Studies on the Response of Nodulated Soybean to Nitrogen Fertilizer*

II. On the seed quality and assimilate partitioning to seeds at different nodal position

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Abstract : A glasshouse pot sand culture experiment was conducted to determine the effects of three levels of combined nitrogen-0N (0), 1N (100), 2N (200 ppm)-on nitrogen nutrition, seed protein and oil content and the distribution pattern of ^{14}C activity in the seed at different nodal position of soybean (*Glycine max* (L) Merr. cv. Akiyoshi). Plants were fed with $^{14}\text{CO}_2$ at the vegetative, flowering, early pod filling and late pod filling stages of growth.

Nitrogen content of plant organs increased with increasing level of supplied nitrogen and declined in the vegetative organs with plant growth. Combined nitrogen had no significant effect on mean seed protein and oil content. No ^{14}C activity was detected in seeds of plants fed at the vegetative and flowering stages. More ^{14}C was retained in seeds of plants fed at late pod filling stage than fed at early pod filling stage. Combined nitrogen had no remarkable effect on the amount and distribution pattern of ^{14}C . Activity of ^{14}C in seeds was in the order of secondary branches < main stem < primary branches. Excessive combined nitrogen had little effects on the seed yield and synthesis of protein and oil components in seeds.

Key words : ^{14}C radioactivity, *Glycine max*, Nitrogen nutrition, Nitrogen fertilizer, Seed oil, Seed protein.

根粒着生ダイズの窒素肥料に対する反応 第2報 子実の品質ならびに節位別子実への同化産物の分配:

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要 旨 : ガラス室において3水準の窒素レベル(0, 100, 200 ppm)で砂耕栽培したダイズを用い、窒素の集積、子実の脂肪とタンパク質含量、ならびに子実における ^{14}C 放射活性の節位別分布に及ぼす影響について調査した。 $^{14}\text{CO}_2$ は栄養生長期、開花期、登熟初期、及び登熟後期に供与した。培養液の窒素レベルが高くなるほど各器官内窒素濃度は高く、栄養器官では生育にともない低下した。窒素レベルは子実の脂肪やタンパク質含量にほとんど影響しなかった。栄養生長期、開花期に $^{14}\text{CO}_2$ を供与しても、子実中に ^{14}C 活性は検出されなかった。登熟後期に供与した場合の方が、登熟前期の場合よりも ^{14}C 活性が強く検出された。窒素レベルは ^{14}C の量とその節位別分布に大きな影響を与えなかった。また ^{14}C 活性は2次分枝<主茎<1次分枝の順であった。これらの結果から、窒素レベルは子実の発達と品質に大きな影響を及ぼさないものと考察した。

キーワード : 子実脂肪、子実タンパク質、 ^{14}C 放射活性、ダイズ、窒素栄養、窒素肥料。

During the period of active nitrogen fixation, it has been estimated that the nodules and supporting root system of grain legumes receive about 15% to 30% of the assimilates of the plant^{12,15}. In the previous report², authors have reconfirmed that though the photosynthesis and respiration of shoot generally reflected the plant size, respiration of roots and nodules was high in the order of 0N > 1N > 2N. Thus, although symbiotic nitrogen fixa-

tion might be economical in terms of cost for dry matter production, acquiring nitrogen by fixation could be at the expense of plant establishment, growth, yield and seed quality. With regard to the partitioning of assimilate produced at different growth stages for seed production of legume crops, there have been many studies on broad bean⁹, *Lupinus*¹³, field bean¹⁸, and soybean^{1,3,8,10,11,14,17,19}, using $^{14}\text{CO}_2$ or $^{13}\text{CO}_2$ labeling experimental methods. The results were mainly concerned with the whole crop carbon economy.

In this study, the nitrogen nutrition, seed protein and oil content of soybean either solely dependent on symbiotic nitrogen fixation or given different levels of combined nitrogen

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were investigated. An additional objective of this work was to analyze the ^{14}C -assimilate partitioning with a special emphasis on seed formation at different nodal position.

Materials and Methods

1. Plant culture and growing conditions

Seedlings of soybean cv. Akiyoshi were transplanted singly into 1/5000 a Wagner pots filled with sand, 7 days after germination. Treatments consisted of three levels of ammonium nitrate designated as 0N (0), 1N (100) and 2N (200 ppm nitrogen). Full experimental details are the same as those in previous report²⁾.

2. Nitrogen and oil analysis

The plant organs were oven dried at 70°C and ground in a Willey mill and their nitrogen content were determined by the modified Kjeldahl technique and oil content of seed by the Soxhlet extraction method.

3. $^{14}\text{CO}_2$ feeding, autoradiography and ^{14}C activity determination

$^{14}\text{CO}_2$ was fed four times, corresponding to the vegetative (I), flowering (II), early pod filling (III) and late pod filling (IV) stages of

growth. Feeding was undertaken in a growth chamber ($10,000\text{ lux}$, 24°C) by enclosing plants in a transparent polyethylene plastic material into which $^{14}\text{CO}_2$ was released by reacting $\text{Na}^{14}\text{CO}_3$ with HCl . After 1 hour of assimilation, excess $^{14}\text{CO}_2$ was evacuated. The polyethylene bag enclosing the plant was removed from the growth chamber and after 48 hours the plant was returned to the glass house.

At maturity, labeled plants were harvested and all pods identified by their nodal positions on the main stem and branches, these identities were maintained during subsequent ^{14}C determination. Cut and smoothed halves of seeds were stuck on a methane foam paper according to their respective positions on the main stem, primary and secondary branches (Fig. 1). The inner surface of the seeds were autoradiographed by placing against Konica X-ray films for 96 hours and the films then developed. The intensities of darkness as they appeared on the developed films, were determined, and the reading value taken as a measure of ^{14}C activity, using a densitometer of diameter 2 mm (Konica model PDA-85).

Fig. 1. An example of seed arrangement (a) and developed X-ray film after exposure (b).

Fig. 2. Nitrogen content in the organs of soybean plants at different growth stages as influenced by combined nitrogen.

I : 26/7, II : 25/8, III : 11/9, IV : 12/10, V : 31/10.

△.....△ : 0N, ○---○ : 1N, ●—● : 2N.

Fig. 3. Nitrogen accumulation in soybean plants as affected by combined nitrogen and stage of growth.

R : Root, N : Nodule, ST : Stem, L : Leaf, PE : Petiole, PO : Pod, SE : Seed.

Results and Discussion

1. Nitrogen nutrition

Generally, the three treatments had common patterns of nitrogen percentage changes throughout plant growth (Fig. 2). Percent nitrogen of the vegetative organs, especially the leaves, during early stages of growth was very high, but thereafter, progressively declined. Percent nitrogen was highest in the nodules and least in the petioles. There was a tendency for combined nitrogen to increase nitrogen percentage in the various organs, especially the vegetative ones.

The total nitrogen content of the plant organs generally reflected dry matter accumulation and showed an increase until around early pod filling and then declined (Fig. 3). This decline in total nitrogen, especially the leaves, may be attributed to the translocation of nitrogen from the vegetative organs into reproductive parts^{4,5,16}; and increasing senescence of leaves from early pod filling stage to maturity. During the vegetative and flowering stages, nitrogen content of the leaves, as a proportion of total plant nitrogen was greater than 40%. At maturity, more than 80% of the total plant nitrogen was allocated to the seeds.

Fig. 4. Effect of combined nitrogen on percent protein of seeds at maturity.

*: Bars with the same letter within the same main stem or branch are not significantly different among treatments at 1% level.

NS: Not significantly different at 1% level.

Total nitrogen per plant was slightly higher in 2N treatment than in 0N and 1N treatments.

2. Seed quality

The mean of percent seed protein, in general, was in the order $2N > 0N > 1N$, while the mean of percent oil content was in the order $1N > 2N > 0N$, though differences among the three treatments were not significant. However, slight differences were also observed between seeds of the main stem, primary and secondary branches (Fig. 4, 5). In the combined nitrogen treatments, high levels of protein were associated with low oil content. This inverse relationship between seed protein and oil contents has been reported by other researchers^{6,7}.

3. Amount and distribution of ^{14}C -assimilate in the seeds at different nodal positions

Autoradiographs revealed no ^{14}C activity in seeds of plants fed at the vegetative stage in all treatments and a very trace amount only in 2N treatments plants, at the flowering stage feeding. This is consistent with the studies on broad bean⁹, *Lupinus*¹³, field bean¹⁸, soybean^{8,14,17}. Pearen et al.¹⁴ reported that when ^{14}C labeled assimilates were taken up before the beginning of grain filling, very small amounts were translocated to the seeds. Akao et al.¹, however, observed that the assimilates fed soon after anthesis affected the composition of seed, and that 50% or more of the labeled carbon in seeds was transported main-

Fig. 5. Effect of combined nitrogen on percent oil of seeds at maturity.

Symbols are the same as those in Fig. 4.

Fig. 6. Total amount and distribution pattern of ^{14}C activity in the seeds of plants fed at early pod filling stage of growth. Symbols are the same as those in Fig. 4.

Fig. 7. Total amount and distribution pattern of ^{14}C activity in the seeds of plants fed at late pod filling stage of growth. Symbols are the same as those in Fig. 4.

Fig. 8. ^{14}C distribution in the seeds expressed as a percentage of total activity. Symbols are the same as those in Fig. 4.

ly from the vegetative organs, by means of a newly developed long-term ^{13}C labeling technique.

Total amount and distribution patterns of ^{14}C in the seeds of plants fed at early and late pod filling are shown in Fig. 6, 7, respectively. Though ^{14}C activity per one seed was the same in the seeds of main stem, primary branch, and secondary branch group, the differences among main stem and branches was slightly clear. And the ^{14}C activity at the different nodal positions were essentially the same among the plants of three nitrogen treatments, though the total amount and distribution of ^{14}C retained changed between the two stages. The results also indicated that seed development changed step-by-step throughout the nodal positions with plant growth. The amount of ^{14}C translocated to and retained in the seeds was greater at late pod filling than at early pod filling. Similarly, Pearen et al.¹⁴⁾ observed that as soybean plants grew older, larger amounts of ^{14}C assimilates were recovered in the seeds. Although the seeds of 2N treatments plants fed at late pod filling had a slightly greater amount of ^{14}C , the combined nitrogen showed little or no effect on the distribution pattern of ^{14}C at the different nodal positions.

The distribution of ^{14}C in the seeds of the main stem, primary and secondary branches expressed as a percentage of total activity is shown in Fig. 8. In general, seeds of the main stem plus the primary branches which

contributed a greater part to the total yield²⁾ also retained more ^{14}C than seeds of the secondary branches. When $^{14}\text{CO}_2$ was assimilated at early pod filling, percent ^{14}C of the main stem seeds tended to be high in 0N treatments and low in other treatments. With late filling, percent ^{14}C of seeds of the secondary branches were less than those treatments at early pod filling feeding.

According to Kokubun et al.¹⁰⁾, the differences in the amount and distribution pattern of ^{14}C might be due to the differences between the photosynthetic properties of the main stem and branch leaves and the ratio of pods (sink) to leaves (source). Percent ^{14}C in seeds of the main stem and branches were remarkably different among the three main stem and branches were remarkably different among the three treatments at late pod filling feeding in this experiment. On the other hand, Yamagata et al.¹⁹⁾ stated that protein carbon in the seeds was relatively more dependent on photosynthate assimilated in the early reproductive stage, but lipid carbon in the later one. This report suggests the necessity of more research on the ^{14}C activity of protein and oil and relationship with the seed development.

The results of this experiment indicated that nitrogen nutrition was essentially similar in those grown with or without nitrogen, and that the combined nitrogen had no remarkable effect on the amount and distribution pattern of ^{14}C in seeds. It was clear that the seed development mainly dependent on atmospheric nitrogen compared favorably with the combined nitrogen fed counterparts. The synthesis of seed protein and oil components of soybean plant may have occurred independently¹⁹⁾. Those results might relate to the process of seed development: seeds which obtained the sink activity developed independently in short term after the late pod filling stage, therefore excessive combined nitrogen had little effects on the seed yield and synthesis of protein and oil components in seeds.

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* In Japanese with English summary.

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