Phosphorus metabolism during the germination of soybean (Glycine max. (L) Merr.)

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INTRODUCTION. — Phosphorylated compounds play an extremely important role in variety of reactions in seed. In light of the importance of various phosphorylated substances in metabolism, different workers have studied the changes in phosphorus compounds during the germination of wheat (PEERS, 1953) cotton (ERGLE & GUINN, 1959), dwarf bean GIBBINE & NORRIS, 1963), oat (HALL & HODGES, 1966) and ground nut (MARCUS & FEELEY, 1962). But no work has been reported in soybean, which is a rich source of protein as well as oil. It has been shown that phospho-proteins occur in seeds (ERGLE & GUINN, 1959; JENNING & MORTON, 1963; and Woo 1919), however their mobilisation and utilization during germination has not been examined in detail. Similarly it is not clear whether phospholipids move from the reserve tissue to the axis or whether they are synthesised *de novo* in the axis. HALL & HODGES (1966) studied the changes in phosphorus containing compounds in oat, but the relative contribution of various phosphorylated compounds needs further confirmation.

It seems desirable to characterise the major phosphorylated substances during the germination process of soybean with respect to the time sequences and the results are reported in this paper.

MATERIAL AND METHODS. — The germination of soybean seeds of variety Pb.No.1 was carried out in washed and sterilised sand. Sand was filled in trays and levelled properly. The sand for sowing was sterlised because the seeds of soybean are susceptible to fungus growth. Seeds for germination were kept at the depth of one inch in sand and the trays were kept in the green house, under normal atmospheric conditions.

Samples of seedlings were taken after 0, 2, 4, 6 and 8 days of germination. Seedlings were washed with distilled water to make them free of adhering sand particles and were then seperated into cotyledons, radicles, plumule and leaves wherever possible. The samples of cotyledons, radicles, plumules

and leaves were dried at low temperature (70° C) and grinded in a grinder and stored in the glass stoppered tubes for further analysis.

The following estimations were carried out in duplicate and recovery was tested in each case and results were calculated on that basis.

Estimation of phosphorus fraction: *Total phosphorus*. — Total phosphorus in the plant material was estimated according to the method of FISKE & SUBBA ROW (1925) after a prior wet digestion.

Extraction procedures for phosphorus fractions. — The various plant parts were extracted according to the modified procedure of Schneider (1945).

i) *Acid soluble phosphorus*. — 20 mg of the plant material was thoroughly homogenized with 0.2 N perchloric acid with the help of pestle and mortar. The extract was taken in a centrifuge tube and kept in ice water for 15 minutes. Then it was centrifuged. The clear supernatent was removed and residue was again extracted twice for 15 minutes in ice cold 0.2 N perchloric acid and clear supernatent removed. The clear acid soluble extracts were combined and analysed for total-P, after drying and digestion with triple acid.

ii) *Lipid phosphorus*. — The acid insoluble residue was extracted three times at room temperature with ethanol: ether: Chloroform (2:2:1, v/v) for 10 minutes each. The cleared extracts after centrifugation were combined and analysed for total-P, after centrifugation were combined and analysed for total-P, after drying and digestion with triple acid.

iii) *Nucleic acid phosphorus*. — The defatted residue left was washed with ice cold 5 per cent trichloroacetic acid for 5 minutes and then extracted with an additional portion of 5 per cent trichloroacetic acid at 90° C for 15 minutes, and centrifuged. The clear extract was removed and residue was again extracted with 5 per cent trichloroacetic acid. The extracts were combined and analysed for total-P after drying and digestion with triple acid.

iv) *Protein phosphorus*. — The hot trichloroacetic acid insoluble fraction was suspended in 1 N sodium hydroxide for 10 minutes. It was then placed in a bioling water bath for another 10 minutes. After cooling it was centrifuged and the clear extract was then analysed for total-P after drying and digestion with triple acid.

The final residue left was analysed for total-P after digestion with triple acid.

RESULTS AND DISCUSSION. — The samples of cotyledons, roots, shoots, leaves and testa, taken after 0, 2, 4, 6 and 8 days of germination of soybean, were analysed quantitatively for their contents of different phosphorus fractions. The results obtained are tabulated in Table 1 and shown graphically in Fig. 1 to 5.

TABLE 1. — *Changes in phosphorus fractions during germination of Soybean.* mg/gm (on dry weight basis).

Plant Part	Time (days)	Total-P	Acid soluble-P	Lipid-P	Nucleic acid-P	Protein-P	Residual-P
Cotyledons	0	7.60	5.25	0.69	1.02	0.52	0.13
	2	8.22	6.13	0.50	1.00	0.50	0.13
	4	7.31	5.44	0.44	0.81	0.38	0.31
	6	6.38	4.88	0.38	0.63	0.31	0.25
	8	5.94	4.56	0.41	0.56	0.13	—
Testa (1)	2	1.63	0.81	0.25	0.38	0.28	—
Roots	2	6.13	4.06	0.69	0.63	0.38	0.25
	4	6.88	4.56	0.81	0.69	0.50	0.28
	6	7.06	4.81	0.88	0.75	0.63	0.09
	8	8.19	5.50	1.13	0.84	0.72	0.06
Shoots	2 (2)	—	—	—	—	—	—
	4	5.59	3.56	0.63	0.56	0.40	0.13
	6	6.56	4.13	0.88	0.75	0.50	0.25
	8	7.25	4.56	1.06	0.81	0.68	0.25
Leaves	6	6.25	4.38	0.63	0.63	0.44	0.13
	8	8.06	5.44	0.75	0.81	0.69	0.13

(1) Testa degraded after 2 days of germination.
(2) The plumule portion was too small and taken together with root at 2 days germination of soybean.

In cotyledons, during germination, it was observed that total-P, content decrease while in roots, shoots and leaves, an increase in total-P content was observed on dry weight basis. In cotyledons, all the phosphorus fractions i.e. acid soluble-P, lipid-P, nucleic acid-P and protein-P, decreased during germina-

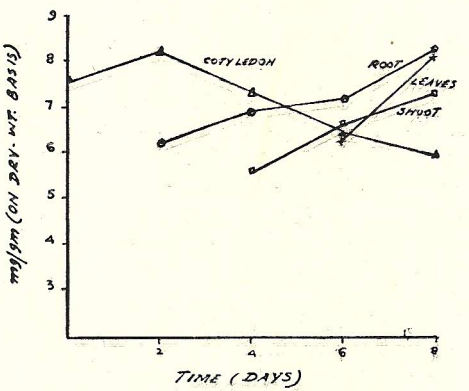

FIG. 1. — Total - P

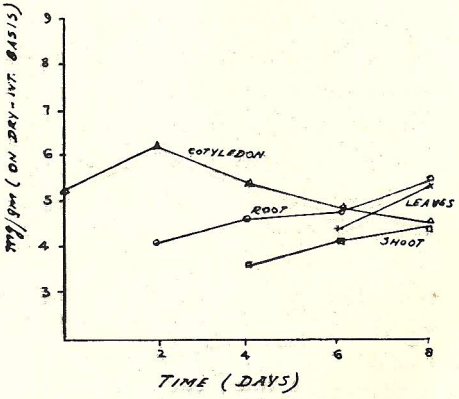

FIG. 2. — Acid-Soluble - P

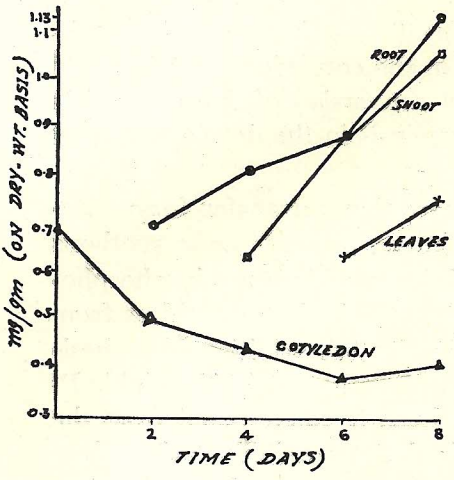

FIG. 3. — Lipid - P

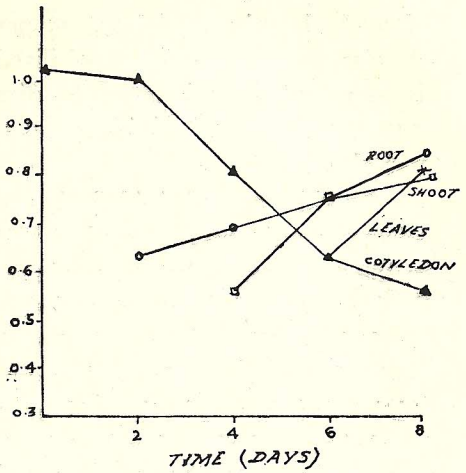

FIG. 4. — Nucleic acid - P

tion. All the axis parts i.e. roots, shoots and leaves, showed an increase in various phosphorus fractions. ERGLE and GUINN (1959) also reported the disappearance of phospho-proteins from cotton seeds during germination. The loss of acid soluble-P from cotyledons and increase in roots and shoots may be due to a stoichometric loss of phytic acid-P from the cotyledons and gain in inorganic-P of the roots and shoots. ROWAN (1966) and VARNE (1965) also pointed out that phytic acid breakdown in the endosperm accounts primarily for the increase in inorganic-P of the roots and shoots.

Although acid soluble fraction represents approximately 75 per cent of the seed phosphorus, the combination of nucleic acid-P, lipid-P and protein-P also make up a sizeable portion

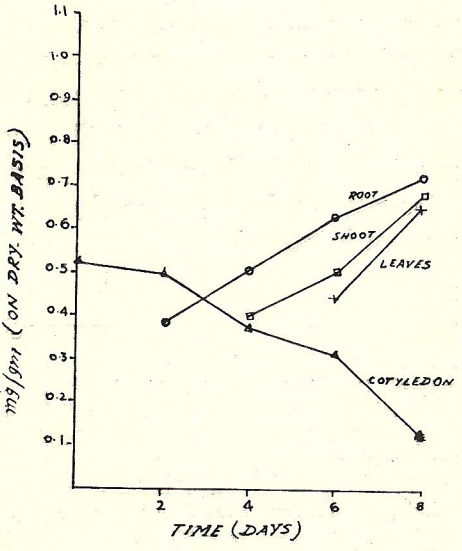

FIG. 5. — Protein - P

of seed phosphorus (about 25 per cent). Further more these substances are also rapidly mobilised in the cotyledons and it would appear that there is nearly a direct conversion of these materials into nucleic acid-P, lipid-P and protein-P in the developing roots and shoots.

In the case of lipid-P, it appears that a transfer from cotyledons 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 cotyledons. The source of phosphate for this additional synthesis might have come from inorganic phosphorus (acid soluble-P) originating from phytic acid or perhaps to some extent from the breakdown of phosphorprotein. The 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 cotyledons. Similar results were obtained by HALL and HODGES (1966) during their study on germination of oat.

The changes in the different phosphorus fractions in relation to total phosphorus present in different parts shows that acid soluble-P as a percentage of total phosphorus increase slightly over the period of sampling in cotyledons and roots whereas in shoots and leaves, slight decrease was observed. The lipid-P as per cent of total-P increases in cotyledons, roots and shoots during germination while in case of leaves it showed a slight decrease. Nucleic acid-P as per cent of total-P decreased in cotyledons, increased in shoots and remained almost constant in roots and leaves. The content of protein-P as per cent of total-P was observed to decrease gradually in cotyledons whereas in roots, shoots and leaves, it increased.

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SUMMARY — The cotyledons, roots, shoots, leaves and testa after 0, 2, 4, 6 and 8 days after germination of soybean were analysed quantitatively for their contents of different phosphorus fractions. Total phosphorus content of soybean cotyledons, was found to decrease with germination time, whereas in roots, shoots and leaves the content of total phosphorus increased. In case of lipid phosphorus along with transfer from cotyledon to the growing axis, *de novo* synthesis in the axis also takes place. Acid soluble phosphorus comprised about 75 per cent of total seed phosphate while the sum of lipid phosphorus, nucleic acid phosphorus and protein phosphorus comprised 25 per cent of the seed phosphate. All the phosphorus fractions were found to decrease in cotyledons and the reserve phosphate materials were mobilised and transfered to the developing axis.

RÉSUMÉ — On a déterminé le contenu des differantes fractions de phosphore dans les cotylédons, racines, bourgeons, feuilles at téguments de soya a distance de 0, 2, 4, 6, et 8 jours de la germination.

Le contenu en phosphore total diminu pendant la germination les cotyledons et augemente dans les racines, dans les bourgeons et dans les feuilles. Le phosphore lipidique augmente pendant qu'il se transfère depuis les cotyledons puisqu'il est syinthétisé aussi le long de l'axe de developpement.

Le phosphore soluble en acides constitue à peu près le 75% du totale contenu dans les graines. Le restant est constitué de phosphore lipidique, (nucleiquet protéique) Toutes les fractions phosproriques diminuent dans les cotylédons, puisque le phosphore de réserve résulte mobilisé et transféré vers l'axe de dévéloppement.

ZUSAMMENFASSUNG — Man hat die Enthaltung der verschiedenen Phosphor Bruchteile in den (keinblätter), Wurzeln, Sprösslinge, Blätter und Hülsen der Soja, 0, 2, 4, 6, und 8 Tage nach der Keimung festgestellt.

Die Enthaltung des totalen Phosphor nimmt ab wärend der Keimung in den Blättern.

Das lipidische Phosphor nimmt zu im Lauf der Versetzung von den (keinblätter) da es auch der Entwicklungs Achse entlang synthetisiert wird.

Das in Säuren lösbare Phosphor, stellt ungerfänr das 75% des ganzen Inhalts der Samen dar. Das übrige besteht aus lipidischem, nukleischem, und proteischem Phosphor.

Alle phosphorischen Bruchteile nehman ab in den (keinblätter), da der Vorbehalt an Phosphor resultiert in Umlauf und gegen die Entwicklungsachse versetzt.

RESUMEN — Ha sido determinado el contenido de las diferentes fracciones de fósforo en los cotiledones, raices, germinaciones, hojas y tegumentos de soja a 0, 2, 4, 6 y 8 días de la germinación.

El contenido de fósforo total va disminuyendo durante la germinación en los cotiledones, mientras que va aumentando en las raices, en las germinaciones y en las hojas. El fósforo lipídico aumenta durante la trasferencia de los cotiledones, puesto que es sintetizado incluso a lo largo del eje del desarrollo.

El fósforo soluble en ácidos presenta casi el 75% del total contenido en las semillas. El resto está constituido por fósforo lipilíco, nucleico y proteico. Todas las fracciones fosfóricas disminuyen en los cotiledones puesto que el fósforo de reserva resulta mobilizado y trasladado hacia el eje de desarrollo.

RIASSUNTO — E' stato determinato il contenuto delle varie frazioni di fosforo nei cotiledoni, radici, germogli, foglie e tegumenti di soia a 0, 2, 4, 6 e 8 giorni dalla germinazione.

Il contenuto in fosforo totale diminuisce durante la germinazione nei cotiledoni e aumenta nelle radici, nei germogli e nelle foglie. Il fosforo lipidico aumenta nel corso del trasferimento dai cotiledoni poiché viene sintetizzato anche lungo l'asse di sviluppo.

Il fosforo solubile in acidi rappresenta circa il 75% del totale contenuto nei semi. Il rimanente è costituito da fosforo lipidico, nucleico e proteico. Tutte le frazioni fosforiche diminuiscono nei cotiledoni, dato che il fosforo di riserva risulta mobilizzato e trasferito verso l'asse di sviluppo.