Comparison of Initial Growth of Hypernodulation Soybean Mutants, NOD1-3, NOD2-4 and NOD3-7, and Their Parent cv. Williams

Sayuri ITO[1], Norikuni OHTAKE[2], Kuni SUEYOSHI[2] and Takuji OHYAMA[2*]

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Summary

Characteristics of the initial growth of hypernodulation soybean (*Glycine max* [L] Merr.) mutants isolatad from cv. Williams, NOD1-3, NOD2-4 and NOD3-7 were compared with that of parent cv. Williams with or without inoculation of *Bradyrhizobium japonicum* at 7 or 8 days after sowing. Total dry weight of each hypernodulation mutant seedling was not significantly different from that of Williams seedling in uninoculation conditions. In inoculation condition, nodule number of the plants at day 8 after sowing was higher in the order NOD1-3 (56) > NOD2-4 (33) > NOD3-7 (19) > Williams (17), but there is no significant difference between NOD3-7 and Williams. The plant dry weight was not different by hypernodulation trait at this stage. In uninoculation condition, root growth of NOD1-3 and NOD3-7 was faster than that of Williams. Stem length and dry weight of NOD3-7 were lower than those of other lines, so shoot growth of NOD3-7 might be different from others. Seedling growth of NOD2-4 was very similar with that of Williams, except nodule number. Therefore, NOD2-4 mutant line may be a best mutant of Williams to investigate autoregulation of nodulation.

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Key words : dry weight, inoculation, hypernodulation mutant, seedling, soybean

Legume plants can form root nodules, which are symbiotic organs with soil bacterium rhizobia, and fix atmospheric nitrogen. However, the number of nodules is strictly regulated by the host plant, because an excess number of nodules may be deleterious due to competition of photosynthates. That negative regulation system is referred to as the autoregulation of nodulation (Caetano-Anolles and Gressoff 1991). Host plants seem to suppress excess nodulation systemically through communications between shoots and roots, by using unknown signal molecules (Olsson *et al.*, 1989; Caetano-Anolles and Gressoff, 1991; Francisco and Harper, 1995, Sato *et al.*, 2002).

Following mutagenesis, several hypernodulating mutant lines of soybean were isolated (Carroll *et al.*, 1985; Gremaud and Harper, 1989; Akao and Kouchi, 1992). The mutant lines can form profuse nodulation in the presence or absence of nitrate compared with their parents. Physiological characterization of these mutants showed some characteristics of plant growth in addition to the hypernodulation trait. Especially, less vigorous plant growth and the small roots had been reported (Carroll *et al.*, 1985; Gremaud and Harper, 1989; Akao and Kouchi, 1992), but it is unclear whether inferiority of plant and root growth occurs as a secondary effect of hypernodulation or not.

Recently, *HAR1* and *GmNARK*, which play an important role in autoregulation of nodulation, were identified as the mutated genes which cause hypernodulation in *Lotus japonicus* and soybean, respectively (Krusell *et al.*, 2002; Nishimura *et al.*, 2002; Searle *et al.*, 2003). However, the role of these genes for autoregulation has not yet been clarified. The investigation of the details of growth characteristic of hypernodulation mutant lines may be important to understand the systemic features of autoregulation mechanism.

The soybean hypernodulation mutants NOD1-3, NOD2-4 and NOD3-7 were isolated from the cv. Williams parent (Gremaud and Harper, 1989). Allelism analysis showed that all NOD mutant lines from cv. Williams are controlled by a single recessive allele (Vuong *et al.*, 1996; Vuong and Harper, 2000). There are some different phenotypes between these mutant lines (Gremaud and Harper, 1989; Ohyama *et al.*, 1993), but the genetic or physiological reason is unclear so far.

The objective of this study was to investigate the characteristics of the NOD mutant lines for early seedling growth. We compared the phenotypes both inoculated seedling and uninoculated seedling.

MATERIALS AND METHODS

Soybean (*Glycine max* [L.] Merr.) seeds of hypernodulation mutant lines, NOD1-3, NOD2-4 and NOD3-7, and of the Williams parent were carefully selected by uniform seed weight. Seeds were sterilized with 0.7L L^{-1} ethanol for 30s and 5g L^{-1} sodium hypochlorite solution for 5min. Then they were thoroughly washed with water and sown on vermiculite bed. In "inoculation experiment" , seeds were inoculated with a suspension of *Bradyrhizobium*

[1] Graduate School of Science and Technology, Niigata University

[2] Faculty of Agriculture, Niigata University

[*] Corresponding author: ohyama@agr.niigata-u.ac.jp

japonicum strain USDA110 (about 10^8cell/mL) for 20 min before sowing, while they were not inoculated and kept in aseptic condition in "uninoculation experiment". They were grown in a growth chamber under the following conditions: 16h photoperiod at 28℃ and 8h dark period at 18℃. They were watered every day and nutrient solution was not supplied.

The plants were harvested at 7 days after sowing in "uninoculation experiment". The plants were harvested at 8 days after sowing, when nodules are appeared on the root, in "inoculation experiment". They were separated to each part, and the number of nodule and lateral root was counted. Separated parts were freeze-dried to analyze the dry weight.

RESULTS

Uninoculation experiment (7 days after sowing)

Table 1 shows that lateral root number, primary root length and dry weight of each part of the hypernodulation soybean mutants, NOD1-3, NOD2-4 and NOD3-7, and their parent cv. Williams. Total dry weight of each mutant line was similar with Williams.

Lateral root number of NOD1-3 and NOD3-7 was higher than that of Williams, and primary root of NOD1-3 and NOD3-7 was slightly longer than that of Williams. These results indicate that root growth of NOD1-3 and NOD3-7 was faster than that of wild type, irrespective of hypernodulation effect. Root dry weight of NOD3-7 was, however, similar with that of Williams while that of NOD1-3 was slightly large. So it might be that root growth of NOD1-3 was more vigorous than wild type at this stage, and that difference of root dry weight between NOD3-7 and Williams was not observed. The root growth characteristics of NOD2-4 was very similar with Williams.

In the shoot growth, dry weight of stem, shoot apex and primary leaf of NOD3-7 was slightly lower than that of Williams, while those of NOD1-3 and NOD2-4 was higher compared with Williams. On the other hand, cotyledon dry weight of NOD3-7 was higher than that of Williams, while those of NOD1-3 and NOD2-4 was lower compared with Williams. Cotyledon is nutrient source for the seedling and withers till day 18 after sowing when grown in this condition. It is considered that small dry weight of cotyledon means early export of storage compound, in cotyledons of NOD1-3 and NOD2-4.

Inoculation experiment (8 days after sowing)

Fig. 1 shows nodule number of NOD1-3, NOD2-4 and NOD3-7, and Williams. Average nodule number was higher in the order NOD1-3 (56) > NOD2-4 (33) > NOD3-7 (19) > Williams (17). There were not significant differences between NOD3-7 and Williams at this stage (8 days after sowing) although NOD3-7 is hypernodulation mutant. This indicates that autoregulation of nodule formation of NOD3-7 is not so

Table 1. Lateral root number, primary root length and dry weight of each part of the hypernodulation soybean mutants, NOD1-3, NOD2-4 and NOD3-7, and their parent cv. Williams. The plants were grown on vermiculite in a growth chamber, without inoculation. They were harvested at 7 days after sowing.

	n	Lateral root number	Primary root length (cm)
Williams	9	41 (11) b	11.9 (2.8) ab
NOD1-3	9	57 (5) a	14.0 (1.8) a
NOD2-4	9	44 (6) b	11.2 (0.9) b
NOD3-7	10	55 (8) a	13.5 (3.0) a

	Dry weight (mg)				
	root	stem plus shoot apex	primary leaf	cotyledon	total
Williams	28 (4) b	24 (2) b	16 (6) ab	82 (5) ab	150 (3) ab
NOD1-3	32 (4) a	26 (2) ab	17 (5) ab	78 (6) b	153 (5) a
NOD2-4	27 (3) b	27 (3) a	19 (4) a	77 (6) b	150 (4) ab
NOD3-7	28 (5) ab	21 (2) c	14 (4) b	85 (5) a	148 (4) b

The number within parentheses indicates standard deviation. Data not followed by the same letter within column are significantly different at 5% level. n, number of plants tested.

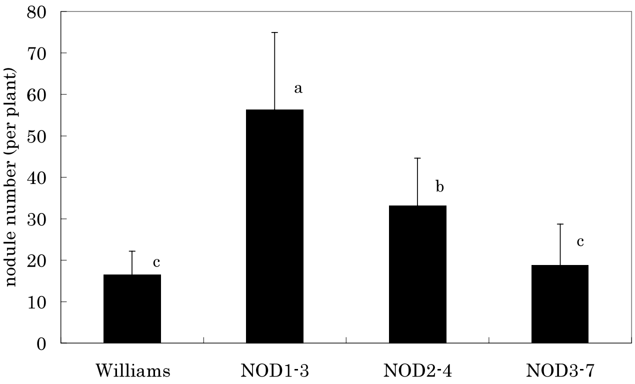

Fig. 1. Comparison of nodule number of the hypernodulation soybean mutants, NOD1-3, NOD2-4 and NOD3-7 and Williams, in inoculation condition at 8 days after sowing. Not followed by the same letter are significantly different at 5% level (n = 10).

much decreased in the initial seedling.

Table 2 shows lateral root number, primary root length, stem length and dry weight of each part of the hypernodulation soybean mutants, NOD1-3, NOD2-4 and NOD3-7, and Williams. Lateral root number and primary root length of each mutant line was not significantly different from those of Williams. These results indicate that root growth was not decreased by profusion of nodule in seedling at day 8 after sowing. The dry weight of the underground part of NOD1-3 and NOD3-7 was slightly higher than that of Williams, but not statistically significant.

In comparison of the shoot growth, stem length and dry weight of NOD3-7 was lower than those of Williams and other mutant lines, so it was shown that stem growth of NOD3-7 was decreased in seedling. Primary leaf dry weight of each mutant line was decreased than that of Williams. Shoot apex dry weight of ND1-3 and NOD3-7 was, however, slightly larger than that of Williams and NOD2-4. Shoot apex growth of NOD1-3 and NOD3-7 might be faster than that of wild type. Dry weight of cotyledon of each mutant line was similar with Williams.

DISCUSSION

In general, it has been reported that plant growth of hypernodulation mutant is less vigorous than that of wild type. In this study, the initial seedling growths of hypernodulation soybean mutants, NOD1-3, NOD2-4 and NOD3-7 were compared with parent cv. Williams with or without inoculation of *B. japonicum* at day 7 or 8 after sowing. It was important to keep uniform seed size because it affects the initial seedling growth. In this study, two growth conditions were designed; one is uninoculation

Table 2. Lateral root number, primary root length and dry weight of each part of the hypernodulation soybean mutants, NOD1-3, NOD2-4 and NOD3-7, and their parent cv. Williams. The plants were grown on vermiculite in a growth chamber, with inoculation of *Bradyrhizobium japonicum*. They were harvested at 8 days after sowing.

	n	Lateral root number	Primary root length (cm)	Stem length (cm)
Williams	10	65 (9) a	15.9 (1.8) a	8.3 (1.0) a
NOD1-3	10	74 (22) a	16.4 (4.6) a	8.5 (0.5) a
NOD2-4	10	63 (8) a	15.5 (1.6) a	8.4 (1.2) a
NOD3-7	10	64 (26) a	15.2 (4.3) a	6.6 (1.1) b

	Dry weight (mg)					
	underground	stem	primary leaf	shoot apex	cotyledon	total
Williams	34 (6) ab	26 (4) a	21 (6) a	1.5 (0.7) b	67 (7) a	149 (8) a
NOD1-3	38 (3) ab	27 (2) a	17 (4) ab	2.2 (0.5) a	66 (5) a	150 (6) a
NOD2-4	33 (4) b	26 (3) a	16 (5) b	1.5 (0.4) b	70 (8) a	147 (4) a
NOD3-7	38 (3) a	21 (4) b	15 (4) b	1.9 (0.8) ab	70 (6) a	145 (6) a

The number within parentheses indicates standard deviation. Data not followed by the same letter within column are significantly different at 5% level. n, number of plants tested.

condition, the other is inoculation condition. It was shown that total dry weight of each hypernodulation mutant seedling was not significantly different from that of Williams seedling in both uninoculation and inoculation conditions. Therefore, the decreases in the growth of hypernodulation mutant become evident after this stage.

In uninoculation experiment, root growth of NOD1-3 was faster than that of Williams. In inoculation experiment, nodule number of NOD1-3 was the largest in all lines, and the root growth was not decreased. It seems that underground (root plus nodule) growth of NOD1-3 in seedling was slightly faster than those of Williams whether inoculated or uninoculated conditions.

Initial seedling growth of NOD2-4 was very similar with that of Williams, but nodule number of NOD2-4 was higher than Williams. Mutation of NOD2-4 affects only autoregulation of nodule formation, but not in plant growth feature.

Nodule number of NOD3-7 was similar with that of Williams although NOD3-7 is identified as hypernodulation mutant. It might be that hypernodulation trait of NOD3-7 is not appeared when they are seedling at very early stage (8 days after sowing). It is also characterized that stem length of NOD3-7 seedling was shorter than other lines.

The NOD2-4 line may be a best mutation line of Williams to investigate autoregulation, because various initial growth characters are similar except for hypernodulation trait.

REFERENCES

Akao, S. and Kouchi, H. 1992. A supernodulating mutant isolation soybean cultivar Enrei. *Soil Sci. Plant Nutr.*, **38**: 183-187.

Caetano-anolles, G. and Gresshoff, P.M. 1991. Plant genetic control of nodulation. *Annu Rev. Microbial.*, **45**: 345-382.

Carroll, B.J., McNeil, D.L. and Gresshoff, P.M. 1985. A supernodulation and nitrate-tolerant symbiotic (*nts*) soybean mutant. *Plant Physiol.*, **78**: 34-40.

Francisco, P.B. Jr. and Harper, J.E. 1995. Translocatable leaf signal autoregulates soybean nodulation. *Plant Sci.*, **107**: 167-176.

Gremaud, M.F. and Harper, J.E. 1989. Selection and initial characterization of partially nitrate tolerant nodulation mutants of soybean. *Plant Physiol.*, **89**: 169-173.

Krusell, L., Madsen, L.H., Sato, S., Aubert, G., Genua, A., Szczyglowski, K. et al. 2002. Shoot control of root development and nodulation is mediated by a receptor-like kinase. *Nature*, **420**: 422-426.

Nishimura, R., Hayashi, M., Wu, G.J., Kouchi, H., Imaizumi-Anraku, H., Murakami, Y. et al. 2002. HAR1 mediates systemic regulation of symbiotic organ development. *Nature*, **420**: 426-429.

Ohyama, T., Nicholas, J.C. and Harper, J.E. 1993. Assimilation of $^{15}N_2$ and $^{15}NO_3^-$ by partially nitrate-tolerant nodulation mutants of soybean. *J. Exp. Botany*, **44**: 1739-1747.

Olsson, J.E., Nakao, P., Bohlool, B.B. and Gresshoff, P.M. 1989. Lack of systemic suppression of nodulation in split root system of supernodulating soybean (*Glycine max* [L.] Merr.) mutants. *Plant Physiol.*, **90**: 1347-1352.

Sato, T., Fujikake, H., Ohtake, N., Sueyoshi, K. and Ohyama, T. 2002. Effect of exogenous salicylic acid supply on nodule formation of hypernodulating mutant and wild type of soybean. *Soil Sci. Plant Nutr.*, **48**: 413-420.

Searle, I.R., Men, A.E., Laniya, T.S., Buzas, D.M., Iturbe-Ormaetxe, I., Carroll, B.J. and Gresshoff, P.M. 2003. Long-distance signaling in nodulation directed by a CLAVATA1-like receptor kinase. *Science*, **299**: 109-112.

Vuong, T.D., Nickell, C.D. and Harper, J.E. 1996. Genetic and allelism analyses of hypernodulation soybean mutants from two genetic backgrounds. *Crop Sci.*, **36**: 1153-1158.

Vuong, T.D. and Harper, J.E. 2000. Inheritance and allelism analysis of hypernodulating genes in the NOD3-7 and NOD2-4 soybean mutants. *Crop Sci.*, **40**: 700-703.

ダイズ根粒超着生変異株 NOD1-3、NOD2-4および NOD3-7とその親株 Williams の幼植物生育の特徴の比較

伊藤小百合[1]・大竹憲邦[2]・末吉　邦[2]・大山卓爾[2*]

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要　約

ダイズ（*Glycine max* [L] Merr.）品種 Williams から分離された根粒超着生変異株 NOD1-3、NOD2-4および NOD3-7について、播種７日後または８日後の幼植物の生育の特徴を、根粒菌非接種および接種条件下で親株の Williams と比較した。根粒菌非接種・接種条件ともに、変異株三系統の総乾物重は Williams との比較で有意な差は認められなかった。非接種条件下では、NOD1-3と NOD3-7の根の生育が Williams より早い傾向にあることが示唆された。根粒菌接種条件下では、根粒数は NOD1-3（56個）＞ NOD2-4（33個）＞ NOD3-7（19個）＞ Williams（17個）の順であったが、NOD3-7と Williams の間に有意な差は認められなかった。NOD3-7は根粒超着生形質を示す変異株であるが、生育の極初期の根粒数に親株との差は認められなかった。幼植物の段階では、NOD1-3および NOD2-4において根粒数が多くても根の生育は減少していなかった。また地上部生育に関して、NOD3-7は茎の生育が他より劣る傾向が認められた。NOD2-4の生育パターンは、根粒数が多いこと以外は Williams に最も近い性質を示した。従って NOD2-4は根粒形成のオートレギュレーションの解析に最も適していると考えられる。

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[1]新潟大学自然科学研究科
[2]新潟大学農学部
*代表著者：ohyama@agr.niigata-u.ac.jp