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Some Aspects to the *in vivo* Nitrate Reductase Activity in *Carex* species

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Abstract

Up to now, there have been done much efforts in regard to nitrate reductase activity (NRA) of dicotyledonous herbs and important crop monocotyledons, but few to wild plants having canopy structure such as *Carex*. The objectives of the present study are to determine: a) the optimum *in vivo* NR assay conditions for leaf samples of *Carex* species, b) changes of NRA according to section within leaf and leaf ages, c) diurnal variations.

Optimized assay media of each *Carex* species were determined. NRA of *C. rostrata* adapted to oligotrophic habitats is readily saturated at lower substrate concentration than those of *C. distans* and *C. gracilis*, adapted to meso- and eutrophic habitats, respectively. All *Carex* species investigated have higher NRA in leaves than in roots. NRA of all species showed maximal values at the middle section of each leaf and in the youngest fully expanded leaves. Compared to *C. gracilis*, NR in leaves of *C. distans* was adapted readily to the light period. On the whole, *Carex* showed rather delayed diurnal variation.

Even if the *in vivo* nitrate reductase assay based on nitrite estimation does not give an accurate estimation of total nitrate reduced, it still serves as a useful tool to find out relative differences in varying environmental conditions. Additionally, *in vivo* NRA measurements are helpful to understand nitrate reduction and basic nitrogen metabolism of *Carex* species having different canopy structure.

Key words – *Carex*, nitrate reductase activity (NRA), *in vivo* NRA assay, diurnal variation,

Introduction

The process of nitrate assimilation is of fundamental biological importance. It occurs in a wide variety of organisms including bacteria, fungi, algae, and higher plants. The conversion of nitrate to ammonium is an 8-electron reduction process that occurs in two steps. The first step is a 2-electron reduction of nitrate to nitrite, catalyzed by the enzyme nitrate reductase (NR). The second step is a 6-electron reduction of nitrite to ammonia, catalyzed by the enzyme nitrite reductase (NiR). The step is coupled to photosynthetic electron transport in algae and higher plants via reduced ferredoxin, a product of the "light" reactions of photosynthesis, which serves as the physiological electron donor for nitrite reductase[25]. The N assimilation from NO_3^- uptake through reduction includes several inducible processes including an apparent induction of the NO_3^- uptake and transport. The regulated step of nitrate assimilation appears to be the initial reaction, catalyzed by NR. This enzyme is considered to be a limiting factor for growth, development, and protein production in plants and other nitrate-assimilating organisms. NR has therefore been intensively studied in

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order to delineate properties related to its catalytic efficiency and overall regulation. In addition, Hageman *et al.*[9] suggested that NRA can be used as an important biochemical measure for improved efficiency of nitrogen utilization. Therefore, much work has been done to define the acitvity of NR as related to nitrate concentration, light, photosynthesis, respiration and carbohydrate supply[4,17].

Principally, nitrate can be reduced in both roots and shoots of plants[1,2]. Differences in the site of reduction in various species are reported to be due to synthesis and activity of NR[22] or to a 'division of labour'[23]. However, there are distinct preferences for one site, but they seem to be limited only to a small number of plant species. Some plants (e.g. species of *Xanthium, Gossypium,* and *Cucumis*) appear to lack root NR and therefore transport all their nitrogen taken up as nitrate[21]. At the other extreme some gymnosperms and members of *Ericaceae* and *Proteaceae*[21,28] reduce nitrate mainly in their roots and transport very little to the shoot. Woody plants as a whole have been considered to translocate very little nitrate in their xylem and show preferential NO_3^- reduction in their roots. In addition, Andrews[3] noted that many of those plants which reduce NO_3^- in the shoot are of tropical or substropical origin, whereas temperate species may be either root or shoot reducers. Moreover, ecological factors such as light, temperature, growth medium, plant age and other environmental conditions may be of importance in determining the predominant nitrate reduction site[22,27].

Following first experiments with halophytes by Stewart *et al.*[31], the NRA has been successfully estabilished as an indicator of NO_3^- supply in ecological studies: the NRA of a plant is assumed to reflect the NO_3^- supply on its habitat. Since the synthesis of this enzyme is suppressed by NH_4^+ and induced by NO_3^- [33], its actual level should reflect adjustment for utilization of nitrate, and the maximum level inducible by ample nitrate supply should indicate the maximum nitrate utilizing capacity. Lee *et al.*[14] give a summary of mean NRA values in leaves of plants from different habitats and climatic regions. These values range from 0.2 μM NO_2/g FW.hr in bog plants up to 4.58 μM NO_2/g FW.hr in plants from ruderal sites and agree with results of mineralization rates from comparable sites. NRA values are available for many plants from different habitats. Al Gharbi and Hipkin[1] found especially high NRA in fast growing plants from ruderal sites, e.g. *Urtica dioica* and *Lamium album.* Very low NRA values have been reported for bog and heath plants especially of the *Ericaceae* and for species from nutrient-poor grassland sites[14]. The level of maximal inducible nitrate reductase activity is generally low in acidophilic plants, and was lowest in the Ericaceae. At first sight, this may be interpreted as the result of higher NH_4^+ availability in acidic (esp. wet-acidic) soils due to reduced nitrification in the soil types; on the other hand, a comparison of nitrate and NRA data of plants from acidic and calcareous habitats shows no strong dependence of nitrate availability on the soil pH *per se*[10,15]. Obviously, great differences in NRA from species to species[30], depending on the age of plants, diurnal rhythmics, water and light supply, contribution of different organs to total NRA, etc. may have not been considered adequately up to now.

There are two basically different modes of construction of herbaceous canopies, depending on the growth pattern of the plant constituents. Dicot herbs grow from an apical meristem. As a results a canopy of erect dicot herbs consists of stems bearing leaves, with youngest leaves receiving highest light intensities. Monocot herbs, on the other hand, develop their leaves from basal meristems. Consequently, when the meristematic tissue are near the ground or below-ground, erect monocot herbs build tillers of long leaves, with the oldest leaf parts receiving the highest light intensities at the top of the canopy[11]. This different canopy may cause different patterns of light and nitrogen distribution within the canopies. High protein content of meristematic tissues may lead to high nitrogen concentration in young leaves or leaf parts[18].

This would imply high nitrogen concentration in the upper layer of canopy in dicot stands, but low total N in the upper layer of canopy in monocot stands.

Up to now, there have been done much efforts in regard to NRA of dicot herbs (e.g. soybean) and important crop monocots (e.g. maize), but few to wild plants having canopy structure such as *Carex*[12,19]. Thus, it was interesting to study the NRA in some monocotyledonous plants in more detail. The objectives of present study are to determine: a) the optimum *in vivo* NR assay conditions for leaf samples of 4 *Carex* species, b) changes of NRA according to section within leaf and leaf ages, c) diurnal variations.

Materials and Methods

4 *Carex* species which are native to wet-acidic oligotrophic (*C. rostrata*), eutrophic (*C. gracilis*), meso-eutrophic flysh (*C. pilosa*) and meso-eutrophic saline (*C. distans*) habitats were selected, and cultivated with 1/10 strength of Knop in the open glass house. After 2 months plant samples were taken for standardization and optimization of the preliminary NRA *in vivo*. In order to obtain reliable results, the cultivation procedure was repeated and the results of the following experiments given as mean values from 3~5 measurements. Statistical analysis (analysis of variance (ANOVA), multiple range test after Scheffe, $P<0.05$) was done using Unistat 1.2.

In vivo nitrate reductase activity (NRA)

NRAwas measured *in vivo* according to Hageman and Reed[8]. Leaf samples (100-150 mg) were sliced into discs of approximately 5 mm^2 and transferred to the incubation medium, vacuum infiltrated twice (2 min each time), and incubated in the dark in a shaking water-bath at 30°C for an hour. Aliquotes (2 ml) were determined by adding an equal volume of a 1 : 1 (v/v) mixture of the naphthylethylenediamine (0.02%) and sulphanilamide reagents (1% in 3 M HCl). After color development for 10 min, the samples were measured at 540 nm.

Results

Optimization

The components of the assay media were optimized for leaves (only reductants for roots, due to difficulties in preparation). The results are shown in Table 1. Different responses of the plant species to each component could be found. Likewise, optimal NO_3^- concentration ranked between 100 to 200 mM, and pH values varied from 6.0 to 8.0. Interestingly, *C. rostrata* adapted to acidic mire habitats showed maximal values at pH 6.0. The medium $H_2PO_4^-$ concentrations ranged from 2 to 200 mM, but there was no particular difference of NRA within the concentration range of $H_2PO_4^-$ mentioned above. To avoid complications, therefore, 200 mM $H_2PO_4^-$ was selected as an optimum concentration in all assay media. In addition, with regard to isopropanol all plant species showed similar responses without great differences, but *C. pilosa* reached maximum at 3% isopropanol, *C. rostrata* at 2%, C. distans at 1% and *C. gracilis* at 0%, respectively. As to substrate NO_3^- concentrations, plant species investigated showed distinct interspecific NRA differences (Data not shown). NRA of *C. rostrata* adapted to oligotrophic habitats is readily saturated at lower substrate concentrations than those of *C. distans* and *C. gracilis*, adapted to meso- and eutrophic habitats, respectively (refer to Table 1).

Moreover, supply of different reductant patterns in the assay medium did not influence on NRA, but *C. pilosa* responded positively to NADPH showing maximum at 100 mM NADPH treatment (Fig. 1). Compared to leaves,

Table 1. Assay optimization for *in vivo* NRA measurement of 4 *Carex* species

Plant species	NO_3 (mM)	PO_4 (mM)	pH	Isopropanol (%)
C. gracilis	200	200	8.0	0
C. distans	200	200	8.0	1
C. pilosa	200	200	8.0	3
C. rostrata	100	200	6.0	2

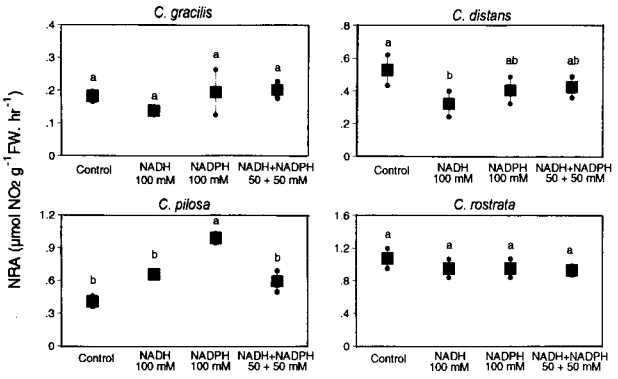

Fig. 1. Effect of various reductants on *in vivo* NRA in the leaves of 4 *Carex* species. For assay medium of each plant species refer to table 1. Error bar means standard deviation.
Values carrying different letter are significantly different at P<0.05.

NRAs in roots were lower but according to reductant supply (esp. NADPH), all plant species except *C. distans* showed rather higher NRA than control (Fig. 2). Thus it is assumed that NADPH (or NADH) being present in the leaves as result of photosynthesis or assimilating processes is not present in saturation concentration and serves as additional electron donor for nitrate reductase

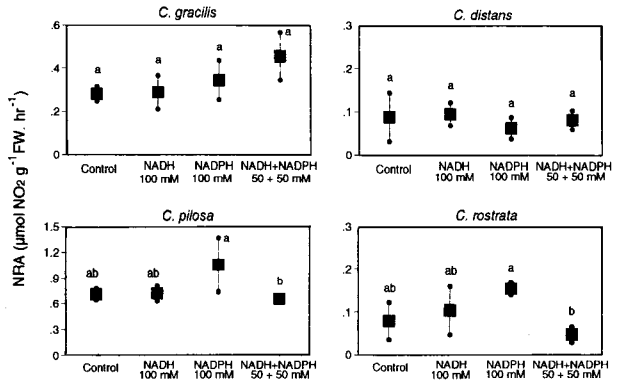

Fig. 2. Effect of various reductants on *in vivo* NRA in the roots of 4 *Carex* species (NADH & NADPH, 100 mM; NADH+NADPH, 50+50 mM; *C. gracilis*, *C. distans* & *C. pilosa* NO_3, 200 mM; *C. rostrata* NO_3, 100 mM; PO_4, 200 mM; *C. gracilis*, *C. distans* & *C. pilosa*, pH 8; *C. rostrata*, pH 6; isopropanol, 0%). Error bar means standard deviation.

especially in C. pilosa.

NRA with regard to leaf section and leaf age

All *Carex* species investigated showed higher NRA in leaves than in roots (Table 2), and remarkable changes according to the leaf section (Fig. 3). In general, all species showed maximal values in the middle section of the middle-aged leaves, *C. gracilis* showed lowest activities in the upper section and *C. distans* in the lower section. However, *C. rostrata*, having relatively short leaves, did not show any remarkable differences in regard to leaf sections. Meanwhile, NRAs in *Carex* species showed distinct differences according to leaf ages, and correlated very well with leaf length (Fig. 4; 0.71^{***} in *C. gracilis*).

Table 2. *In vivo* NRA (μM NO_2 g^{-1} FW.hr^{-1}) in the leaves and roots of 4 *Carex* species

Plant species	Plant part	NRA SD
C. gracilis	Leaf	0.76 ± 0.07
	Root	0.32 ± 0.11
C. distans	Leaf	0.52 ± 0.10
	Root	0.39 ± 0.01
C. pilosa	Leaf	0.73 ± 0.05
	Root	0.31 ± 0.04
C. rostrata	Leaf	1.15 ± 0.07
	Root	0.22 ± 0.01

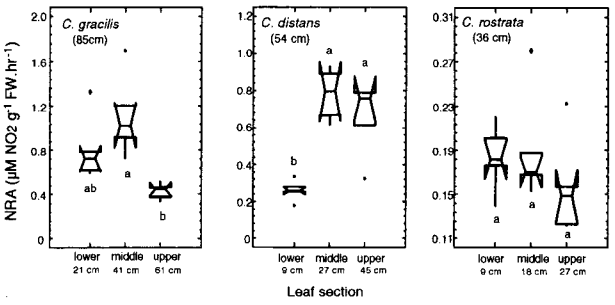

Fig. 3. *In vivo* NRA of 3 *Carex* species according to leaf section (5 replications). In each case, middle-aged leaves (= longest leaves were put together to one sample).
Values carrying different letter are significantly different at P<0.05.

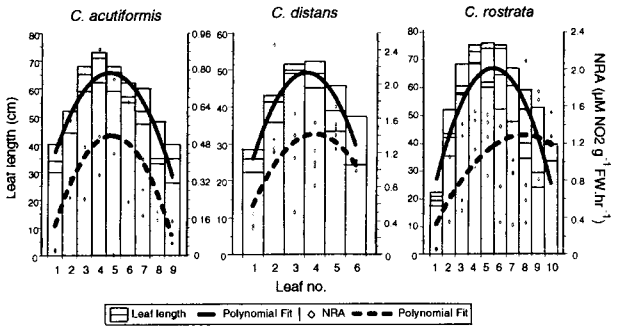

Fig. 4. Nitrate reductase activity of *Carex* species according to the leaf age (1: youngest leaf) and leaf height.

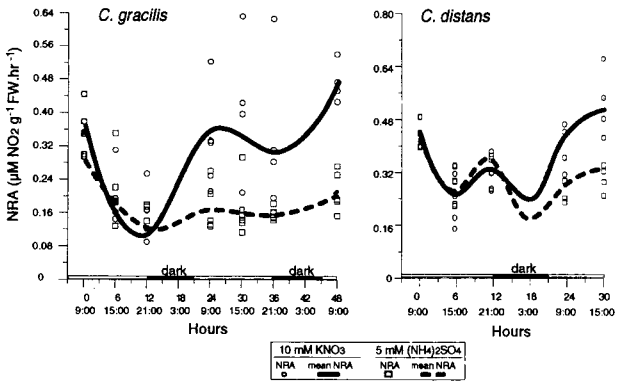

Fig. 5. Diurnal variation of *in vivo* NRA in *Carex* species according to the 2 kinds of nitrogen treatments.

During the ontogenesis of an individual leaf, a typical pattern in NRA is observed. In general, NRA increases in proportion to the leaf length until the rate of leaf expansion is maximal and shows maximal values usually in middle-aged leaves. Thereafter, the activity declines rapidly and in old leaves, NRAs are usually very low. Middle leaves (= middle-aged leaves) show generally higher NRA than younger and older leaves. However, higher NRA rather in outer leaves (= older leaves) than in middle leaves of *C. rostrata* may be connected with differences in canopy architecture, compared to *C. gracilis* and *C. distans* having higher NRA in their middle leaves.

Diurnal variation

Diurnal variations of NRA over a standard 15 hr-20°C day and 9 hr-17°C night temperature is shown in Fig. 5. Prior to start the diurnal profile, plants had received distilled water and low light intensity (5 watts/m^2) for one week. After an initial 3-hr light exposure (at 9:00 a.m.), NRA decreased during the light period. After the end of light period, NRA of 10 mM NO_3^--fed *C. gracilis* increased during the dark period. Upon re-exposure to light, NRA raised up during the first 6 hr of light and decreased again slightly during the next 9 hr of light and 3 hr of dark. Thereafter, activity increased remarkably. On the other hand, plants treated with NH_4^+-N did not show any remarkable diurnal variation after initial 3-hr light exposure. *C. distans* showed a similar diurnal pattern but compared with *C. gracilis*, NRA rate was relatively low. In addition, NR in leaves of *C. distans* was adapted readily to light period and thus showed markedly increased NRA after 22 hrs. Unlike *C. gracilis*. NH_4^+-fed *C. distans* also showed diurnal variation of enzyme activity, utilizing probably NO_3^- stored within cells (probably vacuolar NO_3^-); the relatively high NRA in NH_4^+ nourished plants may also indicate the use of an internal NO_3^- pool. Thus it is suggested that *C. distans* readily utilizes NO_3^--stored for NRA as compared with *C. gracilis*, while *C. gracilis* may lack some factors (probably reductant energy) essential to in vivo NRA.

On the whole, *Carex* species investigated show some phenomena of diurnal variations; however, the exact time pattern as response of light period and NO_3^-/NH_4^+ nutrition or induction is not yet conclusive.

Discussion

General characteristics

It is well known that nitrate reductase (NR) is the rate-limiting enzyme in the reduction process of nitrate to ammonium. Because the activity of NR controls nitrate reduction, this enzyme activity can be used as an important biochemical measure for improved efficiency of

nitrogen utilization[9]. According to optimization results (Table 1), each plant species responded differently to isopropanol concentration. To increase permeability of tissue, *C. pilosa* obviously requires more isopropanol than other species. Unlike the usual pH optimum (ca. 7.5) [24], *C. rostrata* adapted to acidic-oligotrophic habitats shows optimum at low pH of 6, obviously in connection with its ecological demands. In all species investigated, NRA started to increase considerably at 10 mM NO_3^-, but the substrate concentration for maximum NRA varies from species to species. *C. rostrata* adapted to oligotrophic habitats reached its maximum values at a somewhat lower substrate concentration than *C. distans* and *C. gracilis* adapted to meso- and eutrophic habitats, respectively (Data not shown). This is consistent with our findings that NRA in species of oligotrophic origin (e.g. *C. rostrata* and *C. limosa*) is readily inducible reaching higher values than in leaves of meso- and eutrophic species[6]. In addition, this may reflect differences in the rate of uptake of nitrate among species and/or their capacity to mobilize nitrate out of vacuoles to the metabolic pool.

On the other hand, compared to leaf NRA (Fig. 1), the NRA from roots treated with reductants (esp. NADPH) showed rather higher activities than the control (Fig. 2). Compared to shoots, roots generally have lower contents of endogenous reductants which serve as the physiological electron donor for NRA[5]. Thus, additional reductant supply (esp. NADPH) enhances particularly NRA of roots. We recommend therefore to add NADPH (or NADH) routinely to assay medium.

Nitrate reduction site and leaf canopy

The main sites of nitrate reductase in higher plants are the roots and leaves. Similar findings were obtained by Al Gharbi and Hipkin[1,2] and Cruz *et al.*[7]. Studies encompassing a wide range of plant species showed that the NRA ratios between root and shoot are not necessarily constant for each species and may vary with plant growth and age[3,5,35]. As shown in Table 2, all *Carex* species investigated have higher activities in leaves than in roots. *C. rostrata* shows remarkable differences between tissues, which do not fit in the picture presented by Andrews[3] who suggested that perennial species of the temperate zone when grown at low nitrate level develop the highest activities in the roots.

In general, all species showed maximal values in the middle section of middle-aged leaves (Fig. 3), and NR activities increased in proportion to the leaf length until the rate of leaf expansion is maximal (Fig. 4). Similar results are found in soybean[26] and in youngest fully expanded leaves of several other plants[2]. However, in other plant species, NRA reached its maximum even early. For instance, in tobacco, NRA reached a maximum peak when a leaf had expanded to 27% of its final weight and 33% of its final area[34]. It seems likely that these activity dynamics indicate a general loss of protein synthesizing capacity beyond the stage of full leaf expansion[29].

Diurnal variations

In general, NRA shows a diurnal variation. Compared to *C. gracilis*, NR in leaves of *C. distans* was adapted readily to light period and thus showed a marked activity increase after 22 hrs (Fig. 5). It seems likely that the rapid increase of *in vivo* NRA upon illumination was at least partially due to increasing availability of reductant energy [20]. In *C. gracilis*, decrease of NRA in the early light period and late acclimation may be due to an endogenous rhythm in the enzyme activity. Generally, the light stimulation may be associated with increased capacity for protein synthesis in illuminated leaves[32]. Aslam *et al.*[4] suggest that the main effect of light may be to supply photosynthates to support respiration, which in turn drives the induction process. Alternatively or coincidentally light may regulate the availability of nitrate at the induction site (uptake and transport)[13,16].

Even if the *in vivo* nitrate reductase assay based on nitrite estimation does not give an accurate estimation of total nitrate reduced, it still serves as a useful tool to find out relative differences in varying environmental conditions. Additionally, *in vivo* NRA measurements are helpful to understand nitrate reduction and basic nitrogen metabolism of *Carex* species having different canopy structure. However, for a correct application of the *in vivo* NRA test and hence, a full understanding of N assimilation processes on natural habitats, a series of factors including N forms, light, temperature, canopy structure, and their interactions have to be taken into account.

References

1. Al Gharbi, A. and C. R. Hipkin. 1984. Studies on nitrate reductase in British angiosperms. I. A comparison of nitrate reductase activity in ruderal, woodland-edge and woody species. *New Phytol.* **92,** 141-153.
2. Al Gharbi, A. and C. R. Hipkin. 1984. Studies on nitrate reductase in British angiosperms. II. Variation of nitrate reductase activities in natural populations. *New Phytol.* **97**, 629-639.
3. Andrews, M. 1986. The partitioning of nitrate assimilation between root and shoot of higher plant. *Plant Cell Environ.* **9**, 511-519.
4. Aslam, M., R. C. Huffaker and R. L. Travis. 1973. The interaction of respiration and photosynthesis in induction of nitrate reductase activity. *Plant Physiol.* **52**, 137-141.
5. Beevers, L. and R. H. Hageman. 1980. Nitrate and nitrite reduction, pp. 115-168, *In* Miflin, B. J. (ed.), *The Biochemistry of Plants*, Vol. 5, Academic Press, New York.
6. Choo, Y. S. 1995. *Mineral metabolism and organic solute pattern in Carex species of Austria an ecophysiological approach.* pp. 1-339, Ph.D Thesis, University of Wien.
7. Cruz, C. M., M. I. M. Soares, M. A. Martins-Loucao and S. H. Lips. 1991. Nitrate reduction in seedlings of carob (*Ceratonia siliqua* L.). New Phytol. **119**, 413-419.
8. Hageman, R. H. and A. J. Reed. 1980. Nitrate reductase from higher plants. *Method in Enzymol.* **60**, 270-280.
9. Hageman, R. H., E. R. Lengh and J. W. Dudley. 1967. A biochemical approach to corn breeding. *Adv. Agron.* **19**, 45-86.
10. Havill, D. C., J. A. Lee and J. De-Felice. 1977. Some factors limiting nitrate utilization in acidic and calcareous grasslands. *New Phytol.* **78**, 649-659.
11. Hirose, T., M. J. A. Werger and J. W. A. van Rheenen. 1989. Canopy development and leaf nitrogen distribution in a stand of *Carex acutiformis. Ecology* **70**, 1610-1618.
12. Janiesch, P. 1981. *Ökophysiologie Untersuchungen an Carex Arten aus Erlenbruchwäldern*, pp. 1-123, Habilitationsschrift Fachbereich Biologie, Universität Münster.
13. Jones, R. W. and R. W. Sheard. 1975. Phytochrome, nitrate movement and induction of nitrate reductase in etiolated pea terminal buds. *Plant Physiol.* **55**, 954-959.
14. Lee, J. A., S. J. Woodin and M. C. Press. 1986. Nitrogen assimilation in an ecological context, pp. 331-346, *In* Lambers, H., J. J. Neeteson and I. Stulen (eds.), *Fundamental, Ecological and Agricultural Aspects of Nitrogen Metabolism in Higher Plants*, Nijhoff Publ., Dordrecht-Boston-Lancaster.
15. Lee, J. A., R. Harmer and R. Ignaciuk. 1983. Nitrogen as a limiting factor in plant communities, pp. 95-112, *In* Lee, J. A., S. McNeil and I. H. Rorison (eds.), *Nitrogen as an Ecological Factor*, Blackwell Sci. Publ., Oxford.
16. Lillo, C. 1994. Light regulation of nitrate reductase in green leaves of higher plants. *Physiol. Plant.* **90**, 616-620.
17. Meeker, G. B., A. C. Purvis, C. A. Neyra and R. H. Hageman. 1974. Uptake and accumulation of nitrate as a major factors in the regulation of nitrate reductase activity in corn (*Zea mays* L.) leaves: Effects of high ambient CO_2 and malate, pp. 49-58. *In* Bielski, R. L., A. R. Ferguson and M. M. Creswell (eds.), *Mechanisms of Regulation of Plant Growth*, The Royal society of New Zealand, Wellington.
18. Moorby, J. and R. T. Besford. 1983. Mineral nutrition and growth, pp. 481-527, *In* Läuchli, A. and R. L. Bieleski (eds.), *Encyclopedia of Plant Physiology*, Vol. **15B**, Springer-Verlag, Berlin.
19. Müller, E. 1989. *Einfluß der Stickstoffnährung auf die in vivo Nitrat-reduktase-Aktivität von Carex pseudocyperus*

L. in Abhängigkeit von der Tageszeit. pp. 1-112, Diplomarbeit an der Oldenburg.

20. Nicholas, J. C., J. E. Harper and R. H. Hageman. 1976. Nitrate reductase activity in soybeans (*Glycine max* L. cv. Merr.). II. Energy limitations. *Plant Physiol.* **58**, 731-739.
21. Pate, J. S. 1983. Patterns of nitrogen metabolism in higher plants and their ecological significance, pp. 225-255, *In* Lee, J. A., S. McNeil and I. H. Rorison (eds.), *Nitrogen as an Ecological Factor*, Blackwell Scientific Publications, Oxford.
22. Pate, J. S. and C. A. Atkins. 1983. Nitrogen uptake, transport and utilization, pp. 245-298, In Broughton, W. J. (ed.), *Ecology of Nitrogen Fixation*, Vol. **3**, Clarendon Press, Oxford.
23. Radin, J. W. 1978. A physiological basis for the division of nitrate assimilation between roots and leaves. *Plant Sci. Lett.* **13**, 21-25.
24. Runge, M. 1983. Physiology and ecology of nitrogen nutrition, pp. 163-200, *In* Lange, O. L., P. S. Nobel, C. B. Osmond and H. Ziegler (eds.), *Encyclopedia of Plant Physiology*, New Series, Vol. **12C**, Springer-Verlag, Berlin.
25. Salsac, L., S. Chaillou, J. F. Morot-Gaudry and C. Lesaint. 1987. Nitrate and ammonium nutrition in plants. *Plant Physiol. Biochem.* **25**, 805-812.
26. Santoro, L. G. and A. C. N. Magalhaes. 1983. Changes in nitrate reductase activity during development of soybean leaf. *Z. Pflanzenphysiol.* **112**, 113-121.
27. Smirnoff, N. and G. R. Stewart. 1985. Nitrate assimilation and translocation by higher plants: Comparative physiological and ecological consequences. *Physiol. Plant.* **64**, 133-140.
28. Smirnoff, N., P. Todd and G. R. Stewart. 1984. The occurrence of nitrate reduction in the leaves of woody plants. *Ann. Bot.* **54**, 363-374.
29. Srivastava, H. S. 1980. Regulation of nitrate reductase activity in higher plants. *Phytochemistry* **19**, 725-733.
30. Stewart, G. R. and T. O. Orebamjo. 1983. Studies of nitrate utilization by the dominant species of regrowth vegetation of tropical West Africa: a Nigerian example, pp. 167-168, In Lee, J. A., S. McNeil and I. H. Rorison (eds.), *Nitrogen as an Ecological Factor*, Blackwell Sci. Publ., Oxford.
31. Stewart, G. R., J. A. Lee and T. O. Orebamjo. 1972. Nitrogen metabolism of halophytes. I. Nitrate reductase activity in *Suaeda maritima*. *New Phytol.* **71**, 263-267.
32. Travis, R. L. and J. L. Key. 1971. Correlation between polyribosome level and the ability to induce nitrate reductase in dark grown corn seedlings. *Plant Physiol.* **48**, 617-620.
33. Ullrich, W.R. 1983. Uptake and reduction of nitrate: Algae and fungi, pp. 376-397 *In* Läuchli, A. and R. L. Bieleski (eds.), *Encyclopedia of Plant Physiology*, New Series. Vol **15A**, Springer-Verlag, Berlin-New York.
34. Wakhloo, J. and A. Staudt. 1988. Development of nitrate reductase activity in expanding leaves of *Nicotiana tabacum* in relation to the concentration of nitrate and potassium. *Plant Physiol.* **87**, 258-263.
35. Woodin, S. J. and J. A. Lee. 1987. The effects of nitrate, ammonium and temperature on nitrate reductase activity in *Sphagnum* species. *New Phytol.* **105**, 103-115.

초록 : 사초속 식물의 질산환원효소 활성의 특징

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쌍자엽 식물 및 주요 작물의 질산환원효소(nitrate reductase) 활성에 관한 연구는 많이 이루어져 왔으나, 사초속 식물과 같은 야외식물에 대한 연구는 거의 이루어지지 않았다. 본 연구에서는 생육환경이 다른 4종의 사초속 식물을 선택하여, 이들 각 식물에 대한 *in vivo* NR 활성의 최적조건, 잎의 부위 및 연령에 따른 NR 활성 그리고 일변화 양상을 조사하였다.

산성의 빈영양 토양에 적응된 *C. rostrata*의 NR 활성은 영양이 풍부한 장소에 서식하는 *C. distans*와 *C. gracilis* 보다 더 낮은 기질 농도에서도 쉽게 포화되었다. 전반적으로, 조사된 모든 사초속 식물은 뿌리보다는 잎에서 더 높은 NR 활성을 보였으며, 완전히 신장된 새로운 잎의 중앙부에서 최대 활성을 나타내었다. 한편, 질산환원효소의 일주기 반응에서 *C. distans*는 *C. gracilis*에 비해 광주기에 쉽게 적응하였으며, 보편적으로 사초속 식물은 다소 지연된 일변화 양상을 보였다.

Nitrite 측정에 바탕을 둔 *in vivo* NR 활성 분석이 환원되는 nitrate의 총량을 정확히 평가하지 못할지라도, 환경조건의 변화에 따른 상대적인 차이를 규명하는데 있어서 유용한 도구가 되며, 또한 상이한 수관구조를 갖는 사초속 식물의 질산환원 및 기초적인 질소대사를 이해하는데 도움이 된다.