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STUDIES ON THE NITROGEN METABOLISM OF SOYBEANS
I. VARIATION OF METABOLITES DURING THE GROWTH OF YOUNGER PLANTS

By

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Investigations by Oota *et al.* (1) on the metabolism of bean plants have dealt almost exclusively with *Vigna* through short periods of germination at dark. Alvin *et al.* (2) investigated the chemical composition of soybean during maturation. As for the changes during germination, Ergel *et al.* (3) reported for phosphorus compounds of cotton embryos and their changes during germination. Folks *et al.* (4) investigated nitrogen fractions of endosperm during germination, and Simon *et al.* (5) investigated utilization of reserves in germinating *Phaseolus* seeds.

The authors intended to study the metabolism of young soybean plants, till the fall of cytoledon, as the first step of their whole growing process. In the present paper, data of analysis of nitrogenous, carbohydrate and phosphorus compounds are described to elucidate the metabolism of nitrogen in soybean seedlings with respect to the morphological development and the metabolic function in cotyledon during germination.

The results revealed that soybean has an active metabolism period when it develops morphologically. The adult leaf and the cotyledon have homologous functions as foliage leaf in the early stage of germination and as a storage organ. In the intial growth, there occurs a change in dry weight of cotyledon and embyronic organ (shoot and root system) indicating the process of translocation of substances from cotyledon to embryonic organs.

It has been already recognized that the cotyledon of bean plant is more abundant in essential elements for normal growth than the endosperm of other plants. Therefore, we cultured the soybean with no fertilization because it possesses full nutrients. If nutrient is supplied to the sobyean, its morphological differentiation and the growth of bean plant is delayed as long as the cotyledon adheres. On the basis of this work, authors intend to study the effective fertilizational period of

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beans. These results will appear in separate papers.

In this study, the authors have done only a part of the investigation of the metabolic function of intra- and inter-organs of bean plants. On the basis of this experiment, we shall attempt to explain the nitrogen metabolism for all stages of the soybean, especially from the view point of the chemistry of the vegetative stage and of the morphogenesis at stem apexes.

Materials and Methods

Sobyean, *Glycine max* Linne forma Shinmejiro, was used throughout this investigation. Shinemjiro is a summer type and is widely cultivated in the eastern part of Japan (6). The seeds were obtained from Miyagi Agricultural Experiment Station.

Seedlings were grown in sand in a green house for fourteen days from late May to early June, 1965. Conditions of the culture were as follows: seeds were soaked in water for two days, sown in pots which contained sand. No nutrient was supplied. The experiment was done in four runs. The cotyledons were just to fall at the end of the cultural period under these conditions.

Samples were taken at daily intervals during germination. Five to ten plants were taken at random as a sample from each pot. These plants were washed and dried immediately in an air oven at 70–80°C for 30 minutes and then at 60°C for 24 hours. Dried samples were separated into cytoledon and embryonic organ, weighed instantly, then ground into powder with a grinding mill and stored for analysis.

Determinations of nitrogenous, phosphorus substances and carbohydrates were made as follows.

Nitrogenous substances:

The analyses of nitrogenous substances were carried out according to the procedures established with rice plants (7).

Total nitrogen: according to Kjeldhal's method.

Soluble nitrogen: Five grams of the sample was taken in a flask with 80 ml water, 10 ml 10% sodium tungustate and 10 ml 2/3 N sulphuric acid and shaken thoroughly, then extracted overnight. Soluble nitrogen was determined on the filtrate of this extract.

Protein nitrogen: calculated from the difference between the total and soluble nitrogen.

Carbohydrates:

Reducing sugar: determined on the filtrate for analysis of soluble nitrogen colorimetrically by the method of Somogyi (8).

Non-reducing sugar: An aliquot of the filtrate for analysis of the soluble nitrogen was hydrolyzed with dilute sulphuric acid in a boiling water-bath for two hours and determined for soluble sugar. Then the difference between the soluble sugar and reducing sugar was given as non-reducing sugar.

Total sugar: 0.5 gram of sample was taken and added with 50 ml water and 2 ml 30% sulphuric acid and heated for two hours on a boiling water-bath. Amounts of total sugar were also determined colorimetrically.

Crude starch: calculated by the following equation,

Crude starch=(total sugar-soluble sugar)×0.9

Phosphorus substances:

Total phosphorus: according to Allen's method (9).

Inorganic phosphorus: Samples were extracted with 10% trichloroacetic acid and the extracts were analyzed colorimetrically with amidol reagent by the method of Allen.

Lipid-, nucleic- and protein-phosphorus: These fractions were prepared according to the method of Schneider (10). Each fraction was digested with concentrated sulphuric acid and determined colorimetrically by the method of Allen.

Results and Discussion

Fluctuation of dry weight

Determinations of dry weight of cotyledon and embryonic organs of soybean at successive stage of growth were carried out in the period of germination. Changes of the dry weight at successive stage of growth of the seedling are shown in Fig. 1. The cotyledons show a steady decrease in dry weight for the first eight

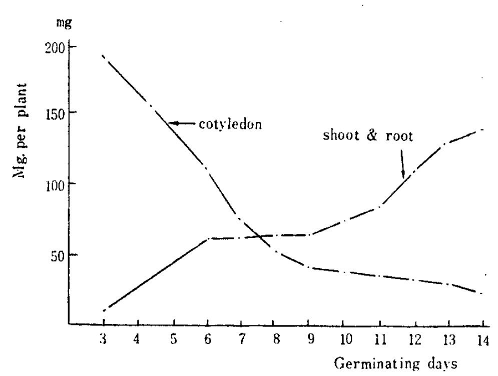

Fig. 1. Changes in dry weight of soybean during germination.

days after seed germination and then show a less severe decrease till the fall of cotyledon, fourteen days after germination. At the same time, there is a steady increase in dry weight in the embryonic organs of soybean.

The decrease of dry weight in cotyledon and the increase of it in the embryonic organs clearly indicate the translocation of metabolites from the cotyledon to the shoot and root system.

Accumulation and translocation of nitrogen.

Total nitrogen:

Determination of total nitrogen contained in the cotyledons and the embryonic organs were carried out at successive stage of their growth. The data are given in Fig. 2.

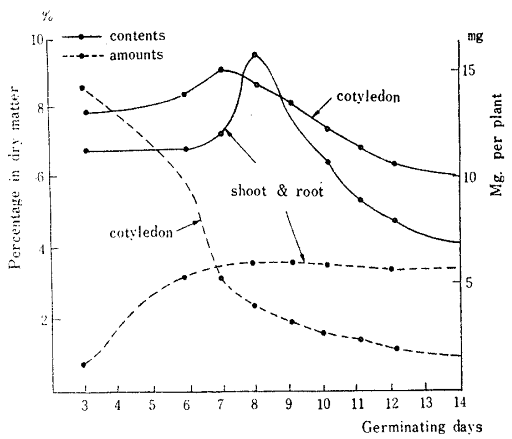

Fig. 2. Changes in total nitrogen of sobyean during germination.

Nitrogen contents in cotyledon increased up to the seventh day after seed germination (this is the middle stage of full adhering period of cotyledon). Afterward, they decreased gradually with the growth of soybean. In the embryonic organs, nitrogen contents increased gradually up to the eighth day and then a rapid decrease was observed.

Nitrogen amount in the soybean was calculated by multiplying the dry weight of each organ and their nitrogen contents. With the growth of bean plants the nitrogen amounts decreased rapidly in the cotyledon but they increased gradually in the embryonic organ.

Nitrogen compounds were available to functional maintenance in the initial stage and soon decomposed remarkably for translocation to the embryonic organ in the cotyledon. In the embyronic organ, these were available for morphological formation of the adult plant as a first step, (this means, when the first leaves developed, the plant enters the initial adult stage of the growth) and then, contributed to the other physiological function as secondary steps. In the soybean, the adult leaf developed on the eighth day after seed germination under the above conditions. The rapid decrease of nitrogen amounts in the cotyledon is also due to translocation of nitrogenous substance to the embryonic organ.

Water-soluble nitrogen:

In both the cotyledon and embryonic organ, contents of water-soluble nitrogen showed a marked tendency as shown in Fig. 3. It shows a peak at the seventh day

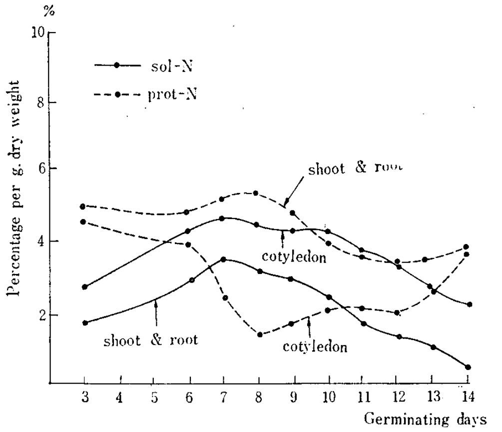

Fig. 3

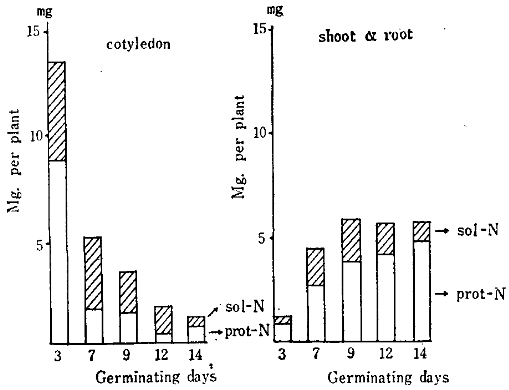

Fig. 4

Fig. 3. Changes in souble and protein nitrogen of soybean during germination.
Fig. 4. Changes in nitrogen fractions of soybean during germination.

after seed germination, like the total nitrogen in cotyledon. The amounts of soluble nitrogen decreased rapidly in the cotyledon and increased rapidly in the embryonic organ up to the nineth day after seed germination (Fig. 4).

Its main components are considered to be peptides and simple nitrogenous compounds such as amides and amino acids as described with barley (4).

Protein nitrogen:

Contents and amounts of protein nitrogen in the cotyledons and embryonic organs are shown in Fig. 3 and Fig. 4.

The protein nitrogen content of cotyledon decreased gradually up to the sixth day after seed germination, afterward, a rapid decrease was observed up to the eighth day which shows the minimum value. Then it increased rapidly till the end of germinating period. The protein content in the embryonic organ increased slowly up to the eighth day, and then decreased gradually with the growth of the soybeans.

Important feature of the results seems to be the difference of protein amounts between the cotyledon and embryonic organ on the eighth day after seed germination. Both cruves showed a contrast tendency. The phenomena suggest that catabolic reactions of protein in cotyledons progressed rapidly, and the breadown products moved from the cotyledons to the embryonic organs. In the embryonic organ, on the contrary, synthetic reactions of protein progressed rapidly till the eighth day after seed germination. After the eighth day, seedlings of soybeans assume an adult form. Protein metabolism will turn to the secondary physiological phase in vivo, after the adult form in the soybean is attained.

Fluctuation of carbohydrates.

Carbohydrates analysis was divided into the total sugar, reducing sugar, non-reducing sugar and crude starch of cotyledon and embryonic organ of the soybean at successive stage of growth.

Total sugar:

Contents and amounts of total sugar in the soybeans are shown Fig. 5.

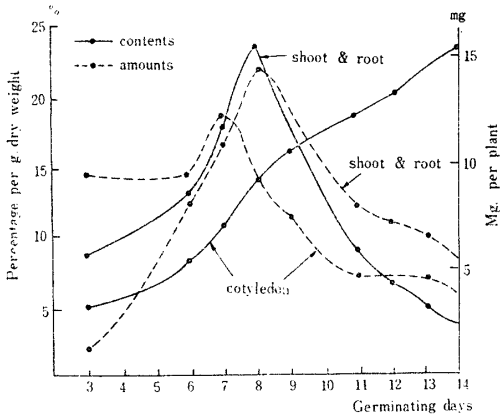

Fig. 5. Changes in total sugar of soybean during germination.

Total sugar contents of the cotyledon increased rapidly after seed germination; at the last stage it was five times the initial stage. Amounts of total sugar of cotyledons decreased very slowly in the initial stage, and it increased rapidly up to the seventh day, afterward it decreased rapidly with the growth of the soybeans. The increase and decrease curves of the contents and amounts in the shoot and root system followed normal distribution cruve. The maximum sugar contents and amounts in the embryonic organ was recognized on the eighth day. When the sugar quantity of those system reached the maxium, the development of adult leaf began almost immediately.

These are the results of accumulation of carbohydrates by acceleration of photosynthesis. But despite the accumulation of carbohydrates, the amounts of total nitrogen and protein nitrogen were shown to be at maximum value eight days after seed germination. It means that there exists a sufficient quantity of effective protein nitrogen for the acceleration of photosynthesis in plant body. On the contrary, it is recognized that there exist the nitrogenous compounds and carbohydrates which are needed for protein synthesis in the development of the adult leaf in soybeans.

Reducing sugar:

Data of the reducing sugar contents and amounts in the soybean are given in Fig. 6.

The reducing sugar contents of cotyledon increased rapidly up to the seventh day, thereafter it decreased up to the thirteenth day and increased again rapidly during the subsequent period. However, in the embryonic organ, the reducing sugar contents decreased gradually up to the eighth day, thereafter, it increased

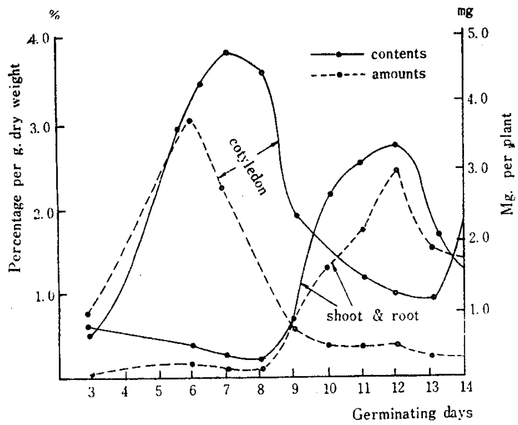

Fig. 6. Changes in reducing sugar of soybean during germination.

rapidly up to the twelfth day and then decreased. The content of reducing sugar was very small in comparison with the content of total sugar or crude starch.

The amounts of reducing sugar increased up to the sixth day and then it decreased in the cotyledon. It decreased gradually up to the eighth day, thereafter, it increased rapidly up to the twelfth day and then decreased in the embryonic organ.

Reducing sugar is an intermediary in carbohydrate transformation and was stored as starch in the initial stage. The amounts of reducing sugar in the embryonic organ are increased, causing a decrease in the amount of carbohydrate. According Takawa *et al.* (11), the amount of reducing sugar is very small at the period of storage and is of little significance as the stored substance. It is used as an energy-source for the plant to live.

The maximum value in carbohydrate content lags well behind the maximum value in protein nitrogen content, that presumably is due to the change from nitrogen to carbon nutrition. It is this period when reducing sugar reaches the maximum value, so that physiological mobility in soybean body is evident.

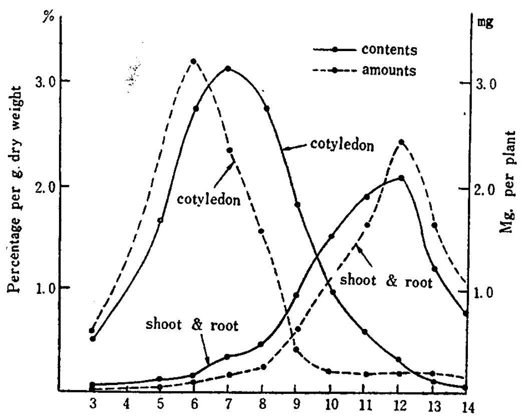

Fig. 7. Changes in non-reducing sugar of sobyean during germination.

Non-reducing sugar:

Fig. 7 shows the non-reducing sugar contents and amounts. They are very small in comparison with total sugar or crude starch. Contents and amounts of non-reducing sugar increased up to the seventh and sixth days. Afterward they decreased rapidly in cotyledon. In the embryonic organ, they increased rapidly up to the twelfth day and then decreased rapidly.

The results of these non-reducing sugar determinations show that the sugar occupies only a small part of the total cabohydrate, but it plays a role in the translocation of carbohydrate in vivo (11).

Crude starch:

Data on the starch contents including amounts in the soybean are given in Fig. 8.

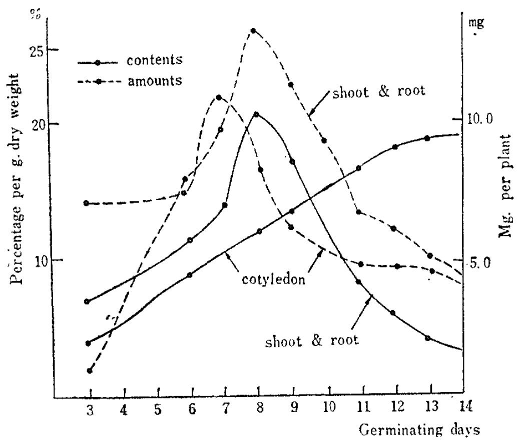

Fig. 8. Changes in crude starch of soybean during germination.

Changes of contents and amounts of crude starch in the cotyledon and embryonic organ are very similar to total sugar behavior. Crude starch occupied the majority of the carbohydrates content in the soybean. Therefore, if we discuss the carbohydrate relation to the growth of the soybean, it is sufficient to deal with the problem of starch only.

As the amounts of total nitrogen increased, the amount of crude starch decreased after development of the adult leaf and a rapid increase in dry weight was observed. These days are periods of increase in nuclear substance and active periods of cell-division. So that, there is no increase in dry weight by accumulation of carbohydrates.

Fluctuation of phosphorus.

Total phosphorus:

Determination of phosphorus contents and amounts in the cotyledon and embryonic organ at successive stages of growth was carried out with other smaples.

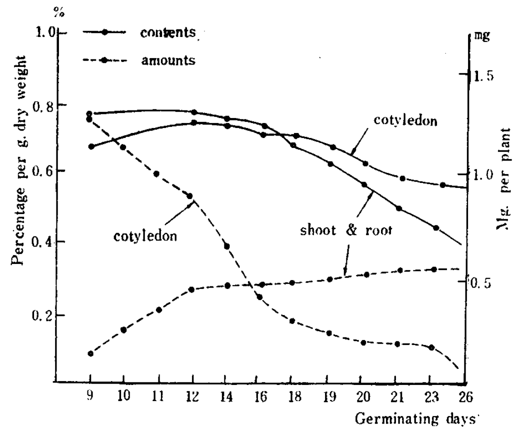

Fig. 9. Changes in total phosphorus of soybean during germination.

The seedlings were cultured during December 1965, under the conditions described above. In this experiment, it took twenty-six days till the cotyledon fell completely. The data are given in Fig. 9.

The amounts in both cotyledon and embryonic organ showed a slight peak on the 16th day. The amounts in cotyeldon decreased rapidly but its quantity in samples during the last sampling days was six times higher than in initial sampling days. But changes of amounts in embryonic organ increased gradually and at the last stage it was three times as much as in the initial stage. Via these experiments, we found that the phosphorus content was directly connected with soybean metabolism.

Phosphorus fractions were analyzed into protein, nucleic, lipid, and inorganic phosphorus. The amounts of phosphorus fractions in sobyean are shown in Fig. 10.

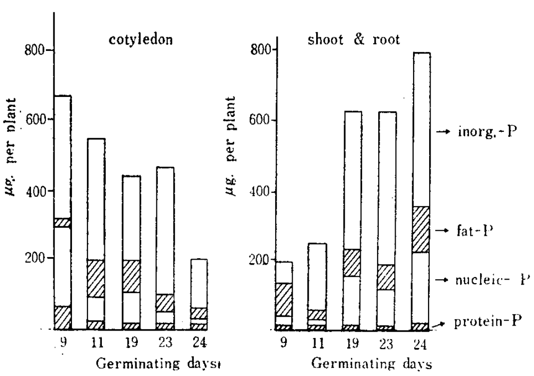

Fig. 10. Changes in phosphorus fractions during germination.

Inorganic phosphorus:

The inorganic phosphorus amounts occupied not less than one-half of the total phosphorus fractions in the cotyledon and embryonic organ. These facts were essentially identical with barely (12), and cotton embryos during germination (13). The amounts of inorganic phosphorus in the cotyledon decreased rapidly, while it increased rapidly in the embryonic organ.

The rate of increase and reduction in them are identical. Although transformation of phosphorus from the organic to inorganic phosphorus 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 phosphorus which was maintained originally in the cotyledon. For exmaple, the amount of nucleic phosphorus in developed seedlings was more than three times and the protein phosphorus double that of the cotyledon.

Lipid phosphorus:

The amounts of lipid phosphorus in the cotyledon increased at the first stage and, thereafter, decreased with the growth of soybean. In contrast, in the embryonic organ, they decreased at the first step, and then increased rapidly. Lipid phosphorus existed in the form of phospholipid phosphate. But since little is known about the phospholipid metabolism of plants, it is difficult to determine the significance of the loss and subsequent recovery of lipid phosphorus.

Nucleic phosphorus:

The amounts of nucleic phosphorus in the cotyledon decreased with the growth of the soybean, but it increased rapidly in the embryonic organ. Oota *et al.* (14) reported that the majority of nucleic acid phosphorus are RNA- and DNA-phosphorus in *Vigna*. Nucleic phosphorus increase is followed by an increase in both cell number and RNA, DNA contents of roots and shoots in the bean. Therefore, the increase of the nucleic phosphorus amounts means a remarkable growth of meristem with ability for division.

Protein phosphorus:

The amounts of protein phosphorus in the cotyledon decreased with the growth of the soybean, but it increased slightly in the embryonic organ. The quantity was less than one-tenth of the total phosphorus amounts. These variations coincided with the changes in protein nitrogen.

Summary

As the first step of the investigation of metabolism in soybean, the variations of nitrogen, phosphorus, and carbohydrate compounds in the seedlings were determined and the nitrogen metabolism during germination was discussed.

Cotyledon has a highly anabolic phase for the growth of tissue itself and for the storage of substances, but then, this activity ceases completely at the maturity of the seed. And when the mature seed germinates, the function of the cotyledon changes to catabolic phase.

Most remarkable change of various substances occurred in the middle stage of germination. This stage coincided morphologically with the formation stage of the adult leaf. Therefore, substances in the cotyledon became available for the formation and the maintenance of the adult leaf at the first step. The phases of active metabolism are discussed as follows.

The decrease of nitrogen in cotyledon in the first stage resulted in the increase of protein in the embryonic organs, and the increase of carbohydrate indicated the increase of photosynthetic products chiefly for an energy-source, because the cotyledon has a homologous function with the leaf in the early stage of germination.

On the changes of substances, nitrogen compounds arose prior to carbohydrate at the middle growth period of the plant. The time when the maximum value of phosphorus content was attained coincided with that of nitrogen.

At the period of cotyledon-fall, enough substances for metabolism of normal growth have been transferred already into the plant body from the cotyledon. Consequently, at least till the first compound leaf develops, external supplies of nutrient are not necessary in soybean.

Acknowledgement

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