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# Nitrogen Partitioning at Low Temperature in Fall-Sowing Species :

## I. Uptake of exogenous N and remobilization of endogenous N

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# 추파 청예작물의 저온 조건하에서 질소의 분배에 관한 연구

## I. 외인성 질소의 흡수 및 내인성 질소의 전이

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## 적 요

저온 재배조건이 추파 청예작물의 외인성 질소의 흡수 및 내인성 질소의 전이에 미치는 영향을 규명하기 위해 저온(5℃) 및 적온(25℃) 조건에서 사초용 유채와 연맥을 수경 재배하였다. $^{15}N$을 labeling을 한 후 온도처리 9일 동안 식물체내 분포된 $^{15}N$의 함량을 분석하여 흡수 및 전이된 질소의 함량을 각각 양적으로 평가하였다. 저온 조건하에서 공시작물의 성장은 유의적으로 감소하였다. 5℃ 처리 9일차의 잎의 질소 함량은 25℃ 처리구에 비해 평균 약 20% 감소하였으나 뿌리의 질소 함량은 약 12% 증가하였다. 5℃ 처리후 9일 동안 연맥 및 사초용 유채가 영양 용액으로부터 흡수한 $NO_3^-$ 함량은 개체당 각각 23.0 및 43.5 mgN으로 25℃ 처리구에 비해 각각 59.3 및 26.1% 감소하였다. 두 종의 공시작물 공히 전 시험기간에 걸쳐 60% 이상의 $^{15}N$이 잎으로 분배되었다. 연맥의 경우 5℃ 및 25℃ 처리후 6일차 잎의 $^{15}N$ 함량은 온도처리일(0일)에 비해 각각 166 및 296 $\mu$g이 각각 증가하여 이 기간중 90 및 163 $\mu$g N이 뿌리로부터 잎으로 전이되었음을 보여주었다. 온도 처리 7일에서 9일차 동안은 각각 75 및 52 $\mu$gN이 잎에서 뿌리로 전이되었다. 사초용 유채의 잎이나 뿌리내 $^{15}N$ 함량은 두 가지 온도처리구에서 공히 일차간 유의적으로 차이가 없었다. 두 종의 공시작물을 비교할 때 연맥이 저온 조건하에서 질소 흡수 및 전이에 있어 사초용 유채보다 더 민감하게 영향을 받았다.


## I. INTRODUCTION

Nitrate leaching from soil profile occurs mainly during autumn and early sping in temperate environment. During these seasons, the mineralization rate of organic nitrogen sources (manure, dead plants and soil microorganisms) are often high. Farming practices that leave the ground bare during winter are also pointed out as a major reason of $NO_3^-$ leaching. Cold soil conditions leads to appreciable accumulation of $NH_4^+$ because the activity of nitrifying bacteria significantly reduce under this condition. Additionally $NH_4^+$ is

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absorbed to soil colloids, while $NO_3^-$ is not absorbed and is much readily leached. Several studies showed that $NH_4^+$ was indeed absorted more readily than $NO_3^-$ when the ions are supplied together at the same concentration, especially at low temperature(Frota and Tucker, 1972; Clarkson and Warner, 1979; Ganmore-Neumann and Kafkafi, 1981; Macduff and Wild, 1989), but that this might be attributable largely to physical changes in different parts of the cell membrane rather than to differences in relative sensitivity of $NO_3^-$ versus $NH_4^+$ uptake to low temperature (Macduff and Wild, 1989). Therefore, it is generally suggested that $NO_3^-$ is more serious in water pollution than $NH_4^+$ when excessive nitrogen leaches from soil profiles.

If the extent of $NO_3^-$ leaching is could be varied with the availability of nitrogen sources in soil and the nitrogen uptake by plant use, the growing catch crops should be considered a suitable farming strategy to decrease $NO_3^-$ leaching. Theoretically, production of 2,000kg of dry matter per ha with a 5% total N content would remove 100kg $NO_3^-$ per ha from the soil profile. Several species within Poaceae, Brassicaceae and Fabaceae family are fall-sown and grown for green fodder production during especially autumn and early spring. Physiological studies concerning $NO_3^-$ uptake are not available for these species. Additionally, regulation of $NO_3^-$ uptake is not yet understood at low temperature because the difficulty seems to arise from the possible existence of a multiphasic system (Breteler and Nissen, 1982), whose activity is modified by numerous external factors such as light (Grant and Turner, 1969), anaerobiosis (Ezeta and Jackson, 1975), the change of membrane permeability (Raison, 1985) and internal concentration in relation to the prior nutritional status (Clarkson, 1985; Lee and Rudge, 1986; Morgan and Jackson, 1988). On the other hand, the partitioning of nitrogen among different organs results from the uptake and transport via xylem and secondly phloem transport. The information of remobilization of endogenous nitrogen, derived from the hydrolysis of organic nitrogen pool such as protein or free amino acids, therefore could constitute a basic understanding about the cycling of nitrogen compounds in plant (Simpson et al., 1986).

The present study was undertaken to quantify $NO_3^-$ uptake and remobilization of endogenous N in winter rye and forage rape at low temperature culture condition.

## II. MATERIALS AND METHODS

### 1. Plant culture

Table 1. Composition of nutrient solution used for the hydroponic culture of winter rye and forage rape.

| | Macro elements (mM) | | Micro elements ($\mu$M) | |
|---|---|---|---|---|
| | Winter rye | Forage rape | | |
| $KNO_3$ | 1.00 | 1.00 | $H_3BO_3$ | 14.0 |
| KCl | 0.25 | | $MnSO_4$ | 5.0 |
| $CaCl_2$ | 0.25 | 3.00 | $ZnSO_4$ | 3.0 |
| $MgSO_4$ | 0.20 | 0.50 | $CuSO_4$ | 0.7 |
| $K_2SO_4$ | | 1.00 | $(NH_4)_6Mo_7O_{24}$ | 0.7 |
| $KH_2PO_4$ | 0.40 | 0.40 | $CoCl_2$ | 0.1 |
| $K_2HPO_4$ | 0.15 | 0.15 | Fe-Na-EDTA | 200.0 |

The sterilized seeds of forage rape (*Brassica napus* L.) and winter rye (*Scale cereale* L.) were germinated on a wet filter paper room temperature (15 ~25℃), and then transferred on wet double-sheets (20 × 20cm) of filter paper to allow elongation of roots and shoots. The seedling were supplied with a complete nutrient solution on double-sheets. Two-week old uniform seedling (15 plants per 10 L plastic pot) were hydroponically grown on a nutrient solution. The compositions of nutrient soultions used for hydroponic culture are given in Table 1. The concentration and composition of some nutrient elements were slightly modified from those employed by Soussana (1986) and Kim et al. (1991) after the preliminary cultivation. A small quantity of $CaCO_3$ was added to each nutrient solution to stabilize pH. The nutrient solutions were continuously aerated and renewed every 5 days. The photo period was controlled to 16 hours (day) and 8 hours (night) with mercury vapor fluorescent at 50~60% of relative humidity.

## 2. Pulse chase labeling of $^{15}NO_3^-$ and calculation of nitrogen flows

Six weeks-old pants were contacted with 1 mM $K^{15}NO_3$ (15.8 atom %$^{15}$N) during 5 days. After labeling the root system were rinsed out several times with 1 mM $CaSO_4$ solution. The plants afterward were placed on non-labeled (1 mM $K^{14}NO_3$) nutrient solution. The uniformly selected plants were separately transferred to the growth cabinets controlled to 25℃ and 5℃. The harvest of samples were carried out on 3, 6 and 9 days after temperature treatment.

The calculations implied 2 assumptions : (1) all endogenous N is uniformly labeled; (2) the return flux from shoots to roots is negligible during the period studied. The apparent change (dN) in nitrogen pool during the periods of temperature treatment is the difference between nitrogen inflow and outflow from this pool:

$$dN = N\,\text{inflow} - N\,\text{outflow} = Nt+dt - Nt \quad \cdots\cdots (1)$$

where $Nt$ and $Nt + dt$ are, respectively, nitrogen contents at time $t$ and $t + dt$. As plants were supplied with $^{14}NO_3$ during temperature treatment, the decrease of $^{15}$N proportion is derived from $^{14}NO_3$ uptake. Therefore, net uptake of $NO_3^-$ (N inflow) can be calculated from $^{15}$N dilution.

$$N\,\text{inflow} = Nt+dt\,(1 - Et+dt/Et) \quad \cdots\cdots\cdots (2)$$

where $Et$ and $Et+dt$ are atom % $^{15}$N excess in this N pool, respectively, at time $t$ and $t+dt$. N outflow from this pool, corresponding to endogenous N remobili-zation during $dt$, can be calculated from equation (1) :

$$N\,\text{outflow} = Nt - Nt+dt+N\,\text{inflow} \quad \cdots\cdots\cdots (3)$$

Substitution from eqution (2) gives :

$$N\,\text{outflow} = (Nt.Et - Nt+dt.Nt+dt) / Et \quad \cdots\cdots (4)$$

The term $Nt.Et - Nt+dt.\ Nt+dt$ represents the net change of $^{15}$N amount in the pool during $dt$.

## 3. Chemical and isotope analysis

Roots and shoots were separately harvested. The freeze dried samples were ground to the fine powder by a vibration mill (Heiko Co. Ltd. Japan), and kept in a freezer until further analysis. The N content and $^{15}$N abundance were deterimined by ANCA-SL (Europa Science, Crewe, Cheshire, UK) using a reference glutamic acid (N % = 9.52, $^{15}$N atom % = 0.362). The ground samples of 2 to 4 mg and glutamic acid of 1 to 2 mg were separately weighted into tin cups, and then set on the ANCA-MS with $CO_2$ removal system. The analytical precision of N content

and $^{15}N$ abundance were within ± 0.5 mg/g DW and ± 0.01%, respectively.

## III. RESULTS AND DISCUSSION

### 1. Growth and nitrogen accumulation

The dry matter production and nitrogen accumulation elaborated by winter rye and forage rape grown at 5℃ and 25℃ are given at Table 2. The growth rate of leaves and roots depressed at 5℃. After 9 days at 5℃, dry weight of roots and leaves decreased to about 18% and 9% for winter rye, and to 8% and 9% for forage rape comparing with the values obtained at 25℃.

Nitrogen content experssed as a unit of mgN per plant allows us to precise the low temperature effcts on nitrogen accumulation in plant tissues (Table 2). After 9 days at 5℃, nitrogen content of leaves decreased to averagely 20% in two species studied although leaf growth decreased only 9% comparing with the plants grown at 25℃. On the contrary, 5℃ treatment induced a higher accumulation of nitrogen than at 25℃ in roots.

Nitrogen in roots of forage rape grown at 5℃ was about 16% highly accumulated while growth of this organ was much severly depressed comparing with that of 25℃ during 9 days. It is suggested that low temperature significantly modifies the distribution of

Table 2. Changes in dry matter and total nitrogen cotent in leaves and roots of plants grown at 25℃ and 5℃ of growth temperature during 9 days. Each value is the mean ± S.E. for n = 3.

| Species | Culture temperature | Organs | Time after temperature treatment (day) | | | |
|---|---|---|---|---|---|---|
| | | | 0 | 3 | 6 | 9 |
| | | | ····· Dry matter (mg/plant) ····· | | | |
| *Secale cereale* L. | 5℃ | Leaves | 781 ± 72 | 1,080 ± 145 | 1,090 ± 69 | 1,280 ± 91 |
| | | Roots | 179 ± 18 | 379 ± 31 | 380 ± 32 | 420 ± 36 |
| | 25℃ | Leaves | 790 ± 68 | 1,050 ± 95 | 1,115 ± 51 | 1,410 ± 66 |
| | | Roots | 175 ± 21 | 308 ± 25 | 320 ± 24 | 510 ± 46 |
| *Brassica* napus L. | 5℃ | Leaves | 1,190 ± 91 | 1,300 ± 106 | 1,430 ± 99 | 1,670 ± 112 |
| | | Roots | 108 ± 11 | 133 ± 15 | 159 ± 21 | 193 ± 17 |
| | 25℃ | Leaves | 1,110 ± 118 | 1,330 ± 94 | 1,590 ± 121 | 1,830 ± 109 |
| | | Roots | 115 ± 18 | 150 ± 13 | 182 ± 22 | 210 ± 19 |
| | | | ····· Nitrogen content (mg/plant) ····· | | | |
| *Secale cereale* L. | 5℃ | Leaves | 34.5 ± 2.8 | 42.3 ± 4.1 | 38.6 ± 6.2 | 45.8 ± 3.9 |
| | | Roots | 4.4 ± 0.4 | 8.7 ± 0.6 | 10.5 ± 0.9 | 13.7 ± 1.2 |
| | 25℃ | Leaves | 35.2 ± 3.8 | 45.1 ± 3.8 | 52.4 ± 5.1 | 57.4 ± 4.9 |
| | | Roots | 4.3 ± 0.3 | 11.0 ± 1.1 | 12.5 ± 0.9 | 14.8 ± 1.9 |
| *Brassica* napus L. | 5℃ | Leaves | 32.5 ± 3.8 | 42.2 ± 4.1 | 50.8 ± 4.8 | 63.2 ± 5.9 |
| | | Roots | 2.8 ± 0.2 | 4.7 ± 0.5 | 6.4 ± 0.5 | 9.7 ± 0.9 |
| | 25℃ | Leaves | 31.1 ± 2.8 | 48.6 ± 3.9 | 60.4 ± 5.9 | 79.2 ± 6.6 |
| | | Roots | 3.0 ± 0.2 | 4.4 ± 0.5 | 5.5 ± 0.4 | 8.2 ± 0.7 |

nitrogen among organs, which well accord with several data obtained from field condition. We previously observed in field condition that nitrogen accumulation occurred during wintering period (Kim et al., 1995) and absorbed nitrogen was incorporated to mainly protein-N in roots of fall-sown forage rape (Chung et al., 1994).

## 2. Dynamics of $NO_3^-$ uptake

The absorbed $NO_3^-$ from medium was quantified by $^{15}N$ labeling and its dynamics were expressed as a cumulated N content per plant during examined time course (Fig. 1.) Total content of absorbed $NO_3^-$ at 5℃ was significantly lower than at 25℃ for both of species.

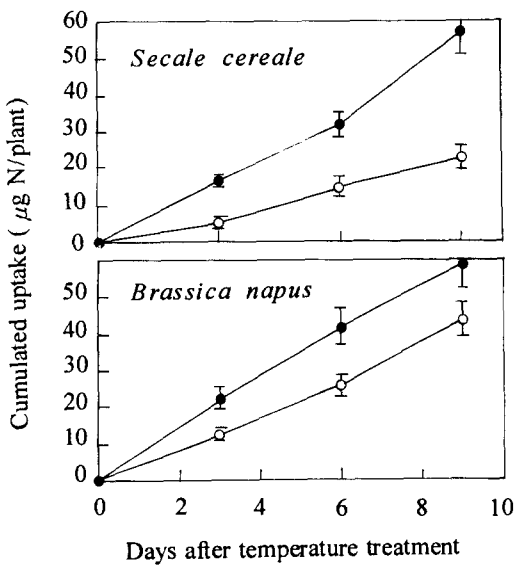


Fig 1. Time course of cumulative $NO_3^-$ uptake by winter rye and forage rape grown at 5℃ (○) and 25℃ (●) of growth temperature. Each value is the mean ± S.E. for n = 3.

Time course showed that exogenous $NO_3^-$ uptake was very quickly responded to low temperature, showing 70.2% and 44.4% lower in winter rye and forage rape than at 25℃ during the first 3 days. Total content of $NO_3^-$ taken up at 5℃ during 9 days decreased to 59.3% and 26.1%, respectively, for winter rye and forage rape in comparison to those estimated at 25℃. In preliminary experiment with $NO_3^-$ depletion method, we observed also a lower activity of $NO_3^-$ uptake at 5℃ than at 25℃ in several overwintering species.

Inhibitory effects of low temperature on nutrient uptake including $NO_3^-$ and $NH_4^+$ has been widely reported. Macduff and Wild (1989) observed in *Brassica napus* the decreases of about 50% for total $NO_3^-$ and $NH_4^+$ ion uptake and 28% of net $NO_3^-$ uptake during 7 days of low temperature treatment (3℃). In our experimental condition, the curves of accumula-ted content of $NO_3^-$ uptake were nearly linear regardless of culture temperature.

This fact could be interpreted that the dynamics of $NO_3^-$ ion uptake per plant is constant. Several results suggested that the uptake of nutrient elements was dependent on the energetic mechanism (Lauchi, 1976). Low temperature reduced the activity of roots with decreasing Q10 value to between 2 and 3 and results in the inhibition of uptake dynamics (Bravo and Uribe, 1981; Macduff et al., 1987). We observed that low temperature led to a nitrogen accumulation in root despite of growth depression (Table 2). The increase of internal N concentration in root seems to be related to the decrease of N uptake at low temperature. Raison (1985) suggested that low temperature changed membrane permeability and the internal concentration was closely linked to the dynamics of nutrient ion uptake.

## 3. Remobilization of endogenous nitrogen

The relative distribution of tracer $^{15}N$ between shoots and roots during time course allows to quantify the apparent remobilization of endogenous nitrogen. The total amount of $^{15}N$ in the plant remained at 1,040

± 42 μg and 1,360 ± 48 μg $^{15}N$ per plant in winter rye and in forage rape, suggesting that there was no significant loss of $^{15}N$ to the medium throughout time course of temperature treatment.

The amount of $^{15}N$ and percentage of currently absorbed nitrogen in leaves and roots of winter rye and forage rape grown at 5℃ and 25℃ is given at Table 3. The distribution of $^{15}N$ into leaves was much higher than that of roots in both of species regardless of culture temperature. In the leaves of winter rye, $^{15}N$ content increased to 166 μg and 296 μg per plant, respectively, in the plants grown at 5℃ and 25℃ during the first 6 days.

Table 3. Changes in amount of $^{15}N$ distributed into leaves and roots of plants grown at 25℃ and 5℃ of growth temperature during 9 days. Each value is the mean ± S.E. for n = 3. The values in parentheses represent the percentage of currently absorbed $^{15}N$ (CNC)*.

| Species | Culture temperature | Organs | Time after temperature treatment (day) | | | |
|---|---|---|---|---|---|---|
| | | | 0 | 3 | 6 | 9 |
| | | | ..... μg $^{15}N$/plant ..... | | | |
| *Secale cereale* L. | 5℃ | Leaves | 624 ± 58 ( 9.5)* | 608 ± 45 ( 7.2) | 790 ± 62 ( 9.7) | 650 ± 39 ( 7.0) |
| | | Roots | 416 ± 32 (58.9) | 431 ± 35 (29.6) | 249 ± 32 (13.0) | 390 ± 34 ( 7.0) |
| | 25℃ | Leaves | 619 ± 38 ( 9.4) | 811 ± 35 ( 8.8) | 915 ± 51 ( 9.0) | 822 ± 60 ( 6.9) |
| | | Roots | 421 ± 28 (58.9) | 229 ± 21 (11.1) | 125 ± 12 ( 4.4) | 218 ± 19 ( 7.2) |
| *Brassica* napus L. | 5℃ | Leaves | 1,115 ± 98 (20.2) | 1,135 ± 79 (15.1) | 1,129 ± 85 (12.0) | 1,115 ± 86 ( 9.1) |
| | | Roots | 244 ± 15 (54.7) | 224 ± 18 (28.2) | 231 ± 24 (21.2) | 244 ± 18 (14.0) |
| | 25℃ | Leaves | 1,110 ± 78 (19.5) | 1,129 ± 84 (12.8) | 1,136 ± 91 ( 9.8) | 1,156 ± 90 ( 7.1) |
| | | Roots | 250 ± 18 (55.9) | 231 ± 21 (32.0) | 224 ± 21 (24.1) | 204 ± 18 (13.7) |

* CNC (%) = ($^{15}N$ atom % of the tissue − 0.366 / $^{15}N$ atom % of labelling solution − 0.366) × 100.

These increased amount of $^{15}N$ corresponded to the decreased amount in roots. From the changes of $^{15}N$ content among organs, it could be estimated that 90 and 163 μgN/plant (c.f equation 4 in calculation of N flows) were transferred from roots into leaves at 5℃ and 25℃. These results clearly indicate that remobillzation via xylem reduces at low temperature condition. The amount of $^{15}N$ decreased from 6 to 9 days after temperature treatment, corresponding 75 μg and 52 μg of outflow from leaves of plants grown at 5℃ and 25℃. This outflow logically means the transport from leaves to roots. The phloem transport therefore slightly accelerated by low temperature. A $^{15}N$ analysis of roots and leaves of wheat showed that 80 to 95% of total absorbed labeled N were transferred to leaves and 10 to 15% of nitrogen in leaves remobilized to roots (Larsson et al., 1991). The similar estimation of phloem transport of barley has been reported (Simpson et al., 1982). On the contrary, there was no signifiant changes in $^{15}N$ distribution into organs of forage rape at both of temperature treatment throughout the entire time course.

In comparison with two species studied, winter rye much sensitively responded to low temperature than forage rape in the uptake of exogenous N (Fig. 1) and the remobilization of endogenous N (Table 3.) The notable differences between winter rye and forage rape for remobilization of endogenous N seems to be

related to the causality with the durability to optimize the utilization of nitrogen within plants. Several reports have shown that the control of inward and outward fluxes might be strongly influenced by morphological parameters such as root surface area, diameter and density (Jackson et al., 1976; Ingemarsson et al., 1987).

Two possible assumptions can be taken from the entire data obtained from this study ; 1) the dynamics of $NO_3^-$ uptake is reduced in relation to the increase of internal N concentration, 2) the remobilization of endogenous N via xylem transport is decreased but that of phloem transport is slightly accelerated by low temperature despite of species specific.

## IV. SUMMARY

A pulse-chase labeling of $^{15}N$ on winter rye (*Scale cereale*) and forage rape (*Brassica napus*) grown at 5℃ and 25℃ was carried out to determine the effects of low temperature on the uptake exogenous N and the remobilization of endogenous N. The growth rate of leaves and roots depressed at 5℃. After 9 days at 5℃, nitrogen content of leaves decreased to 20% on the average while that of roots increased to 12% compared with the plants grown at 25℃. Total content of $NO_3^-$ uptake from medium was 23.0 and 43.5 mg N/plant, respectively, for winter rye and forage rape grown at 5℃ during 9 days. These values were corresponded to 59.3 and 26.1% lower uptake than those of 25℃. A large part of $^{15}N$ was distributed into leaves throughout time course in both of two species. The content of $^{15}N$ in leaves of winter rye at day 6 increased to 166 and 296 $\mu g^{15}N$/plant compared with the initial value (day 0) in the plants grown at 5℃ and 25℃, corresponding to 90 and 163 $\mu g$ N of remobilization from roots into leaves during the first 6 days. From 7 to 9 days, 75 and 52 $\mu g$N of outflow from leaves were occurred at 5℃ and 25℃. However, little remobilization of endogenous N was estimated in forage rape throughout the entire time course regardless of temperature treatment. Comparing two species studied, winter rye was much sensitively influenced by low temperature on the uptake of exogenous N and the remobilization of endogenous N.

## V. LITERATURE CITED

1. Bravo, F.P. and E.G. Uribe. 1981. Temperature dependence of the concentration kinetics of absorption of phosphate and potassium in corn roots. Plant Physiol. 67:815-819.
2. Breteler, H. and P. Nissen. 1982. Effects of exogenous and endogenous nitrate concentration on nitrate utilization by dwart-bean. Plant Physiol. 70:745-759.
3. Clarkson, D.T. 1985. Factors affecting mineral nutrient acquisition by plants. Annu. Rev. Plant Physiol. 36:77-115.
4. Clarkson, D.T. and A.J. Warner. 1979. Relationships between root temperature and the transport of ammonium and nitrate ions by Italian and perennial ryegrass. Plant Physiol. 64:557-561.
5. Ezeta, F.W. and W.A. Jackson. 1975. Nitrate translocation by detopped corn seedlings. Plant Physiol. 56:148-156.
6. Frota, J.N.E. and T.C. Tucker. 1972. Temperature influence on ammonium and nitrate absorption by lettuce. Soil Sci. Soc. Am. Proc. 36:97-100.
7. Ganmore-Neumann, R. and U. Kafkafi. 1980. Root temperature and percentage $NO_3^-/NH_4^+$ effect on tomato plant development. Ⅱ. Nutrient composition. Agron. J. 72:758-761.
8. Grant, B.R. and I.M. Turmer. 1969. Light-stimulated nitrate and nitrite assimilation in several species of algae. Comp. Biochem. Physiol. 29:995-1004.
9. Ingemarsson, B., P. Oscarson, M. Ugglas and C.M. Larsson. 1987. Nitrogen utilization in *Lemna*. Ⅱ.

Studies of nitrate uptake using $^{13}NO_3^-$. Plant Physiol. 85:860-864.

10. Jackson, W.A., K.D. Kwik, R.J. Volk and R.G. Butz. 1976. Nitrate influx and efflux by intact wheat seedlings: effects of prior nitrate nutrition. Planta 132:146-156.
11. Kim, T.H., A. Ourry, J. Boucaud and G. Lemaire. 1991. Changes in source-sink relationship for nitrogen during regrowth of lucerne (*Medicage sativa* L.) following removal of shoots. Aust. J. plant Physiol. 18:593-602.
12. Larsson, C. M., M. Larsson, D.T. Purves and D.T. Clarkson. 1991. Translocation and cycling through roots of recently absorbed nitrogen and sulphur in wheat (*Triticum aestivum*) during vegetative and generative growth. Physiol. Plant. 82:345-352.
13. Lauchi, A. 1976. Symplastic Transport and ion release to the xylem. In Wardlaw I.F. and J.B. Passioura. ed. Transport and transfer processes in plants. Academic press, pp. 101-112.
14. Lee, R.B. and Rudge, K.A. 1986. Effects of nitrogen deficiency on the absorption of nitrate and ammonium by barley plants. Ann. Bot. 50:429-449.
15. Macduff, J.H., M.J. Hopper., A. Wild and F.E. Trim. 1987. Comparison of the effects of root temperature on nitrate ammonium nutrition of oilseed rape (*Brassica napus* L.) in flowing solution culture. Ⅱ. Cation-anion balance. Ibid. 38:1589-1602.
16. Macduff, J.H. and A. Wild. 1989. Interation between root temperature and nitrogen deficiency influence preferential uptake of $NO_3^-$ and $NH_4^+$ by oilseed rape. Ibid. 40:195-206.
17. Morgan, A.M. and W.A. Jackson. 1988. Inward and outward movement of ammonium in root systems: Transient responses during recovery from nitrogen deprivation in presence of ammonium. J. Exp. Bot. 39:179-191.
18. Raison, J.K. 1985. Alterations in the physical properties and thermal response of membrane lipids: correlations with acclimation to chilling and high temperature. In Jone, J. B., J. Berlin and P. C. Jackson. ed. Frontiers of membrane research in agriculture. Rowman & Allanheld, pp. 383-401.
19. Simpson, R.J. 1986. Translocation and metabolism of nitrogen. In Lambers. H., J. J. Neetson and I. Stulin. ed. Fundamental, ecological and agricultural aspects of nitrogen metabolism in higher plants. Martinus Nijhoff, pp. 71-96.
20. Simpson, R.J., H. Lambers and M.J. Dalling. 1982. Translocation of nitrogen in vegetative wheat plant (*Triticum aestivum*). Physiol. Plant. 56:11-17.
21. Sourssana, J.F. 1986. Fixation de $N_2$ et assimilation du nitrate chez la luzerne. Aspects methodologiques. Aspects physiologiques. These de doctorat d'universite, Montpellier, pp. 160.
22. 김태환, 김기원, 정우진, 전해열, 김병호. 1995. 저장탄수화물과 질소의 월동성과 재생활력에 대한 이용성. Ⅱ. 추파유채(*Brassica napus* L.)의 파종밀도가 월동중 저장유기물 함량 및 수량에 미치는 영향. 한초지. 15(4):231-237.
23. 정우진, 김병호, 김태환, 강우성. 1994. 추파유채 (*Brassica napus* L.)의 생육기간중 질소화합물의 함량 변화. 한초지. 14(4):324-330.