Phosphorus Metabolism of Germinating Oat Seeds[1]

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Summary. An investigation has been made of the changes in the major phosphorus containing substances in *Avena sativa* during the first 8 days of dark germination. The endosperm, roots, and shoots were analyzed separately for acid soluble-P, phytic acid-P, inorganic-P, lipid-P, nucleic acid-P, and protein-P. Phytic acid-P comprised 53 % of the total seed phosphate, while the sum of lipid-P, nucleic acid-P and protein-P comprised 27 % of the seed phosphate. All these reserve phosphate materials were mobilized and transferred to the developing axis. The phosphate from phytic acid appeared almost entirely as inorganic-P in the roots and shoots. A close stoichiometry existed between the rate of loss of nucleic acid-P from the endosperm and its rate of appearance in the roots and shoots. Thus no net synthesis of nucleic acid occurred during the 8-day period examined. The rate of synthesis of lipid-P in the roots and shoots exceeded its rate of disappearance from the endosperm during the first 4 days of germination. Protein-P increased in the roots and shoots during germination, but at a rate less than its rate of disappearance from the endosperm. The results provide a relatively complete description of the over-all aspects of phosphorus metabolism associated with germination of oats.

The major metabolic processes associated with seed germination are the mobilization of storage materials in the reserve tissue and their subsequent transfer to and utilization by the developing embryonic axis. This mobilization, transport, and utilization has been studied for a variety of substances (cf. 15, 23), but primarily has been concerned with the various N-containing substances such as proteins and nucleic acids. In light of the importance of various phosphorylated substances in metabolism it seemed desirable to characterize the germination process with respect to the time sequence of changes in the major phosphorylated substances. Although specific aspects of phosphorus metabolism during germination have been examined such as changes in phytic acid, nucleic acid, phospholipids, etc. (1, 3, 11, 20) an over-all description or balance sheet of the changes in these various substances relative to each other during germination has not been made.

Phytate (Ca, Mg, K salt of inositol phosphoric acid) is generally believed to be the primary reserve phosphate in the seed (3, 16). It has been shown that phytase, the enzyme which catalyzes the hydrolysis of phosphate from phytic acid, increases markedly during the first few days of germination (19). The liberated phosphate then

presumably enters into various synthetic reactions occurring in the developing axis. However, the extent of this inorganic-P utilization during dark germination for synthetic reactions is unclear. This stems primarily from the uncertainty of the nature of the mobilization, transfer and utilization of other reserve phosphate containing compounds. For example, some investigators believe that nucleic acids are transported from reserve tissue to axis intact or as the nucleotides (13, 17, 18) while others feel that some de novo synthesis of nucleic acids does occur in the axis (4, 7, 11). Similarly, it is not clear whether phospholipids move from the reserve tissue to the axis or whether they are synthesized de novo in the axis, although the latter appeared to be the case for cotton germination (5). It has also been shown that phosphoproteins occur in seeds (5, 12, 24), however, their mobilization and utilization during germination has not been examined. Thus, the relative contribution of various phosphorylated materials such as nucleic acids, phospholipids, phosphoproteins, etc., in relation to phytic acid for contributing phosphate for the various synthetic processes in the axis needs examination.

In the present work an attempt was made to obtain an over-all description of phosphorus metabolism associated with germination of oats. We have followed the time sequence of changes in acid soluble-P, phytic acid-P, inorganic-P, lipid-P, nucleic acid-P and protein-P in the endosperm, roots, and shoots during the first 8 days of germination.

Materials and Methods

Approximately 100 seeds of *Avena sativa* Var. Goodfield were planted on paper towels saturated with 100 ml of 0.1 mM $CaCl_2$ in 25.4 × 35.6 cm pyrex baking dishes which were covered with perforated Saran wrap. The dishes were placed in a dark germinator at 28° for the desired times. Plants were harvested at 2, 4, 6 and 8 days by carefully removing the lemma and palea and then separating the roots and shoots, with the attached remnants of the embryo, from the remainder of the seed. The remaining part of the seed included both the endosperm and scutellum as well as the other associated tissues. This entire group of tissues will be referred to as 'endosperm'. The roots and shoots were separated at the transition zone with the embryo remnants remaining with the shoot. The tissue was kept on ice until extraction. For data at 0 days, the entire dry seed, excluding lemma and palea, were analyzed. No attempt was made to separate the embryo.

Dry weights were determined by drying the tissue after dissection at 70° for 24 hours. For determination of total-P disappearance from the endosperm and its appearance in the roots and shoots, the various organs were thoroughly homogenized in deionized water with a power-driven, conical glass homogenizer Aliquots of the homogenate were wet washed in 10 N sulfuric acid. Final clearing was accomplished with hydrogen peroxide. The digested samples were diluted and boiled for 10 minutes to break pyrophosphate bonds and then assayed for total-P according to the method of Fiske and SubbaRow (6).

Extraction Procedure. The various plant parts were extracted according to the procedure of Schneider (21) with slight modifications. The 4 fractions examined were the following:

I. Acid Soluble-P. The tissue was homogenized in ice cold 0.2 N perchloric acid with a power driven conical glass homogenizer. The extract was held on ice 15 minutes prior to clearing by centrifugation. The residue was re-extracted twice for 15 minutes in ice cold 0.2 N perchloric acid. The cleared, acid soluble extracts were combined and analyzed for total-P, inorganic-P, and phytic acid-P.

II. Lipid-P. The acid insoluble residue was extracted 3 times at room temperature with ethanol: ether:chloroform (2:2:1 v/v). The cleared extracts were combined and an aliquot removed for total-P determination.

III. Nucleic Acid-P. The defatted residue was washed with ice cold 5 % trichloroacetic acid for 5 minutes and then extracted with an additional portion of 5 % trichloroacetic acid at 90° for 15 minutes. The cleared extracts were combined and a sample taken for total-P determination.

IV. Protein-P. The hot trichloroacetic acid insoluble fraction was suspended in 1 N NaOH and placed in a boiling water bath for 10 minutes. Inorganic phosphate of the cleared extract was taken as a measure of phosphorylated protein (8, 21).

The final residue was analyzed for total-P and never exceeded 0.5 % of the total-P of the tissue. Total-P in each of the fractions was determined as described above for the total-P content of the various organs. Direct estimation of inorganic-P in the acid soluble extract was determined on a sample prior to digestion. Phytic acid-P of the acid soluble extract was determined by the method of Asada and Kasai (2) which first involved bringing the acid soluble fraction to 20 mM with NaEDTA prior to neutralization to prevent coprecipitation of phytic acid (22). The cleared, neutralized extracts were then applied to columns (1.0 × 10 cm) of Dowex 1-X8 chloride resin (200–400 mesh). The column was washed with water and then eluted with a linear gradient of HCl. Six ml fractions were collected and total-P of each fraction was determined. Two major phosphate peaks occurred from the various seed extracts. The first peak (tubes 8–12) was inorganic-P and the second peak (tubes 55–65) eluted at the same place as an authentic sample of Na-phytate (Sigma Chemical Company). The inorganic-P peak was not quantitative since the effluent and wash also contained inorganic-P.

The difficulties encountered in the quantitative extraction and estimation of the various phosphorylated substances in plants are great (7, 9, 10) and as Ingle (10) has pointed out none of the commonly used extraction procedures appears to be completely satisfactory for the estimation of nucleic acids. Thus, the various fractions described above are considered to represent only a semi-quantitative estimate of phospholipids, nucleic acids, phosphoproteins, etc.

Results and Discussion

Figures 1 and 2 show the changes in fresh and dry weight of the various plant parts at the sampling times employed. It is evident from figure 1 that approximately 24 hours were required for the seeds to become fully imbibed. After 24 hours the endosperm (including the scutellum and other associated tissues) decreased in fresh weight until day 4 and then remained fairly constant. The total plant dry weight (fig 2) showed a marked decrease between days 2 and 4 and this corresponds to the most rapid rate of loss of dry weight from the endosperm. By comparing figures 1 and 2 it can be seen that the fresh weight of the shoot material increases much more rapidly than the dry weight.

Disappearance of total-P from the endosperm and its appearance in the roots and shoots are shown in figure 3. These changes correspond quite closely to the changes in dry weight except for the
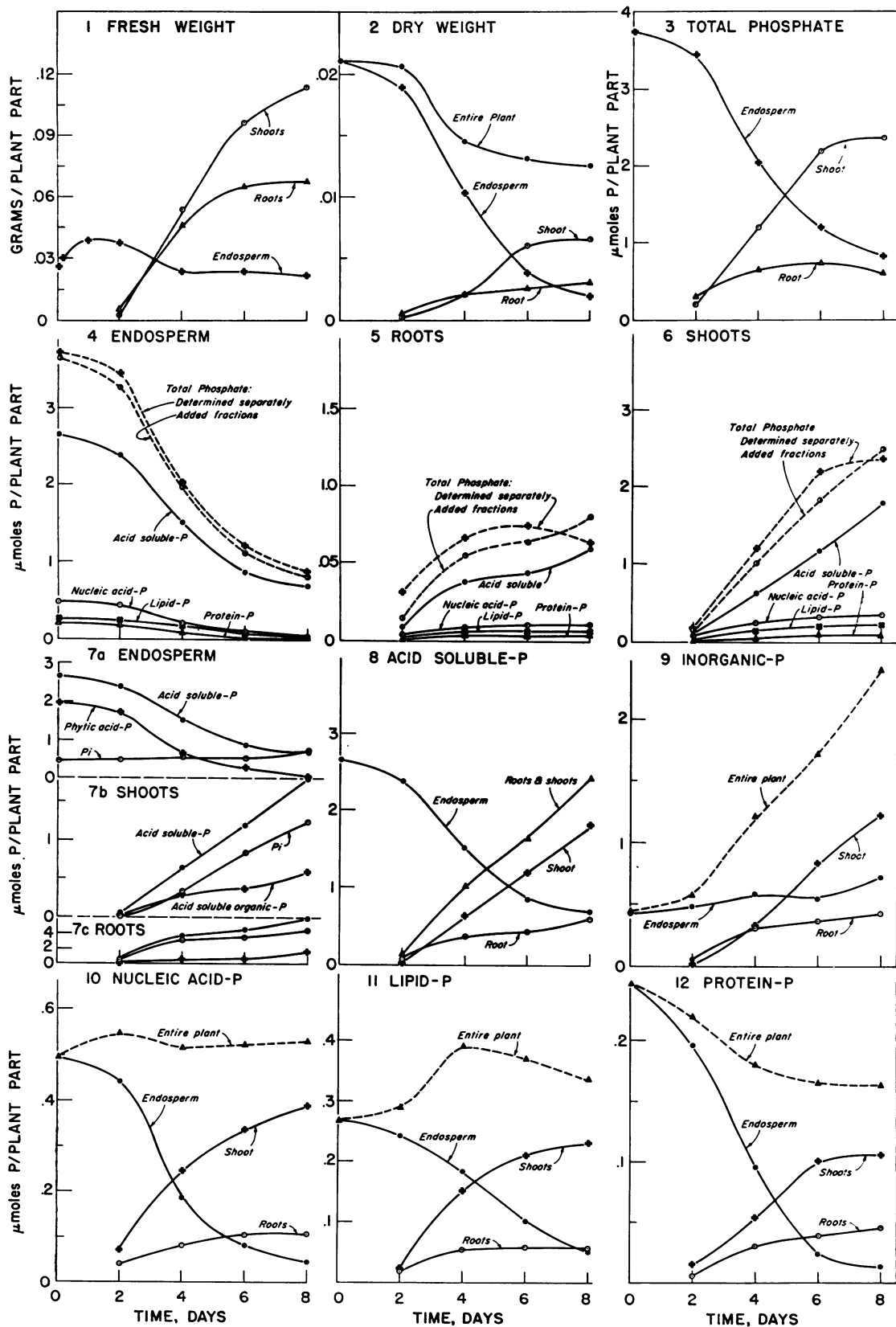
FIGS. 1-12. The changes of various components of the 'endosperm', roots and shoots of oats over an 8-day germination period. The data are averages of from 2 to 6 separate experiments.

rapid increase in phosphate in the shoots which is similar to the rapid increase in fresh weight of the shoots (fig 1). The distribution of total-P between acid soluble-P, lipid-P, nucleic acid-P and protein-P and the time sequence of changes in these fractions in the endosperm, roots, and shoots are shown in figures 4, 5, and 6, respectively. The data are presented in this fashion in order that one can readily compare the relative amounts of these substances. Note, however, that the root data are presented on an expanded scale as compared to the endosperm and shoot data. Also shown in these figures are the total-P determined by homogenization of the tissue in water (fig 3) and the total-P as determined by adding the separate fractions. In general, close agreement was found between these totals except in the case of the root tissue which varied considerably, especially at day 2.

In the case of the endosperm all fractions decreased during the germination period. Most of the phosphate at all sampling times was acid soluble and comprised 75 to 85 % of the total-P. The nucleic acid-P made up about 12 % of the total-P at day 0 and decreased gradually to about 6 % by the eighth day. The lipid-P made up about 6.5 to 8.5 % of the total seed-P and the percentage in the endosperm remained fairly constant during the germination period. The protein-P content decreased from an initial level of 6 % to only 2 % on day 8.

The large amount of acid soluble-P in the endosperm was comprised almost entirely of phytic acid-P and inorganic-P (fig 7a). Phytic acid-P decreased at a rate nearly identical to the decrease in acid soluble-P while the inorganic-P content of the endosperm actually increased slightly during the germination period. In the dry seed, phytic acid-P represents about 74 % of the acid soluble-P or 53 % of the total-P. The percent of phytic acid-P in the endosperm also decreases rapidly during germination indicating its preferential breakdown. Thus, for the 5 sampling times the percent of total-P in phytic acid in the endosperm is 53, 50, 31, 22 and 3 % respectively. Conversely, since the inorganic-P content of the endosperm increases slightly during germination, as a percentage of total-P in the endosperm it increases markedly. Thus by the eighth day nearly all the endosperm phosphate is inorganic-P (cf. figs 4, 7a).

In both the roots and shoots all the phosphate fractions increased with germination time (figs 5, 6). Acid soluble-P accounted for most of the phosphate. On a percentage basis the acid soluble-P increased gradually over the 8 day germination period from 58 % to 74 % in the roots, and 32 % to 72 % in the shoots. The acid soluble-P consisted primarily of inorganic-P in the roots (fig 7c). This was true to a somewhat lesser extent in the shoots (fig 7b). The nature of the acid soluble organic-P (calculated by difference in acid soluble-P and

inorganic-P) was not determined but it presumably consists primarily of sugar phosphates and nucleotides. Neither the roots or shoots were found to contain any phytic acid.

Figures 8 to 12 present these same results in terms of the decrease in specific phosphate fractions of the endosperm tissue and the concomitant increase in these phosphate fractions in both the root and shoot tissue. The increase in acid soluble-P in the roots and shoots appears to occur at the expense of acid soluble-P in the endosperm (fig 8). However, the data in figure 9 as well as figure 7, show that the increase in inorganic-P of the roots and shoots does not occur at the expense of inorganic-P in the endosperm. As already pointed out by others (15, 20, 23) phytic acid breakdown in the endosperm accounts primarily for the increase in inorganic-P of the roots and shoots. This is more clearly depicted in figure 13 which shows a near stoichimetric loss in phytic acid-P from the endosperm and gain in inorganic-P of the roots and shoots. This general pattern has been observed repeatedly by other investigators using different extraction techniques and species (1, 5).

The rate of nucleic acid-P loss from the endosperm occurs at nearly the same rate as nucleic acid-P increases in the roots and shoots (fig 10). The fact that no net gain in nucleic acid-P occurs in the entire plant is different from the results obtained with corn by Ingle and Hageman (11) and for wheat by Matsushita (14). However, it
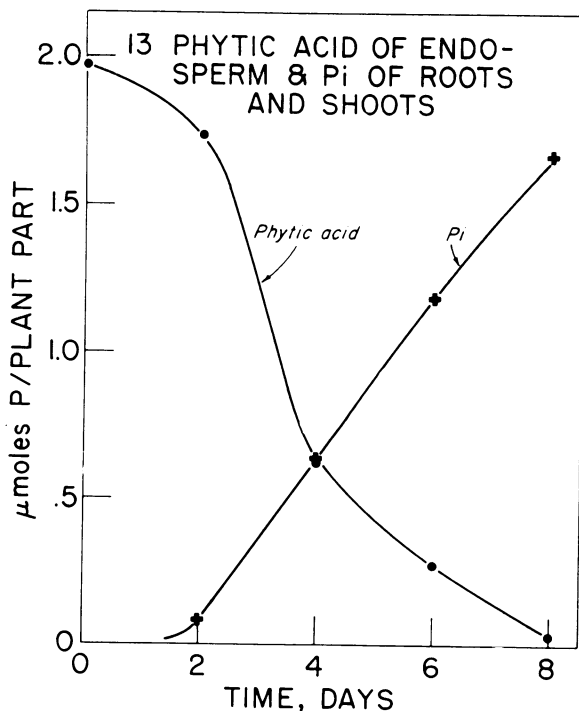

FIG. 13. The changes in phytic acid-P of the 'endosperm' and inorganic-P of the roots plus shoots over an 8-day period of germination.
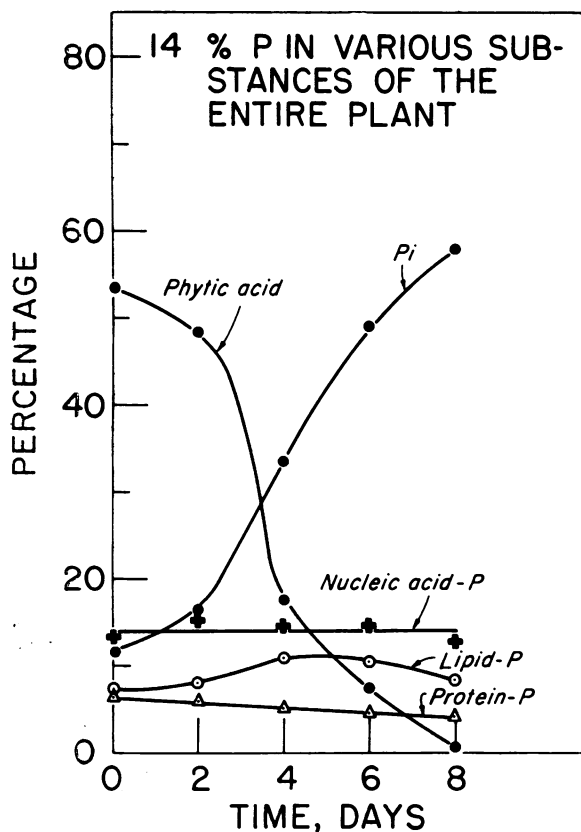
FIG. 14. The changes in percentage of total-P of the entire plant for various phosphorus containing substances over an 8-day period of germination.

is similar to results obtained for barley by Ledoux and Huart (13) and for bean by Oota and Takota (18). These results would then suggest that either nucleic acids moved intact from the reserve tissue into the developing axis or that nucleic acid synthesis in the axis was solely at the expense of nucleotides derived from the reserve nucleic acids. It is possible that some de novo synthesis of nucleotides or nucleic acids may have occurred in the roots and shoots, but the present data do not permit an evaluation of this possibility.

In contrast to the nucleic acid-P, figure 11 shows that a slight net synthesis of phospholipid occurs during the first 4 days of germination. This increase is followed by a gradual decrease in total lipid-P over the next 4 day period but even at the eighth day more total lipid-P exists than was present in the dry seed. Similar results were obtained by Ergle and Guinn (5) for cotton seed germination.

Figure 12 shows the changes in protein-P during the germination period studied. Although the amount of protein-P increases in the roots and shoots the entire plant exhibits an over-all decrease. Although the protein-P of the plant represents a small fraction of the total plant-P it certainly

appears to participate in the metabolism associated with germination.

A summary of the data are shown in figure 14. This figure shows the percentage of total-P of the entire plant for the various fractions examined at each of the sampling times. It is quite apparent that inorganic-P is derived primarily from phytic acid and that a net synthesis of the other phosphorylated substances does not occur except for phospholipids.

Discussion

This study provides a fairly complete balance sheet of the changes which occur in the major phosphorylated substances during the germination of oats. The results confirm that phytic acid represents the primary storage form of phosphate in oat seeds (about 53 % of the total-P). However, the actual participation of the phosphate derived from phytic acid, which appears in the roots and shoots as inorganic-P (fig 14), in various synthetic reactions in the roots and shoots during the dark germination is somewhat questionable. That is, the very fact that the phosphate remains as pools of inorganic-P in the roots and shoots raises the question of its use in synthetic events in the developing axis.

Although phytic acid represents approximately 53 % of the seed-P, the combination of nucleic acid-P, lipid-P and protein-P also make up a sizeable portion of the seed-P (about 27 %). Furthermore these substances are also rapidly mobilized in the endosperm and it would appear that there is nearly a direct conversion of these materials into nucleic acid-P, lipid-P, and protein-P, respectively in the developing roots and shoots. This is especially suggested in the case of nucleic acid (figs 10, 14). Whether the nucleic acids move as macromolecules (13, 17, 18) or are first degraded and resynthesized in the embryonic axis (4, 7, 11, 14) is impossible to ascertain from this type of data. Information on ^{32}P or nucleotide incorporation into nucleotides and nucleic acids are needed to clarify this point. From the variable reports in the literature (4, 7, 11, 13, 14, 17, 18), however, it would appear that some species simply possess the capacity for a net synthesis of nucleic acids during dark germination while others do not.

In the case of lipid-P it also appears that a transfer from endosperm to axis may occur but in addition some de novo synthesis in the axis must also occur since the rate of increase in phospholipid content of the roots and shoots exceeds the rate of loss from the endosperm (fig 11). This synthetic process is most rapid between days 2 and 4 and is most prevalent in the shoots. The source of phosphate for this additional synthesis could have come from inorganic-P originating from phytic acid or perhaps to some extent from the breakdown of phosphoprotein. This latter possibility arises since

the rate of synthesis of protein-P in the roots and shoots does not keep pace with its rate of loss from the endosperm (fig 12). Thus in the case of phosphoproteins a de novo synthesis during dark germination must also be quite limited. The actual function of phosphoprotein in plant metabolism is unknown. Its formation during wheat seed ripening has been shown by Jennings and Morton (12) and Ergle and Guinn (5) have shown its disappearance from cotton seeds during germination. Current studies are directed toward an evaluation of its possible participation in energized ion transport.

As mentioned earlier the fact that the majority of phosphate derived from phytic acid remains as pools of inorganic-P makes one question its metabolic significance in the case of dark germinating oats. This is further suggested by the nearly stoichiometric losses from the endosperm and gains in the roots + shoots of nucleic acids, phospholipids and phosphoproteins. It is possible that plant species such as corn, which possess the capacity for net synthesis of nucleic acids during germination (11) can immediately utilize this source of inorganic-P. In the present case, it is possible that only in the presence of light would the inorganic-P be used for synthesis of organic phosphate substances in the developing axis. A comparative study using species which exhibit a net gain in nucleic acids in the axis (for example corn) and those which do not exhibit a net gain (for example oats) when germinating under light and dark conditions should help to clarify this problem.

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