Korean J Biol Sci 2: 455-462, 1998

Influence of Light Regime on Nitrate Reductase Activity and Organic and Inorganic Solute Composition of Four Sedges (*Carex* spp.)

Yeon-Sik Choo, Roland Albert[1], and Seung-Dal Song*

Department of Biology, College of Natural Sciences, Kyungpook National University, Daegu 702-701, Korea; [1]Coordination Office for Ecology Studies, University of Vienna, A-1091 Wien, Austria

Key Words:
Light regime
Nitrate reductase activity (NRA)
Carex
Mineral metabolism
Organic solutes
Ionic balance

A survey was conducted on the inorganic and organic solute patterns of plants in connection with nitrate metabolism according to different light regimes (1.9, 16.0, 91.5 Wm^{-2}). Besides measuring *in vivo* NRA, we also quantitatively analyzed water-soluble inorganic ions, organic acids, low molecular weight carbohydrates, amino acids and total N (% DW). Among 4 *Carex* species, *C. pilosa* is known as a shade-adapted species and the others as half (*C. gracilis*) to full (*C. rostrata* & *C. distans*) light-adapted species. Compared to species adapted to high light intensity, shade-adapted *C. pilosa* showed reduced productivity under the highest light intensity. In general, nitrate and amino acid levels decreased at higher light intensity, while sugar and organic acid concentrations increased. In *C. pilosa* osmolality tended to rise with increasing light intensity, while in the other species it tended to fall. Under low light intensity, the drop in soluble carbohydrate contents is osmotically compensated for by an enhanced nitrate concentration. It is concluded that competition between nitrate and CO_2 reduction for reductants and ATP from photosynthesis may have important ecological consequences for the adaptation of plants to low or high light conditions. Additionally, the patterns of ionic changes due to increased light intensities were essentially the same in all selected species, indicating similar characteristics of their mineral ion and organic acid metabolism as well as in field-grown *Carex* species.

Photosynthetic energy capture provides green plants with almost all of their chemical energy and is directly and dramatically influenced by the amount of light striking plant leaves. Many investigators have therefore studied how different levels of photosynthetically active radiation affect the CO_2 assimilation process, how plants adapt anatomic-morphologically and physiologically to changing environmental conditions, and how their adaptation processes influence photosynthetic response to the light level (Boardman, 1977; Björkman, 1981; Givnish, 1988). Several features of plant form, physiology, and resource allocation vary with the levels of irradiation to which plants are acclimated and/or ecologically restricted (Boardman, 1977; Björkman, 1981; Givnish, 1987; refer to Bazzaz et al., 1989). In general, it is well established that plants from open, sunny habitats (sun plants) have much greater photosynthetic capacities, which saturate at higher irradiance, than those of plants growing on shady habitats (shade plants) (Boardman, 1977; Björkman, 1981). However, shade plants use low irradiance more effectively for net photosynthetic CO_2 uptake than do sun plants, due to their lower, dark respiration rates (Anderson et al., 1988) and due to complex adaptation mechanisms (Osmond, 1987). In particular, leaf nitrogen content, stomatal conductance and photosynthetic light response show characteristic reactions to irradiance level and are thought to exert a profound influence on plant carbon gain under different light regimes (Björkman, 1981; Gulmon and Chu, 1981). The relationship between photosynthesis and leaf nitrogen is the most important physiological function, because photosynthesis provides the energy and chemical structures necessary for growth, reproduction, or acquisition of additional nitrogen (Field and Mooney, 1986).

In connection with nitrogen, both the intensity and duration of light have long been recognized to have a stimulatory effect on NRA (nitrate reductase activity) in green plants (Nicholas et al., 1976). Nitrate reduction in plants is involved in a basic photosynthetic process, i.e., an important sink for photosynthetically produced reductants (Kaiser and Brendle-Behnisch, 1991), since it either directly or indirectly uses a high proportion of the total photo-generated reducing power in the chlo-

* To whom correspondence should be addressed.
Tel: 82-53-950-5348, Fax: 82-53-953-3066
E-mail: sdsong@bh.kyungpook.ac.kr

Table 1. Range of habitats, morphological characters and light-figure of the investigated *Carex* species.

Species	Range of habitats	Morphological characters	Light figure*
C. rostrata	Found in nutrient-poor, *Sphagnum* bog lakes, as well as in more nutrient-rich lakes, ditches and mesotrophic peat areas.	Height: 0.2-1.0 m Leaf width: 2-7 mm Rhizomes about 2 mm thick and several decimeters long	9
C. distans	Moist meadows, mostly on saline and calcareous soils; salt-containing coastal sand dunes, facultative halophyte.	Height: 0.3-0.6 m Leaf width: 3-5 mm Densely caespitose	9
C. gracilis	A species of nutrient-rich riversides, marshes and other wet places.	High plant: upto 1.5 m Leaf width: 5-10 mm Caespitose, and with long and stout rhizomes	7
C. pilosa	Usually deciduous forest floors, on fresh, mesotrophic, humus-rich soils; sometimes in openings.	Height: 0.2-0.5 m Leaf width: 4-10 mm Rhizomes long and slender	4

*Light-figure is characterized by the indicator value of Ellenberg (1974) (4: between shadow and half-shadow plant, receiving more than 10%, but mostly less than 100%; 7: half-light plant; 9: full-light plant, rarely receiving less than 50%).

roplast (Losada et al., 1981). It has to be expected, therefore, that CO_2 and NO_3^- reduction interact in various ways (Foyer et al., 1995; Evans, 1996). A simple type of interaction would be competition for reductants, and results obtained recently with intact barley plants indicate that higher plants may respond in a similar way (Bloom et al., 1989). This competition may have important ecological consequences for the adaptation of plants to different light conditions (Smirnoff and Stewart, 1985). Evidence for a reverse type of interaction, regulation of nitrate reduction by photosynthesis, is less well demonstrated and understood, although it should be important in helping plants adapt to rapidly changing environmental conditions. NRA, reported to be regulated by NO_3^--flux in the plant (Neyra and Hageman, 1976), is influenced both by the availability of sugars (Aslam et al., 1979) and/or the supply of reducing equivalents (ferredoxin and NADPH) (Foyer et al., 1995; Marschner, 1995). However, a direct relationship between carbon metabolism and NRA in intact plants has not been clearly established.

Until now, many researchers have investigated growth, photosynthetic rate, economics, and nitrogen in the sun-shade response (Nobel, 1976; Patterson et al., 1978; Björkman, 1981; Hesketh et al., 1983; Cowan, 1986), but there are few studies on the patterns of inorganic and organic solutes in connection with light-induced nitrate assimilation.

In the present study, we selected four *Carex* species in order to elucidate the pattern of mineral ions, nitrogen and organic solutes according to light regimes, and additionally to ascertain whether the physiological stability of field-grown *Carex,* which represents a uniform physiotype (Choo and Albert, 1999a), can be also found under controlled conditions.

Materials and Methods

Growth conditions and harvest

Individuals (ramets) of *C. gracilis, C. rostrata, C. distans* (receiving 1/10 strength of Knop solution and grown in an open glass house for 6 months), and *C. pilosa* (collected from the natural habitat) were transferred to 10 L containers and were grown in a nutrient solution containing full strength Knop solution with 5 mM NH_4NO_3 [Note: Because of oxygen deficiency (Table 1: Choo and Albert, 1999b), *C. pilosa* was grown on soil collected from its natural habitat using 1/10 strength of Knop solution]. The plants subsequently were transferred to a growth chamber (20±2℃ during the day and 17±2℃ during the night, day length of 15 h, 80% relative humidity). Irradiation by a metal halide lamp was at 1.9 Wm^{-2} (L1), 16.0 Wm^{-2} (L2) and 91.5 Wm^{-2} (L3). The nutrient solution was renewed every week. Growth was permitted for 6 (*C. rostrata, C. distans,* and *C. gracilis*) and 10 (*C. pilosa*) weeks. All experiments were carried out in 3 replicates. The ecology of the *Carex* species used in this study is shown in Table 1 based on Hegi (1979) and Moog and Janiesch (1989).

After determination of fresh weight (FW), leaf samples were lyophilized for 1 week. The plant water content was evaluated as the difference between fresh and dry weight (DW). The dried plant material was ground to a homogeneous powder and extracted with boiling distilled water for 30 min. In the water extracts, the following groups of substances were quantitatively determined: the soluble fraction of cationic macronutrients (Na^+, K^+, Mg^{2+}, and Ca^{2+}), inorganic anions (Cl^-, NO_3^-, and SO_4^{2-}), the organic anions, inorganic phosphate (Pi), LMWC (low molecular weight carbohydrates as sum of carbohydrates and sugar alcohols), and amino acids.

Chemical analyses

Cations were estimated by atomic absorption spectrophotometry (Perkin Elmer 3030) with 0.1% CsCl as an ionization buffer. Inorganic anions were determined from diluted water extracts by an ion chromatographic

Table 2. Contents of water-soluble inorganic and organic solutes (μeq g^{-1} plant water; TAA & TS, μmol g^{-1} plant water) of *Carex* species under different light intensities for 6 to 10 (*C. pilosa*) weeks after onset of the experiment.

Plant species		K	TC-K	NO_3	TIA-NO_3
C. rostrata	L1	126.89 ± 13.33*	37.39 ± 3.74**	97.77 ± 9.08**	40.00 ± 4.85*
	L2	114.62 ± 13.82*	48.07 ± 6.93*	75.03 ± 6.76**	37.27 ± 3.23**
	L3	144.57 ± 16.67*	51.97 ± 5.27*	42.03 ± 4.37*	32.48 ± 4.87*
C. distans	L1	264.99 ± 14.60**	36.87 ± 4.06*	118.19 ± 8.38**	66.05 ± 7.14*
	L2	164.23 ± 14.81**	35.89 ± 5.88*	106.06 ± 8.10**	52.33 ± 7.59*
	L3	141.61 ± 7.27**	39.26 ± 4.54*	40.87 ± 4.26*	49.76 ± 5.81*
C. gracilis	L1	168.77 ± 15.22**	25.20 ± 3.89*	74.19 ± 8.14*	58.11 ± 4.39**
	L2	149.22 ± 16.82*	35.52 ± 3.70*	111.18 ± 10.95**	24.92 ± 2.91*
	L3	104.11 ± 10.41**	42.56 ± 7.47*	65.73 ± 7.95*	22.03 ± 1.65**
C. pilosa	L1	223.82 ± 21.27**	88.91 ± 10.53*	36.82 ± 1.66**	92.67 ± 8.94**
	L2	212.43 ± 16.23**	80.42 ± 13.74*	9.87 ± 0.65**	49.22 ± 6.78*
	L3	183.19 ± 23.05*	120.40 ± 15.06*	7.90 ± 0.28**	44.57 ± 4.67*
Plant species		TOA	Asn	TAA-Asn	TS
C. rostrata	L1	9.90 ± 0.84**	86.99 ± 3.99**	10.50 ± 0.96**	0.37 ± 0.02**
	L2	23.37 ± 1.75**	30.90 ± 2.17**	10.40 ± 1.27*	37.92 ± 3.82*
	L3	71.59 ± 8.36**	2.44 ± 0.09**	17.09 ± 1.72*	26.96 ± 1.62**
C. distans	L1	29.52 ± 1.46**	47.25 ± 4.33**	14.30 ± 1.51*	16.70 ± 1.76*
	L2	26.84 ± 2.15**	28.77 ± 2.72**	27.99 ± 3.64*	64.44 ± 5.12**
	L3	57.26 ± 2.50**	12.05 ± 1.24*	29.83 ± 3.43*	187.22 ± 25.33*
C. gracilis	L1	5.37 ± 0.48**	125.31 ± 9.46**	9.61 ± 0.60**	11.39 ± 1.04**
	L2	24.22 ± 2.50*	41.31 ± 3.12**	13.20 ± 1.33*	15.33 ± 1.88*
	L3	34.76 ± 3.50*	16.12 ± 1.74*	8.72 ± 0.53**	110.21 ± 11.09*
C. pilosa	L1	103.68 ± 10.99*	1.35 ± 0.14*	6.58 ± 0.70*	81.06 ± 8.54*
	L2	221.46 ± 24.66*	0.02 ± -	13.31 ± 1.06**	172.27 ± 22.42*
	L3	272.60 ± 31.83*	0.82 ± 0.10*	13.79 ± 1.87*	281.73 ± 32.41*

Mean values of 3 replicates with standard errors. Asterisks following the standard errors indicate significant differences ($P<0.05$ (*), $P<0.01$ (**)). L1; 1,9, L2; 16.0, L3; 91.5 Wm^{-2}, TC; total inorganic cation, TIA; total inorganic anions, TOA; total organic acids, Asn; asparagine, TAA; total amino acids, TS; total sugars and sugar alcohols.

method (Wescan conductivity detector, Kontron HPLC-pump and calculation system) under the following conditions: anion exchange column 'Wescan 269001', eluent 5-6 mM potassium hydrogenphthalate with 2.5% methanol, flow rate 2 ml min^{-1}, back pressure 48-55 bars at 40℃ working temperature. However, in this system an injection peak together with two negative system peaks appeared, which interfered with the quantity of Cl^- (Erkelens et al., 1987). Therefore, we used a Buchler Digital Chloridometer (Model, 4-2500) for the accurate measurement of Cl^-.

Carbohydrates, organic acids and phosphate ions were determined by gas-liquid chromatography (Popp, 1974; Albert and Popp, 1977; Englmaier, 1980, 1990), and amino acids were determined by HPLC (Phamacia LKB alpha plus; lithium citrate-method) after Kedenburg (1971).

Total N was determined by the micro-Kjeldahl method. With exception of total N (% DW), all solutes have been analyzed from the hot water extracts and were calculated as μeq (resp. μmol) per gram of plant water which approximates to cell sap concentrations.

Nitrate reductase activity (NRA)

NRA was measured *in vivo* according to Hageman et al. (1971, 1980), but with some modifications. In preliminary experiments, the composition of the assay medium was optimized for all plants under investigation (Choo, 1995), and additionally those leaves (within tillers), showing the highest NRA, were selected for the experiments. Thus, the middle section of the two longest leaves from about 3-5 tillers have been used for NRA measurement (in the case of *C. pilosa*, middle section of the longest 5-7 leaves). Leaf samples (100-150 mg) were sliced into discs of approximately 5 mm and transferred in the incubation medium, vacuum infiltrated twice (2 min each time), and incubated in the dark in a shaking water-bath at 30℃ for an hour. Aliquots (2 ml) were determined by adding an equal volume of a 1:1 (v/v) mixture of naphthylethylenediamine (0.02%) and sulphanilamide reagents (1% in 3 M HCl). After color development for 10 min, the samples were measured at 540 nm. All measurements were repeated 3-5 times.

Results

As shown in Fig. 1, *C. pilosa*, which is adapted to shady habitats, reduced its productivity at the highest light intensity (91.5 Wm^{-2}), whereas all other species showed increased growth at higher light intensities.

All 4 selected species showed similar patterns of cations and anions within leaves under different light conditions (Table 2). As light intensities rise, contents of organic acid increased (factors of 2 to 8), while nitrate levels decreased markedly. Increasing light intensity induced high NRA and stimulated organic acid synthesis to maintain ionic balance (Fig. 2). However, there were no distinct qualitative changes in

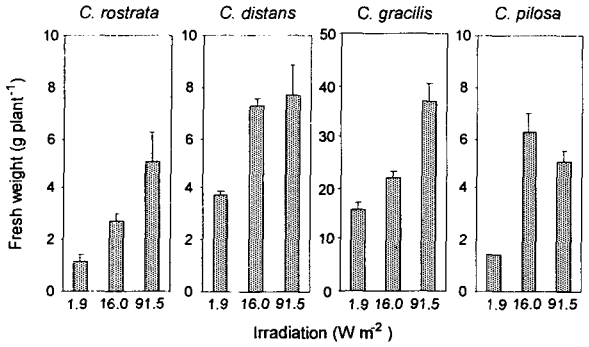

Fig. 1. Plant matter (g FW) of the shoots of *Carex* species grown in solution culture (*C. pilosa* grown on soil collected from its natural habitat) under different light intensities for 6 to 10 (*C. pilosa*) weeks after the onset of the experiment. Note the different scales.

the organic acids produced (data not shown). Regardless of the high light intensity (91.5 Wm^{-2}), all species (except *C. pilosa)* still showed high nitrate contents. Clearly, even the highest light level used in our experiments did not offer the optimal light conditions to which these plants are adapted in the field.

As light intensity increased, total N contents (%) of high-light plants decreased, whereas their NRAs increased markedly (Figs. 2 and 3). In all investigated species, except for *C. pilosa*, ratios of amino-N and nitrate-N to total N dropped considerably, whereas the

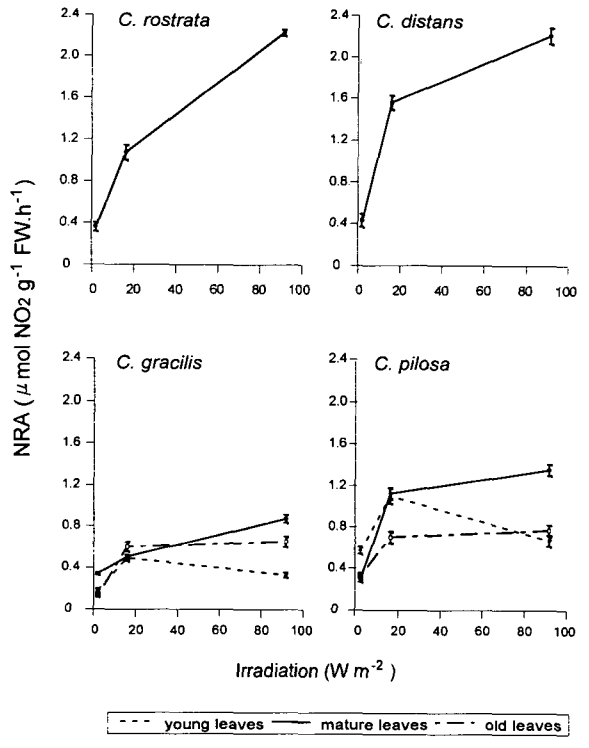

Fig. 2. *In vivo* nitrate reductase activity (NRA, μmol NO_2 g^{-1} $FW.h^{-1}$) in the shoots of the *Carex* species as a function of light intensity. Data represent means ±SE from 3 replicates.

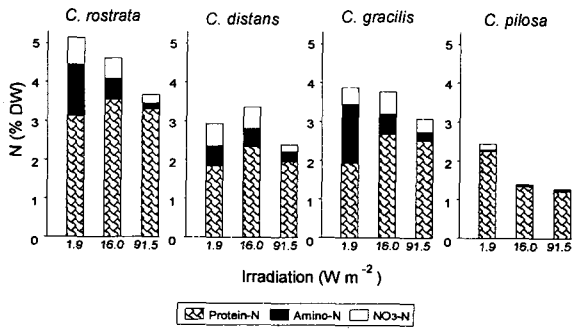

Fig. 3. Contents of nitrogen compounds (% DW) in the shoots of *Carex* species grown in solution culture under different light regimes (*C. pilosa*, 10 weeks). Values are means of 3 replicates.

ratio of protein-N to total N increased. With respect to NRA, *Carex* species showed low NRAs at low light intensity (1.9 Wm^{-2}), but their activities were markedly increased with increasing light intensity (Fig. 2). In general young leaves (of *C. pilosa* and *C. gracilis*) had lower NRAs, and their activities were readily saturated even at low light intensity (16 Wm^{-2}), while NRAs of the mature leaves increased with increasing light intensity until maximum leaf expansion. As leaves grew old, the activity declined rapidly and was usually very low in old leaves (see NRAs of *C. gracilis* & *C. pilosa*).

With the increase of light intensity, all the plants increased their sugar and decreased their amino acid contents (especially asparagine), except *C. pilosa*, whose amino acid contents increased slightly (Table 2 and Fig. 4). Unlike the other species, which contained mostly asparagine (Asn), γ-aminobutyric acid (GABA, data not shown) is the main amino acid in *C. pilosa*. The investigated *Carex* species showed distinct quantitative but not qualitative changes of the main osmotica pattern according to the applied light intensities (Fig. 4). The osmolality of *C. pilosa* (adapted to

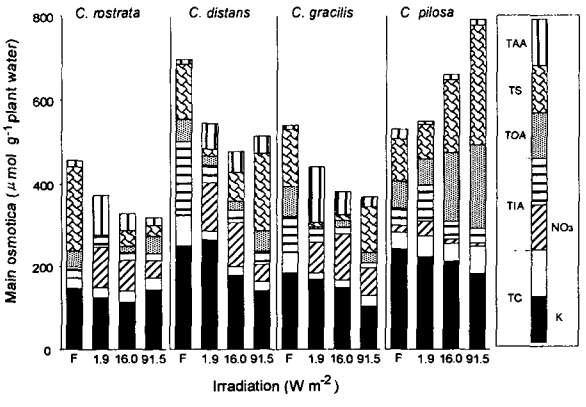

Fig. 4. Contents of main osmotica (μmol g^{-1} plant water) in the shoots of *Carex* species grown in solution culture under different light regimes (*C. pilosa*, 10 weeks). Values are means of 3 replicates. F; field-grown plant species, TC; total inorganic cation, TIA; total inorganic anions, TOA; total organic acids, TAA; total amino acids, TS; total sugars and sugar alcohols.

shady habitats) increased under higher light intensity, while osmolalities of the other species decreased. On the whole, all selected species accumulated more amino acids, but contained less sugars under light intensity that are lower than that occurring usually in their natural habitats. With the increase of light intensity, their nitrate and amino acid levels decreased, while their sugar and organic acid levels increased markedly. With a few exceptions, therefore, sugars are important energy sources and main osmolytes in *Carex* species under natural conditions. At low light intensity, on the other hand, amino acids and nitrate accumulate and may serve as important osmotica. Thus, the decrease in soluble carbohydrate contents is osmotically compensated for by opposite changes in nitrate concentration.

Discussion

Unlike the other species investigated, shade-adapted *C. pilosa* showed marked growth reduction under high light intensity (91.5 Wm^{-2}; Fig. 1). This may be due to the inhibition of photosynthetic activity (i.e. chlorosis), which is consistent with the literature findings (Björkman and Homgren, 1963; Foyer and Hall, 1980; Osmond, 1983).

The changes of ionic patterns with increasing light intensity follow essentially the same trend in all selected species, indicating quite similar characteristics of their mineral ion and organic acid metabolism. Generally, inorganic cations (except K^+) and anions (except NO_3^-) did not respond to changing light intensity. As light intensity rise, the nitrate content decreased, while the organic acid contents increased considerably without changing the qualitative pattern of single components (Table 2 and Fig. 4: Blom-Zandstra and Lampe, 1985). The assimilation of NO_3^- to amino-nitrogen is known to be strictly light-dependent (Canvin and Atkins, 1974). Thus under low light intensity, nitrate is accumulated but organic acids remain relatively low (Hewitt et al., 1976). The same holds true for the *Carex* species. In spite of high nitrate nutrition, NRA remains low and nitrate accumulates within shoots (Fig. 2 and Table 2). Moreover, energy shortages may restrict nitrate reduction. As an overall consequence, the synthesis of organic acids, usually compensating for the buildup of relative cation excess (total inorganic cations-total inorganic anions) in connection with nitrate reduction, is also limited. It should be noted that nitrate is generally phloem immobile, and thus nitrate taken up is stored nearly exclusively in the vacuoles of shoots (Martinoia et al., 1981). Moreover, because vacuolar NO_3^- is metabolically ineffective to function as an inducer for nitrate reductase (NR) (Heimer and Filner, 1971), the release of nitrate from vacuoles into the cytoplasm can become a rate-limiting step for nitrate reduction (Martin, 1973; Rufty et al., 1982).

Compared to the other *Carex* species, which maintained their normal ionic balances even under varying light intensities, *C. pilosa* could do this only under lower light intensity, but at high irradiation, organic acids (especially citrate) accumulated considerably and symptoms of chlorosis occurred, similar to high nitrate-fed *C. pilosa* (Choo and Albert, 1999b).

As light intensity increased, total N (especially nitrate-N and amino-N) decreased gradually, but the ratio of protein-N to total N increased distinctly (Fig. 3). *C. pilosa*, however, showed a remarkable drop in protein-N at higher light intensities. This is presumably due to photoinhibition under intense light, followed by protein degradation. It should be noted that, over a broad range, photosynthetic rates are proportional to leaf nitrogen concentrations (esp. protein-N concentration). This is because most leaf nitrogen is directly involved in photosynthesis as a component of photosynthetic enzymes (e.g. RuBP-Case) and chlorophylls (Evans, 1989). In contrast, the high ratio of amino-N to total N found in all species except *C. pilosa* under low light intensity could be regarded as a stress symptom. This is consistent with some findings that under various stress situations including leaf senescence, soluble nitrogen compounds (esp. amino acids) increase at the cost of proteins (Beevers, 1976; Chu et al., 1976; Huber et al., 1977; Rabe, 1994). However, the qualitative amino acid composition of the three mentioned species remained unchanged and was similar to that under other experimental conditions used in this study (Choo and Albert, 1999b).

Compared to other plants with high amino acid contents (esp. Asn), the low contribution of Asn in *C. pilosa* is probably due to the low nitrogen content in the soil medium (Table 2). Furthermore, the low amino acid contents in *C. pilosa* might be a shade adaptation.

NRAs of expanded leaves were enhanced at higher light levels (Fig. 2). It is assumed that light not only stimulates *de novo* synthesis, but also activates plant tissue NR at the protein level (Lillo, 1994). In green leaves, light induction of NR mRNA and NR protein is apparently mediated through products of CO_2 fixation (Foyer et al., 1995; Lea, 1997). In addition to CO_2 assimilation, therefore, nitrate reduction in leaves is an important sink for photosynthetically produced reductants (Kaiser and Brendle-Behnisch, 1991) and also is closely coupled to net photosynthesis (Kaiser et al., 1992). The general increase of NRA with increasing light is induced by the presence of both nitrate and light (Santos et al., 1992). According to Campbell and Smarrelli (1986), and in contrast to these findings, light directly affects protein synthesis, but plays no direct role in NRA. Until now, its overall regulation by photosynthesis is only poorly understood, although it should be important in adapting to rapidly changing environmental conditions (Kaiser and Brendle-Behnisch, 1991). In the present study, despite high NO_3^- contents

in shoots, low NRA of *Carex* species under low light intensity may be due to the shortage of reductants furnished from photosynthesis.

The light-induced patterns of sugars are in marked contrast to amino acids (Fig. 4). In contrast to amino acids, sugar contents generally increased under higher light intensities. This is partially due to the fact that the pathways of sucrose and amino acid biosynthesis compete for carbon skeletons and energy sources (Champigny and Foyer, 1992). In leaves, NO_3^- assimilation takes place in the same compartments, i.e., cytosol (NO_3^- reduction to NO_2^-) and chloroplasts (reduction of NO_2^- to NH_4^+ and assimilation of the latter into glutamate) as sucrose and starch synthesis, respectively. The reduction of NO_2^- to NH_4^+ uses photochemically generated reducing power, as does the reduction of CO_2 to carbohydrates, and is considered to be an important sink for the products of photosynthetic electron flow (Losada, 1976). Moreover, sucrose synthesis is regulated at the level of partitioning of carbon between organic acid and amino acid synthesis in plant leaves (Champigny et al., 1991). At low light intensity, therefore, increased carbon flux to amino acids is associated with a decrease of phosphoenolpyruvate (PEP) content, the activation of PEP-Case (Champigny and Foyer, 1992) and with low NRA in leaves (Radin et al., 1978). However, shade-adapted *C. pilosa* maintained higher tissue carbohydrate concentrations under low light intensity than species adapted to high light intensity (Table 2 and Fig. 4). It is well known that shade plants have a much higher efficiency in using weak light than sun plants (Osmond et al., 1980). Due to an efficient carbon metabolism under low light intensity, therefore, *C. pilosa* can adapt successfully to shady habitats.

Light intensities below the compensation point lead to starvation with carbohydrates used as a substrate for respiration, but gives rise to considerable NO_3^- accumulation (Tychsen, 1976). Under low light conditions, therefore, the decrease in soluble carbohydrates is osmotically compensated for by an opposite change in nitrate concentration as a result of suboptimal photosynthesis (see above). This is consistent with the literature (ryegrass, Veen and Kleinendorst, 1985; lettuce, Blom-Zandstra and Lampe, 1985; spinach, Steingröver et al., 1986). Thus, nitrate as an osmoticum should also be regarded as a useful adaptation to the variations of carbohydrate synthesis in order to maintain osmotic homeostasis (Ourry et al., 1989). In the investigated *Carex* species, increased light intensity generally causes a distinct shift from nitrate accumulation in the shoots towards accumulation of sugars (mainly sucrose) and organic acids (mainly citrate and malate).

It is concluded that competition between nitrate and CO_2 reduction for reductants and ATP from photosynthesis may have important ecological consequences for the adaptation of plants to low or high light conditions. Additionally, the patterns of ionic changes due to increased light intensities were essentially the same in all selected species, indicating profoundly similar characteristics in their mineral ion and organic acid metabolism as well as in field-grown *Carex* species (Choo and Albert, 1999a).

Acknowledgements

This research was supported by grants from the Austrian Science Foundation (FWF; PO 9807-BIO) and Korea Scientific and Engineering Foundation (971-0811-202-2). The authors would like to thank Prof. H. Kinzel for providing profound comments on the manuscript, and Dr. M. Stachowitsch for correcting the manuscript.

References

Albert R and Popp M (1977) Chemical composition of halophytes from Neusiedler lake region in Austria. *Oecologia* 27: 157-170.

Anderson JM, Chow WS, and Goodchild DJ (1988) Thylakoid membrane organisation in sun/shade acclimation. In: Evans JR, von Caemmerer S, and Adams WW (eds), Ecology of Photosynthesis in Sun and Shade, CSIRO, Australia, pp 11-26.

Aslam M, Huffaker RC, Rains DW, and Rao KP (1979) Influence of light and ambient carbon dioxide concentration on nitrate assimilation by intact barley seedlings. *Plant Physiol* 63: 1205-1209.

Bazzaz FA, Chairiello NR, Corey PD, and Pitelka NF (1989) Allocating resources to reproduction and defence. *BioScience* 37: 58-67.

Beevers L (1976) Nitrogen Metabolism in Plants. Edward Arnolds, London, pp 1-333

Björkman O (1981) Response to different quantum flux densities. In: Lange OL, Nobel PS, Osmond CB, and Ziegler H (eds), Encyclopedia of Plant Physiology, New Series, Vol 12A, Physiological Plant Ecology I, Springer-Verlag, Berlin, pp 57-107.

Björkman O and Holmgren P (1963) Adaptability of the photosynthetic apparatus to light intensity in ecotypes from exposed and shaded habitats. *Physiol Plant* 16: 889-914.

Blom-Zandstra M and Lampe JEM (1985) The role of nitrate in the osmoregulation of lettuce (*Lactuca sativa* L.) grown at different light intensities. *J Exp Bot* 36: 1043-1052.

Bloom A, Caldwell RM, Finazzo J, Warner RL, and Weissbart J (1989) Oxygen and carbon dioxide fluxes from barley shoots depend on nitrate assimilation. *Plant Physiol* 91: 353-356.

Boardman NK (1977) Comparative photosynthesis of sun and shade plants. *Annu Rev Plant Physiol* 28: 355-77.

Campbell WH and Smarrelli J JR (1986) Nitrate reductase biochemistry and regulation. In: Neyra C (ed), Biochemical Basis of Plant Breeding. Vol II, CRC Press, Boca Raton, pp 1-39.

Canvin DT and Atkins CA (1974) Nitrate, nitrite and ammonia assimilation by leaves: effects of light, carbon dioxide and O_2. *Planta* 116: 207-224.

Champigny ML and Foyer C (1992) Nitrate activation of cytosolic protein kinases diverts photosynthetic carbon from sucrose to amino acid biosynthesis. *Plant Physiol* 100: 7-12.

Champigny ML, Valadier MH, van Quy L, and Moyse A (1991) Short-term effects of nitrate on CO_2 photoassimilation in wheat leaves. *C R Acad Sci Ser III* 312: 469-476.

Choo YS (1995) Mineral metabolism and organic solute pattern in *Carex* species of Austria: an ecophysiological approach. Ph. D Thesis, University of Vienna, Austria, pp 1-339.

Choo YS and Albert R (1999a) Mineral ions, nitrogen and organic solute pattern in sedge (*Carex* spp). I. Field samples.

Flora 194: in press.
Choo YS and Albert R (1999b) Mineral ions, nitrogen and organic solute pattern in sedge (*Carex* spp). II.Culture experiments. *Flora* 194: in press.
Chu TM, Aspinall D, and Paleg LG (1976) Stress metabolism. III. Salinity and proline accumulation in barley. *Aust J Plant Physiol* 3: 219-228.
Cowan IR (1986) Economics of cabon fixation in higher plants. In: Givnish TJ (ed), On the Economy of Plant Form and Function, Cambridge University Press, Cambridge, pp 133-170.
Ellenberg H (1974) Indicator Values in Vascular Plants in Central Europe. Scripta Botanica. Vol 9. Verlag E. Goltze KG, Gttingen. pp 1-96.
Englmaier P (1980) Trimethylsilyl-ester pflanzlicher Säuren und ihre Anwendung in der Gaschromatographie. *J Chromatogr* 194: 33-42.
Englmaier P (1990) High resolution-GLC of carbohydrates as their dithioacetal-trimethylsylates and -trifluoroacetates. *J High Resol Chromatogr* 13: 121-125.
Erkelens C, Billiet HAH, de Galan L, and de Leer EWB (1987) Origin of system peaks in single-column ion chromatography of inorganic anions using high pH borate-gluconate buffers and conductivity detection. *J Chromatogr* 404: 67-72.
Evans JR (1989) Photosynthesis and nitrogen relationships in leaves of C_3 plants. *Oecologia* 78: 9-19.
Evans JR (1996) Developmental constraints on photosynthesis: effects of light and nutrition. In: Baker NR (ed), Photosynthesis and the Environment, Kluwer Academic Publishers, Dordrecht, pp 281-304.
Field C and Mooney HA (1986) The photosynthesis-nitrogen relationshi in wild plants. In: Givnish TJ (ed), On the Economy of Plant Form and Function, Cambridge University, Cambridge, pp 25-49.
Foyer CH and Hall DO (1980) Oxygen metabolism in the active chloroplast. *Trends Biochem Sci* 1980: 188-191.
Foyer CH, Valadier MH, and Ferraria S (1995) Co-regulation of nitrogen and carbon assimilation in leaves. In: Smirnoff N (ed), Environment and Plant Metabolism, Bios Scientific Publishers, Oxford, pp 17-33.
Givnish TJ (1987) Comparative studies of leaf form: assessing the relative roles of selective pressures and phylogenetic constraints. *New Phytol* 106: 131-160.
Givnish TJ (1988) Adaptation to sun and shade: a whole-plant perspective. In: Evans JR, von Caemmerer S, and Adams WW III (eds), Ecology of Photosynthesis in Sun and Shade. CSIRO, Australia, pp 63-92
Gulmon SI and Chu CC (1981) The effects of light and nitrogen on photosynthesis, leaf characteristics, and dry matter allocation in the chaparall shrub, *Diplacus aurantiacus. Oecolgia* 49: 207-212.
Hageman RH and Hucklesby DP (1971) Nitrate reductase from higher plants. *Method Enzymol* 23: 491-503.
Hageman RH and Reed AJ (1980) Nitrate reductase from higher plants. *Method Enzymol* 69: 270-280.
Heimer YM and Filner P (1971) Regulation of nitrate assimilation pathway in cultured tobacco cells. III. The nitrate uptake system. *Biochem Biophys Acta* 230: 372-372.
Hegi G (1979) Illustrierte Flora von Mitteleuropa. Vol 2. Paul Parey, Berlin.
Hesketh JD, Larson EM, Gordon AJ, and Peters DB (1983) Internal factors influencing photosynthesis and respiration. In: Dale JE and Miltrope FL (eds), The Growth and Functioning of Leaves, Cambridge Universty Press, Cambridge, pp 381-411.
Hewitt EJ, Hucklesby DP, and Notton BA (1976) Nitrate metabolism. In: Bonner J and Varner JE (eds), Plant Biochemistry, Academic Press, New York, pp 633-681.
Huber W, Kreutmeier F, and Sankhla N (1977) Ecophysiological studies on Indian arid zone plants. IV. Effects of sodium chloride and abscisic acid on amino acid and protein metabolism in leaves of *Phaseolus aconitifolius. Z Pflanzenphysiol.* 81: 234-247.
Kaiser WM and Brendle-Behnisch E (1991) Rapid modulation of spinach leaf nitrate reductase activity by photosynthesis. I. Modulation *in vivo* by CO_2 availability. *Plant Physiol* 96: 363-367.
Kaiser W, Spill D, and Brendle-Behnisch E (1992) Adenine nucleotides are apparently involved in the light-dark modulation of spinach-leaf nitrate reductase. *Planta* 186: 236-240.
Kedenburg CP (1971) A lithium buffer system for accelerated single-column amino acid analysis in physiological fluid. *Anal Biochem* 40: 35-42.
Lea PJ (1997) Primary nitrogen metabolism. In: Dey PM and Harborne JB (eds), Plant Biochemistry, Academic Press, San Diego, pp 273-313.
Lillo C (1994) Light regulation of nitrate reductase in green leaves of higher plants. *Physiol Plant* 90: 616-620.
Losada M (1976) Metalloenzymes of the nitrate-reducing system. *J Mol Catal* 1: 245-264.
Losada M, Guerrero MG, and Vega JM (1981) The assimilatory reduction of nitrate. In: Bothe H and Trebst A (eds), Biology of Inorganic Nitrogen and Sulfur, Springer-Verlag, Berlin, pp 30-63.
Martin P (1973) Nitratstickstoff in Buschbohnenblttern unter dem Gesichtspunkt der Kompartimentierung der Zellen. *Z Pflanzelphysiol* 70: 158-165.
Martinoia E, Heck U, and Wienecken A (1981) Vacuoles as storage compartments for nitrate in barley leaves. *Nature* 289: 292-294.
Marschner H (1995) Mineral Nutrition of Higher Plant. Academic Press, London, pp 1-889.
Moog PR and Janiesch P (1989) Nutrient requirements of *Carex* species in solution culture. *J Plant Nutr* 12: 497-507.
Neyra A and Hageman RH (1976) Relationship between carbon dioxide, malate and nitrate accumulation and reduction in corn (*Zea mays* L.) seedlings. *Plant Physiol* 58: 726-730.
Nicholas JC, Harper JE, and Hageman RH (1976) Nitrate reductase activity in soybeans (*Glycine max* L. Merr.). I. Effects of light and temperature. *Plant Physiol* 58: 731-735.
Nobel PS (1976) Photosynthetic rates of sun versus shade leaves of *Hyptis emoryi* Torr. *Plant Physiol* 58: 218-223.
Osmond CB (1983) Interactions between irradiance, nitrogen nutrition, and water stress in the sun-shade responses of *Solanum dulcamara. Oecologia* 57: 316-321.
Osmond CB (1987) Photosynthesis and carbon economy of plants. *New Phytol* 106: 161-175.
Osmond CB, Björkman O, and Anderson DJ (1980) Physiological Processes in Plant Ecology: Toward a Synthesis with Atriplex. Springer-Verlag, Berlin, pp 1-468.
Ourry A, Gonzalez B, and Boucaud J (1989) Osmoregulation and role of nitrate during regrowth after cutting of ryegrass (*Lolium perenne*). *Plant Physiol* 76: 177-182.
Patterson DT, Duke DO, and Hoagland RE (1978) Effects of irradiance during growth on the adaptive photosynthetic characteristics of velvet leaf and cotton. *Plant Physiol* 61: 402-405.
Popp M (1974) Mineral- und Säurestoffwechsel einiger Kulturpflanzen bei unterschiedlicher mineralischer Ernährung. Ph. D. Thesis. University of Vienna, Austria, pp 1-224.
Rabe E (1994). Altered nitrogen metabolism under environmental stress conditions. In: Pessarakli M (ed), Plant and Crop Stress, Marcel Dekker, Inc, New York, pp 261-276.
Radin JW, Parker LL, and Sell CR (1978) Partitioning of sugar between growth and nitrate reduction in cotton roots. *Plant Physiol* 62: 550-553.
Rufty TW, Jr, Volk PJ, McClure RR, Israel DW, and Raper CD JR (1982) Relative content of $NO3^-$ and reduced N in xylem exudate as an indicator of root reduction of concurrently absorbed $^{15}NO_3$. *Plant Physiol* 69: 166-170
Santos I, Almeida JM, and Salema R (1992) Influence of nitrogen on growth, nitrate reductase and nitrite reductase of seedlings of maize (*Zea may* L. cv. LG12). *J Plant Nutr* 15: 2531-2544.

Smirnoff N and Stewart GR (1985) Nitrate assimilation and translocation by higher plants: comparative physiology and ecological consequence. *Physiol Plant* 64: 133-140.

Steingröver E, Ratering P, and Siesling J (1986) Daily changes in nitrate uptake, reduction and storage on nitrate in spinach, grown at low light intensity. *Physiol Plant* 66: 550-556.

Tychsen K (1976) Irradiance and nitrogen metabolism in spinach. *Acta Agr Scand* 26: 189-195.

Veen BW and Kleindorst A (1985) Nitrate accumulation and osmotic regulation in Italian ryegrass (*Lolium multiflorum* Lam.). *J Exp Bot* 36: 211-218.

[Received September 18, 1998; accepted October 8, 1998]