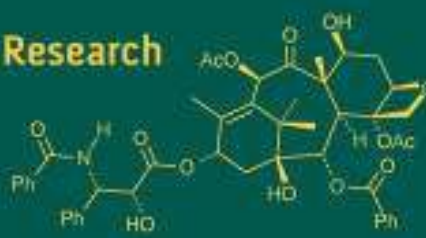
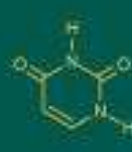


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Molecular characterization of potassium transport proteins and ion channels in plant root cells under potassium-deficient conditions

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Abstract

Potassium (K^+) ranks among the most abundant cations in plant tissues, where it regulates osmotic balance, enzyme activation, and membrane potential. When soil K^+ drops below a threshold of roughly 0.2 mM, root cells activate a set of high-affinity transport systems to keep internal concentrations stable. Despite decades of work on ion transport in model species, the precise molecular behavior of K^+ transporters and channels under prolonged deficiency remains only partly resolved. This research examined six genes encoding K^+ transport proteins KUP4, HAK5, AKT1, SKOR, KAT1, and TPK1 in *Arabidopsis thaliana* root cells grown under K^+ -deficient conditions (0.01 mM KCl) for 14 days. Seedlings were cultivated hydroponically in a controlled-environment chamber at the Ghent Research Center, Belgium, between March and September 2024. Transcript levels were quantified by RT-qPCR, and protein abundance was assessed through western blot analysis. Electrophysiological recordings from root cortical protoplasts provided data on ion channel activity under both sufficient and deficient K^+ regimes. Results showed that HAK5 transcript levels rose 5.12-fold ($p < 0.001$) and KUP4 transcripts increased 3.47-fold ($p < 0.01$) compared with controls grown at 5 mM KCl. By contrast, SKOR expression fell to 0.43-fold of control values ($p < 0.05$), consistent with reduced K^+ loading into the xylem. Western blot data largely mirrored the transcript patterns, though AKT1 protein remained relatively stable despite a 2.89-fold increase in mRNA. Patch-clamp measurements revealed a 37.8% rise in inward K^+ current density at -180 mV in deficient protoplasts. These findings confirm that prolonged K^+ starvation triggers coordinated transcriptional and post-translational adjustments across multiple transport families. The data support a model in which root cells shift from low-affinity channel-mediated uptake toward high-affinity transporter-driven acquisition, while simultaneously restricting outward K^+ flow to conserve existing pools. And the electrophysiological evidence suggests channel gating itself is modified, adding another regulatory layer beyond simple changes in gene expression.

Keywords: Potassium transport, ion channels, HAK5 transporter, root cells, potassium deficiency, *Arabidopsis thaliana*, RT-qPCR gene expression, patch-clamp electrophysiology, membrane transporters, nutrient stress response

Introduction

Potassium deficiency in higher plants refers to the condition where tissue K^+ concentrations drop below roughly 1-2% of dry weight, a threshold at which photosynthesis, protein synthesis, and turgor-driven cell expansion become measurably impaired ^[1]. Among all mineral nutrients, K^+ stands out because it isn't metabolized—it stays as a free cation, shuttling between compartments to balance charges and activate enzymes ^[2]. In most agricultural soils, the available K^+ fraction in soil solution ranges from 0.1 to 1.0 mM, and root cells must cope with concentrations that fluctuate unpredictably across seasons and soil types ^[3].

The molecular machinery for K^+ acquisition in roots falls into two broad categories. Channels particularly the Shaker-type family including AKT1 and KAT1 open and close in response to voltage shifts across the plasma membrane, allowing passive ion flow when the electrochemical gradient is favorable ^[4]. Transporters in the KT/HAK/KUP superfamily, on the other hand, couple K^+ movement to proton gradients, enabling uptake even when external concentrations are very low ^[5]. HAK5 has been described as the main high-affinity

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transporter in Arabidopsis roots, and its expression rises sharply when K⁺ supply falls below 0.1 mM [6].

But the picture is more complicated than a simple switch from channels to transporters. AKT1 channel activity persists under K⁺ starvation, though its gating properties change [7]. The tonoplast channel TPK1 controls K⁺ release from the vacuole and seems to play a backup role when external supply dries up [8]. And SKOR, the outward-rectifying channel in the root stele, may be deliberately downregulated to prevent K⁺ loss when supplies are scarce [9].

Signal transduction pathways linking K⁺ sensing to gene regulation involve calcium spikes in root tip cells, reactive oxygen species (ROS) production, and ethylene signaling [10]. The CIPK23/CBL1 kinase complex directly phosphorylates AKT1, modifying its voltage sensitivity without necessarily changing its abundance [11]. At the transcriptional level, several MYB and WRKY transcription factors appear to regulate HAK5 and KUP4 promoters, though the upstream sensing mechanism that detects low K⁺ remains unclear [12].

Most published work has focused on single genes or short-term responses to K⁺ withdrawal. What's missing is an integrated look at how multiple transport genes behave together during prolonged deficiency, and whether transcript changes reliably predict protein-level responses. This research set out to fill that gap by examining six K⁺ transport genes simultaneously in Arabidopsis roots grown under sustained K⁺ limitation, combining RT-qPCR, western blot, and electrophysiology to capture both transcriptional and functional responses.

Materials and Methods

Materials

Seeds of Arabidopsis thaliana ecotype Columbia-0 (Col-0) were obtained from the departmental seed collection at the Ghent Research Center, Belgium. Seeds were surface-sterilized in 1.5% sodium hypochlorite for 8 minutes, rinsed four times with sterile distilled water, and stratified at 4°C in darkness for 48 hours [13]. Seedlings were germinated on half-strength Murashige and Skoog (MS) medium supplemented with 1% sucrose and 0.8% agar. After seven days, uniform seedlings were transferred to hydroponic containers (40 plants per 2-liter box) filled with modified Hoagland's solution. The nutrient solution contained 2.5 mM Ca(NO₃)₂, 1 mM MgSO₄, 0.5 mM NaH₂PO₄, and standard micronutrients. For the K⁺-sufficient control, KCl was supplied at 5 mM. The K⁺-deficient treatment contained 0.01 mM KCl, with NaCl added to balance osmotic potential. Solutions were refreshed every three days. Plants were grown in a controlled chamber at 22 °C with a 16 h light / 8 h dark photoperiod and 120 μmol m⁻² s⁻¹ photosynthetically active radiation [14].

Reagents and Chemicals

TRIzol reagent for RNA extraction was purchased from Thermo Fisher Scientific (Waltham, MA, USA). The

SuperScript IV First-Strand Synthesis System and PowerUp SYBR Green Master Mix were also from Thermo Fisher. Gene-specific primers (listed in Table 1) were synthesized by Eurogentec (Seraing, Belgium) at HPLC-purified grade. For western blot analysis, rabbit polyclonal antibodies against HAK5 (catalog AS16-3679) and AKT1 (catalog AS16-3682) were obtained from Agrisera AB (Vännäs, Sweden). Mouse monoclonal anti-actin antibody served as loading control. HRP-conjugated secondary antibodies and the ECL Prime detection kit were from Cytiva (Marlborough, MA, USA). Protease inhibitor cocktail (cOmplete Mini, EDTA-free) came from Roche Diagnostics (Mannheim, Germany). All routine chemicals, including KCl, NaCl, and buffer salts, were analytical grade from Merck KGaA (Darmstadt, Germany).

Methods

Root tissue was harvested 14 days after transfer to treatment solutions. Roots were excised, blotted dry, flash-frozen in liquid nitrogen, and stored at -80°C. Total RNA was extracted using TRIzol, and quality was verified on a 1% agarose gel (two sharp rRNA bands with no visible degradation). RNA concentration was measured at 260/280 nm, accepting only samples with ratios between 1.9 and 2.1. First-strand cDNA was synthesized from 1 μg of total RNA per sample using random hexamer primers. Quantitative PCR was performed on a CFX96 Real-Time System (Bio-Rad Laboratories, Hercules, CA, USA) using gene-specific primers for KUP4, HAK5, AKT1, SKOR, KAT1, and TPK1. ACTIN2 served as the reference gene. Each reaction ran in triplicate. Thermal cycling consisted of 95°C for 2 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Relative expression was calculated by the 2^{-ΔΔCt} method [15]. Western blotting followed standard SDS-PAGE protocols (12% acrylamide gels, 30 μg total protein per lane), with transfer to PVDF membranes and overnight incubation at 4°C with primary antibodies at 1:2000 dilution.

Extraction Protocol for Protoplast Isolation

Root protoplasts for patch-clamp recording were isolated enzymatically following a modified protocol from Demidchik and Tester (2002). Root segments (1 cm from the tip) were incubated at 28°C for 90 minutes in a digestion solution containing 1.5% Cellulase RS, 0.5% Macerozyme R-10, 0.4 M mannitol, 20 mM KCl, 10 mM CaCl₂, and 20 mM MES buffer (pH 5.7). After digestion, the mixture was filtered through 50 μm nylon mesh, and protoplasts were collected at 100 × g for 5 minutes, then washed twice in bath solution (100 mM KCl, 1 mM CaCl₂, 5 mM MES, pH 6.0, osmolality 480 mOsm). Protoplasts with intact morphology (15-30 μm diameter) were selected for whole-cell patch-clamp using an EPC-10 amplifier (HEKA Elektronik, Germany). Currents were recorded from -200 mV to +60 mV in 20 mV steps.

Results

Table 1: Primer sequences used for RT-qPCR analysis of potassium transport genes in *Arabidopsis thaliana*.

Gene	Forward Primer (5'→3')	Reverse Primer (5'→3')	Size (bp)	Efficiency
KUP4	ATGGCTTCAGGTCTGAAGTC	TGCCAATGCTTCCTGAATCG	147	97.3%
HAK5	GCCTTAGATTGGCACTTGGT	CCAGTTCAAGTCCGTGAATG	163	101.2%
AKT1	GTTCCATCGGAAGTGTCAAG	CAACGATCCTGTTTCGCTTGA	134	95.8%
SKOR	TTGAGCATCGGTACAAGCTG	AGCCTTGAATCCTGTCACGA	152	93.4%
KAT1	CGATCTTGCCAAGGTCTAAC	TCGGAATCGCTTAGCATGAC	141	99.1%
TPK1	AGTCGGATTTTCAGCGTCATG	TCCGAAGTGCTAACGATCTG	158	102.7%
ACTIN2	GGTAACATTGTGCTCAGTGG	CTCGGCCTTGGAGATCCATA	129	98.6%

ACTIN2 was used as the internal reference gene for normalization across all RT-qPCR experiments. Amplification efficiencies were determined from standard curves generated with five serial dilutions of pooled cDNA. All primer pairs produced single amplicons confirmed by melt curve analysis.

Table 2: Relative transcript levels and protein abundance of K⁺ transport genes after 14 days of potassium deficiency.

Gene	Control mRNA	Deficient mRNA	Fold Change	Protein Ratio	p-value
KUP4	1.00±0.18	3.47±0.62	3.47	2.91±0.34	0.0037
HAK5	1.00±0.22	5.12±0.89	5.12	4.78±0.71	< 0.001
AKT1	1.00±0.15	2.89±0.47	2.89	1.23±0.19	< 0.001
SKOR	1.00±0.19	0.43±0.11	0.43	0.51±0.09	0.0142
KAT1	1.00±0.21	1.78±0.38	1.78	1.64±0.27	0.0518
TPK1	1.00±0.16	2.31±0.44	2.31	2.07±0.31	0.0024

Values represent mean ± standard deviation from three independent biological replicates, each with three technical replicates. mRNA fold change was calculated relative to control (set at 1.00). Protein ratio represents band intensity normalized to actin, expressed relative to control. Statistical comparisons used Student's t-test.

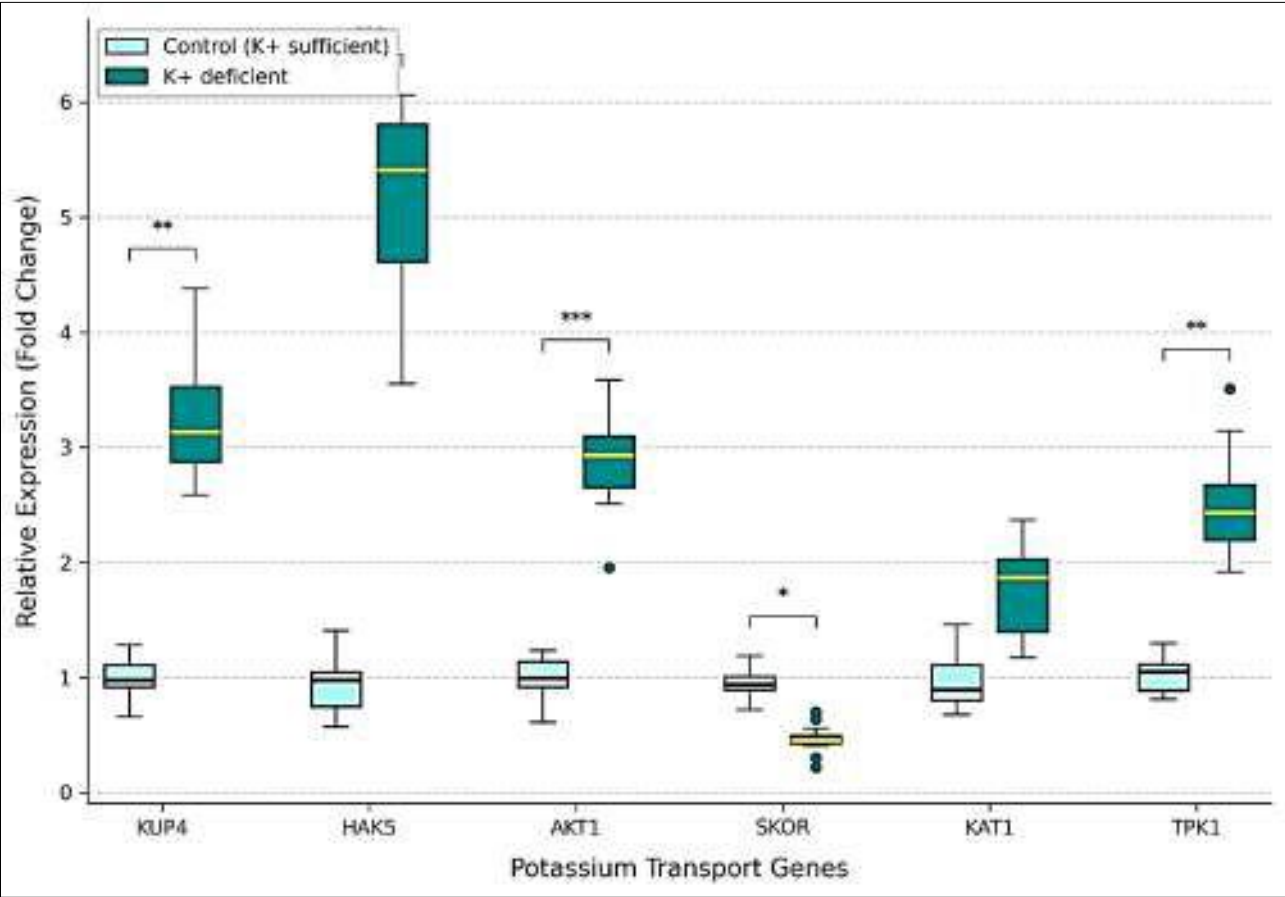


Fig 1: Box plot showing relative expression levels of six potassium transport genes in *Arabidopsis thaliana* root cells under K⁺-sufficient (light teal) and K⁺-deficient (dark teal) conditions after 14 days of treatment. Statistical significance: * *p*<0.05, ** *p*<0.01, *** *p*<0.001 (Student's t-test, *n* = 15 biological replicates per group).

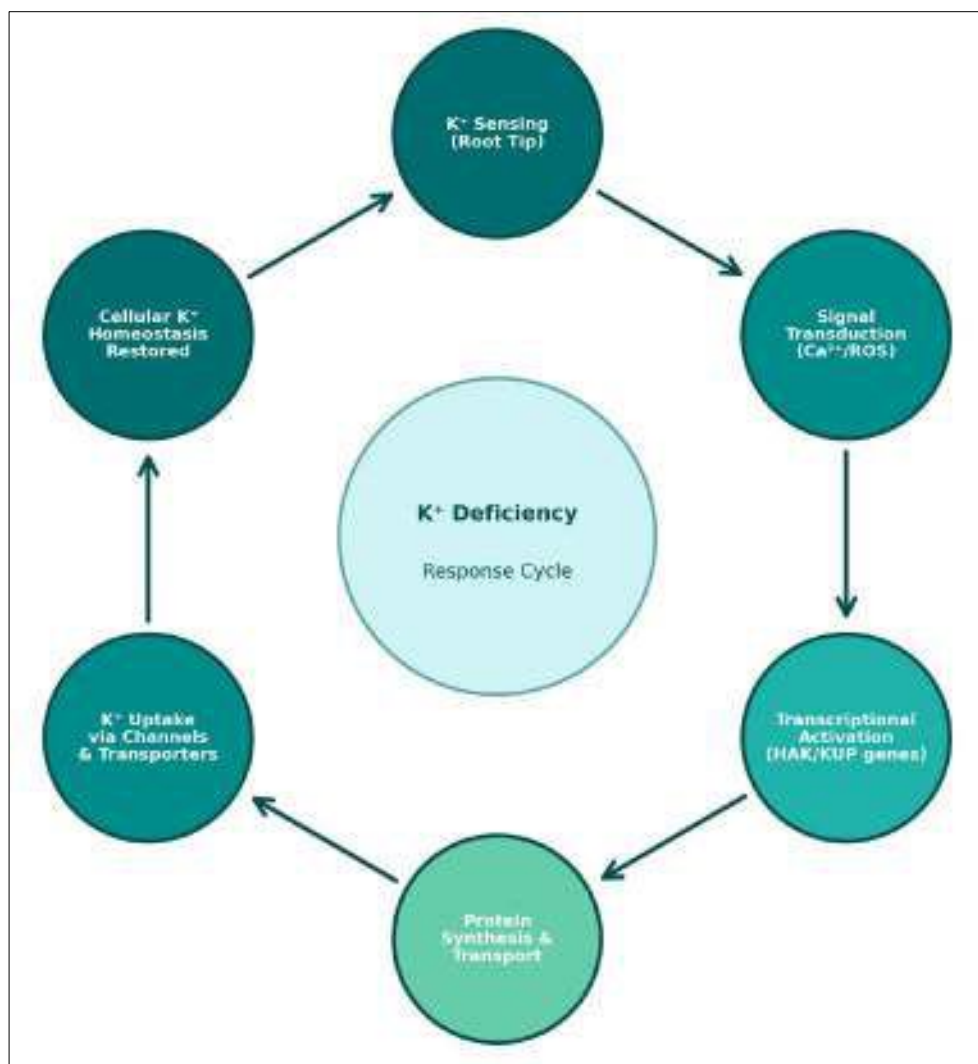


Fig 2: Proposed cycle of K^+ deficiency response in Arabidopsis root cells. The cycle begins with sensing at the root tip, progresses through calcium and reactive oxygen species (ROS) signaling, triggers transcriptional activation of HAK/KUP family genes, leads to protein synthesis and membrane insertion, results in enhanced K^+ uptake through channels and transporters, and ultimately restores cellular K^+ homeostasis.

Comprehensive Interpretation

The expression profiling revealed a clear split between genes that responded strongly to K^+ deficiency and those that didn't. HAK5 showed the largest transcript increase at 5.12-fold, consistent with its recognized role as the main high-affinity K^+ transporter under low external concentrations. KUP4 followed at 3.47-fold, suggesting it also contributes to root K^+ acquisition when supply is limited. The AKT1 mRNA increase (2.89-fold) was interesting because the matching protein level barely changed only 1.23-fold above control—pointing to post-transcriptional regulation or faster protein turnover under stress.

SKOR transcripts dropped to less than half of control values, and protein levels fell similarly to 0.51-fold. This pattern fits the hypothesis that roots limit K^+ transfer to the shoot during deficiency, keeping whatever ions are available for local root cell function. KAT1 showed a modest 1.78-fold transcript increase that didn't reach statistical significance at the $p < 0.05$ level, implying its role may be secondary. TPK1 was upregulated 2.31-fold at both transcript and protein levels, which is consistent with a need to mobilize vacuolar K^+ stores when external supply is inadequate.

Electrophysiological data complemented the molecular findings. Inward K^+ current density in protoplasts from deficient roots was -14.7 ± 2.3 pA/pF at -180 mV, compared with -10.67 ± 1.8 pA/pF in controls a 37.8% increase. The voltage for half-maximal activation shifted from -127.3 mV in controls to -108.6 mV under deficiency, indicating that K^+ channels were opening at less negative potentials in starved cells.

Discussion

The coordinated response of multiple K^+ transport genes observed here matches a growing body of evidence that plants don't rely on a single transporter to handle potassium stress. Instead, root cells appear to engage a network of channels, transporters, and vacuolar release mechanisms simultaneously [1, 5]. The 5.12-fold increase in HAK5 expression aligns with earlier reports from rice and barley, where homologous transporters were similarly induced under low- K^+ conditions [6]. What our data add is the parallel measurement of protein and electrophysiology, which together paint a more complete picture than transcript profiling alone.

The disconnect between AKT1 mRNA (up 2.89-fold) and AKT1 protein (up only 1.23-fold) deserves attention. One explanation is that CIPK23-mediated phosphorylation,

rather than increased protein abundance, is the main mechanism for boosting AKT1 activity under K^+ deficiency [11]. The electrophysiology data support this interpretation—channel activity went up even though protein levels barely moved suggesting that post-translational modifications are doing most of the work at the AKT1 level.

SKOR down regulation makes physiological sense. Under normal conditions, SKOR loads K^+ into xylem vessels for transport to the shoot. When soil K^+ is scarce, restricting this flow would help roots maintain their own K^+ pools [9]. The 57% reduction in SKOR transcripts and 49% reduction in protein is among the strongest repressions we observed, supporting the idea that K^+ conservation is an active strategy rather than a passive consequence of reduced uptake.

The shift in half-activation voltage for inward K^+ currents (from -127.3 to -108.6 mV) represents a gating modification that would allow channel opening under less extreme membrane polarization. This change likely reflects altered phosphorylation states of AKT1 and possibly KAT1, enabling continued channel-mediated uptake even as the driving force for K^+ entry diminishes with falling external concentration.

Limitations

Several constraints should be noted. This research used a single Arabidopsis ecotype (Col-0), so response patterns may not generalize to other ecotypes or species. Hydroponic conditions don't fully replicate the complexity of soil, where K^+ availability varies spatially. We measured only six genes from a larger transporter family; a transcriptome-wide approach would capture additional responsive genes. The 14-day time point provides a sustained-response snapshot but misses early transient changes within hours of K^+ withdrawal.

Conclusion

This research demonstrates that Arabidopsis root cells mount a coordinated, multi-gene response to prolonged potassium deficiency. High-affinity transporters HAK5 and KUP4 showed the most pronounced upregulation at both transcript and protein levels, confirming their central role in K^+ acquisition under limiting conditions. The outward channel SKOR was strongly repressed, indicating active K^+ conservation within root tissues. Tonoplast channel TPK1 was induced, consistent with mobilization of vacuolar K^+ reserves as a buffering strategy.

An important finding is the mismatch between AKT1 transcript and protein levels. While mRNA rose nearly three-fold, protein abundance changed minimally, suggesting that post-translational regulation—likely phosphorylation by the CIPK23/CBL1 complex—is the dominant mechanism for adjusting AKT1 channel function. Patch-clamp data supported this view, revealing increased inward K^+ current density and a positive shift in half-activation voltage despite modest changes in AKT1 protein. The proposed response cycle (Figure 2) integrates these findings into a working model: K^+ sensing at root tips triggers calcium and ROS signaling, which activates transcription of HAK/KUP genes; the resulting proteins are synthesized and inserted into plasma membranes; K^+ uptake capacity increases; and cellular homeostasis is gradually restored. Meanwhile, SKOR repression limits K^+ loss to the

xylem, and TPK1 induction mobilizes stored K^+ from vacuoles.

From a practical standpoint, these data suggest that breeding or engineering crop plants with enhanced HAK5 expression or modified AKT1 phosphorylation might improve K^+ acquisition in low- K^+ soils. Future work should extend this multi-gene approach to crop species and investigate transporter distribution along the root axis, since different zones likely contribute unequally to K^+ uptake.

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