is expressed mainly in the inner, cortical regions of the root (5), where it could function as a scavenging system to recover K⁺ that leaks out of root cells into the apoplasm within the root tissue (for example, through outwardly rectifying K⁺ channels). However, insertion of microelectrodes into cortical cells showed depolarizations by micromolar K⁺ of similar amplitude to those discussed above and these depolarizations are similarly independent of external Na⁺.

Our observations demonstrate that Na⁺ is not essential for high-affinity K⁺ absorption in wheat roots, and hence it seems unlikely that HKTI can make up the major transport system responsible for such uptake. It is therefore more plausible that—as was observed in Arabidopsis thaliana (6)—the principal pathway for high-affinity K⁺ absorption from the soil by wheat is mediated by H+-coupled transport.

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REFERENCES


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Response: In our report, we showed that the high-affinity wheat K⁺ uptake transporter HKTI functions as a Na⁺-K⁺ cotransport system (1). Furthermore, at toxic Na⁺ concentrations, K⁺ uptake by HKTI is inhibited and low-affinity Na⁺ uptake occurs, which correlates to Na⁺ toxicity in plants. With the use of yeast as a screening system, we further developed a genetic strategy to isolate HKTI point mutations that confer Na⁺ tolerance in yeast (1). The increased Na⁺ tolerance of the isolated HKTI mutants was shown to be the consequence of a reduction in Na⁺ uptake and decreased inhibition of K⁺ uptake by toxic Na⁺ concentrations (1). For reducing overall Na⁺ influx into plant cells, this biophysical and genetic approach could be applied to other Na⁺ uptake pathways in plants because many parallel pathways for Na⁺ uptake exist. The cloning of HKTI (2) enables analysis of HKTI homologues in different species involved in plant K⁺ nutrition.

Walker et al. do not clearly distinguish these results, but with foresight go on to address effects of Na⁺-coupled K⁺ transport in roots. They state that (i) Na⁺-cotransport cannot entirely account for high-affinity K⁺ absorption in roots, (ii) that Rb⁺ flux and membrane potential recordings allow quantitative analysis of the underlying transport mechanism, (iii) that one major pathway exists for high-affinity K⁺-absorption from the soils, and (iv) that this is H⁺-K⁺ transport. We agree with (i) but not with (ii) or (iii), and (iv) has not been substantiated by other laboratories.

Plants encounter many different environmental conditions and likely have multiple mechanisms for high-affinity K⁺ uptake. Na⁺-coupling is not the only mechanism for Rb⁺ absorption. The ability to quantitatively dissect transport properties of HKTI (1, 2) has led to recent results that show that the classical experimental methods used by Walker et al. are biophysically insufficient to draw the other conclusions about K⁺ absorption listed, as described below. We observed large Na⁺-coupled K⁺ inward currents in Xenopus oocytes (1), but Na⁺-coupled Rb⁺ inward currents were smaller than the resolution limit, showing an average K⁺ to Rb⁺ selectivity of >35 to 1 (Fig. 1A) (4). Even in yeast, which can compensate for low Rb⁺ conductance because of large proton pump-mediated hyperpolarizations and because of the high membrane resistance of single cells, the K⁺ uptake rate through HKTI is 15-fold larger than the Rb⁺ uptake rate (4). Therefore, in Rb⁺ flux studies in native tissue (3), HKTI activity cannot be detected because the transport of Rb⁺ by HKTI will be masked by Rb⁺ transport through other transporters, known to exist in plants with a lower K⁺ to Rb⁺ selectivity (5) than HKTI.

Walker et al. describe another classical technique of measuring depolarizations in response to micromolar K⁺ that has been instrumental for measuring K⁺ interactions with high-affinity systems (6). However, membrane depolarizations should not be interpreted as showing cation uptake, as assumed by Walker et al. Similar to their results, we found nonspecific wheat root membrane depolarizations in response to micromolar amounts of K⁺, Cs⁺, Rb⁺ (Fig. 1B, n = 31), and Na⁺. Why are high-affinity depolarizations not K⁺-specific? Similar to intact roots (Fig. 1B), in HKTI-expressing oocytes, K⁺, Cs⁺, Rb⁺, and Na⁺ induce nonspecific membrane depolarizations (Fig. 1C). But with the exception of Na⁺, these depolarizations were attributed to inhibition of outward current by HKTI, rather than to stimulation of cation inward currents (4). Even the large depolarizations in Fig. 1C do not allow one to distinguish that K⁺ alone only inhibited outward currents by HKTI (1, 2), while large inward currents were stimulated by Na⁺ (1C) (1, 4). The low specificity among cations in inhibiting HKTI-mediated outward currents has been previously demonstrated [figure 1D in (2)]. Root cortical cell membranes are an electrically complex system, containing pumps, channels, transporters, and multiple cell-cell conductions. Membrane depolarizations clearly do not allow dissection of the functional mechanisms underlying high-affinity K⁺ uptake.

HKTI function has been studied in heterologous systems where the expression is constitutive. When one is studying HKTI function in native tissue, one needs to verify the expression of HKTI. With the use of competitive PCR, we have found that HKTI mRNA is greatly (>10- to 50-fold) induced in 6-day-old seedlings by complete nutrient starvation for 2 days, and strongly suppressed in the presence of 1 mM K⁺ together with other cationic nutrients (7). Walker et al. assume that HKTI is expressed under the conditions employed for their experiments.

To draw their conclusions, Walker et al. implicitly assume that there is one major pathway for high-affinity K⁺ uptake in plants. But our finding that HKTI is a Na⁺-coupled transporter (1) suggests to us that other high-affinity K⁺ transporters with other transport mechanisms are likely to exist. Recent molecular biological studies have hinted at the large extent of nutrient transporter gene families (8, 9). The limited genetic identification of plant Na⁺ excluding Na⁺-tolerant plants points to the fact that redundant pathways for Na⁺ uptake also exist in plant roots (10, 11). HKTI may play an important role in K⁺ nutrition under conditions of low K⁺ and sufficient Na⁺ in soil. Under these conditions, Na⁺ stimulates root growth and K⁺ nutrition [12, and references therein]. A recent study of Arabidopsis has shown doubling of plant growth by weight in the presence of Na⁺ (13).

Walker et al. further suggest that the major mechanism for high-affinity K⁺ uptake in plants is H⁺-coupled K⁺ uptake. They did not analyze whether Rb⁺ uptake was pH-dependent or not. Because some H⁺-coupled transporters can function as Na⁺-coupled transporters and vice versa, we agree with the hypothesis that H⁺-coupled K⁺ transporters provide one of the mechanisms for K⁺ uptake in plants. We found that the activity of HKTI was not
dependent on external pH in the range from pH 5.0 to 8.0 in flux studies or in voltage-clamp experiments (1), and therefore other HKT1 homologs may function as H+-coupled transporters. However, many studies have tested H+-coupled K+ uptake into roots, but supportive evidence has not been found in these analyses (6, 14, 15). Different approaches, such as vibrating extracellular microelectrode techniques and radio tracer flux experiments, have not shown H+-coupled K+ uptake. This is not a problem of experimentally applying extracellular acidification, because other well-known H+-coupled transporters in plants for NH4+, NO3-, and sucrose uptake show a clear stimulation of uptake by external acidification (16–18). These results (6, 14, 15) attest to the difficulty of assessing a single mechanism for high-affinity K+ uptake. In Arabidopsis, subtractive current-voltage curves were used to characterize the mechanism for high-affinity K+ uptake (19). However, this methodology is prone to inaccuracy and is admissible only when important limiting conditions are met, including changes in the external solution affecting only the specific transport system under investigation and the current through the transporter in the absence of substrate being zero (20). The latter condition does not hold for HKT1 (1, 2, 4). Also, other coupled transporters are known to show specific modes of function when extracellular substrates are removed (21). Determination of reversal potential using subtractive current-voltage curves is not generally valid (Fig. 1, D and E). The fact that the current-voltage relations published for the H+-K+ transport current do reverse using this subtraction protocol (19) indicates a possible problem. H+-K+ currents using this protocol in root cells show a low reproducibility [1 of 19 cells (7)]. We do not exclude H+-K+ transport as one of the K+ uptake mechanisms, but until these quantitative issues are resolved, there is sufficient reason not to use subtractive current-voltage curves.

Detailed quantitative biophysical studies are now possible on HKT1 and other K+ transporters that will provide insight into many new physiologically relevant properties of individual transport components (11). Critical errors can arise when Rb+ fluxes, depolarizations, and subtractive current-voltage curves are used for characterization of individual transport components if new quantitative insights (Fig. 1) are not incorporated. The large physiological variation in soil concentrations of K+, Na+, H+, other nutrients, and toxic cations calls for an array of nutrient uptake transporters that allow plant growth (11). Interactions of various transport components under different environmental and stress conditions render the processes of K+ nutrition much more complex and interesting than proposed (3).

**Fig. 1.** (A) Rb+ uptake experiments are not representative of K+ uptake by HKT1. Average steady-state current magnitudes recorded at ~120 mV from uninjected (solid bars, n = 8) and HKT1-expressing oocytes (open bars, n = 10) with 1 mM Na+ + 10 mM K+ (left) or 1 mM Na+ + 10 mM Rb+ (right) in the bath solution. Error bars denote SEM. (B) Exposure of wheat roots to 100-mM concentrations of K+, Cs+, and Rb+ causes nonspecific depolarizations in wheat root cortical cells. (C) Alkaline cations cause nonspecific membrane depolarizations via inhibition of HKT1-mediated outward currents. For quantitative average effects of 1 mM cations on inhibition of outward currents, see also figure 1, C and D, in (2). Depolarizations caused by 100 mM alkaline cations in uninjected (solid bars, n = 3 oocytes for each cation) and HKT1-expressing oocytes (open bars, n = 4 each). Depolarizations relative to control recordings with oocytes exposed to tris-HCl are representative of membrane potential in HKT1-expressing oocytes with tris-HCl was ~129 ± 5 mV, because of the HKT1-mediated outward currents (see text), and ~31 ± 5 mV in control uninjected oocytes. (D and E) Hypothetical reversal potential determination by subtractive current-voltage curve analysis can produce errors. (D) Current-voltage curves of a reversible voltage-independent K+ channel in the absence (solid line) and presence (dashed line) of extracellular K+. (E) Difference current-voltage curve of currents shown in (D) leads to a large systematic error in the hypothetical reversal potential (Vrev). Actual reversal potential can be found in (D).

**REFERENCES AND NOTES**

15. W. Lin and J. E. Hanson, ibid. 58, 276 (1976).
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Response: High-Affinity Potassium Uptake in Plants
Francisco Rubio, Walter Gassmann and Julian I. Schroeder (August 16, 1996)

Editor's Summary

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