Introduction, Development, and Characterization of Supernodulating Soybean Mutant -Shoot Factor Regulation of Nodule Development in Supernodulating Soybean Mutant -

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ABSTRACT

Nodule development was regulated partially by host plant factors originating in the shoots and roots. This study was performed to identify the origin of the factors regulating nodulation in supernodulating soybean (*Glycine max* [L.] Merr.) mutant 'SS2-2' which was isolated recently from ethyl methanesulfonate (EMS) mutagenesis of 'Sinpaldalkong 2'. Self- and reciprocal-grafts were made among three soybean genotypes which consisted of two supernodulating mutants, SS2-2 and 'nts 382', and a normal nodulating Sinpaldalkong 2. Self-grafted supernodulating mutants were characterized by greater nodule number, nodule dry weight, and C_2H_2 reduction activity than self-grafted wild types. They were also characterized by relatively higher nodule to root dry weight. Significant shoot genotypic effects were observed on nodule number, nodule dry weight, and C_2H_2 reduction activity per plant, whereas varying root genotypes had no effects. From this result, it is surmised that supernodulating characters are controlled by a graft-transmissible shoot factor, and mutant SS2-2 may have similar nodulation mechanism to the former supernodulating nts 382. In all grafts, both supernodulating mutants and Sinpaldalkong 2 maintained the similar balance between above ground and below ground parts regardless of significant differences in partitioning of dry matter into root and nodule between supernodulating mutants and Sinpaldalkong 2.

Key words : supernodulation, grafting, autoregulation of nodulation, shoot factor, root factor.

Enhancing symbiotic nitrogen assimilation, requiring the development of a specialized root nodule in legumes, may result in larger inputs of nitrogen into the soil-plant system. Induced mutagenesis resulted in the development of supernodulating legume plant mutants even in the presence of high soil nitrate. Carroll et al. (1985a; 1985b) developed the first supernodulating nts(nitrate tolerant symbiotic) soybean mutants which were isolated from cultivar 'Bragg' mutagenized with EMS. More recently, Akao & Kouchi (1992) isolated a supernodulating mutant En6500 from cultivar 'Enrei'. Hypernodulating soybean mutants, exhibiting nodulation between super- and normal-nodulating types, were also developed independently by Carroll et al. (1985a; 1985b) and Gremaud & Harper (1989). These super- or hyper-nodulating mutants should be used in a breeding program for reducing the nitrogen

fertilizer requirements for crop production as well as for protecting agricultural environments (Lee et al., 1997).

In the previous experiment, we isolated a new supernodulating mutant, SS2-2, from M_2 families generated from the mutagenesis of Sinpaldalkong 2 with 30 mM EMS (Lee et al., 1997). This mutant exhibited a supernodulation character in the presence of high exogeneous nitrate supply which was apparently similar to the nts mutants isolated previously (Lee et al., 1997; Lee, 1998). However, comparison of nodulation between SS2-2 and nts mutants revealed that SS2-2 showed faster nodulation than nts mutants.

The isolation of supernodulating soybean mutant with EMS mutagenesis has stimulated the research on the regulation of nodule development. Reciprocal grafting techniques (Bezdicek et al., 1972) between nodulating mutant and normal wild-type soybean have been widely applied to determine the relative importance of shoot and root factor in the regulation of nodulation. Supernodulating characters are controlled through a graft-transmissible shoot factors in most grafting studies (Delves et al., 1986; Lee et al., 1991; Hamaguchi et al., 1992; 1993). On the other hand, nodulation was dependent on the root in supernodulating 'nod 3' and nodulating resistance 'K5' of pea (*Pisum sativum*), whereas inhibited nodulation of mutant 'k24' was dependent on the root and shoot (Postma et al., 1988).

In this study, grafting among three soybean genotypes, consisting of two supernodulating mutants (SS2-2 and nts 382) and a normal nodulating Sinpaldalkong 2, was made to determine which of the plant parts were essential for the supernodulating characters in mutants. The distribution of plant dry weight was also compared in grafts with Sinpaldalkong 2 and two mutant lines.

MATERIALS AND METHODS

Sinpaldalkong 2 and its supernodulating mutant SS2-2 (Lee et al., 1997) were used in this study. Supernodulating soybean mutant nts 382, previously isolated from cultivar 'Bragg' (Carroll et al. 1985a; 1985b), was also selected for this study. Seeds of nts 382 were supplied by Prof. P.M. Gresshoff, Institute of Agriculture and Center for Legume Research in the Univ. of Tennessee, USA.

Seeds were surface sterilized in 50ml/ℓ NaOCl for 10 min, rinsed several times with distilled water, and planted directly in about 1-ℓ styroform cups filled with river sand. Two plants per pot were grown in the greenhouse.

Seven days after planting, self- and reciprocal grafts among three soybean genotypes were made according to the procedure of Bezdicek et al. (1972). Grafted plants were transferred to a sealed polyethylene chamber maintained with high relative humidity (near 100%) to prevent the grafted region from losing water. After 7 days for adaption, plants were transferred to the greenhouse. The shoots and roots of the three soybean genotypes were grafted and placed in a completely randomized design with three replications (two grafted plants per pot in one replication). Then plants were inoculated with *Bradyrhizobium japonicum* (NITRAGIN, LiphaTech Inc., Milwaukee, Wisconsin, USA). Two or three times a week plants in each pot were fertilized with 50ml of modified Hoagland's solution containing 10 mM NO_3^-.

Eight weeks after grafting, dry weights of each plant part (stem, leaf, root, and nodule) were measured. Nitrogen fixation ability was estimated on the basis of acetylene reduction activity of nitrogenase in the root nodule. The procedure for measuring acetylene reduction activity was described in the previous paper (Lee, 1998). Briefly, sampled root was reacted with 50-cc C_2H_2 for 30 min. A 10-cc aliquot from the jar was injected into a 10-cc vacutainer tube, 0.5-cc aliquot was drawn for gas chromatograph analysis of C_2H_4.

RESULTS AND DISCUSSION

There were no significant effects of root genotype on the all growth and nodulation characters. On the other hand, shoot genotype had significant effects on the all nodulation characters such as nodule number, nodule dry

Table 1. Analysis of variance for nodulation and acetylene reduction activity as affected by shoot and root genotypes in grafting experiments.

Source of variation	df	Mean squares		
		Nodule number	Nodule dry weight	C_2H_2 reduction activity
Shoot (S)	2	46519***	128.0***	24.5***
Root (R)	2	1572	4.1	4.4
S×R	4	15609**	6.7	7.7*
Error	18	772	3.2	1.8

*, **, *** significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

weight, and C_2H_2 reduction activity (Table 1), indicating that supernodulating character was clearly controlled by shoot-derived factor. This was consistent with the previous experiments (Delves et al., 1986; Hamaguchi et al., 1992, 1993; Lee et al., 1991). Significant interaction effects between shoot and root genotypes were also shown in nodule number and C_2H_2 reduction actvity (Table 1). This suggested that differences in nodule number and C_2H_2 reduction activity of grafts derived from three sources of shoot genotype changed significantly over the three sources of root genotype. This was partially in agreement with the previous report by Hamaguchi et al. (1992) that shoot control of supernodulation of En6500 was modified by root factors.

Regardless of root genotypes, differences in nodule number, nodule dry weight, and C_2H_2 reduction activity per plant were observed for grafted plants derived from Sinpaldalkong 2 and supernodulating mutant shoot sources (Table 2). Average nodule number per plant of the three root genotypes was 2.5 and 3.6 times greater for shoot genotypes of SS2-2 and nts 382 than shoot geno-

Table 2. Nodulation and acetylene reduction activity in grafts using Sinpaldalkong 2 and two supernodulating mutants.

Shoot genotype	Root genotype			Mean
	Sinpaldalkong 2	SS2-2	nts 382	
	Nodule number (no. plant^{-1})			
Sinpaldalkong 2	36	43	37	39[c]†
SS2-2	128	101	191	140[a]
nts 382	124	102	71	99[b]
Mean	96[a]†	82[a]	99[a]	
	Nodule dry weight (mg plant^{-1})			
Sinpaldalkong 2	244	294	309	282[b]
SS2-2	419	528	409	452[a]
nts 382	528	494	515	512[a]
Mean	397[a]	439[a]	411[a]	
	C_2H_2 reduction activity (umol h^{-1}plant^{-1})			
Sinpaldalkong 2	4.8	5.7	5.2	5.3[b]
SS2-2	7.7	9.5	6.8	8.0[a]
nts 382	6.5	7.5	10.6	8.2[a]
Mean	6.3[a]	7.6[a]	7.5[a]	

† Within traits, means (column or row) not followed by the same letter are significantly different at P ≤ 0.05 based on LSD.

type of Sinpaldalkong 2. Across all three root genotypes, average nodule dry weight per plant of grafts derived from the shoot of supernodulating SS2-2 and nts 382 was 1.6 to 1.8 times greater than that from Sinpaldalkong 2. The effects of shoot and root genotypes on C_2H_2 reduction activity were similar to the effects on nodule number and nodule dry weight per plant. Across all three root genotypes, C_2H_2 reduction activity per grafted plant with supernodulating mutant shoot sources was about 1.5 times higher than those with Sinpaldalkong 2 shoot sources. However, nodules from self-grafted Sinpaldalkong 2 seemed to be more efficient in fixing nitrogen per unit nodule dry weight than those from self-grafted supernodulating mutants.

Regardless of root genotypes, the relative root to nodule dry weight differed significantly when different shoot genotypes were used as a scion in grafts (Table 3). Across all three root genotypes, the presence of Sinpaldalkong 2 shoots resulted in the nodule containing only 14% of below ground dry weight, whereas supernodulating mutants shoots resulted in nodules containing more than 20% of below ground dry weight.

However, in all grafted plants, the ratio of above to below ground dry weight remained almost constant (Table 4). The main effects of shoot and root on the ratio of

Table 3. Nodule dry weight as a percent of total below ground dry weight (root+nodule) in grafts using Sinpaldalkong 2 and two supernodulating mutants.

Shoot genotype	Root genotype			Mean
	Sinpaldalkong 2	SS2-2	nts 382	
	.... %			
Sinpaldalkong 2	11.2	16.1	15.7	14.3[b†]
SS2-2	23.8	19.6	19.8	21.1[a]
nts 382	27.7	19.1	21.0	22.6[a]
Mean	20.9[a†]	18.3[a]	18.8[a]	

† Within column or row, means not followed by the same letter are significantly different at $P \leq 0.05$ based on LSD.

Table 4. The ratio of top dry weight (stem+leaf) to total below ground dry weight (root+nodule) in grafts using Sinpaldalkong 2 and two supernodulating mutants.

Shoot genotype	Root genotype			Mean
	Sinpaldalkong 2	SS2-2	nts 382	
	.... %			
Sinpaldalkong 2	2.04	1.96	1.99	2.00[a†]
SS2-2	1.93	1.87	1.80	1.87[a]
nts 382	2.22	2.16	1.68	2.02[a]
Mean	2.04[a†]	2.02[a]	1.82[a]	

† Within column or row, means not followed by the same letter are significantly different at $P \leq 0.05$ based on LSD.

above to below ground dry weight were not significant. These data clearly demonstrated that both supernodulating mutants and Sinpaldalkong 2 maintained the similar balance between above ground and below ground parts regardless of significant differences in partitioning of dry matter into root and nodule between supernodulating mutants and Sinpaldalkong 2.

A general model for the autoregulation of nodulation was explained by the interaction between root- and shoot-derived compounds (Gresshoff and Delves, 1986). In wild-type plants, root-derived compound is produced after the root is infected by nitrogen-fixing bacteria. The root-derived compound is translocated to the shoot, and stimulates the production of signal in the shoot. Then the shoot-derived signal moves to the root, and inhibits the nodulation especially in the younger root regions. The absence of a shoot-derived signal inhibiting nodulation in supernodulating soybean mutants was surmised from the shoot control of supernodulation characters in reciprocal grafting experiments of supernodulating nts mutant and its wild type (Delves et al., 1986). This was partially evidenced from the delayed supernodulation of mutant injected with plant extracts from Bragg (Gresshoff et al., 1988).

Based on the present grafting study which confirmed shoot control of supernodulating charcters in SS2-2 mutant as was shown in nts and En6500 mutants, our mutant SS2-2 may have a similar nodulation mechanism as nts and En6500. This suggests that our mutant SS2-2 also lacks the nodulation inhibiting factor derived from the shoot. Genetic studies are currently being conducted to further understand the regulation of supernodulation.

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