

## Calcium Inhibition of Potassium Absorption in Corn Roots<sup>1</sup>

O. E. Elzam<sup>2</sup> and T. K. Hodges

Department of Horticulture, University of Illinois, Urbana, Illinois 61801

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*Summary.* Calcium (or magnesium) sulfate or chloride was found to inhibit energy dependent potassium transport in excised corn roots. This  $\text{Ca}^{2+}$  inhibition of  $\text{K}^+$  transport was most pronounced during the initial phases of transport. As the absorption periods were lengthened the effect of  $\text{Ca}^{2+}$  gradually changed from an inhibition to a typical promotion (after about 30-45 mins) of  $\text{K}^+$  transport. Kinetic analysis indicated the inhibition to be of a non-competitive nature.

Identical experiments with excised barley roots showed that  $\text{CaSO}_4$  had no effect on  $\text{K}^+$  absorption whereas  $\text{CaCl}_2$  had a typical stimulatory effect on  $\text{K}^+$  absorption. Kinetic analysis indicated that both corn and barley have efficient  $\text{K}^+$  transporting systems but barley roots are approximately 5 times more active (on a fr wt basis) than corn roots.

These results illustrate the hazards involved in applying results obtained with 1 (or even several) plant species to all species.

Following the classic work of Viets (20), numerous reports have been made of the promotive effects of  $\text{Ca}^{2+}$  and other polyvalent cations on the absorption of other ions by plant roots. The 'Viets effect' is especially pronounced with  $\text{K}^+$  (4, 9, 14, 20),  $\text{Rb}^+$  (5, 17, 18, 19),  $\text{Br}^-$  (20),  $\text{Cl}^-$  (3, 15),  $\text{SO}_4^{2-}$  (11) and  $\text{PO}_4^{3-}$  (12, 17, 18). Sodium and lithium absorption, on the other hand, are drastically curtailed by  $\text{Ca}^{2+}$  (4, 5, 10, 16). However, the generally promotive effects of  $\text{Ca}^{2+}$  on monovalent cation absorption, plus the finding that  $\text{Ca}^{2+}$  appears to be essential for maintaining the integrity of the selective ion transport mechanisms (4), as well as the fact that  $\text{Ca}^{2+}$  is necessary for the maintenance of cellular membranes (13) has led most investigators to routinely include  $\text{Ca}^{2+}$  in all ion absorption experiments. We have recently found, however, that the inclusion of  $\text{Ca}^{2+}$  under certain experimental conditions results in an inhibition rather than a promotion of  $\text{K}^+$  transport in corn roots. This  $\text{Ca}^{2+}$  (or  $\text{Mg}^{2+}$ ) inhibition of  $\text{K}^+$  transport is greatest during the initial phases of energy dependent  $\text{K}^+$  transport. However, when the absorption periods are lengthened the inhibition of  $\text{K}^+$  transport gradually disappears and finally a typical promotive or 'Viets effect' occurs.

### Materials and Methods

Corn (*Zea mays* L., WF9 X M14) seeds were soaked for 2 minutes in 0.25 %  $\text{H}_2\text{O}_2$ , thoroughly washed with distilled water (all distilled water was passed through a Barnstead Bantam Demineralizer prior to use) and planted on moist cheesecloth over aerated, 0.2 mM  $\text{CaSO}_4$ . Plants were grown in the dark at room temperature (22°) for 4 days. Seedlings were selected for uniformity and washed thoroughly with distilled water. Root samples (0.5 g fr wt) were prepared by excising 1.5 cm root tip sections and used in short-term experiments essentially as described by Epstein, et al. (6).

Barley (*Hordeum vulgare* var. Arivat) seeds were germinated and grown in the dark, at room temperature in aerated 0.2 mM  $\text{CaSO}_4$  as described by Epstein, et al. (6). Root samples (1 g fr wt) were prepared after excising the entire root material from the plants.

Prior to the absorption period the roots were held in aerated, distilled water for 30 minutes at 30°. Experimental solutions contained only KCl labeled with  $\text{Rb}^{86}$  (0.005  $\mu\text{C}/\mu\text{mole K}^+$ ) which was used as the tracer for  $\text{K}^+$ . In experiments dealing with the effect of divalent cations on  $\text{K}^+$  absorption,  $\text{CaCl}_2$ ,  $\text{CaSO}_4$  or  $\text{MgSO}_4$  were added as noted in the text and figure legends. In order to avoid excess depletion of  $\text{K}^+$  during the absorption period, the experimental solutions were sufficiently large to maintain the  $\text{K}^+$  concentration within 1.5 % of its initial value. The pH of the experimental solutions was essentially constant at 5.6 and the experimental temperature was 30°.

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<sup>2</sup> Present address: Biology Department, Case-Western Reserve University, Cleveland, Ohio.

At the end of the absorption period (10 mins except in the time experiments) the roots were rinsed briefly with 250 ml of cold ( $1^{\circ}$ ) 5 mM KCl-0.5 mM  $\text{CaSO}_4$  and then placed in cold ( $1^{\circ}$ ) 5 mM KCl-0.5 mM  $\text{CaSO}_4$  for 30 minutes to remove the diffusible and exchangeable  $\text{K}^+$  ( $\text{Rb}^{86}$ ). The roots were finally rinsed in distilled water, transferred to planchets and ashed by slowly bringing the temperature to  $500^{\circ}$ . The ash was moistened with 1% detergent, dried, and counted with a thin-window, gas-flow counter. Amounts of  $\text{K}^+$  ( $\text{Rb}^{86}$ ) absorbed were obtained by comparison with initial radioactivity of the external solution.

## Results and Discussion

*General Characteristics of  $\text{K}^+$  Absorption by Corn Roots.* Figure 1 shows that  $\text{K}^+$  absorption by corn roots from 0.10 mM KCl is a linear function of time for at least 1 hour. Potassium absorption by corn roots as a function of external  $\text{K}^+$  concentration shows a typical hyperbolic isotherm (fig 2) which can be analyzed by Michaelis-Menton kinetics. From a Lineweaver-Burk plot of the data the  $K_m$  for  $\text{K}^+$  absorption was calculated to be 0.06 mM and the  $V_{max}$  was  $1.51 \mu\text{moles g}^{-1} \text{hr}^{-1}$ . Although the data are not presented here, similar studies in our laboratory with barley roots, over the same concentration range, indicated the  $K_m$  to be 5 times lower (0.012 mM) and the  $V_{max}$  about 4.6 times higher ( $7.0 \mu\text{moles g}^{-1} \text{hr}^{-1}$ ) than in corn roots.

Thus both corn and barley, although quantitatively possessing different absorption affinities and capacities, have an efficient system for absorbing  $\text{K}^+$  from low external concentrations [described by Epstein et al. (6) as mechanism 1] when no other cations are present in the experimental solutions.

*Effect of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  on  $\text{K}^+$  Absorption.* Figure 3 shows the results of 2 separate experiments in which the effect of increasing concentrations of divalent cations on the rate of  $\text{K}^+$  absorption from 0.10 mM KCl was investigated. Both  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  drastically inhibited  $\text{K}^+$  absorption. The absorption time was 10 minutes for both experiments. At  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  concentrations of 0.005 mM (i.e., a concentration 10 times less than that of  $\text{K}^+$  on an equivalence basis)  $\text{K}^+$  absorption was inhibited 28% and 24% respectively. With divalent cation concentrations of 0.20 mM the inhibitions were approximately 70%.

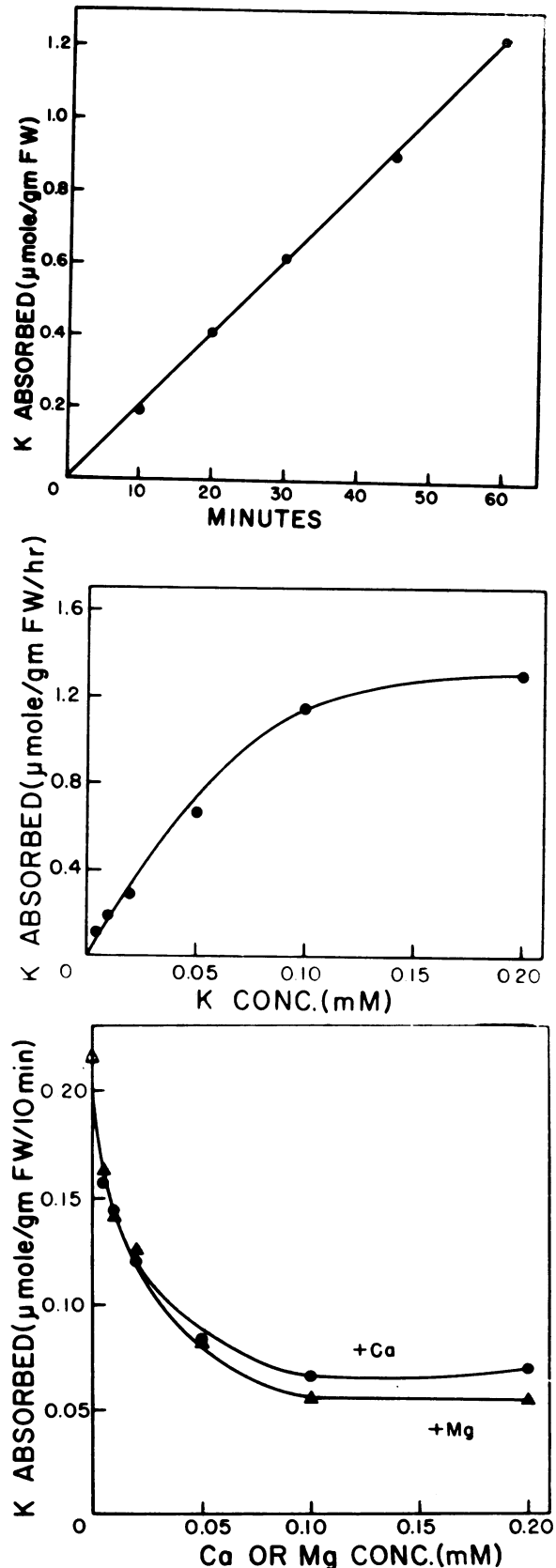


FIG. 1. Potassium absorption by excised corn roots as a function of time. External KCl concentration was 0.10 mM.

FIG. 2. Potassium absorption by excised corn roots as a function of external KCl concentrations.

FIG. 3. The effect of various concentrations of  $\text{CaSO}_4$  and  $\text{MgCl}_2$  on  $\text{K}^+$  absorption by excised corn roots. External KCl concentration was 0.10 mM.

The inhibitions observed must pertain to energy dependent K<sup>+</sup> transport (i.e., active transport of either K<sup>+</sup> or Cl<sup>-</sup>) since all diffusible and exchangeable K<sup>+</sup> (Rb<sup>86</sup>) was removed prior to analysis (See Materials and Methods).

Since this inhibitory effect of Ca<sup>2+</sup> (or Mg<sup>2+</sup>) on K<sup>+</sup> absorption in corn roots was opposite to the results obtained in previous investigations reporting synergistic effects with corn (9) and other plant species (4, 9, 14) we also determined the effect of Ca<sup>2+</sup> on K<sup>+</sup> absorption by barley roots. The results presented in figure 4 show that CaSO<sub>4</sub> up to 2.0 mM had no effect on K<sup>+</sup> absorption by barley roots but again the inhibition of K<sup>+</sup> absorption in corn roots was evident. Hence, the inhibitory effect of Ca<sup>2+</sup> must be characteristic of the species. Short-term preliminary experiments with oats (*Avena sativa* var. Goodfield), like barley, indicated no inhibitory effect of Ca<sup>2+</sup>.

Although Ca<sup>2+</sup> does not inhibit K<sup>+</sup> absorption in barley the fact that no promotive or 'Viets effect' was detected (fig 4) seemed rather unique. However, since the above results were observed using CaSO<sub>4</sub>, it was of interest to investigate the effect of CaCl<sub>2</sub> on K<sup>+</sup> absorption. Figure 5 shows the effect of increasing CaCl<sub>2</sub> concentrations on K<sup>+</sup> absorption from 0.10 mM KCl by both barley and corn roots. With barley roots, Ca<sup>2+</sup> caused a typical promotive effect on K<sup>+</sup> absorption. With corn, however, a Ca<sup>2+</sup> inhibition still occurred (60% inhibition with 0.10 mM CaCl<sub>2</sub>) but at higher Ca<sup>2+</sup> concentrations the inhibition was partially alleviated. These different responses with the SO<sub>4</sub><sup>2-</sup> and Cl<sup>-</sup> salts of Ca<sup>2+</sup> suggest that the promotive effect of CaCl<sub>2</sub> may be due to an increase in absorption of Cl<sup>-</sup> (since the Cl<sup>-</sup> concentrations were increasing) and a consequent increased absorption of K<sup>+</sup>. Further studies of this type may be helpful for clarifying the nature of the 'Viets effect'.

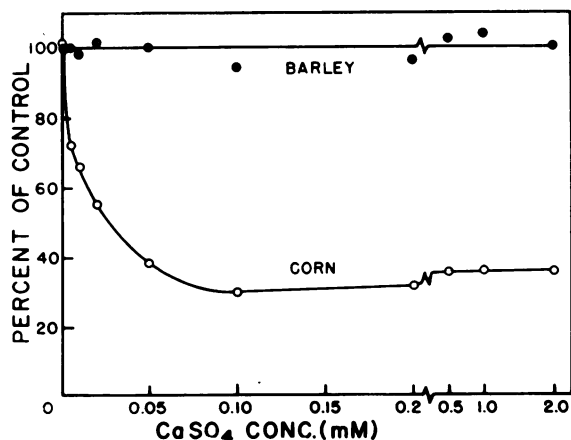


FIG. 4. The effect of various concentrations of CaSO<sub>4</sub> on K<sup>+</sup> absorption by excised corn and barley roots. External KCl concentration was 0.10 mM. Potassium absorption for corn and barley in the absence of Ca<sup>2+</sup> were 0.217  $\mu\text{mole g}^{-1} 10 \text{ min}^{-1}$  and 1.08  $\mu\text{mole g}^{-1} 10 \text{ min}^{-1}$ , respectively.

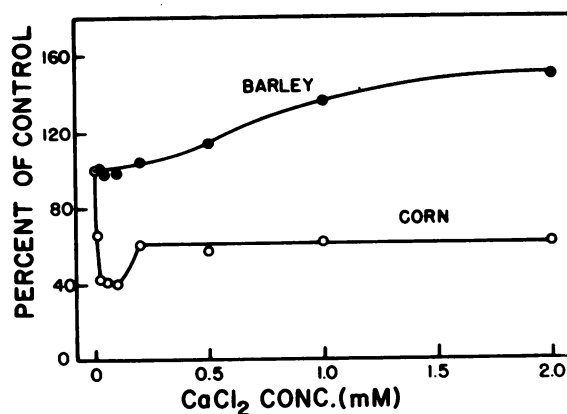


FIG. 5. The effect of various concentrations of CaCl<sub>2</sub> on K<sup>+</sup> absorption by excised corn and barley roots. External KCl concentration was 0.10 mM. Potassium absorption for corn and barley in the absence of Ca<sup>2+</sup> was 0.178  $\mu\text{mole g}^{-1} 10 \text{ min}^{-1}$  and 1.02  $\mu\text{mole g}^{-1} 10 \text{ min}^{-1}$ , respectively.

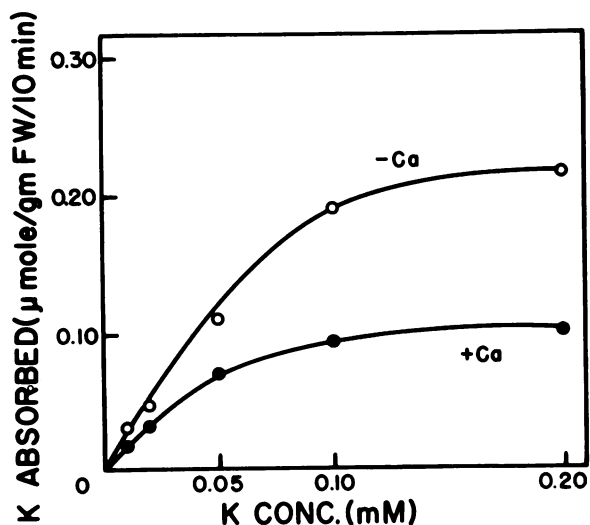


FIG. 6. Potassium absorption by excised corn roots as a function of external KCl concentrations in the absence and presence of 0.05 mM CaSO<sub>4</sub>.

In order to determine the nature of the Ca<sup>2+</sup> inhibition of K<sup>+</sup> transport in corn roots, K<sup>+</sup> absorption from solutions of varying K<sup>+</sup> concentrations was determined in the presence and absence of 0.05 mM CaSO<sub>4</sub>. Figure 6 shows that the inhibition due to Ca<sup>2+</sup> was quite pronounced at all K<sup>+</sup> concentrations investigated (0.005–0.20 mM). A Lineweaver-Burk plot of the data (fig 7) indicated the kinetics to be typical of a non-competitive inhibition. That is, the Km (or apparent affinity) was not altered by the presence of Ca<sup>2+</sup> while the Vmax was lowered from 0.235 to 0.148  $\mu\text{moles g}^{-1} 10 \text{ min}^{-1}$ .

The inhibition of K<sup>+</sup> transport by either Ca<sup>2+</sup> or Mg<sup>2+</sup> observed in the preceding experiments was for absorption periods of 10 minutes. When the absorption periods were lengthened the inhibition gradually

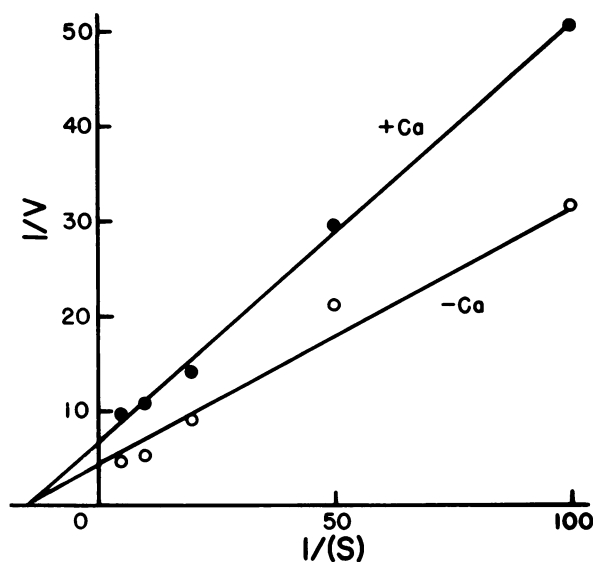


FIG. 7. Lineweaver-Burk plot of the data presented in figure 6.

disappeared and, after 30 to 60 minutes  $\text{Ca}^{2+}$  actually had a promotive effect on  $\text{K}^+$  absorption in corn roots (table I). This transition from inhibition to promotion was especially evident in experiment 3 (table I) where the longest absorption period was 120 minutes. The actual time required for the transition from

inhibition to promotion was somewhat variable. Experiments 2 and 3 were chosen for presentation since they represent the greatest quantitative variation observed in several similar experiments over a period of several months. Experiment 4 (table I) also indicates that the  $\text{Mg}^{2+}$  inhibition of  $\text{K}^+$  absorption is alleviated with time.

### General Discussion

The effect of  $\text{Ca}^{2+}$  on the absorption of other ions by plant roots is obviously quite complicated. In the present case,  $\text{Ca}^{2+}$  (or  $\text{Mg}^{2+}$ ) was found to inhibit the energy dependent transport of  $\text{K}^+$  in corn roots but not in barley or oats. This inhibition was most pronounced when the absorption periods were short and it gradually disappeared as the absorption periods were lengthened. With sufficiently long experimental periods (greater than 30–45 mins) the presence of  $\text{Ca}^{2+}$  resulted in enhanced  $\text{K}^+$  absorption which is similar to results obtained by most other investigators (see Introduction).

A  $\text{Ca}^{2+}$  inhibition of  $\text{K}^+$  or  $\text{Rb}^+$  absorption has been observed previously but always following some severe pretreatment of the roots. For example, exposure of mung bean or soybean roots to ultraviolet light (7, 17), ribonuclease (7, 18), EDTA (7) or in some cases a growing solution devoid of  $\text{Ca}^{2+}$  (19), resulted in increased  $\text{K}^+$  or  $\text{Rb}^+$  absorption. These stimulated absorption rates could then be inhibited by

Table I. *The Effect of Time and Calcium (or Magnesium) on Potassium Absorption by Excised Corn Roots*  
 $\text{CaSO}_4$  and  $\text{MgSO}_4$  concentrations were 0.10 mM.

Experiment	Time mins	K <sup>+</sup> Absorbed		+Ca -Ca × 100
		-Ca	+Ca	
			μmole g <sup>-1</sup>	
1	1.0	0.058	0.014	24.1
	2.5	0.081	0.023	28.9
	5.0	0.129	0.040	30.6
	7.5	0.146	0.050	33.9
	10.0	0.198	0.107	54.0
2	10	0.137	0.080	54.4
	20	0.247	0.187	75.9
	30	0.406	0.374	92.0
	45	0.605	0.514	85.0
	60	0.759	0.997	131.4
3	10	0.144	0.081	56.3
	20	0.261	0.257	98.4
	30	0.390	0.485	124.4
	45	0.620	0.867	139.8
	60	0.837	1.316	157.4
	120	1.968	3.886	197.5
4		-Mg	+Mg	
	10	0.180	0.071	39.4
	20	0.348	0.252	72.9
	30	0.480	0.467	97.3
	45	0.834	0.719	86.2
	60	0.991	1.103	111.1

the addition of  $\text{Ca}^{2+}$  to the experimental solutions. Although these effects of  $\text{Ca}^{2+}$  are similar to those reported here for 'normal' corn roots the results are difficult to compare because of the unknown effects of the various pretreatments.

The  $\text{Ca}^{2+}$  inhibition of  $\text{K}^+$  absorption in corn roots is also similar in some respects to the effect of  $\text{Ca}^{2+}$  on  $\text{Na}^+$  and  $\text{Li}^+$  absorption found in several plant species (4, 5, 10, 16). In these studies the absorption periods ranged from 0 to 3 hours and since  $\text{Na}^+$  and  $\text{Li}^+$  absorption were drastically reduced even after 3 hours of absorption it does not seem likely that these results are like those reported here for  $\text{K}^+$  absorption in corn. Jacobson et al. (10) interpreted their findings to mean that  $\text{Ca}^{2+}$  in some way modified the root surface in such a fashion as to provide an efficient barrier to  $\text{Li}^+$  and  $\text{Na}^+$ . In the presence of  $\text{Ca}^{2+}$ , both  $\text{Li}^+$  and  $\text{Na}^+$  would first have to pass this barrier before the ions could react with the absorption sites. A similar explanation could be advanced to explain the present results except that it seems unlikely that corn and barley roots should not respond similarly to  $\text{Ca}^{2+}$ . There is no consistent evidence that the potential binding of  $\text{Ca}^{2+}$  to exchange sites should be different in corn and barley. In fact, the cation exchange capacities of corn and barley are fairly similar. Drake et al. (2) reported the cation exchange capacities for barley and corn to be 12 and 26 meq/100 g dry wt respectively, whereas Heintze (8) reported them to be 22 for barley and 14 for corn. Thus with the present information one cannot interpret the present results on the basis of different ion binding capacities of the 2 species concerned.

With corn roots, it was shown that  $\text{Ca}^{2+}$  acted as a non-competitive inhibitor of  $\text{K}^+$  transport (figs 6 and 7). This, of course, merely indicates that the action of  $\text{Ca}^{2+}$  must be indirect. That is, if  $\text{K}^+$  is actively transported,  $\text{Ca}^{2+}$  must not compete directly for the  $\text{K}^+$ -carrier site. Or alternately, if  $\text{Cl}^-$  is actively transported and  $\text{K}^+$  is in electrochemical equilibrium across the membrane,  $\text{Ca}^{2+}$  could alter the  $\text{K}^+$  distribution by affecting the active  $\text{Cl}^-$  transfer system. Furthermore,  $\text{Ca}^{2+}$  could also appear to alter energy dependent  $\text{K}^+$  transport (regardless of which ion is actively transported) by decreasing membrane permeability which is indeed one of the classical suggested effects of divalent cations (1). However, in order to explain the different responses of corn and barley to  $\text{Ca}^{2+}$  on the basis of decreased permeability, one must also take into consideration the relative efficiencies of the transport systems of the 2 plant species. Thus, in corn, which relatively has a less efficient transport system than barley, a slight decrease in membrane permeability due to  $\text{Ca}^{2+}$  could explain the initial decrease in  $\text{K}^+$  absorption (i.e. decreased influx). Furthermore, a decreased membrane permeability to  $\text{K}^+$  in the presence of  $\text{Ca}^{2+}$  should not only reduce rates of influx but also rates of efflux (especially after the internal  $\text{K}^+$  concentration became sufficiently high) and the latter phenomena would readily account for the greater  $\text{K}^+$

accumulations (i.e. net flux) after the longer periods of absorption (table I). With barley roots, a slight alteration in membrane permeability due to the presence of  $\text{Ca}^{2+}$ , might be masked by the tremendously efficient ( $K_m = .012 \text{ mM}$ ;  $V_{max} = 7.0 \mu\text{moles g}^{-1} \text{ hr}^{-1}$ ) energy dependent  $\text{K}^+$  transport system and thus no effect of  $\text{Ca}^{2+}$  on  $\text{K}^+$  absorption would be apparent.

Obviously additional information concerning which ions are actively transported, the effect of various ions on the active transport system of a particular ion, and the effect of various ions on membrane permeability is needed before a correct interpretation can be given for these results. However, regardless of the proper interpretation, the finding of species difference with respect to the effects of  $\text{Ca}^{2+}$  on  $\text{K}^+$  absorption should be of help in further studies concerning the mechanism of nutrient absorption. It also serves to emphasize the hazards involved in routinely applying results obtained with one species to other species; unfortunately this has been done especially freely with  $\text{Ca}^{2+}$  ions.

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### Literature Cited

1. COLLANDER, R. 1959. Cell Membranes: Their resistance to penetration and their capacity for transport. In: Plant Physiology—A treatise. F. C. Steward, ed. Academic Press. Vol. II: 3-102.
2. DRAKE, M., J. VENGRIS, AND W. COLBY. 1951. Cation exchange capacity of plant roots. Soil Sci. 72: 139-42.
3. ELZAM, O. E. AND E. EPSTEIN. 1965. Absorption of chloride by barley roots: Kinetics and selectivity. Plant Physiol. 40: 620-24.
4. EPSTEIN, E. 1961. The essential role of calcium in selective cation transport of plant cells. Plant Physiol. 36: 437-44.
5. EPSTEIN, E. 1960. Calcium-lithium competition in absorption by plant roots. Nature 185: 705-06.
6. EPSTEIN, E., D. W. RAINS, AND O. E. ELZAM. 1963. Resolution of dual mechanism of potassium absorption by barley roots. Proc. Natl. Acad. Sci. 49: 684-92.
7. FOOTE, B. AND J. B. HANSON. 1964. Ion uptake by soybean root tissue depleted of calcium by ethylenediamine tetraacetic acid. Plant Physiol. 39: 450-60.
8. HEINTZE, S. G. 1961. Studies on cation-exchange capacities of roots. Plant Soil XIII: 365-83.
9. KAHN, J. S. AND J. B. HANSON. 1957. The effect of calcium on potassium accumulation in corn and soybean roots. Plant Physiol. 32: 312-16.
10. JACOBSON, L., D. P. MOORE AND R. J. HANNAPEL. 1960. Role of calcium in absorption of monovalent cations. Plant Physiol. 35: 352-58.
11. LEGGETT, J. E. AND E. EPSTEIN. 1956. Kinetics of sulfate absorption by barley roots. Plant Physiol. 31: 222-26.

12. LEGGETT, J. E., R. A. GALLOWAY, AND H. G. GAUCH. 1965. Calcium activation of orthophosphate absorption by barley roots. *Plant Physiol.* 40: 897-902.
13. MARINOS, N. G. 1962. Studies on submicroscopic aspects of mineral deficiencies. I. Calcium deficiency in the shoot apex of barley. *Am. J. Botany* 49: 834-41.
14. OVERSTREET, R., L. JACOBSON, AND R. HANDLEY. 1952. The effect of calcium on the absorption of potassium by barley roots. *Plant Physiol.* 27: 583-90.
15. PITMAN, M. G. 1964. The effect of divalent cations on the uptake of salt by beetroot. *J. Exptl. Botany* 15: 444-57.
16. RAINS, D. W. AND E. EPSTEIN. 1967. Sodium absorption by barley roots. Role of the dual mechanisms of alkali cation transport. *Plant Physiol.* 42: 314-18.
17. TANADA, T. 1955. Effects of ultraviolet radiation and calcium and their interaction on salt absorption by excised mung bean roots. *Plant Physiol.* 30: 321-25.
18. TANADA, T. 1956. Effects of ribonuclease on salt absorption by excised mung bean roots. *Plant Physiol.* 31: 251-53.
19. TANADA, T. 1962. Localization and mechanism of calcium stimulation of rubidium absorption in the mung bean root. *Am. J. Botany* 49: 1068-72.
20. VIETS, F. G. 1944. Calcium and other polyvalent cations as accelerators of ion accumulation by excised barley roots. *Plant Physiol.* 19: 466-80.