Plant Physiol. (1969) 44, 1182–1186

Magnesium Uptake by Soybeans

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Received February 4, 1969.

Abstract. Magnesium contents of soybean (*Glycine max*) roots increase and the K and Ca contents decrease with increased $MgCl_2$ concentrations in ambient solutions. The Mg uptake is inhibited when both Ca and K are present in the solution, but not by K or Ca alone. Chloride uptake, which is very low from the $MgCl_2$ solution, is greatly enhanced by the presence of K. The selectivity against Mg imparted by K + Ca appears to be at an external barrier for cation uptake as shown by its dependence on the presence of Ca in the external solution. The Ca content of roots is influenced only slightly by changes in external Ca concentrations from 10^{-4} to 10^{-2} M, but that of shoots is greatly enhanced as the Ca concentration is increased or the K concentration is decreased. These effects on Ca contents are explained as arising from transport to the shoot without involvement of vacuoles of root cells.

Salt uptake by plants involves interactions between the major nutrient cations, K^+, Ca^{2+}, and Mg^{2+}, as well as Na^+ and H^+ under experimental and natural conditions. Predominant attention has been focused on the singly charged cations, K^+ and Na^+, and to a lesser extent, effects of Ca^{2+} presence on them. Collander (2) included doubly charged cations in his study of cation uptake by many species. Moore *et al.* (17) followed the uptake of Mg^{2+} by excised barley roots as influenced by Ca^{2+}. Attention to interactions of several cations, however, is very limited and the interactions are not understood (6, 10).

We have shown previously that Mg^{2+} uptake from single-salt solutions by soybean roots is accompanied by loss of K^+, Ca^{2+}, and Cl^- (13). Observations are reported here on the 3-fold interactions among Mg^{2+}, Ca^{2+}, and K^+.

Materials and Methods

Excised Roots. Roots were obtained from soybean seedlings (*Glycine max* L. Merr. var. Hawkeye) (13). Briefly, seeds were soaked in water 6 hr, then planted on a stainless steel screen suspended over a continuously aerated 0.2 mM $CaSO_4$ solution. The plants were grown in a dark chamber maintained at a temperature of 27°. At the end of 4 days, the roots were excised, cut to 6-cm length from the apex and suspended in a 0.2 mM $CaSO_4$ solution. The elapsed time between root excision and experiment initiation was less than 2 hr.

Excised root samples weighing 0.5 g were used in each liter of test solution. The initial solution pH of 5.6 was not adjusted during the 24-hr uptake experiment. It was more convenient to adjust the root to solution ratios based upon previous experience such that the final pH values would be in the range of 5.1 to 5.5.

At the end of the experimental period, the roots were rinsed, transferred to beakers, and heated in a muffle oven to 480° for 2 hr. The ash was dissolved in 20 ml of 0.1 HNO_3 and 10 % acetic acid solution and reserved for analysis.

Chloride analysis of plant roots after the above dry-ashing procedure was compared with the analysis after several hot water extractions. The Cl^- value was the same for both procedures. However, Cl^- will be lost if the ash is maintained at 480° for more than 8 hr. Selection of the 2-hr period was made on the basis of complete ashing and no Cl^- loss. We did not attempt to relate Cl^- loss more precisely to the ashing period.

Intact Plants. Lots of 5 dark-germinated seeds were placed on gauze in a holder with their roots extending into a 0.2 mM $CaSO_4$ solution. These lots were grown on a 15-hr day at 27° for 7 days when the first trifoliolate leaf was expanding. At this time, the 5 plants had produced about 2 g of roots and 3 to 4 g wet weight of shoots. The lots were transferred after rinsing to experimental solutions as desired. Roots and shoots were separated at the end of the experimental period and prepared for analysis as described for excised roots.

Inorganic Analysis. Cation contents of the plants and solutions were determined by atomic absorption spectrophotometry (4). Chloride contents of the samples were assayed with an Aminco-Cotlove automatic chloride titrator (3).

Results and Discussion

Uptake by Excised Roots. Magnesium uptake by soybean roots from $MgCl_2$ solutions was observed

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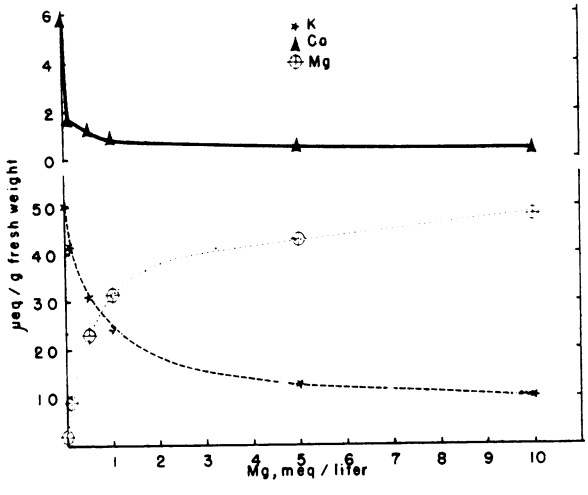

FIG. 1. Influence of the $MgCl_2$ concentration of absorption solutions on the Mg, K, and Ca contents of excised soybean roots. Absorption time, 24 hr.

over a concentration range of 0.1 to 10 mM (Fig. 1). These roots decreased in K^+ and Ca^{2+} content with increase in Mg^{2+} concentration. The attained chloride level was below 1 μeq g^{-1} fresh weight of roots for the 24-hr uptake at all $MgCl_2$ concentrations. Although the Mg^{2+} uptake approximates the loss of K^+ plus Ca^{2+}, we hesitate to infer that Mg^{2+} uptake is dependent only on an equivalent K^+ plus Ca^{2+} exchange because the charge balance of K^+ plus Ca^{2+}, and Cl^- loss is less than the Mg^{2+} uptake for roots previously loaded with KCl (*cf.* Fig. 4 ref 13). Nevertheless, in the absence of Cl^- entry, Mg^{2+} uptake must occur in exchange for other cations.

If the loss of K^+ and Ca^{2+} represented a general cellular "leakiness," a decreased respiration rate might be expected to result from simultaneous loss of substrates. Oxygen consumption and CO_2 production, however, remained constant, independent of the Mg^{2+} treatment (13). The results rather indicate increased membrane permeability only with respect to the inorganic cations.

A further test for the integrity of the cellular structures is afforded by results with 2,4-dinitrophenol (DNP) (table I) which was used as a metabolic inhibitor without regard to its exact mode of action. The observed reduction by DNP of the Mg and K contents in the tissue indicated that the ion levels depended on metabolism. Barker and Koontz (1) in studying DNP and Ca^{2+} uptake concluded that DNP altered the barrier to both DNP and Ca^{2+} transport. In the present report, DNP induced loss of Mg^{2+}, Ca^{2+}, and K^+, whereas the high Mg^{2+} accumulation was correlated only with the loss of Ca^{2+} and K^+. Magnesium accumulation must result from an energy requiring step because of the observed DNP inhibition.

Table I. *Influence of DNP on Ion Retention by the Soybean Root*

Absorption time was 24 hr.

Treatment	Tissue content K	Mg	Ca
		μeq/g fresh wt	
$CaSO_4$ 0.2 mM	55	3	3
$MgCl_2$ 10 mM	5	46	<1
DNP 0.01 mM	0	2	2
$MgCl_2$ 10 mM + DNP 0.01 mM	0	18	<1
H_2O	50	3	2

Some of the influences of cation interactions are shown by results in Fig. 2. The solution used initially contained 10 mM $MgCl_2$ at various pH's. The roots lost both K^+ and Ca^{2+} at each acidity. Potassium losses were greatest under the less acid conditions. Magnesium uptake increased with increasing pH, with the greatest change occurring up to pH 5.

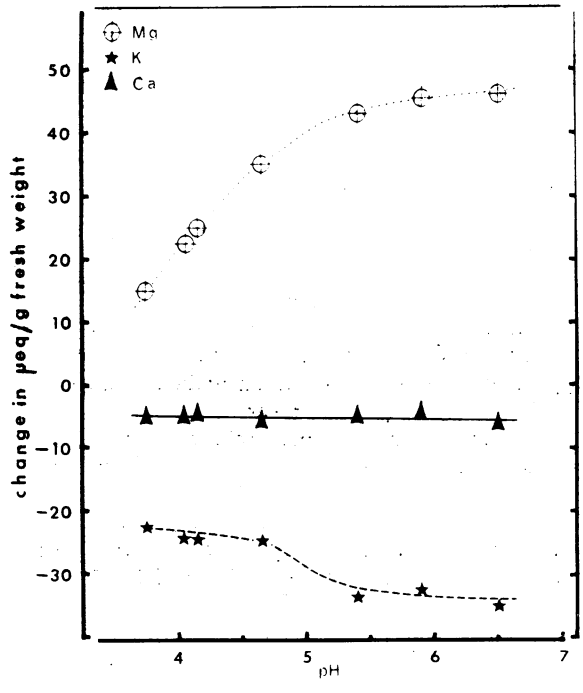

FIG. 2. Influence of H ion concentration on the changes in Mg, K, and Ca contents of excised soybean roots in a 10 mM $MgCl_2$ solution for 24 hr. The roots were grown in 0.2 mM $CaSO_4$.

Attempts to determine the amount of total root Ca^{2+} necessary to induce a change in ion preference have not been successful. The evidence points to the necessity for Ca^{2+} in the external solution to maximize this ion preference (5, 8, 9, 12, 18), although the presence of external Ca^{2+} does not prevent Ca^{2+} loss from the root (13). Anion uptake can also be maximized by addition of Ca^{2+} to the external solution (12, 13, 14). A case for increased prefer-

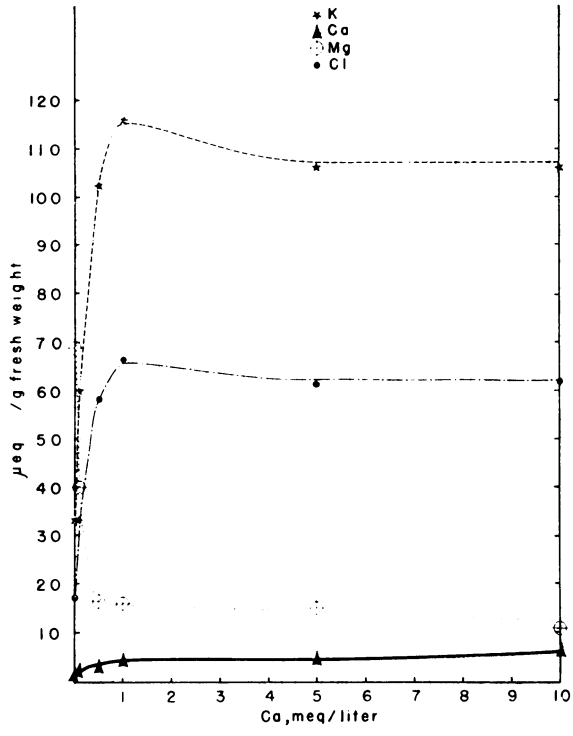

FIG. 3. Influence of $CaCl_2$ concentration of absorption solutions on the Mg, K, Ca, and Cl contents in excised soybean roots. Solution composition: 5 mM KCl + 2.5 mM $MgCl_2$ + 0 to 5 mM $CaCl_2$. Absorption period, 24 hr.

ence in uptake of 1 anion over another is not as obvious as for cations. The increased uptake in the presence of Ca^{2+} reported by Viets (18) seemingly applies to either cation or anion without a dependent coupling of the uptake for each ion.

Calcium in the external solution increased K^+ uptake (5, 8, 9, 12, 14, 18) and decreased Mg^{2+} uptake (10, 17) by barley roots. Although K^+ uptake by soybean roots was increased, the Mg^{2+} uptake was only slightly reduced in the presence of Ca^{2+}. Potassium was also an ineffective inhibitor of Mg^{2+} uptake. Addition of both K^+ and Ca^{2+} to the Mg^{2+} solution drastically reduced Mg^{2+} uptake (Fig. 3). A Ca^{2+} concentration of 0.1 meq/liter was sufficient to invert the $Mg^{2+} - K^+$ ratio value in the root when equivalent amounts of K^+ and Mg^{2+} at a total concentration of 10 meq/liter were in the external solution. The time course of these interactions is shown in Fig. 4. The Mg^{2+} uptake is essentially complete within 1 hr. Likewise the loss of Ca^{2+}, presumably in exchange for Mg^{2+}, occurred during the first hr of the uptake period. Potassium uptake was positive for every sampling time in contrast to the observed loss in the absence of Ca^{2+}. The maximum Ca^{2+} effect near 1 meq/liter (Fig. 3) was also observed in excised barley roots for the uptake of K^+ (5, 8), SO^{2-}_4 (11), and PO^{3-}_4 (12).

The interaction of Mg^{2+}, K^+, and Ca^{2+} leading to the greater preference for K^+ and a restriction of Mg^{2+} uptake occurs at the external solution-root surface interface. We have previously proposed that the interaction takes place at the epidermal cell layer (13), while others consider that intercellular interfaces are involved (9, 15). We find the following features of ion uptake by soybean roots placed in a $K^+ + Ca^{2+} + Mg^{2+}$ chloride solution (A) further Ca^{2+} accumulation in the roots is small (Fig. 3), (B) some changes in cation content take place quickly (Fig. 4), (C) the Cl^- increases parallel those of K^+ (Fig. 3), and (D) the Mg^{2+} accumulation stops after 1 hr (Fig. 4). In these experiments, Ca^{2+} affected the influx of Mg^{2+}, K^+, and Cl^- more than their efflux since the absorbed Mg could not be removed by incubation of Mg-loaded roots in a $Ca^{2+} + K^+$ solution. Mengel and Helal (14) concluded that Ca^{2+} reduces the K^+ efflux rather than increasing the influx thereby providing a greater net accumulation. The $Ca^{2+} - K^+$ mediated effects on selectivity for K^+ uptake relative to Mg^{2+} are not readily explainable by a change in relative flux rates for K^+ uptake alone. Rather, calcium has some distinct influences on membrane properties upon which K^+ effects are superimposed.

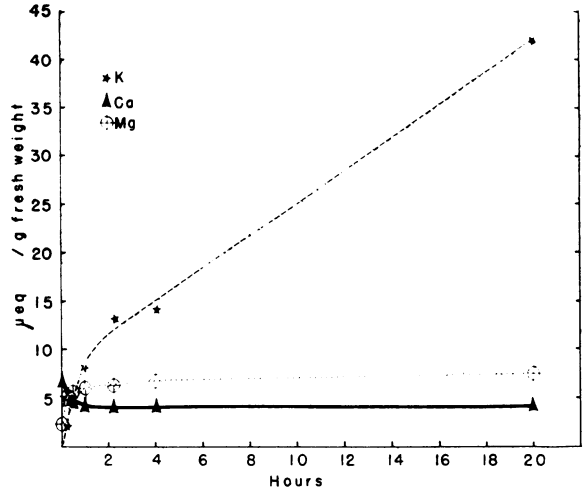

FIG. 4. Cation contents of roots incubated in Ca + Mg + K salt solutions as a function of time. Solution composition: 1 mM $CaCl_2$ + 5 mM KCl + 2.5 mM $MgCl_2$.

Regulation of Mg^{2+} uptake by $Ca^{2+} + K^+$ is clearly evident in the case of solutions of varying $Ca^{2+} - Mg^{2+}$ ratios and constant ionic strength. In a solution with a total ($Mg^{2+} + Ca^{2+}$) salt concentration of 10 meq/liter (Fig. 5) roots maintained their initial K^+ content and increased their Cl^- content in the presence of calcium. In the absence of Ca^{2+}, the K^+ and Cl^- contents approached zero. Calcium contents decreased, as would be expected from completion, as the $Ca^{2+} - Mg^{2+}$ ratio decreased from 1 to 0. The inhibition of Mg^{2+} uptake by Ca^{2+}, however, was negligible as can be determined by comparing Mg^{2+} uptake at equal concentrations in Fig. 1 and 5, and as shown by the slight change in

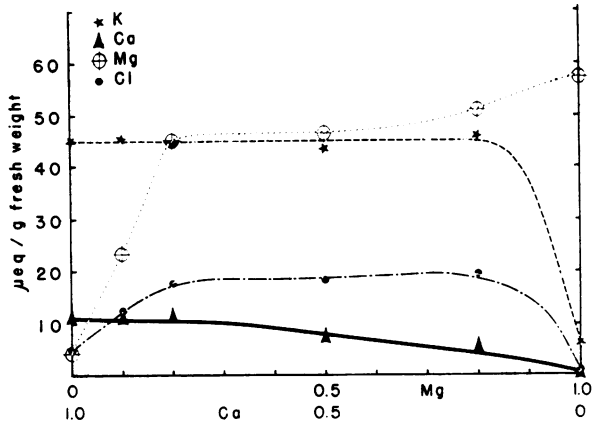

FIG. 5. Influence of Ca/Mg on the Mg, K, Ca, and Cl contents of excised soybean roots in 5 mM (Ca + Mg)Cl_2 solutions for 24 hr.

Mg^{2+} content at intermediate Ca^{2+} — Mg^{2+} ratios (Fig. 5). The Cl^- content of the root remained below 20 meq/kg fresh weight in these experiments in which the Cl^- concentration in the solution was 10 meq/liter and K^+ was not an initial constituent.

Potassium at 1 meq/liter in the 10-meq/liter Ca^{2+} + Mg^{2+} solutions strikingly shifted the root ion contents in the 24-hr experimental period (Fig. 6). The Ca^{2+} contents remained essentially unaffected by the addition of KCl (*cf.* Fig. 5 and 6). The K^+ and Cl^- contents approximately doubled over the range of 20- to 80-mole percent Ca^{2+} in which range K^+:Cl^-:Ca^{2+} ratios within each experiment were relatively constant (*cf.* Fig. 5 and 6). The Mg^{2+} content in the presence of Ca^{2+} and K^+, on the other hand, decreased about 5-fold relative to the level in the absence of K^+. With a Ca^{2+} to Mg^{2+} ratio of 1.0, the Ca^{2+} and Mg^{2+} contents were equal in the presence of K^+ (Fig. 6), but in the absence of K^+, the Mg^{2+} content of the roots exceeded the Ca^{2+} level by about 5-fold (Fig. 5). In the absence of Ca^{2+}, the Mg^{2+} content exceeded K^+ by about

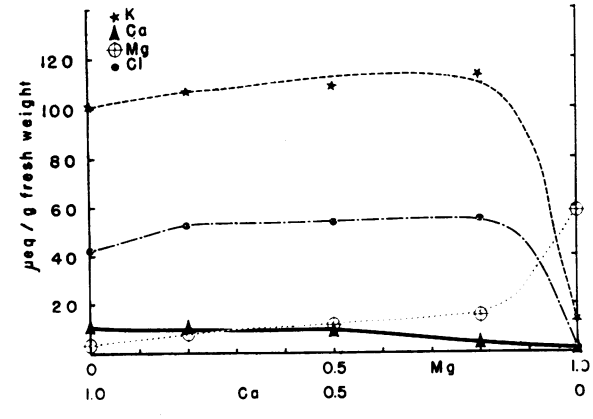

FIG. 6. Influence of Ca/Mg on the Mg, K, Ca, and Cl contents of excised soybean roots after 24 hr in 1 mM KCl and 5 mM (Ca + Mg)Cl_2 solutions. (Note the change in ordinate scale from Fig. 5).

2- and 5-fold (Fig. 3 and 6). For the conditions represented in Fig. 6, the roots actually lost 11 μeq/g of their initial K^+ to the solution. When the Ca^{2+} concentration in the solution was 0.1 meq/liter in the presence of 5 mM KCl + 2.5 mM $MgCl_2$, that is, Ca^{2+}/Mg^{2+} = 0.02, the K^+ and Mg^{2+} contents were about equal (Fig. 3). At Ca^{2+}/Mg^{2+} = 0.1, the K^+ exceeded Mg^{2+} by 7-fold (Fig. 3).

Thus, it is evident that the predominance of K^+ over Mg^{2+} uptake by soybean roots depends on the presence of Ca^{2+} in the external solution. Also, restricted Mg uptake depends on the presence of both K^+ and Ca^{2+} in the external solutions. These controlling interactions appear to be exerted on the solution side of the rate-limiting barrier for uptake, which might be considered as the solution side of a limiting membrane. Time courses of adjustments to initial conditions are correspondingly rapid as shown in Fig. 4. There is a symmetrical exchange of Ca^{2+} and Mg^{2+} which is essentially complete in 30 min, after which the uptake of Mg^{2+} is very low and symmetrical with a small Ca^{2+} loss (Fig. 4). In this 30-min period, the rapid initial change of K^+ content shifts to a steady increase, that is, a constant uptake rate.

Uptake by Intact Plants. Ion uptake by intact plants is affected by growth and transport from the root to the shoot. Kinetics of uptake are best followed on initially low-salt tissue in order to maximize rates of accumulation. Soybean plants can be grown on $CaSO_4$ solutions for about 2 weeks before mineral-deficiency symptoms appear. Roots obtained from either etiolated or green soybean plants cultivated in $CaSO_4$, however, have relatively high salt contents compared with low-salt barley roots. The high level of K^+ in the tissue is due to the high K^+ content of the soybean seed.

Results in table II are for an uptake period of 24 hr from the indicated solutions for plants grown on 0.2 mM $CaSO_4$, with the initial composition as shown in line 1 of the table. The several test solutions contained either nitrate (soln 1), nitrate + sulfate (soln 2), sulfate (soln 3), or chloride as predominant anions. The cation concentrations of both the root and shoot tissues increased by 1.2- to 2.0-fold, indicating that the plants shifted from a relatively low- to a high-salt state. Ion accumulation and transport are reflected rather than growth.

Effects of K^+ + Ca^{2+} on restriction of Mg^{2+} uptake (table II), although greatest in the root tissue, are also clearly evident in the shoot composition. The shoots, which were initially higher in Mg^{2+} than the roots, probably did not approach a steady state for salt accumulation in the 24-hr test period. The Ca concentrations of the roots of the intact plants were similar to the excised ones in being relatively constant in the several solutions. The Ca^{2+} concentrations in the shoot, on the other hand, increased greatly in soln 1 (0.1 Hoagland) and soln 2 ($-K^+$) relative to the initial values. These results indicate a continued uptake by and

Table II. *Cation Content of $CaSO_4$-grown Plants After 24-Hr Uptake Period From Various Salt Solutions*

Treatment	Tissue content: Root K	Root Mg	Root Ca	Root Σ	Shoot K	Shoot Mg	Shoot Ca	Shoot Σ
	μeq/g fresh wt							
	Initial content, prior to 24-hr uptake period							
$CaSO_4$, 0.2 mM	41	4	13	58	64	13	32	109
	Content after 24-hr uptake period							
Soln 1[1] [0.1 Hoagland (7)]	65	13	16	94	99	19	47	165
Soln 2[2] (-K)	29	56	19	104	64	27	88	179
Soln 3[3] (-N)	82	6	10	98	109	23	43	175
$MgCl_2$, 10 mM	40	39	7	86	78	19	35	132
KCl, 10 mM	105	4	9	118	81	17	33	131

[1] Soln 1 = 0.5 mM KNO_3; 0.1 mM KH_2PO_4; 0.5 mM $Ca(NO_3)_2$; 0.2 mM $MgSO_4$.

[2] Soln 2 = 0.05 mM $Ca(H_2PO_4)_2$; 0.5 mM $Ca(NO_3)_2$; 0.2 mM $MgSO_4$.

[3] Soln 3 = 0.25 mM K_2SO_4; 0.05 mM $Ca(H_2PO_4)_2$; 0.2 mM $CaSO_4$; 0.2 mM $MgSO_4$.

movement of Ca^{2+} through the root as would be expected if Ca^{2+} is essentially excluded from the root vacuoles. This is based on the assumption that most of the Cl^- accumulates in the vacuoles and from the observed negligible Cl^- uptake by soybean roots from a $CaCl_2$ solution.

A similar, more direct observation of Ca^{2+} uptake and movement of Ca^{2+} through the root was made by Moore, Mason, and Maas (16). They observed an initial rapid uptake of ^{45}Ca by excised barley roots with a negligible accumulation, while Ca^{2+} in the exudate was 3- to 5-fold the external solution Ca^{2+} concentration. The epidermal and cortical root cells apparently do not accumulate Ca^{2+}. They suggest that the barrier to Ca^{2+} uptake and the concentrating step occurs at the endodermis upon transfer into the conducting vessels. Their results suggest that the accumulation of Ca found in plant shoots probably takes place in the root. One must add that the stem and leaf cells also accumulate Ca^{2+} in contrast to the negligible accumulation by the root cells.

Omission of K^+ (soln 2), while greatly enhancing Mg^{2+} uptake relative to the complete nutrient (soln 1) did not significantly change the total cation content (Σ). Omission of nitrate (soln 3), leaving sulfate as the predominant anion, reduced Mg uptake without essentially changing Σ of cations in roots or shoots. The balance in Σ of cations in the root was effected by the reciprocal changes in K^+ and Mg^{2+}, with the effect of Ca^{2+} being important in the shoot. Absence of nitrate, however, enhanced K^+ contents in both tissues.

We cannot fully assess the anion and cation interactions, which are incidental to the main purpose of the work. There is little doubt, however, that the K^+, Mg^{2+}, and Ca^{2+} uptakes are interdependent and that these interactions are shown both by the excised roots and the intact plant.

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