

## Growth and Maturity in Response to Planting Times in Supernodulating Soybean Mutants

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**ABSTRACT :** This experiment was conducted to investigate the changes of growth and maturity and to clarify the function of supernodulating characters, excessive nodules and high biological nitrogen fixation rate (BNF), on maturity in response to different planting time in supernodulating soybean mutants. Two supernodulating soybean mutants, Sakukei4 and SS2-2, and their parent cultivars, Enrei and Shinpaldalkong2, were planted on May 24 and June 15, 2004. The degrees of the shortening of growth days by the planting time delay were 18 to 22 days in four cultivar, and there were no significant differences among the cultivars. However, four cultivars showed the different maturity properties. Sakukei4, mutated from Enrei, showed later maturity than that of Enrei, and SS2-2, mutated from Shinpaldalkong2, showed earlier maturity than that of Shinpaldalkong2. The plant and nodule dry weights at R6 stage of Sakukei4 showed the smallest decrement and those of SS2-2 was showed the largest decrement by the delay of planting time. The photosynthetic rates of Sakukei4 during the late reproductive growth period were slowly decreased, however those of SS2-2 were steeply decreased in two planting time treatments. Overall, the growth of Sakukei4 was decreased slowly, however the growth of SS2-2 was decreased sharply according to the delay of planting time. The percentage of seed yield of Sakukei4 in June planting plot compared with May planting plot at R8 stage was 92%, which was the lowest decreasing rate of yield among the cultivars, and in the case of SS2-2, it was in 76%, the highest one. These results indicated that the responses of supernodulating mutants by the delay of planting time were very similar to the wild types. This means supernodulating characters in supernodulating soybean mutants might not affect to the maturity property. Additionally, the maturity property could be considered as an important characteristics to decide or to select on the developments of supernodulating soybean mutants, which have a low productivity by an excessive nodules, especially.

**Keywords:** supernodulating soybean mutant, biological nitrogen fixation, planting time, nodules, seed yield, maturity, photosynthetic rates

Soybean is a typical photoperiod sensitive crop. Therefore, the planting time is one of the important production components in soybean cultivation. In Korea, full season soybeans are planted in mid to late of May, and double cropped soybeans are planted in mid to late of June usually depend on cultivation regions. According to the studies about the effect of planting time to the yield, the planting time treatment caused the changes of yield characteristics and harvest index. Therefore, this treatment was used to as a method for clarification of the relationships between physiological characteristics relation to seed yield in soybeans (Park *et al.*, 2000; Soeng, 2002). In recent years, the values of biological nitrogen fixation (BNF) by legumes has been re-evaluated for the establishing environment friendly agricultural systems in an advanced countries. Many researches have focused on application of BNF in sustainable cropping systems (Alves *et al.*, 2003; Hardarson & Atkins, 2003; Jensen *et al.*, 2003; Kelly *et al.*, 2003), and the concerning about high BNF in supernodulating soybean mutants is also extending (Bhatia *et al.*, 2001; Song *et al.*, 1995). Supernodulating soybean mutant, which were characterized as nitrate tolerant symbiotic (nts) mutants, can produce up to 10 times more amount of nodules than that of wild types and showed increased in the rate of N<sub>2</sub> fixation (Zhao *et al.*, 1998). Several hypernodulating mutants were isolated by other research groups and Bhatia *et al.* (2001) reviewed about these mutants. In Korea, Lee *et al.* (1997) isolated a supernodulating mutant, SS2-2, from M<sub>2</sub> families of Shinpaldalkong2 that was mutagenized with 30mM EMS. In Japan, supernodulating mutant, En6500, was isolated from the wild type, Enrei, by treatment EMS (Akao & Kouchi, 1992). A backcrossed supernodulating mutant, Sakukei4, was developed from En6500 recently (Takahashi, unpublished data). This experiment was conducted to investigate the growth and yield characteristics of supernodulating soybean mutants, Sakukei4 and SS2-2, by treatments with planting times, and clarify the function of supernodulating characters, an excessive nodules setting and a high ability of BNF, in response to different photoperiodic environments.

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<Received January 13, 2004>

**MATERIALS AND METHODS**

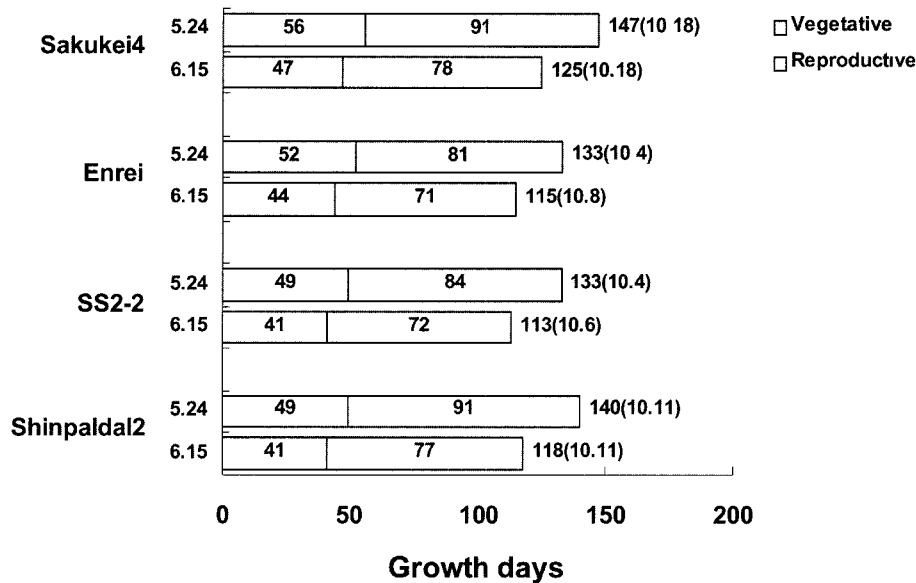
This experiment was carried out at the field of National Institute of Crop Science in Suwon in 2004. Two supernodulating soybean mutants, Sakukei4 and SS2-2, and their parent cultivars, Enrei and Shinpaldalkong2 were used. Planting dates were May 24 and June 15 and the planting density was 60 × 15 cm with 2 seeds per hole. Plot size was 4 m length with 5 rows. Fertilizer, N : P<sub>2</sub>O<sub>5</sub> : K<sub>2</sub>O = 3 : 3 : 3.4 (kg/10a) was incorporated in the soil before planting as basal dressing. Experimental design was split plot arrangement which main plot was planting times and split plot was cultivars with three replications. During the growth period, ten soybean plants per plot were sampled 2 times, one for pod set stage (R3) and the other for full seed stage (R6) stage samples. Each growth stage was identified by Fehr & Caviness (1977). Whole plant included underground parts, root and nodules was carefully scooped from soil. Root and nodules were washed by hand carefully. The cotyledonal node was cutted and the lower part of cotyledonal node was regarded as underground part. To examine the growth characteristics and dry weight, sample was separated into leaf, petiole, stem, pod, root, and nodules, and the each separated part was dried at 75 °C for 48 hours. Nodules activity was measured by nodule number and dry weight per plant. Photosynthesis and transpiration was measured by 5 plants per plot in the morning time at the light intensity about 2,000 μmol/m<sup>2</sup>/s on September 8 and 24 measuring LCA-4 (ADC BioScientific Ltd. UK). At the developmental stage of Full maturity (R8), soybean plants from 2 m row length × 2 rows

of each plot were harvested to determine seed yield and to analyze yield components. The collected data were analyzed using SAS package for ANOVA and GLM.

**RESULTS AND DISCUSSION**

**Changes in growth days**

The changes of growth days and the harvesting dates by the different planting times were shown in Fig. 1. The delay of planting time from May 24 to June 15 caused the growth days of all cultivars got shorten. The degrees of the shortening of growth days were 18 days to 22 days in four cultivars. And there were not appeared the significant differences on the degrees of the shortening of growth days among the cultivars. However, the growth days and the harvesting dates showed the differences among the cultivars. The growth days of Sakukei4, a supernodulating mutant, were 147 days in planting May 24 plot (May plot) and 125 days in planting June 15 plot (June plot), which were revealed the longest growth days among the cultivars. So, their harvesting dates were October 18, the latest harvesting date among the cultivars in two planting time treatments. Otherwise, SS2-2, the other supernodulating mutant, was revealed the shortest growth days and the earliest harvesting date among the cultivars. Interestingly, we found that two supernodulating mutant had the different maturity property and there were also the different maturity between supernodulating mutants and their wild types. Sakukei4, isolated from Enrei, was later maturity than Enrei, and SS2-2, isolated from Shin-



**Fig. 1.** Changes of growth days and harvesting dates by the planting time treatments in supernodulating mutants and the parent soybeans (The numeric values in the bar mean days required during the growth period and the numeric values in the parenthesis mean harvesting date).

paldalkong2, was earlier maturity than Shinpaldalkong2. Therefore, we thought that the different maturity was exist between wild types and the supernodulating mutants, this might be caused by the insertion of the other maturity factor from the other parent into the supernodulating mutant during the backcross breeding processes. And this maturity property was considered as an independent factor separated from the supernodulating characters.

### Changes in biomass accumulation

The delay of planting time caused decrease in plant and nodule dry weight at full seed stage (R6) in all cultivars (Table 1). However, the degrees of their decreasing by delay of planting time were different among the cultivars. On the Sakukei4, belong to late maturity among the four cultivars, the dry weight of June plot was shown 92% of May plot, and on the SS2-2, belong to early maturity among the four

cultivars, it was 58% revealed the marked decrease. Enrei and Shinpaldalkong2, considered as middle maturity among the four cultivars, were revealed the middle range in the decreasing degrees. The changes of nodule dry weight were also similar with the changes of the plant dry weight. Therefore, the similar decreasing patterns between nodule and plant dry weight by the delay of planting time in supernodulating mutants indicated that the supernodulating characters were influenced by the maturity property.

### Changes in photosynthetic rate and WUE

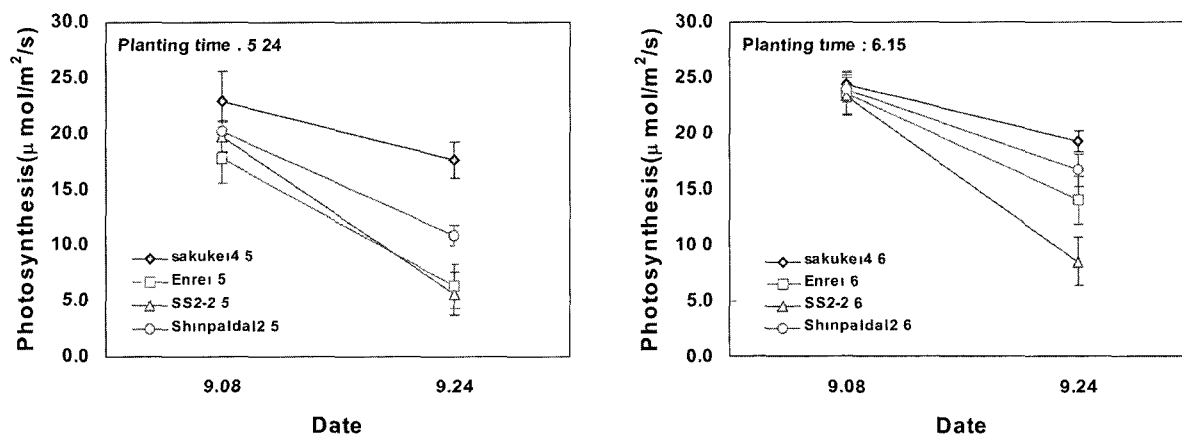
The changes of photosynthetic rate and water use efficiency (photosynthesis/transpiration) during the late reproductive growth period were shown similar results with above one (Fig. 2). In two planting time treatments, the photosynthetic rates of all cultivars were not different at the middle reproductive growth stages (September 8). However,

**Table 1.** Changes of nodule and plant dry weight at full seed stage (R6) by the planting time treatments in supernodulating mutants and the parent soybeans.

Variety	Planting time (Mon date)	Nodules dry weight		Plant dry weight <sup>†</sup>	
		(g/plant)	(%) <sup>‡</sup>	(g/plant)	(%)
Sakukei4	5.24	2.07		32.1	
	6.15	1.87	90	29.4	92
Enrei	5.24	0.69		72.7	
	6.15	0.49	72	44.6	61
SS2-2	5.24	1.56		22.4	
	6.15	0.98	63	13.1	58
Shinpaldal2	5.24	0.31		36.6	
	6.15	0.18	59	22.0	60

<sup>†</sup>Plant dry weight = stem + leaf weight

<sup>‡</sup>Percentages of nodules and plant dry weight in June planting plot to May planting plot



**Fig. 2.** Changes of photosynthetic rate following the full seed stage by the planting time treatments in supernodulating mutants and the parent soybeans

after this stage, the photosynthetic rates of Sakukei4 were slowly decreased, on the other hand, those of SS2-2 were

shown steeply decreased in two planting time treatments. These changes of photosynthetic rates at the late reproduc-

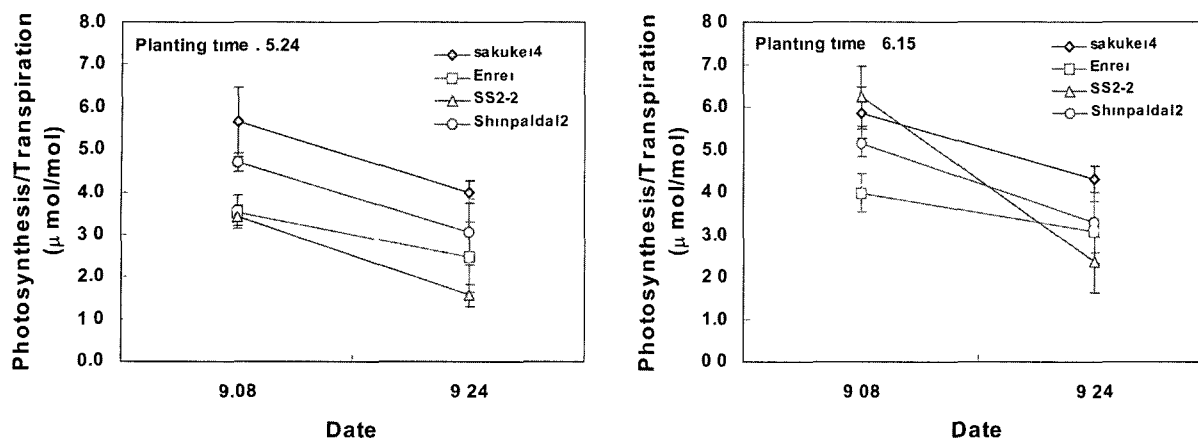


Fig. 3. Changes of water use efficiency following the full seed stage by the planting time treatments in supernodulating mutants and the parent soybeans

Table 2. Comparisons of yield characteristics at full maturity stage (R8) by the planting time treatments in supernodulating mutants and the parent soybeans

Variety	Planting time	Pod number		Seed number		Seed weight		Yield	
		(no /plant)	(%) <sup>†</sup>	(no /plant)	(%)	(g/plant)	(%)	(kg/10a)	(%)
Sakukei4	5.24	57		91		28.4		300	
	6.15	44	77	77	85	23.4	82	278	92
Enrei	5.24	102		181		49.3		379	
	6.15	65	64	118	65	33.9	69	328	86
SS2-2	5.24	56		121		22.9		337	
	6.15	39	68	81	67	14.0	61	256	76
Shinpaldal2	5.24	49		121		22.5		359	
	6.15	45	91	100	83	19.5	87	314	88
LSD <sub>0.05</sub>		14.1		25.9		9.51		34.8	

<sup>†</sup>Percentages of pod number, seed number, seed weight, and yield in June planting plot to May planting plot, respectively

Table 3. Comparisons of 100 seeds weight at full maturity stage (R8) by the planting time treatments in supernodulating mutants and the parent soybeans.

Variety	Planting time	100 seeds weight		
		(g)	LSD <sub>0.05</sub>	(%) <sup>†</sup>
Sakukei4	5.24	32.4		
	6.15	32.2	ns	99
Enrei	5.24	33.1		
	6.15	31.8	ns	96
SS2-2	5.24	22.1		
	6.15	20.3	0.94	92
Shinpaldal2	5.24	21.7		
	6.15	20.9	ns	96

<sup>†</sup>Percentage of 100 seeds weight in June planting plot to May planting plot

tive growth period were related with the senescence of vegetative organ, and were considered as influences by maturity property.

### Changes in yield components and yield

The changes of yield components and yield per unit area at full maturity stage (R8) were shown similar to the previous results (Table 2). The yield of June plot was 92% of May plot in Sakukei4, which was the highest percentage among the cultivars, and it was 76% in SS2-2, the lowest one. On the changes of 100 seeds weight, the similar results were shown that the 100 seeds weight of June plot was significantly decreased in SS2-2 (Table 3).

All the data of the growth and yield characteristics by the planting time treatment in supernodulating mutants were shown consistent results that the growth of Sakukei4 was decreased slowly and that of SS2-2 was decreased sharply according to delay of planting time. These results indicated that the responses of supernodulating mutants by the delay of planting time were very similar to the wild types. Therefore, we assumed that the supernodulating characters, an excessive nodules setting and a high ability of BNF, were not affected to maturity property, and these two characteristics were independent to each other. Also, the maturity property was considered as an important selection characteristics on the developments of supernodulating soybean mutants, which have a low productivity by an excessive nodules, especially.

### ACKNOWLEDGMENTS

This work was supported by postdoctoral fellowships course from Nation Institute of Crop Science, RDA. The author is grateful to staffs of National Institute of Crop Science of Japan, for their supply of soybean seeds, Sakukei4 and Enrei, and a professor Suk Ha, Lee of Seoul National University for their supply of soybean seeds, SS2-2.

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