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Effects of Nitrate Gradients on Growth and Nitrogen Economy of Soybean Plant

Kim, Sung-Jun, Yeon-Sik Choo and Seung-Dal Song
(*Dept. of Biology, College of Natural Science, Kyungpook National University*)

대두의 생장 및 질소 경제에 미치는 Nitrate 구배의 영향

김성준 · 추연식 · 송승달
(경북대학교 자연대 생물학과)

ABSTRACT

Soybeans(*Glycine max* Merr. cv. Kwanggyo), inoculated with *Rhizobium japonicum* 110 and then sand-cultured with nitrate gradients (0, 1, 3, 10 and 30 mM KNO_3), were studied on the growth analysis, nitrogen fixation and nitrogen economy during the growing period.

The maxium values of total leaf area, biomass and nitrogen quantity were increased 139%, 122% and 161%, respectively with higher concentration of nitrate treatment. Nodulation showed significant linear correlation with leaf area growth for each treatment of nitrate concentration increased. The more nitrate concentration increased, the more distribution ratios of dry matter and nitrogen to nodule decreased, and the more T/R ratios, CGR and N content increased. On the other hand, F/C ratios and RGR showed little changes. The amounts of nitrogen fixation of soybean alloted to 0, 1, 3, 10 and 30 mM nitrate treatments were 100, 46, 14, 0.1 and 0.004% for the total nitrogen assimilation, respectively. The nitrogen utility of soybean plant was smaller than that of other plants and ranged from 23 to 30 at varying nitrate gradients.

INTRODUCTION

Nitrogen economy of plant is characteristic for species and determines the adaptation limit of plant growth to the environmental nitrogen sources. Many investigations on the nitrogen economy such as dynamics of pool size, mobility and turnover rate of nitrogen in plants have been reported in the level of several plant communities and populations (Song and Monsi, 1974; Hirose and Monsi, 1975; Song, 1977; Libardi and Harper, 1982).

All the higher plants need nitrogen sources of inorganic NO_3^- and NH_4^+ fertilizers supplied by the biological or nonbiological fixation from atmospheric nitrogen gas. However, certain nodule bearing leguminous or nonleguminous plants could grow with nitrogen gas by the symbiosis with effective N_2-fixing organisms (Sprent, 1979; Postgate, 1982; Song, 1983). While, the nodulation and N_2 fixation activity of these symbiotic systems are affected significantly by the combined nitrogen fertilizer and other environmental factors (Wever, 1966; Hatfield *et al.*, 1974; Semu and Hume, 1979; Schweitzer and Harper, 1985; Streeter, 1985).

Although the growth of nodulating plants is promoted by the nitrogen fertilization, the combined nitrogen in the circumference repressed the formation of nodules and the nitrogenase activity of the symbiotic organisms (Allos and Bartholomew, 1959). It is necessary to understand quantitatively the influences of environmental nitrogen compounds on the growth charateristic of nodulating plant, N_2-fixing activity of symbiotic system and the dynamics of nitrogenous substances in plant.

The present study was designed to examine the effects of various nitrate gradients on the growth analytical characteristics, nodulation, N_2-fixation activity, N assimilation rates, distribution patterns of total nitrogen and the nitrogen utility of soybean plant during the growing period.

MATERIALS AND METHODS

Seeds of soybean (*Glycine max* Merr. cv. Kwanggyo) were selected as mean sizes and planted in plastic pot of 20 cm diameter. Plants were inoculated with ***Rhizobium japonicum*** strain 110 enriched in the minimal medium and grown in sand culture with density of 10 seedlings per pot. Treatments of nitrate gradients were made up of 0, 1, 3, 10 and 30 mM KNO_3 with N-free Boysen-Jensen medium. Each pot was watered daily with 200 ml of culture medium of the respective treatment.

Plant sampling was carried out for each nitrate treatment every two weeks during the growing period, and measured height, leaf area, and fresh and dry weight of each organ. Total leaf area was determined by the intergration of each leaf area measured by multiplication of length/width of the leaf. Triplicate samplings were carried out for the determination and analysis of plant growth and nitrogen contents.

During the growing period plants grown at various nitrate gradients were analyzed for the allocation ratio to each organ, F/C ratio, T/R ratio, RGR, NAR and CGR in dry matter and nitrogen quantity by growth analytical methods (Blackman, 1919; Buttery, 1969).

The nitrogen fixation activity of root nodules treated with nitrate gradients was measured by the acetylene reduction method with gas chromatography equipped with a H_2-frame ionization detector and a column of Porapak R (182×0.32 cm). N_2-fixation rate was calculated with conversion ratio of 1.5 : 1.0(acetylene reduced : N_2-fixed) (Evans *et al.*, 1973). Total nitrogen content was determined by the micro-Kjeldahl method (Song and Monsi, 1974). The amount of N_2-fixation of symbiotic plant treated with various nitrate gradients was determined by the total nitrogen quantity of N-free treated plant multiplied by the ratio of nodule activity of nitrate treated plant to that of N-free treated plant. The nodule activity was calculated by multiplication the nodule amounts by the specific activity of nodules. The nitrogen utility (NU) was compared as the ratio of biomass to the total amount of nitrogen quantity of the plant (Hirose, 1971).

RESULTS AND DISCUSSION

Changes of leaf area growth of soybean treated with various nitrate gradients showed sigmoid curves with little differences among treatments during the growing period (Fig. 1). The maximum leaf area for the treatments of 0, 1, 3, 10 and 30 mM of nitrate gradients were 4,020, 4,450, 5,200, 5,620 and 5,590 cm^2 per plant, respectively in the 98th day after sowing. The plants of control and 1 mM NO_3^- treatment plots showed shedding phenomenon in the lower part of leaves and significantly diminished leaf area as compaired with the plants of higher nitrate gradients. And the maximum heights of plants were 73, 77, 84, 80 and 74 cm in the 98th day, respectively for 0, 1, 3, 10 and 30 mM of nitrate gradients. Statistical analysis of all data showed no significant differences among treatments.

The changes of standing crop showed also little variations among nitrate gradients, but continued the increases with the seed ripeness even after the peak growth of leaf area and height of plant. The maximum biomass of soybean plant was 26.6, 29.8, 31.1, 32.2 and 32.5 g dw per plant, respectively for 0, 1, 3, 10 and 30 mM of nitrate gradients (Fig. 2).

The average total nitrogen content of soybean changed gradually from 6% in the earlier growth to 4% in the later growth. And the average total nitrogen content with higher nitrate gradients was a little higher than that of the lower nitrate gradients. Fig. 3 illustrates the seasonal changes of total nitrogen content in each organ of soybean plant treated with various nitrate gradients.

Seasonal changes of the total nitrogen quantity of soybean treated with nitrate gradients

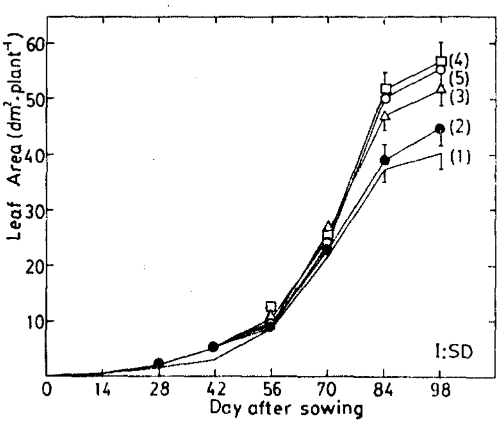

Fig. 1. Effect of nitrate gradients on leaf area growth of soybean during the growing period: (1), 0; (2), 1; (3), 3; (4), 10; (5), 30 mM NO_3^-.

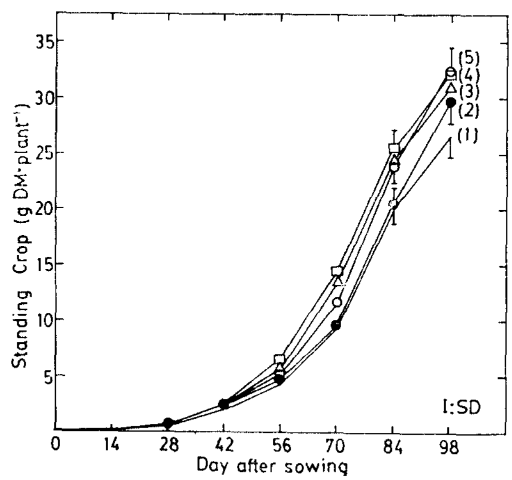

Fig. 2. Effect of nitrate gradients on standing crop of soybean during the growing period: (1), 0; (2), 1; (3), 3; (4), 10; (5), 30 mM NO_3^-.

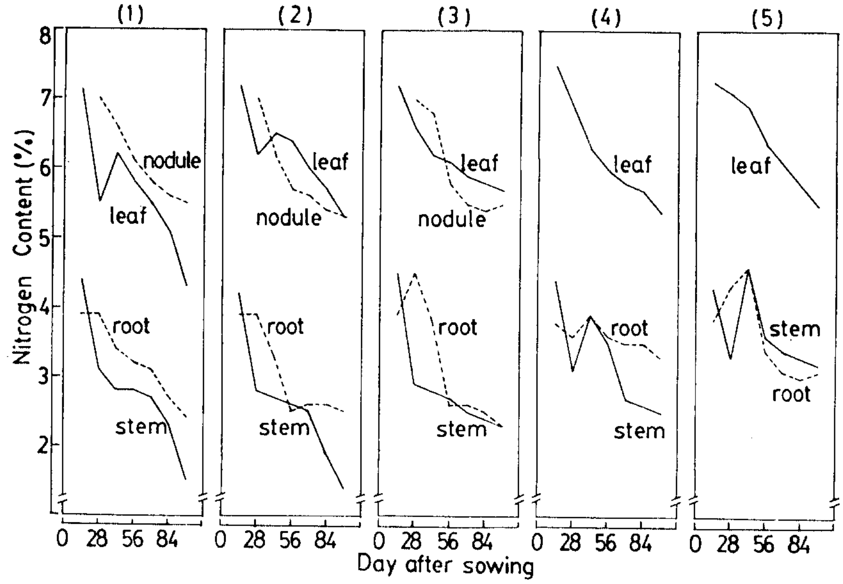

Fig. 3. Effect of nitrate gradients on the N content in each organ of soybean during the growing period: (1), 0; (2), 1; (3), 3; (4), 10; (5), 30 mM NO_3^-.

are shown in Fig. 4. The maximum amounts of total nitrogen quantity were 884, 1,036, 1,224, 1,230 and 1,419 mg N per plant, respectively for 0, 1, 3, 10 and 30 mM of nitrate gradients. The total nitrogen quantity was accumulated more in plants with higher nitrate

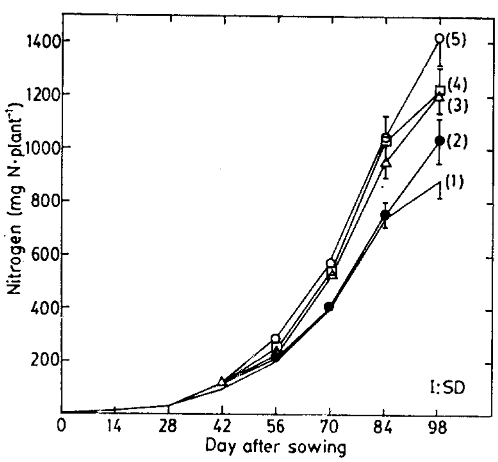

Fig. 4. Effect of nitrate gradients on total N amount of soybean during the growing period: (1), 0; (2), 1; (3), 3; (4), 10; (5), 30 mM NO_3^-.

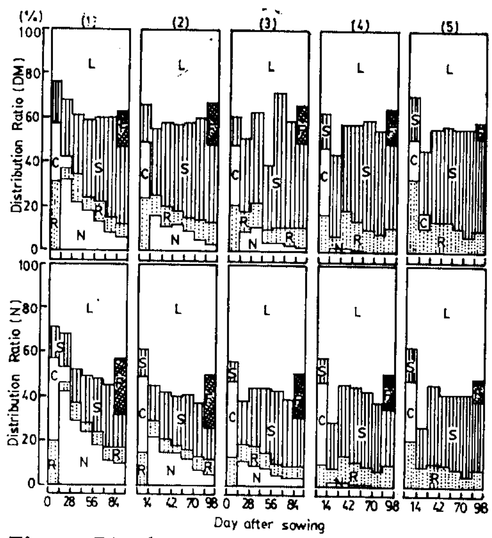

Fig. 5. Distribution ratios of the dry matter and the total N quantity to each organ of soybean treated with nitrate gradients during the growing period. L, S, R, N and F stand for leaves, stems, roots, nodules and fruits, respectively: (1), 0; (2), 1; (3), 3; (4), 10; (5), 30 mM NO_3^-.

gradients.

During the plant development the distribution patterns of dry matter and total nitrogen to each organ were illustrated in Fig. 5. The allocation ratio of dry matter to leaf increased from 25% in the earlier growth to 41% in the exponential growth, whereas that to stem increased continuously to the maximum ratio of 45% in the later growth. And both of them decreased during the ripening of fruit. On the other hand, the distribution ratio to root showed only little change, and that to nodule showed the maximum of 30% in the earlier growth. On the treatments with higher nitrate gradients, the allocation ratio to leaf was higher than that of the control plot in the earlier growth, but that to nodule was significantly diminished. On the total nitrogen quantity, the allocation ratio to nodule and leaf was remarkably high during the growing period in comparison with that of dry matter. And that ratios to stem and root were relatively low. Similary with higher nitrate gradients the allocation ratio of total nitrogen showed increase for leaf and decrease for nodule, stem and root.

Table 1 summarized the growth characteristics of F/C ratio, T/R ratio, RGR, NAR and CGR of dry matter and total nitrogen quantity of soybean plant treated with various nitrate gradients during the exponential growth. The ratio of photosynthetic organ to nonphotosynthetic one (F/C) increased from 0. 5 to 1. 2 in dry matter and from 0. 5 to 2. 7 in total nitrogen quantity by the treatment of higher nitrate gradients. It is explained by the rapid development of photosynthetic organ with the supply of combined nitrogen. The ratio of aboveground parts to underground ones (T/R) increased also from 1. 2 to 13. 0 in dry matter and from 1. 2 to 11. 8 in total nitrogen quantity by the treatments of higher nitrate gradients. The growth of underground organs was significantly inhibited by higher nitrate gradients. The relative growth rate (RGR) showed little effects of nitrate gradients and attained the highest value of 0. 09 g $dw \cdot g^{-1} \cdot day^{-1}$ in the earlier growth and the lowest value of 0. 02 g $dw \cdot g^{-1} \cdot day^{-1}$ in the later growth for the control plant. The net assimilation ratio (NAR) showed also the maximum value of 45. 3 mg $dw \cdot dm^{-2} \cdot day^{-1}$ in the earlier growth and the minimum value of 10 mg $dw \cdot dm^{-2} \cdot day^{-1}$ in the later growth for the control plant. The crop growth rate (CGR) was obtained the maximum value of 763 mg $dw \cdot dm^{-2} \cdot$

Table 1. Effect of nitrate gradients on the growth characteristics of soybean in dry matter (DM) and nitrogen quantity (N)

Growth Characteristics	DM					N				
	NO_3^- treatment (mM)					NO_3^- treatment (mM)				
	0	1	3	10	30	0	1	3	10	30
F/C (28th day)	0. 5	0. 8	1. 0	1. 3	1. 2	0. 5	1. 2	1. 7	2. 4	2. 7
T/R (28th day)	1. 6	2. 9	4. 7	13. 0	4. 7	1. 2	2. 4	4. 8	11. 8	9. 9
RGR* (42th day)	0. 09	0. 11	0. 09	0. 08	0. 10	0. 08	0. 10	0. 08	0. 08	0. 11
NAR** (42th day)	45. 3	41. 7	32. 5	30. 7	38. 4	2. 20	1. 94	1. 52	1. 46	2. 20
CGR*** (84th day)	763	792	796	801	900	24. 4	25. 1	30. 6	32. 5	36. 8

RGR*: $g \cdot g^{-1} \cdot day^{-1}$, NAR**: $mg \cdot dm^{-2} \cdot day^{-1}$, CGR***: $mg \cdot dm^{-2} \cdot day^{-1}$

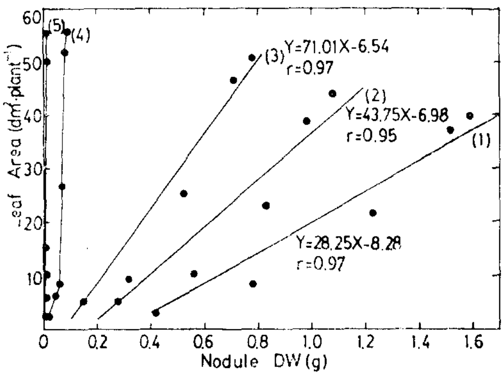

Fig. 6. Relationships between leaf area (Y) and nodule dry weight (X) of soybean treated with nitrate gradients: (1) 0; (2) 1; (3) 3; (4) 10; (5) 30 mM NO_3^-.

day^{-1} in the later growth for the control plant. Growth analyses of RGR, NAR and CGR on the total nitrogen assimilation showed similar patterns with those of the dry matter and greater differences among nitrate gradients (Table 1).

The relationship between leaf area and nodule dry weight of soybean showed a satisfied linear equation with high correlation for each treatment of nitrate gradients (Fig. 6).

The changes of nitrogen fixation by nodules of soybean plant treated with nitrate gradients are shown in Table 2. Treatment with higher nitrate gradients deteriorated the nitrogen fixation efficiency by hindering the nodulation and the specific activity of nitrogenase. The nodules began to be formed in two weeks after planting and increased exponentially during the active growth of biomass. The maximum nodule amount was 1,590, 1,050, 780, 90 and 8 mg dw per plant, respectively for 0, 1, 3, 10 and 30 mM of nitrate gradients in the 98th day. The specific activity of N_2-fixation of nodules attained the maximum rate of 25.0, 18.0, 6.7, 0.9 and 0.4 nM $C_2H_4 \cdot mg \cdot dw^{-1} \cdot hr^{-1}$, respectively for each treatment of nitrate gradients in the 28th day. The total activity of acetylene reduction of soybean increased continuously by the remarkable increment of nodule amount despite of the decrease of specific activity of N_2-fixation with age. The maximum values were 81.3, 35.5, 15.7, 0.1 and 0.004 μM $C_2H_4 \cdot plant^{-1}$, respectively for each treatment of nitrate gradients in the later growth.

Table 2. The amounts of nitrogen fixation by nodules of soybean treated with nitrate gradients during the growing period

Period (day)	Nodule activity (μM $C_2H_4 \cdot Plant^{-1} \cdot Hr^{-1}$)					Amount of fixed N ($mg\text{-}N \cdot Plant^{-1}$)				
	NO_3^- treatment (mM)					NO_3^- treatment (mM)				
	0	1	3	10	30	0	1	3	10	30
~28	12.60	4.55	1.07	0.06	0.001	19.9	7.19	1.69	0.09	0.002
~42	30.85	14.00	3.62	0.10	0.002	66.6	30.22	7.81	0.22	0.004
~56	44.50	23.60	7.50	0.09	0.004	102.5	54.36	17.28	0.21	0.009
~70	62.40	30.45	12.40	0.10	0.006	199.7	97.29	39.62	0.32	0.020
~84	76.80	34.15	15.65	0.10	0.005	341.3	151.8	69.55	0.44	0.020
~98	81.30	35.50	15.65	0.10	0.004	143.6	62.70	27.64	0.18	0.007

The total amount of nitrogen assimilation was supplied either by symbiotic N_2-fixation from nodules or by absorption of combined nitrogen from soil during the plant growth. Seasonal changes of the N_2-fixation and absorption showed remarkable differences by nitrate gradients as illustrated in Fig. 7. The total amount of nitrogen increased during the growth

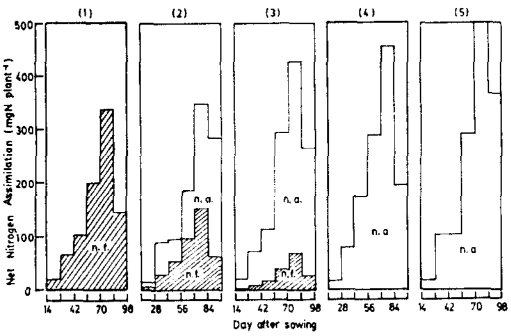

Fig. 7. Seasonal changes of nitrogen fixation (n.f.) and nitrogen absorption (n.a.) in soybean treated with nitrate gradients: (1) 0; (2) 1; (3) 3; (4) 10; (5) 30 mM NO_3^-.

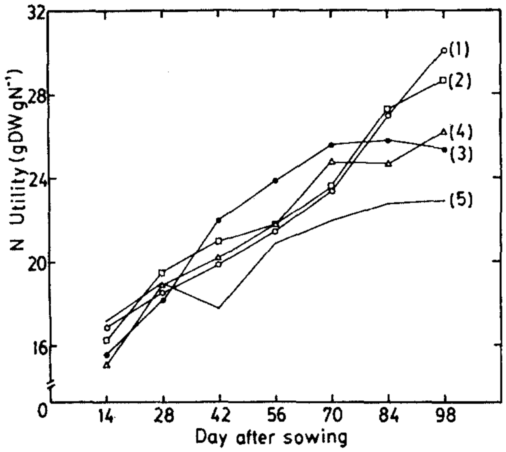

Fig. 8. Effect of nitrate gradients on nitrogen utility (NU) of soybean during the growing period: (1) 0; (2) 1; (3) 3; (4) 10; (5) 30 mM NO_3^-.

of control plant was 884 mg N·plant^{-1} indicating the amount of nitrogen fixation only by nodules. The percentage of N_2-fixation amount for the total nitrogen assimilation accounted for 45.6% of 1,025 mg N·plant^{-1} in the 1 mM of nitrate treatment, 13.5% of 1,210 mg N·plant^{-1} in the 3 mM of nitrate treatment, 0.1% of 1,216 mg N·plant^{-1} in the 10 mM of nitrate treatment and less than 0.01% of 1,419 mg N·plant^{-1} in the 30 mM of nitrate treatment.

The nitrogen utility (NU) of soybean plant showed very low values without regard to nitrate gradients in the earlier growth and increased in the later growth (Fig. 8). The values of NU increased from 16.0 for all treatments in the earlier growth to 30.1, 28.7, 25.8, 26.2 and 22.9, respectively for 0, 1, 3, 10 and 30 mM of nitrate gradients in the later growth. As Hirose (1978) reported in the study of a *Solidago altissima* population, the NU became larger with the progress of plant growth and had variations according to environmental conditions. And each species had a characteristic NU range to adapt its environment. Since the NU is an important determinant to limit the developmental process of plant, the species having a small NU will have a certain competitive advantage in the habitat of higher nitrogen availability, whereas the species having a large NU will grow well in the poor nitrogen habitat. In conclusion, the symbiotic nitrogen fixing plant like soybean maintained very low value of the NU through the growing period, but was effected little to nitrogen gradients accumulated in the soil and showed a characteristic pattern of nitrogen economy satisfying in the habitat of poor nitrogen environment.

摘 要

대두품종 "광교"(*Glycine max.* Merr. cv. Kwanggyo)에 질소고정균 ***Rhizobium japonicum*** 110을 접종하고 0, 1, 3, 10 및 30 mM 질산구배의 배양액으로 사경 재배하여 생육기간 중 생장해석과 질소고정 및 질소경제를 분석하였다.

고농도의 질산처리구에서 총엽면적, 현존량 및 총질소량은 대조구에 비해 각각 139%, 122% 및 161%의 증가를 보였고 근류의 형성은 각 처리구에서 엽면적 생장과 유의한 직선상관을 보였다.

NO_3^- 구배가 클수록 건물과 질소량에 대한 기관별 분배율은 근류에서 감소하고, T/R비, CGR 및 N함량은 증가하였으며, F/C비와 RGR에는 큰 변화가 없었다. 질소고정량은 NO_3^- 농도구배에 따라 급격히 감소되어 총질소 동화량에 대해 각각 100, 46, 14, 0.1 및 0.004%의 질소고정을 보였다. NU값은 타 식물에 비해 낮은 값으로서 구배처리에 따라 23~30의 범위에서 변화를 보였다.

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