Revta brasil. Bot., São Paulo, V.24, n.4, p.407-413, dez. 2001

Effects of different ammoniacal nitrogen sources on N-metabolism of the atmospheric bromeliad *Tillandsia pohliana* Mez

VÍVIAN TAMAKI[1] and HELENICE MERCIER[1,2]

(received: January 11, 2001; accepted: July 19, 2001)

ABSTRACT - (Effects of different ammoniacal nitrogen sources on N-metabolism of the atmospheric bromeliad *Tillandsia pohliana* Mez). Increasing levels of atmospheric ammonia from anthropogenic sources have become a serious problem for natural vegetation. Short-term effects of different ammoniacal sources on the N metabolism of *Tillandsia pohliana*, an atmospheric bromeliad, were investigated. One-year-old, aseptically grown plants were transferred to a modified Knudson medium lacking N for three weeks. Plants were subsequently transferred to Knudson media supplemented with 0.5, 1.0, or 1.5 mM of N in the forms of NH_3 or NH_4^+ as the sole N source. The activities of glutamine synthetase (GS) and glutamate dehydrogenase (GDH-NADH) were determined after 40 h. The GS activity was stimulated significantly by increasing the levels of the gaseous form. The GDH-NADH activity increased significantly under increasing N concentrations with NH_3, while no significant differences were observed with NH_4^+ as a N source. These results may reflect a faster NH_3 absorption by *T. pohliana* compared to NH_4^+ uptake. The increased activity of GDH-NADH in NH_3 treatment may play a role in protecting the cells from the toxic effects of increased endogenous level of free ammonium. A raise in the concentration of N, especially in the form of NH_3, greatly increased the content of free amino acids and soluble proteins. A possible utilisation of *T. pohliana* to evaluate the changes of atmospheric gaseous ammonia is proposed.

RESUMO - (Efeitos de diferentes fontes amoniacais sobre o metabolismo de nitrogênio da bromélia atmosférica *Tillandsia pohliana* Mez). As fontes antropogênicas de amônia atmosférica têm se tornado um problema sério para a vegetação natural. No presente trabalho foram analisados os efeitos da aplicação de diferentes fontes amoniacais sobre o metabolismo do nitrogênio de *Tillandsia pohliana*, uma bromélia atmosférica. As sementes foram germinadas *in vitro* e as plantas cresceram em meio de cultura de Knudson por um ano. Após esse período, elas foram transferidas para o meio de Knudson sem as fontes nitrogenadas originais. Três semanas depois, as plantas foram colocadas nesse mesmo meio de cultura, porém, suplementado com 0,5, 1,0 ou 1,5 mM de N nas formas de NH_3 ou NH_4^+ como fontes únicas de nitrogênio. O tempo de exposição a essas fontes foi de 40 h. As atividades enzimáticas da glutamina sintetase (GS) e da glutamato desidrogenase (GDH-NADH) foram analisadas. Os teores endógenos de amônio, de aminoácidos livres e de proteínas solúveis, presentes nos eixos caulinares dessa bromélia, também, foram determinados. As atividades da GS e da GDH-NADH foram estimuladas, significativamente, com o aumento do nitrogênio na forma gasosa. Esses resultados podem refletir a rápida absorção de NH_3 pela planta. Entretanto, a atividade aminante da GDH não apresentou diferença significativa em resposta ao aumento do N na forma de NH_4^+. Provavelmente, o incremento na atividade da GDH-NADH, no tratamento com NH_3, está relacionado com a proteção das células aos possíveis efeitos tóxicos e altas concentrações endógenas de NH_4^+. O aumento na concentração do nitrogênio, especialmente na forma de NH_3, elevou os conteúdos de aminoácidos livres e de proteínas solúveis. Uma possível aplicação prática para o presente trabalho é a utilização de *T. pohliana* como indicadora da variação da concentração de amônia atmosférica.

Key words - Bromeliaceae, NH_3, NH_4^+, GS, GDH-NADH

Introduction

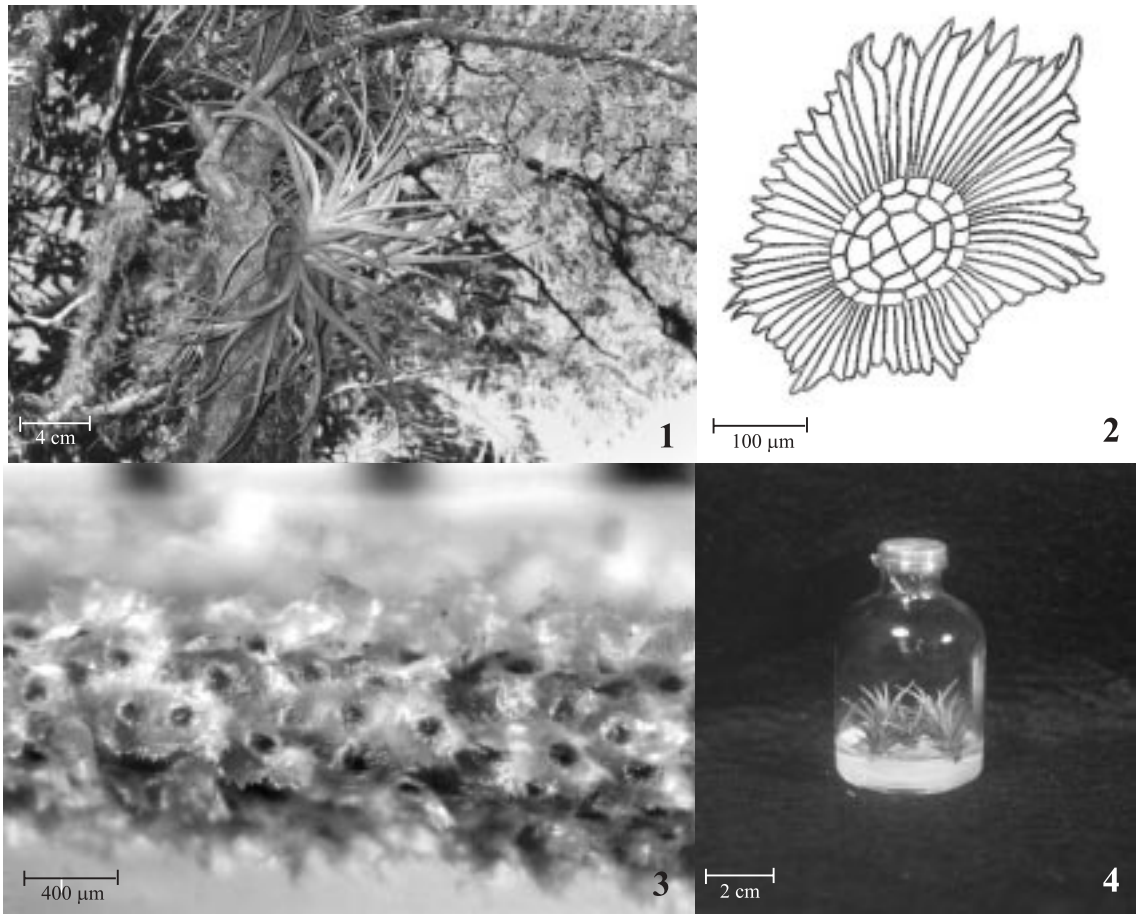
Nitrogen (N) can be released from the soil in the form of ammonia gas (NH_3). NH_3 is the most important alkaline component of the atmosphere (Hanstein & Felle 1999) and most NH_3 emission occurs in areas of intense agricultural activities, where large quantities of fertilisers are employed (Schjørring 1998). It is estimated that about 60% of the global NH_3 emission comes from anthropogenic sources. Emitted NH_3 returns to the surface mainly in the form of dry deposition of NH_3 and wet deposition of NH_4^+ (Asman *et al*. 1998). It is possible that throughfall is rich in NH_4^+ as it was observed in forests in the polluted regions of The Netherlands, with concentrations of 0.32 to 2.4 mM (Van Breemen *et al*. 1982). In several European countries, it was observed that 50% of the N compounds released into the environment consisted of NH_3/NH_4^+, and this indicates the importance of these compounds for vegetation (Pearson & Stewart 1993). Besides being released from the soil, NH_3 can be emitted from senescing

1. Universidade de São Paulo, Instituto de Biociências, Laboratório de Fisiologia Vegetal, Caixa Postal 11461, 05422-970 São Paulo, SP, Brasil.
2. Autor para correspondência: hmercier@usp.br

plant parts (Hanstein & Felle 1999). In addition, plants give off N-NH_3, through the stomata, as has been observed in rice (*Oryza sativa* L.) (Silva & Stutte 1981), wheat (*Triticum aestivum* L.) (Parton *et al*. 1988), and pea (*Pisum sativum* L.) (Betelsen & Jensen 1992), as well as in several wild species, such as *Mercurialis perennis* L., *Rubus fruticosus* L., and *Trientalis europaea* L. (Pearson *et al*. 1998). There is strong evidence that increased N level in the atmosphere is beneficial to epiphytic plants up to a certain limit (Baxter *et al*. 1992). Levels above a limit can poison epiphytes; this particularly applies to lower plants such as lichens and mosses (Baxter *et al.* 1992).

GS is a central biological catalyst in N metabolism. It catalyses the reaction of NH_4^+ with glutamate to glutamine with the aid of ATP (Choi & Kwon 1998). GDH-NADH also operates in the assimilation of NH_4^+ thereby complementing the enzymes of the GS-GOGAT cycle in the synthesis of glutamate, especially under conditions of stress (Srivastava & Singh 1987).

Tillandsia pohliana (figure 1), which belongs to Bromeliaceae, is an atmospheric epiphytic species, which possesses succulent leaves totally covered with epidermic trichomes (figures 2 and 3). The roots operate as holdfast devices, and nutrition occurs through the foliar trichomes, which absorb both water and nutrients (Benzing *et al.* 1976, Nyman *et al.* 1987). The importance of trichomes is also associated with light reflection, which, when raised, act as efficient reflectors, softening the thermic action of sun rays (Benzing *et al.* 1978).



Figures 1-4. 1. *Tillandsia pohliana* in its natural habitat; 2. A schematic view of an individual trichome; 3. View of the abaxial portion of *Tillandsia pohliana* leaf (400x); 4. *Tillandsia pohliana* cultived *in vitro*.

In this study we present a comparison of ammoniacal N utilisation (NH_3 or NH_4^+) by shoots of *T. pohliana* cultivated *in vitro*. The activities of the GS and GDH-NADH enzymes as well as the endogenous concentrations of free NH_4^+, free amino acids and total soluble proteins were verified. The results obtained have allowed us to build up a picture of the nitrogen metabolism occurring in this atmospheric bromeliad in the context of increasing atmospheric N.

Material and methods

In vitro plant cultivation - Seeds of the atmospheric epiphytic bromeliad *Tillandsia pohliana* Mez were aseptically germinated *in vitro*. The medium used contained Knudson's basic salts (1946) and was supplemented with Murashige & Skoog's micronutrients (1962), 2% gelrite, and 3% sucrose. The culture flasks were kept on shelves illuminated with fluorescent daylight lamps (40 $\mu mol.m^{-2}.s^{-1}$) under a 12 h photoperiod. The growth room temperature was 26 ± 2 °C. *In vitro* germination and aseptic bromeliad culture were necessary to avoid contamination by nitrifying organisms and the conversion of ammonium to nitrate.

Utilization of different forms of ammoniacal nutrition - One-year-old plants of *T. pohliana* were transferred to a modified Knudson medium lacking N. Three weeks later, one group of plants was transferred to a modified Knudson medium supplemented with $(NH_4)_2SO_2$ as the only N source. Three concentrations of this salt were used in order to obtain 0.5, 1.0 and 1.5 mM of N.

Another group of plants was transferred to flasks containing modified Knudson media lacking N. NH_3 was injected into these flasks (figure 4) through a rubber lid which avoids leakage and prevents the exchange of gases with the outside air. The same three N concentrations (0.5, 1.0, and 1.5 mM N) of that gas were used. The ratio of NH_3 dissolution and protonation in the medium surface is dependent on two factors: gelling agent concentration and exposure time. NH_4^+ formation was considerably decreased when raising the gelrite concentration from 2 to 10% (data not shown). Above 10% the medium was very solid and plant inoculation was difficult. Therefore, 10% of gelrite was used in the experiments where NH_3 was the only N source. Figure 5 shows NH_3 concentration changes after its injection into the flasks, which contained only gellified medium without any plants. NH_3 uptake by the medium with consequent NH_4^+ formation was less than 10% 40 hours after its injection into the flasks. No NH_3 was detected in the flasks atmosphere after two weeks. Based on these preliminary experiments, 40-hour exposure period was chosen as being sufficient time for foliar absorption with minimal decrease in NH_3 concentration in the flasks.

Shoots of *T. pohliana* of both experimental groups were collected after a 40-hour exposure to NH_3 gas (the fumigation was initiated during the dark period when the stomata were open) or $(NH_4)_2\ SO_4$ salt and then frozen in liquid N_2 and subsequently stored at -20 °C.

Free ammonium analysis - Extracts were obtained from 1 g of shoots homogenized with 5 ml of water and centrifuged at 11,000 g for 15 min. The supernatant was collected and used for the free-NH_4^+ analysis.

Free- NH_4^+ was determined by the phenol-hypochlorite reaction in which 1 ml of the extract was used (Weatherburn 1967). The reaction used a 5 ml of reagent A (containing 10 g phenol and 50 mg sodium nitroprusside in 1 liter of H_2O) and 5 ml of reagent B (containing 5 g NaOH and 8.4 ml NaOCl 5-6%). The reaction tubes were incubated at 25 °C for 30 min and the absorbance was read at 625 nm. The NH_4^+ concentration was expressed as nmol NH_4^+ g^{-1} FM.

The process of NH_4^+ determination described above (the phenol-hypochlorite reaction) was used for the analysis of NH_4^+ in the gellified medium (20 µl of the medium were used).

Enzyme extraction - One gram of shoots was ground in liquid N_2 and centrifuged (at 12,000 g for 40 min at 4 °C) with 5 ml of a buffer solution (0.05 M imidazole and 5 mM DTT, pH 7.9). The supernatant was centrifuged at 12,000 g for 40 min at 4 °C. After that, the supernatant was maintained at 4 °C and used for all the enzymatic assays (Cammaerts & Jacob 1985).

Glutamine synthetase (GS) assay - GS (EC 6.3.1.2) activity was measured as described by Pérez-Soba *et al.* (1994) and Farnden & Robertson (1980) with modifications for *T. pohliana*.

The reaction mixture with a final volume of 500 µl contained 1.2 M hydroxylamine, 0.32 M glutamate, 40 mM $MgCl_2$, 50 mM ATP (pH 7.5) and 150 µl of the plant extract. The tubes were incubated at 30 °C for 40 min. The final step of the reaction was the addition of 1 ml of a reagent containing 0.37 M $FeCl_3$, 0.20 M trichloroacetic acid, and 0.67 M HCl. The absorbance of the samples was measured at 540 nm. Blank controls were analysed with a 0.5 ml imidazole buffer (0.1 M, pH 7.5) and 1 ml of the reagent described above. The GS activity was expressed as µmol γ-GHA.min^{-1} g^{-1}. FM.

Glutamate dehydrogenase - aminating (GDH-NADH) assay - GDH-NADH (EC 1.4.1.2) activity was determined spectrophotometrically at 340 nm as described by Cammaerts & Jacobs (1985), modified for *T. pohliana*. The activity was determined according to NADH oxidation.

The assay mixture consisted of 0.15 M $(NH_4)_2\ SO_4$, 1 mM $CaCl_2$, 0.14 mM NADH, 0.02 M 2-oxoglutarate, 0.5 ml 0.1 M Tris-HCl pH 8.2, and 0.5 ml of plant extract. The Sephadex G-25 column was essayed, but the enzyme activity did not show any difference. The reaction was incubated at 30 °C for 10 min. Blank controls without the plant extract and 0.02 M

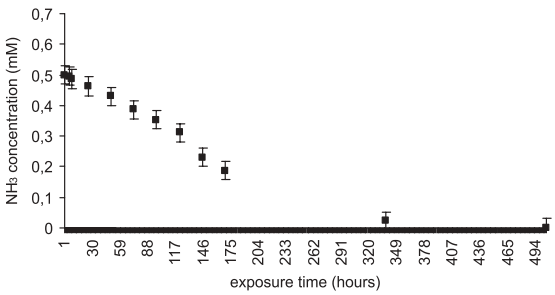


Figure 5. NH_3 concentration changes in the atmosphere of the flasks after NH_3 injection.

2-oxoglutarate were used. The GDH-NADH activity was expressed as µmol NADH. min^{-1}. g^{-1} FM.

Total free amino acids analysis - One gram of shoots was homogenised with 20 ml of 80% ethanol. The homogenate was passed through four layers of gauze and one layer of Whatman Nº 1 filter paper. Pigments and proteins were extracted by partition with chloroform and water as described by Fernandes (1983), and the total free amino acids were assayed by the ninhydrin method (Moore & Stein 1954). The data were expressed as µmol leucine. g^{-1} FM.

Protein analysis - The proteins were extracted as previously described and determined according to the procedure proposed by Bradford (1976). The levels of soluble proteins were expressed as mg albumin. g^{-1} FM.

Statistical analysis - Data were subjected to analysis of variance. The Tukey test was used to compare the means. Significant differences were inferred when $P < 0.05$.

Results and Discussion

The results of the endogenous levels of free NH_4^+ in the shoots showed a faster absorption of N in the form of NH_3 in comparison with NH_4^+ (figure 6). In concentrations higher than 0.5 mM, *T. pohliana* absorbed NH_3 faster than NH_4^+. *T. pohliana*'s shoots were fumigated with NH_3 during the dark period when its stomata were open, since it is a CAM bromeliad (Tamaki & Mercier 1997). This procedure favored a net influx of NH_3 into the leaves, which caused rapid changes in N metabolism. This bromeliad does not seem to be susceptible to damage after 40 h of exposure to NH_3. The use of another epiphytic bromeliad, *Tillandsia usneoides,* as an air pollution indicator has shown that this species is an efficient accumulator of atmospheric mercury (Malm *et al.* 1998). It was highlighted by these authors that since *T. usneoides* is a CAM bromeliad, its tolerance to drought stress makes it more appropriate than bryophytes and lichens for biomonitoring tropical environments.

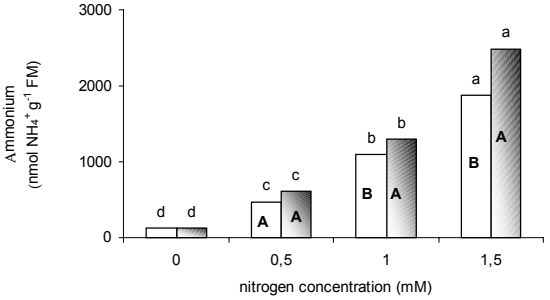


Figure 6. Free ammonium levels in the shoots of *Tillandsia pohliana* plants cultivated for 40 h in different N concentrations in ionic or gaseous ammoniacal forms. Different lower case letters correspond to significant differences for the same N-source, and different upper case letters correspond to significant differences for the same N concentration (Tukey test, $P < 0,05$) (□ NH_4^+ and ■ NH_3).

Plant leaves can take up not only nitrate and ammonium, but also atmospheric gaseous NH_3 and utilise it as a source of N (Farquhar *et al.* 1980, Schjørring *et al.* 1993). NH_3 and NH_4^+ can enter plants through the stomatal openings, by micropores that exist on the cuticule that are readily permeable to solutes (Marschner 1995), and by the special foliar trichomes that exist in bromeliads (see figure 2 and 3). NH_3 is highly reactive and forms soluble products inside the leaves as can be exemplified by the following equation: $NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$. The major determinants for NH_3 deposition are stomatal conductance and environmental conditions, particularly humidity and light that increase the rate of deposition (Van Hove *et al.* 1987).

When NH_3 enters the leaves it gets dissolved in the apoplasmic solution, and NH_4^+ formation is a very rapid process. Its transport into the cytoplasm of leaf cells may occur by carrier-mediated exchanges and an increase in NH_4^+ concentration in the leaves would be expected after fumigation with NH_3 (Yin *et al.* 1996). That increase in NH_4^+ concentration was confirmed in *T. pohliana* shoots (figure 6). The build-up of free NH_4^+ in the plant tissues may not necessarily inhibit photosynthesis or growth on a short-term basis. The acid-base regulation in cells depends on intrinsic features of the plant's metabolism (Pearson & Stewart 1993).

The results obtained for *T. pohliana* clearly demonstrated that the GS enzyme was stimulated by increasing the concentrations of N in the gaseous form (NH_3). At the concentration of 1.5 mM, the gas induced the highest GS activity (figure 7A). The aminating activity of the GDH enzyme did not show any variation as a result of the increase in the N concentration in the form of NH_4^+ (figure 7B). However the GDH-NADH activity increased with a rise in NH_3, which suggests that the aminating function of this enzyme is restricted to the use of this form of N. The increased activity of GDH-NADH may play a role in protecting the cells from the toxic effects of increased levels of free NH_4^+. Such increase in GDH activity as a function of an increase in N level has also been reported in *Arabidopsis thaliana* by Cammaerts & Jacobs (1985) and in *Vitis vinifera* by

Loulakakis & Roubelakis-Angelakis (1992). On the other hand, GDH was not found to be modulated by N concentration in rice (Magalhães & Huber 1991) and *Kalanchöe lateriria* (Santos & Salema 1992). Perez-Soba *et al.* (1994) observed that exposure of *Pinus sylvestris* L. shoots to NH_4^+ caused increases in the concentrations of free amino acids and soluble proteins, but did not affect GDH-NADH activity.

Levels of amino acids and soluble proteins in shoots of *T. pohliana* increased regardless of the N form used (figures 8A and B). The gaseous ammoniacal source was the most inductive. The influence of different N compounds on the production of amino acids in plants has been widely studied. In the case of various plant materials it has been shown that a co-relationship exists between the increase of N availability and the production of free amino acids and proteins (Fernandes 1983, Santos & Salema 1991, Perez-Soba *et al.* 1994, and Leport *et al*. 1996). The amino acid, which is often present in greater proportions, as a result of an increase in N concentration, is glutamine, as reported by Barneix *et al*. (1984) in barley (*Hordeum vulgare* L. cv. Sonja), and by Sugiharto & Sugiyama (1992) in maize (*Zea mays* L.).

A possible practical application of the present work could be the use of *T. pohliana* plants as ammoniacal pollution indicators. Their morphology and physiology (as observed in the N-metabolism characterisation presented in this study) make them suitable for handling since they are totally independent from the soil, and for transplanting to different areas where air quality measurements are necessary.

Ackowledgments - The first author thanks the CNPq for a graduate-level scholarship, and we thank MSc Cristiane Kalife for the schematic view of an individual trichome.

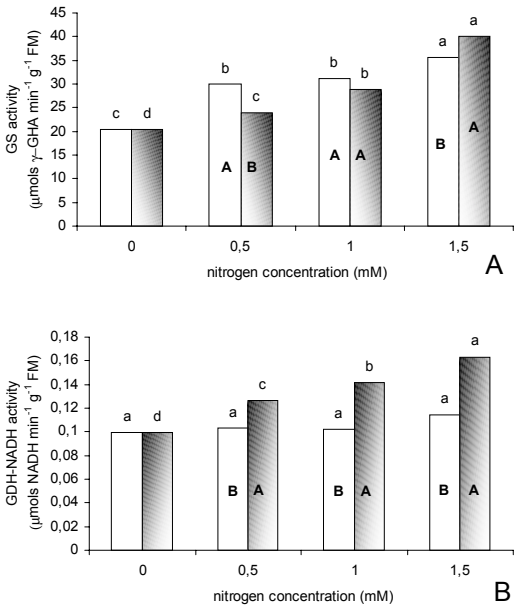


Figure 7. GS (A) and GDH-NADH (B) activities in the shoots of *Tillandsia pohliana* plants cultivated for 40 h in different N concentrations in ionic or gaseous ammoniacal forms. Different lower case letters correspond to significant differences for the same N-source, and different upper case letters correspond to significant differences for the same N concentration (Tukey test, $P < 0,05$) (□ NH_4^+ and ■ NH_3).

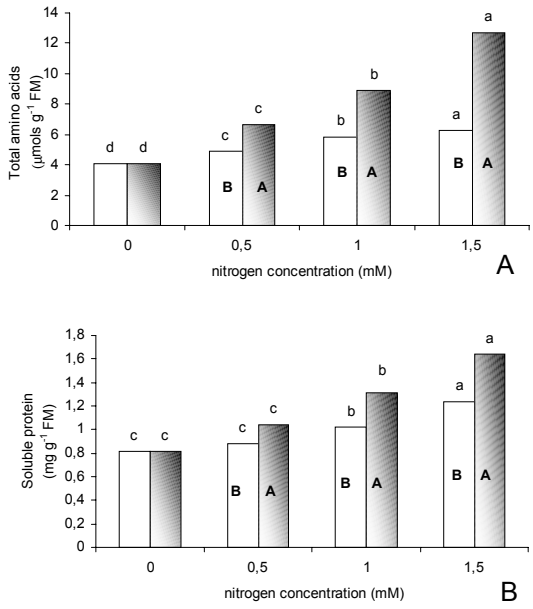


Figure 8. Total free amino acids (A) and soluble protein (B) levels in the shoots of *Tillandsia pohliana* plants cultivated for 40 h in different N concentrations in ionic or gaseous ammoniacal forms. Different lower case letters correspond to significant differences for the same N-source, and different upper case letters correspond to significant differences for the same N concentration (Tukey test, $P < 0,05$) (□ NH_4^+ and ■ NH_3).

References

ASMAN, W.A.H., SUTTON, M.A. & SCHJØRRING, J.K. 1998. Ammonia emission, atmospheric transport and deposition. New Phytologist 139:27-48.

BARNEIX, A.J., JAMES, D.M., WATSON, E.F. & HEWITT, E.J. 1984. Some effects of nitrate abundance and starvation on metabolism and accumulation of nitrogen in barley (*Hordeum vulgare* L. cv Sonja). Planta 162:469-476.

BAXTER, R., EMES, M.J. & LEE, J.A. 1992. Effects of an experimentally applied increase in ammonium on growth and amino-acid metabolism of *Sphagnum cuspidatum* Ehrh. Ex. Hoffm. from differently polluted areas. New Phytologist. 120:265-274.

BENZING, D.H., SEEMANN, J. & RENFROW, A. 1978. The foliar epidermis in Tillandsioideae (Bromeliaceae) and its role in habitat selection. American Journal of Botany 65:359-365.

BENZING, D.H., HENDERSON, K., KESSEL, B. & SULAK, J. 1976. The absorptive capacities of bromeliad trichomes. American Journal of Botany 63:1009-1014.

BETELSEN, F. & JENSEN, E.S. 1992. Gaseous nitrogen losses from field plots grown with pea (*Pisum sativum* L.) and spring barley (*Hordeum vulgare* L.) estimated by ^{15}N mass balance and acetylene inhibition techniques. Plant and Soil 142:287-295.

BRADFORD, M..M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72:248-254.

CAMMAERTS, D. & JACOBS, M. 1985. A study of the role of glutamate dehydrogenase in the nitrogen metabolism of *Arabidopsis thaliana*. Planta 163:517-526.

CHOI, Y.A. & KWON, Y.M. 1998. Comparative characterization of glutamine synthetase isoforms from *Canavalia lineata* in biochemical and immunological aspects. Plant Science 134:171-180.

FARNDEN, K.J.F. & ROBERTSON, J.G. 1980. Methods for studying enzymes involved in metabolism related to nitrogenase. *In*: Bergsen, F.J., ed. Methods for evaluating biological nitrogen fixation. John Wiley & Sons Ltd., p.265-307.

FARQUHAR, G.D., FIRTH, P.M., WETSELAAR, R. & WEIR, B. 1980. On the gaseous exchange of ammonia leaves and the environment: Determination of the ammonia compensation point. Plant Physiology 66:710-714.

FERNANDES, M.S. 1983. N-carriers, light and temperature influences on the free amino acid pool composition of rice plants. Turrialba 33:297-301.

HANSTEIN, S. & FELLE, H.H. 1999. The influence of atmospheric NH_3 on the apoplastic pH of green leaves: a non-invasive approach with pH-sensitive microeletrodes. New Phytologist 143:333-338.

KNUDSON, L. 1946. A new nutrient solution for germination of orchid seed. American Orchid Society Bulletin 15:214-217.

LEPORT, L., KANDLBINDER, A., BAUR, B. & KAISER, W.M. 1996. Diurnal modulation of phosphoenolpyruvate carboxylation in pea leaves and roots as related to tissue malate concentrations and to the nitrogen source. Planta 198:495-501.

LOULAKAKIS, K. & ROUBELAKIS-ANGELAKIS, K.A. 1992. Ammonium-induced increase in NADH-glutamate dehydrogenase activity is caused by de-novo synthesis of the a-subunit. Planta 187:322-327.

MAGALHÃES, J.R. & HUBER, D.M. 1991. Response of ammonium assimilation enzymes to nitrogen form treatments in different plant species. Journal of Plant Nutrition 14:175-185.

MALM, O., FONSECA, M.DE F., MIGUEL, P.H., BASTOS, W.R. & PINTO, F.N. 1998. Use of epiphyte plants as biomonitors to map atmospheric mercury in a gold trade center city, Amazon, Brazil. Science of the Total Environment 213:57-64.

MARSCHNER, H. 1995. Mineral nutrition of higher plants, 2nd edn. London: Academic Press Ltd.

MOORE, S. & STEIN, W.H. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. Journal of Biological Chemistry 211:907-913.

MURASHIGE, T. & SKOOG, F. 1962. A revised medium for rapid growth and bioassays with tabacco tissue cultures. Physiologia Plantarum 15:473-497.

NYMAN, L.P., DAVIS, J.P., O'DELL, S.J., ARDITTI, J., STEPHENS, G.C. & BENZING, D.H. 1987. Active uptake of amino acids by leaves of an epiphytic vascular plant, *Tillandsia paucifolia* (Bromeliaceae). Plant Physiology 83:681-684.

PARTON, W.J., MORGAN, J.A., ALTENHOFER, J.M. & HARPER, L.A. 1988. Ammonia volatilization from spring wheat plants. Agronomic Journal 80:419-425.

PEARSON, J., CLOUGH, E.C.M., WOODALL, J., HAVILL, D.C. & ZHANG, X.H. 1998. Ammonia emissions to the atmosphere from leaves of wild plants and *Hordeum vulgare* treated with methionine sulphoximine. New Phytologist 138:37-48.

PEARSON, J. & STEWART, G.R. 1993. The deposition of atmospheric ammonia and its effects on plants. New Phytologist 125:283-305.

PÉREZ-SOBA, M., STULEN, I. & VAN DER EERDEN, L.J.M. 1994. Effect of atmospheric ammonia on the nitrogen metabolism of Scots pine (*Pinus sylvestris*) needles. Physiologia Plantarum 90:629-636.

SANTOS, I. & SALEMA, R. 1991. Nitrogen nutrition and the level of crassulacean acid metabolism in *Kalanchöe lateritia* Engl. Plant, Cell and Environment 14:311-317.

SANTOS, I. & SALEMA, R. 1992. Effect of nitrogen nutrition on nitrate and nitrite reductase, glutamine synthetase, glutamate synthase and glutamate dehydrogenase in the CAM plant *Kalanchöe lateritia* Engl. Plant Science 84:145-152.

SCHJØRRING, J.K. 1998. Atmospheric ammonia and impacts of nitrogen deposition: uncertainties and challenges. New Phytologist 139:59-60.

SCHJØRRING, J.K., KYLLINGSBAEK, A., MORTENSEN, J.V. & BYSKOV-NIELSEN, S. 1993. Field investigations of ammonia exchange between barley plants and the atmosphere. I. Concentrations profiles and flux densities of ammonia. Plant, Cell and Environment 16:161-167.

SILVA, P.R.F.DA & STUTTE, C.A. 1981. Nitrogen loss in conjunction with transpiration from rice leaves as influenced by growth stage, leaf position, and N supply. Agronomic Journal 73:38-42.

SRIVASTAVA, H.S. & SINGH, R.P. 1987. Role and regulation of L-glutamate dehydrogenase activity in higher plants. Phytochemistry 26:597-610.

SUGIHARTO, B. & SUGIYAMA, T. 1992. Effects of nitrate and ammonium on gene expression of phosphoenolpyruvate carboxylase and nitrogen metabolism in maize leaf tissue during recovery from nitrogen stress. Plant Physiology 98:1403-1408.

TAMAKI, V. & MERCIER, H. 1997. Diurnal variation in vacuolar acidity and PEP-carboxylase activity in *Tillandsia pohliana* Mez (Bromeliaceae) grown in vitro. Bromélia 4:16-23.

VAN BREEMEN, N., BURROUGH, P.A., VELTHORST, E.J., VAN DOBBEN, H.F., WIT, T., RIDDER, T.B. & REIJNDERS, H.F.R. 1982. Soil acidification from atmospheric ammonium sulphate in forest canopy throughfall. Nature 299:548-550.

VAN HOVE, L.W.A., KOOPS, A.J., ADEMA, E.H., VREDENBERG, W.J. & PIETERS, G.A. 1987. Analysis of the uptake of atmospheric ammonia by leaves of *Phaseolus vulgaris* L. Atmospheric Environment 21:1759-1763.

WEATHERBURN, M.W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Analytical Chemistry 39:971-974.

YIN, Z.-H., KAISER, W.M., HEBER, U. & RAVEN, J.A. 1996. Acquisition and assimilation of gaseous ammonia as revealed by intracellular pH changes in leaves of higher plants. Planta 200:380-387.