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Relationship between Nodulating Characters and Yield Components in Supernodulating Soybean Mutants

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ABSTRACT : This experiment was conducted to clarify the functions of supernodulating characters on seed yield determination through the comparison of agricultural traits of supernodulating soybean mutants, Sakukei4, SS2-2, and their parent cultivars, Enrei and Shinpaldalkong2. The plant dry weights of supernodulating mutants, Sakukei4 and SS2-2, were 52% and 61% of their wild type parents at full seed stage (R6). However, the relative growth rate (RGR) from the pod set stage (R3) to R6 of Sakukei4 was 0.022 g/g/day and that of SS2-2 was 0.016 g/g/day, which were higher than those of their parents. Nodule number and dry weight were increased in two supernodulating mutants by the R6 stage. The nitrogen concentrations of leaf, petiole and stem of Sakukei4 were higher than those of Enrei. SS2-2 showed higher nitrogen concentration in petiole than Shinpaldalkong2 had. The positive correlations were appeared between nodule dry weight, plant dry weight and pod number, in two supernodulating mutants during the period from R3 to R6 stage. Although all of the yield components and seed yield were lower in two supernodulating mutants than their parents at the stage of full maturity (R8), the harvest index was higher in supernodulating mutants. The increasing rates of pod number to stem dry weight in two supernodulating mutants showed the higher than those of two their parents at R8 stage. In conclusion, the relative growth rates during the early to the middle reproductive growth period were higher in supernodulating mutants than the wild types. This could be resulted in an increase in pod number. The increase of relative growth rate was the result of the successive supplement of nitrogen source from biological nitrogen fixation (BNF) of nodules during the middle reproductive growth period in supernodulating mutants.

***Keywords*:** supernodulating soybean mutant, biological nitrogen fixation, nodules, seed yield, harvest index, relative growth rate

Soybean, like other legumes, has the ability of atmospheric nitrogen fixation into ammonium nitrogen that can be used by the plant. Therefore, biological nitrogen fixation (BNF) by soybean has been re-evaluated as an important component of nitrogen re-cycling in environment friendly agricultural system among the advanced countries (Alves *et al.*, 2003; Bhatia *et al.*, 2001; Jensen *et al.*, 2003; Song *et al.*, 1995). The main constraint to utilization of BNF was the inhibition of nodulation and nitrogen fixation in the presence of soil nitrate (Harper, 1987; Streeter, 1988). To solve this problem, a soybean mutant that nodulated in the presence of high nitrate concentration was first isolated by Caroll *et al.* (1985) from cultivar 'Bragg' by the ethyl methanesulfate (EMS) treatment. Thereafter, several hypernodulating mutants were isolated by the several reseach groups and Bhatia *et al.* (2001) reviewed these mutants. In Korea, Lee *et al.* (1997) isolated a supernodulating mutant, SS2-2, from M_2 families of Shinpaldalkong2 which was mutagenized with 30 mM EMS. Although supernodulating mutants have an advantage of a high ability of BNF than wild type, they showed low productivity what was a weak point as an cultivar in terms of seed production. In addition, an excessive nodulation has been attributed to physiological growth inhibition (Day *et al.*, 1986; Ha & Lee, 2001). Copeland & Romanov (1995) was described that as much as 50% of the daily photosynthetic product may be consumed by nodules during the peak nitrogen fixing period. Zhao *et al* (1998) suggested that a backcrossed intermediate supernodulating line has fixed more atmospheric nitrogen and yielded as much as wild type. In our previous study, supernodulating mutants, SS2-2 and Sakukei4, which was developed from En6500 (Takahashi, unpublished data), revealed low vegetative growth and seed yield, but showed higher harvest index compared with their parent cultivars. Also, nodule number and weight per plant was extended at the stage of full seed (R6) in two supernodulating mutants. Therefore, we thought that the extended nodule life time to middle reproductive growth period in supernodulating mutant was an distinct physiological feature in relation to seed yield production. This experiment was conducted to clarify the functions of the supernodulating characters in seed yield determination by the comparison of agricultural traits of supernodulating

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soybean mutants with those of their parent cultivars.

MATERIALS AND METHODS

This experiment was carried out at the field of National Institute of Crop Science in Suwon province in 2004. Two supernodulating soybean mutants, Sakukei4 and SS2-2, and their parent cultivar, Enrei and Shinpaldalkong2, were tested. The chemical properties of soil in experimental plot showed the Table 1. Plantings were done at May 24 and June 15 and planting density was 60 × 15 cm with 2 seeds per hole. Plot size was 4 m in length with 5 rows. Fertilizer, N : P_2O_5 : K_2O = 3 : 3 : 3.4 (kg/10a) was incorporated in the soil before planting as basal dressing. Experimental design was randomized complete block arrangement with three replications. During the growth period, ten soybean plants per plot were sampled at the growth stages of pod set (R3) and full seed (R6). Each growth stage was identified by the method proposed by Fehr & Caviness (1977). Whole plant included under ground parts, root and nodule were scooped by spade. Root and nodule were washed with tap water by hand. The lower part of cotyledonal node was regarded as under ground part. To examine the growth characteristics and dry weight, the plant sample was separated into leaf, petiole, stem, pod, root, and nodule, and the each part was dried at 75 °C for 48 hours. The dried samples were milled and carbon and nitrogen concentration of each part were measured by CNS analyzer. Nodule activity was measured by nodule number and dry weight per plant. The rates of photosynthesis and transpiration were measured in 5 plants per plot in the morning time at the light intensity about 2,000 μmol/m^2/s at September 8 and 24 by LCA-4, ADC BioScientific Ltd. UK. At the growth stage of full maturity (R8), soybean plants from 2 m row length × 2 rows of each plot were harvested for determining seed yield and yield components. The collected data were subjected to statistical analyses using SAS package for ANOVA and GLM.

RESULTS AND DISCUSSION

Changes in biomass and growth rate

The changes of plant dry weight and nodule characteristics during the middle reproductive growth period in supernodulating soybean mutants and their parent cultivars are shown in Table 2. Plant dry weights of supernodulating mutants, Sakukei4 and SS2-2, at full seed stage (R6) were 30.8 g/plant and 17.8 g/plant and their parents, Enrei and Shinpaldalkong2, were 58.7 g/plant and 29.3 g/plant. Thus, the plant dry weight of supernodulating mutants, Sakukri4 and SS2-2 were 52% and 61% of their parent, respectively. This meaned that the dry matter accumulation in vegetative organs were markedly lower in supernodulating mutants compared with their parents by the middle reproductive

Table 1. Chemical properties of experimental soil before planting

pH	Organic matter	Total nitrogen	P_2O_5	K	Ca	Mg	Na
(1:5)	(%)	(%)	(ppm)	(cmol$^+$/kg)			
7.1	2 99	0.158	247	1.2	6.5	2.1	0 4

Table 2. Changes of plant dry weight, relative growth rate and nodule characteristics from pod set stage (R3) to full seed stage (R6) stage of supernodulating mutants and the parent soybeans

Variety	Growth stage	Plant† dry weight	Relative growth rate (RGR)	Nodule number		Nodule dry weight	
		(g/plant)	(g/g/day)	(no /plant)	(ratio)‡	(g/plant)	(ratio)
Sakukei4	Pod set (R3)	16.0		1680		1 64	
	Full seed(R6)	30.8	0.022	1833	1.09	1.97	1 21
Enrei	Pod set (R3)	33.1		151		0 60	
	Full seed(R6)	58.7	0.020	110	0.73	0 59	0 98
SS2-2	Pod set (R3)	10.2		929		1.10	
	Full seed(R6)	17.8	0 016	1195	1.29	1.27	1.15
Shinpaldal2	Pod set (R3)	19.3		102		0 27	
	Full seed(R6)	29.3	0.011	81	0 79	0 25	0.92
$LSD_{0.05}$		8 83		339.6		0.313	

†Plant dry weight = stem + leaf weight
‡Ratio of R6 plot to R3 plot

growth period. This was a consistent result with other researches, which reported the supernodulating mutants shower lower in dry matter accumulation (Ha & Lee, 2001).

However, the relative growth rates (RGR) during the stage of pod set (R3) to full seed (R6) in two supernodulating mutants were 0.022 g/g/day in Sakukei4 and 0.016 g/g/day in SS2-2. These are higher than those of their parents. Therefore, we found that although dry matter was higher in wild types, the dry matter accumulation rate was higher in supernodulating mutants during the early to middle reproductive growth period. This is a critical factor in determination of seed yield in soybean. Many researches of seed yield determination in soybean have revealed that the growing of vegetative growth and crop growth rate during the early reproductive growth period were important factor in determination of pod and seed number per unit area (Board & Tan, 1995; Egli & Yu, 1991; Jiang & Egli, 1995; Park *et al.*, 2000; Seong, 2002).

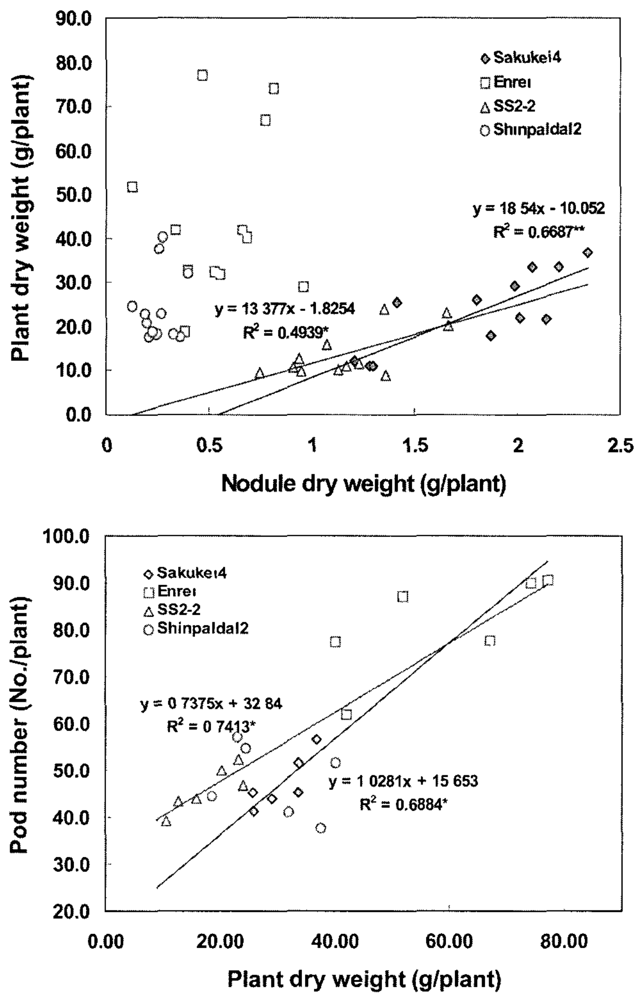

Fig. 1. Relationships between nodule dry weight, plant dry weight and pod number, respectively, at pod set stage (R3) and full seed stage (R6) stage in supernodulating mutants and the parent soybeans

The nodule activity, nodule number and dry weight were increased from R3 stage to R6 stage in two supernodulating mutants. However, the wild type parents of mutants showed a decrease in nodule activity, nodule number and dry weight. Therefore, we identified that an expanding of nodule life to R6 stage in supernodulating mutants was a distinct nodules characteristics compared with the wild types.

The relationships between nodule dry weight, plant dry weight and pod number at R3 and R6 stage were shown in Fig. 1. Two supernodulating mutants showed a positive correlations between nodule dry weight, plant dry weight and pod number. On the other hand, these were no correlations between these characteristics in their parents. Assuming that an expand of nodule life to R6 stage might be related to dry matter accumulation rate, it could effect to pod and seed development during the middle reproductive growth period in supernodulating mutants.

Changes in photosynthetic ability

The changes in photosynthetic rate after R6 stage are presented in Fig. 2. By the R6 stage, photosynthetic rates were not showen the significant differences in all cultivers. Thus, we thought that these similar abilities of the CO_2 assimilation rates in all cultivars by the R6 stage did not affect to the changes of the relative growth rates (Table 2). And these similar abilities of CO_2 assimilation rates between supernodulating mutants and their parents could be explained the reasons of low dry matter accumulations of supernodulating mutants by the unusage CO_2 assimilates to making dry matter and the transportation of CO_2 assimilates to excessive nodule more compared with those of their wild types.

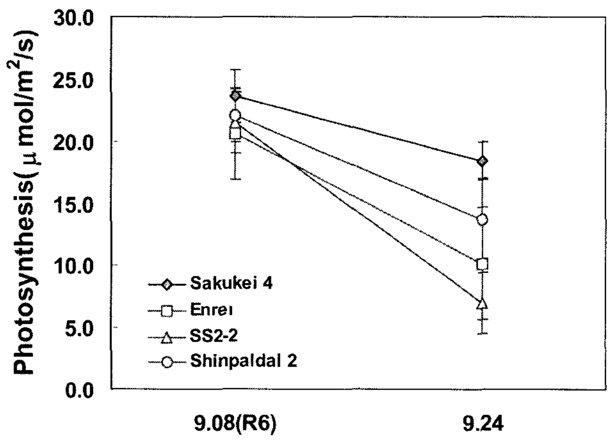

Fig. 2. Changes of photosynthetic rate at late reproductive growth stage in supernodulating mutants and the parent soybeans.

Table 3. Comparisons of percentages of carbon and nitrogen in each vegetative plant part at full seed stage (R6) in supernodulating mutants and the parent soybeans.

Source	Variety	Leaf	Petiole	Stem
		(%)		
Carbon(C)	Sakukei4	50.0	45.8	48.3
	Enrei	49.6	45.8	48.1
	SS2-2	50.6	46.0	49.2
	Shinpaldal2	49.9	45.0	47.9
	$LSD_{0.05}$	ns	ns	0.96
Nitrogen(N)	Sakukei4	5.1	2.4	2.3
	Enrei	4.4	1.3	1.9
	SS2-2	4.4	2.2	1.7
	Shinpaldal2	4.3	1.4	1.7
	$LSD_{0.05}$	0.15	0.31	0.37

Table 4. Comparisons of yield characteristics and harvest index at full maturity stage (R8) in supernodulating mutants and the parent soybeans.

Variety	Pod number	Seed number	Seed dry weight (A)	Plant dry[†] weight (B)	Harvest index (A/A+B)	Yield	
	(no./plant)		(g/plant)			(kg/10a)	(%)
Sakukei4	51	84	25.9	20.5	0.56	289	83
Enrei	84	150	41.6	38.1	0.53	350	
SS2-2	48	101	18.5	11.3	0.63	295	88
Shinpaldal2	47	110	21.0	16.4	0.57	335	
$LSD_{0.05}$	10.0	18.3	6.72	3.99	0.036	24.6	

[†]Plant dry weight = stem + pod weight

Distributions of carbon and nitrogen

The distributions of carbon and nitrogen source in each vegetative part were presented in Table 3, which are shown the concentrations of carbon and nitrogen in leaf, petiole and stem at R6 stage. Carbon concentrations of each part were not significantly different in all cultivars. However, nitrogen concentration was different. Nitrogen concentrations of all parts were higher in Sakukei4 than Enrei. In the case of SS2-2, nitrogen concentration was higher in petiole than that of Shinpaldalkong2. Higher nitrogen concentration in petiole was caused by high ureides (data not shown), which was a transport form of nitrogen compounds produced from BNF of nodules in soybean. Herridge & Peoples (1990) have been published from their several research papers that the measurement of BNF was conducted by determination of relative ureides in petiole and xylem sap. Therefore, higher nitrogen concentrations of petioles in two supernodulating mutants could be meaned that nitrogen source was supplied from nodules to plant organs by R6 stage successively, and this corresponded with the delay of nodule number and dry weight (Table 2). Based on this result, we believe that an expand in nodule life may be physiologically meaningful. Expand in nodules life allows longer supplies of nitrogen source from nodules to vegetative organs until the middle reproductive periods.

Yield characteristics and harvest index

At the stages of full maturity stage (R8), seed yield characteristics and harvest index (HI) are presented in Table 4. All of the yield components were lower, however, the HI was higher in two supernodulating mutants than those of their parents. The increase of HI in supernodulating mutants was presented in Fig. 3. The percentages of seed weight and pod number of supernodulating mutants compared with those of their parents were higher than those of plant dry weights. This indicated that although seed yield was lower caused by the decrease of plant dry weight in supernodulating mutants compared with their parent, the seed yield in supernodulating mutants was compensated indirectly by the increase of pod number and relative growth rate (Table 2)

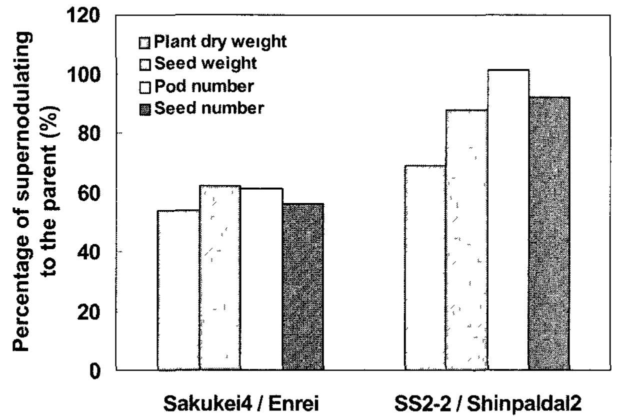

Fig. 3. Percentages of supernodulating mutants to the parent soybeans on yield characteristics at full maturity stage (R8).

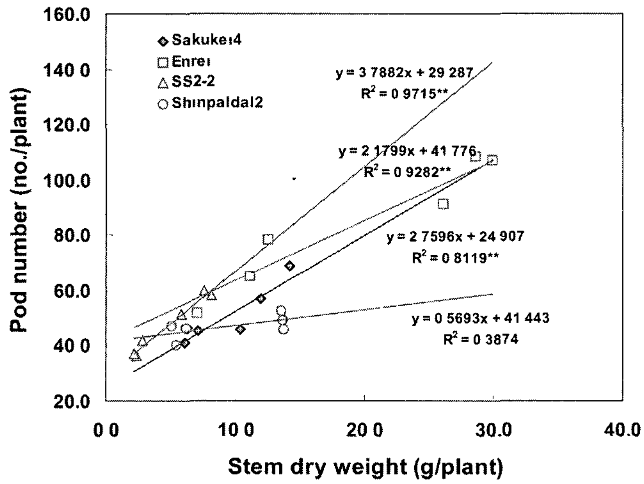

Fig. 4. Relationships between pod number and stem dry weight at full maturity stage (R8) in supernodulating mutants and the parent soybeans.

from the early to middle reproductive growth periods (Fig. 2). The relationship between stem dry weight and pod number at R8 stage in Fig. 4 are revealed that the increasing rates of pod number to stem dry weight were higher in two supernodulating mutants than their parents.

In conclusion, we are assumed that the relative growth rate (RGR) from the early to the middle reproductive growth period was higher in supernodulating mutants than the wild types and this could be related to increasing pod number. The increase of RGR could be resulted from the successive supplement of nitrogen source from BNF of nodule by the middle reproductive growth period in supernodulating mutants.

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