ASPECTOS FISIOLÓGICOS, AGRONÓMICOS Y AMBIENTALES EN LA NUTRICIÓN MINERAL DE LAS PLANTAS

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Effect of increasing ammonium concentrations on rhizospheric and foliar pH of tomato and pea seedlings

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Introduction

Although nitrate (NO₃⁻) and ammonium (NH₄⁺) are the major nitrogen (N) sources for plants, species differ in their relative adaptations to these two N sources (Kronzucker et al 1997, 1999). In fact, most plant species show reduced growth, smaller leaves and a stunted root system when exposed to high NH₄⁺ concentrations, and in severe cases this leads to necrosis or even death (Bloom et al 1992; Lasa et al 2002).

The proton coupled transport process for ammonium results in equivalent efflux of H⁺ from the cytoplasm after membrane repolarization (Britto et al 2001). Ammonium uptake is cytosolic alkalization and rhizospheric acidification process. For this reason ammonium uptake poses a challenge to mechanisms maintaining cytosolic pH (Stitt et al 2010).

Our working hypothesis is that plants' ability to maintain the pH of the rhizosphere and that of the leaf surface reflects its tolerance to NH₄⁺ nutrition. In order to check this hypothesis, two crop species with different degrees of NH₄⁺ sensitivity were grown with increasing NH₄⁺ concentrations (0.5, 1, 5 and 10 mM) and the changes in their rhizospheric and foliar surface pH monitored. The two crops used were tomato (*Lycopersicon esculentum*), which is NH₄⁺ sensitive (Lasa et al 2002) and pea (*Pisum sativum*), which is NH₄⁺ tolerant (Cruz et al 2006).
Materials and Methods

Seeds of tomato (Lycopersicon esculentum L. cv. ACE 55 VF) and pea (Pisum sativum L. cv snap pea) were grown in Petri plates (14 cm diameter) with increasing NH$_4^+$ concentrations. Plants were grown in a solid modified Hoagland solution with 1 mM of MES buffer and 10 g/L of agar. The N sources were (NH$_4$)$_2$SO$_4$ and NH$_4$Cl in the following concentrations, 0.5, 1, 5 and 10 mM N. The pH of the growth media solutions was adjusted to 5.8.

Seeds (6 tomato seeds or 4 pea seeds) were placed on the surface of the cooled growing medium along an imaginary diameter line (Fig. 1). Controls consisted of Petri plates containing solely the growth medium, without seeds. Each treatment was replicated in 4 Petri plates.

Plants were grown under controlled conditions: light intensity was approximately 250 mE m$^{-2}$ s$^{-1}$, with a day/night cycle of 14h/10h at 22 °C/18 °C. After 21 days of growth, surface pH of the growing medium was measured (Crison pH-metro PH 25 DL) along two imaginary lines, parallel to the diameter where the seeds were placed, one corresponding to the roots influence (4 cm lower), and the other, to the shoots (4 cm upper). Each zone of each Petri plate was measured in three points along the lines (Fig. 1). Changes in surface pH of the growing mediums (pH$_1$) were calculated by subtracting the average surface pH of the control Petri plates (pH$_C$ - Fig. 2).

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\text{Surface } \Delta \text{pH} = \text{pH}_1 - \text{pH}_C
\]

For two contrasting NH$_4^+$ concentrations, 0.5 and 5 mM, adaxial and abaxial leaf surface pH was also measured (Crison pH-metro PH 25 DL) in one second fully developed leaf per plant, three plants per plate (12 per treatment).

Results and Discussion

Tomato and pea plants responded differently when grown with increasing NH$_4^+$ concentrations in terms of pH changes in the growth medium (Fig. 2) and in the leaf surface (Fig. 3).

The pH of the growth medium where tomato plants were grown showed a higher acidification with increasing NH$_4^+$ concentrations (irrespective of the NH$_4^+$ salt- ammonium sulphate or ammonium chlorite) than that of peas (Fig. 2). This acidification was more pronounced in the root zone than in the shoot zone (see Fig. 1).
When grown with ammonium concentrations between 1 and 10 mM tomato plants acidified the medium in ± 2 pH units, while at 0.5 mM the acidification was of ± a pH unit (Fig. 2). These distinct levels of acidification of the root zone may be related with the $\text{NH}_4^+$ influx. At concentrations up to 0.5 mM ammonium uptake occurs through the high affinity transport system, at higher ammonium concentrations the influx is mainly through the low affinity transport system and occurs in a concentration dependent way (Wang et al 1993). No significant effects of the accompanying ion on the medium pH changes were observed.

Fig. 2. Changes in growth medium’s surface pH in relation to the controls (no plants) in tomato (left) and pea (right) both in the root and in the shoot zone (see Fig. 1). Plants were grown with 0.5, 1, 5 and 10 mM of $(\text{NH}_4)_2\text{SO}_4$ or $\text{NH}_4\text{Cl}$. 
Taking in consideration that root NH$_4^+$ concentration in tomato plants increases from 3 to 10 mM and that part of this ammonium is transported to the shoots (Cruz et al 2006), it implies that at higher ammonium concentrations the leaf surface pH can also be affected. Since plant leaves can emit or absorb ammonia (NH$_3$ - Farquhar et al 1980), the equilibrium between NH$_4^+$ and NH$_3$ may have contributed to the observed pH changes. The NH$_3$ concentration at which emission and uptake are balanced is called the stomatal NH$_3$ compensation point. The influence of N nutrition on stomatal NH$_3$ compensation point has been demonstrated in several studies (Herrmann et al 2002) that show that NH$_3$ emission is higher in NH$_4^+$ grown plants than in NO$_3^-$ grown ones. Indeed, the abaxial (where stomata density is higher - Gay and Hurd, 1975) leaf surface of tomato plants also decreased with increasing NH$_4^+$ concentrations. It is interesting to notice that for low 0.5 mM NH$_4^+$, NH$_4$Cl tends to cause lower pHs (adaxial and abaxial) than (NH$_4$)$_2$SO$_4$, while for 5 mM NH$_4^+$ there is no difference between NH$_4^+$ salts and leaf surface (Fig. 3).

![Fig. 3. Changes in adaxial and abaxial leaf surface pH in tomato (left) and pea (right) plants grown with 0.5 and 5 mM of (NH$_4$)$_2$SO$_4$ or NH$_4$Cl.](image)

As expected, and contrary to tomato, rhizospheric (Fig. 2) and foliar surface (Figs 2 and 3) pH of pea plants did not show significant acidification with increasing NH$_4^+$ concentrations. Thus, the present data confirms that plants’ ability to maintain the pH reflects its tolerance to NH$_4^+$ nutrition. Moreover, it highlights the role of pH unbalances in the NH$_4^+$ toxicity syndrome.

Finally, leaf surface pH may be further assessed as an indicator of the ‘NH$_4^+$’ status of the plant.
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References


