Potassium Influx into Maize Root Systems

INFLUENCE OF ROOT POTASSIUM CONCENTRATION AND AMBIENT AMMONIUM

Received for publication December 14, 1986 and in revised form May 15, 1987

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ABSTRACT

Potassium influx into roots of dark-grown decapitated maize seedling (Zea mays L., cv. Pioneer 3369A) was examined in presence and absence of ambient ammonium and at various root potassium concentrations. Six-day old seedlings which were dependent on the endosperm reserves for their energy source were exposed to KCl (labeled with 86Rb) ranging from 5 to 200 micromolar. At both low (13 micromoles per gram fresh weight) and high (100 micromoles per gram fresh weight) root potassium concentration, isotherms indicated two potassium influx systems, one approaching saturation at 50 to 100 micromolar potassium and an additional one tentatively considered to be linear. A mixed-type inhibition by ammonium for the low-concentration saturable system was indicated by a concomitant decrease in \( V_{max} \) and increase in \( K_m \). High root potassium concentration decreased \( V_{max} \) but had little effect on \( K_m \) of this system. The rate constant for the second quasilinear system was decreased by ambient ammonium and by high root potassium status. Transfer of high potassium roots to potassium-free solutions resulted in an increase in influx within 2 hours; by 24 hours influx significantly exceeded that of roots not previously exposed to potassium. In roots of both low and high root potassium concentrations, potassium influx was restricted progressively as ambient ammonium increased to about 100 micromolar, but there was little further inhibition as ammonium concentrations increased beyond that to 500 micromolar. The data imply that potassium influx has two components, one subject to inhibition by ambient ammonium and one relatively resistant.

Conflicting results have been reported for the influence of ammonium on the uptake of potassium by plant tissue. Smith and Epstein (25) indicated that ammonium was a competitive inhibitor of potassium influx whereas Deane-Drummond and Glass (4) reported a mixed-type inhibition which implies both competitive and noncompetitive elements. For the opposite effect, Scherer et al. (24) observed a mixed-type inhibition of net ammonium uptake by potassium whereas no inhibition of ammonium uptake by potassium occurred with rice (19).

It is possible that root potassium concentration may affect the nature and magnitude of the influence of ammonium on potassium influx. A restricting effect of internal root potassium on potassium influx in root tissue has been observed consistently (3, 6, 8–10, 15). To our knowledge, the combined effects of potassium ions at the internal surface and ammonium ions at the external surface on potassium influx have not been examined, although the restriction in net potassium uptake by ambient ammonium was similar in both potassium-loaded and potassium-depleted tomato and prune (23). The present experiments were designed to examine the influences of both ambient ammonium and internal potassium on potassium influx by maize roots. Different internal potassium concentrations were obtained either by pretreating the roots with potassium for selected periods of time or by exposure to potassium-free solutions after prior exposure to potassium.

MATERIALS AND METHODS

Plant Culture. Seeds of corn (Zea mays L., cv. Pioneer 3369A) were placed in paper rolls soaked in 0.1 mM CaSO4 and germinated in a dark chamber maintained at 30°C and 98% RH. On d 3, seedlings were selected for uniformity, and all roots except the primary axis were excised. Groups of four seedlings (a culture) were transferred to holders consisting of plastic stoppers with holes through which the primary roots were threaded. The cultures were transferred to 15-L tanks (15 cultures tank⁻¹) containing the basal growth nutrient solution which consisted of 0.5 mM CaSO4 and micro-nutrients at 40% of the concentration of Hoagland solution (12). The growth solution was adjusted to pH 6.0 with Ca(OH)2, and the tanks were placed in the dark chamber. Seeds in each culture were covered with cotton moistened with 0.1 mM CaSO4. The solutions were aerated continuously and were replaced daily.

On d 5, approximately 125 h after germination was initiated, the etiolated shoots were excised 2 cm above the seed. Root systems of each culture of detopped seedlings were placed in an expanded polystyrene support (6 x 6 cm) and floated either on the basal growth solution or on pretreatment solution, according to the experiment involved. A piece of dry cotton was placed over the cut mesocotyls to absorb the xylem exudate and was changed periodically. From this time on, the decapitated seedlings were kept under room conditions (25 ± 1°C), where pretreatment and influx measurements were performed.

Pretreatment of Root Systems. Pretreatments were designed to produce roots of different potassium status. Roots grown and pretreated in the basal growth nutrient solution (potassium-free) are referred to as low potassium roots. Prior to initiating potassium influx measurement from the basal solution plus 200 \( \mu \)M KCl with and without 200 \( \mu \)M NH4Cl, root systems were pretreated for 0, 1, 3, 5, 8, 16, and 24 h in the basal solution plus 25 mM KCl, or pretreated for 16 h in the basal solution plus 25 mM KCl following which they were placed in the basal (i.e. potassium-free) solution for 0, 2, 6, and 24 h. In a third experiment, root systems were pretreated for 0 or 16 h in the basal solution plus 25 mM KCl and then subjected to potassium influx measurements from the basal solution plus 200 \( \mu \)M KCl with NH4Cl ranging from 0 to 500 \( \mu \)M. For the fourth experiment, root systems were pretreated exactly as for the third experiment with subsequent influx being measured from the basal solution
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plus KCl ranging from 5 to 200 μM with and without 200 μM NH₄Cl.

All pretreatment solutions were adjusted to pH 6.0 with Ca(OH)₂. Solution acidity remained within ±0.5 pH units of that value during all pretreatment periods. Constant aeration was also maintained. Prior to initiating influx measurements, the roots were placed in fresh aerated basal nutrient solution for two sequential 5-min periods to remove free space K⁺ ions.

K⁺ (⁶⁷ᵐ⁺Rb) Influx Measurement. On d 6, after appropriate pretreatment of the root system, short-term (10 min) K⁺ (⁶⁷ᵐ⁺Rb) influx measurements were performed. It has been shown that ⁶⁷ᵐ⁺Rb is a suitable analog of potassium for measuring influx into corn roots (4, 16, 18). The volume of the influx medium was chosen such that ambient ammonium and potassium concentrations during the influx period did not decrease by more than 5%.

Influx was terminated by removing the culture from the influx medium, dripping twice in distilled water and then desorbing for 5 min in cold (<7°C) basal solution plus 200 μM KCl. At the end of the desorption period, decapitated corn seedlings from each culture were blotted and divided into roots and seeds plus mesocotyls. Fresh weights were recorded, the tissues were oven-dried at 60°C for 3 h, and the roots and seeds plus mesocotyls were ashed at 480°C for 12 h. The ash was dissolved in 10 ml 1.0 N HCl. Aliquots were taken in duplicate and counted by liquid scintillation. Potassium influx values were determined by dividing the sum of the activity in all tissue by the ambient specific activity. Influx values are given in μmol K⁺ per g root fresh weight per h and represent the means of four replicate cultures.

Determination of Root Potassium Concentration. At the same time that cultures were selected for potassium influx measurement, four cultures representing each pretreatment were harvested for analysis of root potassium concentration. These cultures were processed in exactly the same way as cultures harvested after the desorption period. Potassium in the root tissue extracts was analyzed by flame photometry and values are expressed as μmol K⁺ per g root fresh weight.

RESULTS

Effect of Ambient Ammonium on K⁺ (⁶⁷ᵐ⁺Rb) Influx by Roots Differing in Potassium Status. Figure 1 shows K⁺ (⁶⁷ᵐ⁺Rb) influx from 200 μM KCl as a function of root potassium concentration in the absence and presence of 200 μM NH₄Cl. In the absence of ammonium, potassium influx was inhibited consistently only at root concentrations in excess of 80 μmol g⁻¹ fresh weight. The presence of 200 μM ambient ammonium decreased potassium influx appreciably and did not alter the pattern of response to increasing root potassium concentrations.

Figure 2 shows the change in potassium influx after root systems, previously pretreated for 16 h with 25 mM KCl (and containing 98 μmol K⁺ g⁻¹ fresh weight), were transferred to potassium-free solution for different periods of time. In the absence of ambient ammonium, potassium influx increased significantly (P = 0.05) after 2 h in potassium-free solution. With additional time it increased further, reaching 10.9 μmol g⁻¹ fresh weight h⁻¹ after 24 h. This is a significantly higher rate than obtained when roots had not been pretreated (Fig. 1). When assayed in presence of ambient ammonium, potassium influx was decreased by about 70%, and this low influx was maintained up to 6 h after transfer to potassium-free solution. During the following 18 h, influx increased about 3-fold as the potassium concentration of roots declined to 70% of the initial level.

Effect of Increasing Ambient Ammonium Concentration on Potassium Influx by Roots Differing in Potassium Concentration. With both low and high root potassium concentrations (13 and 98 μmol g⁻¹ fresh weight, respectively), potassium influx was increasingly inhibited by increasing ambient ammonium to

![Fig. 1. Relationship between K⁺ (⁶⁷ᵐ⁺Rb) influx and root K⁺ concentration in decapitated maize seedlings pretreated with 25 mM KCl for 0 to 24 h (as shown on the upper horizontal axis); influx media (A) with 200 μM KCl and (C) with 200 μM KCl plus 200 μM NH₄Cl. Influx media were adjusted to pH 6.0. The vertical and horizontal bars indicate ±1 standard deviation (four replicates) when they exceed the size of the symbol.](https://www.plantphysiol.org/content/95/3/1417/F1.large.jpg)

![Fig. 2. Effect of ambient ammonium on K⁺ (⁶⁷ᵐ⁺Rb) influx after maize roots had been pretreated with 25 mM KCl for 16 h and then exposed to potassium-free solution (basal growth solution) for different periods of time; influx media (A) containing 200 μM KCl and (C) containing 200 μM KCl plus 200 μM NH₄Cl; (Δ) root potassium concentration after each pretreatment time. Influx media were adjusted to pH 6.0. The vertical bars indicate ±1 standard deviation (four replicates) when they exceed the size of the symbol.](https://www.plantphysiol.org/content/95/3/1417/F2.large.jpg)

...about 100 μM (Fig. 3). Exponential regression of potassium influx on external ammonium concentration in this ammonium concentration range gave (a) \( Y = 6.532e^{-0.696X} \) (\( r^2 = 0.97 \)) for low potassium roots, and (b) \( Y = 4.64e^{-2.688X} \) (\( r^2 = 0.99 \)) for high potassium roots, where \( Y \) is potassium influx and \( X \) is ambient ammonium concentration. Therefore, the decrease in potassium influx per unit increase in external ammonium concentration was the same in low and high potassium roots. With both low and high potassium roots, there was little further reduction of potassium influx beyond 100 μM ammonium. The decrease in potassium influx due to high root potassium concentrations was similar at all ambient ammonium concentrations.

Potassium Influx Isotherms—Effect of Ambient Ammonium
and Root Potassium Concentration. Potassium influx isotherms in the absence and presence of ambient ammonium are shown in Figures 4 and 5 for low- and high-potassium roots, respectively. A single isotherm based on Michaelis-Menten kinetics did not describe well the relation between influx and external potassium concentration in the range 0 to 200 μM K⁺. This conclusion is supported by Hofstee (13) plots (Figs. 4 and 5, insets) in the absence of ambient ammonium showing a linear relationship from 0 to 50 or 100 μM which then curved upward. The limited number of external potassium concentrations used was insufficient for a thorough analysis of the most appropriate model to describe these data sets. They therefore were treated assuming the 0 to 50 μM K⁺ range to represent a saturable component with the 50 to 200 μM K⁺ range being considered separately.

For the low concentration saturable component, kinetic parameters were determined using nonlinear regression procedures (27). The values are presented in Table I. Linear regressions of potassium influx on external potassium from 50 to 200 μM were used to characterize potassium influx in this range and the values for the first-order rate coefficient (k) for this component are also presented in Table I. Ambient ammonium altered the low concentration saturable component of potassium influx (Figs. 4 and 5) at both low and high root potassium concentration. This effect of ammonium was exerted through a significant (P < 0.05) decrease of Vₘₐₓ values and concomitant increase of Kₘ values in both tissues. The first-order rate coefficient of the second system also was decreased by ambient ammonium.

Increasing the root potassium concentration from 13 to 102 μmol g⁻¹ fresh weight affected both the low concentration saturable component and the additional component of potassium influx (Table I). For the saturable component, the primary effect of high root potassium concentration was to decrease Vₘₐₓ significantly (P < 0.05); the decrease occurred both in the absence and presence of ambient ammonium. The Kₘ was not altered significantly in either case. The rate coefficient of the second component was decreased to about one-half by high root potassium concentration regardless of whether ambient ammonium was present or not (Table I).

**DISCUSSION**

Transitions in the influx isotherms of both low- and high-potassium roots occurred at ambient potassium concentrations of 50 to 100 μM (Figs. 4 and 5). The data imply the existence of a low concentration saturable phase of potassium influx and an additional linear phase. However, the limited concentration range employed (0–200 μM) does not permit excluding the possibility of a biphasic system involving one phase transition (11,
between was evident. Therefore, inhibition by ammonium was a saturable phase of potassium influx as seen in the experimental data (17). This phase may reflect influx into epidermal cells whereas the second phase may represent a diffusion-limited access to cortical cells (17). Alternatively, the two phases could reflect longitudinal separation of influx sites with apical and basal regions exhibiting different kinetics (21).

Inhibition by ammonium of the low concentration saturable phase of potassium influx resulted from a concomitant decrease in $V_{\text{max}}$ and increase in $K_m$, indicating a mixed-type inhibition for both low- and high-potassium roots (Table 1). A mixed-type inhibition has been noted previously in barley (4). Inhibition by ammonium was unaffected by increases in root potassium concentration until the latter exceeded the concentration (about 80 uM g$^{-1}$ fresh weight) at which its own inhibitory effect was exerted (Fig. 1). At the higher root potassium concentrations the magnitude of the inhibition by ammonium declined but was still evident. The consequence was an extremely limited potassium influx in roots of high potassium status in presence of ambient ammonium (Fig. 1).

The root potassium concentration (about 80 uM g$^{-1}$ fresh weight; Fig. 1) beyond which a marked decline in potassium influx occurred in these dark-grown maize seedlings was significantly higher than that in barley (10, 14) or in light-grown maize seedlings (3). Substantial differences among species have been noted previously in the root potassium concentrations at which inhibition of potassium influx occurred (15), but the difference between the present data and those of light-grown maize (3) are especially notable. Influx was significantly restricted in the latter at 50 to 60 uM g$^{-1}$ fresh weight whereas in the dark-grown seedlings no inhibition occurred at these concentrations (Fig. 1). These dissimilarities could reflect a differential sensitivity of potassium influx to allosteric regulation by cytoplasmic potassium to vacuoles and xylem (1, 2, 5, 26). A relatively small pool of internal potassium appears to be responsible for the restriction because the inhibition diminished after only 2 h in potassium-free solution (Fig. 2).

The inhibitory effect of high root potassium concentration on the low concentration saturable phase was exerted only by a decrease in $V_{\text{max}}$ (Table 1), implying a limitation in number or functioning of transport sites. Failure of a significant change in $K_m$ implies no influence of internal potassium in altering the initial interaction of potassium with the system, a result counter to observations with barley (8, 9), and with ryegrass and white clover (6), in which the inhibitory effect was exerted through an increase in $K_m$ as well as a decrease in $V_{\text{max}}$.

The magnitude of the inhibition by high root potassium concentrations was similar as ambient ammonium increased from nil to 500 uM (Fig. 3), indicating that inhibition by internal potassium was independent of inhibition by external ammonium under these conditions. However, interactions between the two inhibitory actions did occur under other conditions. For example, there was a decrease in the magnitude of the inhibition by ammonium as root potassium concentration increased progressively beyond 80 uM g$^{-1}$ fresh weight where the internal effect of potassium was exerted (Fig. 1). Moreover, following transfer to potassium-free solutions, relief from inhibition by internal potassium was delayed when inhibition by ambient ammonium also was occurring (Fig. 2).

Another perspective of the influence of ambient ammonium and root potassium concentrations can be invoked. Potassium influx from 200 uM decreased as ambient ammonium increased to about 100 uM and the decrease (about 3.2 uM g$^{-1}$ fresh weight h$^{-1}$) was similar in both low- and high-potassium roots (Fig. 3). However, further increases in ambient ammonium to 500 uM were without additional effect indicating a component resistant to inhibition by ambient ammonium within this concentration range. Potassium influx by the resistant component was 4.5 and 1.5 uM g$^{-1}$ fresh weight h$^{-1}$ by the low- and high-potassium roots, respectively (Fig. 3). Thus, in this view high root potassium concentrations decreased the influx component which was resistant to ambient ammonium whereas the component sensitive to such inhibition was not altered significantly.

Roots without potassium pretreatment consistently had potassium concentrations of 12 to 14 uM g$^{-1}$ fresh weight. In these roots influx from 200 uM potassium was within the range 6.7 to 7.7 uM g$^{-1}$ fresh weight h$^{-1}$ (Figs. 1 and 4). Although it is unlikely that these low potassium roots would be inhibited by internal potassium, it appears that their influx system was not fully expressed. This is because prior exposure to potassium followed by 24 h in potassium-free media resulted in significantly higher influx (10.9 uM g$^{-1}$ fresh weight h$^{-1}$; Fig. 2) than in roots not previously exposed to potassium. This high rate occurred when the root potassium concentration had decreased to 68 uM g$^{-1}$ fresh weight h$^{-1}$ (Fig. 2), a concentration which resulted in a significantly lower value (7.5 uM g$^{-1}$ fresh weight h$^{-1}$) when influx was measured immediately after pretreatment (Fig. 1). A stimulation in influx capacity due to prior exposure to potassium, as suggested by Pettersson and Jensen (22), is implied.

Acknowledgment—The technical assistance of P. Longmire is sincerely appreciated.

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