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Responses of a Supernodulating Soybean Genotype, Sakukei 4 to Nitrogen Fertilizer

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Abstract : The supernodulating soybean genotype Sakukei 4 is potentially high-yielding. We characterized its leaf nitrogen (N) content, photosynthesis and growth at different developmental stages and under different dosages and types of N fertilizer, and compared it with its parental cultivar Enrei and the non-nodulating line En1282. At the pod-expansion and seed-filling stages, the N contents per leaf dry weight and per leaf area, and apparent photosynthetic rates (AP) were higher in Sakukei 4 than in the normal and the non-nodulating genotypes. The nodule activity per plant was also higher in Sakukei 4 than in Enrei during the reproductive stage. These traits varied less with the growing condition (field- or pot-grown) and dose or type of N fertilizer applied in Sakukei 4 than in the other genotypes. The superior ability of Sakukei 4 to maintain high leaf N and AP, however, did not enhance its growth performance, which tended to be inferior to that of Enrei. Further studies are needed to define the cultivation conditions optimal for an exploitation of the favorable traits of Sakukei 4.

Key words : *Glycine max*, Leaf nitrogen, Nitrogen response, Photosynthesis, Soybean, Supernodulation.

Soybean (*Glycine max* (L.) Merr.) has been grown as a nutritionally valuable crop since ancient times in Asian countries. Recently, there have been a number of reports showing that soybean contains several medicinal substances such as isoflavones and saponins (Fukushima, 2000). Therefore, the future demand for soybean is expected to increase worldwide. Despite these prospects, the production of soybean in major eastern Asian countries has substantially decreased in recent decades, because of the low profitability of soybean farming as compared with other major crops such as rice or vegetables. Thus, an increase in the productivity of soybean is required to render its cultivation more lucrative.

Soybean requires more N than gramineous crops because of its specific physiological characters (Sinclair and de Wit, 1975). Cultivated soybean has three N sources : fertilizer N, soil N and symbiotically fixed N_2. Since application of fertilizer N hinders symbiotic N_2 fixation, total N absorption is not likely to be enhanced by increasing fertilizer N (Harper, 1987). In addition, overuse of N fertilizer can cause environmental problems. Therefore, soybean productivity should be improved by maximizing the amount of symbiotically fixed N_2, particularly in soils of low fertility.

The genetic improvement of symbiotic N_2 fixation is an option to enhance the N absorption capability of soybean. Supernodulation would be a feasible way of enhancing N_2 fixation capability, and several supernodulating soybean mutants have been isolated (Carroll et al., 1985a, 1985b; Gremaud and Harper, 1989; Akao and Kouchi, 1992). However, most supernodulating lines bred so far grow slowly, and therefore are agronomically inferior compared to conventional cultivars (Herridge and Rose, 2000; Kokubun, 2001). The poor growth performance appears to be due to excessive consumption of photosynthates to form and maintain a large number of nodules, and to a low capacity to absorb nutrients and water due to smaller root systems (Gremaud and Harper, 1989; Hansen et al., 1989; Ohyama et al., 1993; Takahashi et al., 1995). The supernodulating genotype Sakukei 4 (formerly named En-b0-1-2) used in the present study has a high-yielding potential comparable to its parental cultivar Enrei (Takahashi et al., 2003). Clarification of the physiological characteristics of this genotype should help to utilize it as a high-yielding cultivar, and to further improve it genetically.

The objectives of this study were to characterize and compare the N content in the leaf, photosynthetic rate and growth of the supernodulating genotype Sakukei 4 with those of the two related nodulating and non-nodulating lines under different conditions of fertilization.

Materials and Methods

1. Pot experiment (Exp. 1)

(1) Plant material

The supernodulating genotype Sakukei 4 (formerly En

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Abbreviations : AP, apparent photosynthetic rate; ARA, acetylene reduction activity; DAS, days after seeding; NAR, net assimilation rate; PPFD, photosynthetic photon flux density.

-b0-1-2), its parent cultivar Enrei and the non-nodulating line En1282 were used in this experiment. Sakukei 4 is an improved line which was selected from the progeny of Enrei×En6500 (Takahashi et al., 2003). En1282 is a non-nodulating mutant derived from Enrei provided by Dr. S. Akao, National Institute of Agrobiological Sciences, Japan. We used pot-grown plants in Exp. 1, because the effects of the nitrogen amount would be more clearly expressed in the physically restricted rhizosphere than in the field. Four seeds per pot (16 cm in diameter, 19 cm tall) were sown on 29 May 2001 and thinned to two plants per pot after emergence. Plants were grown in greenhouses until V1 according to Fehr et al. (1971), then they were transferred to open air with adequate irrigation. Prior to seeding, fertilizer N was applied at four rates (0, 0.2, 0.6, 1.5 g pot^{-1}) as ammonium sulfate. Other fertilizers were applied at fixed rates; 0.6g P_2O_5, 0.6g K_2O and 5g of slaked lime per pot. The soil type was a fine-textured Terrace Yellow soil, clayey (Classification Committee of Cultivated Soils, 1996), containing small amounts of nitrogen and humus. It did not seem necessary to inoculate the soil with *Bradyrhizobium japonicum* because it had been used for growing soybean previously. Each fertilizer was applied to 10 pots per treatment, and each pot (two plants) was considered an experimental unit.

(2) Measurement of growth

Five pots (ten plants) per treatment were collected at the pod-expansion stage (R3 according to Fehr et al., 1971; 73−81 DAS) and again at maturity (146−165 DAS). After the measurement of acetylene reduction activity (ARA), leaves, stems with petioles, and roots including nodules were separated. The leaves were photocopied and the area of the copies was estimated using a computer. Then the samples were oven dried at 80°C for more than two days, and weighed.

(3) Measurement of photosynthetic rate and analysis of leaf nitrogen content

The apparent photosynthetic rate (AP) of the recently expanded terminal leaflet in five plants of each treatment was measured with an LI6400 Portable Photosynthesis System (LI-COR, NE, USA). The measurement was carried out during 1000 and 1300 hr in the plants at the pod-expansion (73-76 DAS) and the seed-filling stage (113 DAS). The flow rate of air in the leaf chamber was controlled at 500 $\mu mol\ s^{-1}$, and the CO_2 concentration was maintained at 350 $\mu mol\ mol^{-1}$. The irradiance on the measured leaves (6 cm^2) was regulated at 1,500 $\mu mol\ m^{-2}\ s^{-1}$ PPFD. The temperature of the chamber was kept at 25°C. After the photosynthesis measurement, the leaflets were excised, oven dried at 80°C for more than two days and weighed. The dried samples were ground in a mill and the N content was analyzed by the Kjeldahl-Gunning procedure (Kimura, 1995).

(4) Acetylene reduction assay

At the pod-expansion stage (73-79 DAS), three plants from three pots were selected and separated into shoots and roots. Immediately after the separation, the nodulated root system was enclosed in 900-mL glass bottles with rubber caps, and then incubated with 30-mL acetylene at 25°C. Samples (1 mL) were collected after 20 and 30 min of incubation and injected into a Gas Chromatograph HITACHI 163 (Hitachi Corp., Tokyo, Japan) equipped with a Porapack N glass column with N_2 as carrier. Ethylene produced during 10 min from the 20 th to 30 th min of incubation was quantified from the ratio of the peak area of samples to that of control ethylene gas. The 2-time-point assays showed that the rate of acetylene reduction was generally linear for all the samples. After the determination of ARA, nodules were detached from the roots, counted and weighed.

2. Field experiment (Exp. 2)

(1) Plant material

The same three genotypes as in Exp.1 were seeded in the experimental field of Tohoku University on 25 May 2001 at a spacing of 70×15 cm. The soil was the same type as in Exp. 1. N was applied as either of three types; urea, LP-70 and LP-100. The latter two are commercial coated fertilizers (Chisso Asahi Fertilizer Co. Ltd., Tokyo, Japan) that release N over prolonged periods (at 25°C in water; LP-70 and LP-100 release 80% of their total N in 70 and 100 days, respectively). The amount of N, P_2O_5, and K_2O applied was 3, 10, and 10 g m^{-2}, respectively. All fertilizers were applied prior to seeding. Nine plots (3 genotypes treated with either of three fertilizer types) were arranged randomly, each plot occupying 7.5 m by 2.1 m. Each plant was considered an experimental unit.

(2) Measurement of growth

Ten plants were collected at maturity (DAS 156−171) and five plants each at the flowering stage (R2, DAS 66−68) and at the pod-expansion stage (R4, DAS 91−96). Roots and nodules were carefully collected from a rhizosphere of a 15-cm radius from a plant and 15 cm deep from the soil surface. Leaf area measurement and dry weight determination were performed as described above.

(3) Measurement of AP, analysis of leaf N content and ARA

AP and leaf N contents were measured at the pod-expansion and the seed-filling stage, while ARA at the flowering and the pod-expansion stage, respectively, in the same way as described for Exp. 1.

3. Statistical analysis

Differences between values were tested by Fisher's LSD ($P<0.05$).

Results

1. Leaf nitrogen content

In pot-grown Enrei and Sakukei 4 plants (Exp. 1), leaf N contents per leaf dry weight at the pod-expansion

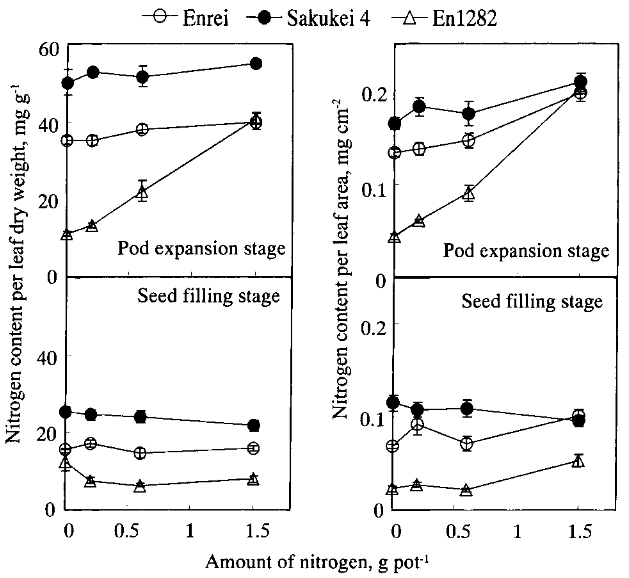

Fig. 1. Dependence of leaf nitrogen content per leaf dry weight (left) or per leaf area (right) on the amount of N applied to pot-grown plants (Exp. 1) of three soybean genotypes (Enrei, Sakukei 4, En1282). Samples were taken at the pod-expansion stage and the seed-filling stage. Each point represents the mean±SE (n=4 or 5).

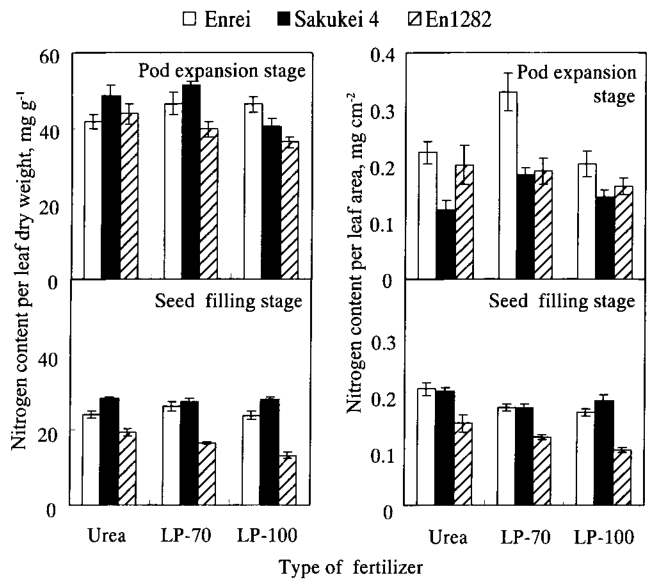

Fig. 2. Effects of different fertilizer types on leaf nitrogen contents per leaf dry weight (left) or per leaf area (right) in different soybean genotypes (Enrei, Sakukei 4, En1282) grown in the field (Exp. 2). Measurements were made at the pod-expansion stage and the seed-filling stage. Each point represents the mean±SE (n=4 or 6).

and the seed-filling stage were not changed by the amount of fertilizer applied (Fig. 1, left). By contrast, leaf N content per leaf dry weight at the pod-expansion stage in En1282 increased with increasing amount of N fertilizer although that at the seed-filling stage did not. The N contents per leaf area at the pod-expansion stage tended to increase with increasing fertilization in all genotypes (Fig. 1, right), but the response was only moderate in Sakukei 4. At the seed-filling stage, no such dependence on fertilizer dosage was observed. Leaf N content expressed per dry weight or per leaf area was the highest in Sakukei 4, intermediate in Enrei, and lowest in En1282. Generally, leaf N content was higher at the pod-expansion stage than at the seed-filling stage.

When urea or LP-70 was applied, leaf N content per dry weight at the pod-expansion stage tended to be higher in Sakukei 4 than in Enrei and En1282, but when LP-100 was applied, it was higher in Enrei (Fig. 2, left). Enrei had the highest leaf N content expressed on leaf area basis at the pod-expansion stage irrespective of the type of fertilizer (Fig. 2, right). At the seed-filling stage, leaf N content (both per leaf dry weight and per leaf area) of En1282 was significantly lower than that of the other two genotypes. At this stage, leaf N level seemed to be slightly higher in Sakukei 4 than in Enrei. Comparing the effects of the different N fertilizers, only En1282 showed a general tendency; leaf N contents were the highest when fertilized with urea, followed by LP-70, and LP-100, in this order.

2. Photosynthesis

In pot-grown plants (Exp.1), the photosynthetic rate

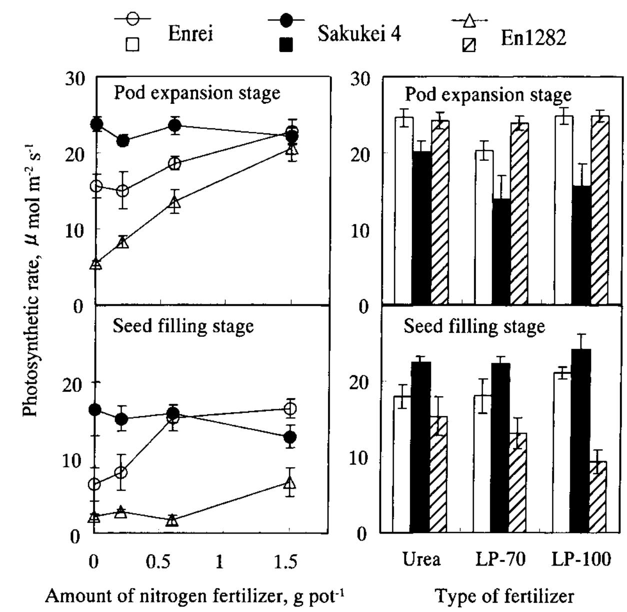

Fig. 3. Effects of the amount of nitrogen fertilizer applied (left; Exp. 1) or of the type of fertilizer (right; Exp. 2) on photosynthetic rate in different soybean genotypes (Enrei, Sakukei 4, En1282) at the pod-expansion and seed-filling stage. Each point represents the mean±SE (n=5). Experimental details as in Figs. 1 and 2.

responded to the amount of N applied (Fig. 3, left) in a genotype-dependent pattern similarly to that of leaf N content (compare Fig. 1). In field-grown plants (Exp. 2), Sakukei 4 showed the lowest photosynthetic activity among all genotypes at the pod-expansion stage, but the highest activity at the seed-filling stage (Fig. 3, right).

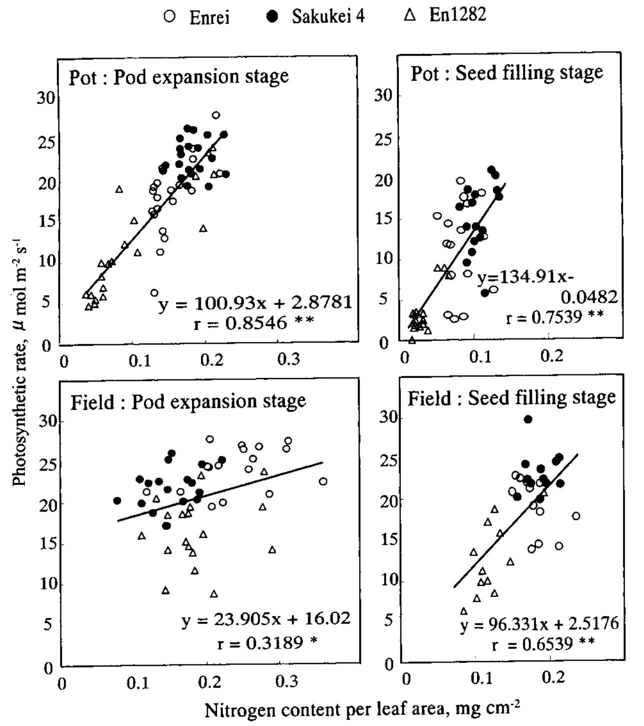

Fig. 4. The relationship between leaf nitrogen contents per leaf area and photosynthetic rate of three soybean genotypes (Enrei, Sakukei 4, En1282) at the pod-expansion stage (left) and at the seed-filling stage (right). Equations are regression functions describing the dependence of photosynthetic rate on leaf nitrogen content, with correlation coefficient (r). * and ** : significant at 5% and 1% level, respectively.

Table 1. Effects of the amount of nitrogen fertilizer applied on total dry weights and seed yields of different soybean genotypes (Enrei, Sakukei 4, En1282) grown in pot, Exp. 1.

Genotype	Amount of nitrogen applied (g pot^{-1}) 0	0.2	0.6	1.5
	g pot^{-1}			
Total dry weight (including root) at the pod expansion stage				
Enrei	63.2a*	67.9ab	76.8b	69.0ab
	(100)	(107)	(121)	(109)
Sakukei 4	51.3a	63.8b	66.3b	67.3b
	(100)	(125)	(129)	(131)
En1282	17.4a	33.9b	48.0c	70.4d
	(100)	(195)	(276)	(405)
Total dry weight (stem+pod+seed) at the maturity				
Enrei	58.4b	60.5b	62.4b	52.0a
	(100)	(104)	(107)	(89)
Sakukei 4	50.7a	54.0ab	56.8b	52.3ab
	(100)	(106)	(112)	(103)
En1282	5.8a	11.1b	25.1c	49.0d
	(100)	(191)	(430)	(841)
Seed yield				
Enrei	28.4b	28.7b	28.7b	22.3a
	(100)	(101)	(101)	(79)
Sakukei 4	26.2b	26.6b	26.4b	21.2a
	(100)	(102)	(101)	(81)
En1282	1.3a	2.3b	7.2c	19.1d
	(100)	(172)	(549)	(1459)

*Values followed by the same letter within one line are not significantly different at the 0.05 probability level. Values given are means of five pots. Figures in parentheses indicate percentages to the value in the plants without application of nitrogen fertilizer.

Fig. 4 shows the relationship between leaf N content per leaf area and AP in the three genotypes. By calculating regressions using the values pooled for all the genotypes, we observed a linear relation between the two parameters. Generally, the variances of leaf N contents per leaf area and AP were smallest in Sakukei 4 and greatest in En1282; Enrei was intermediate. The variance of leaf N contents per leaf area of Enrei was larger when grown in the field (Exp. 2) than when grown in pots (Exp. 1). By contrast, the variances of the two parameters were independent of growth conditions in Sakukei 4. In general, leaf N content and AP were higher in field-grown than in pot-grown plants, with the exception of the genotype Sakukei 4 at the pod- expansion stage. The AP of Sakukei 4 tended to exceed that of Enrei and En1282 at a given leaf N content, particularly at the seed-filling stage.

3. Growth

Table 1 summarizes the growth and seed yields in pot-grown plants (Exp. 1). The dry weight of En1282 was markedly low in the absence of fertilizer and increased dramatically with increasing amounts of N fertilizer. A fertilizer-dependent dry weight increase was observed also in Enrei and Sakukei 4, but the differences between treatments were not statistically significant. The highest dry weight was reached in the genotype Enrei fertilized with 0.6 g N per pot. Up to this dose, dry weight was generally highest in Enrei, intermediate in Sakukei 4, and lowest in En1282; treatment with 1.5 g N per pot yielded mixed results. There was no significant difference in seed yields between Enrei and Sakukei 4, and both substantially exceeded the yield of En1282. Seed yield in En1282 showed a strong dependence on fertilizer dosage, but that in the other genotypes did not.

Dry weight did not significantly vary with the fertilizer type in any of the genotypes (Exp. 2, Table 2). Seed yield of En1282, but not that of Enrei and Sakukei 4, depended on the type of fertilizer applied. The yield was highest in urea-treated plants. Reflecting the difference in dry weight, seed yield was significantly higher in Enrei than in Sakukei 4.

4. Root dry weight, nodule dry weight, N fixation activity and leaf area

The root dry weight (Exp. 1) increased with increasing dose of N fertilizer in all genotypes, whereas the nodule dry weight significantly declined at higher dosages of N (Table 3). ARA per plant declined at the highest amount of N applied. Nodule dry weights and ARA per plant tended to be greater in Sakukei 4 than in Enrei. ARA per nodule dry weight did not vary signifi-

Table 2. Effects of different types of nitrogen fertilizer on total dry weights and seed yields of different soybean genotypes (Enrei, Sakukei 4, En1282) grown in field, Exp. 2.

Genotype	Type of fertilizer Urea	LP-70	LP-100
	g plant $^{-1}$		
Total dry weight (including root) at the flowering stage			
Enrei	64.3ns	63.3ns	64.1ns
Sakukei 4	36.5ns	28.2ns	33.3ns
En1282	43.7ns	43.0ns	52.9ns
Total dry weight (including root) at the pod expansion stage			
Enrei	133.9ns	122.7ns	160.3ns
Sakukei 4	90.0ns	84.4ns	81.5ns
En1282	89.9ns	85.1ns	105.6ns
Total dry weight (stem+pod+seed) at the maturity			
Enrei	108.4ns	111.5ns	122.5ns
Sakukei 4	81.5ns	78.6ns	83.0ns
En1282	79.7c	62.6b	49.8a
Seed yield			
Enrei	50.9ns	51.2ns	57.9ns
Sakukei 4	40.4ns	40.0ns	42.5ns
En1282	34.0c	22.6b	15.3a

*Values followed by the same letter within one line are not significantly different at the 0.05 probability level. Values given are means of five to ten plants.

Table 3. Effects of the amount of nitrogen fertilizer applied on root and nodule dry weights and on ARA in different soybean genotypes (Enrei, Sakukei 4, En1282) grown in pot at the pod-expansion stage, Exp. 1.

Genotype	Amount of nitrogen applied (g pot $^{-1}$) 0	0.2	0.6	1.5
Root dry weight (g pot $^{-1}$)				
Enrei	8.0a	7.8a	10.8b	10.6b
Sakukei 4	6.9a	8.3ab	8.6b	10.5c
En1282	6.2a	9.5b	11.0b	11.3b
Nodule dry weight (g pot $^{-1}$)				
Enrei	2.9b	2.6b	2.8b	1.0a
Sakukei 4	6.7c	7.7c	5.4b	1.8a
ARA per plant (C_2H_4 μ mol h $^{-1}$ plant $^{-1}$)				
Enrei	34.7ab	24.8ab	46.6b	16.2a
Sakukei 4	59.4b	51.3b	47.6b	13.2a
ARA per nodule dry weight (C_2H_4 μ mol h $^{-1}$ g $^{-1}$)				
Enrei	25.4ns	21.2ns	29.8ns	30.3ns
Sakukei 4	17.4ns	13.8ns	17.2ns	15.6ns

*Values followed by the same letter within one line are not significantly different at the 0.05 probability level. Values given are means of five pots.

cantly with the N dosage, and generally was lower in Sakukei 4 than in Enrei.

Root dry weight (Exp. 2) did not depend on the fertilizer type applied at the flowering stage in any of the

Table 4. Effects of different types of nitrogen fertilizer on root and nodule dry weights and on ARA in different soybean genotypes (Enrei, Sakukei 4, En1282) grown in field at the flowering stage and the pod-expansion stage, Exp. 2.

Genotype	Type of fertilizer Urea	LP-70	LP-100
Flowering stage			
Root dry weight (g pot $^{-1}$)			
Enrei	4.2ns	4.2ns	3.7ns
Sakukei 4	2.9ns	2.8ns	3.0ns
En1282	3.9ns	4.1ns	4.6ns
Nodule dry weight (g pot $^{-1}$)			
Enrei	0.3a	0.4a	0.8b
Sakukei 4	2.1b	1.3a	2.5b
ARA per plant (C_2H_4 μ mol h $^{-1}$ plant $^{-1}$)			
Enrei	3.1ns	5.6ns	5.9ns
Sakukei 4	10.2a	5.1a	23.4b
ARA per nodule dry weight (C_2H_4 μ mol h $^{-1}$ g $^{-1}$)			
Enrei	8.8ns	31.3ns	8.2ns
Sakukei 4	4.7a	4.4a	9.1b
Pod expansion stage			
Root dry weight (g pot $^{-1}$)			
Enrei	10.1ab	8.5a	12.4b
Sakukei 4	8.9ns	8.4ns	9.1ns
En1282	7.3a	8.5ab	10.8b
Nodule dry weight (g pot $^{-1}$)			
Enrei	0.7a	0.8a	1.4b
Sakukei 4	4.4ns	3.4ns	3.8ns
ARA per plant (C_2H_4 μ mol h $^{-1}$ plant $^{-1}$)			
Enrei	14.4ns	22.6ns	45.3ns
Sakukei 4	51.8ns	33.7ns	44.4ns
ARA per nodule dry weight (C_2H_4 μ mol h $^{-1}$ g $^{-1}$)			
Enrei	23.0ns	27.8ns	27.8ns
Sakukei 4	15.1ns	10.2ns	13.0ns

*Values followed by the same letter within one line are not significantly different at the 0.05 probability level. Values given are means of five to ten plants.

genotypes (Table 4). However, root dry weight at the pod-expansion stage in Enrei and En1282 was significantly heavier in the plants fertilized with LP-100. In Sakukei 4, nodule dry weight and ARA at the flowering stage were higher in the plants fertilized with LP-100, but at the pod-expansion stage there was no significant difference. ARA per plant measured at the pod-expansion stage was significantly higher than that at the flowering stage in Enrei and Sakukei 4. At the pod-expansion stage, ARA per plant tended to be higher in Sakukei 4 than in Enrei, but the reverse relationship was observed in ARA per a nodule dry weight.

Leaf area per pot (Exp. 1) was increased by N application with the exception of Enrei treated with 1.5 g N (Table 5). Leaf area of Sakukei 4 was always lower than that of Enrei with the exception of the dose of 1.5 g N. The same tendency was observed in Exp. 2.

Table 5. Effects of dosage and type of nitrogen fertilizer on leaf area in different soybean genotypes (Enrei, Sakukei 4, En1282) at the flowering stage (Exp. 2) and the pod-expansion stage (Exp. 1, 2).

Pot [Exp.1]	Amount of nitrogen applied (g pot^{-1})			
Genotype	0	0.2	0.6	1.5
Pod expansion stage		100 cm^2 plant^{-1}		
Enrei	80.1ab	89.0bc	93.1c	73.1a
Sakukei 4	62.0a	77.1b	81.4b	79.2b
En1282	11.4a	24.2b	48.2c	73.3d

Field [Exp.2]	Type of fertilizer		
Genotype	Urea	LP-70	LP-100
Flowering stage		100 cm^2 plant^{-1}	
Enrei	83.3a	81.3a	109.0b
Sakukei 4	46.4b	30.0a	37.1ab
En1282	58.3ns	57.2ns	63.7ns
Pod expansion stage			
Enrei	135.1ns	107.9ns	113.9ns
Sakukei 4	80.6ns	79.1ns	65.8ns
En1282	72.5ns	78.1ns	74.8ns

*Values followed by the same letter within one line are not significantly different at the 0.05 probability level. Values given are means of five to ten plants.

Discussion

Our experiments showed that the supernodulating genotype Sakukei 4 possessed higher N contents per leaf dry weight as well as per leaf area, and higher photosynthetic rates than related normal or non-nodulating genotypes. These characteristics of Sakukei 4 were not significantly affected by the growing condition (field or pots), or the dosage or type of N fertilizer employed. In non-nodulating line En1282, the N contents per leaf dry weight and the AP drastically changed with the N dose applied.

In the pot experiment (Exp. 1), the rhizosphere was severely restricted, resulting in significantly different AP and N content per leaf area among the three genotypes tested. Under field conditions (Exp. 2), these differences were less clearly expressed. Apparently, Enrei and En1282 could absorb more N in the field than in the pots. The non-nodulating genotype En1282 utilizes N only from soil and fertilizer without fixing atmospheric N_2. The N content of En1282 at the pod-expansion stage was slightly higher than that of Sakukei 4, indicating that En1282 absorbed much N from the soil (Fig. 2, right). Under field conditions, En1282 extended its root system more vigorously than Sakukei 4 (Table 4), which probably contributed to its high capacity for N uptake from the soil. Root growth of Sakukei 4 was poor compared with the other two genotypes, which might restrict N absorption in the field.

It is well-known that gramineous crops are more responsive to N application than legumes. In this study, non-nodulating En1282 responded to applied N similarly to gramineous plants. On the contrary, the nodulating genotypes Enrei and, in particular, Sakukei 4 did not show a pronounced N response that is typical of leguminous crops. It is well documented that the application of a large amount of nitrogen fertilizer to soybean inhibits the formation and activity of nodules, resulting in little enhancement of total nitrogen uptake (Harper, 1987).

The genotypic difference in N content became more significant at the seed-filling stage (Fig. 4). Sakukei 4 contained more N per leaf area and showed a higher AP than Enrei and En1282, both in pots and in the field. It is well-known that in normally nodulating soybeans, the nitrogen fixing capability generally declines during seed filling (Sinclair and de Wit, 1975). The N content per leaf area, however, sharply declined in En1282 at the seed-filling stage. These results suggest that Sakukei 4 is capable of maintaining a high N level in leaves by continuous N-fixation until late growth stages. Consequently, Sakukei 4 is less likely to exhibit self-destructive N translocation which is commonly observed in soybean (Sinclair and de Wit, 1975).

There was a positive correlation between leaf N content and photosynthetic rate regardless of the dosage and the type of N fertilizer (Fig. 4). A similar correlation has been observed previously (Lugg and Sinclair, 1981). Leaf N content was also found to correlate with AP in F_3 progenies of crosses of various soybean cultivars (Ojima and Kawashima, 1970). Moreover, AP has been reported to correlate positively with soluble protein content of leaves in the studies comparing two (Hesketh et al., 1981) and 29 cultivars (Boon-Long et al., 1983). Sinclair and Horie (1989) quantified the correlation between AP and leaf N content, and found a nearly linear correlation between them in the range between 0.10 and 0.24 mg N cm^{-2}. In our study, it is noteworthy that at a given leaf N content per leaf area, Sakukei 4 had higher photosynthetic rates than the other two genotypes (Fig. 4). The reasons for this phenomenon remain to be elucidated.

Nodule dry weight and ARA decreased with increasing dose of N fertilizer in all genotypes (Table 3). However, nodule dry weights and ARA in most cases were higher in Sakukei 4 than in Enrei, and the difference between the two genotypes was greater at lower N levels. A similar genotypic difference was observed at the flowering stage when plants were fertilized with LP-100 which retains parts of its N load over prolonged periods (Table 4). These results indicate that Sakukei 4 is likely to maintain higher nodule activity at low N availability in the soil. Since nitrogen acquisition in Sakukei 4 seems to rely greatly on nodule activity, growth of Sakukei 4 is expected to depend on soil conditions suitable for nodule activity.

The nodule activity (ARA) per plant was higher in Sakukei 4 than in Enrei in many cases. However, the relationship was reverse in ARA per nodule dry weight (Tables 3, 4). This phenomenon, which also was observed in the parental mutant En6500 (Francisco et al., 1992), is probably caused by insufficient photosynthate supply to the nodules, or by excess nodule formation.

In a previous study with Sakukei 4 and Enrei grown in Ibaraki, Sakukei 4 exhibited a superior yield in some cases, particularly when the overall yield level was low (Takahashi et al., 2003). In the present study, however, the high photosynthetic ability of Sakukei 4 did not result in better growth performance and seed yield. This may be attributed to the cooler growing environment in Sendai. The growth performance of crops can be characterized by two factors; leaf area and net assimilation rate (NAR). The NAR of Sakukei 4 was higher than that of Enrei during the flowering to pod-expansion stage in our study (data not shown), whereas leaf area of Sakukei 4 was always lower (Table 5). Thus, Sakukei 4 appeared to accumulate N in the leaf rather than to utilize it for the expansion of the leaf.

In conclusion, the supernodulating genotype Sakukei 4 is characterized by high AP and high leaf nitrogen contents which are maintained during advanced developmental stages, regardless of the dosage and type of N fertilizer applied. Despite this desirable character, the growth performance was inferior to that of the normal nodulating genotype Enrei. In the practical use of Sakukei 4, increased planting density is probably one of the measures to maximize growth and yield. This genotype also has a potential to grow under conditions of low N availability. Field experiments should be conducted to evaluate these possibilities.

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*In Japanese with English summary.

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