

根粒非着生ダイズにおける 葉中窒素含量と子実収量の関係

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Leaf Nitrogen Content and Seed Yield Correlation in Non-nodulating Soybean

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摘 要

ダイズは種子貯蔵タンパク質を合成するために多くの窒素を必要とする。登熟期の栄養器官に貯えられている窒素が増収のために重要であることが知られている。窒素固定するダイズとしないダイズの子実収量が葉中から移動する窒素にどれくらい依存するかを比較することは、大気中の窒素の固定、土壌中からの取り込み、葉組織からの再分配といった3種の窒素源の相互作用を明らかにする上で大変興味深い問題である。本研究の目的は、根粒非着生ダイズを異なる窒素施肥下で栽培した場合の、葉中の窒素含量と子実収量の関係を明らかにすることである。実験の結果、葉中の窒素含量と収量の関係は遺伝子型と年次によって変動した。窒素固定能を欠き、吸収窒素と葉中窒素の再移動に依存している非着生ダイズの子実収量は、施肥した窒素量に反応し、葉中の窒素含量と収量の高い相関関係がみられた。子実収量が高く、葉中窒素の移動を促進するような良好な栽培年次には、子実収量の葉中窒素への依存が、とくに着生ダイズにおいて小さくなった。すなわち、窒素が十分に供給される場合、種子は葉中から再移動した窒素よりむしろ、固定窒素や根から吸収した窒素を直接利用すると考えられた。

キーワード：葉中窒素・窒素施肥・根粒非着生・子実収量・ダイズ

Abstract : Soybean requires a large amount of N to support the synthesis of seed storage protein. The amount of N stored in the vegetative parts at seed filling stage is clearly important for development of a large seed yield. Comparison of the dependence of

seed yield on mobilized leaf N in the presence and absence of N_2 fixation is an interesting study to verify the interactive roles of the three N sources, direct assimilation of atmospheric and soil N, and from redistribution in leaf tissue. The objective of

this study was to investigate the correlation between leaf N content and seed yield in non-nodulating genotypes grown at different levels of N fertilizer. The leaf N content and seed yield correlation varied with genotypes and growing seasons. The non-nodulating genotypes lacking the N₂ fixation ability and depending their seed yield more on N absorption and leaf N re-mobilization showed higher responses to the levels of N fertilizer and higher coefficients of determination between leaf N content and the yield. When the growing conditions were favorable to increase the yield and mobilizable leaf N, the dependence of seed yield on leaf N was greatly reduced in the genotypes especially in the nodulating genotype. Therefore, if the supply of N is abundant, the seed may prefer to use the N directly supplied by N₂ fixation and root absorption rather than remobilizing the leaf N.

Key words : Leaf nitrogen content, Nitrogen fertilizer, Non-nodulating, Seed yield, Soybean.

Soybean (*Glycine max* [L.] Merr.) requires a large amount of N to support the synthesis of seed storage protein. The N stored in the vegetative parts of soybean has an important role in providing the N requirements. About 50% of the N required for seed protein synthesis can come from the mobilized vegetative N and the greater proportion of this is from leaf tissue (Hanway and Weber, 1971 ; Loberg et al., 1984; Egli and Leggert, 1985). The amount of N stored in the vegetative pool at seed filling stage is important to seed yield. There are genotypic variation in the amount of N accumulated in vegetative tissues and in the proportion of vegetative N mobilized for seed development (Jeppson et al., 1978; Zeiher et al., 1982; Loberg et al., 1984 ; Guffy et al., 1992). The relationship between potentially mobilizable leaf N and seed yield capacity has been studied and either weak or inconsistent correlation was reported (Zeiher et al., 1982 ; Loberg et al., 1984; Vasilas et al., 1995).

Usually, soybean plants can have the three main sources of N for seed production from direct assimilation of atmospheric and soil N, and from redistribution in vegetative tissue. On the other hand, non-nodulating soybeans are restricted the N sources to soil N and its redistribution due to the lack of ability of N₂ fixation. While comparison of the dependence of seed yield on mobilized leaf N in the presence and absence of symbiotic N₂ fixation is an interesting study to verify the interactive roles of atmospheric N₂ fixation and mobilization on seed development, there are limited reports on the vegetative N content and seed traits in non-nodulating soybeans.

It is known that the vegetative N content in non-nodulating soybeans is strongly dependent on the N fertilizer levels (Weber, 1966). Comparison of mobilization of plant N to seeds in nodulating and non-nodulating isolines by Jeppson et al. (1978) indicated that adding N fertilizer increased whole plant weight and the percent N in non-nodulating soybeans, but which were less than those in nodulating counterparts. Previously we investigated variation for seed size in nodulating and non-nodulating soybeans grown at different N levels over five years and found that non-nodulating soybeans are highly responsive to N fertilizer and climatic conditions (Manalo et al., 1998). These results suggest that the relationship between potentially mobilizable leaf N and seed yield capacity may differ between nodulating and non-nodulating genotypes due to differential responses to the quantity of soil N. The objective of this study was to investigate the correlation between leaf N content and seed yield in non-nodulating genotypes grown at different levels of N fertilizer.

Materials and Methods

1. Plant materials

The non-nodulating lines were developed from the cross between the nodulating cultivar Toyosuzu (TS) and the non-nodulating line To1-0, which is a

breeders line produced at Hokkaido Prefecture Tokachi Agricultural Experiment Station (Sasaki and Sunada, 1978) and carries the non-nodulating allele, *zz*, derived from T201 soybean. Three homozygous non-nodulating derivatives, TS1, TS2 and TS3 were selected and advanced by single seed descent to F₁₄ generation. These three lines were chosen in a preliminary evaluation for differences in response of seed yield to N fertilizer levels. TS was used as a reference genotype of nodulating in this experiment.

2. Experiment

A two-year field trial was conducted at the experimental field of Obihiro University of Agriculture and Veterinary Medicine on a static fluffy brown andosols (Entice Hapludans) in 1996 and 1998. The experimental plots were randomized using split plot design with three replications. The main and subplots comprised fertilizer treatment and genotypes, respectively. Subplots consisted of three rows of 2 m in length. Four levels of N fertilizer treatments, 0, 2, 10, and 20 g m⁻² (0N, 2N, 10N, 20N), implemented by ammonium sulfate were applied. Phosphorous, potassium and magnesium were also applied as a basal dressing to all plots at the rates of 18.0, 9.0 and 2.3 g m⁻², respectively. The 20N treatment was carried out in that only half was applied before sowing and the other half was sidedressed at the R1 stage. Plots were overseeded in late May and thinned to two plants per hill. Planting density was 20 cm between hills and 60 cm between rows.

Two plants were harvested from the center row at the R5 stage. All leaves collected were dried at 70 °C for 48 hours and weighed. The leaves were grounded finely and about 0.3-0.5 g of samples were analyzed for N concentration by Kjeldahl method. Total leaf N content was estimated by the product of leaf dry matter and leaf N composition (%). Seed yield was determined by harvest of four plants in plots. The data on leaf dry matter, total leaf N content and seed yield were transformed to gram

per square meter.

Results

The leaf dry matter in each genotype was higher in the second year than that in the first year (Table 1), indicating better growing conditions in 1998. In both years, the non-nodulating genotypes increased leaf dry matter with the levels of N fertilizer, whereas the response of TS was slight in the 2N to 20N treatments. There was a significant difference between the non-nodulating genotypes over two

Table 1. Leaf dry matter of the non-nodulating genotypes and nodulating TS soybean at four levels of N fertilizer in two years

Genotype	N levels (g m ⁻²)				Mean
	0	2	10	20	
1996	— g m ⁻² —				
TS1	43	85	154	262	136
TS2	59	74	115	131	94
TS3	45	91	141	173	112
TS	46	82	86	99	78
	LSD (0.05) = 24.6				106
1998					
TS1	120	131	224	294	192
TS2	91	109	155	216	142
TS3	101	125	155	184	141
TS	115	128	146	163	138
	LSD (0.05) = 28.6				154

years, as TS1 was the highest in dry matter and its response to N fertilizer.

Leaf N composition ranged from 3.2% to 5.5% (Table 2). Variation was small and not significantly differed between years. The genotypes tended to

Table 2. Nitrogen composition of the non-nodulating genotypes and nodulating TS soybean at four levels of N fertilizer in two years

Genotype	N levels (g m ⁻²)				Mean
	0	2	10	20	
1996	— % —				
TS1	3.4	3.8	4.6	5.5	4.3
TS2	3.6	3.8	4.8	5.4	4.4
TS3	3.7	3.7	4.4	4.8	4.2
TS	3.2	4.5	4.6	5.1	4.4
	LSD (0.05) = 0.47				4.3
1998					
TS1	3.7	3.8	3.9	4.0	3.8
TS2	3.9	4.8	4.9	5.3	4.7
TS3	3.3	3.4	4.4	4.3	3.9
TS	4.7	4.7	4.9	5.4	4.9
	LSD (0.05) = 0.14				4.3

increase leaf N composition with higher N fertilizer levels and genotypic difference was revealed in the second year. The consistently low composition of TS1 in 1998 was compensated by its high leaf dry matter.

The mean total leaf N content in 1998 was 7.0 g m⁻² which was significantly higher than 5.0 g m⁻² in 1996 (Table 3). This was obviously due to higher leaf dry matter in the second year and no difference in leaf N

Table 3. Total leaf N content of the non-nodulating genotypes and nodulating TS soybean at four levels of N fertilizer in two years

Genotype	N levels (g m ⁻²)				Mean
	0	2	10	20	
1996	— g m ⁻² —				
TS1	1.5	3.2	7.4	15.0	6.8
TS2	2.2	2.9	5.7	7.4	4.5
TS3	1.7	3.5	6.4	8.7	5.1
TS	1.6	3.8	4.1	5.3	3.7
	LSD (0.05) = 1.29				5.0
1998					
TS1	4.6	5.2	9.2	12.2	7.8
TS2	3.7	5.4	7.9	11.8	7.2
TS3	3.5	4.4	7.0	8.3	5.8
TS	5.7	6.2	7.4	9.1	7.1
	LSD (0.05) = 1.30				7.0

composition between years. At 0N in 1996, all genotypes seemed to suffer from N deficiency stress since the N content in leaves was about 5 g m⁻² or less which was around 30-40 % of the year mean. In both years, while genotypes increased total leaf N content with the levels of N fertilizer, the responses of the three non-nodulating genotypes were higher than the response of TS. At the lower levels of N fertilizer (0N and 2N), total leaf N content was almost comparable between genotypes in both years. At the higher N fertilizer levels (10N and 20N), on the other hand, the non-nodulating genotypes produced more leaf N than TS. This is remarkable in TS1. Leaf N production of TS2 was affected by years due to differential responses to N fertilizer especially to 20N (7.4 g m⁻² in 1996 vs. 11.8g m⁻² in 1998).

Seed yields of the three non-nodulating genotypes and TS were presented in Table 4. This trait followed a similar trend of responses as that of leaf dry matter

Table 4. Seed yield of the non-nodulating genotypes and nodulating TS soybean at four levels of N fertilizer in two years

Genotype	N levels (g m ⁻²)				Mean
	0	2	10	20	
1996	— g m ⁻² —				
TS1	114	206	298	408	267
TS2	96	190	312	373	256
TS3	56	93	157	118	106
TS	213	258	306	194	267
	LSD (0.05) = 34.2				218
1998					
TS1	277	373	386	430	366
TS2	267	350	312	491	355
TS3	166	179	243	280	218
TS	376	486	427	462	438
	LSD (0.05) = 56.2				344

and total leaf N content. There was a large difference between years. The year mean in 1998 was about 60% higher than that in 1996. Furthermore, at 2 N which is the standard level in our area for soybean cultivation, the nodulating TS produced 486 g m⁻² yield in the second year which was as near twice as 258 g m⁻² in the first year.

While seed yields of the non-nodulating genotypes generally increased with N fertilizer levels in both years, the nodulating TS scarcely responded to N fertilizer application. At the lower N fertilizer levels in 1996, the seed yields of non-nodulating TS1 and TS2 were significantly lower than those of TS, then started to become equal at 10 N. Finally these two non-nodulating genotypes gave higher yields than TS at 20 N. In the 1998 experiment, the highest yields of TS1 and TS2 were recorded at 20N, but their yield increases were not proportional to N fertilizer levels. TS3 showed a consistently and significantly lower performance than the other non-nodulating genotypes over N fertilizer treatments and years.

Since total leaf N content and seed yield were varied largely year to year, relationships between these two characters were analyzed in each year. In all genotypes, a close correlation was detected in the first year. The coefficient of determination (r²) values of the four genotypes ranged from 0.52 to 0.72, meaning that about 50 to 70 % of variation in

seed yield were associated with or explained by the variation in total leaf N content. The r^2 of the nodulating TS ($r^2 = 0.66$) was within the range of r^2 values of the non-nodulating genotypes. This indicated that the contribution of re-mobilized N from leaves to seed yield was high and almost equal in nodulating and non-nodulating genotypes. On the other hand, all genotypes showed greatly reduced relationships in the second year. TS was a remarkable genotype as no relationship between the characters was found.

Discussion

The present study showed that leaf dry matter, total leaf N at the R5 stage and seed yield varied year to year and genotype to genotype, while leaf N composition did not vary largely. Since total leaf N content was estimated by the product of leaf dry matter and leaf N composition, variation in the total amount of N pool in leaves was found to associate strongly with variation in the amount of leaves. The amount of leaves can be a good indicator of the crop's photosynthetic potential (Boon-Long et al., 1983 ; Buttery and Buzzell, 1988). Therefore, it would be safe to assume that the higher leaf production in 1998 generated higher photosynthesis, although we did not actually measure the photosynthesis. If this is the case, one might expect a correlated responses of seed yield and total leaf N content (or leaf dry matter) to N fertilizers. Certainly, high coefficients of determination in the 1996 experiment confirmed the correlated responses of these characters. However, under the high levels of seed yield in the 1998 experiment, this hypothesis is cancelled out by the observation of the occurrence of lower relationships between seed yield and total leaf N content. Therefore, when the growing conditions were favorable to increase the yield and mobilizable leaf N, the dependence of seed yield on leaf N was greatly reduced in the genotypes especially in TS.

This observation can be partly explained by

considering physiological roles of leaves. The leaves are important not only as a source of N for developing seeds, but also as a main function providing the energy needed by plants through photosynthesis. The higher photosynthesis may accelerate the direct N assimilating processes, N_2 fixation and N absorption by the root system, because these are photosynthate dependent processes (Ursino et al., 1982). We noted the possible interactions of the three N sources for developing soybean seeds. For the nodulating TS in 1998, the N fertilizer application contributed to leaf dry matter and total leaf N content but not to yield increase, suggesting that the N from the leaf reserved pool may be no longer or less necessary for seed development due to a lot of N available directly from N_2 fixation and root absorption. It seems therefore that if the supply of N is abundant, the seed may prefer to use the N directly supplied by N_2 fixation and root absorption rather than remobilizing the leaf N. This may support the Shibles and Sunberg (1998)'s conclusion. They pointed out that vegetative N pool is clearly important and needed for the development of a large yield, but secondary source that is drawn upon variably in different environments depending on the plant's capacity to assimilate N directly.

The present study showed the varying degree of correlation between leaf N content and grain yield depending on genotypes and growing seasons. As mentioned above, non-nodulating genotypes lacking the N_2 fixation ability have lesser sources of N available for seed development. Thus it is reasonable to hypothesize that non-nodulating genotypes depend their seed yield more on N absorption and leaf N re-mobilization. This may explain the higher responses to the levels of N fertilizer and higher coefficients of determination between leaf N content and the yield in non-nodulating soybeans. TS3 showed a consistently and significantly lower performance than the other non-nodulating genotypes over N fertilizer treatments and years. This

was as expected because TS3 was chosen as a low response genotype to N fertilizer (Manalo et al., 1998). The yield responses of the non-nodulating TS1 and TS2 were not proportional to N fertilizer levels in the 1998 experiment. Thus, the lack of N₂ fixation ability alone can not fully explain the responsiveness of the non-nodulating genotypes to N fertilizer application.

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