybean plants are grown in nutrient cultures with widely varying levels of P and Zn. The accumulation of phytic acid in P-tolerant and P-sensitive soybean isolines treated with varying levels of nutrient P, including a toxic P level, was also studied. In addition to the frequently studied relationships between phytic acid P and other seed P fractions (13, 17, 18), leaf P was determined to assess the P status of the plants under study and to learn whether this parameter also has value for predicting the phytic acid level of mature seeds.

MATERIALS AND METHODS

Phosphorus and Zinc Nutrient Treatments. 'Williams 79' soybeans (*Glycine max* [L.] Merr.) were inoculated with *Rhizobium japonicum*, sown in 19-L pots filled with silica sand, and thinned to two plants per pot following emergence. The study was located outside at Urbana, IL during the summer of 1982. Each pot was fertilized twice weekly with 1 L of an initial nutrient solution containing 10 mg P l^{-1} as KH_2PO_4, 75 mg N l^{-1} as $Ca(NO_3)_2 \cdot 4H_2O$, 10 mg N l^{-1} as $(NH_4)_2SO_4$, 50 mg K l^{-1} as KCl, 2 mg Fe l^{-1} as Sequestrene 138Fe (Ciba-Geigy) and Hoagland micronutrients (14) containing 0.05 mg Zn l^{-1} as $ZnSO_4 \cdot 7H_2O$. Pots were watered as needed with tap water. Forty two days after planting, approximately 10 d prior to the onset of flowering, the KH_2PO_4 and $ZnSO_4 \cdot 7H_2O$ concentrations of the initial nutrient

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solution were modified to provide nine different nutrient solutions representing a three by three factorial combination of P (2, 10, or 50 mg l^{-1}) and Zn (0.05, 5.00, or 50.00 mg l^{-1}). An additional nutrient treatment was included that contained 22.4 mg P l^{-1} and 0.05 mg Zn l^{-1}. Nutrient treatments were assigned to pots in a completely random design, with three replications (pots) of treatments containing 0.05 mg Zn l^{-1}, and two replications of treatments containing 5.0 or 50.0 mg Zn l^{-1}.

Sampling and Analysis. Leaf samples were collected at 7 and 35 d following flowering. The outermost leaflet of the third or fourth fully unfurled trifoliolate from the apex of the main stem was harvested, dried (48 h at 60°C), and stored in a desiccator until analysis. Seeds were harvested at maturity, and the number of seeds (seed set) per plant was recorded. Seed total P, Zn, and crude protein concentrations were determined as previously described (24). Leaf total P and Zn concentrations were determined with the same methods using 250 mg of oven-dried tissue. Phytic acid was isolated using a modification (24) of the acid extraction and iron precipitation methods of deBoland *et al.* (7), and P content of the precipitate was determined colorimetrically after acid digestion. Phytic acid was calculated by multiplying phytic acid P values by 3.55. To determine Pi, 100 mg of oven-dried tissue was extracted twice with 4 ml of 12.5% (w/v) TCA in 0.025 M $MgCl_2$ in a cold Ten Broeck ground glass tissue homogenizer. Each extract was centrifuged at 10,000*g* for 10 min and filtered through Whatman No. 1 filter paper. The filtered extracts were combined, diluted to 12.5 ml, and Pi was determined colorimetrically (6).

Phosphorus-Sensitive and P-Tolerant Genotypes. Seeds of the P-sensitive (gene *np*) soybean cultivars 'Harosoy' (maturity group II) and 'Clark' (maturity group IV) and their respective P-tolerant (gene *Np*) near-isogenic lines L66-704 (Harosoy background) and L63-1677 (Clark background) were provided by Dr. R. L. Bernard, Urbana, IL. The parentage of L63-1677 is Clark[6] × Chief and the parentage of L66-704 is Harosoy[6] × (Clark[6] × Chief). The study was conducted in the greenhouse during the spring of 1982. Incandescent lights maintained a minimum light intensity of approximately 175 $\mu E\ m^{-2}\ s^{-1}$ for a 14-h daylength. Seeds of each cultivar or isoline were inoculated with *R. japonicum*, germinated in sand, and seedlings were transplanted to 19-L pots filled with silica sand. Pots were fertilized three times weekly with an initial nutrient solution containing 15 mg P l^{-1}, with the other salts (including 0.05 mg Zn l^{-1}) as in the first experiment. Flowers first appeared on Harosoy and L66-704 at 34 d after planting and at 36 d on Clark and L163-1677. Thirty-five days after planting, the KH_2PO_4 concentration of the initial nutrient solution was modified to provide three nutrient solutions containing either 2.5, 25.0, or 100.0 mg P l^{-1}. Experimental design was a random complete block with two replications of each cultivar or isoline at each level of P. Replicate one contained three plants per pot, and replicate two contained two plants per pot. Leaf samples were collected at 14 and 49 d after flowering, and seeds were harvested at maturity. Methods of sample collection, preparation, and analysis were the same as in the first experiment.

Paper Electrophoresis. Samples of dry soybean flour (200 mg) from selected treatments were extracted with 5 ml 0.4 M HCl in a Ten Broeck glass homogenizer, which was then rinsed with 1 ml of extractant. The extract plus rinse was centrifuged (10,000*g*, 10 min). The supernatant fluid was evaporated under reduced pressure and dissolved in 1 ml of 0.4 M HCl. A 5-μl aliquot of this concentrated extract was subjected to paper electrophoresis (0.1 M oxalic acid, pH 1.6; 23 v/cm for 2 h; Whatman No. 1 paper, Ref. 24). Standards were KH_2PO_4, sodium phytate (Sigma Chemical Co.), *myo*-inositol 2-monophosphate (Sigma Chemical Co.), and a mixture of *myo*-inositol polyphosphates produced by partial hydrolysis of phytic acid as described by Desjobert and Petek (8). Phosphates were detected with Hanes-Isherwood reagent (2). The standards and their electrophoretic mobilities compared to that of picric acid (R_{picric} values) were as follows: Pi (0.58), *myo*-inositol monophosphate (0.78), *myo*-inositol bisphosphate (1.05), *myo*-inositol trisphosphate (1.19), *myo*-inositol tetrakisphosphate (1.36), *myo*-inositol pentakisphosphate (1.55), phytic acid (1.68).

Ion-Exchange Chromatography. Samples of dry soybean flour from selected treatments (250 mg if seeds contained moderate to high phytic acid and 500 mg if seeds contained low phytic acid) were extracted in 15 ml hot (50–60°C) 0.4 M HCl with a Polytron tissue homogenizer for 3 min. The extract was centrifuged (10,000*g* for 10 min) and vacuum-filtered through Whatman No. 1 paper. Ten ml of filtered extract was diluted to 50 ml with glass-distilled H_2O and passed through a 0.7- × 13-cm column containing 5 ml of Dowex 1-X8 (formate) resin, pH 5.2. The effluent collected during column loading was tested for P to insure complete binding. Duplicate samples of tissue from selected treatments were extracted, and duplicate columns were prepared. One column was eluted at 1 ml/min with a linear gradient of ammonium formate, pH 7.0 (0.24–1.0 M) to a total of 400 ml, and 5-ml fractions were collected. The second column was eluted batchwise by a two-step procedure consisting of 25 ml of 0.24 M ammonium formate (pH 7.0), followed by 25 ml of 1.2 M ammonium formate (pH 7.0). Phosphorus was detected colorimetrically in the various fractions after digestion of dried samples with concentrated H_2SO_4 (11).

RESULTS

Effects of Nutrient P and Zn Treatments on Williams 79 Soybeans. An initial study was conducted in the summer of 1981. Plants were watered (3 L/week) with one of four possible nutrient solutions representing a factorial combination of two levels of P (2.5 or 25 mg l^{-1}) and two levels of Zn (0.05 or 5.0 mg l^{-1}). The treatments caused seed Pi to vary from 165 to 311 $\mu g\ g^{-1}$, phytic acid P to vary from 1.8 to 5.4 mg g^{-1} (equivalent to 6.3 to 19.1 mg g^{-1} phytic acid), and seed Zn to vary from 62 to 88 $\mu g\ g^{-1}$. Consequently, the phytic acid to Zn molar ratio varied from 7 to 31. Phosphorus treatment had little effect on seed Zn, and Zn treatment had little effect on seed total P or phytic acid. These results were confirmed and extended by a second study conducted the following summer.

A positive, highly linear relationship between nutrient P and seed phytic acid P concentration (when nutrient Zn was held at 0.05 mg l^{-1}) was observed in the follow-up study (Fig. 1). At the lower levels of nutrient P, the concentrations of phytic acid P ranged from 1.2 to 1.8 mg g^{-1} (equivalent to 4.2 to 6.5 mg g^{-1} phytic acid), only one-fourth to one-third of those observed at the highest level of nutrient P and less than one-half of those present in field-grown seed of the same cultivar (Ref. 24; Table I). Figure 1 illustrates that virtually all of the increase in seed

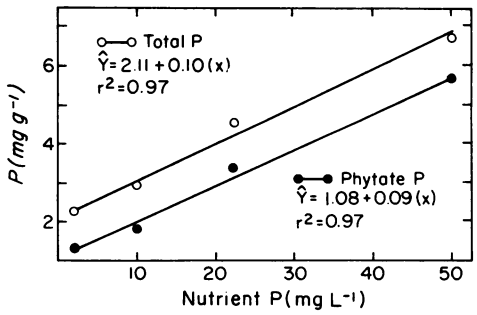

FIG. 1. Effects of nutrient P on soybean seed total P and phytic acid P. Data represent treatments which contained 0.05 mg Zn l^{-1}. Each point represents the mean of three replications, using the cv Williams 79.

Table I. *Effects of Nutrient P and Zn Treatments on Soybean Seed and Leaf Traits*

Soybeans (*Glycine max* [L.] Merr. cv Williams 79) were grown to maturity in sand culture with nine nutrient treatments representing a combination of three levels of P and three levels of Zn.

Treatment			Seed						Leaf[a]		
No.	P	Zn	Phytic acid	Pi	Zn	Phytic acid:Zn	Seed set	Dry wt	Total P 7 d	Total P 35 d	Zn (35 d)
	mg l^{-1}		mg g^{-1}	$\mu g\ g^{-1}$		M ratio	seeds $plant^{-1}$	mg $seed^{-1}$	mg g^{-1}		$\mu g\ g^{-1}$
1	2	0.05	4.7	129	76	6.2	55	147	1.4	0.9	159
2	2	5.00	6.5	133	89	7.2	44	157	1.4	0.9	281
3	2	50.00	4.2	183	116	3.6	13	110	1.0	0.8	512
4	10	0.05	6.8	130	62	10.9	125	187	2.4	1.5	156
5	10	5.00	7.0	156	91	7.7	115	175	2.4	1.3	345
6	10	50.00	4.4	175	121	3.6	22	139	1.1	0.8	519
7	50	0.05	19.2	301	56	33.8	187	166	6.2	4.1	142
8	50	5.00	18.6	282	87	21.1	195	174	4.9	3.5	330
9	50	50.00	17.2	233	167	10.2	163	159	3.8	2.6	640
	LSD 0.05		1.6	7	17	8.0	22	17	0.5	0.5	71

[a] The outermost leaflet of the third or fourth fully unfurled trifoliolate from the main stem apex was harvested 7 and 35 d after the onset of flowering, and analyzed for total P and Zn.

total P resulting from increasing nutrient P was as phytic acid P. Consequently, there was no detectable effect of nutrient P treatment on the non-phytic acid P fraction of mature seeds (23). Seed Pi did increase more than 2-fold as nutrient P was raised from 10 to 50 mg l^{-1}, but Pi represented only 5% of seed total P, a quantitatively negligible fraction when compared with phytic acid P.

Nutrient Zn levels above that of the standard Hoagland solution (0.05 mg l^{-1}) resulted in an increase in seed Zn from the 60 to 70 $\mu g\ g^{-1}$ range to well over 100 $\mu g\ g^{-1}$. The highest nutrient Zn treatment tended to cause a decrease in seed phytic acid levels, at all P levels. High nutrient Zn in combination with high nutrient P resulted in dramatically high seed Zn (Table I, treatment 9). Fluctuations in seed phytic acid and Zn resulted in a 9-fold change in the phytic acid to Zn ratio when low nutrient P-high Zn treatments are compared with high nutrient P-low Zn treatments (Table I).

Protein values ranged from 366 to 427 mg g^{-1} for the nine nutrient treatments, and the analysis of variance indicated that these values were not significantly different (23). Seed set was reduced by the high (50 mg l^{-1}) Zn treatment as well as by decreasing the nutrient P level, so there was a drastic reduction in yield of plants given reduced P together with the highest level of Zn (Table I). Reductions in seed yield were due mainly to altered numbers of seeds per plant, but there was some effect on seed weight, particularly at the highest level of Zn.

There were large increases in leaf P and Zn levels in response to increases of these elements in the nutrient solutions (Table I). The highest nutrient Zn treatment resulted in significant reductions in leaf P. A close relationship was observed between leaf P concentration and several seed fractions (total P, phytic acid, and Pi), with correlations ranging from 0.88 to 0.97 (Table II). Similarly, seed Zn concentration closely reflected leaf Zn concentrations. With the exception of the high Zn treatments, the effects of P treatment on leaf and seed P and phytic acid appeared to be independent of the effects of Zn treatment on leaf and seed Zn.

Effects of Nutrient P Treatment on P-Sensitive and P-Tolerant Genotypes. The results for Clark, Harosoy, and their respective P-tolerant near-isogenic lines are given in Table III and Figure 2. Overall, the relationship between nutrient P level and seed P and phytic acid P was similar to that observed in the first experiment. Phytic acid P accounted for most of the variation in seed P, with little effect of P treatment on the non-phytic acid P fraction of seeds. The concentrations of leaf P at 49 d and phytic acid P in Harosoy and its P-tolerant isoline L66-704 were generally higher than those found in Clark and L63-1677 at any level of P. The seed phytic acid and leaf P concentrations for tolerant plants did not differ from values for sensitive plants at the low and intermediate levels of nutrient P. However, differences did appear at the highest level of nutrient P. When treated with high nutrient P, both sensitive lines had considerably higher concentrations of leaf P at 14 d than their respective tolerant isolines. This difference persisted to 49 d for the Harosoy isolines, and cv Harosoy also had a significantly higher concentration of seed phytic acid than its P-tolerant isoline. For Clark and its P-tolerant isoline, the difference in leaf P concentration observed at 14 d in plants treated with high nutrient P was no longer evident at 49 d and no difference in phytic acid concentration in the mature seeds was observed.

Table II. *Simple Correlations between Leaf and Seed Phosphorus and Zinc Parameters*

	Leaf[a]		
	Total P (mg g^{-1})		Zn ($\mu g\ g^{-1}$)
	7 d	35 d	35 d
		r[b]	
Seed			
Total P (mg g^{-1})	0.96**	0.97**	0.01
Phytate P (mg g^{-1})	0.95**	0.97**	0.00
Nonphytate P (mg g^{-1})[c]	0.12	0.01	0.16
Pi ($\mu g\ g^{-1}$)	0.88**	0.91**	0.12
Zn ($\mu g\ g^{-1}$)	−0.21	−0.16	0.96**

[a] d refers to days after flowering.

[b] (**), Significant at the 0.01 level of probability.

[c] Non-phytate P is calculated by subtracting phytate P from seed total P.

Confirmation of the Iron Precipitation Procedure. Paper electrophoresis and ion-exchange chromatography confirmed the results of phytic acid determinations based on the iron precipitation procedure. A single spot was detected after electrophoresis of acid extracts from high and low phytic acid seeds (Harosoy treated with 100 mg l^{-1} P, L66-704 treated with 2.5 mg l^{-1} P), and the R_{picric} values (1.65 and 1.71, respectively) were not

Table III. *Effects of Nutrient P Treatments on Seed Traits of P-Tolerant and P-Sensitive Soybean Near-Isogenic Lines*

The P-sensitive soybean cultivars Harosoy and Clark, and their P-tolerant near-isogenic lines L63-1677 (Clark background) and L66-704 (Harosoy background) were grown to maturity in sand culture with three levels of nutrient P (2.5, 25.0, and 100 mg l^{-1}).

P Treatment	Genotype	Phytic Acid	Seed Set	Dry Wt	Yield
mg l^{-1}		*mg g^{-1}*	*no. plant^{-1}*	*mg seed^{-1}*	*g plant^{-1}*
2.5	Clark	8.4	43	229	9.8
	L63-1677	8.1	47	213	9.9
	Harosoy	13.8	31	252	7.8
	L66-704	10.5	32	258	8.3
25.0	Clark	15.7	115	210	24.1
	L63-1677	16.9	119	190	22.7
	Harosoy	21.4	76	225	17.1
	L66-704	21.1	80	213	17.2
100.0	Clark	22.8	116	197	22.7
	L63-1677	21.6	96	197	18.9
	Harosoy	30.3	68	164	11.2
	L66-704	24.2	80	223	17.8
LSD 0.05		4.5	21	32	5.0

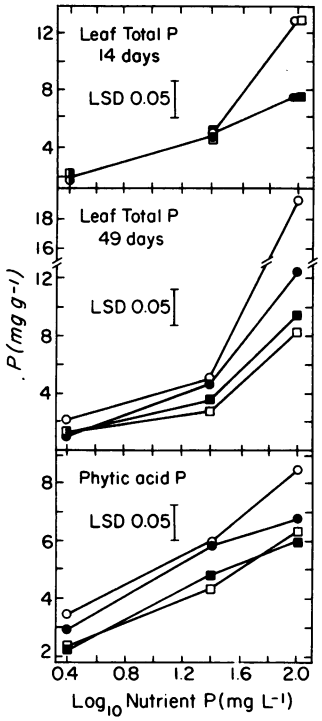

FIG. 2. Effects of nutrient P on leaf total P and phytic acid P in P-sensitive and -tolerant soybean genotypes. The cultivars Harosoy (○) and Clark (□) are sensitive to high nutrient P. Their near-isogenic lines L66-704 (●, Harosoy background) and L63-1677 (■, Clark background) are tolerant to high nutrient P. Leaf P was measured at two dates, 14 and 49 d after flowering. Each point represents the mean of two replications.

different from that of the phytic acid standard. Both extracts contained a major peak that corresponded to the phytic acid standard after elution from ion-exchange resin with an ammonium formate gradient, and the Harosoy extract contained much more phytic acid as expected (Fig. 3). Partially phosphorylated

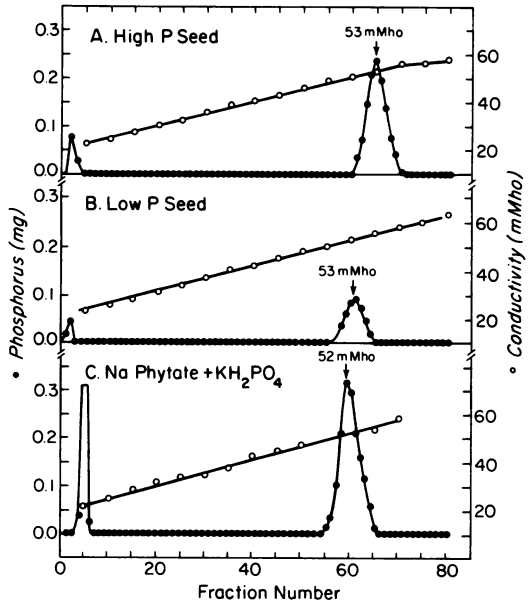

FIG. 3. Ion-exchange chromatography of acid extracts of soybean flour from selected treatments. The ammonium formate gradient and other experimental conditions are described in "Materials and Methods." A, The column was loaded with 10 ml of extract equivalent to 167 mg seed flour from Harosoy plants fed with 100 mg l^{-1} nutrient P. B, The column was loaded with 10 ml of extract equivalent to 333 mg seed flour from L66-704 plants fed with 2.5 mg l^{-1} nutrient P. C, The column was loaded with 6.4 mg Na phytate (equivalent to 1.3 mg Pi) and 0.5 mg Pi.

inositols were not detected on the electrophoresis papers or in the column effluents. The phytic acid peaks of Figure 3 represent 89% (Harosoy) and 70% (L66-704) of the phytic acid present in the same seed lots as determined by the standard iron precipitation procedure. Similar results were obtained when phytic acid was eluted from the resin by a stepwise increase in ammonium formate: 95% recovery from Harosoy and 71% recovery from L66-704.

DISCUSSION

These experiments indicate that the phytic acid level, and to a lesser extent the Pi, of mature soybean seeds is very responsive to altered concentrations of nutrient P, but there is little or no change in seed protein, Zn, or the other major P-containing fractions. Phytic acid levels varied over a 4-fold range as a result of the different nutrient P treatments, and at a standard level of nutrient Zn (0.05 mg l^{-1}), these two variables displayed a linear relationship between 2 and 50 mg nutrient P l^{-1}. The increase in seed Pi that accompanies high nutrient P (Table I) may be related to the observed increase in phytic acid accumulation. Phosphoinositol kinase is located at the branch point in the pathway leading to phytic acid (4, 5), and Pi may directly stimulate the activity or biosynthesis of this enzyme. A precedent is the activation of invertase by Pi (15). Alternatively, increased Pi might lead to enhanced enzyme activity due to increased levels of its substrates (1 L-*myo*-inositol-1-P and ATP) (4, 5).

Phytic acid concentration appears to be a function of plant P status during soybean seed development. This is in agreement with the findings of Early and Deturk (9), who demonstrated a requirement for nutrient P during maize kernel development. Asada *et al.* (1) demonstrated the positive relationship between nutrient P and phytic acid accumulation in rice. Recently, in a study with wheat grown in a potting soil medium, Michael *et al.* (18) also demonstrated the effectiveness of late P fertilization (at or after flowering) as a means of inducing variation in phytic

acid content.

The modulation of nutrient P uptake that is characteristic of P-tolerant soybean lines when treated with high nutrient P (12) was clearly reflected in the leaf P concentrations of both Harosoy and Clark isolines at 14 d after flowering. A significant difference in seed phytic acid concentration resulting from this modulation of nutrient P uptake was only observed between the Harosoy isolines, however. Results with the P-sensitive cultivar Harosoy are especially striking, since high nutrient P resulted in a seed phytic acid concentration nearly twice that observed when plants of this cultivar were grown in a fertile soil (24). It is not known why the significant difference in leaf P concentration observed at 14 d after flowering between Clark and L63-1677 did not persist to 49 d after flowering and was not reflected in the phytic acid concentration of the mature seed. In any case, variation in seed phytic acid concentration among lines and cultivars within a given level of nutrient P clearly paralleled variation in leaf P concentration observed at 49 d after flowering.

Results of this study support nutrient culture as an effective method to produce mature soybean seed with varying levels of phytic acid and Zn. The variation in phytic acid content reported here is greater than that reported for rice (1) and wheat (18), several times greater than the range reported for field-grown peas and snap beans given various levels of P (22), and equivalent to that reported for immature and mature soybean seeds where the level of Zn (but not P) was varied in nutrient culture (27). The highest phytic acid to Zn molar ratio obtained here (33.8) is similar to the values observed for soybeans grown in the field with adequate P (24), and the lowest ratio (3.6) is considerably below what is found for field-grown seeds. Ratios of 11.4 to 38.6 were observed in a recent survey of 32 cultivars representing nine cereal and legume species (16), and Zn bioavailability was favored by a lower ratio.

Electrophoresis and ion-exchange chromatography revealed that partially phosphorylated intermediates do not appear when phytic acid accumulation is greatly reduced by limiting the nutrient P or when accumulation is greatly accelerated by excess P. Mung bean phosphoinositol kinase preferentially phosphorylates the higher homolog when it is incubated with various pairs of inositol phosphates that differ by one phosphate (5), and such a regulatory mechanism may have prevented the appearance of intermediates here. The results also indicate that the iron precipitation procedure is adequate for measuring phytic acid over the range encountered.

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