Photosynthesis sensitivity to NH_4^+-N change with nitrogen fertilizer type

A. Nasraoui-Hajaji, H. Gouia

Research Unit of Nitrogen Nutrition and Metabolism and Stress-related Proteins (99/UR/C 09-20), Tunisian Faculty of Sciences, University of Tunis El Manar, El Manar, Tunisia

ABSTRACT

N-fertilization type affected differently tomato growth. In the field experiment, hydroponic cultures were conducted using NO_3-N (5 mmol); mixture of KNO_3-N (3 mmol) and $(NH_4)_2SO_4$-N (2 mmol); NH_4^+-N (5 mmol) or urea (5 mmol) as nitrogen source. Compared to nitrate, ammonium and urea had negative effects on morphology and dry matter production. Effects of the different nitrogen forms were investigated by measuring several photosynthesis parameters and chl *a* fluorescence. Two different significant types of reaction were found. When nitrogen was added as ammonium or urea, dry weight, chlorophyll tenor, transpiration rate, stomatal conductance and photosynthetic activity were inhibited. Supply of ammonium or urea, reduced the ratio (F_v/F_m), photochemical quenching and enhanced the non photochemical quenching. These data suggest that the adverse decrease in tomato growth under ammonium or urea supply may be related principally to inhibition of net photosynthesis activity. The high non photochemical quenching shown in tomato fed with ammonium or urea indicated that PS II was the inhibitory site of NH_4^+-N which was directly uptaken by roots, or librated via urea hydrolysis cycle.

Keywords: ammonium; chlorophyll fluorescence; gas exchange; tomato; *Solanum lycopersicon*

Plants can use various forms of nitrogen from soils, most importantly the inorganic ions ammonium (NH_4^+) and nitrate (NO_3^-). Nevertheless, urea constitutes another nitrogen source widely used by plants. In fact to provide crops production urea is intensively used as a nitrogen fertilizer. Urea nitrogen enters the plant either directly, or in the form of ammonium or nitrate after urea degradation (Byrnes and Freney 1995). Urease is the enzyme responsible of urea hydrolysis producing ammonium (Guettes et al. 2002). NH_4^+-N is one of the major nutrients for plants, and a ubiquitous intermediate in plant metabolism (Von Wiren et al. 2000). Because NH_4^+-N assimilation requires less energy than that of NO_3^--N, it is usually expected to be preferred by plants (Britto et al. 2001). However, the ammonium ion is notorious for its toxic effects on many, if not all, plant strains: only a few strains perform well when NH_4^+ is the only, or predominant, nitrogen source (Kronzucker et al. 1997, Li et al. 2009). In reality, ammonium is markedly present in agricultural soils as a result of fertilizers use, nitrogen cycle, or pollution. This additional NH_4^+-N input affected species composition: even local species extinction, and large-scale forest decline was attributed directly to the ammonium (Dai et al. 2008). Numerous studies demonstrated that different N forms significantly influenced plant growth, but contrasting results were observed depending on the plant species used. Some plants such as maize, wheat, tobacco, bean, preferred nitrate to ammonium nutrition (Walch et al. 2000, Guo et al. 2002). These plants would suffer ammonium toxicity when supplied with high ammonium in the root medium as the sole N source. Having a higher ammonium assimilation capacity than other plant species, those plants could avoid ammonium toxicity and exhibited a preference for ammonium nutrition (Britto et al. 2004, Guo et al. 2007). Although the toxicity of ammonium regardless its origin, has been observed for more than one hundred years (Britto et al.

2001), most reports concentrated on the study of animal (Alonso and Camargo 2003, Arauzo 2003), bacterium (Muller et al. 2006), and higher plants (Gerendás 1997, Britto and Kronzucker 2002).

In the present study, it was suggested that ammonium directly added in nutrient solution or indirectly produced by hydrolysis of urea affected growth of plants through photosynthesis inhibition. When ammonium was added simultaneously with nitrate, deleterious effects on growth were reduced and photosynthesis activity was maintained.

MATERIAL AND METHODS

Plant material and growth conditions. Seeds of tomato (*Solanum lycopersicon*) were germinated in Petri dishes at 25°C in darkness. Uniform seedlings were transferred to continually aerate nutrient solution containing low concentration of KNO_3 (0.1 mmol). Ten days later, the seedlings were supplied with nutrient solutions containing KNO_3 (5 mmol), mixture of KNO_3 (3 mmol) and $(NH_4)_2SO_4$ (2 mmol), $(NH_4)_2SO_4$ (5 mmol) or urea (5 mmol). After 14 days of treatment, 24-day-old plants were ready for the measurement of different parameters converted by this study.

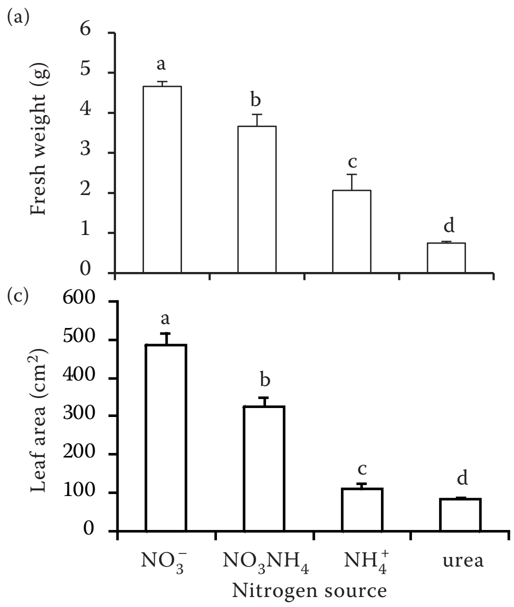
Determination of chlorophyll content. Chlorophyll *a*, *b* and total contents were determined as described by Arnon (1956).

Measurements of gas exchange and chlorophyll fluorescence. Measurements of net assimilation rate (A_{max}), transpiration rate (E) and stomatal conductance (G_W) were made with a CIRAS-1 gas exchange system (PP Systems, Hitchin, UK). Chlorophyll fluorescence emission from the upper surface of the leaves of intact plants was measured by modulated fluorimeter (MINI-PAM) photosynthesis yield analyser (Walz, Effeltrich, Germany). Leaves previously selected for measurement of stomatal conductance were used for fluorescence measurements. The minimal (F_0) and maximal chl *a* fluorescence (F_m) emissions were assessed in leaves after 30 min of dark adaptation and the maximum quantum efficiency of PS II photochemistry was calculated as $F_v/F_m = (F_m – F_0)/F_m$. The parameters were estimated following Baker and Rosenquist (2004). Non-photochemical quenchm-ing of fluorescence (NPQ), which is proportional to the rate constant of thermal energy dissipation was calculated following Bjorkman and Demmig (1987). The photochemical quenching (q_p) was calculated following Van Kooten and Snel (1990). The intrinsic efficiency of open PS II (Φ_{exc}) (or efficiency of excitation energy capture by open PS II reaction centres) was calculated following Harbinson et al. (1990).

Statistical analysis. All values reported are the means of six replicates per treatment (± SE), each experiment being conducted in duplicate. Data were processed by the variance analysis (ANOVA) and significance levels were accepted at $P \leq 0.05$ at all tests.

RESULTS AND DISCUSSION

Many plant species show growth depression when ammonium was supplied as a sole nitrogen form Claussen and Lenz (1999). Pure ammonium nutrition has a negative effect on different growth parameters such as leaf area, chlorophyll content and fresh matter yield (Figure 1) (Errebhi and Wilcox 1990, Raab and Terry 1994). It was suggested that the toxicity of NH_4^+-N was correlated with low pH value of growth medium resulting from the stoichiometry of excess H^+ production Raven (1986). Acidification of the rhizosphere due to assimilation of NH_4 restricted cation uptake compared with plants receiving mixture of nitrate and ammonium (Basra and Goyal 2002). In fact, presence of NO_3^--N played also an important role as osmoticum besides its essential function in counter-ion for cation translocation in the xylem. Thus, there are reports indicating that NH_4^+-grown wheat contains lower Ca^{2+}, Mg^{2+} and K^+ concentrations than plants supplied with NO_3^- (Marschner 1995). In our previous study, we demonstrated that *Arabidopsis* growth was negatively affected by ammonium when added at excess or deficient doses. In contrast, the supply of average quantity of ammonium enhanced the growth of plants (Nasraoui et al. 2013). Compared to seedlings-fed with NO_3^--N as sole form of N, a lower CO_2 assimilation rate, stomatal conductance and transpiration rate were found for ammonium or urea-supplied tomato plants. Moreover, the reduction degree of these parameters, was alleviated by addition of both nitrate and ammonium (Figure 2). Such results may indicate tomato tolerance capacity to low ammonium supply parallel with high proportion of NO_3^--N (Claussen and Lenz 1999). These results suggested that some of the adverse effects of ammonium nutrition on plant growth



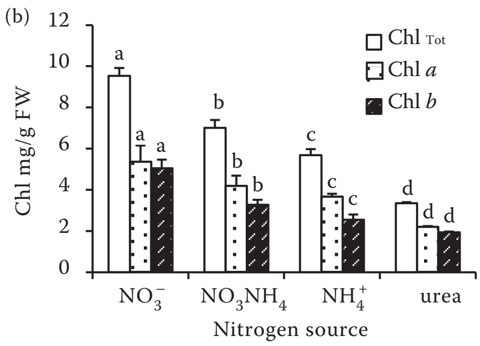
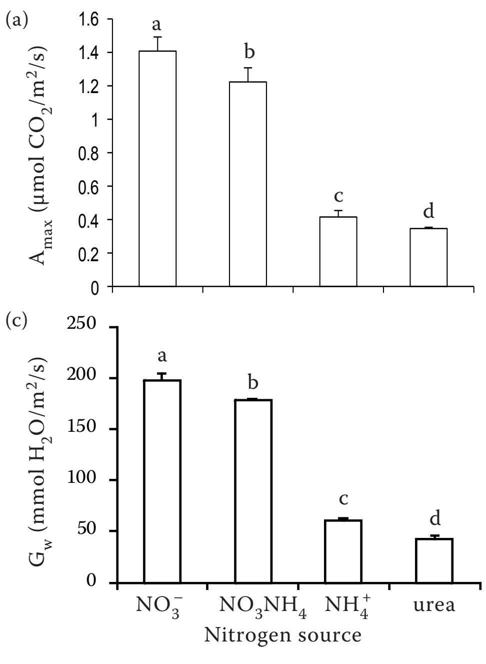


Figure 1. Changes in fresh weight (a), Chl *a*,*b* and total contents (b) and leaf area (c) of tomato seedlings grown in nitrogen solutions containing either nitrate, mixture of nitrate and ammonium, ammonium or urea. Data are means ± confidence limits (*n* = 6)

were related to photosynthesis. In contrast to nitrate supply, the use of ammonium directly furnished or produced by hydrolysis of urea caused a reduction of leaf expansion (Marschner 1995), leading to a higher ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) amount and activity, chlorophyll content and density (Guo et al. 2000, 2007). To explain how nitrogen form influences photosynthesis, photo-energy consumption and reductant supply were observed. The differences in photo-energy consumption and reductant supply between nitrate and ammonium-grown plants were discussed previously (Gerendás et al. 1997). It is generally accepted that the discrepancies observed were related to the different assimilatory pathways of nitrate (in shoots) and ammonium (in roots). Briefly, the total cost in each N form absorption, transport, reduction, and assimilation varied largely (Raven 1985). Compared to photosynthetic CO_2 assimilation, the N assimilation into glutamate is a very important sink for redox equivalents from the photosynthetic electron flow (Champigny and Foyer 1992). Generally, ammonium is toxic to plants and its toxic effect was reduced by assimilating the ammonium into organic compounds in the roots via the glutamine



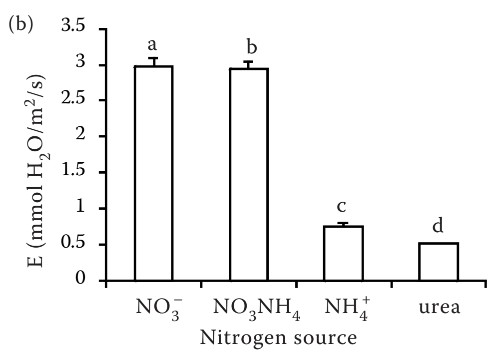
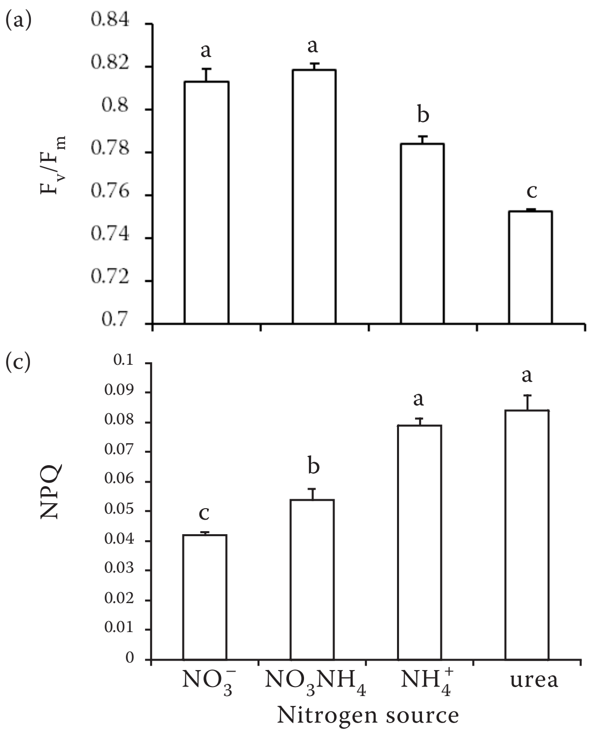


Figure 2. Changes in CO_2 assimilation rate (A_{max}) (a), transpiration (E) (b) and stomatal conductance (G_W) (c) in leaves of tomato seedlings grown in nitrogen solutions containing either nitrate, mixture of nitrate and ammonium, ammonium or urea. Data are means ± confidence limits (*n* = 6)

synthetase/glutamate synthetase (GS/GOGAT) (Nasraoui et al. 2010). The suppression of CO_2 assimilation rate could be due to the competition for carbon skeletons and photogenerated reductant between the photosynthetic CO_2 fixation and NH_4^+-N assimilation (Elrifi et al. 1988). It was suggested that NH_4^+-N assimilation had a much higher requirement ratio for ATP/NADPH than for CO_2 and NO_3^- assimilation (Turpin and Bruce 1990). The thylakoid bound NAD(P)H dependent PQ reductase activity-NAD(P)H dehydrogenase mediate cyclic electron transport around PS I (Mi et al. 2001).

It seems that the main factor limiting net photosynthesis (expressed as A_{max}) by NH_4^+-N was stomatal closure, which occurred when high ammonium concentration or urea nitrogen were added in culture medium. The large diminution in G_w suggested that stomatal closure was one of the most important factors contributing to depress photosynthetic assimilation rate. These results in tomato agreed with those of Lawlor and Cornic (2002), who showed that stomatal closure was the main factor in the reduction in leaf photosynthesis during abiotic stress. The other reason that may be involved to explain why NH_4^+-N or urea should induce lower G_W was the poorer osmotic adjustment. Moreover, a lower content of different cations and especially K^+ may affect stomatal function (Laporte et al. 2002). Alternatively, earlier root senescence caused by NH_4^+-N may be the cause of the lower G_W in these plants (Britto and Kronzucker 2001, Basra and Goyal 2002). Declines in the different gas exchanges due to NH_4^+-N source were accompanied by significant differences observed for the chlorophyll fluorescence parameters studied in this work (F_v/F_m, q_p and NPQ).

In these data, study of chlorophyll fluorescence parameters indicated that the efficiency of the photochemistry of PS I1 was affected by ammonium stress. In fact, ammonium was supplemented in nutrient solution alone but at high dose, simultaneously with nitrate or produced by hydrolysis urea cycle. Indeed, the F_v/F_m ratio determined on the fully expanded leaves was inhibited in tomato plants that received NH_4^+-N alone or urea as nitrogen source. Thus the changes observed in F_v/F_m ratio values help us to explain the decrease in photochemical quenching coefficient q_p (Figures 3a,b). This decline in q_p indicated that the primary electron acceptor of PS II, QA, was less oxidized. This suggested that in stressed plants the photochemical conversion and the capacity of the electron transport for the reduction of NADP were affected. On the other hand, the quantum yield of photochemical efficiency of PS I1 was affected, thus indicating that ammonium interfere with the light reactions of photosynthesis. The rise of non-photochemical quenching coefficient indicated that a higher proportion of absorbed



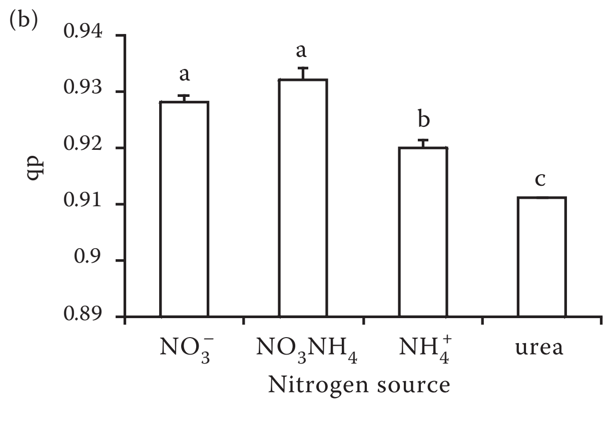


Figure 3. Changes in F_v/F_m ratio (a), photochemical quenching (qp) (b) and non photochemical quenching (NPQ) (c) in leaves of tomato seedlings grown in nitrogen solutions containing either nitrate, mixture of nitrate and ammonium, amonium or urea. Data are means ± confidence limits (*n* = 6)

photons was lost as heat instead of being used to drive photosynthesis) (Figure 3c).

We can conclude that regardless ammonium origin (nitrogen source or liberated by different metabolic cycles as urea hydrolysis), high NH_4^+-N level present in plant tissues affected plants growth. This negative effect was generated as a consequence of photosynthesis process perturbation and depended mainly on the content of photosynthetic pigments. Anomaly of light energy dissipation as fluorescence was not only indicative of lower chlorophyll content, but also photochemical energy conversion decrease. The enhancement of photosynthesis process and decline of chl fluorescence intensity together with pigment accumulation in mixture fed- tomato, revealed that partial substitution of NH_4^+-N by NO_3^--N was beneficial to the growth of this plant. However, addition of high NH_4^+-N dose alone or urea can easily and significantly damage growth plants.

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Correspomding author:

Dr. Afef Hajaji Nasraoui, University of Tunis El Manar, Tunisian Faculty of Sciences, El Manar, Tunisia
e-mail: hajajiafef@yahoo.fr