Plant Signaling & Behavior 8:7, e24799; July 2013; © 2013 Landes Bioscience

Complexity of potassium acquisition
How much flows through channels?

Devrim Coskun and Herbert J. Kronzucker*

Department of Biological Sciences; University of Toronto; Toronto, ON Canada

Keywords: barley, *Arabidopsis*, roots, ammonium, radiotracer, influx, transporters, respiration, anion

The involvement of potassium (K^+)-selective, Shaker-type channels, particularly AKT1, in primary K^+ acquisition in roots of higher plants has long been of interest, particularly in the context of low-affinity K^+ uptake, at high K^+ concentrations, as well as uptake from low-K^+ media under ammonium (NH_4^+) stress. We recently demonstrated that K^+ channels cannot mediate K^+ acquisition in roots of intact barley (*Hordeum vulgare* L.) seedlings at low (22.5 μM) external K^+ concentrations ($[K^+]_{ext}$) and in the presence of high (10 mM) external NH_4^+, while the model species *Arabidopsis thaliana* L. utilizes channels under comparable conditions. However, when external NH_4^+ was suddenly withdrawn, a thermodynamic shift to passive (channel-mediated) K^+ influx was observed in barley and both species demonstrated immediate and dramatic stimulations in K^+ influx, illustrating a hitherto unexplored magnitude and rapidity of K^+-uptake capacity and plasticity. Here, we expand on our previous work by offering further characterization of channel-mediated K^+ fluxes in intact barley, with particular focus on anion effects, root respiration and pharmacological sensitivity and highlight key additions to the current model of K^+ acquisition.

The potassium (K^+)-selective Shaker-type channel, AKT1, has been shown to mediate both high- and low-affinity K^+ acquisition in roots of higher plants,[1-3] and a sophisticated model involving its molecular regulation and relative contribution to K^+ uptake has emerged, based largely on the work in the model system *Arabidopsis* (*Arabidopsis thaliana* L.).[4-7] According to this view, approximately 80% of high- and low-affinity K^+ uptake in *Arabidopsis* can be attributed to the sum of functions of AtHAK5 (a member of the HAK/KUP/KT family of transporters)[8] and AtAKT1, while the remaining ~20% is mediated by as yet unidentified components.[5] Of special interest and the source of some controversy, has been the involvement of AtAKT1 in mediating K^+ uptake from low (micromolar) external K^+ concentrations ($[K^+]_{ext}$) in the presence of high (millimolar) external ammonium (NH_4^+).[1,9] Under such conditions, high-affinity K^+ uptake is severely suppressed at the functional level, which, in *Arabidopsis*, has been directly linked to AtHAK5 inhibition.[10] It has been shown that under such conditions, AtAKT1 can conduct the majority of K^+ uptake from $[K^+]_{ext}$ as low as 10 μM.[1] However, such findings have been difficult to reconcile with thermodynamic considerations that suggest channel-mediated K^+ acquisition at such low $[K^+]_{ext}$ is generally not feasible.[11,12] Our recent study[13] showed that the *Arabidopsis* model of K^+ acquisition is not a universally applicable one, and that, in particular, it may not apply to cereals. Our work demonstrated that in roots of intact barley (*Hordeum vulgare* L.) seedlings grown under high (10 mM) NH_4^+, K^+ channels could not conduct K^+ acquisition when $[K^+]_{ext}$ was low (22.5 μM), but at intermediate (112.5 μM) $[K^+]_{ext}$, channels operated jointly with high-affinity transporters. At high (5 mM) $[K^+]_{ext}$, K^+ channels dominated and stimulations of K^+ influx by anions chloride (Cl^-) and nitrate (NO_3^-) were observed. K^+ efflux was found to be channel-mediated at low and intermediate $[K^+]_{ext}$, albeit with differing pharmacological profiles and no K^+ efflux was found at high $[K^+]_{ext}$. When external NH_4^+ was withdrawn, significant stimulations in K^+ influx were observed (176% increase compared with control), coincident with a thermodynamic shift from active to passive conditions that permitted channel-mediated K^+ influx even at low $[K^+]_{ext}$. This ammonium-withdrawal effect, termed AWE, was also observed in wild-type and mutant lines of *Arabidopsis* (*athak5*, *atakt1* and *athak5 atakt1*). AWE was suppressed by high (1–5 mM) levels of external calcium (Ca^{2+}) at low and intermediate, but not at high, $[K^+]_{ext}$, which was attributed to Ca^{2+}-sensitivity of AKT1. The effect was additionally enhanced by the presence of Cl^- and NO_3^- at high $[K^+]_{ext}$, conditions under which we observed some of the highest trans-plasma-membrane K^+ fluxes ever reported [~36 μmolg (root fresh weight)$^{-1}$h^{-1}]. Furthermore, AWE was sustainable (and at times indeed increasing) over a 24 h period, at low and intermediate $[K^+]_{ext}$ and persisted for up to ~8 h at high $[K^+]_{ext}$. This resulted in significant total-tissue K^+ accrual at all $[K^+]_{ext}$ over 24 h. These results raise a suite of interesting questions about the nature of channel-mediated K^+ influx in intact plants. Although we showed that K^+ channels cannot "do it all," as suggested by early thermodynamic consid-

*Correspondence to: Herbert J. Kronzucker; Email: herbertk@utsc.utoronto.ca
Submitted: 04/19/13; Revised: 04/24/13; Accepted: 04/24/13
Citation: Coskun D, Kronzucker HJ. Complexity of potassium acquisition: How much flows through channels? Plant Signal Behav 2013; 8: e24799; http://dx.doi.org/10.4161/psb.24799

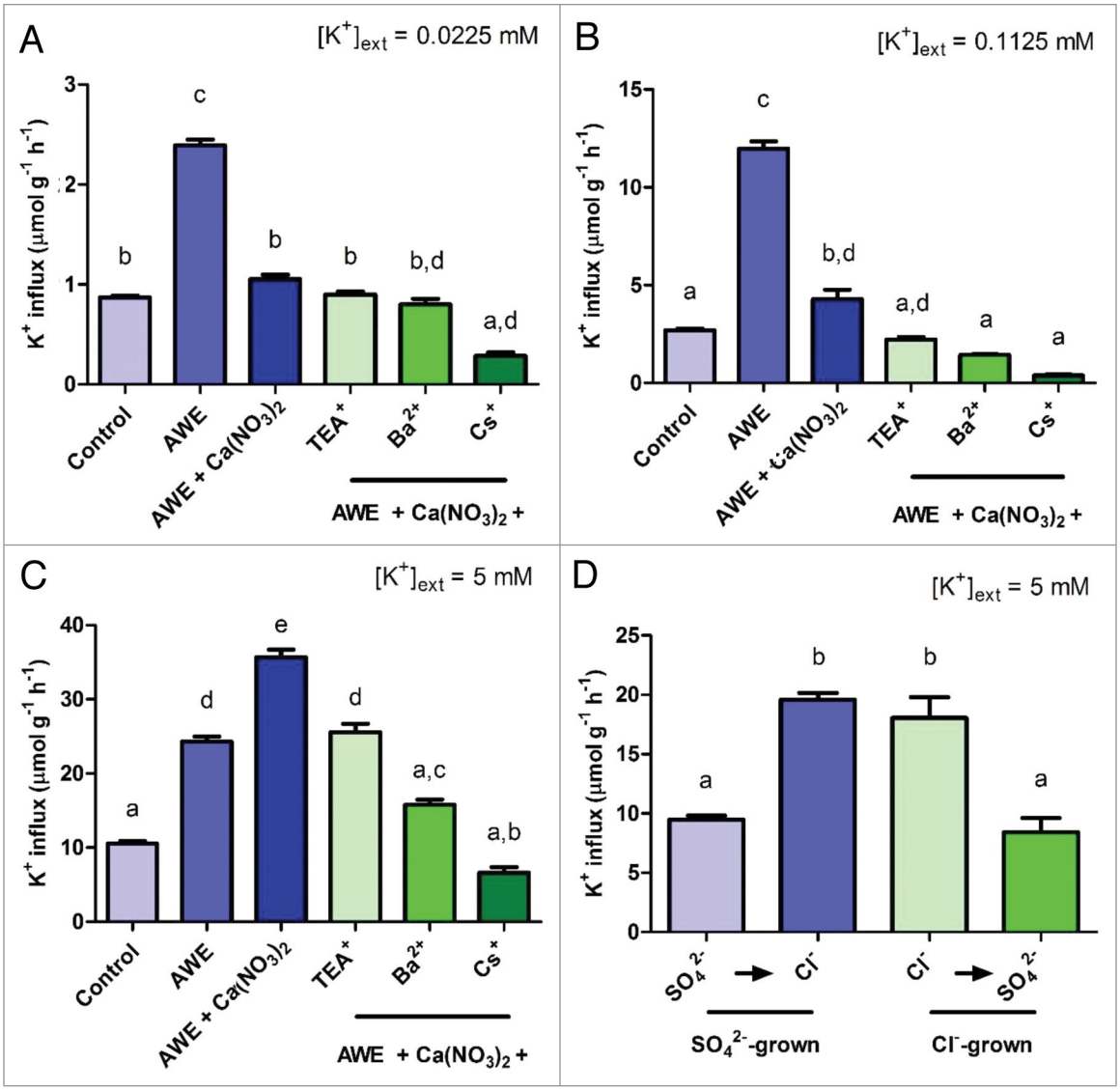

Figure 1. K^+ influx into intact 7-d old barley (*Hordeum vulgare* L.) seedlings. (**A–C**) Steady-state (control) flux in plants grown in a full nutrient medium (0.25-strength Johnson's solution)[13] with 10 mM NH_4^+ and either 0.0225 (**A**), 0.1125 (**B**) or 5 mM (**C**) K^+ (as sulfate salts) and the effect of ammonium-withdrawal (AWE) ± $Ca(NO_3)_2$ (5 mM) and AWE + $Ca(NO_3)_2$ ± TEA^+ (10 mM), Ba^{2+} (5 mM), or Cs^+ (10 mM) (as chloride salts). All treatments involved 5 min incubation prior to $^{42}K^+$ uptake (5 min protocol).[13] (**D**) Influx into plants grown in a full nutrient medium (as above) with 5 mM K^+ and 10 mM NH_4^+ (both as either SO_4^{2-} or Cl^- salts) and the effect of switching (K^+ and NH_4^+) counter-ions (10 min incubation prior to uptake, as above). Influx measured on a per g (root fresh weight) basis. Error bars indicate ± SEM of minimum four replicates. Letters in each panel denote significantly different means ($p < 0.05$, one-way ANOVA with Tukey post-hoc test).

erations,[11] our work sheds light on the wide-ranging and complex operation of K^+ channels in planta.

Here, we expand on our previous work by further characterizing channel-mediated K^+ uptake in roots of barley. **Figure 1** illustrates the varying sensitivity of AWE to $Ca(NO_3)_2$, which is clearly a function of $[K^+]_{ext}$: Ca^{2+}-induced suppressions are seen at low and intermediate $[K^+]_{ext}$ (**Fig. 1A and B**), while a NO_3^--induced stimulation is seen at high $[K^+]_{ext}$ (**Fig. 1C**). Interestingly, under all AWE + $Ca(NO_3)_2$ combinations, we observed effective suppression by the K^+-channel inhibitors[14] tetraethyl ammonium (TEA^+), barium (Ba^{2+}) and cesium (Cs^+) (**Fig. 1A–C**). Thus, although Ca^{2+} can block K^+ channels (specifically AtAKT1)[13] under some AWE conditions, it appears that K^+ channels continue to operate under such suppressed conditions, resulting in a higher flux compared with control (NH_4^+-background) conditions (**Fig. 1A and B**). This is consistent with previous thermodynamic analyses[13] that showed AWE + $Ca(NO_3)_2$ hyperpolarizes root plasma-membrane potentials away from the equilibrium potential for K^+ (E_{K^+}) at low and intermediate $[K^+]_{ext}$. At high $[K^+]_{ext}$, it is evident that NO_3^--induced stimulations in K^+ influx are also linked to K^+ channels (**Fig. 1C**), illustrating their complexity of function and regulation (also see below). Interestingly, these elevations in K^+ influx compared with control (albeit minor at low and intermediate $[K^+]_{ext}$) are sufficient to noticeably increase tissue-K^+ content over 24 h at all three $[K^+]_{ext}$ (data not shown), similar to what AWE entails on its own.[13]

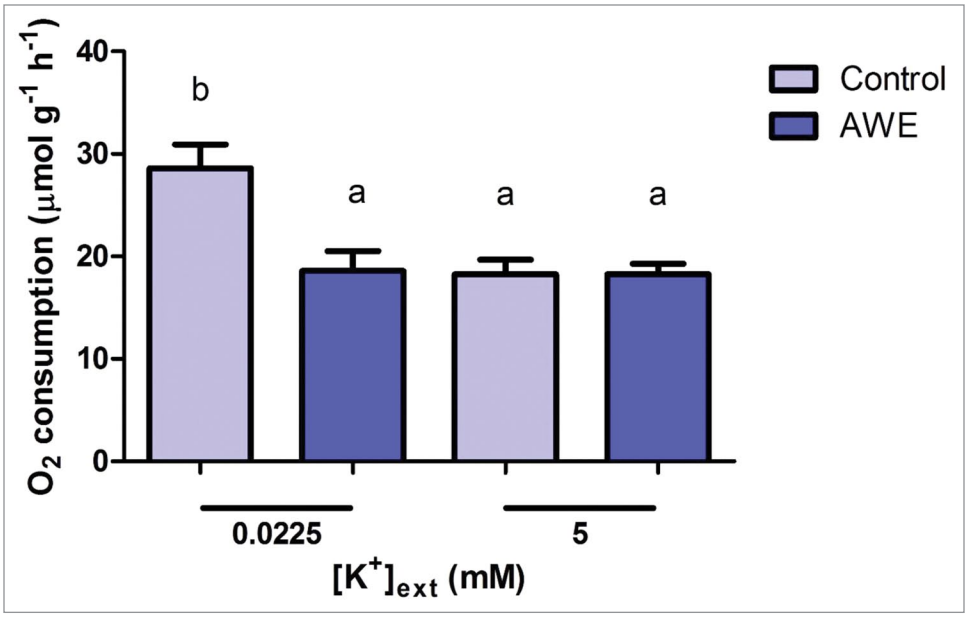

Figure 2. Steady-state (control) root O_2 consumption, as measured using a Hansatech oxygen electrode and Oxygraph control system (Hansatech Instruments), in intact barley (*Hordeum vulgare* L.) seedlings grown in a full nutrient medium (**Fig. 1**) at 0.0225 or 5 mM K^+ and 10 mM NH_4^+ (both as sulfate salts) and the effect of sudden (5 min treatment) NH_4^+ withdrawal (AWE). O_2 flux measured on a per g (root fresh weight) basis. Error bars indicate ± SEM of 4 replicates. Letters denote significantly different means ($p < 0.05$, one-way ANOVA with Tukey post-hoc test).

Expanding on the anion effects observed at high $[K^+]_{ext}$, we also found that switching the counterions for K^+ and NH_4^+ from sulfate (SO_4^{2-}) to Cl^- (a 10 min treatment was used in SO_4^{2-} grown plants) approximately doubles K^+ influx (from ~10 to 20 $\mu molg^{-1}h^{-1}$) and the reverse scenario (switching from Cl^- to SO_4^{2-}, in Cl^--grown plants), reduces influx by half (from ~20 to 10 $\mu molg^{-1}h^{-1}$, **Fig. 1D**). This suggests that anion effects related to K^+-channel functioning are highly effective in both the short (10 min) and long (steady-state) term. These findings are consistent with early reports[15,16] demonstrating higher low-affinity K^+ influx in the presence of Cl^- over SO_4^{2-}, possibly as a result of a coupling of the more rapidly absorbed anion (Cl^-) with K^+ influx. Surprisingly, there has been little, if any, advance in understanding this phenomenon. It does, however, raise interesting questions about the mechanism and regulation of channel-mediated K^+ influx. For one, when one considers that the fluxes of all anions tested (Cl^-, SO_4^{2-} and NO_3^-) are coupled to H^+ influx and are electrogenic (net positive),[17-19] anion fluxes (particularly those of NO_3^- and Cl^-, which show high rates of uptake)[20] should theoretically decrease the gradient for K^+ uptake, i.e. working against what is observed for rates of influx (**Fig. 1C and D**; refs. 15 and 16). Moreover, to our knowledge, there are no demonstrations of possible allosteric modulations of Shaker-type K^+ channels by anions in the plant literature;[7,21] by contrast, some evidence exists in the animal literature[22] that such allosteric modulation may occur. This warrants further investigation. Lastly, the reasons why Cl^-/NO_3^--induced stimulations are only observed at high (low-affinity-range) $[K^+]_{ext}$ remain largely unknown. Reports on the allosteric modulation of Shaker-type K^+ channels by $[K^+]_{ext}$ appear exclusive to outward rectifiers,[23] and studies on the post-translational modification (e.g., phosphorylation/dephosphorylation networks) of inward rectifiers are restricted to low-$[K^+]_{ext}$ sensing.[24-26] Thus, it is clear that as yet insufficiently understood mechanisms exist regarding the regulation of K^+ channels by anions, a phenomenon that can have profound effects on rates of K^+ acquisition.

We also observed that NH_4^+ withdrawal (for 5 min) results in significant reductions in root respiration at low $[K^+]_{ext}$ (from ~30 to 20 $\mu molg^{-1}h^{-1}$, as measured by oxygen $[O_2]$ consumption; **Fig. 2**). Based on earlier suggestions, this may be attributable to a reduction in futile NH_4^+ cycling at root-cell plasma membranes, an energetically demanding scenario linked to NH_4^+ toxicity in barley,[27] or other, more generic respiratory stress responses when NH_4^+ levels are high. Since futile cycling of NH_4^+ can be reduced by high $[K^+]_{ext}$,[28] as evident in the significantly lower steady-state root respiration at high $[K^+]_{ext}$ (**Fig. 2**), this may explain the lack of any further respiratory drop upon NH_4^+ withdrawal. Given the substantial increases in K^+ influx (from ~10 to 25 $\mu molg^{-1}h^{-1}$) (**Fig. 1C**), it is interesting that no change in root respiration was observed, in keeping with the contention that the energy cost per K^+ transported is significantly lower for channels than for high-affinity transporters,[29] i.e., plants already engaging channels may show no further significant energetic requirement upon NH_4^+ withdrawal even when K^+ influx is greatly enhanced. Thus, AWE carries no major cost to the plant, while a significant benefit in terms of net K^+ accumulation in tissue is seen (see above). Indeed, at low $[K^+]_{ext}$, barley expends significantly less energy than prior to NH_4^+ withdrawal, while accruing large quantities of K^+. This highlights an important feature of engaging channels episodically in the acquisition of potassium that may be of

importance under fluctuating nutrient conditions,[30] especially in NH_4^+-dominated systems that normally suppress K^+ uptake,[31-33] which, integrated over an extended time frame, may result in significant growth benefits.[34]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank R. Pasuta and M. Butler at the McMaster Nuclear Reactor for ^{42}K provision. This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canada Research Chair (CRC) program and the Canadian Foundation for Innovation (CFI).

References

1. Hirsch RE, Lewis BD, Spalding EP, Sussman MR. A role for the AKT1 potassium channel in plant nutrition. Science 1998; 280:918-21; PMID:9572739; http://dx.doi.org/10.1126/science.280.5365.918
2. Chérel I. Regulation of K^+ channel activities in plants: from physiological to molecular aspects. J Exp Bot 2004; 55:337-51; PMID:14739260; http://dx.doi.org/10.1093/jxb/erh028
3. Gierth M, Mäser P, Schroeder JI. The potassium transporter AtHAK5 functions in K(+) deprivation-induced high-affinity K(+) uptake and AKT1 K(+) channel contribution to K(+) uptake kinetics in Arabidopsis roots. Plant Physiol 2005; 137:1105-14; PMID:15734909; http://dx.doi.org/10.1104/pp.104.057216
4. Dennison KL, Robertson WR, Lewis BD, Hirsch RE, Sussman MR, Spalding EP. Functions of AKT1 and AKT2 potassium channels determined by studies of single and double mutants of Arabidopsis. Plant Physiol 2001; 127:1012-9; PMID:11706182; http://dx.doi.org/10.1104/pp.010193
5. Gierth M, Mäser P. Potassium transporters in plants--involvement in K^+ acquisition, redistribution and homeostasis. FEBS Lett 2007; 581:2348-56; PMID:17397836; http://dx.doi.org/10.1016/j.febslet.2007.03.035
6. Alemán F, Nieves-Cordones M, Martínez V, Rubio F. Root K(+) acquisition in plants: the *Arabidopsis thaliana* model. Plant Cell Physiol 2011; 52:1603-12; PMID:21771865; http://dx.doi.org/10.1093/pcp/pcr096
7. Dreyer I, Uozumi N. Potassium channels in plant cells. FEBS J 2011; 278:4293-303; PMID:21955642; http://dx.doi.org/10.1111/j.1742-4658.2011.08371.x
8. Mäser P, Thomine S, Schroeder JI, Ward JM, Hirschi K, Sze H, et al. Phylogenetic relationships within cation transporter families of Arabidopsis. Plant Physiol 2001; 126:1646-67; PMID:11500563; http://dx.doi.org/10.1104/pp.126.4.1646
9. Spalding EP, Hirsch RE, Lewis DR, Qi Z, Sussman MR, Lewis BD. Potassium uptake supporting plant growth in the absence of AKT1 channel activity: Inhibition by ammonium and stimulation by sodium. J Gen Physiol 1999; 113:909-18; PMID:10352038; http://dx.doi.org/10.1085/jgp.113.6.909
10. Qi Z, Hampton CR, Shin R, Barkla BJ, White PJ, Schachtman DP. The high affinity K^+ transporter AtHAK5 plays a physiological role in planta at very low K^+ concentrations and provides a caesium uptake pathway in Arabidopsis. J Exp Bot 2008; 59:595-607; PMID:18281719; http://dx.doi.org/10.1093/jxb/erm330
11. Kochian LV, Lucas WJ. Can K^+ channels do it all? Plant Cell 1993; 5:720-1; PMID:12271082
12. Maathuis FJM, Sanders D. Mechanism of high-affinity potassium uptake in roots of *Arabidopsis thaliana*. Proc Natl Acad Sci USA 1994; 91:9272-6; PMID:7937754; http://dx.doi.org/10.1073/pnas.91.20.9272
13. Coskun D, Britto DT, Li M, Oh S, Kronzucker HJ. Capacity and plasticity of potassium channels and high-affinity transporters in roots of barley and Arabidopsis. Plant Physiol 2013; In press; PMID:23553635; http://dx.doi.org/10.1104/pp.113.215913
14. Bertl A, Reid JD, Sentenac H, Slayman CL. Functional comparison of plant inward-rectifier channels expressed in yeast. J Exp Bot 1997; 48:405-13; PMID:21245219; http://dx.doi.org/10.1093/jxb/48.Special_Issue.405
15. Epstein E, Rains DW, Elzam OE. Resolution of dual mechanisms of potassium absorption by barley roots. Proc Natl Acad Sci USA 1963; 49:684-92; PMID:16591089; http://dx.doi.org/10.1073/pnas.49.5.684
16. Kochian LV, Xin-Zhi J, Lucas WJ. Potassium transportin corn roots .4. Characterization of the linear component. Plant Physiol 1985; 79:771-6; PMID:16664490; http://dx.doi.org/10.1104/pp.79.3.771
17. Sanders D. The mechanism of Cl^- transport at the plasma membrane of *Chara corallina*.1. Co transport with H^+. J Membr Biol 1980; 53:129-41; http://dx.doi.org/10.1007/BF01870581
18. Lass B, Ullrich-Eberius CI. Evidence for proton/sulfate cotransport and its kinetics in *Lemna gibba* G1. Planta 1984; 161:53-60; http://dx.doi.org/10.1007/BF00951460
19. Ullrich WR, Novacky A. Nitrate-dependent membrane potential changes and their induction in *Lemna gibba* G1. Plant Sci Lett 1981; 22:211-7; http://dx.doi.org/10.1016/0304-4211(81)90233-9
20. Lee RB. Selectivity and kinetics of ion uptake by barley plants following nutrient deficiency. Ann Bot (Lond) 1982; 50:429-49
21. Gambale F, Uozumi N. Properties of *shaker*-type potassium channels in higher plants. J Membr Biol 2006; 210:1-19; PMID:16794778; http://dx.doi.org/10.1007/s00232-006-0856-x
22. Adams DJ, Oxford GS. Interaction of internal anions with potassium channels of the squid giant axon. J Gen Physiol 1983; 82:429-48; PMID:6315855; http://dx.doi.org/10.1085/jgp.82.4.429
23. Johansson I, Wulfetange K, Porée F, Michard E, Gajdanowicz P, Lacombe B, et al. External K^+ modulates the activity of the Arabidopsis potassium channel SKOR via an unusual mechanism. Plant J 2006; 46:269-81; PMID:16623889; http://dx.doi.org/10.1111/j.1365-313X.2006.02690.x
24. Li L, Kim BG, Cheong YH, Pandey GK, Luan SA. A Ca(2)+ signaling pathway regulates a K(+) channel for low-K response in Arabidopsis. Proc Natl Acad Sci USA 2006; 103:12625-30; PMID:16895985; http://dx.doi.org/10.1073/pnas.0605129103
25. Lee SC, Lan WZ, Kim BG, Li L, Cheong YH, Pandey GK, et al. A protein phosphorylation/dephosphorylation network regulates a plant potassium channel. Proc Natl Acad Sci USA 2007; 104:15959-64; PMID:17898163; http://dx.doi.org/10.1073/pnas.0707912104
26. Xu J, Li HD, Chen LQ, Wang Y, Liu LL, He L, et al. A protein kinase, interacting with two calcineurin B-like proteins, regulates K^+ transporter AKT1 in Arabidopsis. Cell 2006; 125:1347-60; PMID:16814720; http://dx.doi.org/10.1016/j.cell.2006.06.011
27. Britto DT, Siddiqi MY, Glass AD, Kronzucker HJ. Futile transmembrane $NH_4(+)$ cycling: a cellular hypothesis to explain ammonium toxicity in plants. Proc Natl Acad Sci USA 2001; 98:4255-8; PMID:11274450; http://dx.doi.org/10.1073/pnas.061034698
28. Szczerba MW, Britto DT, Balkos KD, Kronzucker HJ. Alleviation of rapid, futile ammonium cycling at the plasma membrane by potassium reveals K^+-sensitive and -insensitive components of NH_4^+ transport. J Exp Bot 2008; 59:303-13; PMID:18203690; http://dx.doi.org/10.1093/jxb/erm309
29. Glass ADM. Plant nutrition. An introduction to current concepts. Boston, MA: Jones and Bartlett Publishers, Inc, 1989
30. Hodge A. The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytol 2004; 162:9-24; http://dx.doi.org/10.1111/j.1469-8137.2004.01015.x
31. Sasakawa H, Yamamoto Y. Comparison of the uptake of nitrate and ammonium by rice seedlings: influences of light, temperature, oxygen concentration, exogenous sucrose, and metabolic inhibitors. Plant Physiol 1978; 62:665-9; PMID:16660579; http://dx.doi.org/10.1104/pp.62.4.665
32. Kirk GJD, Kronzucker HJ. The potential for nitrification and nitrate uptake in the rhizosphere of wetland plants: a modelling study. Ann Bot 2005; 96:639-46; PMID:16024557; http://dx.doi.org/10.1093/aob/mci216
33. Kronzucker HJ, Siddiqi MY, Glass ADM. Conifer root discrimination against soil nitrate and the ecology of forest succession. Nature 1997; 385:59-61; http://dx.doi.org/10.1038/385059a0
34. Balkos KD, Britto DT, Kronzucker HJ. Optimization of ammonium acquisition and metabolism by potassium in rice (*Oryza sativa* L. cv. IR-72). Plant Cell Environ 2010; 33:23-34; PMID:19781010