Journal of Plant Physiology 168 (2011) 1617–1626

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

NH_4^+-stimulated low-K^+ uptake is associated with the induction of H^+ extrusion by the plasma membrane H^+-ATPase in sorghum roots under K^+ deficiency

Juan Carlos Alvarez-Pizarro[a], Enéas Gomes-Filho[a,*], José Tarquínio Prisco[a], Maria Fátima Grossi-de-Sá[b], Osmundo Brilhante de Oliveira-Neto[b,c]

[a] Departamento de Bioquímica e Biologia Molecular and Instituto Nacional de Ciência e Tecnologia em Salinidade (INCTSal/CNPq), Universidade Federal do Ceará, Caixa Postal 6039, 60455-900 Fortaleza, Ceará, Brazil
[b] Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica, Final WS, Asa Norte, 70770-900 Brasília, DF, Brazil
[c] Centro Universitário Unieuro, Av das Nações Trecho 0, Conjunto 5, Brasília, DF, Brazil

ARTICLE INFO

Article history:
Received 8 December 2010
Received in revised form 25 February 2011
Accepted 1 March 2011

Keywords:
H^+-ATPase
Low-K^+ net uptake
Plasma membrane
Roots
Sorghum bicolor

ABSTRACT

The effect of external inorganic nitrogen and K^+ content on K^+ uptake from low-K^+ solutions and plasma membrane (PM) H^+-ATPase activity of sorghum roots was studied. Plants were grown for 15 days in full-nutrient solutions containing 0.2 or 1.4 mM K^+ and inorganic nitrogen as NO_3^-, NO_3^-/NH_4^+ or NH_4^+ and then starved of K^+ for 24, 48 and 72 h. NH_4^+ in full nutrient solution significantly affected the uptake efficiency and accumulation of K^+, and this effect was less pronounced at the high K^+ concentration. In contrast, the translocation rate of K^+ to the shoot was not altered. Depletion assays showed that plants grown with NH_4^+ more efficiently depleted the external K^+ and reached higher initial rates of low-K^+ uptake than plants grown with NO_3^-. One possible influence of K^+ content of shoot, but not of roots, on K^+ uptake was evidenced. Enhanced K^+-uptake capacity was correlated with the induction of H^+ extrusion by PM H^+-ATPase. In plants grown in high K^+ solutions, the increase in the active H^+ gradient was associated with an increase of the PM H^+-ATPase protein concentration. In contrast, in plants grown in solutions containing 0.2 mM K^+, only the initial rate of H^+-pumping and ATP hydrolysis were affected. Under these conditions, two specific isoforms of PM H^+-ATPase were detected, independent of the nitrogen source and deficiency period. No change in enzyme activity was observed in NO_3^--grown plants. The results suggest that K^+ homeostasis in NH_4^+-grown sorghum plants may be regulated by a high capacity for K^+ uptake, which is dependent upon the H^+-pumping activity of PM H^+-ATPase.

Introduction

K^+ uptake by roots is a key physiological process for plant growth, as well as development and survival in environmentally stressful conditions, such as salinity and drought (Maathuis and Sanders, 1996; Maathuis and Amtmann, 1999). In soils with low-K^+ availability, transport of this ion across the plasma membrane (PM) is mediated by a high-affinity transport system whose kinetic, energetic and regulatory aspects have been characterised in detail (Epstein et al., 1963; Maathuis and Sanders, 1996; Véry and Sentenac, 2003).

High-affinity K^+ uptake was first characterised by Epstein et al. (1963) in barley roots, and its kinetic parameters were fitted to Michaelis–Menten's kinetic model. Since then, several studies have demonstrated that high-affinity K^+ influx exhibits K_m values in the micromolar range, shows no discrimination between Rb^+ and K^+ and is sensitive to Na^+ and NH_4^+ (Epstein et al., 1963; Vale et al., 1987; Bañuelos et al., 2002). Furthermore, high-affinity K^+ uptake is tightly regulated by K^+ status in tissues (Glass, 1975). When plants are subjected to K^+ starvation, tissue K^+ content decreases and K^+ influx rates are rapidly induced (Glass, 1975; Fernando et al., 1990; Martínez-Cordero et al., 2005). Under these conditions, a number of high-affinity K^+ carriers of the KUP/HAK/KT and HKT families are rapidly up-regulated (Santa-María et al., 1997; Wang et al., 1998). These transporters are believed to move K^+ via coupling transport to the H^+ or Na^+ gradient (Maathuis and Sanders, 1994; Rubio et al., 1995). However, the AKT1 K^+ channel has also been identified as an important component of the K^+ uptake system, even in micromolar concentrations of external K^+ (Hirsch et al., 1998; Spalding et al., 1999). Electrophysiological measurements have demonstrated the capacity of plant cells to develop highly negative membrane

Abbreviations: PM, plasma membrane; t_0, plants grown for 15 d in full nutrient solutions; t_1, t_2 and t_3, plants subjected to K^+ starvation for 24, 48 and 72 h, respectively; TE, transport efficiency; UE, uptake efficiency.

* Corresponding author at: Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará, Caixa Postal 6039, 60455-900 Fortaleza, Ceará, Brazil. Tel.: +55 85 3366 9405; fax: +55 85 3366 9829.
E-mail address: egomesf@ufc.br (E. Gomes-Filho).

0176-1617/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jplph.2011.03.002

potentials required for passive K^+ uptake to occur through a channel (Sentenac et al., 1992; Hirsch et al., 1998).

Exposure of roots to NH_4^+-rich environments can affect the contribution of each system (K^+ transporters and K^+ channels) to high-affinity K^+ uptake. A NH_4^+-sensitive component, probably mediated by transporters of the KUP/HAK/KT family, contributes to K^+ uptake in the absence of NH_4^+, while a NH_4^+-insensitive component, mediated by AKT K^+ channels, operates when NH_4^+ is present in the growth solution (Santa-María et al., 2000; Rubio et al., 2008; Szczerba et al., 2008). The physiological role of both K^+ transport systems for K^+ nutrition and plant growth has been demonstrated (Pyo et al., 2010; Rubio et al., 2010), but the relative contribution of each system in controlling K^+ influx may vary considerably between plant species. For instance, it has been demonstrated that the NH_4^+-sensitive component dominates high-affinity K^+ uptake by tomato roots (Nieves-Cordones et al., 2007), while both components operate in pepper and *Arabidopsis* (Martínez-Cordero et al., 2005; Rubio et al., 2008). Although the responses to external NH_4^+ and K^+ availability are of great interest for our understanding of K^+ uptake in plants, little is known about their effects on the energisation mechanisms of secondary transport systems.

PM H^+-ATPase (EC 3.6.1.35) plays a critical role in the plant response to nutrient deficiency. Its proton pumping activity results in the formation of an electrochemical gradient and is believed to activate and regulate secondary solute transport across the root surface. Previous investigations have shown the close relationship between PM H^+-ATPase activity and the uptake of nutrients such as nitrate (Santi et al., 2003), iron (Dell'orto et al., 2000) and phosphorus (Shen et al., 2006). Correlative evidence for PM H^+-ATPase activity and K^+ influx was suggested by Amtmann et al. (1999), who observed that apoplastic acidification may cause changes in K^+ uptake rates via specific channels. However, in spite of the large increase in K^+ influx observed in K^+-starved roots in barley (Fernando et al., 1990), the total amount of PM H^+-ATPase was not altered, and the induction of specific isoforms was suggested (Samuels et al., 1992). Recently, *LeHAK5* transcript levels have been proposed to be positively regulated by the hyperpolarisation of PM potential in tomato roots (Nieves-Cordones et al., 2008). Thus, the role of this enzyme would be essential for high-affinity K^+ influx.

In this study, we carried out a kinetic comparison of the K^+ uptake from low-K^+ solutions by sorghum roots on the influence of the tissue K^+ content and the presence of different combinations of inorganic nitrogen in the growth solution. The H^+-pumping and ATP hydrolysis activities and the isoform expression (by immunoblotting) of the PM H^+-ATPase in sorghum roots were also investigated. The results obtained show that the external NH_4^+ increases the active H^+-transport by the PM H^+-ATPase under conditions of K^+ deprivation, which can explain, at least in part, the stimulation of low-K^+ uptake. Moreover, the increase in the PM H^+-ATPase activity could involve one or more specific isoforms. Together, the results show important characteristics of K^+ uptake and its regulation in the presence of NH_4^+.

Materials and methods

Plant growth and treatments

Seeds of sorghum [*Sorghum bicolor* (L.) Moench], genotype CSF 20, were surface sterilised for 5 min using a 1% solution of commercial bleach and then washed several times in distilled water. Seeds were germinated in plastic cups containing vermiculite moistened with distilled water. After 4 d, fifteen seedlings were placed in 10.0 L containers filled with modified one-fourth Hoagland solutions, which were formulated to contain two concentrations of K^+ (0.2 and 1.4 mM), and three inorganic nitrogen sources (NO_3^-, NO_3^-/NH_4^+ and NH_4^+) at a final concentration (total nitrogen) of 4.0 mM. K^+ was supplied as KCl and nitrogen as either $Ca(NO_3)_2$, NH_4NO_3 or NH_4Cl. In nutrient solutions containing NO_3^- as the sole nitrogen source, the following macronutrients were supplied: 0.25 mM $MgSO_4$ and 0.2 mM NaH_2PO_4. In nutrient solutions containing NO_3^-/NH_4^+, the following macronutrients were added: 0.25 mM $MgSO_4$, 0.2 mM NaH_2PO_4 and 2.0 mM $CaCl_2$. In nutrient solutions containing NH_4^+ as the sole nitrogen source, the following macronutrients were supplied: 0.25 mM $MgSO_4$, 0.2 mM $(NH_4)_2HPO_4$ and 2.0 mM $CaCl_2$. Micronutrients were similar in all tested nutrient solutions: 50 μM $CaCl_2$, 12.5 μM H_3BO_3, 1 μM $MnSO_4$, 1 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 0.1 μM H_2MoO_4 and 10 μM Fe–EDTA. Constant aeration was also maintained. The pH of the growth solutions was maintained at 5.5–6.0 and adjusted as needed with 1 M NaOH or HCl. The K^+ concentration of nutrient solutions was monitored daily and maintained at established values. Solutions were exchanged for fresh nutrient solutions on days 9 and 12 to ensure that plants remained at a nutritional steady state. On day 15 (t_0), plants were transferred to identical solutions lacking in K^+ and subjected to K^+ deprivation for 24 (t_1), 48 (t_2) and 72 (t_3) h. Plants were grown in a greenhouse with mean values for air temperature and relative humidity of 32.0 °C and 67.5% (daytime) and 22.0 °C and 90.0% (nighttime), respectively.

K^+ content and uptake and transport efficiencies

Five plants were harvested from each treatment at t_0, t_1, t_2 and t_3, and their roots were immersed in deionised water for 10 min. Roots and shoots were then separated and dried in an oven at 60 °C for 72 h. K^+ was extracted from 20 mg of finely powdered root and shoot samples with 2 mL deionised water and centrifuged at 3000 × *g* for 10 min. K^+ content was determined by flame photometry and expressed on a dry weight (DW) basis. K^+ uptake (UE) and transport (TE) efficiencies were estimated from the data corresponding to t_0 by the following equations:

$$\mathrm{UE\,(mmol\ K^+\ g^{-1}\ DW)} = \frac{\text{total } K^+ \text{content in plant}}{\text{root DW}}$$

$$\mathrm{TE\,(\%)} = \frac{K^+ \text{content in shoot}}{\text{total } K^+ \text{content in plant}} \times 100$$

K^+ depletion experiments

The K^+ uptake from low-K^+ solutions was estimated by the rate of depletion of K^+, as described by Claassen and Barber (1974). Assays were carried out in a controlled-environment room with air temperature and relative humidity of 24.8 ± 0.7 °C and 69.8 ± 5.5%, respectively. Light was provided by three fluorescent lamps, which were placed at a distance of 50 cm over the plants. In the early morning (7:00 am), plants maintained in their respective growth solutions were transferred to the room to acclimate to the described conditions. After 3 h of acclimation, roots were rinsed in deionised water for 10 min, and then the plants were individually placed in plastic pot containing 130 mL of uptake solution. This solution consisted of the following macronutrients: 2.0 mM $Ca(NO_3)_2$, 0.25 mM $MgSO_4$, 0.2 mM NaH_2PO_4 and 0.1 mM KCl. The micronutrient composition was similar to that of the growth solutions. The pH of the uptake solution was adjusted at 5.8. During depletion assays, constant aeration was supplied. 1 mL samples were taken at 30 min intervals for 5.5 h, and K^+ concentration was determined by flame photometry. After each sampling, deionised water was added to the pot to maintain a constant solution volume. After completion of the experiment, roots were rinsed for 10 min in distilled water, then excised and dried in an oven for 3 d. The data of the K^+ concentrations during the first 90 min for each deple-

tion curve were fitted by simple linear regression, and the slopes obtained were used to estimate the initial rates of low-K^+ net uptake (μmol K^+ min^{-1} g^{-1} DW_{root}).

Plasma membrane isolation

Sorghum roots (15 g) harvested at t_0 and t_2 were rinsed with cold, distilled water and homogenised with mortar and pestle for 5 min in 50 mL of homogenising buffer containing 75 mM Tris–HCl, pH 8.0, 250 mM sucrose, 2 mM EGTA, 2 mM $MgSO_4$, 1 mM phenylmethylsulfonyl fluoride, 2 mM dithiothreitol (DTT), 10% glycerol, 0.5% bovine serum albumin (BSA) and 3% polyvinylpyrrolidone. The homogenate was filtered through three layers of cheesecloth and subjected to sequential centrifugation, first centrifuging at 9000 × *g* for 15 min, then discarding the pellet and centrifuging the supernatant at 20,000 × *g* for 1 h. The microsomal pellet was carefully resuspended in 1.5 mL of buffer containing 5 mM KH_2PO_4, pH 7.5, 250 mM sucrose, 1 mM DTT and 0.1 mM EDTA. The PM vesicles were purified by the aqueous polymer two-phase partitioning system described by Widell et al. (1982). Resuspended membranes were added to an 8.0 g phase mixture to produce a 10.0 g aqueous polymer two-phase system with a final composition of 5 mM KH_2PO_4, pH 7.5, 250 mM sucrose, 6.2% (w/w) dextran T500 (Sigma), 6.2% (w/w) polyethylene glycol (PEG 3350, Sigma) and 5 mM KCl. The samples were thoroughly mixed by inversion 20 times and centrifuged at 1000 × *g* for 5 min. The upper phase (U1) was transferred to a second tube containing a new lower phase obtained from the centrifuged phase system. The procedure of mixing and centrifugation was repeated twice to obtain U3. This upper phase was removed, diluted with 5 mM Tris–Mes, pH 7.5, 250 mM sucrose and 1 mM DTT and then centrifuged at 80,000 × *g* for 1 h. The pellet was resuspended in buffer containing 1 mM Tris–Mes, pH 7.5, 20% glycerol and 1 mM DTT and was either used immediately or stored at −20 °C. All steps were performed at 4 °C. The protein concentration was determined according to Bradford (1976) using BSA as a standard.

H^+-ATPase activity

PM fractions (2–4 μg of protein) were incubated in 0.5 mL of reaction medium containing 30 mM Mes–Tris, pH 6.5, 5 mM $MgSO_4$, 50 mM KCl, and 0.05% (w/v) polyoxyethylene cetyl ether (Brij 58). The reaction was initiated by adding 5 mM ATP and carried out for 30 min at 30 °C. Inorganic phosphate content was determined spectrophotometrically at 820 nm according to Fiske and Subarrow (1925). To assess the homogeneity of the PM fraction preparations, ATP hydrolysis was assayed using inhibitors specific for mitochondrial (1 mM sodium azide), vacuolar (50 mM potassium nitrate), and PM (0.5 mM sodium orthovanadate) ATPases and for unspecific acid phosphatase (0.1 mM ammonium molybdate) (Yan et al., 1998). PM ATPase activity was determined by the difference in activity between the assays in the presence and absence of 0.5 mM sodium orthovanadate.

Proton pumping

Proton uptake into inside-out vesicles was spectrophotometrically measured as the decrease in absorbance at 495 nm of the ΔpH-sensitive probe acridine orange (AO) as described by Palmgren and Sommarin (1989). The reaction medium (1.0 mL) contained 10 mM Mes–Tris, pH 6.5, 5 mM $MgSO_4$, 50 mM KCl, 0.05% (w/v) Brij 58, 10 μM AO and 75 μg of membrane protein. After equilibration of the vesicles with the dye for 10 min at 25 °C, 2 mM ATP was added and the decrease in absorbance was monitored. After 6.5 min, the protonophore gramicidin was added to a final concentration of 2 μM. Passive proton movement through membrane vesicles was determined in reaction medium without ATP (Klobus and Janicka-Russak, 2004).

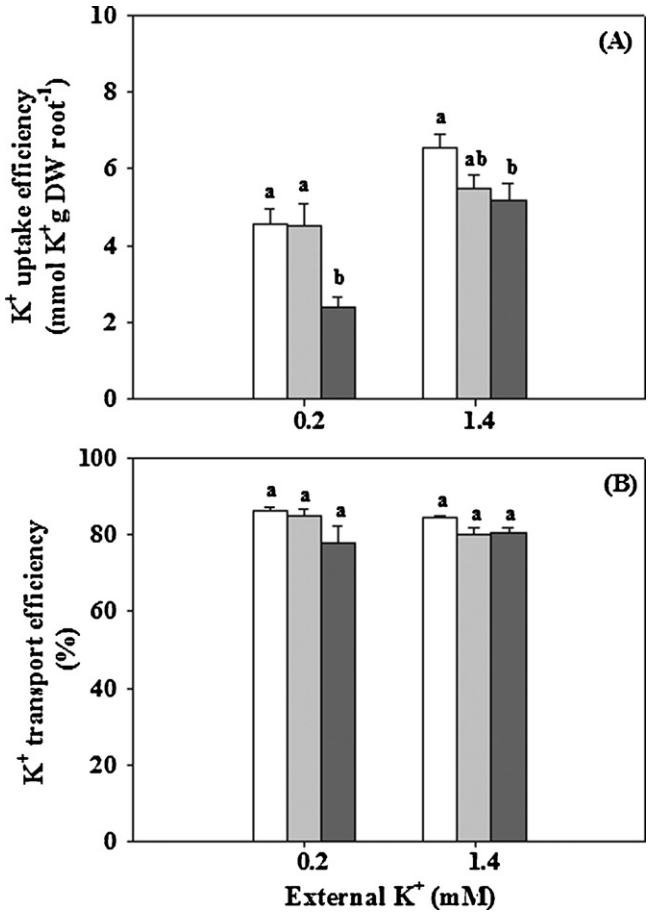

Fig. 1. Effect of inorganic nitrogen sources and K^+ levels on K^+ uptake and transport efficiencies of sorghum plants. Plants were grown for 15 d in complete nutrient solutions containing 0.2 mM or 1.4 mM K^+ and three inorganic nitrogen sources, NO_3^- (white bars), NO_3^-/NH_4^+ (light gray bars) or NH_4^+ (dark gray bars). Values represent the mean ± SE for five independent measurements. Data were subjected to one-way ANOVA and compared using Tukey's test ($P \leq 0.05$). Significant differences due to nitrogen sources tested, at the same K^+ concentration, are indicated with different lowercase letters.

SDS–PAGE and protein gel blot analyses

20 μg of membrane proteins were precipitated with 10% cold trichloroacetic acid and washed in cold acetone as described by Lefevbre et al. (2007). Samples were solubilised in standard sample buffer containing 7 M urea and 2 M thiourea and separated by SDS–PAGE (10% acrylamide) according to Laemmli (1970). Polypeptides were electrophoretically transferred to a nitrocellulose membrane (Hybond-C Extra, Amersham) using a semidry blotting system. Transfer buffer contained 25 mM Tris, 192 mM glycine and 20% (v/v) methanol. Electrophoretic transfer was carried out at 10 mV for 50 min. BSA (3%) in 20 mM Tris–HCl, pH 7.5, 150 mM NaCl and 0.05% (v/v) Tween 20 was used as blocking reagent. The blot was incubated with a polyclonal antibody raised against the N-terminal (residues 6–51), the central (residues 340–650) and the C-terminal (residues 851–949) domains of *Arabidopsis thaliana* PM H^+-ATPase (*AHA3*), diluted 1:2000 in 20 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1% BSA and 0.05% (v/v) Tween 20. The incubation system was gently shaken overnight at 4 °C. After rinsing three times in 20 mM Tris–HCl, pH 7.5, 150 mM NaCl and 0.05% (v/v) Tween 20, the blot was incubated at room temperature for 1 h with a 1:3000 dilution of

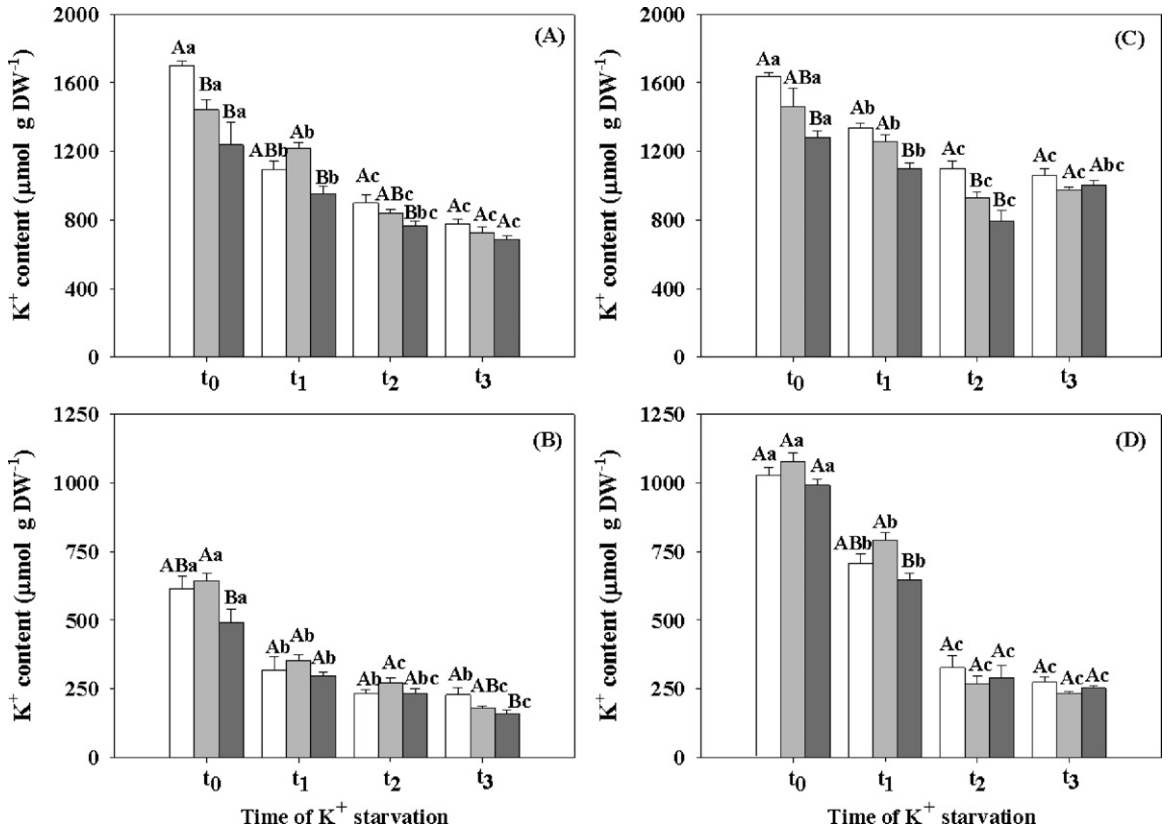

Fig. 2. Effect of K^+ starvation and inorganic nitrogen sources on K^+ content of sorghum shoot (A and C) and roots (B and D). K^+ content was determined using plants grown for 15 d (t_0) in complete nutrient solutions containing 0.2 mM (A and B) or 1.4 mM (C and D) K^+ and three inorganic nitrogen sources, NO_3^- (white bars), NO_3^-/NH_4^+ (light gray bars) or NH_4^+ (dark gray bars), and starved of K^+ for 24 (t_1), 48 (t_2) and 72 (t_3) h. Values represent the mean ± SE of five independent measurements. Data were subjected to one-way ANOVA and compared using Tukey's test ($P \leq 0.05$). Significant differences due to the nitrogen source tested, at the same starvation time, are indicated with different capital letters. Significant differences due to K^+ deficiency, at the same level of K^+ and nitrogen source, are indicated with different lowercase letters.

secondary antibody (alkaline phosphatase-conjugated anti-rabbit IgG, Sigma). For the development of alkaline phosphatase reaction, a standard 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium (BCIP/NBT) protocol (Bio-Rad, Hercules, CA, USA) was used.

Results

Effect of NH_4^+ and K^+ concentration on K^+ uptake and transport efficiencies

The long-term effects of K^+ levels and inorganic nitrogen sources in the growth solution on K^+ absorption and transport to the shoot were studied in sorghum plants. At t_0, the presence of NH_4^+ as the sole nitrogen source severely affected K^+ uptake efficiency by sorghum roots grown in nutrient solution containing K^+ at 0.2 mM, compared to those grown in solutions containing NO_3^- and NO_3^-/NH_4^+ (Fig. 1A). Increasing the external K^+ concentration to 1.4 mM lessened the severity of the inhibitory effect of NH_4^+ on K^+ uptake (Fig. 1A). The capacity to translocate K^+ to the shoot was not significantly affected by NH_4^+ in nutrient solution (Fig. 1B).

Effect of NH_4^+ and K^+ starvation on K^+ tissue content

At t_0, sorghum plants grown in the presence of K^+ at 0.2 mM and NH_4^+ as the sole nitrogen source accumulated less K^+ in shoot and roots than those cultivated with NO_3^- and NO_3^-/NH_4^+ (Fig. 2A and B). When the K^+ level in the growth solution was increased (1.4 mM), the root K^+ content did not differ between treatments with different nitrogen sources (t_0, Fig. 2D), while the shoot K^+ content remained lower in plants grown with NH_4^+ compared with plants grown with NO_3^-, as the sole nitrogen source (t_0, Fig. 2C).

Transfers of plants to K^+-free solutions resulted in obvious reductions in root and shoot K^+ contents (Fig. 2). At t_1 and t_2, the K^+ content of the aerial parts of plants grown in the presence of NH_4^+ and previously cultivated in 1.4 mM K^+ was significantly lower than in plants grown with NO_3^- (Fig. 2C), while this effect was only observed at t_2 in plants previously cultivated in 0.2 mM K^+ (Fig. 2A). At t_3, the K^+ content of shoots did not differ between treatments with different inorganic nitrogen sources (Fig. 2A and C). On the other hand, root K^+ content of plants grown in nutrient solutions containing 0.2 mM K^+ and three inorganic nitrogen sources reached similar values at t_1 and t_2, while at t_3 the K^+ content was lower in plants grown with only NH_4^+ (Fig. 2B). In plants grown in higher levels of K^+, the K^+ contents were similar at t_2 and t_3 (Fig. 2D).

Effect of NH_4^+ and K^+ starvation on the low-K^+ net uptake by sorghum roots

Plotting the external K^+ concentration in the uptake solution vs. time showed that the rates of K^+ net depletion increased with the time of K^+ starvation (Figs. 3 and 4). At t_0, plants grown in nutrient solutions containing 1.4 mM K^+ did not show a K^+ net depletion (Fig. 4A), while those cultivated in solutions supplied with 0.2 mM K^+ depleted approximately 10% of the initial level of K^+ (100 µM) (Fig. 3A). Plants starved of K^+ for 24 (t_1) and 48 (t_2) h and grown in the presence of NH_4^+ (NO_3^-/NH_4^+ and NH_4^+) exhibited higher capacities to deplete micromolar concentrations of external K^+ than plants grown with NO_3^- as the sole nutrient source. This response was independent of the K^+ concentration in which plants were

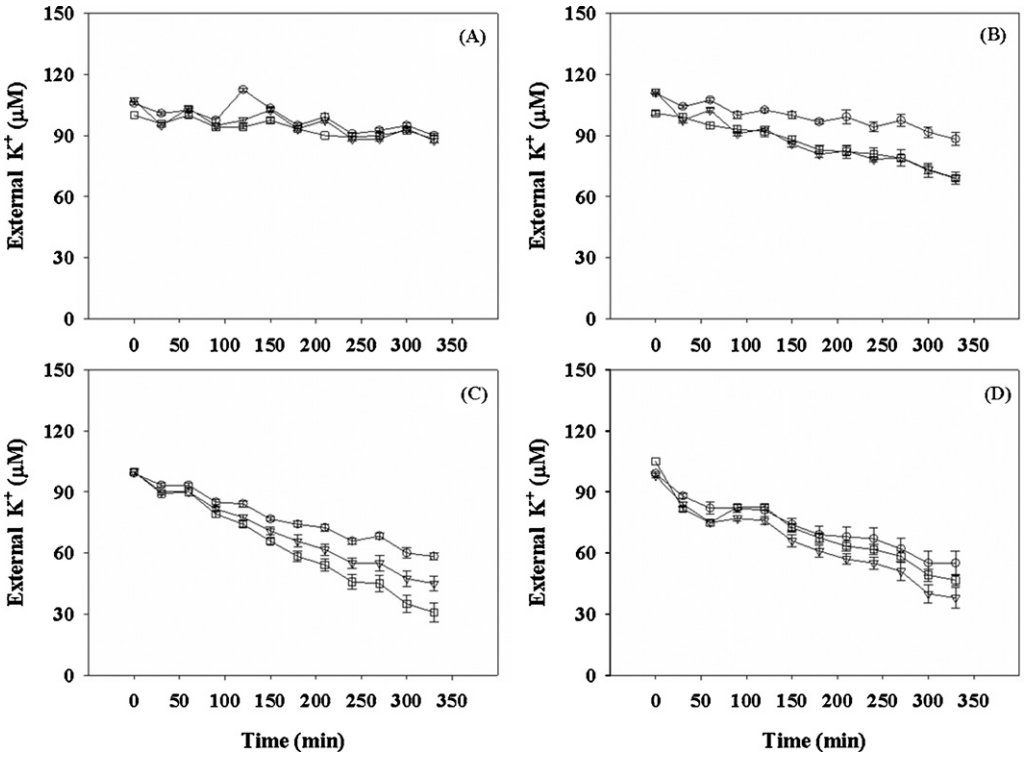

Fig. 3. Effect of inorganic nitrogen sources and K^+ starvation on rates of external K^+ depletion by sorghum roots. Depletion curves were determined using plants grown for 15 d (A) in complete nutrient solutions containing 0.2 mM K^+ and three inorganic nitrogen sources, NO_3^- (circles), NO_3^-/NH_4^+ (triangles) or NH_4^+ (squares), and starved of K^+ for 24 (B), 48 (C) and 72 (D) h. After K^+ desorption, plants were transferred to a solution containing K^+ at 100 μM. External solution aliquots were taken at 30 min intervals and the concentration of K^+ was determined. Each curve was drawn with data obtained from six plants. K^+ concentration at each sampling time represents the mean ± SE.

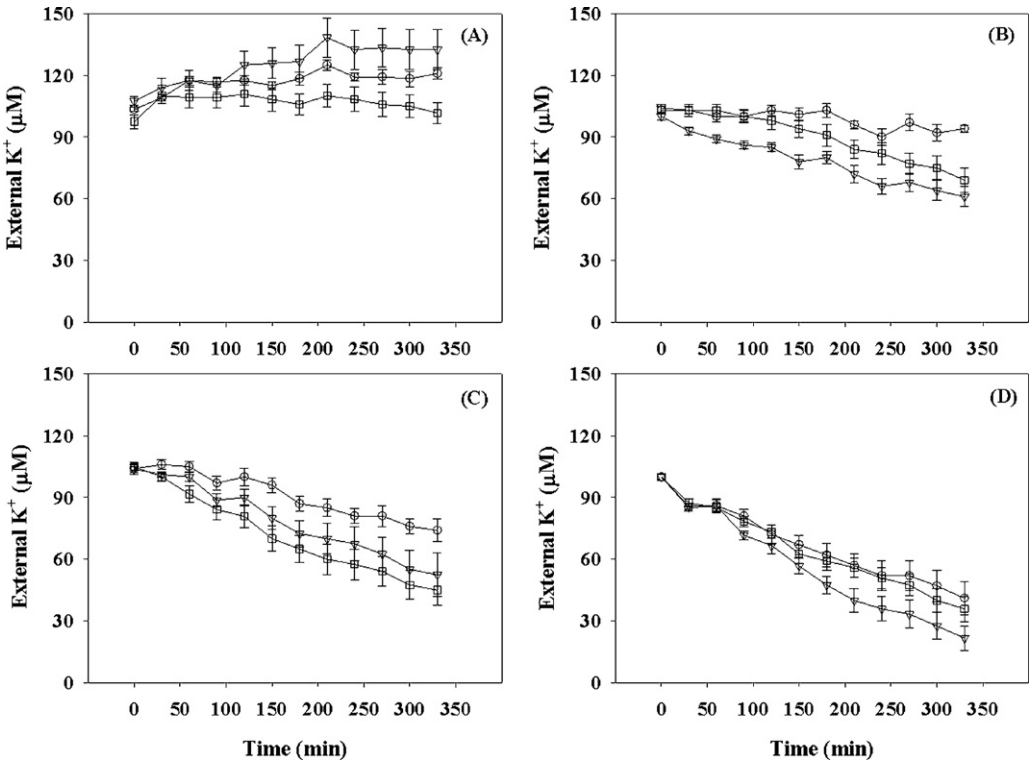

Fig. 4. Effect of inorganic nitrogen sources and K^+ starvation on rates of external K^+ depletion by sorghum roots. Depletion curves were determined using plants grown for 15 d (A) in complete nutrient solutions containing 1.4 mM K^+ and three inorganic nitrogen sources, NO_3^- (circles), NO_3^-/NH_4^+ (triangles) or NH_4^+ (squares), and starved of K^+ for 24 (B), 48 (C) and 72 (D) h. After K^+ desorption, plants were transferred to a solution containing K^+ at 100 μM. External solution aliquots were taken at 30 min intervals and the concentration of K^+ was determined. Each curve was drawn with data obtained from six plants. K^+ concentration at each sampling time represents the mean ± SE.

Table 1
Effect of K^+ starvation and external nitrogen sources on the initial rate of low-K^+ net uptake (100 μM) by sorghum roots. Initial rates were determined using plants grown for 15 d (t_0) in complete nutrient solutions containing 0.2 mM or 1.4 mM K^+ and three inorganic nitrogen sources, and starved of K^+ for 24 (t_1), 48 (t_2) e 72 (t_3) h.

K^+ (mM)	Time of K^+ starvation	Initial rate (μmol K^+ min^{-1} g^{-1} DW_{root})		
		NO_3^-	NO_3^-/NH_4^+	NH_4^+
0.2	t_0	−1.01 ± 1.28 Ab	−2.28 ± 1.19 Ac	−4.66 ± 0.99 Ac
	t_1	3.02 ± 1.35 Bb	−3.52 ± 0.63 Ac	−4.79 ± 1.94 Ac
	t_2	−7.96 ± 1.32 Ba	−9.33 ± 0.73 ABb	−11.35 ± 0.74 Ab
	t_3	−10.50 ± 1.70 Ba	−13.42 ± 0.88 Ba	−17.56 ± 0.98 Aa
1.4	t_0	11.74 ± 1.14 Ac	11.09 ± 2.80 Ab	7.46 ± 4.01 Ac
	t_1	3.81 ± 3.42 Bb	−4.93 ± 1.43 Aa	0.16 ± 1.38 ABb
	t_2	3.30 ± 3.06 Bb	−1.13 ± 0.77 ABa	−4.73 ± 1.89 Aab
	t_3	−6.02 ± 0.94 Aa	−6.47 ± 0.90 Aa	−7.05 ± 0.74 Aa

Values represent the mean ± SE of six independent measurements. Data were subjected to one-way ANOVA and compared using Tukey's test ($P \leq 0.05$). Significant differences due to the nitrogen source tested, at the same starvation time, are indicated with different capital letters. Significant differences due to K^+ deficiency, at the same level of K^+ and nitrogen source, are indicated with different lowercase letters.

grown. For instance, at t_2, sorghum roots grown in the presence of NH_4^+ and NO_3^-/NH_4^+ and cultivated previously with 0.2 mM K^+ reduced the external K^+ to about 69% and 55% of the initial levels, respectively, while roots grown with NO_3^- reduced external K^+ to around 42% (Fig. 3C). Similar results were also obtained with plants grown in solutions containing 1.4 mM K^+ (Fig. 4C). At the end of the K^+ uptake experiment (5.5 h), a slight increase in the pH of the depletion solution was observed only in assays with plants grown with NO_3^- as the sole nitrogen source (Supplementary Data). However, values reached were within the optimum pH range of nutrient uptake. No significant change in the pH of the depletion solution was observed in the assays with plants grown with NO_3^-/NH_4^+ or NH_4^+ (Supplementary Data).

At t_0 and at both K^+ concentrations, no significant differences were observed in the initial rates of low-K^+ uptake regardless of the nitrogen sources used (Table 1). However, as the time of K^+ starvation increased, the initial rates of K^+ uptake were higher in the roots of plants grown with NH_4^+ or NO_3^-/NH_4^+ than plants grown with NO_3^- as the sole nitrogen source. This was clearly evident in the sorghum plants grown in nutrient solutions containing a low K^+ level (0.2 mM) and subjected to three periods of K^+ starvation (Table 1). For instance, the initial rate of low-K^+ uptake in plants grown with NH_4^+ alone was 42.6% and 67.2% higher than those grown with NO_3^- alone at t_2 and t_3, respectively. In contrast, plants grown in nutrient solutions containing a high-K^+ level (1.4 mM) and grown with NH_4^+ showed higher initial rates of low-K^+ uptake at t_2, compared with plants grown with NO_3^- as the sole nitrogen source (Table 1). The initial rates of low-K^+ uptake tended to converge toward a steady value at t_3 of K^+ starvation (Table 1).

Activities and immunoblotting of PM H^+-ATPase

The basal ATPase activity of membrane fractions from sorghum roots isolated by aqueous polymer two-phase partitioning was not affected by the addition of azide and nitrate but was strongly sensitive to 0.5 mM vanadate, indicating the high purity of preparations with plasmalemma (Table 2). However, a slight inhibition in the total ATPase activity by molybdate was observed, indicating the presence of unspecific acid phosphatases (Table 2).

K^+ starvation did not stimulate the ATP hydrolysis activity of PM H^+-ATPase in sorghum roots grown in the three different nitrogen sources tested (Fig. 5). Moreover, the effect of the nitrogen source during K^+ starvation was observed only in the roots of plants grown

Table 2
Marker enzyme activities in plasma membrane preparations from sorghum roots isolated by aqueous polymer two-phase partitioning. Values are expressed as a percentage of the total ATPase activity measured in absence of inhibitors.

Enzymatic marker	Inhibitor	Reduction of total ATPase activity
Unspecific acid phosphatase	Molybdate (0.1 mM, pH 6.5)	9.8%
P-H^+-ATPase	Vanadate (0.5 mM, pH 6.5)	85.7%
V-H^+-ATPase	Nitrate (50 mM, pH 7.5)	Not inhibited
F_0F_1-ATPsynthase	Azide (1 mM, pH 7.5)	Not inhibited

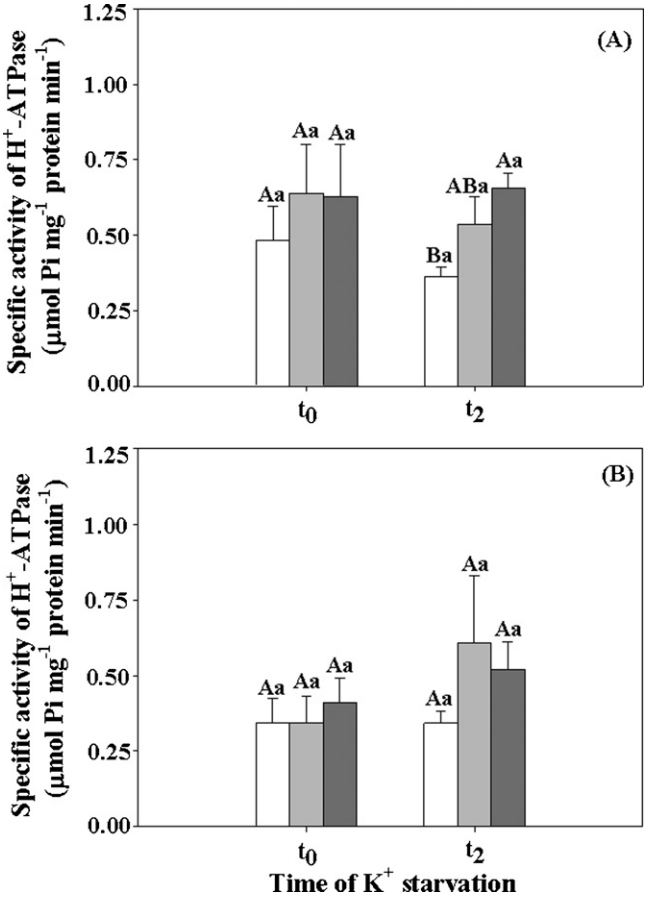

Fig. 5. Effect of inorganic nitrogen sources and K^+ starvation on ATP hydrolytic activity of plasma membrane H^+-ATPase from sorghum roots. Membrane vesicles were isolated from plants grown for 15 d (t_0) in complete nutrient solutions containing 0.2 mM (A) or 1.4 mM (B) K^+ and three inorganic nitrogen sources, NO_3^- (white bars), NO_3^-/NH_4^+ (light gray bars) or NH_4^+ (dark gray bars), and starved of K^+ for 48 (t_2) h. Values represent the mean ± SE of three independent experiments. Data were subjected to one-way ANOVA and compared using Tukey's test ($P \leq 0.05$). Significant differences due to the nitrogen source tested, at the same starvation time, are indicated with different capital letters. Significant differences due to K^+ deficiency, at the same level of K^+ and nitrogen source, are indicated with different lowercase letters.

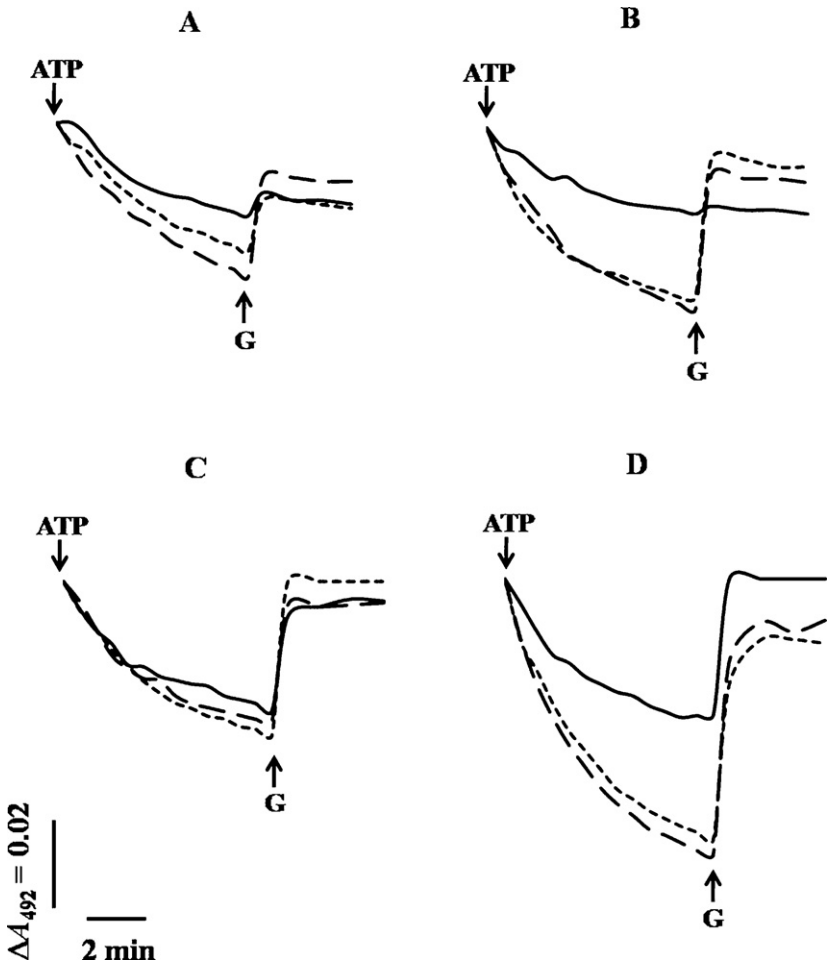

Fig. 6. Effect of inorganic nitrogen sources and K^+ starvation on H^+ pumping activity of plasma membrane H^+-ATPase from sorghum roots. Membrane vesicles were isolated from plants grown for 15 d in complete nutrient solutions containing 0.2 mM (A) or 1.4 mM (C) K^+ and three inorganic nitrogen sources, NO_3^- (–), NO_3^-/NH_4^+ (– –) or NH_4^+ (···), and starved of K^+ for 48 h (B, plants previously grown with 0.2 mM K^+ and D, with 1.4 mM K^+). The reaction was started by adding of 5 mM ATP. The H^+ gradient was dissipated by addition of 2 μM gramicidin (G). Traces represent the quenching of AO absorbance and were obtained from averaged data from three independent experiments.

in solutions containing 0.2 mM K^+ and exposed to K^+ deficiency for 48 h (Fig. 5A). The presence of NH_4^+ in the nutrient solution significantly stimulated ATP hydrolysis.

Two parameters, initial rate and pH gradient, were used to characterise H^+ pumping by PM H^+-ATPase. At t_0, the nitrogen source had no significant effect on the initial rate of H^+ pumping in plants grown in high K^+, but it was significantly higher in plants grown with NO_3^-/NH_4^+ than in those grown with NO_3^- in plants grown at low K^+ (Fig. 6 and Table 3). Under K^+ starvation (t_2) and in the presence of NO_3^- as the sole nitrogen source, no change in initial rate of H^+ pumping was observed, whereas it was increased by the presence of NH_4^+ in plants from solutions with 0.2 and 1.4 mM K^+ (Fig. 6 and Table 3). On the other hand, the net proton transport across the PM (pH gradient) was steeper in plants grown in the presence of NH_4^+ than in those grown in nutrient solutions containing NO_3^- as the sole nitrogen source at t_0 (Fig. 6 and Table 3). After 48 h of K^+ starvation (t_2), the H^+ transport was increased twofold in plants grown in solutions containing 1.4 mM K^+ and NH_4^+, compared to the values at t_0 (Fig. 6 and Table 3).

Table 3
Effect of K^+ starvation and external nitrogen sources on active H^+ transport catalyzed by plasma membrane H^+-ATPase from sorghum roots. Initial rates and H^+ gradient formation were determined using plants grown for 15 d (t_0) in complete nutrient solutions containing 0.2 mM or 1.4 mM K^+ and three inorganic nitrogen sources, and starved of K^+ for 48 (t_2) h.

K^+ (mM)	Time of K^+ starvation	Initial rate (ΔA_{492} min^{-1})			H^+ gradient (ΔA_{492} mg^{-1} min^{-1})		
		NO_3^-	NO_3^-/NH_4^+	NH_4^+	NO_3^-	NO_3^-/NH_4^+	NH_4^+
0.2	t_0	0.004 ± 0.001 Ba	0.011 ± 0.001 Ab	0.008 ± 0.001 ABb	0.049 ± 0.002 Ca	0.079 ± 0.003 Aa	0.066 ± 0.001 Ba
	t_2	0.006 ± 0.001 Ca	0.016 ± 0.000 Ba	0.019 ± 0.001 Aa	0.044 ± 0.001 Ba	0.095 ± 0.002 Aa	0.089 ± 0.008 Aa
1.4	t_0	0.013 ± 0.000 Aa	0.014 ± 0.001 Ab	0.012 ± 0.001 Ab	0.061 ± 0.005 Ba	0.083 ± 0.005 Ab	0.079 ± 0.004 Ab
	t_2	0.014 ± 0.000 Ca	0.027 ± 0.000 Aa	0.023 ± 0.000 Ba	0.072 ± 0.001 Ba	0.144 ± 0.004 Aa	0.136 ± 0.002 Aa

Values represent the mean ± SE of three independent experiments. Data were subjected to one-way ANOVA and compared using Tukey's test ($P \leq 0.05$). Significant differences due to the nitrogen source tested, at the same starvation time, are indicated with different capital letters. Significant differences due to K^+ deficiency, at the same level of K^+ and nitrogen source, are indicated with different lowercase letters.

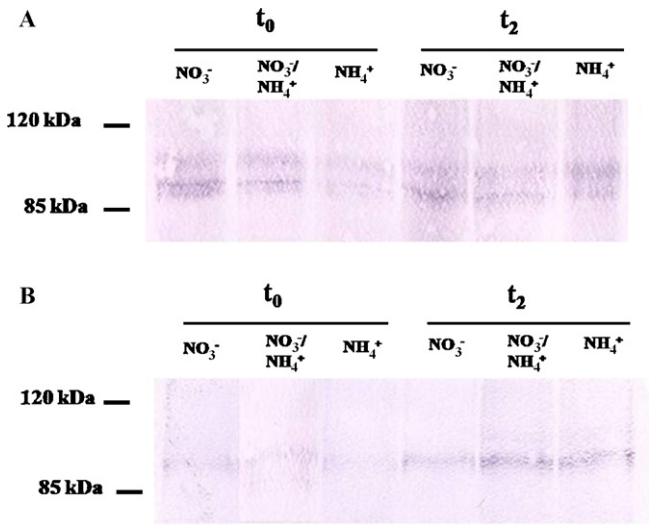

Fig. 7. Immunoblot of PM H^+-ATPase from sorghum roots. Membrane vesicles were isolated from plants grown for 15 d (t_0) in complete nutrient solutions containing 0.2 mM (A) or 1.4 mM (B) K^+ and three inorganic nitrogen sources, and starved of K^+ for 48 (t_2) h. The blots were probed with antibodies against AHA3 (a PM H^+-ATPase isoform of *Arabidopsis thaliana*), which was a gift from Dr. R. Serrano.

Protein blots obtained with membrane preparations from sorghum roots grown in nutrient solutions containing 1.4 mM K^+ (t_0) or under K^+ deficiency for 48 h (t_2) showed that polyclonal antibody specific against PM H^+-ATPase crossreacted with a single band of about 100 kDa (Fig. 7B). The H^+-ATPase content was slightly increased in plants grown with NO_3^- as the only nitrogen source after starved of K^+ for 48 h (Fig. 7B). However, the band of PM H^+-ATPase from plants grown in the presence of NH_4^+ and starved of K^+ was more intense than that from plants grown in complete nutrient solution and with the same nitrogen source. Interestingly, the polyclonal antibodies against *Arabidopsis thaliana* PM H^+-ATPase used in this work produced two bands of similar intensity for membrane preparations from roots grown in nutrient solutions containing 0.2 mM K^+ or starved of K^+ for 48 h (t_2), irrespective of the nitrogen source (Fig. 7A).

Discussion

The growth of sorghum plants in nutrient solutions containing different K^+ concentrations and inorganic nitrogen sources revealed differences in the capacity of the root system to take up micromolar K^+ concentrations. In comparison to NO_3^-, the presence of NH_4^+ in the growth solution stimulated high rates of K^+ depletion by the sorghum roots that were K^+ starved (Figs. 3 and 4). The sensitivity to external NH_4^+ of low-concentration K^+ influx is one of the more conspicuous effects of NH_4^+ toxicity in plants (Vale et al., 1987; Martínez-Cordero et al., 2005; Nieves-Cordones et al., 2007; Santa-María et al., 2000; Szczerba et al., 2008). However, the stimulation of K^+ depletion by the presence of NH_4^+ during plant growth has also been documented (Nieves-Cordones et al., 2008). In this work, the different chemical forms of inorganic nitrogen did not produce any significant effects on root growth (data not shown) or plant health at t_0 and during K^+ starvation that could have influenced measurements of K^+ uptake. Moreover, no important alteration was observed in the pH of the depletion solutions (Supplementary Data), and the K^+ concentrations and pH in the growth solutions were strictly maintained at the established values. Thus, the observed differences in K^+ depletion capacity by sorghum roots can be attributed to influence of nitrogen source at the root ambient and K^+ tissue level.

Our results show that the high capacity of low-K^+ depletion by sorghum roots grown with NH_4^+ and starved of K^+ (at t_2, Figs. 3 and 4) occurred in parallel with the induction of H^+ extrusion by the PM H^+-ATPase (Fig. 6). The increased activity of this enzyme may be an important response under growth conditions that alter K^+ homeostasis in plants, because the establishment of more negative PM potentials and steeper H^+ gradients constitutes the driving force to move K^+ into plant cells (Maathuis and Sanders, 1996; Rodriguez-Navarro, 2000), as well as modulating factor of the activities of some cytoplasmic components for sensing and signalling K^+-deficiency (Wang and Wu, 2010). Recently, the role of negative PM potentials in the regulation of *LeHAK5* transcript levels and plants' capacity to deplete external K^+ in NH_4^+-grown tomato roots has been proposed (Nieves-Cordones et al., 2008). In studies with *HvHAK1*-expressing yeast cells, the effect of membrane hyperpolarisation on changes in Rb^+ influx has also been suggested (Fulgenzi et al., 2008). In this study, the increase of H^+-ATPase activity in sorghum roots by high NH_4^+ concentrations may have resulted in PM hyperpolarisation of root cells, favouring high rates of K^+ net uptake. It is well known that in the presence of NH_4^+, voltage-dependent AKT K^+ channels provide a pathway for high-affinity K^+ uptake, which may sustain plant growth and development in low K^+ environments (Hirsch et al., 1998; Rubio et al., 2008, 2010). If the high rates of K^+ net uptake in NH_4^+-treated sorghum plants are mediated through K^+ channels, the concomitant increase in PM H^+-ATPase activity becomes essential. It has been suggested that the contribution of AtAKT1 channels to mediate K^+ uptake above its concentrative capacity in NH_4^+-treated *Arabidopsis* roots probably depends on the development of negative membrane potentials (Rubio et al., 2008). Thus, the findings described in this work lend support to the essential role of PM H^+-ATPase in the acquisition of low-concentration of K^+ in the presence of NH_4^+. However, the discrepancy observed between the increased activity of H^+ transport (t_2, Fig. 6 and Table 3) and a significant induction of K^+ uptake (t_2, Table 1) in the NO_3^-/NH_4^+-treated plants also suggests the participation of other regulatory mechanisms that could act directly on the K^+ transport system, which could be activated when NH_4^+ is the only nitrogen source in the external medium.

Notably, although the K^+ root content has been considered to act as a regulatory factor of the rate of high-affinity K^+ uptake (Glass, 1975; Vale et al., 1987), the results of this study seem to indicate that the level of control of K^+ uptake in K^+-starved sorghum plants exposed to different sources of inorganic nitrogen might have been exerted by the K^+ shoot content (t_2, Fig. 2). Previous studies have shown that the quantity of K^+ re-translocated from the shoot to the root via the phloem may convey the shoot demand for K^+ and, in turn, influence the uptake rates of K^+ (Drew et al., 1990; White, 1997). In agreement with this hypothesis, the greater decrease in the shoot K^+ content of plants grown with NH_4^+ than that of plants grown with NO_3^- (t_2, Fig. 2A and C) suggests that a positive feedback mechanism, based on K^+ recirculation, could be a primary signal increasing the K^+ net uptake in sorghum plants grown with NH_4^+ (Table 1, Figs. 3 and 4). Low-K^+ stress signalling pathways involving shoot K^+ concentration, as well as metabolic signals originating from the breakdown of cellular homeostasis has also been observed in other species (Véry and Sentenac, 2003; Martínez-Cordero et al., 2005; Amtmann et al., 2006). It is also remarkable that despite the reduction in the K^+ accumulation in the tissues of sorghum plants grown with NH_4^+ (Fig. 2), its relative distribution (K^+ transport efficiency) within the plant was not significantly affected (Fig. 1B). In contrast, seedlings of barley and rice grown under high levels of NH_4^+ and low-K^+ conditions showed significant decreases in the translocation rate of K^+ to the shoots (Santa-María et al., 2000; Szczerba et al., 2008). The possible effect of NH_4^+ on SKOR K^+ transporters that mediate the root-to-shoot K^+ translocation has also been suggested (Szczerba et al., 2008). Interestingly,

experiments with tobacco plants fed with NH_4^+ showed that the amount of xylem-transported K^+ was similar to that of NO_3^-- and NO_3^-/NH_4^+-fed plants and that it was mainly the result of massive export from the leaves and cycling of the K^+ in the phloem (Lu et al., 2005). Likely, the transport mechanisms involved in xylem loading may have been activated to enable a more efficient K^+ translocation from the root to shoot in sorghum plants under NH_4^+ nutrition.

Long-term exposure to different concentrations of K^+, 0.2 mM (high-affinity range) and 1.4 mM (low-affinity range), induced qualitative changes in the PM H^+-ATPase expression pattern (Fig. 7). The induction of two isoforms in plants grown at low levels of K^+ can be interpreted as a response to nutritional deficit for the purpose of increasing uptake capacity in K^+-poor soil. Interestingly, these isoforms were induced irrespective of the inorganic nitrogen source and were not affected by K^+ deficiency (Fig. 7A, t_0 and t_2). The correlation between the induction of specific H^+-pump isoforms and nutritional deficiency has previously been established in maize (Santi et al., 2003) and rice (Chang et al., 2009). On the other hand, the sole isoform detected in PM from roots grown at K^+ 1.4 mM was induced by K^+ starvation (Fig. 7B, t_0 and t_2). Contrary to the results presented here, other studies have shown no change in the amount of PM H^+-ATPase of roots after they were subjected to K^+ withdrawal (Samuels et al., 1992).

These data reveal a close link between the induction of PM H^+-ATPase and higher low-K^+ uptake rates by K^+-starved sorghum roots under NH_4^+ nutrition. Under such growth conditions, stimulation of this enzyme activity can lead to changes in the electrochemical H^+ gradient across the PM and increase K^+ transport via specific channels and carriers. The results could be interpreted as an effective mechanism to regulate K^+ homeostasis in plant tissue, which is impaired by the presence of NH_4^+.

Acknowledgements

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). We thank Dr. Ramón Serrano (Instituto de Biología Molecular y Celular de Plantas, Universidad Politécnica de Valencia, Spain) for kindly providing the antibody against PM H^+-ATPase.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jplph.2011.03.002.

References

Amtmann A, Jelitto TC, Sanders D. K^+-selective inward-rectifying channels and apoplastic pH in barley roots. Plant Physiol 1999;119:331–8.
Amtmann A, Hammond JP, Armengaud P, White PJ. Nutrient sensing and signaling in plants: potassium and phosphorus. Adv Bot Res 2006;43:209–57.
Bañuelos MA, Garciadeblas B, Cubero B, Rodriguez-Navarro A. Inventory and functional characterization of the HAK potassium transporters of rice. Plant Physiol 2002;130:784–95.
Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein–dye binding. Anal Biochem 1976;72:248–54.
Chang CH, Hu Y, Sun S, Zhu Y, Ma G, Xu G. Proton pump OsA8 is linked to phosphorus uptake and translocation in rice. J Exp Bot 2009;60:557–65.
Claassen N, Barber SA. A method for characterizing the relation between nutrient concentration and flux into roots of intact plants. Plant Physiol 1974;54:564–8.
Dell'orto M, Santi S, De Nisi P, Cesco S, Varanini Z, Zocchi G, et al. Development of Fe-deficiency responses in cucumber (*Cucumis sativus* L.) roots: involvement of plasma membrane H^+-ATPase activity. J Exp Bot 2000;51:695–701.
Drew MC, Webb J, Saker LR. Regulation of K^+ uptake and transport to the xylem in barley roots; K^+ distribution determined by electron probe X-ray microanalysis of frozen-hydrated cells. J Exp Bot 1990;41:815–25.
Epstein E, Rains D, Elzam O. Resolution of dual mechanism of potassium absorption by barley roots. Proc Natl Acad Sci U S A 1963;49:684–92.
Fernando M, Kulpa J, Siddiqi MY, Glass ADM. Potassium-dependent changes in the expression of membrane-associated proteins in barley roots. Plant Physiol 1990;92:1128–32.
Fiske CH, Subarrow H. The colorimetric determination of phosphorus. J Biol Chem 1925;66:375–400.
Fulgenzi FR, Peralta ML, Mangano S, Danna CH, Vallejo AJ, Puigdomenech P, et al. The ionic environment controls the contribution of the barley HvHAK1 transporter to potassium acquisition. Plant Physiol 2008;147:252–62.
Glass A. The regulation of potassium absorption in barley roots. Plant Physiol 1975;56:377–80.
Hirsch R, Lewis BD, Spalding EP, Sussman MR. A role for the AKT1 potassium channel in plant nutrition. Science 1998;280:918–21.
Klobus G, Janicka-Russak M. Modulation by cytosolic components of proton pump activities in plasma membrane and tonoplast from *Cucumis sativus* L. roots during salt stress. Physiol Plant 2004;121:84–92.
Laemmli UK. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature 1970;227:680–5.
Lefevbre B, Furt F, Hartmann MA, Michaelson LV, Carde JP, Sargueil-Boiron F, et al. Characterization of lipid rafts from *Medicago truncaulta* root plasma membranes: a proteomic study reveals the presence of a raft-associated redox system. Plant Physiol 2007;144:402–18.
Lu YX, Li CJ, Zhang FS. Transpiration, potassium uptake and flow in tobacco as affected by nitrogen forms and nutrient levels. Ann Bot 2005;95:991–8.
Maathuis FJM, Amtmann A. K^+ nutrition and Na^+ toxicity: the basis of cellular K^+/Na^+ ratios. Ann Bot 1999;84:123–33.
Maathuis FJM, Sanders D. Mechanism of high-affinity potassium uptake in roots of *Arabidopsis thaliana*. Proc Natl Acad Sci U S A 1994;91:9272–6.
Maathuis FJM, Sanders D. Mechanism of potassium absorption by higher plant roots. Physiol Plant 1996;96:158–68.
Martínez-Cordero M, Martínez V, Rubio F. High-affinity K^+ uptake in pepper plants. J Exp Bot 2005;56:1553–62.
Nieves-Cordones M, Martínez-Cordero M, Martínez V, Rubio F. An NH_4^+-sensitive component dominates high-affinity K^+ uptake in tomato plants. Plant Sci 2007;172:273–80.
Nieves-Cordones M, Miller AJ, Alemán F, Martínez V, Rubio F. A putative role for the plasma membrane potential in the control of the expression of the gene encoding the tomato high-affinity potassium transporter HAK5. Plant Mol Biol 2008;68:521–32.
Palmgren M, Sommarin M. Lysophosphatidilcholine stimulates ATP dependent proton accumulation in isolated oat root plasma membrane vesicles. Plant Physiol 1989;90:1009–14.
Pyo YJ, Gierth M, Schroeder JI, Cho MH. High-affinity K^+ transport in *Arabidopsis*: AtHAK5 and AKT1 are vital for seedling establishment and postgermination growth under low-potassium conditions. Plant Physiol 2010;153:863–75.
Rodriguez-Navarro A. Potassium transport in fungi and plants. Biochim Biophys Acta 2000;1469:1–30.
Rubio F, Gassmann W, Schroeder J. Sodium-driven potassium uptake by the plant potassium transporter HKT1 and mutations conferring salt tolerance. Science 1995;270:1660–3.
Rubio F, Nieves-Cordones M, Alemán F, Martínez V. Relative contribution of AtHAK5 and AtAKT1 to K^+ uptake in the high-affinity range of concentration. Physiol Plant 2008;134:598–608.
Rubio F, Alemán F, Nieves-Cordones M, Martínez V. Studies on *Arabidopsis athak5*, *atakt1* double mutants disclose the range of concentration at which AtHAK5, AtAKT1 and unknown systems mediate K^+ uptake. Physiol Plant 2010;139:220–8.
Samuels AL, Fernando M, Glass ADM. Immunofluorescent localization of plasma membrane H^+-ATPase in barley roots and effects of K nutrition. Plant Physiol 1992;99:1509–14.
Santa-María G, Rubio F, Dubcovsky J, Rodriguez-Navarro A. The *HAK*1 gene of barley is a member of large gene family and encodes a high-affinity potassium transporter. Plant Cell 1997;9:2281–9.
Santa-María GE, Danna CH, Czibener C. High-affinity potassium transport in barley roots ammonium-sensitive and -insensitive pathways. Plant Physiol 2000;123:297–306.
Santi S, Locci G, Monte R, Pinton R, Varanini Z. Induction of nitrate uptake in maize roots: expression of a high-affinity nitrate transporter and plasma membrane H^+-ATPase isoforms. J Exp Bot 2003;54:1851–64.
Sentenac H, Bonneaud N, Minet M, Lacroute F, Salmon JM, Gaymard F, et al. Cloning and expression in yeast of a plant potassium ion transport system. Science 1992;256:663–5.
Shen H, Chen J, Wang Z, Yang C, Sasaki T, Yamamoto Y, et al. Root plasma membrane H^+-ATPase is involved in the adaptation of soybean phosphorus adaptation. J Exp Bot 2006;57:1353–62.
Spalding EP, Hirsch RE, Lewis DR, Qi Z, Sussman MR, Lewis BD. Potassium uptake supporting plant growth in the absence of AKT1 channel activity. J Gen Physiol 1999;113:909–18.
Szczerba MW, Britto DT, Ali SA, Balkos KD, Kronzucker HJ. NH_4^+-stimulated and -inhibited components of K^+ transport in rice (*Oriza sativa* L.). J Exp Bot 2008;59:3415–23.
Vale FR, Jackson WA, Volk RJ. Potassium influx into maize root systems. Plant Physiol 1987;84:1416–20.

Véry A, Sentenac H. Molecular mechanism and regulation of K^+ transport in higher plants. Annu Rev Plant Biol 2003;54:575–603.
Wang TB, Gassmann W, Rubio F, Schroeder JI, Glass ADM. Rapid up-regulation of *HKT1*, a high-affinity potassium transporter gene, in roots of barley and wheat following withdrawal of potassium. Plant Physiol 1998;118: 651–9.
Wang Y, Wu WH. Plant sensing and signaling in response to K^+-deficiency. Mol Plant 2010;3:280–7.
White PJ. The regulation of K^+ influx into roots of rye (*Secale cereale* L.) seedlings by negative feedback via the K^+ flux from shoot to root in the phloem. J Exp Bot 1997;48:2063–73.
Widell S, Lundborg T, Larsson C. Plasma membrane from oats prepared by partition in an aqueous polymer two-phase system. Plant Physiol 1982;70:1429–35.
Yan F, Feuerle R, Schäffer S, Fortmeier H, Schubert S. Adaptation of active proton pumping of plasmalemma ATPase activity of corn roots to low root medium pH. Plant Physiol 1998;117:311–9.