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Nitrogen Fixation and Metabolism in Soybean Plants

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Abstract
Results of the studies, including relevant methodologies, on nitrogen fixation and metabolism in soybean plants which have been undertaken in Japan in the past 10 years are reviewed in this paper. Three methods, i.e. $^{15}N_2$ gas feeding, ^{15}N dilution and ^{15}N natural abundance, were employed for estimating an amount of fixed nitrogen. In the two isogenic line of soybean: non nodulating (T201) and nodulating (T202) lines, the ratio of symbiotically fixed N in the total absorbed N were estimated to be 76% by the 15N dilution method, while it was 52% by the ^{15}N natural abundance method. Contribution of the following three N sources; fertilizer, N_2 fixation and soil-N, was estimated with a ^{15}N tracer technique in combination with ^{15}N natural abundance and with low level ^{15}N application. Nodulating soybean plants obtained 13±10% of their N from fertilizer, 66±8% from N_2 fixation and 21±10% from soil N in Andosol. In the experiment in which nodules attached to the intact roots was exposed to $^{15}N_2$, it was observed that the fixed N was rapidly translocated from bacteroids to plant cell cytosol in the form of ammonia, and assimilated by the GS/GOGAT system in the latter.

Discipline: Soils, fertilizers ad plant nutrition
Additional key words: bacteroid and cytosol, N_2 fixation, natural ^{15}N abundance, nitrogen assimilation

Introduction

Soybean plants demand for plenty of nitrogen and nutrient, absorbing 8–10 kg of N in producing 100 kg of grain yields. Approximately 40–70% of N requirements of the plants are met by symbiotic nitrogen fixation during the whole growing period. In recent years, grain yields of about 3 t/ha are produced in Japan by well-experienced producers. The amount of N necessary for maintaining this yield level is estimated at approximately 270 kg/ha. From the viewpoint of economy of energy and environmental preservation, it might not be appropriate to depend on further increase in the input of N chemical fertilizer to meet N requirement. Therefore, perspective of increasing the contribution of N fixation to total absorbed nitrogen is of great significance. The use of ^{15}N has been a powerful tool in research on nitrogen fixation in general, and its quantitative and metabolism analyses in crop plants in particular.

In the last 10 years, a great number of research papers containing various information on the biological N_2 fixation and the metabolism of fixed N using ^{15}N tracer techniques have been presented in Japan. This paper attempts to review the results obtained in those studies.

Estimation of symbiotically fixed N by ^{15}N tracer methods

Estimation of fixed N with a ^{15}N tracer technique has been made by the following three approaches; $^{15}N_2$-feeding, ^{15}N dilution and natural ^{15}N abundance methods. The $^{15}N_2$-feeding method, in which the ^{15}N-labelled N_2 gas is supplied to rhizosphere, is called "a direct method". It demonstrates determination of the N distribution in a plant and provides an insight into the assimilation processes of the fixed N. Its applicability, however, is limited due to two reasons: one is the difficulties in sealing N_2 gas released into the experimental system; and the other is the unavoidable contamination with non-labelled N_2 gas in air. On the other hand, ^{15}N dilution and natural ^{15}N abundance methods have been widely employed in field tests.

Nitrogen-fixing activity of plants has been conventionally determined by an acetylene-reducing method. Theoretically speaking, the conversion ratio of N fixation from acetylene reduction is three, but it is known that the actual values obtained differ from the theoretical value. The conversion factor was measured by Kanamori et al.[3] and Kumazawa et al.[6] in order to estimate accurately an amount of N fixation from that of acetylene reduction. Kanamori et al. indicated that the conversion factor varied between 3.9 and 11.1 (Table 1), and Kumazawa et al. reported that the determination of evolved H_2 was essential to calculate the amount of N fixation. Taking that H_2 into account, the following relationship was formulated (Table 2):

$$^{15}N_2 \text{ fixation} \times 3 + H_2 \text{ evolution} = C_2H_2 \text{ reduction.}$$

Although it is difficult to adopt a $^{15}N_2$ gas method under field conditions since it requires a closed system, this method is usable for the studies on partitioning and metabolism of fixed N and determination of C_2H_2/N_2 ratio. Examples of application of the $^{15}N_2$ gas method to a field test were presented by

Table 1. Relationship between N_2 fixation and C_2H_2 reduction by intact soybean plants

N fertilizer (g/m^2)	$^{15}N_2$ fixed (mg/pot·day)	C_2H_2 reduction (μM/pot·hr)	C_2H_2/N_2 ratio
0	2.71	28.76	7.13
1	4.74	46.85	6.64
4	6.47	37.59	3.90
8	2.43	40.12	11.09

Source: Kanamori et al. (1983)[3].

Table 2. Relationships among C_2H_2 reduction, $^{15}N_2$ fixation and H_2 evolution

(Unit: μmol/hr·g nodule fresh weight)

Sequence of treatments	C_2H_2 production	$^{15}N_2$ fixation	H_2 evolution
$^{15}N_2$ fixation ↓ C_2H_2 reduction	9.73	2.97	9.51
C_2H_2 reduction ↓ $^{15}N_2$ fixation	20.54	1.79	4.34

Source: Kumazawa et al. (1983)[6].

Yoshida and Yoneyama[22], and Akao and Ishii[1]. They supplied $^{15}N_2$ gas, using a specifically designed chamber and pot for gas-tight plant growth. Yoshida et al. investigated the amount of N fixed by microorganisms in the rice rhizosphere. These results are outlined in detail by Marumoto[7]. Akao et al. conducted an experiment, in which $^{15}N_2$ gas was put into buried pots in a field for five days at three stages each; flowering, early pod-filling and middle pod-filling. Their findings indicate that the amount of fixed N is the highest at the early pod-filling stage, followed by that at the flowering stage and the middle pod-filling stage in this order. Fixed N accumulated mostly in the leaves at the flowering stage, in the stems at the early pod-filling stage, and in the grains at the middle pod-filling stage.

The isotope dilution technique is based on the assumption that each of the test plant and the non-nodulating reference plant absorbs the same proportion of soil N and applied N during the growing period. For the purpose of comparison, a nodulating genotype and a non-nodulating one in the same plant species have been used in general. Kanamori et al.[3] estimated the symbiotically fixed N with multisplit application of ^{15}N-labelled ammonium sulfate, using the following two isogenic lines of soybean: i.e. non-nodulating (To1-1) and nodulating (To1-0). The data obtained indicated that 76% in total N absorbed throughout the growing period was derived from symbiotically fixed N. Ichita[2] estimated the contribution of symbiotic fixation to the total plant N under a basal application of ^{15}N-labelled ammonium sulfate at a rate of 24 kg/ha, using non-nodulating (T201) and nodulating (T202) soybean lines. It was found that the relative proportion of symbiotic N in the total N increased from 25% at the flowering stage to 40% at the pod-filling stage (Fig. 1). In his study which dealt with hydroponically grown soybean supplied with ^{15}N-labelled nitrate (3.32 atom %, 50 ppm N), Kato[5] found that the daily rate of N_2 fixation increased with vegetative growth of plants and reached a maximum at the early pod-filling stage, when the amount of N derived from symbiotic N_2 fixation became nearly equal to that from nitrate. During the middle and late pod-filling stages, N_2-fixing activity declined sharply while nitrate uptake continued at an almost maximal rate until the late pod-filling stage (Fig. 2). These results indicate that the proportional contribution of fixed N reaches

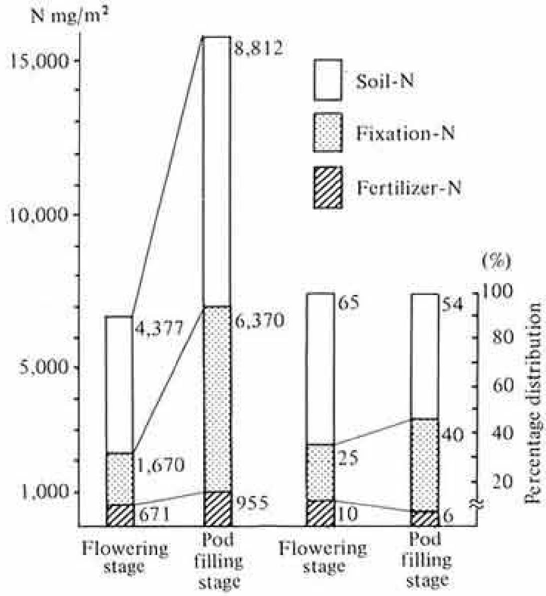

Fig. 1. Distribution of three N-sources at the flowering and pod-filling stages
Source: Ichita (1986)[2].

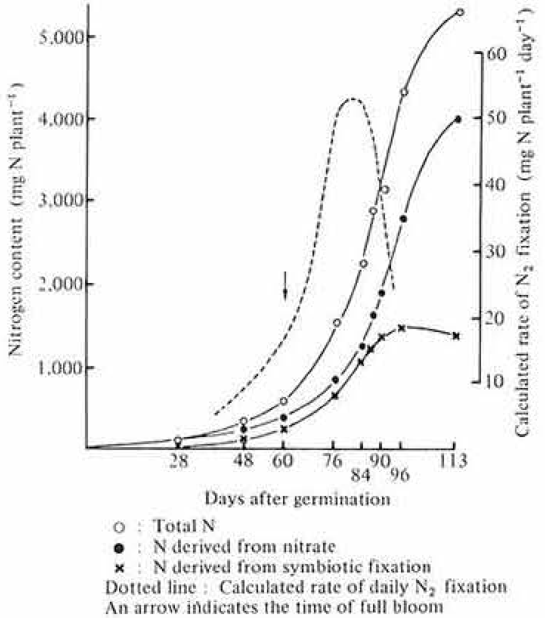

Fig. 2. Changes in nitrogen content and daily rate of N_2 fixation of soybean plants
Source: Kato (1981)[5].

its peak at the pod-filling stage.

Some studies utilizing a natural ^{15}N abundance technique have also been conducted in Japan[4,13,20], since it was reported that the natural ^{15}N abundance of N_2 fixing plants was generally lower than that of non-nodulating plants[14,17,19]. This technique has a great potential for estimating the amount of N fixation under a field condition. The difference in natural ^{15}N abundance between legumes and non-legumes is based on the ^{15}N abundance in soil N, which is derived from organic matters and is generally higher than that of atmospheric N_2. Yoneyama et al.[16,20,21] investigated variations in natural ^{15}N abundance of field grown soybean grains in Japan. It was recognized that $\delta^{15}N$ values of the nodulating soybean varied among locations, and the values were lower than those of the non-nodulating soybean in each location (Tables 3 & 4). On the basis of these fundamental studies, Yoneyama[16] concludes that 52 $\pm 26\%$ of the total N content of soybean in Japan is derived from N_2 fixation. On the other hand, in a field experiment using natural isotopic abundances and a low level ^{15}N tracer technique simultaneously, Wada et al.[13] show that use of the low level ^{15}N tracer technique at natural abundance levels can provide several advantages, such as estimation of the contributions of three N-sources (fertilizer, N_2 fixation and soil N), economy of expensive ^{15}N and applicability to a large-scale field experiment. The experiments were undertaken at the sites of Andosol Brown (a dry volcanic ash soil), Andosol Black (a wet volcanic ash soil) and Alluvial soil. It was reported that the nodulating soybean line obtained $13 \pm 10\%$ of its N from fertilizer, $66 \pm 8\%$ from N_2 fixation and $21 \pm 10\%$ from soil N in Andosol Brown soil; 30, 16 and 54% in Andosol Black soil; 7, 77 and 16% in Alluvial soil, respectively (Table 5).

Metabolism of symbiotically fixed N in plants

The ^{15}N gas pulse feeding experiment is the most effective method for the studies of the assimilation process of fixed N[8–11]. While the use of ^{15}N-labelled nitrate and/or ammonia as a tracer is also an effective method for the studies pertaining to uptake, distribution and redistribution of absorbed N (soil and fertilizer N) in distinction from fixed N[12,15,18]. Ohyama et al.[9] and Matsumoto et al.[8] investigated the incorporation of ^{15}N into various fractions of soluble nitrogen in soybean plants after exposing the

Table 3. Natural ^{15}N abundance of soybean grains harvested in farmers' fields in the Kanto District

Location (Basal fertilizer)**	Non-nodulating* $\delta^{15}N$(0/00)	Nodulating		
		Cultivar	$\delta^{15}N$(0/00)	%Ndfa
Yuuki (c)	+0.7	Enrei	−0.9	70
Johoku (c)	+11.6	Miyagioojiro	0.0	88
Hokota (c)	0.0	Enrei	−0.6	37
Minori (c)	+2.8	Enrei	−0.5	75
Ryugasaki (b)	+1.1	Enrei	−0.7	67
Shimodate (b)	−0.7	Enrei	−0.9	22
		Suzuyutaka	−0.9	22
		Miyagioojiro	−1.0	33
Ishige (c)	+2.6	Enrei	−0.8	81
Naganochushin (a)	+1.9	Enrei	−0.2	60

* T201 was used as non-nodulating reference.
** Basal fertilizers were applied at the levels of (a): 1-4-4, (b): 2-8-8, and (c): 3-12-12 of N-P_2O_5-K_2O in kg per 10 a.
Source: Yoneyama (1987)[16].

Table 4. Natural ^{15}N abundance of soybean grains harvested in farmers' fields in the Hokkaido District

Location	Soil $\delta^{15}N$(0/00)	Non-nodulating* $\delta^{15}N$(0/00)	Nodulating		
			Cultivar	$\delta^{15}N$(0/00)	%Ndfa
Taiki	+6.5	+2.3	Kitamusume	−0.2	63
Shimizu	+6.0	+3.9	Toyosuzu	+1.5	44
Toyokoro	+4.9	+1.9	Kitamusume	−0.6	71
Toshibetsu	+4.6	+3.6	Toyosuzu	+1.5	40

* Tol-0 was used as non-nodulating reference.
Source: Yoneyama (1987)[16].

Table 5. Estimates of the contributions of three N-sources to field grown soybean plants (var.: Kitamusume) at 30 kg N/ha

Soil type	Percent contribution^a)			
	Ndff	Ndfa	Ndfs	Ndfa by N yield^b)
Andosol Brown soil				
1980	0.12	0.60	0.28	0.60
1981	0.14	0.72	0.14	0.81
Andosol Black soil				
1981	0.30	0.16	0.54	0.25
Alluvial soil				
1981	0.07	0.77	0.16	0.70

a): Ndff, Ndfa and Ndfs denote nitrogen derived from fertilizer, atmosphere, and soil, respectively.
b): Ndfa was estimated on the basis of the difference of N yields of the isogenic lines (Tol-0, Non-nod and Tol-1, Nod) in the same fields under the fertilization of 30 kg N/ha.
Source: Wada et al. (1986)[13].

nodulated intact soybean plants to $^{15}N_2$ gas. Their findings indicate that there are several compartments of ammonia in nodules, and that one of them is closely associated with the N_2 fixation process. According to Ohyama et al.[10,11] who presented some details of the primary pathway of ammonia assimilation in soybean nodules, it is indicated that the fixed N is rapidly translocated from bacteroids to plant cell cytosol in a form of ammonium, and assimilated by the GS/GOGAT system in the latter.

The recent report of Yanagisawa et al.[15] refers to the translocation of C and N among the various organs of soybean plants in the double-label experiments which used ($^{13}CO_2 + {}^{15}N_2$) and $^{13}CO_2 + {}^{15}NO_3$) at the flowering, pod formation and pod-filling stages. It shows that ^{15}N is mainly distributed to nodules and leaves, then it is gradually redistributed to seed, with an exception that the ^{15}N incorporated at the flowering stage is essentially immobile.

Allantoin and allantoic acids are the major forms in the translocation of fixed N from nodules to plant shoots. The pathway of allantoin biosynthesis in soybean nodules was investigated by Ohyama & Kumazawa[11] through the administration of ^{15}N-labelled compounds (ammonia, glutamine, glycine and alanine). They demonstrated that allantoin was synthesized via purine degradation in the nodules.

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