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Effects of Low Temperature on Nitrate Uptake and Accumulation of Nitrogenous Compound in Fall-Sowing Species

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추파 청예작물의 저온 조건이 질산태 질소의 흡수와 식물조직내의 질소 화합물의 함량에 미치는 영향

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적 요

추파 청예작물의 저온 조건하에서 질산태 질소의 흡수 활력을 규명하기 위해 사초용 유채, 보리 및 연맥을 수경 재배하였다. 저온(℃) 및 적온(25℃) 조건에서 성장한 공시작물의 질산태 질소의 흡수력 및 식물조직내의 질소 화합물의 함량을 각각 분석하였다. 3종의 공시작물을 비교할 때 5℃ 및 25℃의 온도처리구에서 공히 유채의 질산태 질소의 흡수 활력이 가장 높았다. 5℃ 처리후 9시간 동안 유채, 보리 및 연맥에 의해 흡수된 질산태 질소의 농도는 25℃ 온도처리구에 비해 각각 31, 50 및 27% 감소하였다. 저온 처리에 따른 식물조직내의 질소 화합물의 함량의 변화는 뿌리 조직이 잎 조직에 비해 훨씬 민감하게 영향을 받았다. 5℃ 처리후 9일차의 유채, 보리 및 연맥의 뿌리내 질산태 질소의 함량은 25℃ 온도처리구에 비해 각각 48.3, 38.7 및 53.5% 증가하였다. 암모니움태 질소의 함량의 변화는 다른 질소 화합물에 비해 온도 처리에 따른 영향이 상대적으로 적었다. 5℃ 처리구 잎 조직의 아미노산태 질소의 함량은 25℃ 처리구에 비해 약간 감소하였으나 뿌리 조직은 높은 폭으로 증가하였다. 온도 처리에 따른 단백질태 질소의 함량은 아미노산태 질소와 비슷한 경향이었으나 변화 비율은 낮았다. 이러한 결과들은 저온 조건하에서 질산태 질소와 흡수는 감소되며 흡수 활력의 감소는 뿌리 조직내 질산태 및 아미노산태 질소 농도의 증가에 의해 밀접한 관계가 있음을 제시한다.

I. INTRODUCTION

It is generally suggested that the excessive NO_3^- leaching from soil profiles is very serious in water pollution. The NO_3^- leaching is closely related to the availability of nitrogen sources in soil and the extent of nitrogen use by plant uptake.

Manure, dead plants and soil microorganisms are important sources of nitrogen retuned to soil, but most of this nitrogen is insoluble and not immediately available for plant use. Organic nitrogen should be firstly ammonified by soil to be availably taken up by plant. In warm, moist soils with near neutral pH, NH_4^+ ions are further oxidized by bacteria to NO_3^-.

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The successful use of these ions as a N source depends on the ability to assimilate most of inorganic N and the assimilation dynamics is affected by the availability of these ions. In many soils especidally in agriculture, there can be a variation with soil pH(Rains et al., 1964; Bassioni, 1971) or with season(Clarkson, 1985). 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 absorbed to soil colloids, while NO_3^- is not absorbed and is much readily leached.

Several studies showed NH_4^+ was indeed absorbed 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). Taking overall respects into consideration, it is easily accepted NO_3^- leaching from the soil may be more serious than NH_4^+ leaching in water pollution.

If development of accelerated net uptake of NO_3^- by plant use might be an important strategy to decrease the leaching rate from soil, there is little informations for estimating the dynamics NO_3^- uptake by overwintering crops. In additionally regulation of NO_3^- uptake is not yet understood under low temperature circumstance. Its complexity seems to arise from the possible correlation among various external factors such as light (Grant and Turner, 1969), anaerobiosis (Ezeta and Jackson, 1975), temperature (Macduff and Hopper, 1986; Macduff et al., 1987) and prior nutritional status (Lee and Rudge, 1986). Also it has been reported that uptake of most nutrient ions including NO_3^- and NH_4^+ depends on internal concentration (Morgan and Jackson, 1988; Clarkson, 1985) in relation to the change of membrane permeability (Raison, 1985).

Our interests focussed on an agrophysiological practices for improving NO_3^- use by overwintering plants to reduce NO_3^- leaching from soils during late autumn and early spring. The present study was undertaken to investigate the influence of low culture temperature on 1) the net NO_3^- uptake by some overwintering species, 2) the accumulation on NO_3^- and reduced nitrogen into plant tissues.

II. MATERIALS AND METHODS

1. Plant culture

The seeds of forage repe (*Brassica napus* L., cv. Swiss), barley (*Hordeum vulgare* L.) and winter rye (*Scale cereale* L.) were sterillzed with 80% ethanol and twin 20. They were germinated on a wet filter paper at room temperature (15~25℃), and transferred on wet double-sheets (20 × 20cm) of filter paper to allow elongation of roots and shoots. The seedling were supplied with a compete nutrient solution on double-sheets. Two-week old uniform seedling (15 plants per 10 L plastic pot) were hydroponically on a nutrient solution. The composition of nutrient solution used for hydroponic culture of grasses(barley and winter rye) and for forage rape is given in Table 1. 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. Measurement of NO_3^- uptake

Two days before uptake experiment, the plants were transferred to a fresh nutrient solution under at ambient green house circumstance. The roots of uni-

Table 1. Composition of nutrient solution used for the hydroponic culture of grasses (*Hordeum vulagare, Scale cereale*) and *Brassica* genus.

	Macro elements (mM)		Micro elements (μ M)	
	grasses	*Brassica* genus		
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

formly selected plants were rinsed several time with distilled water and were immersed in a uptake buffer solution for 5 min prior to uptake experiment in order to saturate the free apace of root cell wall. Two plants were transplanted on 10 mL of 10 mM MES hydrate-KOH buffer, pH 5.5, containing 10 mg/L N-KNO_3. The prepared plants were separately transferred to the growth cabinets controlled to 25℃ and 5℃ after 3 hours of photoperiod. The uptake solution was sampled at 1, 2, 4, 6 and 9 hours after temperature treatment. The NO_3^- concentration of uptake solution was measured spectrophotometrically by the method of Cawse (1967) with a modification in part. One mL of uptake solution sample was added to 0.2 mL of 2.5% (W/V) sulfamic acid and to 1.3 mL of 5% (V/V) perchloric acid. Absorbance at 210 nm was read after 10 min of incubation at ambient condition. The change of volume caused by evaporation and sampling for analysis was corrected by addition of distilled water according to the difference of weight comparing with the initial weight of container with plant at each sampling time. NO_3^- uptake was determined by the disappearance of NO_3^- from the uptake buffer solution. This flux was experessed as μmol NO_3^- taken up per g fresh weight.

3. Chemical analysis

Temperature treatment was prolonged until 9 days to analyse the contents of NO_3^- and reduced N in plant tissues. After 9 days of temperature treatment, shoots and roots were separately harvested and stored at deep freezer. About 250 mg of freeze dried sample was separated to the soluble and insoluble N fraction by two successive extraction with boiling water-ethanol-chloroform according to Pace et al. (1982). The separation of soluble fraction from the ethanol-water phase was performed by filtration. The chloroform phase was added to insoluble N residue. Nitrate and amino N of the soluble fraction were further separated using Dowex 50 H+ column as described by Yoneyama et al. (1987).

Nitrate was measured by a colorimetric method after reduction to nitrite on CdCu column (Ferrari and Varner, 1971). The fraction of insoluble N and amino acids N were mineralized by a Kjeldahl procedure. The nitrogen determination of mineralized aliquots was done by microdiffusion and nesslerization as described by Kim and Kim (1996).

III. RESULTS AND DISCUSSION

1. NO_3^- uptake

Concentration of NO_3^- taken up by three species under 2 regimes of culture temperature represents in Figure 1. Comparing with three species examined, the highest NO_3^- uptake by *Brassica napus* was observed at both of culture temperature during an entire time course. The cumulative concentration of NO_3^- taken up during 9 hours was 1.8 and 1.4 times higher at 5℃ and 1.3 and 1.5 times higher at 25℃ than those of *Hordeum vulgare* and *Secale cereale*, respectively. Uptake rates experssed with μmol/gFW/h of *Brassica napus*, *Hordeum vulgare* and *Secale cereale* were 1.22, 0.66 and 0.89 at 5℃ and 1.78, 1.44 and 1.22, respectively, at 25℃ atter 9 hours of temperature treatment. The observation of a higher NO_3^- uptake by *Brassica napus* from our experimental condition well confirms the results of Laine et al. (1993) showed the highest V_{max} value comparing uptake of NO_3^- by several catch crops within Hydrophyllaceae, Poaceae, Brassicaceae and Fabaceae family.

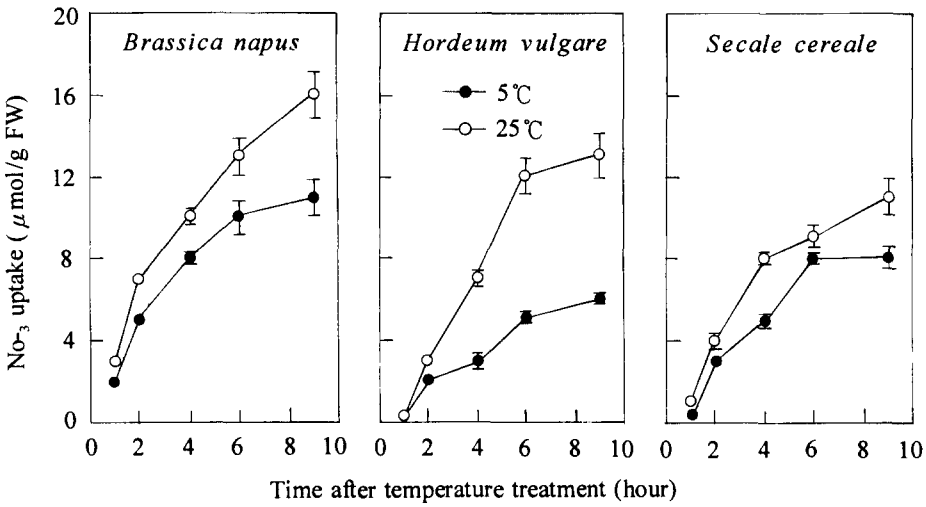

Fig 1. Time course of nitrate uptake at 5℃ and 25℃ of culture temperature. Values are given as means ± S.E. for n = 5.

NO_3^- uptake significantly decreased in plants grown at 5℃ comparing with those grown at 25℃ in all of three species examined. Nine hours after temperature treatment, the NO_3^- uptake by *Brassica napus*, *Hordeum vulgare* and *Secale cereale* at grown at 5℃ decreased to about 31, 50 and 27%, respectively, compared with those grown at 25℃. Macduff and Wild (1989) observed the decrease 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 *Brassica napus*. Inhibitory effects of low temperature on nutrient uptake including NO_3^- and NH_4^+ has been widely reported. Comparative studies of NO_3^- and NH_4^+ suggest that NO_3^- uptake is more severely inhibited than NH_4^+ uptake at low temperature (Bloom and Chapin, 1981; Macduff and Wild, 1989; Smart and Bloom, 1991). In preliminary experiment, we observed that low temperture led to a depression of growth and nitrogen accumulation in root. The increased internal 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.

2. NO_3^- content in plant tissues

After 9 days of temperature treatment, dry weight of leaves of plants grown at 5℃ decreased averagely to 12% and those of roots decreased to 18% compared with 25℃ (data not shown). In comparison with the plants at 25℃, total nitrogen contents in leaves decreased at 5℃ for all of three species examined while low temperature induced an increase of total nitrogen content in roots Root nitrogen of *Brassica napus, Hordeum vulgare* and *Secale cereale* grown at 5℃ were increased to 24.3, 14.3 and 9.1% than those of 25℃, respectively (data not shown).

NO_3^- contents in leaves and roots of three plant species after 9 days of temperature treatment are given in Fig. 2. When same temperature was applied, no significant difference of NO_3^- content in leaves was observed among three species. However, NO_3^- content in root significantly affected at 5℃. NO_3^- accumulation in roots of *Brassica napus* at 5℃ was the highest among three species examined. NO_3^- contents in roots of *Brassica napus, Hordeum vulgare* and *Secale cereale* grown at 5℃ were 48.3 38.7 and 53.5% higher than those of 25℃, respectively. These results suggest that NO_3^- reduction in roots may be inhibited by low temperature. In the further experiment of a series of this study, we determinend 32.5% lower activity of *nitrate reductase* in roots of *Brassica napus* grown at 5℃ than at 25℃. Therefore, the distribution and reduction of exogenous NO_3^- in plant tissue could be linked to the status of internal NO_3^- concentration and plant growth (Crafts-Brandner and Harper, 1982; Gojon et al., 1991).

3. Reduced nitrogen content in plant tissues

Reduced nitrogen contents in leaves and roots of three plant species after 9 days of temperature treatment are given in Table 2. The contents NH_4^+-N in leaves and roots of three species were not significantly affected by the culture temperature. Leaf tissues contained higher NH_4^+-N than roots. Comparing with the plants grown at 25℃, amino acids-N content in leaves at 5℃ was slightly lower while that of roots highly increased.

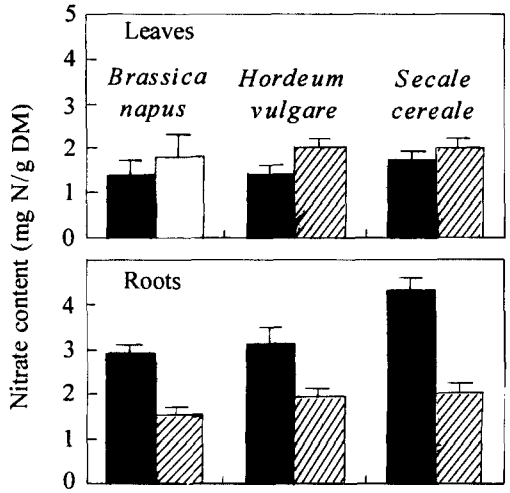

Fig 2. Nitrate content in leaves and roots grown at 5℃ (■) and 25℃(▨) during 9 days.
Values are given as means ± S.E. for n = 5.

Amino acids-N contents of in roots of *Brassica napus, Hordeum vulgare* and *Secale cereale* grown at 5℃ were 50.8, 40.9, 41.7% higher than those of 25℃, respectively. Protein-N was the largest pool of nitrogen representing about 55 to 70% of total nitrogen. Although the temperature influence on protein-N content showed a similar pattern with that amino acids-N, it was much less sensitive. Although we could not give a direct eviodence from this experimental condition, the high increase of NO_3^- (Fig. 2) and amino acids (Table 2) in roots are closely related to the decrease of NO_3^- uptake at low treatment. Several reports suggsted that some of NO_3^- inducible membrane proteins might

Table 2. Content of soluble reduced(NH_4^+-N, amino acid-N) and protein-N in plant grown at 25℃ and 5℃ of culture temperature during 9 days. Nitrogen source was supplied with 1mM KNO_3. Values are given as means ± S.E. for n=5.

Species		Culture temperature	Nitrogen fraction		
			NH_4^+-N	amino acids-N	Protein-N
			mg N/g DW		
Brassica napus L.	Leaves	25℃	1.3 ± 0.4	8.4 ± 0.8	30.5 ± 2.6
		5℃	1.7 ± 0.5	7.7 ± 0.8	27.4 ± 2.1
	Roots	25℃	2.3 ± 0.3	5.9 ± 0.5	16.5 ± 1.3
		5℃	2.9 ± 0.2	12.0 ± 1.3	21.1 ± 1.4
Hordeum vulgare L.	Leaves	25℃	1.2 ± 0.2	8.2 ± 0.9	30.1 ± 1.8
		5℃	1.4 ± 0.2	7.4 ± 0.6	26.7 ± 2.4
	Roots	25℃	2.5 ± 0.4	6.2 ± 0.7	17.6 ± 1.4
		5℃	2.7 ± 0.3	10.5 ± 1.0	18.5 ± 1.9
Secale cereale L.	Leaves	25℃	1.2 ± 0.2	8.0 ± 0.6	29.5 ± 1.9
		5℃	1.5 ± 0.2	7.2 ± 0.5	26.1 ± 2.1
	Roots	25℃	2.4 ± 0.2	6.0 ± 0.9	17.4 ± 1.0
		5℃	3.0 ± 0.2	10.3 ± 1.1	17.7 ± 1.4

have a role in NO_3^- uptake. If low temperature change membrane permeability (Raison, 1985), the increased internal concentration of NO_3^- and amino acids in roots could have a strong influence on the low temperature response of NO_3^- (Macduff et al., 1987; Macduff and Wild, 1989). In addition, inhibition of NO_3^- may be controlled by the molecular signal of foliar origin. Cooper and Clarkson (1989) postulated that nutrient ion uptake was regulated by the pool of composed amino acids circulating between roots and leaves. The diminution of amino acids utilization in leaves under low temperature stress induces to a depression of xylem transport of amino acids and to an increase of internal concentration. The inhibition of uptake seems to arise from the changes of these physiological factors.

We are still in vagueness for the interpretation of metabolic pathways resulting in an increase of internal NO_3^- and amino acids concentration in roos at low temperature. The possible assumptions are; 1) the increase of root flows from exogenous NO_3^-, 2) the decrease of reduction of exogenous or endogenous NO_3^-, 3) relative decline of reduced N transport at low temperature. Further studies therefore necessary with an enzyme assay to estimate the activity of NO_3^- reduction and an isotope ^{15}N labeling for investigating the internal cycling of exogeous and endogenous NO_3^-.

Ⅳ. SUMMARY

The experiments were undertaken to investigate the dynamics of NO_3^- uptake at low temperature with forage rape(*Brassica napus*), barle(*Hordeum vulgare*) and winter rye (*Scale cereale*). A net NO_3^- uptake and the contents of nitrogenous compound in tissues at

low temperature (5℃) were compared with normal temperature (25℃). The net uptake of NO_3^- by *Brass ica napus* was the highest among three species at both temperature applied. During 9 hours after temperature treatment, NO_3^- uptake of *Brassica napus, Hordeum vulgare* and *Scale cereale* grown at 5℃ decreased to about 31, 50 and 27% compared with those grown at 25℃. NO_3^- contents in roots were much sensitively affected by low temperature than those of leaves. Comparing with the plants grown at 25℃, NO_3^- contents in roots of increased to 48.3, 38.7 and 53.5%, repectively, for *Brassica napus, Hordeum vulgare* and *Scale cereale* during 9 days. A relatively less changes in the content of NH_4^+-N were observed according to the temperature treatment. The ontents of amino acids-N in leaves at 5℃ slightly decreased while that of roods remarkably increased compared with 25℃. Protein-N was the largest pool of nitrogen and its contents in leaves and roots showed a similar response on the temperature treatment. These results suggested that NO_3^- uptake was inhibited at low temperature. The dynamics of NO_3^- uptake could be closely related to the increased concentration of NO_3^- and amino acids in roots.

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