Comparison of the Growth and Nitrogen Fixation Activity of the Hypernodulation Soybean Mutant NOD1-3 and its Parent cv. Williams in Field Cultivation

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ABSTRACT. The hypernodulation soybean mutant NOD1-3 and its parent cv. Williams were transplanted in the sandy dune field of Faculty of Agriculture of Niigata University at planting density 2 plantsm^{-2}. The plants and root bleeding xylem sap were sampled at 54, 61 (R1), 81 (R3), 102 (R5), and 130 (R7) days after sowing. The concentration of major nitrogenous compounds (ureide-N, amide-N, nitrate-N) in xylem sap was measured by colorimetric method and capillary electrophoresis. The concentration of ureide-N was consistently higher in NOD1-3 than Williams. The amide-N concentration was similar between Williams and NOD 1- 3, and it increased at R7. The nitrate-N concentration in NOD1-3 was higher than Williams after R1, showing a peak at R5. According to the simple relative ureide method, nitrogen fixation rate and nitrogen absorption rate were estimated. The highest nitrogen fixation activity and nitrogen absorption rate was observed from R3 to R5 stage, and it decreased after R5 in both Williams and NOD1-3. Throughout the growth period, nitrogen absorption rate and N_2 fixation rate was always higher in Williams than NOD1-3. After flowering dry weight of NOD1-3 was severely depressed and almost a half of Williams. This way be due to the imbalance of photosynthate supply due to excess nodulation, as well as inferior water and nutrient absorption by small root system. The 58% and the 65% of N was derived from N_2 fixation in Williams and NOD1-3 respectively.

One example of Williams was carefully dug out without loosing the roots and nodules. It had 17 branches, 178 total nodes, 3.44 pods per node, 2.74 seeds per pod and 1687 total number seeds. The plant didn't show a succulent growth in spite of the vigorous growth. The length of main stem was 87.5 cm and the diameter of the stem was 25 mm. Because the basement of the stem and root were extremely strong, it didn't lodge. There were 1874 nodules per plant and the total nodule fresh weight was 45 g. The dry weight per plant reached 564 g and the seed yield was estimated at 7.16 t ha^{-1}. However, the final yield was 2.2 t ha^{-1} due to the severe damage by insects.

Key words: Soybean, relative ureide method, N_2 fixation rate, nitrogen absorption rate

INTRODUCTION

Soybean have root nodules which is a symbiotic system with a micro symbiont *Baradyrhizobium japonicum* and fixes nitrogen in air, and they can utilize the nitrogen for their nutrition. The nitrogen fixation ability of soybean is relatively high among leguminous plants, and it is estimated that it depends from about 40% to 70% of all the nitrogen assimilation on nitrogen fixation in Japanese cultivation (FUJII et al. 1987). The contribution of nitrogen fixation is higher under a high yield cultivation, and it was about 60~80% of total accumulation N of the plants cultivated in drained paddy field in Niigata (TAKAHASHI and OHYAMA 1999). In addition to N_2 fixation, soybean is able to use the nitrogen from soil origin and fertilizer nitrogen. However it is well known that nodulation is decreased by nitrogen fertilization, especially nitrate-N severely depresses nodule formation, growth and N_2 fixation activity (HASHIMOTO 1981, HARPER 1989, KUWABARA 1986).

The seed yield of soybean depends on the dry matter production of shoots (TAMURA et al. 1993), and it requires a continuous supply of a large amount of nitrogen. Soil mineralized nitrogen supply amounts to 50-100 kgN ha^{-1}, and only soil N produces less than 1t ha^{-1} seeds. For higher yield such as 4-6 t ha^{-1}, nitrogen fixation is fundamentally important. It is necessary for the maximum yield of soybean to use nitrogen fixation and absorbed nitrogen from roots (HARPER, J. E. 1974). Furthermore, the N supply from soil or fertilizer is necessary during young seedling stage before commencement of N_2 fixation from 2 to 4 weeks after sowing and after pod setting and seed filling stages when the competition of photosynthesis between pods and nodules some times results in severe decrease of N_2 fixation. Therefore, a continuous supply of low level of N from soil organic matter or fertilizers is beneficial for soybean growth and yield as well as maintenance of nodulation and N_2 fixation.

TAKAHASHI et al. developed a new fertilization technique for soybean to supplement N during seed filling stage without depressing N_2 fixation by deep placement of slow release N fertilizer coated urea. The highest yield was about 6 t ha^{-1} when the climate condition was good (TAKAHASHI et al. 1991, 1992). The bacteroids symbiotic forms of rhizobia in soybean nodules fix atmospheric dinitrogen to ammonia, which is rapidly excreted to plant cytosol and converted to ureides (allantoin and allantoic acid) in the nodules, and the ureides transported to the shoot through the root and stem xylem (OHYAMA and KUMAZAWA 1978). The nitrogen absorbed from the soil by the root is transported to the shoot in the forms of nitrate and amino compounds, especially asparagine, and used for nitrogen metabolism in shoot (OHYAMA et al. 1989). The relative ureide-N concentration in total N (ureide-N+amide-N+nitrate-N) in xylem sap is a good indicator for the estimation of the dependence on nitrogen fixation and nitrate absorption of field grown soybean (TAKAHASHI et al. 1993).

Previously, SATO et al. (1998) reported the changes in xylem sap composition of hypernodulation soybean mutant lines (NOD1-3, NOD2-4, NOD3-7) and their parent Williams, and the mutant En 6500 and its parent Enrei cultivated in 1995 in the dune field of Faculty of Agriculture, Niigata University. The results indicated that the concentration of ureides and nitrate in xylem sap decreased with plant age, but the asparagine concentration is increased during maturing stage, in both hypernodulation and parent lines. The concentration of ureides and asparagine were higher, and the nitrate concentration was lower in the mutant lines than in their parents, possibly due to the higher dependence on N_2 fixation than NO_3^- utilization. However in this experiment, the changes in total N wasn't measured to estimate daily N_2 fixation activity and N absorption rate.

In this report, we compared the growth and nitrogen fixation activity and N absorption rate of the hypernodulation soybean mutant NOD1-3 and its parent cv. Williams in field cultivation by the simple relative ureide method analyzing xylem sap composition and total N accumulation changes.

MATERIALS AND METHODS

A hypernodulation soybean mutant NOD1-3 and its parent cv. Williams were used in this study. Seeds were sterilized with 0.7 L L^{-1} ethanol for a minute, and 5 g L^{-1} sodium hypochrolite solution for 5 min, then thoroughly washed with a tap water. They were inoculated with *Bradyrhizobium japonicum* strain USDA110, and sown in vermiculite at May 15 th, 2000, and grown in a green house under natural conditions. At 8 days after sowing, the hypernodulation soybean mutant NOD1-3 and its parent cv. Williams were transplanted in the sandy dune field of Faculty of Agriculture of Niigata University. The planting density was 20,000 plants ha^{-1}. 20 kgN ha^{-1} of ammonium sulfate, 80 kg P_2O_5 ha^{-1} of phosphoric acid, 80 kg K_2O ha^{-1} of potassium and 1t ha^{-1} of calcium carbonate were supplied as a basal dressing. The xylem sap was periodically collected from the cut basal stem on 54, 61 (R1), 81 (R3), 102 (R5), and 130 (R7) d after sowing (DAS), where "R" indicates the reproductive growth stage proposed by Fehr et al. (1971). Water was supplied by sprinkler every day.

The concentrations of ureide-N and amino-N were analyzed by a colorimetric method based on the Young-Conway's ninhydrin method (TAKAHASHI et al. 1993) and nitrate-N was analyzed by the capillary electrophoresis. The detached shoots and roots were immediately frozen in liquid nitrogen. After the plants were dried for 3 days in a ventilator oven at 80℃ and separated to the shoot, roots and nodules, then the dry weight and nodule number were measured. Total nitrogen content of the plant was determined by the Kjeldahl digestion method (OHYAMA et al. 1991).

The growth observation of a Williams plant was conducted at R5. The root system was carefully dug up using running water to washing off the sand from fine root. Almost all the roots and nodules were recovered, and the number of the stem, nodes, pods and seeds, and nodules were counted. Leaf area was measured, fresh weight and dry weight of each part was determined.

RESULTS AND DISCUSSION

The changes in concentration of the total ureide-N, amide-N, and nitrate-N in the xylem bleeding sap of Williams and NOD1-3 are shown in Fig. 1. In Williams, the level of total ureide-N was highest at 54 DAS and decreased significantly at R1. It kept at about 150 mg N L^{-1} until R5, finally it decreased to 30 mg N L^{-1} at R7. In NOD1-3, the level of total ureide-N kept high around 400 mgN L^{-1} until R3, then rapidly decreased at R5. This may be due to the result that the number of active nodules had decreased

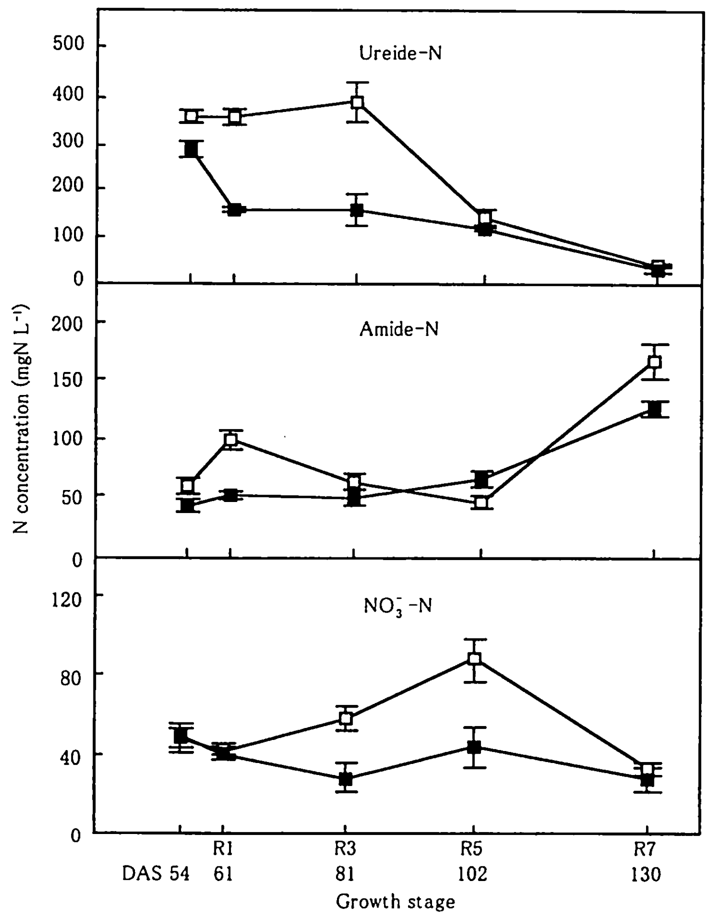

Fig. 1 Concentration of ureide-N, amide-N, and nitrate-N in xylem sap collected from Williams and NOD1-3.
■ : Williams, □ : NOD1-3

from R3 to R5. And it decreased to 30 mgN L^{-1} in R7 similar to Williams. The concentration of ureide-N was consistently higher in NOD1-3 than Williams throughout the growth stage. The nitrate-N concentration in sap was relatively constant in Williams about 40 mgN L^{-1} from 54 DAS to 130 DAS (R7). In NOD1-3, the nitrate-N concentration was higher than Williams after R1 stage, showing a peak at R5. Different from the concentration of ureide-N and nitrate-N, the amide-N concentration increased from R5 to R7 in Williams. In NOD1-3, the amide-N concentration once increased at R1 stage and decreased till R5 stage, then rapidly decreased at R7 stage like Williams. The conspicuous increase in amide-N at maturing stage was observed in non-nodulated isoline T201 and nodulated T202 with various N fertilization (OHYAMA et al. 1994). The amide-N increase at R7 may be derived from protein degradation of roots and nodules.

Fig 2 shows the percentage of relative ureide-N in the xylem sap by the equation, ureide-N/(ureide-N + amide-N + nitrate-N) × 100. In Williams relative ureide-N was about 70% until R3 stage, then decreased to 20% at R7, while in NOD1-3 relative ureide-N was about 80% until R3 and higher than Williams, but decreased from R5 to R7 similar to Williams.

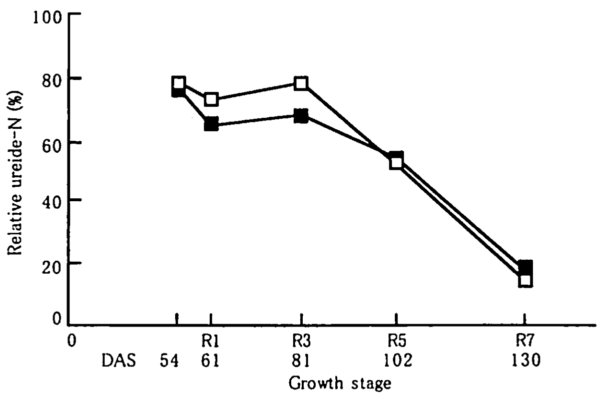

Fig. 2 Changes in the percentage of relative ureide-N in xylem sap calculated by the equation, ureide-N/(uraide-N + amide-N + nitrate-N) ×100.
■ : Williams, □ : NOD1-3

Table 1. The estimation of the amount of fixed N and absorbed N during two succussive sampling

DAS (Sampling time)	Nitrogen acquisition during two successive sampling (kgN ha^{-1})		Average percent of relative ureide-N		Amount of N from N_2 fixation (kgN ha^{-1})		Amount of N from N absorption (kgN ha^{-1})	
	Williams	NOD1-3	Williams	NOD1-3	Williams	NOD1-3	Williams	NOD1-3
54 – 61 (R1)	5.70	6.12	75.9	77.3	4.32	4.73	1.38	1.39
61 (R1) – 81 (R3)	13.88	3.59	69.8	74.7	9.70	2.68	4.19	0.91
81 (R3) – 102 (R5)	62.82	39.92	65.6	74.4	41.20	29.68	22.70	10.23
102 (R5) – 130 (R7)	119.55	53.43	60.1	63.9	71.87	34.16	47.68	19.27
130 (R7)	46.74	13.57	35.6	33.2	16.63	4.51	30.11	9.06

Nitrogen fixation activity and N absorption rate was calculated by the simple relative ureide method (TAKAHASHI et al. 1993), combined relative ureide percent (Fig. 2) and the total acquisition of N between successive sampling times (Table 1). The nitrogen content of the plant and the amount of fixed nitrogen were consistently higher in Williams than NOD1-3 throughout the growth stages. This is attributed to the inferior growth of NOD1-3 to Williams, although the relative ureide-N percent in xylem sap was higher in NOD1-3 than Williams.

Fig 3 shows daily nitrogen fixation rate and nitrogen absorption rate. Both nitrogen absorption rate and nitrogen fixation rate were shown to be high from R3 to R5 stages and decreased after R5 both in Williams and NOD1-3. In every growth period, nitrogen absorption rate and nitrogen fixation rate was higher in Williams than NOD1-3. In this way, the change of the ureide-N concentration in xylem sap that decreased from R5 to R7 may be due to the decline of the nodule activity of both soybean lines.

Table 2 shows the estimation of the overall nitrogen fixation dependence to total N. Total amount of N from N_2 fixation was 144 kgN ha^{-1} in Williams and 76 kgN ha^{-1} in NOD1-3, and that of N absorption were 106 kgN ha^{-1} in Williams and 41 kgN ha^{-1} in NOD1-3. This means 58% and 65% of N derived from N_2 fixation in Williams and NOD1-3 respectively. Although the dependence on nitrogen fixation differs by the cultivation condition, the hypernodulation mutant NOD1-3 depended more on N_2 fixation than the parent Williams.

Table 3 shows the dry weight of shoots, roots and nodules, total plants and nodule number of Williams and NOD1-3. The shoot dry weight increased rapidly from R3 to R5. The root dry weight increased from Rl to R3 in Williams, from R3 to R5 in NOD1-3. Although the number of nodules in Williams was almost about 500 in the middle growth period, those of NOD1-3 continued to increase till R5, reached to about 1600. But nodule number in Williams and NOD1-3 decreased rapidly at R7. The nodule dry weight increased to 3.9 g in Williams and 5.8 g in NOD1-3 till R5, decreased to about 1.0 g at R7.

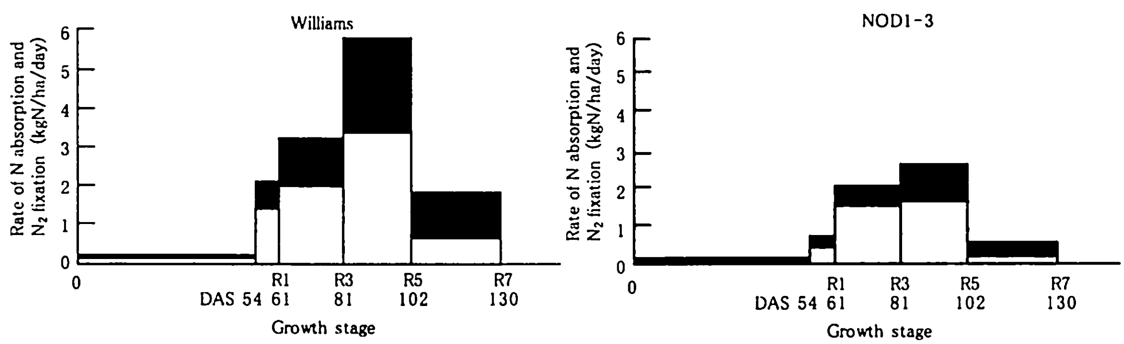

Fig. 3 Evaluation of nitrogen absorption and nitrogen fixation rate by simple ureide method
■ : Nitrogen absorption rate, □ : Nitrogen fixation rate

Table 2. Estimation of the despendence on nitrogen fixation

Rine	Total nitrogen content (kgN ha^{-1})	Total amount of nitrogen from N_2 fixation origin (kgN ha^{-1})	Dependence on nitrogen fixation (%)
Williams	250	144	58
NOD1-3	116	76	65

Table 3. The measurement of the dry weight of shoots, roots and nodules, and nodule number

Line	DAS (Sampling time)	Shoot (g plant⁻¹)	Root (g plant⁻¹)	Nodule (g plant⁻¹)	Total weight (g plant⁻¹)	Nodule number
Williams	54	7.32	1.62	0.51	9.45	151
	61 (R1)	23.50	4.61	1.35	29.46	396
	81 (R3)	122.86	18.26	2.32	143.44	434
	102 (R5)	308.15	24.63	3.90	336.68	535
	130 (R7)	358.98	20.82	1.14	380.94	148
NOD1-3	54	5.63	1.44	0.85*	7.92	437*
	61 (R1)	9.04**	1.95**	1.37	12.36**	655
	81 (R3)	60.52*	1.95**	4.31**	66.78*	1215**
	102 (R5)	142.61**	9.46*	5.84	157.91**	1702*
	130 (R7)	162.72**	7.88**	1.07	171.67**	285*

* Data for comparison of the NOD1-3 and Williams are different at $p=0.05$ levels.
** Data for comparison of the NOD1-3 and Williams are different at $p=0.01$ levels.

Table 4. The daily relative growth rate of each part

DAS (Sampling time)	Shoot (%)		Root (%)		Nodule (%)		Total plant (%)	
	Williams	NOD1-3	Williams	NOD1-3	Williams	NOD1-3	Williams	NOD1-3
54 – 61 (R1)	21.45	8.22	19.04	5.17	17.53	8.16	20.85	7.69
61 (R1) – 81 (R3)	9.09	10.52	7.51	0	2.89	6.22	8.69	9.82
81 (R3) – 102 (R5)	4.71	4.38	1.51	8.21	2.65	1.54	4.36	3.92
102 (R5) – 130 (R7)	0.57	0.49	−0.62	−0.67	−4.45	−6.10	0.46	0.31

Table 5. Nitrogen concentration and total nitrogen content

DAS (Sampling time)	Nitrogen concentration (g g⁻¹)		Total nitrogen content (g Plant⁻¹)	
	Williams	NOD1-3	Williams	NOD1-3
54	0.030	0.038	0.285	0.306
61 (R1)	0.033	0.039	0.979	0.486
81 (R3)	0.029	0.034	4.120	2.471
102 (R5)	0.028	0.033	9.541	5.153
130 (R7)	0.030	0.034	11.49	5.831

Fig. 4 Potographs of a Williams at R5 on August 30, 2000 in sandy dune field of Faculty of Agriculture of Niigata University. A scale indicates 10 cm.

Table 4 shows the daily growth rate of each part calculated by the equation, $a(x+1)^{n-1}=b$, where, x is daily increase percent of DW, a and b are dry weight of successive sampling, and n is the days between sampling. It is interesting to note that in all parts the growth rate of NOD1-3 from 54 DAS to 61 DAS (R1) was lower in comparison with Williams. It was possible that the severe competition of photosynthate occurred because there were excess number of nodule in NOD1-3, and the nourishment absorption was lowered because the growth of root was depressed remarkably at flowering stage. It was suggested that the growth around R1 was very important in plant growth, nitrogen fixation activity and nitrogen absorption rate. Table 5 shows nitrogen concentration and total nitrogen content throughout growth stage. Although the nitrogen concentration in NOD1-3 was always higher than Williams, total nitrogen content in NOD 1- 3 was lower than Williams.

One example of Williams was carefully taken from sandy field without loosing roots and nodules (Fig. 4). In this cultivation plant growth was vigorous due to low planting density. It had 17 branches, 178 total nodes, 3.44 pods per node, 2.74 seeds per pod and 1687 total seeds. The length of main stem was restrained at 87.5 cm and the diameter of the stem was 25 mm. Because the basement of the stem and root were extremely strong, it didn't lodge in spite of the vigorous growth. There were 1874 nodules per plant and the nodule flesh weight was 45 g. The dry weight per plant reached 564 g (leaf: 100 g, stem: 204 g, pod: 220 g, root: 41 g, nodule: 9 g) and the yield was estimated to be at 7.16 t ha^{-1}. It was reported that the yield of soybean depended on total pods and total nodes number per plant (TAMURA et al. 1993). Although the rate of effective pods number and nodes number were about 70% respectively, the final yield was 2.2 t ha^{-1}. It was considered that the decrease of yield resulted from the damage of insect.

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圃場栽培におけるダイズ品種 Willams と根粒超着生変異株 NOD1-3の生育と窒素固定活性の比較

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（平成12年11月20日受付）

摘　　要

ダイズ根粒超着生変異株 NOD1-3とその親株である品種 Williams を新潟大学農学部砂丘圃場において栽培密度2.0株 m^{-2}で栽培し、播種54、61（R1）、81（R3）、102（R5）、130（R7）日後に試料採取を行った。導管液中の主な窒素化合物（ウレイド態窒素・アミド態窒素・硝酸態窒素）の濃度は、比色法及びキャピラリー電気泳動装置を用いて分析した。ウレイド態窒素濃度は、Williams よりも NOD1-3の方が全生育を通して常に高かった。アミド態窒素は、Williams、NOD1-3共に R5期までは一定値を示し、R7期に急上昇した。NOD1-3の硝酸態窒素濃度は、R1以降 Williams よりも常に高く、R5で最も高い濃度を示した。単純相対ウレイド法により、どちらの株も窒素固定速度・窒素吸収速度が、R3から R5にかけて最も高くなり、それ以降は減少した。全生育期間を通して Williams の方が乾物生産が2倍程度多く、窒素固定速度、窒素吸収速度ともに常に高かった。NOD1-3では、開花期頃に生長の抑制が認められ、これは、根粒が過剰に着生することによる光合成産物供給のアンバランスと、根の生長が抑制されたことによる養水分の低下が主な原因と考えられる。また、窒素固定依存率は、Williams で58%、NOD1-3では、65%と NOD1-3が高かった。

Williams において粒肥大期後期に根や根粒をできるだけ完全に回収して生育調査を行った。一株の分枝数17本、総節数178個、節当たり莢数3.44個、莢当たり粒数2.74個で、総種子数1874個に及んだ。極めて旺盛な生育にも関わらず、主茎長87.5cm に抑えられ、茎径25mm と茎と根の基部が極めて強靱で倒伏もなかった。この時、株当たりの根粒数は1874個もあり、根粒生体重は45g に及んだ。また植物体乾物重は564g で、推定収量は7.16t ha^{-1}であったが、害虫の被害のため最終的な収量は2.2t ha^{-1}にとどまった。

キーワード：ダイズ、相対ウレイド法、窒素固定速度、窒素吸収速度