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Growth, Nitrogen Metabolism, and Nodulation of Hypernodulating Soybean Mutant Affected by Soil Fertility

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ABSTRACT : This study was performed to evaluate the growth and nodulation characters of hypernodulating soybean mutant, SS2-2, and to know the growth and yield performance of the mutant in infertile soil. Soil fertility was adjusted by mixing the different ratios of soil components including clay, river sand, and horticultural bed, which resulted in fertile and infertile soil. Dry weight, nitrogen concentration, and leaf nitrate reductase of each plant were measured around V6 stage (47 days after planting) and around R3 stage (82 days after planting). There were significant effects of soil fertility and soybean genotype on the total dry weights including root, nodule, stem, leaf, and pod dry weight at V6 and R3 stages. Total dry weight of hypernodulating mutant, SS2-2, was clearly less than that of its wild type, Sinpaldalkong 2. However, nodule development on the roots of SS2-2 was much greater than that of Sinpaldalkong 2, regardless of soil fertility. Though SS2-2 was smaller in plant size than Sinpaldalkong 2, genotypic difference in total nitrogen content was not significant at both V6 and R3 stages because SS2-2 fixed more nitrogen biologically than its wild type in the root nodule. The SS2-2 mutant showed lower plant yield in both infertile and fertile soil. The SS2-2 contained more crude seed protein than Sinpaldalkong 2, and was characterized with reduced top and root growth.

Keywords : soybean, hypernodulation, soil fertility, nitrogen concentration, nitrate reductase.

Legume plants have two main sources of nitrogen including combined nitrate or ammonia nitrogen and fixed nitrogen from the atmosphere. Much emphasis has been placed on the utilization of nitrogen derived from biological nitrogen fixation in the root nodule.

Supernodulating soybean mutant, isolated from ethyl methanesulfonate (EMS) mutagenesis, fixed more nitrogen in the root nodule than normal nodulating cultivars (Eskew *et al.*, 1992; Hansen *et al.*, 1989; 1990). In addition, supernodulating soybean mutants have their greatest proficiency in nodulation regardless of soil nitrate concentration. Generally, the lower the soil nitrate level, the greater the proportion of N derived from biological N_2 fixation in normal nodulating soybean plant (Eskew *et al.*, 1989). Under high nitrate levels, normal nodulating cultivars showed decreased nodule production, whereas supernodulating or hypernodulating mutants continued nodule formation and N_2 fixation (Hansen *et al.*, 1989).

Supernodulation in soybean actually appears to retard plant growth 30 days after planting due to increased energy requirements needed to support the growth and maintenance of additional nodules (Lee *et al.*, 1991; Eskew *et al.*, 1989). Factors that cause this phenomenon are unknown, but due to increased nodulation, less energy is available for plant growth (Lee *et al.*, 1991). Day *et al.* (1986, 1987) concluded that higher respiration rate by the root and nodule may reflect on the great energy cost of nodulation and N_2 fixation activity. The higher N_2 fixation capacity and reduced plant size results in a higher plant N concentration, particularly in the leaves and nodules (Hansen *et al.*, 1989; Herridge and Rose, 1994). The increased N concentration may result in a higher seed protein content, which may also be the reason for increased vigor of supernodulating plants at the early stages of growth (Day *et al.*, 1986,1987; Hansen *et al.*, 1989; Herridge and Rose, 1994).

Hypernodulating soybean mutant, SS2-2, was isolated from M_2 families of Sinpaldalkong 2 mutagenized with 30 mM EMS (Lee *et al.*, 1997). This mutant was re-evaluated for its nodulation characters and renamed as hypernodulating rather than supernodulating, as this mutant showed greater nodulation than normal nodulating soybean and less than supernodulating soybean. Response of this hypernodulating mutant to NO_3^- concentration in a green house study revealed that nodulation of SS2-2 even in the presence of high exogenous nitrate was not inhibited when compared to that of wild type soybean genotype, Sinpaldalkong 2 (Lee *et al.*, 1998).

In this study, hypernodulating soybean mutant, SS2-2 was compared with its wild type Sinpaldalkong 2 in growth, nitrogen metabolism, and nodulation characters in response to soil fertility.

MATERIALS AND METHODS

Two soybean genotypes, Sinpaldalkong 2 and SS2-2, were

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used for this experiment. Five seeds per pot were sown on May 28, 1999 in the PVC pipe pot, which was 20 cm in diameter and 60 cm in height. Pots were filled with clay, river sand, and horicultural bed (Baroku, Seoul Agricultural Materials Co., Ltd.), in which soil fertility was adjusted by changing the ratio of these soil components. Fertile soil was made by increasing the proportion of horticultural bed (clay : river sand : horticultural bed=1:1:1), whereas infertile soil was made by increasing the proportion of clay and river sand (clay : river sand:horticultural=6:3:1). In addition, the fertilizer was applied in fertile soil treatment at a ratio of 4-7-6 kg/10a (N-P_2O_5-K_2O), whereas it was not applied in infertile soil treatment. The 2 × 2 factorial treatment combinations of two soybean genotypes and two soil fertility levels were laid out in a completely randomized design with four replications. All plants were thinned to be two healthy seedlings per pot 20 days after planting.

Dry weights of root, shoot, and leaf, and pod of each plant were measured around V6 stage (47 days after planting) and around R3 stage (82 days after planting). At the same time, nitrogen concentration was measured by the macro-Kjeldhal method (Kjeltec Auto Sampler System 1035 Analyzer, Tecator AB, Sweden). Crude seed protein concentration was calculated by multiplying nitrogen content by 6.25. Yield and yield components were also measured at harvest.

Leaf nitrate reductase activity was estimated by measuring the amount of nitrite being produced from nitrate. Fully expanded leaves from the top at the around V6 and R3 stage were cut into pieces (squares of about 5 mm^2). Approximately 0.2 g of the leaves was put into 5 ml of incubation mixture containing 0.1M potassium phosphate buffer, pH 7.7, 0.1M KNO_3, and 1%(v/v) isopropanol. After the samples were incubated in the dark for 30 min, the nitrite being produced from nitrate were reacted with 0.02%(w/v) N-(1-naphtyl)ethylenediamine dichloride. The resulting complex was pink in color, and absorbance was measured at 540 nm[$E^{1\,mM}_{1\,cm}$ nitrite complex (540 nm)=55].

RESULTS AND DISCUSSION

Nodulation characters were compared between hypernodulation soybean mutant, SS2-2, and its wild type, Sinpaldalkong 2. There were significant effects of soybean genotype on the nodule dry weight and number per plant (Table 1). Nodules on the root system of Sinpaldalkong 2 were largely confined to the upper portion of the root system, whereas those of SS2-2 were to the entire root system. Nodule development on the roots of SS2-2 was much more prolific than that of Sinpaldalkong 2. Both nodule number and nodule mass of the mutant were several-fold higher than those of the wild type (Fig. 1).

Table 1. Analysis of variance for nodulation characters as affected by soil fertility and soybean genotype.

Source of variation	df	Mean squares	
		Nodule dry wt	Nodule number
		47 days after planting	
Soil fertility(S)	1	$0.1^{ns\dagger}$	101921*
Soybean genotypes(G)	1	1.0**	349577**
S × G	1	0.1^{ns}	2627^{ns}
Error	12	0.1	13725
		82 days after planting	
Soil fertility(S)	1	0.6^{ns}	20093^{ns}
Soybean genotypes(G)	1	62.6**	6263758*
S × G	1	1.4^{ns}	31595^{ns}
Error	12	2.1	911946

†ns, * and **: Not significant, significant at 0.05 and 0.01 levels, respectively.

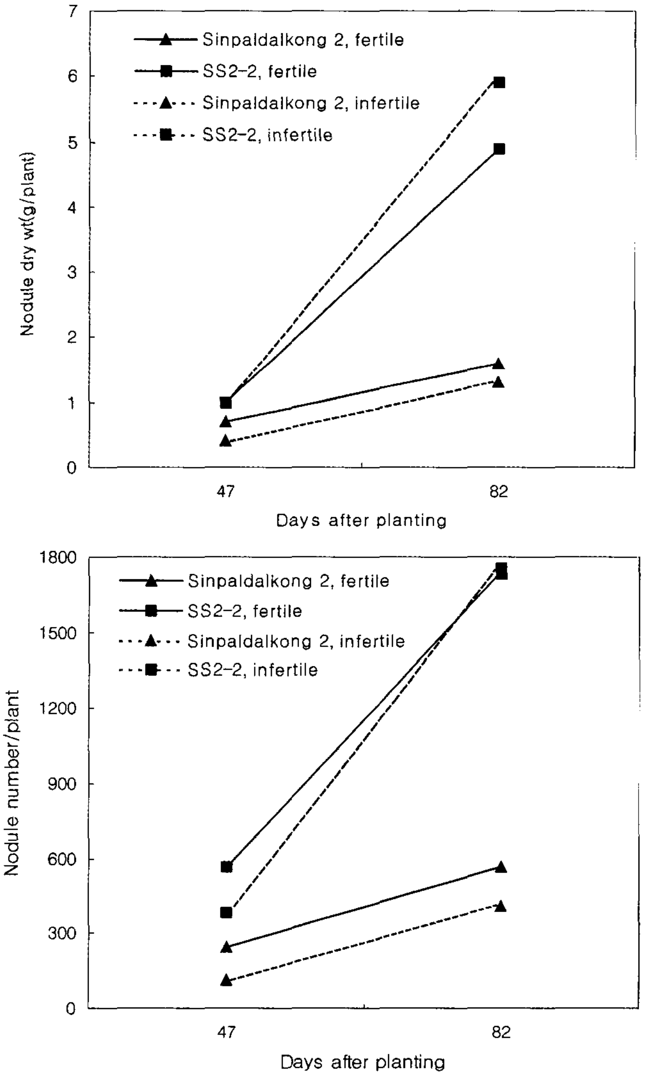

Fig. 1. Nodulation characters of Sinpaldalkong 2 and SS2-2 in response to soil fertility.

Table 2. Genotypic difference in dry weight, nitrogen concentration, and total nitrogen content of each plant part as affected by soil fertility.

Plant part	Soil fertility	Dry weight (g)		Mean	N concentration (mg/g)		Mean	Total N content (g/plant)		Mean
		Sinpaldal-kong	2 SS2-2		Sinpaldal-kong 2	SS2-2		Sinpaldal-kong 2	SS2-2	
					---------------47 days after planting---------------					
Leaf	Infertile	2.5	2.3	2.4^b	38.9	46.7	42.8^a	97.5	109.2	103.4^b
	Fertile	5.6	3.9	4.8^a	39.6	49.3	44.5^a	219.1	189.7	204.4^a
	Mean	4.1^a	3.1^b		39.3^b	48.0^a		158.3^a	149.5^a	
Stem	Infertile	1.7	1.2	1.5^b	12.7	18.0	15.4^a	22.0	22.4	22.2^b
	Fertile	4.6	2.6	3.6^a	14.7	20.3	17.5^a	66.4	53.2	59.8^a
	Mean	3.2^a	1.9^b		13.7^b	19.2^a		44.2^a	37.8^a	
Root	Infertile	1.7	1.0	1.4^b	14.3	15.2	14.8^a	24.0	14.4	19.2^b
	Fertile	3.5	1.4	2.5^a	13.7	13.7	13.7^b	48.2	19.2	33.7^a
	Mean	2.6^a	1.2^b		14.0^a	14.5^a		36.1^a	16.8^b	
Nodule	Infertile	0.4	1.0	0.7^a	56.7	48.1	52.4^a	21.2	48.8	35.0^a
	Fertile	0.7	1.0	0.9^a	54.2	47.4	50.8^a	37.1	48.7	42.9^a
	Mean	0.6^b	1.0^a		55.5^a	47.8^b		29.2^b	48.8^a	
Total	Infertile	6.3	5.5	5.9^b	122.7	127.9	125.3^a	164.8	194.9	179.9^b
	Fertile	14.4	8.9	11.7^a	122.2	130.8	126.5^a	370.8	310.7	340.8^a
	Mean	10.4^a	7.2^b		122.5^b	129.4^a		267.8^a	252.8^a	
					---------------82 days after planting---------------					
Leaf	Infertile	13.0	7.8	10.4^b	30.6	34.0	32.3^b	394.3	262.7	328.5^b
	Fertile	21.2	15.3	18.3^a	34.5	36.4	35.5^a	729.4	555.9	642.7^a
	Mean	17.1^a	11.6^b		32.6^b	35.2^a		561.9^a	409.3^b	
Stem	Infertile	18.7	9.7	14.2^b	10.9	13.0	12.0^b	202.9	123.7	163.3^b
	Fertile	31.5	18.9	25.2^a	12.4	20.2	16.3^a	388.8	379.1	384.0^a
	Mean	25.1^a	14.3^b		11.7^b	16.6^a		295.9^a	251.4^a	
Root	Infertile	9.1	4.3	6.7^b	13.9	10.2	12.1^a	125.3	43.5	84.4^a
	Fertile	12.8	6.1	9.5^a	13.2	11.7	12.5^a	172.1	72.8	122.5^a
	Mean	11.0^a	5.2^b		13.6^a	11.0^b		148.7^a	58.5^b	
Nodule	Infertile	1.3	5.9	3.6^a	44.9	42.7	43.8^b	59.5	250.7	155.1^a
	Fertile	1.6	4.9	3.3^a	47.3	48.9	48.1^a	73.7	241.2	157.5^a
	Mean	1.5^b	5.4^a		46.1^a	45.8^a		66.6^b	246.0^a	
Total	Infertile	45.4	31.2	38.3^b	100.2	99.8	100.0^b	884.0	794.8	839.4^b
	Fertile	77.2	51.7	64.5^a	107.3	117.1	112.2^a	1711.2	1491.9	1,601.6^a
	Mean	61.3^a	41.5^b		103.8^a	108.5^a		1,297.6^a	1,143.4^a	

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

Generally, exogenous supply of high nitrate levels suppress the symbiosis, as shown by decreased nodule mass as well as decreased nitrogenase activity per unit of nodule mass. However, nodule number in fertile soil condition was greater than that in infertile soil at 47 days after planting regardless of soybean genotypes. This may be due to the fact that at least small amount of nitrate is needed to stimulate nodulation at an early stage as a starter-nitrogen for nodulation. Nitrate, which is the primary source of nitrogen available from the soil, may be too deficient in infertile soil treated in this study. Nitrogen fixation is normally initiated in the root system of soybean plants 20 to 30 days after planting (Hardy *et al.* 1971). Thus, nitrogen demand at a seedling stage must be met through utilization of nitrogen from the seed as well as nitrogen from the soil.

Significant effect of soybean genotypes was present on the dry weight of each plant part (Table 2). In spite of greater nodulation, SS2-2 was characterized by smaller leaf, shoot, root, and total dry weight than its wild type, Sinpaldalkong 2 (Table 2). This was not in a good agreement with other study by Eskew *et al.* (1989) and Maloney *et al.* (1997) that biomass yield at R3 and R7 growth stages were not different between supernodulating and normal nodulating genotypes. On the other hand, Day *et al.* (1986) found that growth of a supernodulating mutant was greater than its wild type soybean plant up to 13 days after planting. After nodulation occurred, they also reported greater growth of the wild type soybean genotype than supernodulating soybean mutant.

There were significant effects of soil fertility on the leaf, shoot, root, and total dry weight per plant (Table 2). At 82 days after planting, there were no interaction effects between soil fertility and soybean genotype on the dry weight of each plant part. Total dry weight grown under fertile soil was greater than that under infertile soil. Of specific interest was the smaller difference in total dry weight between SS2-2 and Sinpaldalkong 2 when grown in infertile soil, but the greater difference in fertile soil, suggesting that hypernodulating soybean mutant could be a unique biological material that might be useful for growing in infertile soil.

Due to the greater nodulation, hypernodulating soybean mutant, SS2-2 can fix more nitrogen in the root nodule than Sinpaldalkong 2. Higher nitrogen concentration was observed in leaf and shoot of hypernodulating soybean mutant, whereas lower or equal nitrogen concentration was in nodule and root (Table 2). It indicates that nitrogen fixed in the nodule is translocated to the top part in hypernodulating soybean mutant. When compared to SS2-2, Sinpaldalkong 2 showed greater nitrogen content in leaf, shoot, and root, while SS2-2 contained greater nitrogen content in nodule. Although greater total dry weight was shown in Sinpaldalkong 2 than SS2-2, SS2-2 had similar total plant nitrogen content to Sinpaldalkong 2. This is clearly due to the fact that SS2-2 with larger number of nodules fixed more nitrogen than Sinpaldalkong 2.

The level of leaf nitrate reductase activity is generally in parallel with the concentration of nitrate in the leaf tissue over the entire growing season. A precipitous drop in both parameters was noted within 2-3 weeks after flowering (Harper *et al.*, 1972), which was also consistent with our study. The content of nitrate in the youngest fully expanded trifoliolate leaves at 45 days after planting were relatively higher than that at 82 days after planting. As the plants approached the reproductive stage, a rapid decline in the content of nitrate was noted. Higher leaf nitrate reductase activity was observed in fertile soil, whereas lower leaf nitrate reductase activity was in infertile soil. Harper *et al.* (1972) also reported the similar results that plants grown on the higher nutrient treatment had significantly higher reductase activity than the plants on the lower nutrient treatment.

Table 3. The activity of leaf nitrate reductase in uppermost fully expanded leaves of Sinpaldalkong 2 and SS2-2.

Soil fertility	Soybean genotypes		Mean
	Sinpaldalkong 2	SS2-2	
	---μmol NO2$^-$ hr$^-$1gFW$^-$1 ----		
	47 days after planting		
Infertile	1.4	1.3	1.4^{b†}
Fertile	4.1	11.9	8.0^{a}
Mean	2.8^{b}	6.6^{a}	
	82 days after planting		
Infertile	0.6	0.4	0.5^{b}
Fertile	1.0	0.6	0.8^{a}
Mean	0.8^{a}	0.5^{b}	

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

Table 4. Yield and yield components of Sinpaldalkong 2 and SS2-2 as affected by soil fertility.

Genotype	Soil fertility		Mean
	Infertile	Fertile	
	---------- Plant height (cm) ----------		
Sinpaldalkong 2	47	55	51^{a†}
SS2-2	35	47	41^{b}
Mean	41^{b}	51^{a}	
	--------- Pod number/plant ----------		
Sinpaldalkong 2	75	101	88^{a}
SS2-2	47	67	57^{b}
Mean	61^{b}	84^{a}	
	--------- Seed number/plant ----------		
Sinpaldalkong 2	115	169	142^{a}
SS2-2	73	100	87^{b}
Mean	94^{b}	135^{a}	
	--------- Plant seed yield (g) ----------		
Sinpaldalkong 2	21	33	27^{a}
SS2-2	15	23	19^{b}
Mean	18^{b}	28^{a}	

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

Regardless of soybean genotypes, higher soil fertility resulted in increased plant height, pod and seed number per plant, and plant yield (Table 4), which was against the hypothesis that hypernodulating soybean mutant can maintain its growth and yield using biologically fixed nitrogen in the root nodule even in the absence of soil nitrogen. Lower yield of hypernodulating mutant in infertile soil can be partially explained from the fact that infertile soil in this experiment may have also low level of other soil nutrient components as well as nitrogen.

There were significant effects of soybean genotypes on

Table 5. Seed protein content of Sinpaldalkong 2 and SS2-2 as affected by soil fertility.

Genotype	Soil fertility		Mean
	Infertile	Fertile	
	-------------- mgN/g --------------		
Sinpaldalkong 2	371	373	372[b†]
SS2-2	396	386	391[a]
Mean	384[a]	380[a]	

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

the plant height, pod and seed number per plant, and plant yield. Average plant yield of the hypernodulating genotype over two soil fertility levels was 30% lower than that of Sinpaldalkong 2. The ability of the hypernodulating soybean mutant to develop more root nodules and to have greater N_2 fixation did not result in increased grain yields compared with its normal nodulating parent. Our result was similar to those reported by Pracht *et al.* (1994), in which grain yield of the wild-type soybean plant was significantly higher than that of supernodulating mutant. From this result, it could be concluded that hypernodulating soybean mutant needed more leaf photoassimilates from the top for nodule growth and maintenance. This caused the reduced yield of hypernodulating soybean mutant when compared to the wild type. On the other hand, SS2-2 was significantly greater in seed protein concentration than Sinpaldalkong 2, regardless of soil fertility (Table 5).

The results from this study provide an evidence for greater nodulation of hypernodulating soybean mutant, when compared to its wild type. The data also indicate that hypernodulating soybean mutant was characterized with smaller plant growth and yield as well as similar total plant nitrogen content to wild type soybean. Further studies should be needed to characterize the agronomic use of this mutant in infertile soil.

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