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Dry Matter, Nitrogen Distribution and Organic Reserves Accumulation as Affected by Nitrate Supply Level in Alfalfa (*Medicago sativa* L.)

Tae-Hwan Kim, Woo-Jin Jung, Bok-Rye Lee, Dae-Hyun Kim and Kil-Yong Kim*

질산태 질소의 공급수준에 따른 알팔파의 건물, 질소의 분포 및 저장영양소의 축적

김태환 · 정우진 · 이복례 · 김대현 · 김길용*

ABSTRACT

To investigate the effects of the exogenous NO_3^- supply level on the nitrate assimilation and growth during the vegetative growth stage, and on the accumulation of organic reserves during the successive regrowth period, dry matter (DM), the amount of nitrogenous compounds, total N and starch in alfalfa plants grown hydroponically with 0.2, 1.0 and 3.0 mM KNO_3 was estimated, respectively, during vegetative growth period and two cycle of regrowth. When compared with DMs and N contents in various N compounds in the organs grown with 1.0 mM NO_3^-, N starvation symptoms were found in 0.2 mM and a depressive effect was observed in 3.0 mM after 10 weeks of vegetative growth. Total starch content in root system grown with 0.2, 1.0 and 3.0 mM NO_3^- during the first regrowth was 50.96, 15.47 and 6.37 mg $plant^{-1}$, respectively. Starch was contained mainly in taproots. The starch content was not significantly changed by 24 days of the second regrowth with 1.0 mM NH_4NO_3. Total nitrogen content in root system grown with 0.2, 1.0 and 3.0 mM NO_3^- during the first regrowth was 6.66, 8.43 and 11.09 mg $plant^{-1}$, respectively. Nitrogen was contained mainly in lateral roots; 80% (in 0.2 mM), 74% (1.0 mM) and 76% (3.0 mM) of total nitrogen in root system. Total N content in root system at the end of the second regrowth also closely affected by the NO_3^- supply level during the first regrowth. These results suggest that the level of NO_3^- may strongly influence the accumulation of organic reserves in root system, and that the initial level of organic reserves for the successive regrowth was one of the determinants for shoot regrowth.

(**Key words** : Alfalfa, NO_3^- supply level, Growth, N assimilates, Starch)

I. INTRODUCTION

Nitrogen appears to be the major nutrient limiting primary production of forage plants. Nitrate is usually the major form of inorganic nitrogen available to higher plants. Under natural

전남대학교 농업생명과학대학 동물자원학부 & 농업과학기술연구소(Department of Animal Science, Institute of Agricultural Science & Technology College of Agriculture and Life Science, Chonnam National University, Gwangju 500-757, Korea)

* 전남대학교 농업생명과학대학 농화학과(Department of Agricultural Chemistry, College of Agriculture and Life Science, Chonnam National University, Gwangju 500-757, Korea)

conditions it commonly occurs at concentrations of 1 mM or less in the interstitial water of soils (Andrews, 1986), although in arid areas greater levels may build up (Russell. 1973). In agricultural soils nitrate concentration can be as high as 20 mM because of the addition of N fertilizer (Reed and Hageman, 1980; Andrews, 1986).

Within the plant, nitrate must first be reduced to ammonium before being assimilated into amino acids (Beevers and Hageman, 1983). In healthy plants grown on nitrate, both nitrite and ammonium assimilation are rapid, with neither form of inorganic N playing a major role in storage or intercellular transport (Schrader and Thomas, 1981; Pate, 1980). It is commonly considered that, regardless of growth conditions, most nitrate assimilation is carried out in the roots of some genera, e.g. *Lupinus* and *Vicia*, and in the shoots, in particular leaves, of others, e.g. *Xanthium* and *Stellarid* (Beevers, 1981; Pate, 1980; Smirnoff and Stewart, 1985). It have been well established the cellular NO_3^- is separated with two pools, one metabolizable pool in cytoplasm and the other stored pool in vacuole (Ferrari et al., 1973; Martinoia et al., 1981). Most of cellular NO_3^- in vacuolar pool, while only small part of NO_3^- in the cytoplasmic pool and in the xylem flow are readily available for reduction (Shaner and Boyer, 1976).

For the past 20 years, attention has been given to N reserves mobilization with respect to regrowth dynamics in alfalfa. About 60 to 80 % of the total N in regrowing shoots is derived from N reserves in roots from the first 10 days of regrowth (Kim et al., 1991; 1993b). Amino N seems to be the most readily available form of N (Kim et al., 1993a; Hendershot and Volenec, 1993) and protein N the largest storage form (Ourry et al., 1989; Kim et al, 1991).

A question arises from the these data how does respond the nitrate assimilation in whole plant in relation to the exogenous NO_3^- supply level during the vegetative growth stage, and how dose it affect consequently on the accumulation of organic reserves. To answer these question, dry matter, the amount of nitrogenous compounds during vegetative growth period, and total N and starch accumulation in root system during two cycle of regrowth were estimated.

Ⅱ. MATERIALS AND METHODS

1. Plant Culture and Experimental Design

Alfalfa seeds (*Medicago sativa* L. cv. Vernal) were sterilized and germinated on sand, 15 seedlings were transplanted into 9 L culture pot when the primary trifoliate leaves had developed. Plants were grown hydroponically in a continuously aerated nutrient solution as described previously by Kim et al. (1991). The natural light was supplemented with fluorescent tubes (150 μmol m^{-2} s^{-1} at height of the canopy) for 16 h per day. The thermoperiod was 23℃ (day) and 18℃ (night).

Plants were grown in a complete nutrient solution containing 0.2, 1.0 and 3.0 mM KNO_3 with 9 replications with different pot until the early flowering stage (about 10 weeks old). Three pots from each treatment were harvested. The harvested plants were separated into lateral roots, taproots, crown leaves, crown stems, principal stems, secondary stems and leaves. Samples were dried at 60℃ for 48 h for the determinations of dry weight and nitrogenous compound during vegetative growth period. The shoots in remaining 6 pots were cut to a height

of 6 cm above taproot level. They were then regrown with a same level of KNO_3 for 24 days and root samples were harvested from 3 pots to estimate the accumulated organic reserves. After the second cutting, the last 3 pots of each treatment were regrown with 1 mM NH_4NO_3 for 24 days and root samples were prepared for determination of organic reserves.

2. Chemical analysis

The soluble and insoluble N fractions were separated by 2 successive extractions with boiling water-ethanol-chloroform according to Pace et al., (1982). Nitrate and amino N of the soluble fraction were further separated using a Dowex 50 H+ column. Nitrate was measured after reduction to nitrite on CdCu columns by the colorimetric method. Insoluble N (protein-N) and amino N were mineralized by a Kjeldahl procedure. After materialization in Conway dishes, colorimetric determination of ammonium was performed with Nessler's reagent (Kim and Kim, 1996).

Ⅲ. RESULTS AND DISCUSSION

1. Dry Matter and Nitrogenous Compounds during Vegetative Growth Period

The growth of different organs in response to the exogenous nitrate supply level was estimated by the biomass produced during 10 weeks of vegetative growth. Dry matters of each organ of the plants grown with 0.2, 1.0 and 3.0 mM KNO_3 are given at Fig. 1. The dry matter of all organs exposed to 0.2 mM was largely lower than that of other 2 nitrate levels, suggesting

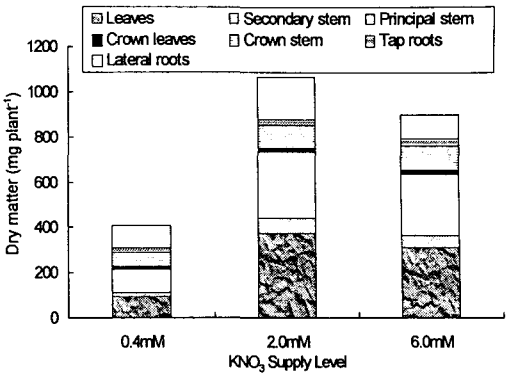

Fig. 1. Effects of nitrate supply level on the dry matter accumulation in different organs of 10 weeks old non-nodulated alfalfa plants.

nitrate nutrition with 0.2 mM is insufficient for the proper growth of alfalfa. By comparison with the plants grown with 1.0 mM, it could be found that the N starvation in 0.2 mM NO_3^- caused to reduction of 80% leaf mass and 50% root mass. The reduction of stem number has also been found. When compared with the plants grown with 1.0 mM NO_3^-, 3.0 mM NO_3^- nutrition showed a depressive effect in dry matter production, showing 14% of total dry mass reduction. In plants grown with 3.0 mM NO_3^-, about 17% and 10% of reduction in dry matter of leaves and stems occurred, respectively, compared with the plot of 1.0 mM. Dry matter of crown leaves and stems was not significantly different between 1.0 mM and 3.0 mM KNO_3 supply. Dry mass of lateral roots grown with 3.0 mM largely decreased (43% in comparison with 1.0 mM), while that of taproots was not significantly changed. The depression of fibrous roots development and growth under excessive N supply has been reported in pine trees (Fraig and Mohr, 1992) and forage legumes (Pate, 1980).

The content of N compounds in alfalfa organs

supplied with different NO_3^- supply levels are summarized in Table 1. N contents of all 3 compound in most organs for the plants supplied with 0.2 mM NO_3^- was significantly lower than the plots exposed to 1.0 and 3.0 mM. Proteins-N was found to be the largest pool of N in all organs examined, representing 83~87% of total N in leaves, 58~80% in stems and 63~82% in root system. The contents of proteins-N in leaves, taproots and lateral roots grown with 0.2 mM was 31%, 38% and 20% lower than those of 1.0 mM. Protein-N in stems and crown was not significantly different between 0.2 mM and 1.0 mM NO_3^-. When compared with the proteins-N content in all organs of the plants grown 1.0 mM and 3.0 mM NO_3^-, non-significant increases in proteins-N was found by increasing NO_3^- supply level to 3.0 mM. It was noteworthy that nitrate-N in leaves, taproots and lateral roots was significantly increased by increasing NO_3^- supply level to 3.0 mM, showing 2, 5 and 2 times higher content, respectively. These results suggest that an excessive inflow of NO_3^- over the N demands for protein synthesis leads NO_3^- to be stored in vacuole without a further assimilation. Taproot, representing the highest nitrate-N, seems to be one of N storage organs. Our results indicate that the shoot contribution to whole plant NO_3^- reduction increases at high NO_3^- concentration in the solution, and nearly equal to the root contribution at 3.0 M external NO_3^-. Hence, NO_3^- reduction in alfalfa at full vegetative growth appears to be primarily limited by the xylem flux of NO_3^-. This conclusion is in good agreement with the hypothesis that distribution

Table 1. Effects of nitrate supply level on the composition of nitrogenous compound in different organs of 10 weeks old non-nodulated alfalfa plants. Each value is the mean±s.e. for n=3.

KNO_3 Level	N Compounds	Leaves	Stems		Crown	Roots	
			Principal	Secondary	Stem	Tap	Lateral
0.2 mM	P-N*	2.7±0.21	0.9±0.05	1.4±0.08	0.7±0.06	1.0±0.06	2.0±0.11
	AA-N	0.3±0.01	0.2±0.01	0.3±0.02	0.2±0.06	0.2±0.08	0.2±0.04
	NO_3^--N	0.1±0.01	0.02±0.0	0.02±0.0	0.01±0.0	0.02±0.0	0.4±0.03
1.0 mM	P-N	3.9±0.24	1.1±0.05	1.8±0.06	1.0±0.08	1.6±0.17	2.5±0.16
	AA-N	0.5±0.03	0.3±0.02	0.3±0.02	0.2±0.01	0.3±0.02	0.4±0.02
	NO_3^--N	0.2±0.01	0.5±0.04	0.7±0.05	0.1±0.01	0.2±0.02	0.4±0.01
3.0 mM	P-N	3.9±0.31	1.1±0.04	2.0±0.12	1.1±0.08	2.2±0.12	2.8±0.16
	AA-N	0.4±0.03	0.2±0.01	0.4±0.03	0.2±0.08	0.2±0.01	0.8±0.02
	NO_3^--N	0.4±0.01	0.5±0.03	1.0±0.08	0.2±0.06	1.0±0.05	0.9±0.03

* P-N : proteins-N; AA-N: amino acids-N; NO_3^--N: nitrate-N

of NO_3^- reduction between roots and shoots in mainly dependent on the ability of the roots to export NO_3^- to the shoots (Radin, 1978; Gojon et al., 1991).

2. Starch and Nitrogen Accumulation in Root System during Regrowth Period

Starch accumulation in root system as affected by different NO_3^- supply levels during the first regrowth and by the same level of exogenous nitrogen (1.0 mM NH_4NO_3) during the second regrowth is presented in Fig. 2. Total starch content in root system was strongly increased by reducing NO_3^- supply level during the first regrowth. Total starch content in root system grown with 0.2, 1.0 and 3.0 mM NO_3^- during the first regrowth was 50.96, 15.47 and 6.37 mg $plant^{-1}$, respectively. Starch was contained mainly in taproots; 51% (in 0.2 mM), 59% (1.0 mM) and 71% (3.0 mM) of total starch in root system. There was no significant difference in accumulation between the beginning and the end of the second regrowth, suggesting that a full cycle of depletion - reconstitution of starch occurred within 24 days. Total content of starch in root system at the end of the second regrowth showed a very similar pattern with that of the end of the first regrowth, although a same level of N was supplied for all three treatments during 24 days of regrowth. The starch content in taproots at the end of the second regrowth (T1: 0.2 mM NO_3^- during the first regrowth) was 33.67 mg $plant^{-1}$. This starch content in T2 and T2 at the end of the second regrowth was decreased to 22.3% and 14.6% of T1. This effect of low N supply on starch accumulation was previously noted by Fishbeck and Phillips (1981). They showed that

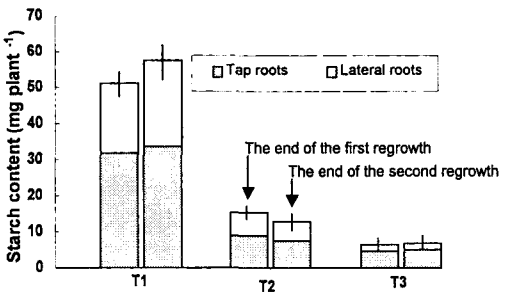

Fig. 2. Effects of NO_3^- level applied during the first regrowth on starch in taproots and lateral roots after shoot removal for two successive cycles of regrowth. The first regrowth occurred on a medium containing 0.2, 1.0 and 3.0 mM KNO_3 for T1, T2 and T3, respectively. The second regrowth occurred for all treatments on a medium containing 1.0 mM NH_4NO_3. Vertical bars give the s.e. for n=3.

in nodulated alfalfa, greater availability of combined N decreased the starch content in roots and crown tissues but increased shoot yield. These results indicate that the NO_3^- supply level has a significant influence on the accumulation of starch in root system, and that the initial starch level for the second regrowth (accumulated until the end of the first regrowth) is a major determinant for the starch accumulation during the second regrowth.

Nitrogen accumulation in root system as affected by different NO_3^- supply levels during the first regrowth and by the same level of exogenous nitrogen (1.0 mM NH_4NO_3) during the second regrowth is presented in Fig. 3. Total nitrogen content in root system grown with 0.2, 1.0 and 3.0 mM NO_3^- during the first regrowth was 6.66, 8.43 and 11.09 mg $plant^{-1}$, respectively. Nitrogen was contained mainly in lateral roots; 80% (in 0.2 mM), 74% (1.0 mM) and 76% (3.0 mM) of total nitrogen in root

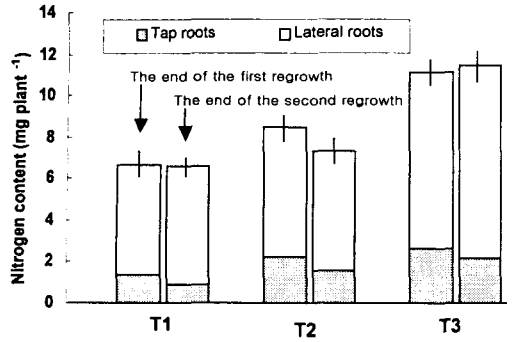

Fig. 3. Effects of NO_3^- level applied during the first regrowth on nitrogen accumulation in taproots and lateral roots after shoot removal for two successive cycles of regrowth. The first regrowth occurred on a medium containing 0.2, 1.0 and 3.0 mM KNO_3 for T1, T2 and T3, respectively. The second regrowth occurred for all treatments on a medium containing 1.0 mM NH_4NO_3. Vertical bars give the s.e. for n=3.

system. Total N content in root system at the end of the second regrowth also significantly increased with increasing the NO_3^- supply level during the first regrowth. Total N content in taproots at the end of second regrowth increased proportionally with the initial N reserves, which previously accumulated during the first regrowth in relation to NO_3^- supply level. There was no significant difference in N content in lateral roots between T1 and T2 at the end of second regrowth, while significantly increased in T3. This indicates that N reconstitution in root system was closely associated with the level of N reserves previously accumulated. This suggestion is consistent with the results of Ourry et al. (1994) who reported in non-nodulated alfalfa that higher availability of N reserves in response to increased N supply during the first regrowth allowed higher mobilization mainly from the remaining organs after shoot removal. They provided a direct evidence using ^{15}N tracing method that the amount of exogenous or endogenous N translocated to shoots was proportional to the availability of N reserves at the beginning of regrowth. It could be summarized from our results that the starch accumulation in root system was negatively responded to exogenous NO_3^- level during the first regrowth, but nitrogen was accumulated proportionally.

The data obtained from the present experiments suggest that the level of NO_3^- may strongly influence the accumulation of organic reserves (starch and N in this experiment) in root system, and that the previously stored organic reserves play a major role in shoot yield and reconstitution of organic reserves during the next regrowth.

Ⅳ. 요 약

질산태 질소의 공급수준이 알팔파의 영양생장기 동안의 질소동화와 성장 및 재생기간 동안의 뿌리조직내 전분과 질소 축적에 미치는 영향을 규명하고자 0.2, 1.0 및 3.0 mM KNO_3 하에서 10주 동안의 영양생장 후 건물 및 질소화합물의 식물조직내의 분포를 분석하고, 24일간 1차 및 2차 재생을 각각 시킨 후 뿌리조직에 축적된 전분과 질소 함량을 분석하였다. 10간의 영양생장 후 건물과 질소화합물의 함량을 1.0 mM 처리구를 기준으로 비교한 결과, 0.2 mM 처리구에서는 질소결핍 현상이 그리고 3.0 mM 처리구에서는 질소과잉에 의한 억제 효과가 나타났다. 0.2, 1.0 및 3.0 mM NO_3^- 하에서 24일간 재생 후 주근과 지근에 축적된 전분의 총 함량은 개체 당 각각 50.96, 15.47 and 6.37 mg이었다. 전 처리구 공히 1.0 mM NH_4NO_3하에서 24일간 재생 후 전분의 함량과 1차 재생

후와 비교할 때 유의적인 차이가 없었다. 1차 재생 후 뿌리조직에 축적된 전 질소 함량은 개체 당 각각 6.66, 8.43 and 11.09 mg 이었으며, 지근에 주로(뿌리조직의 총 질소 함량의 70% 이상) 분포되어 있었다. 이상의 결과들은 재생기간 중 질산태 질소의 공급수준이 증가할수록 뿌리조직내의 전분축적은 감소하나, 질소축적은 비례적으로 증가한다는 것을 보여준다. 뿐만 아니라, 1차 재생기간 중 축적된 저장 유기물의 수준은 차기 재생활력 및 유기물의 뿌리내 재축적에 영향을 미치는 중요한 생리적인 요인 중의 하나임을 보여준다.

(**검색어** : 알팔파, NO_3^- 공급수준, 영양생장, 재생, 저장유기물 축적)

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