Studies on Nitrogen Metabolism of Soybean Plants

IV. The dynamic aspect of leaf nitrogen and its relation to protein turnover*

Yasumasa KATO and Subaru KITADA

(Faculty of Textile Science and Technology, Shinshu University, Ueda, Nagano, 386)

Received September 7, 1978

The patterns of accumulation and re-distribution of nitrogen in soybean plants have been studied by many investigators^{3,4,5,16,18,20}. These results have demonstrated that a considerable fraction of the nitrogen accumulated by each organ underwent net export to various degrees to the developing seeds. Since leaves, among vegetative organs, are known to make the greatest contribution in this respect^{3,4,5,20}, it is important to evaluate their metabolic function in relation to nitrogen storage, turnover, and transport etc. which may determine the nitrogen supplying capacity of the leaf. In the studies reported so far, however, soybean leaves were analyzed collectively and a clear understanding of the nitrogen economy of the individual leaves therefore remains for further inquiries.

The authors are of the opinion that the simultaneous influx and efflux of nitrogen may always exist for a mature soybean leaf, nitrogen being constantly replaced^{7,9}. Nitrogen released from a mature leaf associated with such an efflux, if occurred at all, would possibly help to meet the current needs in sink organs, just like the nitrogen released from senescing leaves. In extending the concept of redistribution to implicate contributions from latent efflux such as this, one would necessarily have to assign a much greater role in nitrogen supplying function to leaves.

This study was undertaken to prove this idea as well as to provide some details of

dynamic aspect of nitrogen within the soybean plant, and also to see what the effect of sink removal is on the dynamics of nitrogen. Special emphasis was placed on the manifestation of the extent to which nitrogen in individual leaves could be replaced during the course of leaf ontogenesis. The turnover of leaf proteins has been examined in anticipation that it might be related to the turnover of leaf nitrogen. For the experimental convenience, young soybean plants were used in this study.

Materials and Methods

Soybean (*Glycine max* (L.) Merr. cv. Enrei) seeds were sterilized with a 5% chlorox, then set to germinate on the hydroponic equipment (Fig. 1) which was devised by one of the authors (Y. Kato). The main part of this equipment consists of a piece of long polyester cloth folded in two to form a shallow "bag" or "vessel" of 5.4 m long with a depth of 30 cm. The outside the "vessel", except its opening end, is enveloped with a polyethylene sheet which in turn is covered with an opaque, thermal insulating material. Nutrient solution is applied to the upper end of the cloth through a plastic

Fig. 1. Diagram of hydroponics system. C: cloth, P: pump, Pp: plastic pipe, Ps: polyethylene sheet, T₁, T₂: nutrient solution tanks. For further explanation, see "Materials and Methods".

*This investigation was supported by a grant from the Ministry of Education (No. 848029) given to the first author, and part of this work was presented at the 162nd Meeting of the Crop Science Society.

pipe. The plant roots are allowed to develop inside the "vessel" and they absorb nutrients from the solution as it flows down the wet surfaces of the cloth. The texture of the cloth, therefore, must be fine enough to prevent it from piercing by the plant roots. Excess solution freed from absorption is collected, pumped up, and used repeatedly. This type of hydroponics has advantage in that a large number of vigorous plant materials could be obtained without extra aeration devices.

In our experiments, 8 such "vessels" were arranged in rows in a green house, 4 for ^{14}N feeding and another 4 for ^{15}N feeding. The nutrient solution used was half-strength Hoagland's solution with iron tartarate replaced by iron EDTA. The pH of the nutrient solution was maintained at about 6.0 and the concentration of the solution was adjusted frequently by measuring its electric conductivity.

The experimental plants, otherwise grown on the ^{14}N medium, were transferred to the ^{15}N medium (3.94 atom %, used as nitrate- ^{15}N) during one of the following periods; 0–8 days (period-I, day 0 is taken at the start of germination), 8–14 days (period-II), 14–20 days (period-III), 20–26 days (period-IV), and 26–33 days (period-V). In addition to these, another group of plants were grown on the ^{15}N medium throughout the experimental period (0–33 days). This group was used to follow the fate of cotyledon-derived nitrogen within the plant.

At 8 days after germination, half of the experimental plants were subjected to topping treatment through which the apical growing region immediately above the primary leaf node was removed.

Samplings were made at the end of each period, 10 plants from each feeding series at the first 3 samplings, and 5 at the later 2 samplings. The harvested plants were separated into cotyledons, roots, stems plus petioles, primary leaves, and in the control plants further into individual trifoliate leaves. All samples other than primary leaves were oven-dried (85°C) and analysed for total nitrogen using a Kjeldahl method modified

to include nitrate nitrogen. Analysis of ^{15}N was carried out with an emission spectrometer, JASCO, NIA-15 analyzer according to the procedure described by KUMAZAWA¹⁴⁾.

The fresh primary leaves were extracted by grinding with 2 volumes of 0.05M phosphate buffer, pH 7.0 (containing 0.05M sodium ascorbate and 0.001 M MgCl_2) and quartz sand in a chilled mortar. The resulting homogenate was centrifuged at 20,000 g for 20 minutes. The pellet was washed twice by centrifugation and the combined supernatant was used for the fractionation of Fraction 1 protein, and also for the determination of total soluble protein.

Total soluble protein was precipitated from an aliquot of the supernatant by adding an equal volume of 20% trichloroacetic acid, the precipitate was then washed with the same precipitant and Kjeldahl digested for analysis of nitrogen content and of isotopic abundance.

Insoluble nitrogen content and its isotopic abundance were measured on the preparations obtained from Kjeldahl digestion and subsequent distillation of the pellets. In the text, this fraction will be called as "insoluble protein".

For fractionation of Fraction 1 protein, the combined supernatant was brought to saturation by the stepwise addition of ammonium sulphate, the precipitate collected by centrifugation, and redissolved in 0.05 M phosphate buffer, pH 7.0, containing 0.05 M sodium ascorbate. The solution was then dialyzed for 24 hours against 4 changes of 50 volumes of 0.05 M phosphate buffer, pH 7.0, containing 0.025 M sodium ascorbate. The dialyzed protein solution was passed through a Sephadex G-100 column, 70×2.5 cm, and 5 ml samples were collected at a flow rate of 20 ml/hr. All procedures were carried out below 4°C. The method described above is essentially a modification of the method of KAWASHIMA et al.¹²⁾

The concentration of protein in the fractionated eluates was measured on an aliquot of each sample by the method of LOWRY et al.¹⁵⁾ The samples corresponding to the first giant peak, known as the Fraction 1 protein, were pooled and the protein was

obtained by the method described in the analysis of total soluble protein. The analysis of ^{15}N in the Fraction 1 protein was carried out on the dry powdered protein. The method is essentially the same as described by YONEYAMA and KUMAZAWA²¹⁾.

Results

Since few investigators hitherto have tried to discriminate seedling nitrogen of cotyledonal origin from that of external medium origin, we attempted to follow these components separately over a period of 33 days after germination of soybean seeds. In the text, the signs **Nc** and **Nm** will be used often to denote the plant nitrogen came from cotyledons and from external medium, respectively.

1. Balance sheet for cotyledonary nitrogen during germination

During the first 8 days of germination (period-I), the axis gained 11.43 mg of total nitrogen of which 9.27 mg was **Nc** and 2.16 mg was **Nm**, while the cotyledons showed a net loss of only 6.96 mg (Fig. 2). Examination of isotopic abundance in the cotyledonary nitrogen revealed that 15.6% or 2.16 mg of the total nitrogen in the cotyledons at day 8 was of external medium

Fig. 2. Balance sheet for utilization of cotyledonary nitrogen by the axis during and after germination of soybean seeds. Shaded part indicates medium nitrogen (^{15}N) taken up in cotyledons. Axis Nc: cotyledon-derived N in axis. Medium ^{15}N taken up by the axis is omitted.

origin (Fig. 2). It is thought, therefore, that actually 9.12 ($6.96 + 2.16$) mg of nitrogen must have been lost from the cotyledons, the amount being approximately equal to that of **Nc** recovered in the axis (9.27 mg).

Fig. 3. Patterns of accumulation and distribution of cotyledon-derived and medium-derived nitrogen within the young soybean plant.

□ : cotyledon-derived N, ■ : medium-derived N.

From 8 to 14 days (period-II), the loss of nitrogen from the cotyledons exceeded the gain of **Nc** in the axis and the total cotyledonary nitrogen in the whole seedling decreased gradually after 8 days, while **Nc** in the axis reached its maximum at 20 days and thereafter a net loss of **Nc** occurred (Fig. 2). A similar loss of cotyledonary nitrogen from total seedling has been reported for *Pisum sativum* by BEEVERS et al.¹⁹

2. Distribution of **Nc** and **Nm** within the plant

The results are shown in Fig. 3. During the period-I (0–8 days), the seedling (less cotyledons) received 82% of its total nitrogen from the cotyledons and 18% from the medium. The former (**Nc**) was distributed into the primary leaves, stem, and root in the ratio 41:28:31. Supply of nitrogen from cotyledons further continued through the next period from 8 to 14 days, but the distribution this time was principally to the first and second trifoliate leaves. Thus the function of cotyledons in the latter half of their life is directed predominantly towards nourishing new leaves. Although the third trifoliate leaf might have received a few from cotyledons prior to latter's abscission, it may be concluded that the second trifoliate leaf is practically the final one to which cotyledonary nitrogen is directly supplied in substantial amount.

From the 20th day onwards, the redistribution of **Nc** from lower to upper leaves was clearly seen (Fig. 3). As a result, **Nc** became more or less uniformly distributed between the leaves excepting very young ones. At the end of the experiment (33 days), a traces amount of **Nc** was detected in the uppermost 8th trifoliate leaf which was very small and folded in the apical bud.

On the other hand, the absorption of medium nitrogen by the seedling increased progressively with time and at the 20th day of germination 61% of the total nitrogen in the axis was of external medium origin.

The rate of absorption was greatest during the period-IV (20–26 days) and somewhat decreased during the period-V (26–33 days) presumably because of slight nitrogen starvation in the nutrient solution during the latter period. In any event, a major

portion (about 60%) of the medium nitrogen absorbed in each period went into the whole leaves with the then most rapidly expanding leaf obtaining the largest share.

3. Detailed patterns of accumulation and redistribution of nitrogen in each organ

(1) Leaves (Fig. 4)

At the end of the experiment, each plant was carrying a pair of primary leaves and additional 8 trifoliate leaves including small folded ones at the apex. Within the range of this experimental period, however, only the primary leaves could have completed their probable total growth period, hence their result will be described below in somewhat detail.

In Fig. 4A, the outermost curve represents the changes with time in total nitrogen content of the primary leaves and the innermost curve, the changes in the amount of nitrogen came from cotyledons (**Nc**). The blackened areas indicate the amounts of nitrogen taken up from the external medium (**Nm**) during the respective pulse feeding period (marked Roman numerals in Fig. 4). The subsequent fate of any specified **Nm** may be traced by the changing width of the whitened area immediately following the blackened area.

The total nitrogen content of the primary leaves increased almost linearly up to the 14th day, reached a maximum at around the 20th day, and then decreased towards the end of the experiment. Some 80% of the total nitrogen received by the primary leaves during the first 8 days came from cotyledons (Fig. 4A). The **Nc** increased up to the 14th day and decreased thereafter. From the comparison of these two curves, it will be noted that the decline of **Nc** commenced about a week earlier than that of the total nitrogen. Earlier decline of **Nc** was also found in the first trifoliate leaf (Fig. 4B). Likewise, in the fourth trifoliate leaf (Fig. 4E), both period-I **Nm** and period-III **Nm** (the **Nm** taken up during the period-I, and period-III, respectively) were shown to redistribute while its total nitrogen was still increasing. It seems likely, therefore, that a still expanding, but near-mature, leaf can already redistri-

bute some of its nitrogen from the leaf.

The period-I **Nm** incorporated into the primary leaves exhibited a brief rise over the period-II notwithstanding the labelled nitrogen had been removed from the medium (Fig. 4A, also see Fig. 10B). After this time, however, the period-I **Nm** decreased steadily with further aging of the primary leaves. The observed brief rise of period-I **Nm** after the pulse feeding might have been due to a temporary release from the root (Fig. 5B, also see Fig. 10B).

On the contrary, the medium nitrogen assimilated into the primary leaves during the period-II, III, and IV showed immediate decrease after the respective pulse feedings (Fig. 4A).

The rate at which primary leaves took up medium nitrogen was very rapid during the latter period of their expansion but decreased after maturity. It must be stressed here, however, that the primary leaves at all times did show their ability to take up nitrogen throughout the entire period of their life span (Fig. 4A). In fact, even at their stage of senescence where export of nitrogen was overwhelming, there still was a considerable flow of nitrogen entering the primary leaves (Fig. 4A, period-V).

As stated earlier, every component part of the total nitrogen (excepting the period-V **Nm**) in the primary leaves, regardless of its origin or the time of feeding, was finally subjected to redistribution, but none was completely exhausted from the leaves. This naturally would result in the stepwise increase with time in number of the component parts constituting the total nitrogen of the primary leaves, the number being added by one for each period passed. Thus, at the end of the experiment the total nitrogen of the primary leaves was consisted of six component parts, i.e. the **Nc** and the period-I, II, III, IV and V **Nm** s.

The statement presented for the primary leaves will hold also for the first trifoliate leaf without substantial alterations (Fig. 4B). Only difference that should be pointed out here may be that the nitrogen arriving this leaf at its earliest life was made up of three components (**Nc**, period-I **Nm**, and period-

II **Nm**) rather than two (**Nc** and period-I **Nm**) as was the case with the primary leaves. Since the first trifoliate leaf started to grow during the period-II, any period-I **Nm** found in this leaf must have been translocated from the older parts of the plant, presumably the root in this case (see Fig. 10B). The same holds true for the second and third trifoliate leaves because these leaves also started to grow during the period-II (Fig. 4C and D).

A similar situation has been shown for all the younger leaves; an inspection of Fig. 4E-I will immediately reveal that the nitrogen initially delivered to any leaf at its youngest stage invariably comprises, along with the nitrogen currently absorbed from the medium, all the possible counterparts of the nitrogen that had been taken up by the plant in every period in the past in its life history by the time of emergence of the leaf in question. That these latter components were all derived from redistribution within the plant will need no stressing.

From these results, it can be said that the higher the acceptor leaf is on the main stem, the more will its composition of nitrogen (in terms of the feeding period) become complex from the very beginning of its growth period. In most leaves examined, this redistributed nitrogen constituted a major portion of the total nitrogen of the leaves at their earliest stage of growth (Fig. 4, also see Table 2), suggesting an important role of this nitrogen in the early growth of the leaf tissues.

A sharp decline of total nitrogen content during the period-V (26–33 days) observed in the first, second, and third trifoliate leaves (Fig. 4B, C and D) might be due to a slight nitrogen deficiency incidentally developed in the nutrient solution at that time.

On the whole, the present data provided convincing evidence that the nitrogen in mature soybean leaf is undergoing a constant partial renewal.

For later formed leaves such as 5th trifoliate leaf or those at higher positions, the experiment could cover only the earliest

Fig. 5. Changes in total nitrogen and its constituent parts during the early growth of soybean stem (5A) and root (5B). Further details as in Fig. 4.

phase of their development, hence no redistribution was found from these leaves (Fig. 4F, G, H and I).

(2) *Stem and root* (Fig. 5)

The distribution pattern obtained for the stem (Fig. 5A) was very similar to that obtained for the root (Fig. 5B), both being strikingly different, however, from those for the individual leaves (Fig. 4).

The delivery of nitrogen from cotyledons to the root had completed by the 8th day of germination, and after that time neither loss nor gain of root **Nc** occurred (Fig. 5B). The stem also received most **Nc** during the first 8 days but thereafter there was a small net gain in **Nc** up to the end of the experiment (Fig. 5A).

The behaviors of **Nm** in the stem and root were much the same as those of **Nc**. Once incorporated, **Nm** also tended to remain relatively constant in the stem and in the root throughout the rest of the experimental period, although a temporary release of **Nm** immediately after the pulse feeding may occur in the root (Fig. 5B).

From these results it may be concluded that the stem and root nitrogen in the young soybean plant was not susceptible to redistribution.

4. *Effects of sink removal on the turnover of*

nitrogen and proteins in the primary leaves

Topping treatment was performed at the 8th day of germination when the primary leaves were about half expanded. This treatment was done in an attempt to find out whether sink and source relationship for nitrogen exists between the apical growing region and the mature leaf.

(1) *Turnover of nitrogen*

Topping greatly altered the patterns of nitrogen economy of the primary leaves (Fig. 6A, cf. Fig. 4A). The most remarkable difference between the control and topped plants was an almost complete cessation of export of nitrogen from the latter leaves. Fig. 6A shows that all component parts of the total nitrogen that had been incorporated into the primary leaves were retained after the topping treatment. Another outstanding feature characteristic of the topped plant concerns a pronounced enhancement of the rate of uptake of medium nitrogen by the primary leaves.

As the combined results of these two effects, the nitrogen content of the primary leaves of topped plant increased markedly at a linear rate over the whole experimental period. When compared at the end of the experiment, the primary leaves of topped plant contained total nitrogen more than

Fig. 6. Changes in total nitrogen and its constituent parts during the growth of (A) primary leaves, (B) stem and (C) root of topped soybean plant. Arrows indicate the time of topping treatment. Otherwise as in Fig. 4.

Fig. 7. Changes in Fraction 1 protein (○), total soluble protein (△), and insoluble protein (□), contents during the growth of primary leaves of (A) control plant, and (B) topped plant. Arrows indicate the time of topping treatment.

four times that of the control primary leaves (Fig. 6A, cf. Fig. 4A). Thus the redistribution of nitrogen from the leaf could be apparently prevented by the removal of apical sinks.

In spite of these differences found between the leaves of control and topped plants, there were many similarities between their fundamental distributional patterns for stems (Fig. 5A and Fig. 6B) and also for roots (Fig. 5B and Fig. 6C). The finding that

nitrogen once fixed in the stem or root of control plant remained rather stationary was also true for the stem and root of topped plant. This close agreement may lend validity to the aforementioned view that no appreciable redistribution of nitrogen may be normally occurring from both the stem and root of the young soybean plants.

(2) Turnover of proteins

Fig. 7A shows the changes with time of various protein components in the primary

Fig. 8. Effects of topping treatment on uptake and subsequent fate of ^{15}N pulse-fed into various protein fractions at different times of growth of primary leaves. A: insoluble protein, B: total soluble protein, C: Fraction 1 protein; control plant ($\circ, \triangle, \square$), topped plant ($\bullet, \blacktriangle, \blacksquare$). ^{15}N pulse feeding was made during the period-I, II, III, respectively. Thick line means uptake of ^{15}N during pulse feeding, and thin line (solid, broken, or dotted) means its subsequent fate. Arrows indicate the time of topping treatment.

leaves. All protein fractions rose and fell in closely parallel with the total nitrogen (cf. Fig. 4A). Furthermore, the effects of topping on the contents of various protein fractions were in most way similar to the effects on total nitrogen (Fig. 7B, cf. Fig. 6A).

The Fraction 1 protein, total soluble protein, and insoluble protein of the primary leaves were pulse labelled with ^{15}N at different times of the leaf development and the fate of the labels were compared between the control and topped plants (Fig. 8A, B, and C).

In the control leaves, the incorporation of the label into proteins and its subsequent release from them were shown to occur in each of these successive pulse feedings conducted during the period-I, II and III, respectively (Fig. 8, control). Assuming that the incorporation of label into proteins as a measure of protein synthesis and its release as a measure of protein breakdown, proteins would be in a state of turnover.

On the other hand, while the primary leaves of topped plant showed a remarkably increased incorporation of the label into

all the protein fractions, almost no subsequent release of the label was found (Fig. 8, topped), indicating that protein breakdown may be largely regulated by the presence or absence of the apical sinks where much of the protein breakdown products should be continuously consumed.

Discussion

Most work on redistribution of nitrogen has been done with the senescent organs where net losses of nitrogen were occurring. In such cases, the amount of nitrogen redistributed from a leaf, for example, has been estimated from the difference between the nitrogen content of the leaf at its maximum and its senescent minimum. However, since the present work has demonstrated that at least a part of nitrogen in the mature leaf is being constantly replaced (Fig. 4A, B, C and D), the real amount of nitrogen redistributed should be much greater than the amount estimated from the conventional method of calculation.

In our data, the total nitrogen of a leaf is analyzed into the nitrogen of cotyledonal origin and that of external medium origin,

Table 1. Comparison of the amounts of nitrogen redistributed from the leaves estimated by the present method and conventional method. (N/mg/plant)

Method* of estimation		Primary leaves	First trifoliolate leaves	Second trifoliolate leaves	Third trifoliolate leaves	Fourth trifoliolate leaves
Present method	Nc	2.30	1.96	2.02	0.65	0.06
	period-I Nm	0.59	0.17	0.20	0.06	0.06
	period-II Nm	0.88	1.16	1.06	0.26	—
	period-III Nm	0.78	1.72	2.42	1.09	0.52
	period-IV Nm	0.58	0.59	0.35	0.02	0.06
	Total	5.13	5.60	6.05	2.08	0.70
Conventional method		2.75	3.48	3.49	1.37	0

*See "Discussion"

Fig. 9. Effects of topping treatment on uptake and subsequent fate of ^{15}N pulse-fed into total N fraction (●, ▲, ■), and total protein N fraction (○, △, □) at different times of growth of primary leaves. A: control plant, B: topped plant. Other details are as in Fig. 8.

the latter being further divided into five component parts each differing in terms of the time of feeding. Furthermore, the accumulation and redistribution of each constituent has been followed in the experiments. So we can now calculate the amount of nitrogen redistributed from the leaf by adding up the net losses of nitrogen occurring in each of all these constituents over the entire period of the leaf ontogeny, instead of taking the net loss occurring only during the stage of leaf senescence.

The amounts of nitrogen redistributed from various leaves which have been estimat-

ed by these two different methods are shown in Table 1. In all leaves examined, the present method gives consistently higher values (almost as much as twice) as compared to those estimated by the conventional method. Also, it must be remembered that, in this study the isotope was pulse-fed for 6 or more days, extremely long to detect any redistribution which might have occurred in association with those nitrogenous compounds with rapid turnover rates. It may be therefore quite possible that shortening the pulse feeding period might lead to a finding of even greater nitrogen supplying

Table 2. Percentage contribution of redistributed nitrogen to the total nitrogen of various leaves at their youngest stage of growth (%).

Nitrogen sources	Pr L	1 L	2 L	3 L	4 L	5 L	6 L	7 L	8 L	Mean
Redistributed	82.8	76.6	79.6	84.4	47.0	53.1	67.0	83.1	76.9	72.3
Directly from medium	17.2	23.4	20.4	15.6	53.0	46.9	33.0	16.9	23.1	27.7

Pr L: Primary leaves 1 L—8 L: 1st trifoliate leaf—8th trifoliate leaf.

Fig. 10. Accumulation and successive distribution within the young soybean plant of (A) cotyledon-derived N, and (B) medium-derived ^{15}N pulse-fed during the period-I (0—8 days). Pr L (primary leaves), 1L—8L (1st—8th trifoliate leaves).

function of soybean leaves.

The pulse chasing experiment on various protein fractions in the primary leaves (Fig. 8) has suggested that all the protein fractions examined may be being turned over. That the Fraction 1 protein is a crude RuDPCase has been now well documented^{6,13}, and its lack of turnover has been generally proposed from ^{14}C feeding experiments^{6,19}. KAWASHIMA and WILDMAN¹³ may be the few of those who support the view that the Fraction 1 protein is under constant turnover. In the present study using ^{15}N as a tracer, the results were obtained that is in keeping with the latter's view. The uptake and release of the label into and out of the Fraction 1 protein was observed simultaneously (Fig. 8C, day 14—20). However, we wish to reserve judgement in this respect because our Fraction 1 protein specimen might have not achieved a sufficient purification.

Fig. 9 shows how close parallelism exists between the results of pulse chasing experi-

ment obtained for total nitrogen and for total protein nitrogen (total soluble protein-N plus insoluble protein-N) in the primary leaves. It will be noted that every time the leaf takes up nitrogen there is always a simultaneous synthesis of leaf protein, and if uptake of nitrogen is increased as in the topped plant, the corresponding increase in protein synthesis is shown to occur in the leaf. Conversely, the export of nitrogen from the leaf is invariably accompanied by the simultaneous breakdown of leaf protein (Fig. 9A) and when the export of nitrogen is prevented by a sink removal (Fig. 9B), the breakdown of leaf protein also ceases.

These close similarities in kinetics of nitrogen and protein turnover would suggest that there may exist in the leaf some physiological and structural connections which would appear to link nitrogen-supplying system with protein anabolic system on the one hand, and nitrogen-draining system with protein catabolic system on the

other.

Moreover, Fig. 9 indicates that most of the label in the total nitrogen fraction (generally more than 70%) resides in the total protein fraction; in other words, most of the nitrogen taken up by the leaf is utilized for the synthesis of new proteins.

All these results have led authors to the speculation that, in the intact soybean leaves, protein synthesis is proceeding all the time primarily utilizing the recently delivered nitrogenous compounds as nitrogen sources, and that most of the nitrogenous compounds set free after the breakdown of leaf proteins are transported from the leaf without being reutilized in the protein synthesis in that leaf. The possibility of the limited reutilization of protein breakdown products within the same leaf has been shown by earlier studies in this laboratory^{8,9}.

However, the nitrogen thus exported from a leaf can be reutilized by other leaves. Fig. 10 was reproduced from the results shown in Fig. 4–5 to illustrate more vividly the pattern of redistribution of tagged nitrogen within the young soybean plant. These graphs clearly demonstrate the ways in which both the **Nc** (Fig. 10A) and period-I **Nm** (Fig. 10B) are transferred acropetally through a series of sink-to-source changeover taking place in turn in the successive leaves. Contrary to leaves, the stems and roots showed less movement, excepting the abrupt loss of root period-I **Nm** observed immediately after the pulse feeding (Fig. 10B). This period-I **Nm** from the root evidently went into the lowest three leaves. The observed temporary release from the root, however, might simply be explained by a migration of the label which was *in transit* to the shoot, and therefore might not be due to “redistribution” as evidenced by the shape of its time course curve with an abrupt change. As mentioned earlier, the stem and root nitrogen tended to be retained over the experimental period. The relative immobility of root nitrogen has been reported also for field bean²³.

Although the present data tell nothing about the nature or the forms of the nitrogenous compounds exported from the leaf,

OGHOGHORIE and PATE¹⁷, studying with field pea, have put forward an interesting remark in this respect; they stated that “this stream of nitrogen, carrying amino compounds in almost correctly balanced proportions for direct incorporation into protein, is regarded as being an extremely important source of nitrogen for the growing points of the plant”. A positive evidence of translocation of various amino acids from soybean leaves has been indicated by one of us (Y.K.)¹⁰.

In accordance with this view, our results showed that the redistributed nitrogen was the main component of the total nitrogen of the leaves at their youngest stage, constituting up to 84.4% with an average of 72.3% (Table 2). It may be expected from these high percentage values that this form of nitrogen may be useful for the growth of young leaves in much the same way as the cotyledonary nitrogen is useful for the growth of the embryo.

Based on these results and foregoing considerations, a mature soybean leaf may be depicted as a “transformer” through which inorganic or organic nitrogenous compounds of lower metabolic forms, and therefore presumably “indigestible” for meristematic tissues, could be changed by the process of protein turnover into more elaborated, well-balanced, higher metabolic forms which might be “digestible” for the growth of the embryonic sinks. This function of the leaf as a “transformer” could be only operative when acceptor organs (sink organs) are present.

Considering from the nutritional point of view, the role of this nitrogen supplying function of the leaf would be expected to become increasingly important at the time of seed protein formation. But what seems more important from the practical considerations would be a finding that this function may be regulated, in some manner, by the sink organs themselves. In this connection, the problem of whether soybean leaves might possess any potentiality which enable them to increase their nitrogen supplying capacity in response to the increased metabolic activities or demands in

the part of the sink organs is of particular interest, and we have some evidence to suggest its possibility¹¹⁾. Further approach to an understanding of this regulation mechanism is needed.

Summary

The fate of nitrogen that came from cotyledons and nitrogen (¹⁵N) pulse-fed at 5 different times during the growth of young soybean plants has been studied over a 33-day period after germination. Cotyledons furnished nitrogen to the primary leaves, stem, and root for the first 8 days but thereafter principally to the 1st and 2nd trifoliate leaves. Redistribution of cotyledon-derived nitrogen from primary leaves commenced from 14th day of germination when their total nitrogen was still increasing. At the end of the experiment, cotyledon-derived-nitrogen was distributed approximately uniformly between the 6 expanded leaves, and very small amounts were found in the 3 immature leaves. Soybean leaves were shown to take up ¹⁵N (via the root) throughout the entire period of their life, and from their near-mature stage onwards, uptake and redistribution of nitrogen were observed simultaneously. Thus the nitrogen in the mature leaf was in a constant partial renewal. Considering this fact, the nitrogen supplying capacity of a soybean leaf was estimated about two times greater than that estimated conventionally from a net loss of nitrogen during its senescence. The turnover of leaf nitrogen was closely related to the turnover of total leaf protein. Influx of nitrogen was invariably accompanied by the simultaneous synthesis of leaf proteins, and conversely, efflux by the simultaneous breakdown of leaf proteins. A sink removal (topping treatment) prevented breakdown of leaf proteins (as measured from the rate of release of label after the pulse feeding) as well as export of nitrogen from the leaf. The nitrogen supplying function of soybean leaves was discussed in relation to nitrogen and protein turnover of leaves.

References

1. BEEVERS, L. and E. S. GUERNSEY 1966. Changes in some nitrogenous components during the germination of pea seeds. *Plant Physiol.* **41**: 1455—1458.
2. COOPER, D. R., D. G. HILL-COTTINGHAM and C. P. LLOYD-JONES 1976. Absorption and redistribution of nitrogen during growth and development of field bean, *Vicia faba*. *Physiol. Plant.* **38**: 313—318.
3. DERMANN, B. D., D. C. RUPP and L. D. NOODEN 1978. Mineral distribution in relation to fruit development and noncarpoc senescence in Anoka soybeans. *Amer. J. Bot.* **65**: 205—213.
4. EGLI, D. B., J. E. LEGGETT and W. G. DUNCAN 1978. Influence on N stress on leaf senescence and redistribution in soybeans. *Agron. J.* **70**: 43—47.
5. HANWAY, J. J. and C. R. WEBER 1971. Accumulation of N, P, K by soybean (*Glycine max* (L.) Merrill) plants. *Agron. J.* **63**: 406—408.
6. HUFFAKER, R. C. and L. W. PETERSON 1974. Protein turnover in plants and possible means of its regulation. *Ann. Rev. Plant Physiol.* **25**: 363—392.
7. KATO, Y. and Y. YANAGISAWA 1961. Studies on nitrogen metabolism of soybean leaves. 1. Changes in nitrogen content of the leaves as affected by girdling their petioles. *Proc. Crop Sci. Soc. Japan* **29**: 421—424. (in Japanese with English summary)
8. ——— and ——— 1962. Studies on nitrogen metabolism of soybean leaves. 2. Protein metabolism of petiole-girdled leaves with special reference to the behavior of nitrogen liberated by protein breakdown. *Proc. Crop Sci. Soc. Japan* **30**: 249—252. (in Japanese with English summary)
9. ——— 1973. Studies on nitrogen metabolism of soybean plants. I. Transport and metabolism of labelled and non-labelled nitrogen. *Proc. Crop Sci. Soc. Japan* **42**: 154—163. (in Japanese with English summary)
10. ——— 1975. Studies on nitrogen metabolism of soybean plants. III. Effects of topping on the export of nitrogen and amino acids from the leaves. *Proc. Crop Sci. Soc. Japan* **44**: 172—177. (in Japanese with English summary)
11. ——— 1977. Nitrogen nutrition and metabolism of soybeans. *JARQ* **11**: 141—144.

12. KAWASHIMA, N., A. IMAI and E. TAMAKI 1967. Studies on protein metabolism in higher plants. III. Changes in the soluble protein components with leaf growth. *Plant & Cell Physiol.* **8**: 447—458.
13. ————— and S. G. WILDMAN 1970. Fraction 1 protein. *Ann. Rev. Plant Physiol.* **21**: 325—358.
14. KUMAZAWA, K. 1970. Determination of ^{15}N enrichment of small samples by emission spectroscopy and application to plant nutrition research. *Kagaku to Seibutu* **8**: 246—252. (in Japanese)
15. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193**: 265—275.
16. de MOOY, C. J., J. PESEK and E. SPALDON 1973. Mineral nutrition. In *Soybeans: Improvement, production, and uses*. (Ed.) B. E. Caldwell, Amer. Soc. Agron. Inc. Madison, 267—352.
17. OGHOGHORIE, G. C. O. and J. S. PATE 1972. Exploration of the nitrogen transport system of a nodulated legume using ^{15}N . *Planta* **104**: 35—49.
18. OHLROGGE, A. J. 1960. Mineral nutrition of soybeans. *Advan. Agron.* **12**: 229—263.
19. PETERSON, L. W., G. E. KLEINKOPF and R. C. HUFFAKER 1973. Evidence for lack of turnover of ribulose-1,5-diphosphate carboxylase in barley leaves. *Plant Physiol.* **51**: 1042—1045.
20. TOGARI, Y., Y. KATO and M. EBATA 1955. Studies on the yield analysis of soybeans. (I.) Changes in principal chemical constituents of the soybean plant in relation to its growth. *Proc. Crop Sci. Soc. Japan* **24**: 103—107. (in Japanese with English summary)
21. YONEYAMA, T. and K. KUMAZAWA 1974. A kinetic study of the assimilation of ^{15}N -labelled ammonium in rice seedling roots. *Plant & Cell Physiol.* **15**: 655—661.

〔和 文 摘 要〕

大豆のチッソ代謝に関する研究

第4報 大豆の葉におけるチッソの動態と葉のタンパク質代謝との関連について

加 藤 泰 正・北 田 寿 美

(信州大学繊維学部)

大豆植物体内のチッソを子葉に由来する部分と、生育各期に与えた肥料チッソ (^{15}N) に由来する部分に分け、各器官、とくに葉におけるそれらの分布や転流状況を発芽後 33 日間追跡した。

発芽初期の 8 日間、幼植物は大部分 (約 80%) のチッソを子葉から獲得し、それらは根、茎、初生葉に分布したが、その後子葉のチッソは第 1、第 2 本葉に送られるようになり、根、茎への供給はほとんど止むか、極めてわずかにすぎなかった。発芽後 14 日目、初生葉中の子葉由来チッソは、初生葉全チッソがなお増加中にもかかわらず、早くも再転流を開始した。20 日目以降 (子葉はすでに脱落)、子葉由来チッソは第 1、第 2 本葉などからも顕著に再転流を行い、実験終了時には下部より 6 枚の展開葉中にはほぼ均等に分布し、なお上位 3 枚の未展開葉中にも微量が検出された。一方 ^{15}N は葉の全生育期間にわたって絶えず葉に流入し、葉の完全展開直前頃より流入と流出が同時に観察されるようになる。この点を考慮すると、大豆葉のチッソ転流能力は従来の推定を約 2 倍上まわるものと考えられる。このような葉のチッソの動態は、葉の全タンパク質の示す代謝回転と密接な関係にあり、葉におけるチッソの流入とタンパク質の合成、およびチッソの流出とタンパク質の分解は、それぞれがつねに同時に進行し、摘心処理によって地上部の生長部分を除去すると、葉に対するチッソの流入とタンパク質合成はともに増加するが、葉のタンパク質分解とチッソの流出は何れもほぼ完全に停止することが観察された。