

Symbiotic Effectiveness of *Bradyrhizobium japonicum* USDA 110 in Supernodulating Soybean Mutant SS2-2

Puji Lestari***, Kyujung Van*, Moon Young Kim*, and Suk-Ha Lee*†

*Department of Plant Science, Seoul National University, Seoul, 151-921, Korea

**Research Institute for Food Crops Biotechnology, Bogor, 16111, Indonesia

ABSTRACT : In the absence of exogenous nitrogen supply, evaluation of a symbiosis effectiveness of *Bradyrhizobium japonicum* USDA 110 in a supernodulating soybean mutant, SS2-2, its wild type, Sinpaldalkong 2, and control genotype, Jangyeobkong, was conducted in this study. Nodules in SS2-2 were initially white and similar to its wild type, Sinpaldalkong 2. At the late stage, the wild type nodules became dark pinkish by maturation, by contrast, mature nodules in SS2-2 remained light green to pinkish, indicating a lack of leghemoglobin. Tap root length was short in nodulated symbiotic SS2-2 than that of its wild type and the control genotype. Nodulated root length and nodule density on root length were significantly increased by *B. japonicum* inoculation, but no significant increase was observed on root length and percentage of nodulation to total root length. Regardless of *Bradyrhizobium* inoculation, SS2-2 showed higher nodule dry weight and higher acetylene reduction activity (ARA) when compared with its wild type and the control genotype. Inoculation of *B. japonicum* led the increase of ARA in 47 days after planting (DAP), in part because of nodule development. Supernodulating mutant, SS2-2, less responded to *B. japonicum* induction in terms of nitrogen fixation and nodulation characteristics than its wild type. Thus, interaction of supernodulating soybean mutant with *Bradyrhizobium* had less symbiotically associated response than normal nodulating soybean.

Keywords: *Bradyrhizobium japonicum*, nitrogen fixation, soybean, supernodulation

Symbiotic N₂-fixation is a complex physiological process influenced by the interaction of genetic elements between higher plants and rhizobia. The development of N₂-fixing nodules on legume roots upon invasion of *Rhizobium* is subject to regulation by factors both external and internal to the plant host (Delves *et al.*, 1986). Interruption of invasion is related to the effectiveness of the host-*Rhizobium* association (Carroll *et al.*, 1985) as well as to other internal factors (Pierce & Bauer, 1983). In the absence of externally

supplied nitrogen, nodulation is tightly regulated with the number of infections greatly exceeding the final number of mature nodules (Bauer, 1981). The formation of effective nodules in soybean with compatible rhizobia leads to fixation of atmospheric nitrogen.

Genetic variation for N₂-fixation ability has been reported involving both soybean and *Rhizobium* components of the symbiotic association (Hungria & Bohrer, 2000; Sanginga *et al.*, 2000; Sinclair *et al.*, 1991; Pulver *et al.*, 1985). Various methods, including acetylene reduction (Denison *et al.*, 1983) and xylem ureide assay (McClure *et al.*, 1980), have been used for determining N₂-fixation. Recently, non-destructive method, excised into halves of fresh nodule was established, allowing for rapid screening of soybean for N₂-fixation effectiveness (Gwata *et al.*, 2003). It is a simple and reproducible method for identifying early juvenile soybean genotypes effective in N₂-fixation under nitrogen-free growth medium.

According to previous studies, supernodulating soybean mutants fixed more nitrogen in the root nodule than normal nodulating cultivars (Eskew *et al.*, 1992; Hansen *et al.*, 1990). Day *et al.* (1987) reported that higher respiration rate by the root and nodule may reflect on the great cost of nodulation and N₂-fixation activity. The higher N₂-fixation capacity and reduced plant size in the mutants results in a higher plant N concentration, especially in the leaves and nodules (Hansen *et al.*, 1989; Herridge & Rose, 1994). Moreover, initial assessments of nitrogen fixation in the supernodulating mutants showed increased C₂H₂ reduction (N₂-fixation) activity but yield was decreased than that of the wild type (Carroll *et al.*, 1985).

The yield of supernodulating mutant, SS2-2, generated from Sinpaldalkong 2 mutagenized with EMS (Lee *et al.*, 1997), was not changed a lot in supply of nitrogen fertilization, although Sinpaldalkong 2 showed increase in yield by nitrogen supply (Lee & Lee, 1998). Furthermore, SS2-2 and *nts382* grown without induction of *Rhizobium* showed more nodules and nodule mass, and greater C₂H₂ activity than their wild types, regardless of the level of exogenous nitrogen supply (Ha *et al.*, 1999). The information of symbiotic effectiveness in terms of nodulation between supernodulat-

†Corresponding author. (Phone) +82-2-880-4545 (E-mail) sukhalee@snu.ac.kr

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ing mutant and *B japonicum* is still limited. Therefore, this study was conducted to evaluate the symbiotic effectiveness of *B japonicum* USDA 110 in a supernodulating soybean mutant, SS2-2, and to compare symbiotic effectiveness with its wild type under the absence of exogenous nitrogen supply.

MATERIALS AND METHODS

Three genotypes of soybean, supernodulating mutant, SS2-2, its wild type, Sinpaldalkong 2 and control genotype, Jangyeobkong, were used in this study. Soybean seeds were surface-sterilized by 70% ethanol for 15 min followed by 7% hydrogen peroxide for 5 min. After thoroughly rinsing with sterile distilled water, seeds were inoculated by imbibition (Hansen & Akao, 1991) in suspensions of *B japonicum* USDA 110 containing 10^8 CFU/ml (Delves *et al.*, 1986). After one pathogen-free seed was placed in a hole 1 cm-deep in the center of small plastic pot filled with sterilized vermiculite (Gwata *et al.*, 2003), pots were kept in the greenhouse. Non-inoculated (control) plant was included to assess effective nodulation. Nitrogen-free Jensen's reagent (Fang & Hirsch, 1998) was applied to each pot twice a week. At six weeks after emergence, each nodule was excised and observed its color.

For determining nodulation characteristics, soybean seeds were planted in sand. The 3×2 factorial treatment combinations of three soybean genotypes and two inoculation levels (inoculated and non-inoculated by *B japonicum*) were laid out in a completely randomized design with 3 replications. The dry weight of each plant part, nodule number on root system, tap root nodulation pattern and acetylene reduction activity (ARA) were measured at 47 days after planting (DAP).

Dry weight of plant parts was measured by incubating the samples at 70 °C for 3 days. Nitrogenase activity was determined by ARA, which was measured with removed root from the whole plant which was placed in a 1 liter jar, and sealed with a lid containing a serological stopper. Using a syringe, a 50-cc aliquot of air was removed from the jar, and the same amount of C_2H_2 was then injected into the sample jar. The root system was allowed to remain in the jar with C_2H_2 for 30 min, after a 10-cc aliquot was withdrawn from the jar, the aliquot was injected into a 10-cc vacutainer tube. From this 10-cc tube, 0.5-cc aliquot was later drawn for gas chromatography/GC analysis (Denison *et al.*, 1983).

RESULTS AND DISCUSSION

Determination of effective nodulation in early juvenile

After six weeks of growth under nitrogen-free conditions, *B japonicum* induced effective nodule on all genotypes, a supernodulating mutant, SS2-2, its wild type, Sinpaldalkong

2 as well as control genotype, Jangyeobkong. Inoculated plants, SS2-2 and its wild type, showed green leaves and high turgor, while all non-inoculated soybean plants were distinctly chlorotic, yellow and stunted. Chlorotic plants with yellow leaves, were visually distinguishable from vigorous plants with green leaves, indicating availability of nitrogen derived from the nitrogen fixation because the plants were grown in a nitrogen free medium (Fig. 2A). Nitrogenous compounds resulting from N_2 -fixation were exported from root nodules in the form of ureides, translocated to the leaves where catabolized (Winkler *et al.*, 1987), and used for the biosynthesis of chlorophyll and other proteins essential for photosynthesis.

The nodule type induced by *B japonicum* in a supernodulating mutant was categorized as functional nodulating like normal soybean. Functional nodulation means the symbiotic association formed effective nodules capable of N_2 -fixation after inoculation and soybean plants had green leaves without nitrogen fertilization (Gwata *et al.*, 2003; Pulver *et al.*, 1985).

The nodules of SS2-2 were smaller than those of its wild type and nodules on the root system of Sinpaldalkong 2 were clustered near root crown (Fig. 1). This study showed that non-inoculated soybeans grown in nitrogen-free condition were able to form nodule, indicating there was induction of *Rhizobium* in the soybean host. The rhizobia might come from irrigating water and allowed the nodules to fix atmospheric dinitrogen, even in low level. Using cross section technique of intact nodule, clear differences in the color of nodular tissue between inoculated and non-inoculated plant were observed in term of effectiveness of nodulation among SS2-2, Sinpaldalkong 2 and Jangyeobkong (Fig. 2B). The ineffective nodules from non-inoculated plant were white to light green inside, while the effective nodules were characteristically pinkish-brown. Different internal nodule colors suggested that there were significant differences in N_2 -fixation capabilities as well as effective nodulation in response to *B japonicum* among three genotypes.

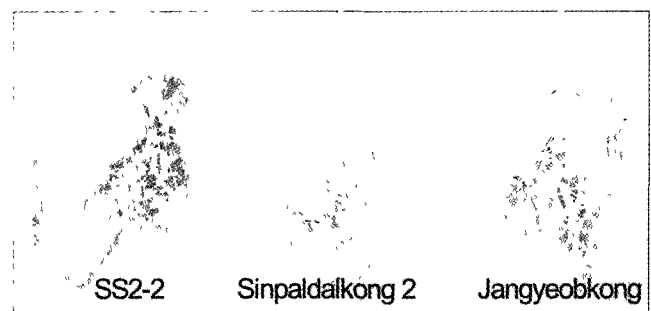


Fig. 1. Nodulation types of supernodulating mutant, its wild type and control genotype inoculated with *B japonicum* at 47 days after planting.

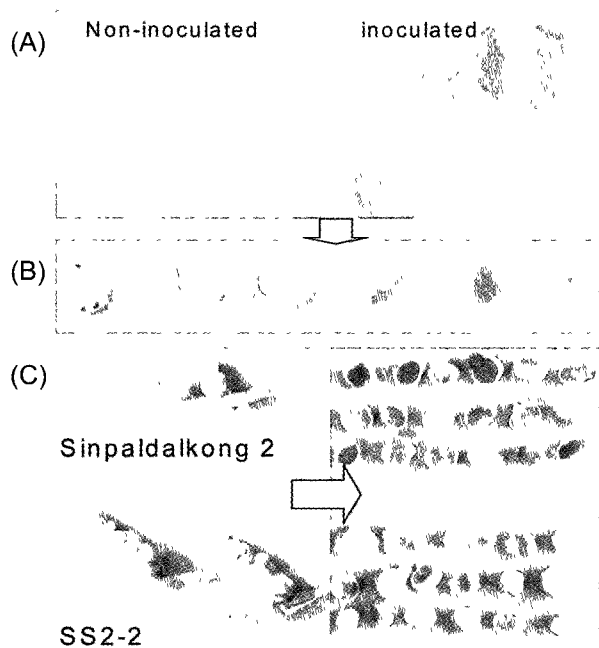


Fig. 2. Effectiveness of nodulation in soybeans at 6 weeks after emergence. (A) Differences in leaf color, (B) Intact and cross-section of root nodules from early juvenile soybean plants, and (C) Cross section nodules of Sinpaldalkong 2 and SS2-2.

Interestingly, SS2-2 had light pinkish-brown nodules with less leghemoglobin compared to its wild type (Fig. 2C). Nodule color in Sinpaldalkong 2 was changed from white to dark pinkish by maturation. But, mature nodules in SS2-2 remained light green to pinkish, although initial nodule color was white like the wild type. These results were supported by previous studies that leghemoglobin showed abundant expression in wild type, Gifu plant (*Lotus japonicus*) but not detected in the mutant, *alb1*, throughout nodule development (Szczyglowski *et al.*, 1997). Moreover, *ENOD40*, known as one of early nodulin genes, was expressed very low (Imaizumi-Anraku *et al.*, 2000) in the mutant *alb1*. Some early and late nodulin genes (leghemoglobins) are likely to be involved in the nodule development (Trevaskis *et al.*, 2002), including cell division, structural protein of the cell wall (Lobler & Hirsch, 1993; Scheres *et al.*, 1990), and leghemoglobin content (Appleby, 1984). Thus, some nodulin genes might play a role in defective autoregulatory control for nodule development/differentiation in the supernodulating mutant.

Characterization of nodulation

The size and number of nodules between SS2-2 and normal nodulating soybeans, Sinpaldalkong 2 and Jangyeo-

Table 1. Tap root nodulation pattern of N₂-dependent soybean genotypes at 47 days after planting.

Tap root parameter	Genotype	Inoculated	Non-inoculated	Mean
Root length (cm)	SS2-2	14.8	14.6	14.7 ^{bf}
	Sinpaldalkong 2	26.3	23.1	24.7 ^a
	Jangyeobkong	24.9	27.1	26.0 ^a
	Mean	22.0 ^{af}	21.6 ^a	
Nodulated root length (cm)	SS2-2	10.5	9.5	10.0 ^a
	Sinpaldalkong 2	5.5	5.7	5.6 ^b
	Jangyeobkong	5.0	3.2	4.1 ^c
	Mean	7.0 ^a	6.1 ^b	
Percentage of nodulated to total root length (%)	SS2-2	70.9	58.2	64.5 ^a
	Sinpaldalkong 2	20.9	13.9	17.4 ^b
	Jangyeobkong	20.1	22.9	21.5 ^b
	Mean	37.3 ^a	31.7 ^a	
Nodule density on root length (nodules/cm)	SS2-2	8.2	7.6	7.9 ^a
	Sinpaldalkong 2	0.49	0.31	0.4 ^b
	Jangyeobkong	0.47	0.33	0.4 ^b
	Mean	3.0 ^a	2.7 ^b	
Acetylene reduction activity ($\mu\text{mol h}^{-1} \text{plant}^{-1}$)	SS2-2	3.95	5.20	4.57 ^a
	Sinpaldalkong 2	1.62	4.00	2.81 ^b
	Jangyeobkong	1.43	3.90	2.67 ^c
	Mean	2.33 ^b	4.37 ^a	
Specific acetylene reduction activity ($\mu\text{mol h}^{-1} \text{g}^{-1} \text{nodule dry weight}$)	SS2-2	15.17	17.60	16.38 ^b
	Sinpaldalkong 2	18.00	37.04	27.52 ^a
	Jangyeobkong	18.26	43.82	31.04 ^a
	Mean	17.14 ^b	32.82 ^a	

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

Table 2. Responses of three genotypes of soybean to *B japonicum* USDA 110 induction in nitrogen-free condition at 47 days after planting.

Parameter	Ratio of inoculated/non-inoculated treatment		
	SS2-2	Sinpaldalkong 2	Jangyeobkong
Nodule number	1.166	1.296	1.237
Nodule dry weight	1.126	1.200	1.136
Shoot dry weight	1.344	1.147	1.127
Root dry weight	1.395	1.709	1.608
ARA	1.316	2.469	2.727

bkong were clearly distinguishable, but morphology and exterior color of intact nodules among them could not be discriminated. Under given condition, 47-day-old SS2-2 plants had considerably higher nodule number than its wild type and control genotype (Fig. 1). These results were consistent with the conclusion of that the supernodulators were producing relatively more nodular tissue than normally-nodulating types in the absence of nitrogen (Hansen *et al.*, 1992). Tap root length was short in supernodulated symbiotic SS2-2 plants than that of its wild type and control genotype grown under identical conditions (Table 1). The nodulated root length is defined as the distance between the upper most and lower most nodule on the tap root. The nodulated root length was extended and nodule density was increased in SS2-2. Nodule density, expressed on the total tap root length was almost 20 times higher in SS2-2 than Sinpaldalkong 2 and Jangyeobkong. It showed that inoculation of *B japonicum* increased nodulated root length and nodule density on root length significantly than non-inoculated treatment, but not significantly to root length and percentage of nodulation to total root length (Table 1).

Supernodulating mutant gave higher ARA in comparison with wild type and control genotype, positively correlating with nodule dry weight (Table 1). *B japonicum* caused the increase of ARA at 47 DAP, in part because of nodule development. But, ARA started to decline at later stage of growth, because of not only nodule senescence but also the steady increase in nodule dry weight. This result was supported previous studies, that *B japonicum* was effective to induce nodulation and increase nitrogen fixation (Israel *et al.*, 1986; Yelton, 1983).

Otherwise, as the symbiosis progressed, specific nitrogenase activity was the lowest in SS2-2 among three genotypes (Table 1). Day *et al.* (1989) reported lower specific nitrogenase activity of supernodulating mutant was mainly due to the reduced amount of symbiotic tissue which was characterized by smaller nodule cells and lowered leghemoglobin content per nodule. So, although nodule emergence and subsequent nodule growth were coordinately controlled in SS2-2, specific nitrogenase activity is not coordinately regulated with this nodule development parameter in the

mutant (Carroll *et al.*, 1985).

Inoculation of *B japonicum* led an increase in nodule number, nodule dry weight, root dry weight as well as ARA among three soybean genotypes. SS2-2 showed less increase in these characteristics than those of its wild type (Table 2). In contrast, the increasing of shoot dry weight in SS2-2 by *B japonicum* was higher than its wild type as well as control genotype. Thus, shoot growth of the wild type and the mutant were identical under symbiotic condition, while root growth still lagged behind (data not shown). These results supported that the development of the soybean-*Bradyrhizobium* symbiosis was under the control of both shoot and root factors and that these factors interact (Delves *et al.*, 1986; Kosslak & Bohlool, 1984). Furthermore, rapid regulatory response in soybean is reproducible elicited by *Rhizobium japonicum* (Pierce & Bauer, 1983) and autoregulatory control mechanism (van Workum *et al.*, 1998).

So, SS2-2 showed less response to *B japonicum* induction in terms of nitrogen fixation and nodulation characters. The nodulation in supernodulating mutant in the absence of nitrogen suggested a mutational alteration of the autoregulation system, indicating closed relationship between nitrogen fixation and autoregulation of nodule development (Delves *et al.*, 1986). SS2-2 is a mutant in the regulation of nodule initiation and nodule development, and the autoregulation mechanism normally controlling nodulation in wild type soybeans (Pierce & Bauer, 1983) is anomalous in this mutant. Supernodulating mutant might maintain its growth by biologically fixed nitrogen (Delves *et al.*, 1986; Carroll *et al.*, 1985). Therefore, interaction of rhizobia and supernodulating soybean mutant had less symbiotically associated response than normal nodulating soybean.

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REFERENCES

- Appleby, O. 1984. Leghemoglobin and *Rhizobium* respiration. *Annu. Rev. Plant Physiol.* 35 : 443-478.
- Bauer, W. D. 1981. Infection of legumes by rhizobia. *Annu. Rev. Plant Physiol.* 32 : 407-449.
- Carroll, B. J., D. J. McNeil, and P. M. Gresshoff. 1985. A supernodulation and nitrate-tolerant symbiotic (*nts*) soybean mutant. *Plant Physiol.* 78 : 34-40.
- Day, D. A., B. J. Carroll, A. C. Delves, and P. M. Gresshoff. 1989. Relationship between autoregulation and nitrate inhibition of nodulation in soybeans. *Physiol. Plant.* 75 : 37-42.
- Day, D. A., G. D. Price, K. A. Schuller, and P. M. Gresshoff. 1987. Nodule physiology of a supernodulating soybean (*Glycine max*) mutant. *Aust. J. Plant Physiol.* 14 : 527-538.
- Delves, A. C., A. Mathews, D. A. Day, A. S. Carter, B. J. Carroll, and P. M. Gresshoff. 1986. Regulation of the soybean-*Rhizobium* nodule symbiosis by shoot and root factors. *Plant Physiol.* 82 : 588-590.
- Denison, R. F., T. R. Sinclair, R. W. Zobel, M. N. Johnson, and G. M. Drake. 1983. A non-destructive field assay for soybean nitrogen fixation by acetylene reduction. *Plant Soil.* 70 : 73-182.
- Eskew, D. L., P. M. Gresshoff, M. Doty, and C. Mora. 1992. Sealed-tube combustion for natural N^{15} abundance estimation of N fixation and application to supernodulating soybean mutants. *Can. J. Microbiol.* 38 : 598-603.
- Fang, Y. and A. M. Hirsch. 1998. Studying early nodulin gene *ENOD40* expression and induction by nodulation factor and cytokinin in transgenic alfalfa. *Plant Physiol.* 116 : 53-68.
- Gwata, E. T., D. S. Wofford, K. J. Boote, and H. Mushoriwa. 2003. Determination of effective nodulation in early juvenile soybean plants for genetic and biotechnology study. *African J. Biotech.* 11 : 417-420.
- Ha, B. K., J. S. Lee, K. I. Yun, H. S. Lee, E. H. Park, Y. W. Kim, and S. H. Lee. 1999. Isolation and molecular characterization of hypernodulating soybean mutant. *Korea Soybean Digest.* 2 : 47-56.
- Hansen, A. P. and S. Akao. 1991. Early nodule development of soybean cv. Bragg and some nodulation mutants in response to different levels of inoculation. *J. Plant Physiol.* 138 : 501-506.
- Hansen, A. P., P. Martin, B. R. Buttery, and S. J. Park. 1992. Nitrate inhibition of N_2 -fixation in *Phaseolus vulgaris* cv. OAC Rico and a supernodulating mutant. *New Phytologist.* 122 : 611-615.
- Hansen, A. P., M. B. Peoples, P. H. Brown, B. J. Carroll, and P. M. Gresshoff. 1990. Nitrogen partitioning during early development of supernodulating soybean (*Glycine max* [L.] Merrill) mutants and their wild-type parent. *J. Exp. Bot.* 41 : 1239-1244.
- Hansen, A. P., M. B. Peoples, P. M. Gresshoff, C. A. Atkins, J. S. Pate, and B. J. Carroll. 1989. Symbiotic performance of supernodulating soybean (*Glycine max* [L.] Merrill) mutants during development on different nitrogen regimes. *J. Exp. Bot.* 40 : 715-724.
- Herridge, D. F. and I. A. Rose. 1994. Heritability and repeatability of enhanced N fixation in early and late inbreeding generations of soybean. *Crop Sci.* 34 : 360-367.
- Hungria, M. and T. R. J. Bohrer. 2000. Variability of nodulation and dinitrogen fixation capacity among soybean cultivars. *Biol. Fertil. Soils.* 31 : 45-52.
- Imaizumi-Anraku, H., H. Kouchi, K. Syono, S. Akao, and M. Kawaguchi. 2000. Analysis of *ENOD40* expression in *alb1*, a symbiotic mutant of *Lotus japonicus* that forms empty nodules with incompletely developed nodule vascular bundles. *Mol. Gen. Genet.* 264 : 402-410.
- Israel, D. W., J. N. Mathis, W. M. Barbour, and G. H. Elkan. 1986. Symbiotic effectiveness and host-strain interactions of *Rhizobium fredii* USDA 191 on different soybean cultivars. *Appl. Environ. Microbiol.* 51 : 898-903.
- Kosslak, R. M. and B. B. Bohlool. 1984. Suppression of nodule development on one side of a split-root system of soybean caused by prior inoculation of the other side. *Plant Physiol.* 75 : 125-130.
- Lee, H. S., Y. A. Chae, E. H. Park, Y. W. Kim, K. I. Yun, and S. H. Lee. 1997. Introduction, development, and characterization of supernodulating soybean mutant. I. Mutagenesis of soybean and selection of supernodulating soybean mutant. *Korean J. Crop Sci.* 42 : 247-253.
- Lee, H. S. and S. H. Lee. 1998. Introduction, development and characterization of supernodulating soybean mutant: Nitrate inhibition of nodulating and nitrogen fixation in supernodulating soybean mutant. *Korean J. Crop. Sci.* 43 : 23-27.
- Lobler, M. and A. M. Hirsch. 1993. A gene that encodes a proline-rich nodulin with limited homology to *PsENOD12* is expressed in the invasion zone of *Rhizobium meliloti*-induced alfalfa root nodules. *Plant Physiol.* 103 : 21-30.
- McClure, P. R., D. W. Israel, and R. J. Volk. 1980. Evaluation of the relative ureide content of xylem sap as an indicator of N_2 -fixation. *Plant Physiol.* 66 : 720-725.
- Pierce, M. and W. D. Bauer. 1983. A rapid regulatory response governing nodulation in soybean. *Plant Physiol.* 73 : 286-290.
- Pulver, E. L., E. A. Kueneman, and V. Raga-Rao. 1985. Identification of promiscuous nodulating soybean efficient in nitrogen fixation. *Crop Sci.* 25 : 660-663.
- Sanginga, N., G. Thottappilly, and K. Dashiell. 2000. Effectiveness of rhizobia nodulating recent promiscuous soybean selections in the moist savanna of Nigeria. *Soil Biol. Biochem.* 32 : 127-133.
- Scheres, B., F. van Engelen, and E. van der Knaap. 1990. Sequential induction of nodulin gene expression in the developing pea nodule. *Plant Cell.* 2 : 687-700.
- Sinclair, T. R., A. R. Soffer, K. Hison, S. L. Albrecht, and P. L. Phaffler. 1991. Genotypic variation in soybean nodule number and weight. *Crop Sci.* 31 : 301-304.
- Szczyglowski, K., D. Hamburger, P. Kapranov, and F. J. de Bruijn. 1997. Construction of a *Lotus japonicus* late nodulin expressed sequence tag library and identification of novel nodule-specific genes. *Plant Physiol.* 114 : 1335-1346.
- Trevaskis, B., G. Colebatch, G. Desbrosses, M. Wandrey, and S. Wienkoop. 2002. Differentiation of plant cells during symbiotic nitrogen fixation. *Comparative and Functional Genomics.* 3 : 151-157.
- van Workum, W. A., T. S. van Slageren, A. A. N. van Brussel, and J.

- W. Kijne. 1998. Role of exopolysaccharides of *Rhizobium leguminosarum* bv *viciae* as host plant-specific molecules required for infection thread formation during nodulation of *Vicia sativa*. *Molecular Plant-Microbe Interaction*. 111 : 1233-1241.
- Winkler, R. D., D. G. Blevins, J. C. Pollaco, and D. D. Randall. 1987. Ureide catabolism in soybeans. II Pathway of catabolism in intact leaf tissue. *Plant Physiol*. 83 : 585-591
- Yelton, M. M., S. S. Young, S. A. Edie, and S. T. Lim. 1983. Characterization of an effective salt-tolerant, fast-growing strain of *Rhizobium japonicum*. *J. Gen. Microbiol*. 129 : 1537-1547.