

PHOSPHORUS COMPOUNDS OF COTTON EMBRYOS AND THEIR CHANGES DURING GERMINATION¹

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The phosphorus compounds of cotton embryos, particularly ribonucleic (RNA) and deoxyribonucleic (DNA) acid, and their metabolism during germination have not been intensively investigated with modern methods of analysis. In the period 1913 to 1918 Anderson (1) and Rather (9) elucidated the true nature of phytic acid, the principal form of seed phosphorus. Although Anderson obtained the hexaphosphate of inositol, Rather found only the pentaphosphoric acid derivative due to the activity of the enzyme phytase. In 1946 Fontaine and co-workers (5) presented results showing an increase in the percentage of phosphorus in the inorganic form during germination. Pons et al (8) developed an analytical system for analysis of the phosphorus fractions of plant materials and in 1953 reported data on the phytin-, inorganic-, lipid-, ester-, and nucleic acid-P contents of cotton embryos. More recently Ergle and Eaton (3) reported on the amounts of similar fractions, plus protein-P, in various cotton tissues including 10-day-old ovules and mature embryos.

In the present paper information on the phosphorus compounds of cotton embryos has been extended to include RNA and DNA and the changes which they and the above mentioned phosphorus fractions undergo during germination and early seedling development. Results on Paymaster 54B, Deltapine TPSA, and Mesilla Valley Acala, representative of early, intermediate, and late maturing cottons, respectively, are compared.

MATERIALS AND METHODS

GERMINATION: Lots of 300 acid-delinted seeds of Mesilla Valley Acala, Paymaster 54B, and Deltapine TPSA cotton varieties were germinated in the dark at 28 to 30° C in moist, autoclaved vermiculite for 1, 2, and 4 days. To obtain 6-day-old seedlings, 4-day-old seedlings from the vermiculite were transferred to 2-gallon glazed jars filled with 0.005 M CaSO₄ solution and grown in the dark for 2 additional days with aeration. The seedlings were supported on waxed hardware cloth. Similarly, seeds of the 3 varieties were germinated and grown in sunlight in the greenhouse for harvest only at the end of the 6-day period.

HARVEST AND SAMPLE PREPARATION: The embryos from air dry seed, representing 0-day germination, were separated from the seed coats and ground in a small Wiley mill 1st to 20-mesh and later (after being defatted) to 80-mesh fineness before analysis. The seedlings, after removal of vermiculite and any adhering seed coats, were lyophilized and ground to 80-mesh fineness. The cotyledons were separated mechanically from the remainder of the seedling and analyzed separately in most of the experiments.

ANALYSIS: The analytical methods used in the separation of (a) lipid-, phytin-, inorganic-, and ester-P and (b) RNA- and DNA-P were basically those described by Pons et al (8) and Ogur and Rosen (7), respectively. However, since the 2 methods were combined into a single analytical system (plus some modification) in the present work, the procedure used is briefly described. Except when noted, the phosphorus contents of the separated fractions were determined after wet ashing with a minimum amount of H₂SO₄ by the method of Fiske and Subbarow (4).

Lipid-P: Five grams of embryos or 3 g of the germinated tissue were extracted at a rapid rate for 8 hours each time in a Soxhlet apparatus; 1st with petroleum ether (B.P. 30 to 65° C) and then with an alcohol : benzene azeotrope (32.4 % : 67.6 %, by weight, B.P. 68.2° C). The phosphorus contents of the evaporated extracts were determined separately.

Phytin-P: Phytin was extracted from a 1 g sample of the lipid-free residue above with a 2 % HCl-10 % Na₂SO₄ solution at room temperature. Phytin was precipitated from a suitable aliquot of the extract with FeCl₃ as iron phytate, washed, and converted to the soluble Na-salt by treatment with dilute NaOH. After separation of the Fe(OH)₃ precipitate by filtration and washing, phytin-P was determined on an aliquot of the filtrate plus washings made to volume.

Total acid soluble-P: A 500 mg sample (if high in inorganic-P) or a 1 g sample (if low in inorganic-P) of the residue from the lipid-P extraction was shaken on a mechanical shaker for 1 hour with 0.75 N trichloroacetic acid (TCA). The residue was then separated by centrifuging and washed free of soluble-P with small amounts of the same reagent. Usually 4 or 5 washings were necessary with tissues of high soluble-P contents. Care was taken to retain quantitatively the residue in the centrifuge tube since it was used for the separation of subsequent

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fractions. Phosphorus was determined on a digested aliquot of the filtered TCA extract. The value obtained was used only to calculate ester-P.

Inorganic-P: This fraction was obtained by extracting an aliquot of the TCA extract with isobutyl alcohol which was then determined directly, without digestion, by the isobutyl alcohol colorimetric method (8).

Ester-P: Ester-type phosphorus was calculated as the difference between total acid soluble-P and the sum of the values for phytin- and inorganic-P.

RNA-P: The tissue residue from the TCA extraction was extracted with 1 *N* perchloric acid at 3° C 3 times at intervals of about 40 hours. Phosphorus was determined in an aliquot of the combined extracts after digestion.

DNA-P: The phosphorus extractable from the RNA-P residue above with 0.5 *N* perchloric acid at 70° C.

Protein-P: The phosphorus remaining in the residue after separation of DNA-P.

Total P: Sum of the separate fractions. When checked, the sum of the fractions agreed well with the values for total phosphorus determined directly.

The data are expressed on the basis of the initial tissue weights when dried at 75° C.

RESULTS

GERMINATION IN THE DARK: The concentrations (mg/g) of the various phosphorus fractions in the embryos and seedlings of the 3 cotton varieties are given in table I. Before germination (day 0) the embryos of the 3 cottons differed principally in their phytin-P concentrations and dry weights, being in the order Acala > Paymaster > Deltapine. During germination of all varieties there were successive

decreases with time in phytin-P and corresponding increases (except day 1) in the concentration of inorganic-P. Lipid-P increased from day 0 to 2 and then declined but again exceeded its initial concentration by day 6. Ester-P changes were for the most part irregular but tended except in the Acala variety to exceed the original concentration at all times. RNA-, DNA-, and protein-P fractions in general increased in concentration with time of germination.

Changes, during germination, in the amounts (μ g) of the above phosphorus fractions per seedling, using the data for Paymaster in table I, are illustrated in figure 1. To facilitate graphing, RNA- and DNA-P were plotted as the sum instead of separately since they were of similar magnitude.

On the basis of differential solubility in petroleum ether (Pet) and alcohol-benzene azeotrope (AB) there appeared to be at least 2 phospholipid fractions present in the cotton embryos and seedlings (fig 2). At the start of germination these 2 forms were present in a ratio of about 3:1 but after day 2 they tended to become and remain of equal magnitude.

Separation of 6-day-old seedlings of the 3 cotton varieties into cotyledons and hypocotyls plus roots showed that the various phosphorus fractions except phytin-P and RNA-P are fairly evenly distributed between the 2 plant parts (table II). In the varieties tested little or no phytin-P was in the hypocotyl plus roots and the RNA-P content was only about one sixth that of the cotyledons.

On the basis of mean values of the 3 cottons, phytin-P was found to comprise about 83 %, inorganic-P 4 %, lipid-P 7 %, ester-P 3 %, and RNA-, DNA-, and protein-P each 1 % of the 0-day embryo phosphorus (table III). Germination of the seeds and growth of the seedlings for 6 days in the dark were accompanied by a redistribution of phosphorus among

TABLE I

CHANGES IN DRY WEIGHTS AND THE PHOSPHORUS FRACTIONS OF SEEDLINGS OF ACALA, PAYMASTER AND DELTAPINE VARIETIES OF COTTON DURING GERMINATION AND GROWTH IN THE DARK*

TIME	DRY WEIGHT	PHYTIN	INORG	TOTAL LIPID	ESTER	RNA	DNA	PROTEIN	TOTAL
days	mg/seedling	mg	mg	mg	mg	mg	mg	mg	mg
ACALA									
0	82.7	9.58	0.43	0.73	0.42	0.11	0.11	0.10	11.48
1	82.6	9.28	.37	.85	.32	.09	.09	.10	11.10
2	80.6	8.78	1.23	.89	.17	.16	.13	.15	11.51
4	78.9	4.81	4.79	.60	.48	.25	.22	.31	11.46
6	76.9	3.49	6.26	.87	.54	.39	.41	.24	12.20
PAYMASTER									
0	61.0	8.61	0.44	0.71	0.32	0.12	0.11	0.11	10.42
1	59.0	8.49	.29	.81	.41	.11	.11	.10	10.32
2	57.1	7.15	1.87	.87	.40	.15	.12	.16	10.72
4	56.8	4.00	4.77	.50	.58	.25	.21	.28	10.59
6	56.0	1.97	7.02	.85	.42	.39	.44	.26	11.35
DELTAPINE									
0	57.3	6.49	0.29	0.71	0.14	0.11	0.11	0.11	7.96
1	57.1	6.24	.20	.74	.18	.11	.11	.11	7.69
2	55.8	4.53	1.54	.80	.40	.18	.14	.18	7.77
4	54.9	2.77	3.30	.56	.49	.29	.23	.30	7.94
6	52.9	1.29	4.92	.80	.44	.42	.46	.23	8.56

* Phosphorus as mg/g of dry weight.

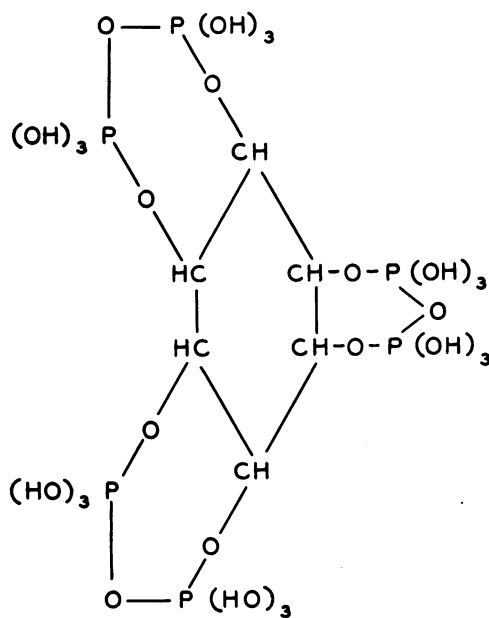
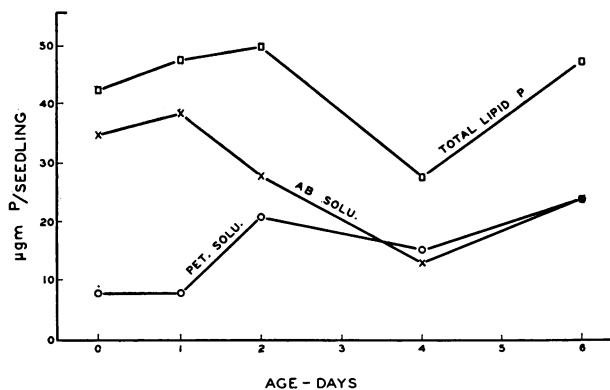
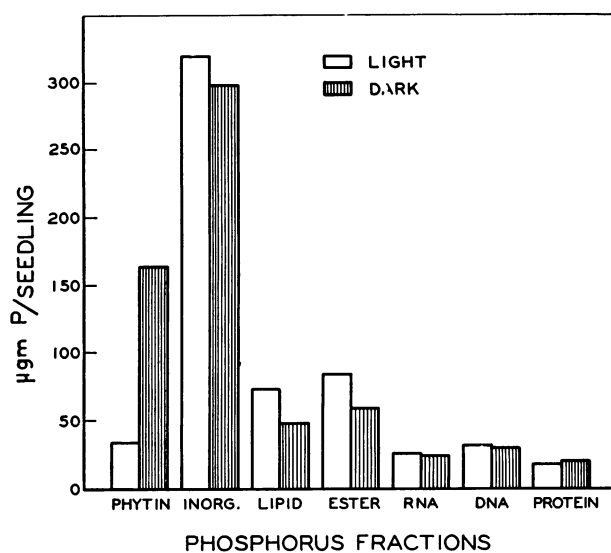
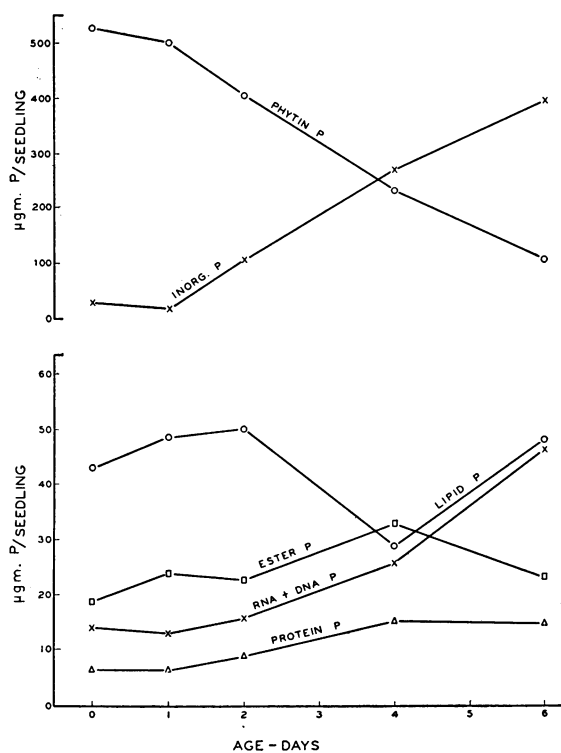


FIG. 1 (*upper left*). Changes in the phosphorus fractions of dark-grown seedlings of Paymaster variety during development.

FIG. 2 (*lower left*). Variations in the petroleum ether (Pet) and alcohol-benzene (AB) soluble lipid-P fractions during development of dark-grown seedlings of Paymaster variety.

FIG. 3 (*upper right*). Influence of light on phosphorus fractions after germination and growth of seedlings for 6 days.

FIG. 4 (*lower right*). Phytic Acid, the principal form of embryo phosphorus.

TABLE II

DISTRIBUTION OF DRY WEIGHT AND PHOSPHORUS FRACTIONS BETWEEN COTYLEDONS AND HYPOCOTYLS PLUS ROOTS OF 6-DAY-OLD SEEDLINGS OF THE THREE COTTON VARIETIES GERMINATED AND GROWN IN THE DARK *

CONSTITUENTS	ACALA		PAYMASTER		DELTAPINE	
	COTYLEDONS	HYPOCOTYL PLUS ROOT	COTYLEDONS	HYPOCOTYL PLUS ROOT	COTYLEDONS	HYPOCOTYL PLUS ROOT
	mg	mg	mg	mg	mg	mg
Dry weight/seedling	46.1	32.4	29.8	27.8	28.7	27.3
Phosphorus fractions:						
Phytin	6.54	0.00	4.52	0.00	1.91	0.00
Inorganic	4.24	5.31	5.15	6.13	2.68	4.53
Lipid A**	.30	.25	.28	.23	.29	.21
Lipid B***	.52	.51	.45	.46	.47	.49
Total Lipid	.82	.76	.73	.69	.76	.70
Ester	.70	.92	.87	1.11	1.34	.71
RNA	.61	.10	.69	.07	.68	.08
DNA	.42	.49	.53	.46	.55	.42
Protein	.28	.29	.34	.32	.40	.28
Total	13.61	7.87	12.83	8.78	8.32	6.72

* Phosphorus as mg/g of dry weight.

** Alcohol-benzene soluble.

*** Petroleum ether soluble.

most of its fractions with phytin- and inorganic-P exhibiting the greatest changes. Lipid-P showed comparatively little net gain and ester-P only slightly more. RNA- and DNA-P, however, each increased more than 3-fold and protein-P, 2-fold.

GERMINATION IN THE LIGHT: Data from the light experiment are given in table IV. Separation of the seedlings into cotyledons and hypocotyls plus roots for analysis in this experiment was done primarily for the purpose of determining whether there was a differential effect of light and darkness on phytin- and RNA-P contents of the hypocotyls plus roots. A comparison of the data in tables II and IV shows there was none; phytin-P was absent and RNA-P remained low and essentially the same in both instances. However, there was an influence of light on certain of the other P-fractions as illustrated in figure 3 in which the mean values of the 3 cotton varieties of table II (dark), calculated on the whole seedling basis, are compared with those of table IV (light). As shown, light germinated seedlings con-

tained substantially less phytin- and more inorganic-, lipid-, and ester-P than the dark germinated ones after 6 days. On the other hand RNA-, DNA-, and protein-P contents were essentially the same under the 2 conditions.

DISCUSSION

Phytin is the calcium, magnesium and potassium salt of phytic acid (fig 4) and the foregoing results show that more than 80 % of the embryo phosphorus, irrespective of variety, resides in this single molecular species. Seeds of some other crop plants (8) have equally high phytin-P contents. Another notable feature of phytin, as demonstrated in the current study, is the rapidity with which its phosphorus is liberated, presumably through the action of phytase, during germination. As shown in table I, dephosphorylation was evident as early as day 1 after planting and by day 6 about 60 % of the original phytin-P content had reappeared as inorganic-P (table III). The liberated phosphorus was roughly equally distributed between cotyledons and hypocotyls plus roots of dark-grown seedlings.

Although mobilization of phosphorus from the organic to inorganic-P was the primary reaction in terms of amounts involved, about 10 % of the latter phosphorus was used in the synthesis of additional amounts of organic-P containing compounds originally present in the embryo. For example the RNA- and DNA-P contents of the 6-day-old seedlings were each more than 3 times, and protein-P double that of the embryos. Although there had resulted small net increases in ester- and lipid-P by the 6th day, there was also evidence of small phosphorus loss earlier from these 2 sources. There was a substantial decrease in amounts per seedling of total lipid-P between days 2 and 4, and ester-P between days 4 and 6 (figs 1, 2).

TABLE III

DISTRIBUTION OF PHOSPHORUS AMONG THE PHOSPHORUS FRACTIONS IN EMBRYOS AND 6-DAY-OLD SEEDLINGS *

PHOSPHORUS FRACTIONS	EMBRYOS	6-DAY-OLD SEEDLINGS
	%	%
Phytin	82.9	22.0
Inorganic	3.8	56.0
Total Lipid	7.1	7.7
Ester	3.0	4.4
RNA	1.1	3.7
DNA	1.1	4.0
Protein	1.0	2.2
Total	100.0	100.0

* Results as mean percentages of the total phosphorus contents of the 3 cotton varieties germinated and grown in the dark.

Utilization of phosphorus from the latter source is not surprising since this group of compounds comprise, among others, adenosine triphosphate, uridine triphosphate and other nucleotides with energy-rich phosphate bonds that mediate energy transfer within the cell. Since little is known about the phospholipid metabolism of plants it is difficult to determine the significance of the loss and subsequent recovery of the lipid-P. Actually, as shown in figure 2, the alcohol-benzene (AB) soluble lipid-P fraction declined after day 1 but this loss is accounted for by a corresponding increase in the fraction soluble in petroleum ether (Pet). Thereafter changes in the 2 fractions were similar. It is not known at this time whether these 2 fractions represent respectively cephalin and lecithin, the dominant phospholipids of the great majority of plant tissues. Lecithin often constitutes about one third and cephalin two thirds of the total plant phospholipids. In the present instance this proportion, on the basis of lipid-P, was approached in the respective Pet and AB soluble extracts of the embryos but not of the seedlings.

Separation of 6-day-old dark-grown seedlings into cotyledons and hypocotyls plus roots for analysis showed some differences in the phosphorus metabolism of the 2 organs. The absence of phytin-P in the 6-day-old hypocotyls plus roots (also in those from a 3-day germination through the data are not reported) suggest that it is not translocated from the cotyledons as such. Instead, dephosphorylation occurs principally in the cotyledons and substantial amounts of the resulting inorganic-P then move into the hypocotyls plus roots. How much of the translocated phosphorus was used in the synthesis of the other organic fractions is beyond the scope of this paper. The results showed that the organic-P compounds, with the exception of RNA-P, accumulated in the hypocotyls plus roots in amounts similar to those found in the cotyledons. In-

asmuch as RNA is generally regarded as a template for the incorporation of amino acids into protein (2), this difference may be a reflection of characteristic differences in protein levels of the 2 organs, being slightly higher in the cotyledons. Regardless of the site of synthesis, it is of interest that the hypocotyls plus roots of the 3 varieties contained only about one-sixth to one fifth as much RNA- as DNA-P whereas they occurred in the cotyledons in nearly equal amounts.

In the experiment in which germination was conducted in sunlight, the seedlings, as in the dark experiment, started emerging the 4th day after planting. Thus it is estimated that the light-germinated seedlings probably received a total of 24 to 30 hours of light for photosynthesis. It was shown that light (table IV) under these conditions caused (a) a further increase in rate of phytin hydrolysis, with a concomitant increase in inorganic-P, and (b) the synthesis of additional amounts of ester- and lipid-P compounds, compared to germination in the dark (table II). The other phosphorus compounds, RNA, DNA, and protein, were essentially unaffected. The increase in ester-P appears to be logical since phosphoglyceric acid is known to be the major initial product of photosynthesis followed by the phosphorylated sugars and still other ester-P compounds. Though less is known of the influence of light on lipid metabolism, it is pertinent that Smith (10) also reported a rapid synthesis of ether-soluble phosphorus compounds and lipoidal material when etiolated barley seedlings were illuminated, and that phospholipids are among the components of chloroplastic material.

Turning to differences among the 3 cotton varieties in their mobilization and utilization of phosphorus, it was noted that phosphorus content and metabolism were roughly correlated with embryo and seedling weight. That is, the heavier the embryo the more of

TABLE IV

DRY WEIGHTS AND PHOSPHORUS FRACTIONS OF COTYLEDONS AND HYPOCOTYL PLUS ROOTS OF 6-DAY-OLD SEEDLINGS OF ACALA, PAYMASTER AND DELTAPINE GERMINATED AND GROWN IN THE LIGHT *

CONSTITUENTS	ACALA		PAYMASTER		DELTAPINE	
	COTYLEDONS	HYPOCOTYL PLUS ROOT	COTYLEDONS	HYPOCOTYL PLUS ROOT	COTYLEDONS	HYPOCOTYL PLUS ROOT
	mg	mg	mg	mg	mg	mg
Dry weight/seedling	61.4	18.8	41.2	17.5	38.5	17.8
Phosphorus fractions:						
Phytin	1.00	0.00	0.59	0.00	0.46	0.00
Inorganic	6.34	5.29	5.36	3.79	3.07	3.44
Lipid A**	.63	.31	.69	.31	.57	.30
Lipid B***	.66	.48	.57	.46	.52	.53
Total Lipid	1.29	.79	1.26	.77	1.09	.83
Ester	1.80	.68	1.53	.31	1.20	.71
RNA	.56	.09	.55	.08	.54	.08
DNA	.47	.43	.49	.44	.50	.47
Protein	.29	.25	.28	.29	.30	.28
Total	11.75	7.53	10.06	5.68	7.16	5.81

* Phosphorus as mg/g of dry weight.

** Alcohol-benzene soluble.

*** Petroleum ether soluble.

each phosphorus fraction was found. However phytin-P, and consequently total-P, was found to vary significantly with variety even when the results were expressed on a concentration basis (mg/g). This fraction (table I) was always (before and during germination) present in higher concentrations in the late (Acala) than in the 2 early maturing varieties (Paymaster and Deltapine). The significance of this, if any, as a biochemical distinction between earliness and lateness in cottons will, of course, have to be determined by further study. This deficit of phosphorus was certainly of no disadvantage in early development as all 3 varieties were observed to grow equally well (hypocotyl plus root elongation data obtained but not reported).

Although earliness in cotton may be influenced by environmental factors it is primarily under genetic control. Of the 7 phosphorus containing compounds reported in this study, DNA is the 1 most frequently found to be associated with heritable characters and is often referred to as the "carrier" of heredity. In this study the concentrations of DNA in the 3 varieties, as indicated by its phosphorus content, were remarkably similar before and during germination. No differences should be expected since the DNA content of cells, except when dividing, tends to be constant (6). Differences in DNA, which have been found to characterize the source, are usually found in the nucleotide composition of the DNA molecule itself: DNA acids of different species of organisms have different nucleotide compositions (6).

SUMMARY

Successive changes in the amounts and concentrations of the phosphorus containing compounds of 2 early- and 1 late-maturing varieties of cotton during a 6-day germination period were followed analytically.

The dominant process of phosphorus metabolism in the germinating seed was the dephosphorylation of phytin with the simultaneous accumulation of relatively large amounts of inorganic-P. However, germination was also marked by the synthesis and accumulation of additional amounts (beyond those of the embryos) of RNA-, DNA-, protein-, ester- and lipid-P compounds.

Light, compared to darkness, increased the rate of phytin hydrolysis and the synthesis of lipid- and ester-P compounds during germination and early seedling

growth but had no appreciable effect on the other phosphorus compounds.

In the partition of phosphorus, the hypocotyls plus roots of 6-day-old seedlings differed mainly from the corresponding cotyledons in that the former contained little or no phytin- and only about one sixth as much RNA-P.

Varietal difference was limited to phytin-P which was present in the embryos and seedlings of the late variety in substantially greater amounts and concentrations than in the 2 early-maturing cottons.

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