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Evaluation of Nitrogen Fixation between Supernodulating Soybean Mutants and their Wild-Types Using ^{15}N in Field Conditions

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Abstract

Improvement of N_2 fixation by symbiotic rhizobia is considered an effective means for enhancing its productivity without high input of nitrogen (N) fertilizer. Several methods to improve N_2 fixation have been proposed including the use of supernodulating mutants. The objective of this research was to identify the varietal difference in N and N_2-fixation ability among the soybeans, with different nodulation abilities using ^{15}N in field conditions. The dry weight (DW) was higher in wild-type soybeans. The distribution rate of DW in each plant part was high in seeds of supernoduating and wild-type soybeans but high in stems and leaves of non-nodulating mutants. Although the supernodulating mutants had a low DW rate at maturity, they showed a similar N_2 fixation ability compared with wild-type. Supernodulating mutant plants mainly obtained N from N_2 fixation, while soil N was the main resource for obtaining N in non-nodulating mutants. The percentage of N derived from atmospheric dinitrogen (*Ndfa*) was higher in supernodulating mutants than in wild-type and relatively high in seeds between plant parts at maturity. In particular, supernodulating mutants showed higher N content in roots than those of wild-type and non-nodulating mutants. It was considered that supernodulating mutants have the advantage of saving nitrate in soil and being beneficial for N absorption of subsequent crops due to their conserving more N in the field and releasing considerable amounts of N from roots and leaves fallen to the soil.

Key words: nitrogen, N_2 fixation, nodule, supernodulating mutant, soybean

Introduction

In agricultural practice, it is considered that maximum soybean (*Glycine max* L. Merr.) seed yields could be achieved by the optimum use of both N_2 fixation by the nodules and N absorption from roots (Harper 1974, 1987). Soybean plants need to obtain a large amount of N by alternative sources from biological N_2 fixation or N fertilizers. However, a high dose of chemical fertilizer severely depresses nodule formation and N_2 fixation activity either by basal dressing or by top dressing. It sometimes causes over-luxuriant growth and a small increase or even decrease in seed yield, despite the additional cost and labor (Weber 1966).

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Improvement of N_2 fixation by symbiotic rhizobia is considered as the effective means for enhancing its productivity without high input of N fertilizer and consequent heavy pollution load on the environment. Several methods to improve N_2 fixation have been proposed including the selection of rhizobial strains with high energy efficiency and high N_2 fixation capacity. Top dressing of N fertilizer at the flowering stage and the use of slow-release N fertilizers have also been tried to improve N utilization, so as not to inhibit nodulation and N_2 fixation through an increased soil inorganic N status (Takahashi et al. 2003). Another approach to enhance N_2 fixation is the use of supernodulating mutants isolated from various soybean varieties (Carroll et al. 1985; Gremaud and Harper 1989). Such mutants can form a large number of nodules in soils with a wide range of inorganic N levels.

Many supernodulating soybeans including nts1007 (Carroll et al. 1985), En6500 (Akao and Kouchi 1992), and SS2-2 (Lee et al. 1997) were developed by ethyl methanesulfonate (EMS) mutagenesis. Takahashi et al. (2003) developed the supernodulating cultivar Sakukei 4 from crosses using a supernodulating line En6500. Supernodulating lines were initially expected to be useful not only to gain a better understanding of autoregulation mechanisms of nodulation, but also to enhance soybean productivity through a higher N_2 fixation capacity. However, the so-called supernodulating or extremely supernodulating lines, which have more than 10 to 20 times as many nodules as the parent lines, are in fact inferior in growth and seed yield, and do not seem to have any agronomic benefit (Hansen et al. 1989). Although the hypernodulation trait is caused by a single recessive gene mutation (Carroll et al. 1985; Kokubun and Akao 1994), phenotypes of the nodulating mutants were different. For example, plant growth at the early growth stage is less vigorous than that of the parents. Especially, root growth of the mutant is much less vigorous than that of the parents (Gremaud and Harper 1989; Ha and Lee 2001; Ohyama et al. 1993; Sato et al. 1999).

However, only a few genotypes that are intermediate in nodulation and form about twice as many nodules as parental lines sometimes have similar yield as their parents and have been found to be of some agricultural use (Herridge and Danso 1995; Pracht et al. 1994; Zhao et al. 1998; Herridge and Rose 2000). Clearly, they showed higher N-fixing ability than parental lines. Recently developed supernoduating cultivar, Sakukei 4, also showed vigorous growth (Takahashi et al. 2003). When the NOD mutant lines were grown in the absence of nitrate and compared with the Williams parent at 28 days after planting, the nodule number was about 2.5-fold higher, while the nodule weight and acetylene reduction activity (ARA) per plant were about 1.4- and 1.8-fold higher, respectively (Gremaud and Harper 1989). Some super- or hypernodulating soybean genotypes showed high N-fixing ability only in the early growth stage (Day et al. 1986; Eskew et al. 1989; Wu and Harper 1991) and in the late growth stage (Takahashi et al. 2005).

Meanwhile, N yield, N difference, ^{15}N, acetylene (C_2H_2) reduction, and xylem solute (Ureide) methods have all been used for measuring N_2 fixation (Herridge and Rose 2000). Although there is no single best method for measuring soybean N_2 fixation, the use of ^{15}N has been a powerful tool in research on N_2 fixation in general, and its quantitative and metabolism analysis in particular (Akao and Kouchi 1992). However, the N_2 fixation ability has not been recently investigated in SS2-2 and Sakukei 4, which were developed as supernodulating genotypes in Korea and Japan, respectively.

Therefore, the objective of this research was to identify the varietal differences in N and N_2 fixation ability among the soybean plants with different nodulation abilities using ^{15}N in field conditions.

Materials and Methods

Plant materials and cultivation

Two supernodulating soybeans, Sakukei 4, bred from a cross of 'Enrei'/'En6500'//'Tamahomare' in Japan, and SS2-2, as an EMS mutant from Sinpaldalkong 2 in Korea, were used to compare with their wild-types, Enrei and Sinpaldalkong 2. For the calculation of the fraction of N_2 fixation, two non-nodulating control soybeans, T201 (Weber 1965) and En1282, were used. The experiment was conducted in the field of the National Institute of Crop Science, RDA, Korea in 2004 and 2005. The soil was artificial sandy loam of 80-120 cm in depth based on Hwadong Series (fine, mixed, mesic family of Aquic Hapludalfs). Chemical properties of the soil were as follows: pH (H_2O) 7.1; organic matter 29 (g kg^{-1}); total N content 0.16% in 2004, pH (H_2O) 7.0; organic matter 25 (g kg^{-1}); total N content 0.20% in 2005. The experiment was conducted as randomized complete block with three replications. The soybeans were sown at high density and then plants were thinned to adjust to 22 plants m^{-2} at the second node stage (V2), which is the conventional planting density in Korea. Plot size was 5 × 4.2 m. In particular, the PVC pipes of 30 cm in diameter and 50 cm in height were set up on each row in the plot to apply ^{15}N enriched $(NH_4)_2SO_4$ (5 atom%). In all years, fertilizer was supplied at 0.42 g N, 0.42 g P_2O_5, 0.48 g K_2O per PVC pipe. Plant management including weeds, diseases, and insects followed recommended practices.

Sample collection

Plants were collected from the experimental plots at the maturity stage (R8). Whole shoots and the central part of roots were taken out from the soil. Each plant was compartmented into root, stem + leaf, pod, and seed, and dried in an oven at 80 °C for 48 h. Samples were weighed and ground in a hammer mill, passing through a 0.5 mm sieve and stored at -5 °C in bags until analysis. Total N content and ^{15}N atom% of the plant samples were measured by stable isotope mass spectrometer (Isoprime-EA, Micromass, UK), and the amount of N derived from atmospheric dinitrogen (*Ndfa*), from fertilizer (*Ndff*) and from soil (*Ndfs*) were calculated by using ^{15}N dilution method. The method was based on the assumption that nodulating legumes and reference plants absorbed the same rates of *Ndfs* and *Ndff*, and the calculation was as follows (Hardarson and Danso 1993):

%*Ndfa* = (1-(atom% excess ^{15}N of nodulating legume/ atom% excess ^{15}N of non-nodulating legume)) x 100

%*Ndff* = (atom% excess ^{15}N of nodulating legume/ atom% excess ^{15}N of fertilizer) x 100

%*Ndfs* = 100 - %*Ndfa* - %*Ndff*

Results

Dry matter accumulation

Fig. 1 shows the dry weight (DW) of each plant part of soybean varieties at R8 stage. The highest total dry weight was observed in Enrei (51.1 and 47.1 g plant^{-1}) in both 2004 and 2005. The DW of various plant parts in each soybean genotype also showed similar pattern to the value of the total DW.

The DW distribution rate of each plant part in SS2-2 and Sakukei 4 was relatively constant between experimental years, with an average value of 47.9 and 45.6% in seeds, 20.4 and 21.3% in pods, 22.9 and 23.9% in stems and leaves, and 8.8 and 9.2% in roots, respectively. The percentage of distribution of DW in Sinpaldalkong 2 and Enrei was 43.4 and 40.2% in seeds, 19.9 and 19.2% in pods, 28.6 and 32.7% in stems and leaves, and 8.1 and 8.0% in roots, respectively. The percentage of distribution of DW in En1282 and T201 was 28.4 and 33.2% in seeds, 10.8 and 15.1% in pods, 46.9 and 40.7% in stems and leaves, and 13.8 and 11.1% in roots, respectively.

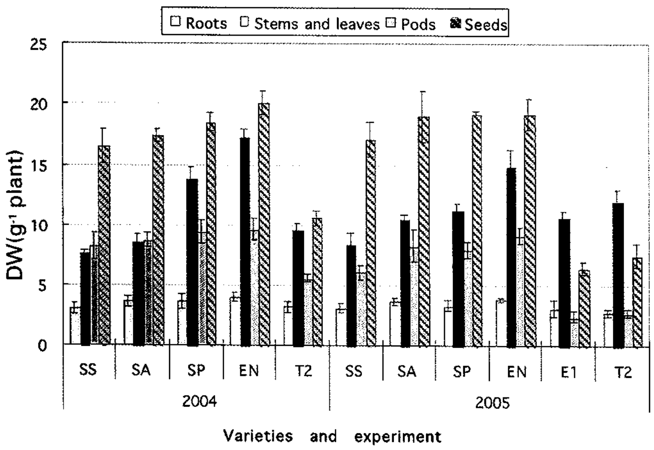

Fig. 1. Dry matter accumulation in various plant parts of soybeans in 2004 and 2005. Column and bars on top denote the mean and standard deviation three replications, respectively. SS: SS2-2, SA: Sakukei 4, SP: Sinpaldalkong 2, EN: Enrei, T2: T201, E1: En1282.

Table 1. N concentration of various plant parts of soybeans.

Year	Nodulation type	Varieties	Nitrogen concentration (mg N g^{-1} DW)			
			Roots	Stems and leaves	Pods	Seeds
2004	Super	SS2-2	17.8^{ab}	8.8^{ab}	7.3^{a}	66.8^{a}
		Sakukei 4	20.0^{a}	11.8^{a}	8.3^{a}	63.6^{a}
	Normal	Sinpaldalkong 2	11.7^{bc}	8.6^{ab}	5.4^{a}	64.5^{a}
		Enrei	13.8^{bc}	10.1^{a}	7.3^{a}	61.6^{a}
	Non	T201	9.3^{c}	4.9^{b}	5.2^{a}	46.6^{b}
2005	Super	SS2-2	14.0^{ab}	5.1^{b}	7.1^{bc}	67.3^{a}
		Sakukei 4	14.9^{a}	7.3^{a}	9.5^{a}	66.9^{a}
	Normal	Sinpaldalkong 2	10.1^{cd}	4.3^{bc}	6.9^{bc}	64.4^{a}
		Enrei	10.7^{cd}	4.2^{bc}	8.8^{ab}	65.6^{a}
	Non	En1282	7.9^{d}	3.6^{c}	5.9^{c}	56.2^{b}
		T201	11.7^{bc}	4.0^{c}	5.9^{c}	46.3^{c}

Values followed by the same superscripted letter within a column of the same year are not significantly different by LSD ($P < 0.05$).

Table 2. N content of various plant parts of soybeans.

Year	Nodulation type	Varieties	Nitrogen content (g N plant^{-1})				Total
			Roots	Stems and leaves	Pods	Seeds	
2004	Super	SS2-2	0.05^{b}	0.07^{cd}	0.06^{a}	1.10^{a}	1.28^{b}
		Sakukei 4	0.07^{a}	0.10^{bc}	0.07^{a}	1.11^{a}	1.35^{b}
	Normal	Sinpaldalkong 2	0.04^{bc}	0.12^{b}	0.05^{ab}	1.20^{a}	1.41^{ab}
		Enrei	0.06^{ab}	0.18^{a}	0.07^{a}	1.24^{a}	1.54^{a}
	Non	T201	0.03^{c}	0.05^{d}	0.03^{b}	0.50^{b}	0.60^{c}
2005	Super	SS2-2	0.04^{ab}	0.04^{c}	0.04^{b}	1.16^{a}	1.29^{b}
		Sakukei 4	0.06^{a}	0.08^{a}	0.08^{a}	1.27^{a}	1.49^{a}
	Normal	Sinpaldalkong 2	0.03^{bc}	0.05^{c}	0.05^{b}	1.24^{a}	1.38^{ab}
		Enrei	0.04^{b}	0.06^{b}	0.08^{a}	1.26^{a}	1.45^{a}
	Non	En1282	0.02^{c}	0.04^{c}	0.01^{c}	0.36^{b}	0.44^{c}
		T201	0.03^{bc}	0.05^{c}	0.02^{c}	0.34^{b}	0.44^{c}

Values followed by the same superscripted letter within a column of the same year are not significantly different by LSD ($P < 0.05$).

N concentration and content

Tables 1 and 2 show N concentration (mg N g DW^{-1}) and N content (mg N plant^{-1}) in various plant parts of soybean varieties in 2004 and 2005. There was a significant difference in the N concentration in all plant parts among soybean varieties. The N concentration in all plant parts was significantly lower in non-nodulating soybeans than supernodulating and wild-type soybeans. In addition, the N concentration in roots was higher in supernodulating mutants (14-20 mg N g DW^{-1}) than wild-types (12-15 mg N g DW^{-1}). However, a significant difference was not observed in the N concentration in leaves and stems, and pods and seeds between two different nodulation genotypes.

The total N content per plant was highest in wild-type soy-

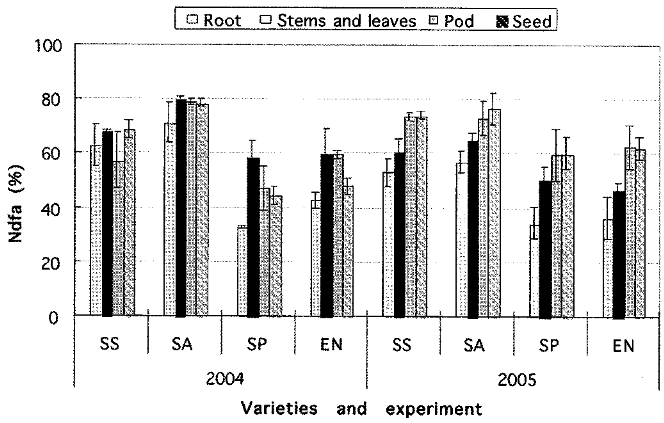

Fig. 2. The percentage of N derived from atmosphere (*Ndfa*) in various plant parts of soybeans. Column and bars on top denote the mean and standard deviation of three replications, respectively. SS: SS2-2, SA: Sakukei 4, SP: Sinpaldalkong 2, EN: Enrei.

Table 3. Amount of N derived from atmosphere (*Ndfa*) in various organs of soybeans.

Year	Nodulation type	Varieties	Amount of *Ndfa* (mg N plant^{-1})				Total
			Roots	Stems and leaves	Pods	Seeds	
2004	Super	SS2-2	40^{b}	46^{b}	34^{ab}	759^{a}	880^{b}
		Sakukei 4	60^{a}	81^{ab}	57^{a}	869^{a}	$1,068^{a}$
	Normal	Sinpaldalkong 2	19^{c}	69^{ab}	25^{b}	531^{b}	645^{c}
		Enrei	29^{bc}	107^{a}	42^{ab}	599^{b}	777^{b}
2005	Super	SS2-2	23^{ab}	26^{b}	32^{b}	858^{ab}	939^{ab}
		Sakukei 4	31^{a}	50^{a}	58^{a}	979^{a}	$1,118^{a}$
	Normal	Sinpaldalkong 2	11^{c}	24^{b}	33^{b}	748^{b}	816^{b}
		Enrei	15^{bc}	30^{b}	50^{a}	778^{ab}	872^{b}

Values followed by the same superscripted letter within a column of the same year are not significantly different by LSD ($P < 0.05$).

Table 4. Estimation of the percentage of N originating from various N sources based on the ^{15}N dilution method on the soybeans.

Year	Nodulation type	Varieties	*Ndfa*	*Ndfs*	*Ndff*
			---	% ---	
2004	Super	SS2-2	68.4^{b}	30.0^{c}	1.5^{b}
		Sakukei 4	78.9^{a}	21.0^{d}	1.0^{b}
	Normal	Sinpaldalkong 2	45.8^{c}	51.6^{b}	2.6^{b}
		Enrei	50.2^{c}	47.3^{b}	2.5^{b}
	Non	T201		95.0^{a}	5.0^{a}
2005	Super	SS2-2	72.9^{a}	26.4^{c}	0.7^{c}
		Sakukei 4	74.8^{a}	24.5^{c}	0.7^{c}
	Normal	Sinpaldalkong 2	59.1^{b}	39.9^{b}	1.0^{c}
		Enrei	60.4^{b}	38.5^{b}	1.0^{c}
	Non	En1282	-	97.9^{a}	2.1^{b}
		T201	-	96.8^{a}	3.2^{a}

Values followed by the same superscripted letter within a column of the same year are not significantly different by LSD ($P < 0.05$).

bean, Enrei (1.54 g) in 2004 and in supernodulating mutant, Sakukei 4 (1.49 g) in 2005 (Table 2). But, the N content was not significantly different between supernodulating and wild-type soybeans except for the N content per plant in 2004 and was smaller in Sakukei 4 than in its parent type. There was no significant difference in N content of seeds between supernodulating and wild-type soybeans, resulting in an increase of the N concentration in supernodulating soybeans despite low plant DW. In non-nodulating soybeans, T201 and En1282, the N content in various plant parts was lower than that in nodulating soybeans, which resulted in a decrease in plant DW and N concentration compared with that in nodulating soybeans.

The average distribution rate of N content per plant in SS2-2 and Sakukei 4 was 87.8 and 83.8% in seeds, 4.0 and 5.3% in pods, 4.3 and 6.4% in stems and leaves, and 3.8 and 4.5% in roots, respectively. In Sinpaldalkong 2 and Enrei, the rates were 87.4 and 83.9% in seeds, 3.8 and 5.1% in pods, 6.0 and 7.8% in stems and leaves, and 2.8 and 3.2% in roots, respectively. In T201 and En1282, the rates were 82.5 and 80.3% in seeds, 3.3 and 4.3% in pods, 8.6 and 9.4% in stems and leaves, and 5.6 and 6.1% in roots, respectively. Unlike the distribution pattern of DW in plant parts, the distribution rate of N content in seeds make up more than 80%; no difference was observed in each plant part between supernodulating and wild-type soybeans. However, non-nodulating soybeans showed a slightly higher value in stems, leaves, and roots, while nodulating soybeans produced fewer seeds.

Percentage and amount of N_2 fixation

The percentage and amount of N originating from N_2 fixation are shown in Fig. 2 and Table 3. The percentage of N derived from *Ndfa* was significantly higher in the supernodulating mutants than in wild-type soybeans. The average percentage of *Ndfa* in SS2-2 and Sakukei 4 was 71.4 and 77.5% in seeds, 65.4 and 75.9% in pods, 64.2 and 72.2% in stems and leaves, and 57.9 and 64.2% in roots, while in Sinpaldalkong 2 and Enrei, it was 52.4 and 54.9% in seeds, 53.5 and 61.1% in pods, 54.2 and 53.6% in stems and leaves, and 33.5 and 39.5% in roots, respectively. The considerable difference in percentage of *Ndfa* between supernodulating and wild-type soybeans was shown in roots among various plant parts. The percentage of N originating from N_2 fixation in wild-type soybeans was only 73.3-70.9% (seeds), 81.8-80.5% (pods), 84.5-74.2% (stem and leaves), and 57.9-61.6% (roots) of that in supernodulatilng soybeans.

There was a significant difference in the amount of *Ndfa* among soybean varieties of various plant parts. In seeds and roots, the amount of *Ndfa* was significantly higher in the supernodulating soybeans (759-979 and 23-60 mg N plant^{-1}) than in the wild-type soybeans (531-778 and 11-29 mg N plant^{-1}). In all treatments, the highest amount of *Ndfa* was found in seeds, followed by shoots. The average distribution rate of fixed N in SS2-2 and Sakukei 4 was 88.8 and 84.5% in seeds, 3.7 and 5.2% in pods, 4.0 and 6.0% in stems and leaves, and 3.5 and 4.2% in roots, respectively. In Sinpaldalkong 2 and Enrei, it was 87.0 and 83.1% in seeds, 4.0 and 5.6% in pods, 6.8 and 8.6% in stems and leaves, and 2.2 and 2.7% in roots, respectively. The distribution pattern of fixed N in each plant part was similar to that of N accumulation.

Percentage and amount of three N sources

Tables 4 and 5 showed the estimation of the percentage and amount of N originating from various N sources based on the ^{15}N dilution method. It was found that the soybean plants obtained 66.4-78.9% of N from N_2 fixation, 21.0-30.0% from soil N, and 0.7-1.5% from fertilizer in the supernodulating soybeans; in the wild-type soybeans it was 45.8-60.4% of N from N_2 fixation, 38.5-51.6% from soil N, and 1.0-2.6% from fertilizer, while 95.0-97.9% was obtained from soil N and 2.1-5.0% from fertilizer in the non-nodulating soybeans. These values showed that the major portion of total N content in the supernodulating soybean plants was derived from N_2 fixation and the percentage of *Ndfa* was very high in the supernodulating mutants compared with wild-type and non-nodulating soybeans. However, the percentages of *Ndfs* and *Ndff* were higher in non-nodulating soybeans, followed by wild-type, and supernodulating soybeans.

The amount of *Ndfa* was significantly higher in SS2-2 (0.88-0.94 N plant^{-1}) and Sakukei 4 (1.07-1.12 N plant^{-1}) than in Sinpaldalkong 2 (0.65-0.82 N plant^{-1}) and Enrei (N plant^{-1}). It suggests that supernodulating soybeans accumulate fixed N more than wild-type ones despite lower growth due to the high percentage of *Ndfa*. On the other hand, the amount of *Ndfs* was the highest in wild-type soybeans, followed by non-nodulating, and supernodulating soybeans. The amount of *Ndff* was not different among soybean varieties.

Daily N_2 fixation activity

The daily N_2 fixation activity and N absorption rate during whole growth period was calculated by a ^{15}N dilution method (Table 6). The N2 fixation activity was higher in supernodulating mutants (7.46-8.92 mg plant^{-1} day^{-1}) than in wild-type (5.33-7.70 mg plant^{-1} day^{-1}). Among soybean varieties, the highest N-absorption rate was found in wild-type soybeans (4.96-6.35 mg plant^{-1} day^{-1}), followed by non-nodulating (4.20-5.11 mg plant^{-1} day^{-1}), and supernodulating mutants (2.17-3.43 mg plant^{-1} day^{-1}).

Table 5. Estimation of the amount of N originating from various N sources based on the ^{15}N dilution method on the soybeans.

Year	Nodulation type	Varieties	*Ndfa*	*Ndfs*	*Ndff*	Total N
			g plant^{-1}			
2004	Super	SS2-2	0.88^{b}	0.38^{c}	0.02^{ab}	1.28^{b}
		Sakukei 4	1.07^{a}	0.27^{d}	0.01^{b}	1.35^{b}
	Normal	Sinpaldalkong 2	0.65^{d}	0.73^{a}	0.04^{a}	1.41^{ab}
		Enrei	0.78^{c}	0.73^{a}	0.04^{a}	1.54^{a}
	Non	T201	-	0.57^{b}	0.03^{ab}	0.60^{c}
2005	Super	SS2-2	0.94^{ab}	0.39^{c}	0.01^{a}	1.34^{b}
		Sakukei 4	1.12^{a}	0.47^{bc}	0.01^{a}	1.59^{ab}
	Normal	Sinpaldalkong 2	0.82^{b}	0.71^{a}	0.01^{a}	1.54^{ab}
		Enrei	0.87^{b}	0.75^{a}	0.01^{a}	1.63^{a}
	Non	En1282	-	0.44^{bc}	0.01^{a}	0.45^{c}
		T201	-	0.53^{b}	0.01^{a}	0.55^{c}

Values followed by the same superscripted letter within a column of the same year are not significantly different by LSD ($P < 0.05$).

Table 6. Estimation of N2 fixation activity and N absorption rate based on the data by ^{15}N dilution method.

Year	Nodulation type	Varieties	N_2 fixation activity (mg plant^{-1} day^{-1})	N absorption rate (mg plant^{-1} day^{-1})
2004	Super	SS2-2	7.46^{a}	3.43^{c}
		Sakukei 4	8.09^{a}	2.17^{c}
	Normal	Sinpaldalkong 2	5.33^{c}	6.35^{a}
		Enrei	6.27^{b}	6.19^{ab}
	Non	T201	-	5.11^{b}
2005	Super	SS2-2	8.92^{a}	3.30^{b}
		Sakukei 4	8.49^{ab}	2.80^{b}
	Normal	Sinpaldalkong 2	7.20^{b}	4.96^{a}
		Enrei	7.70^{ab}	5.06^{a}
	Non	En1282	-	4.20^{a}
		T201	-	4.23^{a}

Values followed by the same superscripted letter within a column of the same year are not significantly different by LSD ($P < 0.05$).

Discussion

Many researchers have reported that plant growth of supernodulating mutants was lower than wild-type soybeans (Day et al. 1986; Takahashi et al. 1995; Matsunami et al. 2004). This phenomenon may be due to very strong competition of carbon between shoots and many nodules. In the present study, the supernodulating mutants, SS2-2 and Sakukei 4, were compared with its wild-type (Sinpaldalkong 2) and parent type (Enrei) at R8. The DW of each plant part in supernodulating mutants was lighter than that in wild-type soybeans and heavier than that in non-nodulating ones (Fig. 1). Also, the ratio of dry-matter partitioning to seeds was generally higher in seeds of SS2-2 and Sakukei 4 and that to roots and stems and leaves showed high in En1282 and T201. The higher percentage of DW in supernodulating mutants might be due to the poor shoot growth comparison with total DW, while higher values in roots and stems and leaves percentage of DW in non-nodulating mutants were observed because of the poor seeds DW caused by the compensatory effect of root growth on N deficiency associated with the lack of N_2 fixation.

The supernodulating soybean lines, NOD1-3, NOD2-4, and NOD3-7 (Ohyama et al. 1993), En6500 (Takahashi et al. 1995), and Sakukei 4 (Matsunami et al. 2004), assimilated smaller amounts of nitrate than their wild-type soybeans. But our results (Table 2) suggest a different story. Although Sakukei 4 was significantly lower than Enrei, in general SS2-2 and Sakukei 4 showed similar amounts of N in each plant part compared to its wild-type (Sinpaldalkong 2) and pollen parent (Enrei). It is well known that the amount of N accumulation is largely affected by the dry matter weight and N concentration. But, high N concentration in roots and seeds of supernodulating mutants at maturity were detected (Table 1), although DW was less than that of wild-type soybeans. In other words, Takahashi et al. (2005) reported that the N concentration and content in Sakukei 4 was greater than that of Enrei at maturity which is in agreement with our data. Meanwhile, soybeans are hypothesized to be "self-destructive" because they apparently need to translocate large amounts of N from vegetative tissues during seed-filling to sustain seed growth (Sinclair and de Wit 1976). Our results also explained that supernodulating mutants showed weak self-destruction compared to wild-type soybeans. The non-nodulating soybeans showed the lowest N concentration and content, caused by slow growth and N deficiency associated with the lack of N_2 fixation.

It is expected that N_2 fixation activity in supernodulating or hypernodulating lines would be higher in the supernodulating genotypes than in their parent cultivar or pollen parent (Hansen et al. 1989; Francisco et al. 1992; Takahashi et al. 1995, 2005), but in some reports, they did not clearly show high ability in the late growth stages (Day et al. 1986; Eskew et al. 1989; Wu and Harper 1991). Some scientists estimated the fraction of symbiotically fixed N obtained by isotope dilution technique, showing 76% (Kanamori et al. 1987), 26-78% (Yoneyama 1987), 40% (Ichita 1986), 54-77% (Wada et al. 1986), 44-53% (Coale 1985), and 60% (Tewari et al. 2005). In this study, N originating from various N sources was estimated at maturity based on the ^{15}N dilution method on the soybean plants. The supernodulating mutants showed higher percentage (68.4-78.9%) and amount of *Ndfa* compared with wild-type soybeans (Tables 4 and 5). By the comparison of the percentage of *Ndfa* between plant parts (Fig. 2), supernodulating mutants showed the highest values in seeds (71.4-77.5%) and the lowest in roots (57.9-64.2%), but wild-types showed much lower values than in supernodulating ones and similar values between plant parts except for relatively low values in roots. Unlike the pattern of the distribution ratio of DW in each plant part (Fig. 1), the distribution ratio of N and *Ndfa* content in seeds was more than 80%, with no difference in each plant part between supernodulating and wild-type soybeans (Tables 3 and 5).

Akao and Ishi (1987) reported that the fixed N accumulated mostly in leaves and stems before the middle pod-filling stage, but after that stage the accumulation is higher in seeds. Takahashi et al. (2005) concluded that the high ARA due to the higher N-fixation capability may possibly be due to an increase in N content and the fraction of fixed N in supernodulating genotype, Sakukei 4, at maturity. These reports support our results that the percentage of *Ndfa* was higher in supernodulating than in wild-type and relatively high in seeds between plant parts at maturity. The daily N_2-fixation activity was higher in supernodulating than in wild-type soybeans, although the daily N absorption rate was low in supernodulating mutants (Table 6). But, the daily N-assimilation rate was also high in supernodulating mutants (data not shown). On the other hand, the supernodulating mutants showed a higher N concentration and content in roots than wild-type and non-nodulating soybeans (Tables 1 and 2), and a low percentage and amount of *Ndfs* and *Ndff* per plant than wild-type and non-nodulating ones (Tables 4 and 5). By our results, it can be considered that the supernodulating soybeans may have the advantage of saving nitrate in soil and be beneficial for N absorption of succeeding crops due to a nitrate-sparing effect, which saved more N in the field. In addition, a considerable amount of N may be released from the roots and leaves fallen to the soil (Table 2). Ohyama et al. (1993) and Hansen et al. (1989) also exhibited a restricted root growth and absorbed less nitrate N than their parent cultivar due to the small root mass, which restricted overall plant growth and total N accumulation but saved more N in the field.

From our field experiments, we reconfirmed that supernodulating soybeans might have high or similar N content compared to their wild-type lines due to larger N_2-fixation ability and daily N assimilation rate. Also, it was considered that they have the advantage of saving N in soil and releasing larger amounts of N to the soil, if fallen leaves including roots were not removed from the field.

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