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How high do ion fluxes go? A re-evaluation of the two-mechanism model of K^+ transport in plant roots

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ABSTRACT

Potassium (K^+) acquisition in roots is generally described by a two-mechanism model, consisting of a saturable, high-affinity transport system (HATS) operating via H^+/K^+ symport at low (<1 mM) external $[K^+]$ ($[K^+]_{ext}$), and a linear, low-affinity system (LATS) operating via ion channels at high (>1 mM) $[K^+]_{ext}$. Radiotracer measurements in the LATS range indicate that the linear rise in influx continues well beyond nutritionally relevant concentrations (>10 mM), suggesting K^+ transport may be pushed to extraordinary, and seemingly limitless, capacity. Here, we assess this rise, asking whether LATS measurements faithfully report transmembrane fluxes. Using $^{42}K^+$-isotope and electrophysiological methods in barley, we show that this flux is part of a K^+-transport cycle through the apoplast, and masks a genuine plasma-membrane influx that displays Michaelis–Menten kinetics. Rapid apoplastic cycling of K^+ is corroborated by an absence of transmembrane $^{42}K^+$ efflux above 1 mM, and by the efflux kinetics of PTS, an apoplastic tracer. A linear apoplastic influx, masking a saturating transmembrane influx, was also found in *Arabidopsis* mutants lacking the K^+ transporters AtHAK5 and AtAKT1. Our work significantly revises the model of K^+ transport by demonstrating a surprisingly modest upper limit for plasma-membrane influx, and offers insight into sodium transport under salt stress.

1. Introduction

The two-mechanism model of potassium (K^+) acquisition in roots of higher plants is a standard of transport physiology and molecular biology [1–5]. In this model (Fig. 1A), the high-affinity transport system (HATS) predominately functions at external K^+ concentrations ($[K^+]_{ext}$) below 1 mM, and is mediated by secondarily active K^+/H^+ symporters of the HAK/KUP/KT family, including AtHAK5 in Arabidopsis (*Arabidopsis thaliana*; [4]) and HvHAK1 in barley (*Hordeum vulgare*; [6]). By contrast, the low-affinity transport system (LATS), which functions at $[K^+]_{ext}$ above 1 mM, is less well understood, although it is widely accepted to be a thermodynamically passive process mediated by K^+-selective (Shaker-like) channels (*e.g.* AtAKT1 and HvAKT1 in Arabidopsis and barley, respectively; [3–5,7]). Studies using heterologous expression systems and knock-out mutants in Arabidopsis have furthermore shown that AtAKT1 can also operate under some high-affinity conditions [8–11]. Moreover, recent investigations in the Arabidopsis double-knock-out mutant, *athak5 atakt1*, have shown that there is an additional back-up system ("BUS") that can contribute to K^+ uptake in the LATS range [12–14]. Although genetically uncharacterized, it has been suggested that BUS operates via non-selective cation channels (NSCCs), which may be gated by cyclic nucleotides [13].

One commonly observed feature of the K^+ LATS is its linearly rising kinetic response to increasing $[K^+]_{ext}$, which contrasts sharply with the saturating pattern typical of the HATS [15–19] (see also Fig. 1). From the outset, it is worth noting that this model is based solely on unidirectional fluxes measured using radiotracer methodology, and although many aspects have been corroborated by other experimental methods, the linearity of the low-affinity flux is one aspect that has not. Any attempt to investigate this important concept must therefore also employ radiotracer techniques. Moreover, although many studies have reported on a linear LATS, it is important to note that some studies have reported low-affinity K^+ influxes that saturate [1,20,21]. It is interesting to note that such studies often employ longer absorption and desorption times (*e.g.* 10-30 min each), compared to many studies that report linear fluxes, which may result in closer approximations to net K^+ fluxes, rather than unidirectional uptake (see below).

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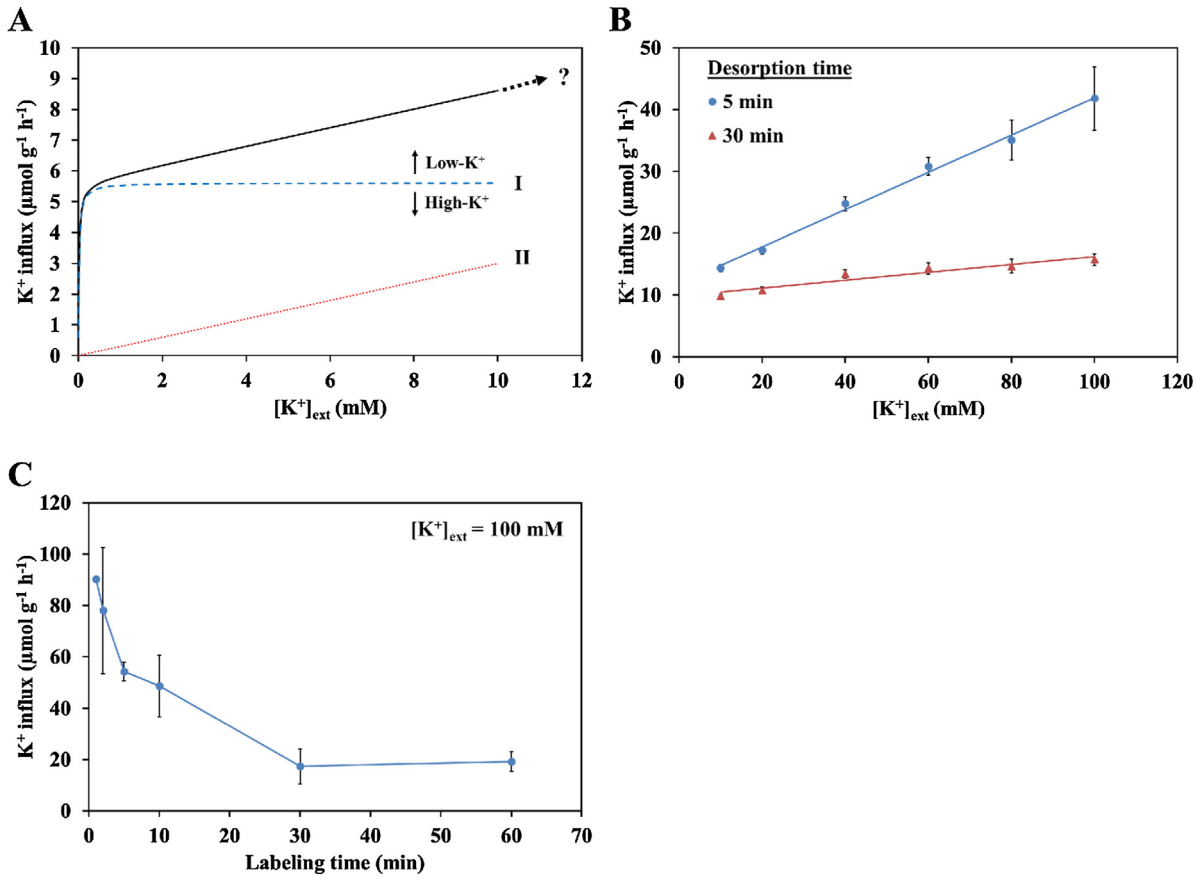

Fig. 1. The two-mechanism model of K^+ acquisition in roots of higher plants and its extension into the saline range. (A) The standing model of K^+ transport. Influx (solid black line), as a function of external $[K^+]$ ($[K^+]_{ext}$), is described as the sum of activities of two distinct transport systems: (I) the saturable high-affinity transport system (HATS; blue dashed line), which follows Michaelis–Menten kinetics and is regulated by internal K^+ status; and (II) the low-affinity transport system (LATS; red dotted line), which is linear. The HATS is primarily governed by HAK/KUP/KT transporters, whereas the LATS is governed by AKT1-channel complexes and unknown back-up system(s). Here we ask whether influx will continue to rise linearly beyond nutritionally relevant $[K^+]_{ext}$ (*i.e.* > 10 mM, and into the saline range). Redrawn from [3,63]. (B) K^+ influx into roots of intact barley seedlings, as a function of $[K^+]_{ext}$, and its dependence on desorption time (5 min, blue circles; 30 min, red triangles). Seedlings were grown on full-nutrient media supplemented with 0.1 mM K^+. Labeling time = 5 min. (C) Influx as a function of labeling time, in barley seedlings grown and measured under 100 mM K^+. Desorption time = 5 min. Throughout, error bars represent ± SEM ($n \geq 4$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Furthermore, a linear LATS is not unique to K^+, but has been observed with many ions, including sodium (Na^+), ammonium (NH_4^+), chloride (Cl^-), and nitrate (NO_3^-) [18,22–25], as well as with organic solutes such as amino acids and sugars [26–28]. Such fluxes are generally considered to be channel-mediated, and typically show no signs of saturation at external substrate concentrations between 10 and 50 mM. Only a few studies have examined fluxes beyond this range because such conditions are often considered physiologically or ecologically irrelevant, and, moreover, potentially damaging to cells. An important exception is that of Na^+ fluxes in the context of salinity stress, where the measurement of fluxes at very high substrate concentrations (*e.g.* >100 mM) is both appropriate and routine. In this case, extraordinarily high fluxes are frequently reported, and although believed to be mediated by NSCCs, their mechanistic underpinnings remain poorly defined [29]. By contrast, much more is known about K^+ transport into roots and within the plant [5,30,31]; thus, we are better equipped to investigate the nature of the K^+ LATS, and use results from this model system to gain insight into Na^+ transport at high external concentrations.

Another critical aspect of low-affinity transport in general involves the efflux of ions, which occurs simultaneously with influx, and appears to rise more steeply than influx does, as external concentrations increase [32]. Such patterns have been observed for all major nutrient ions, including K^+, Na^+, Cl^-, NO_3^-, NH_4^+, and sulfate (SO_4^{2-}), and efflux in the low-affinity range frequently achieves rates approximating those of influx (*i.e.* efflux:influx ratios become close to unity). This futile cycling of ions typically carries with it a potentially substantial energetic burden on root systems, given that efflux or influx components of the cycle may occur against significant thermodynamic gradients [25,32,33].

High rates of efflux make the accurate measurement of low-affinity unidirectional influx, by use of tracer methods, a challenging prospect [34]. In order to limit the effect of efflux occurring during tracer-influx measurement protocols, many researchers advocate the use of very short labeling times (*e.g.* 2–5 min; [25,35,36]). For the same reason, short desorption times (*i.e.* "washes" in non-labeled solutions following uptake, to remove extracellular tracer; see Section 2.2) are often prescribed. Although such protocols have become standard in the measurement of low-affinity fluxes (*e.g.* see [29]), the assumptions that they minimize tracer efflux across the plasma membrane, and clear the roots of extracellular tracer, are rarely tested, and indeed may be untenable in some cases. For example, we recently showed that K^+ efflux across the plasma membrane in roots of intact barley seedlings ceases above a $[K^+]_{ext}$ of 1 mM (*i.e.* in the low-affinity range), and concluded that tracer release from pre-labeled roots was extracellular (apoplastic) in origin [37]. The implications from this study for low-affinity influx measurements require further exploration.

Here, we ask three fundamental questions regarding the nature of low-affinity K^+ influx in roots of higher plants: (1) Does influx