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Localization of the Ca-Mediated Apparent Ion Selectivity in the Cross-Sectional Volume of Soybean Roots

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Summary. A major portion of the calcium in a soybean root appears to be in less than 10 % of the root volume. Specifically, Ca is considered to be almost entirely localized in the epidermal cell layer. This relationship was established from consideration of rates and extent of ion absorption and ion interactions during the absorption process.

Presence of calcium at the root-solution interface was associated with a change in the apparent selectivity of K over Mg by soybean roots. Accumulation of calcium by soybean roots was negligible.

Essentiality of Ca for cellular growth and survival has long been recognized. The range in observations includes the Ca reversal of KCl-induced paralysis in *Tubifex rioulorum* (24), a Ca-induced increase of potential differences across muscle (4), a decrease in the yeast cell volume in the presence of Ca (13), Ca or Mg associated shrinkage of smooth muscle (3), and increased rate of cation (8, 20, 23, 26), and anion (10, 15) absorption in plant tissue, as well as an increased preference for a particular cation (8) and changes in the internal pH of mitochondria (5).

Calcium is often considered to react with the plant root near the periphery of individual root cells both in its absorption and its influence on other ions (18, 19). These deductions rest solely on the necessity for the presence of Ca in the external solution in order to obtain an increased K absorption. However, the influence of Ca is not fully recognized as to its function in transport as is evident by use of single salt in experiments with varying salt concentrations or ones including a constant amount of Ca. In either case, the interaction of ions, dependent on their ratios and concentrations, can be observed. During the course of recent absorption studies, we have deduced the localization of the root Ca and a Ca influence on absorption of ions. Observations were made on the levels and rate of change of salt composition of soybean roots in various solutions. The data indicate that a large percentage of the Ca is located in less than 10 % of the root volume occupied by K. Although K appears to be uniformly distributed in the root, the Ca is localized in 10 % of the root volume near the external solution-root interface. Calcium is capable of rapid modification of this interface and results in quick changes in both individual and relative ion absorption rates as well as in ion retention levels.

Methods

Excised Roots. Soybean seeds (150 g of *Glycine max* L. Merr., var. Hawkeye) were soaked in aerated water for 6 hours and distributed on cheesecloth supported on a stainless steel screen. The screen was placed on top of a polyethylene tray (13 × 11 × 5 inches) containing 2×10^{-4} M $CaSO_4$ solution at a level 1 cm below the screen. A stainless steel screen covered with cheesecloth was placed over the seeds. The tray was covered with a sheet of plastic and placed in a constant temperature chamber (27°) with solution being continually aerated. Forty-eight hours after planting, the top cheesecloth and steel cover were removed. Four days after planting, the roots were excised, cut to a 6 cm length, measured from the root apex and suspended in a 2×10^{-4} M $CaSO_4$ solution.

The excised roots were removed from the solution and blotted on absorbent tissue to remove adhering solution. Root samples of 0.5 g portion were weighed, rinsed 3 times with water and transferred to 1-liter volumes of test solution (chloride salts). At the end of the absorption period, the roots were removed, rinsed 3 times with water and transferred to 30 ml pyrex beakers. The samples were placed in a muffle oven and heated to 480° for 2 hours. After cooling, the ash was dissolved in 20 ml of a solution containing 0.1 N HNO_3 and 10 % (v/v) acetic acid.

Inorganic Analysis. Potassium, sodium, calcium, magnesium, and chloride were determined in both root samples and absorption solutions. The cations were determined by usual techniques of

flame emission for K and Na (11), or by atomic absorption for K, Na, Ca, and Mg (7). Chloride content of root samples and absorption solutions was measured by conductometric titration (6). Solutions were analyzed at the conclusion of the absorption period to verify any excess removal of ions and as a check on the original composition.

The pH was determined at the beginning and at the end of an absorption period on a small aliquot of the solution. In the observations reported here, the difference between initial and final pH values were less than 0.2 unit. This change from pH 5.5 was not considered to be significant for the ion absorption under study and we, therefore, did not make periodic adjustments.

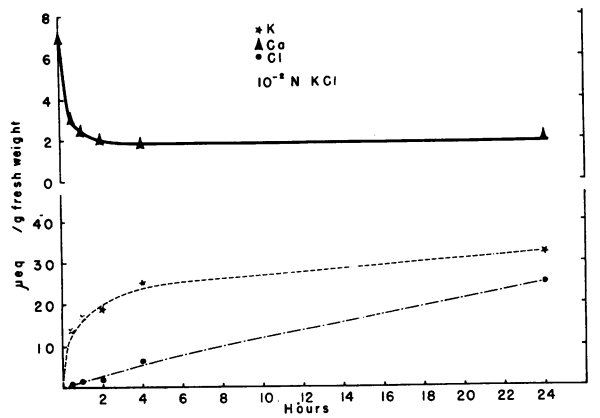

FIG. 1. Influence of 10^{-2} N KCl on the net uptake of K and Cl and the loss of Ca by soybean roots as a function of time.

Results

The interaction of a plant root with its environment can be evaluated from observations of ion movements between the 2 systems. Thus, one finds that excised roots placed in a KCl solution lose Ca and accumulate K and Cl (fig 1). These values represent net uptake of K and Cl and the Ca content at the indicated times. It is readily apparent that one is observing a dynamic system and hence must proceed cautiously with interpretations founded on observations of a particular ion. The high rate and extent of Ca loss was unexpected. Loss of Ca by soybean roots was not peculiar to this KCl concentration. However, as to be expected, the Ca loss decreased with decreasing KCl concentrations with the loss to 10^{-4} N KCl being 0.5 of that in 10^{-2} N KCl. Excised roots in water for an equivalent time period as in salt solutions did not lose Ca. Thus, one must conclude a negligible association of a Ca-salt in the apparent free space of the root under these experimental conditions.

The initial time course of Ca loss from soybean roots placed in a $MgCl_2$ solution was found to be similar to the loss in KCl. Nevertheless, Ca loss was greater for roots placed in $MgCl_2$ than a KCl solution (figs 2, 3, 4). The data in figures 2, 3, and 4 are for the amount of the indicated element present in the root at the time of measurement. Entry of Mg is always equal or slightly greater than the Ca loss at all times of measurement. In general, uptake of Cl from a $MgCl_2$ solution does not occur to any measurable extent. Although the entry of Mg can approach that of K, the Cl uptake does not appear to be associated nor mediated by Mg.

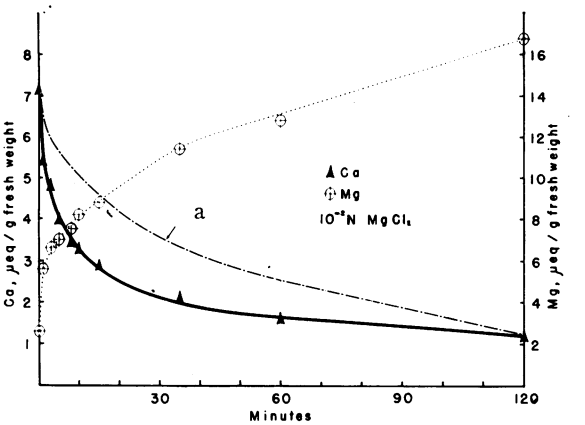

FIG. 2. Influence of 10^{-2} N $MgCl_2$ on the gross content of Mg and loss of Ca by soybean roots as a function of time. Line a: Theoretical loss of Ca by diffusion where $D = 3 \times 10^{-8}$ cm^2 sec^{-1} and r = 0.3 mm.

The loss of Ca from excised roots to a solution containing both Mg and K is similar to that observed in the absence of K. Data of figure 3 were obtained on roots placed in a 5×10^{-3} N $MgCl_2$ solution for various time periods. One observes an immediate loss of Ca and an accumulation of K and Cl. Potassium loss was apparent

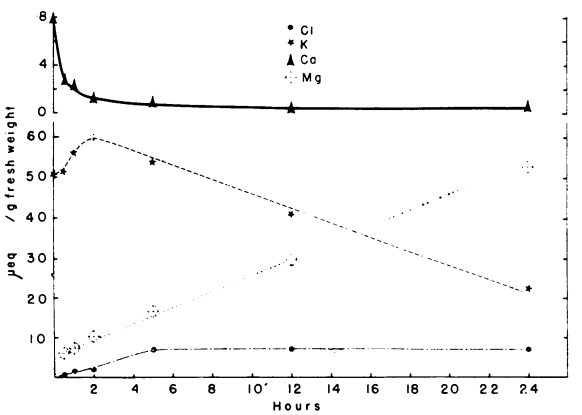

FIG. 3. Change in the gross content of K and Ca and net uptake of Mg and Cl of soybean roots in a 5×10^{-3} N KCl + 5×10^{-3} N $MgCl_2$ solution as a function of time.

at times exceeding 2 hours and continuing to the last observation at 24 hours.

Soybean plants were grown in a 5×10^{-3} N KCl plus 2×10^{-4} M $CaSO_4$ solution for 24 hours prior to root excision in order to examine Ca loss from high-salt roots. These roots were placed in 1×10^{-2} N $MgCl_2$ and at various times up to 24 hours examined for Ca, K, Cl, and Mg content (fig 4). The loss of Ca began immediately, an observation in agreement with the previous data. As was the case in figure 3, the loss of K became very evident near the 2-hour period where the Ca loss was asymptotically approaching zero. The chloride content of these roots decreased nearly to zero after being in $MgCl_2$ for 24 hours. Examination of data in figure 3 and 4 also indicates that the rates of K loss and Mg entry were greater from the $MgCl_2$ solution than in the $MgCl_2$ plus KCl solution without a noticeable influence on the Ca loss.

No attempt is made to evade the fact that soybean roots may lose K because they are damaged in some manner as a result of Mg uptake. This damage may be explained on the basis of a Ca loss which is directly associated with the structural changes of membranes as observed by Marschner and Gunther (21). However, damage as expressed by the K loss was not evident as assayed by respiration rates. The rate of O_2 consumption and CO_2 production was observed to be approximately 4 and 3 μmole hr^{-1} g^{-1}, respectively, under all experimental conditions. Respiration of the Mg-treated roots was slightly higher than for those in Ca, thereby eliminating a possibility of a Mg impairment of the respiratory enzymes or loss of substrates to the external solution through a leaky membrane.

The fact that root damage does occur under certain of these experimental conditions may be beneficial with respect to attempts at relative localization of ions within a root. Thus, we observed the rates of loss of particular ions as well as the ratio of their loss as an indication of their distribution in the root. The shift in ion content of the KCl-loaded roots during Mg uptake in the 0- to 2-hour time period was an accumulation of Mg and a loss of Ca, K, and Cl of 13.9, 5.2, 7.0, and 1.2 μeq g^{-1}, respectively (fig 4). Expressed as a percent of the initial content, the Ca decreased by 87 % and the K by 9 %. These results present evidence of a heterogenic distribution of a major portion of the Ca with the K of the root. However, the remaining Ca, K, and Cl would seem to be distributed homogeneously within the root volume because each decreased at the rate of 9 % every 2 hours after the second hour of the experiment.

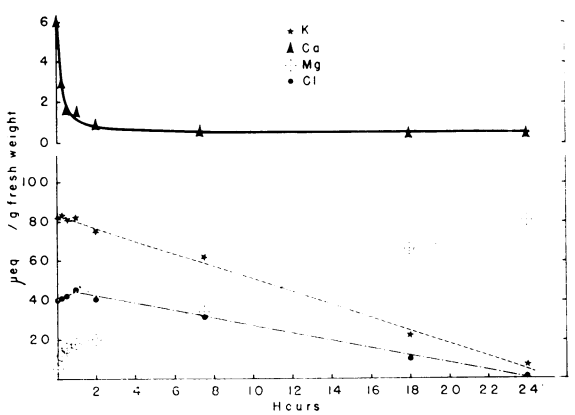

FIG. 4. Influence of 10^{-2} N $MgCl_2$ on the gross content of Mg, Ca, K, and Cl of soybean roots as a function of time. Root pretreated for 24 hours prior to excision in a 5×10^{-3} N KCl + 2×10^{-4} M $CaSO_4$ solution.

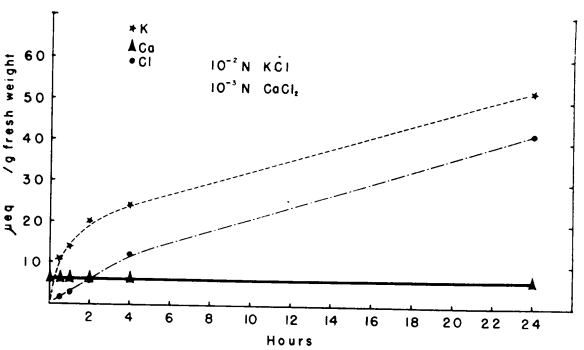

FIG. 5. Influence of 10^{-2} N KCl + 10^{-3} N $CaCl_2$ on the net uptake of K and Cl and the gross content of Ca of soybean roots as a function of time.

Both potassium and chloride uptake were increased by the presence of Ca in the external solution (see figs 1 and 5). Values reported for K and Cl represent net uptake, whereas those for Ca represent total content at indicated times. The increase for both K and Cl uptake in the presence of Ca was approximately 1.6-fold at the end of 24 hours. Calcium increased in some manner both the rate and amount of Cl uptake beginning at zero time. As for K, an increase in rate and amount of uptake was more pronounced between the 4- and 24-hour time period. That the influence of Ca on the uptake of K and Cl must be at an interface bathed by the external solution is concluded from these observations because (1) the effect of Ca was immediately observed, and (2) the Ca content of the root did not increase during an uptake period of 24 hours (fig 5).

The influence of Ca on the preference of uptake between 2 cations may also indicate the localization of the interaction. Soybean roots accumulate relatively more Mg than K from a solution containing equivalent concentrations of Mg and K (table I). However, addition of Ca to such a solution reverses the uptake ratio, i.e. the roots accumulate more K than Mg. Further increases in Ca concentration resulted in a slight increase in the preference for

Table I. *Effect of Ca on K, Mg, and Cl Absorption by Excised Soybean Roots*

Absorption solution: KCl = $MgCl_2$ = 5 × 10^{-3} N. Absorption period: 24 hours. Initial content: K = 46; Ca = 6; Mg = 3; Cl = 0.2 μeq/g.

Solution conc Ca	Tissue content K	Mg	Cl	Ca
meq/l		μeq/g fr wt		
0	35	69	17	0.3
0.1	60	40	34	1.0
0.5	101	17	59	1.3
1.0	113	16	66	2.0
5.0	103	16	61	4.3
10.0	106	12	62	5.8

K as well as an increase in the level of Ca. This relationship was surprising because the addition of 0.5 me/liter of Ca induced an infinite net change relative to absence of Ca in the K-Mg ratio, while the root content of Ca had decreased 4.7 μeq/g below that of the initial value. Because Ca in the external solution induced such a change without a major change in the root Ca content suggests a drastic modification of the interface between the external solution and the root.

The rate of removal of Ca and K from roots was determined for roots placed in 0.1 N HCl to further elucidate the proximity of root Ca to the external solution. It was observed that in 10 seconds the roots lost 40 % and 3 % of the initial content of Ca and K, respectively (fig 6). The loss of Ca was initially fast followed by a slower rate approaching that observed for K. Potassium content of the root initially decreased as a linear function of time as if it had a homogenous distribution in the root. The distribution of K relative to that of Ca is heterogenous and the external solution must be readily accessible to the Ca because of its initial rapid loss.

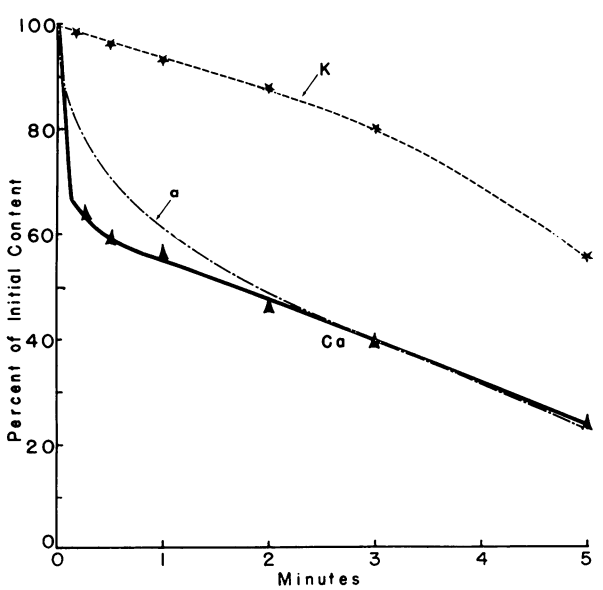

FIG. 6. Influence of 0.1 N HCl on the gross content of Ca and K of soybean roots as a function of time. Line a: Theoretical loss of Ca by diffusion where D = 5 × 10^{-7} cm^2 sec^{-1} and r = 0.3 mm.

Calcium Loss—Actual and Theoretical Diffusion Rates. Investigations of ion uptake by excised roots usually leads to consideration of separation of accumulated ions from those associated in the apparent free space (AFS). This free space contains the cations and anions in the same concentration as the external solution as well as some exchangeable cations (22). The salt in the free space is readily lost to water or solution of a different salt. Half-time of this reaction was reported to be only a few seconds (1). The exchangeable cation will be removed at a rate controlled by those limitations imposed upon a diffusion exchange reaction.

The data presented for Ca loss probably does not contain a significant fraction of the diffusible Ca-salt in the free space of the soybean roots. This conclusion was drawn from the observation that excised roots did not lose a measurable amount of Ca nor K to distilled water in 24 hours following excision and 3 water rinses.

A major portion of the Ca in a root is considered to be held in an exchangeable form (9) and is not accumulated to any extent by the individual cells (18). The exchangeable fraction must therefore exist either near the root surface, in the cell walls, or distributed in some ratio between these 2 locations. The possibility exists that one can use the equation describing diffusion from a cylinder of infinite length as an aid in the localization of Ca in a soybean root. It is necessary at this point to agree (1) that diffusion rather than exchange will be the rate-limiting step, (2) that the total volume of the root be considered, and (3) that although Ca probably is confined to the cell wall region, the overall distribution can be treated as being uniform throughout the cylinder.

Comparison of the time course of Ca loss with the theoretical loss by diffusion from a cylinder where the average concentration C, in the cylinder of radius, r_o, whose initial concentration had been C_i, while its final concentration is C_f, is given by the series (12):

$$\frac{C - C_f}{C_i - C_f} = 4\frac{1}{Z_1^2} e^{-DtZ_1^2/r_o^2} + \frac{1}{Z_2^2} e^{-DtZ_2^2/r_o^2} + \ldots$$

Z_1 and Z_2 are series coefficients. Defining the ion content of root in terms of concentration is at best an approximation, hence the left side of the equation is taken to be F, the fraction of the initial ion content still remaining in the root at time, t.

The above equation was used to calculate line *a* of figures 2 and 6. A value for the diffusion coefficient, D, was determined for a 0.06 cm diameter root and the value for F selected at 120 minutes and 3 minutes from figures 2 and 6, respectively. The calculated values for D were 3×10^{-8} cm^2 sec^{-1} for figures 2, and 5×10^{-7} cm^2 sec^{-1} for the data in figure 6. These values are to be compared to 5×10^{-6} cm^2 sec^{-1} for tritiated water in a maize root (27) and 3 to 4×10^{-7} cm^2 sec^{-1} as diffusivity of K and Na in barley root (22). On the basis of these reported values, use of our calculated apparent diffusion coefficients appears to be a realistic assumption to obtain a theoretical curve.

It has been assumed that Ca was in the AFS of a plant root and that the loss of exchangeable Ca was rate limited by diffusion. However, we find that the initial loss of Ca in both cases (figs 2 and 6) was greater than the theoretical value as obtained by use of the equation. If one attempts to fit the equation to the initial portion of the curve, then the latter portion of the curve is not even close to the experimental data. The values of D selected to describe a tangent to the initial time course experimental curve was 5×10^{-7} cm^2 sec^{-1} and 1×10^{-5} cm^2 sec^{-1} for data in figures 2 and 6, respectively. Using these values for D, the theoretical loss would be 90% complete in 10 minutes for data of figure 2, and 29 seconds for data of figure 6. If one selects smaller values of D, the curve will be above line *a* with the initial reaction being much less prominent.

The initial loss of Ca is not adequately described as a diffusion-limited process, although the loss at longer time periods appears to be more nearly typical of diffusion out of a cylinder. Inspection of the theoretical and experimental curves indicates that the initial loss of Ca was more rapid than predicted by the equation. Thus, this indicates a shorter path length for Ca diffusion than the 1 assumed and that this amount of Ca must be associated near the root surface relative to the total Ca. The Ca loss at the longer time periods appears to be described by the diffusion equation, with the implication that this fractional Ca content is uniformly distributed in the root.

Bernstein and Nieman (1) placed the free space region exterior to the endodermis of a root, while Pitman (22) and Woolley (27) did not find any evidence of a diffusion barrier at the endodermis. The uptake data on intact and separated cortex and stele of corn roots by Yu and Kramer (28) also contains evidence that the endodermis doesn't restrict movement of ions. Since these conclusions were from data on the movement of both cations and an anion, we assumed that they are also valid for Ca movement throughout the root.

The evidence presented indicates that the Ca loss measured in these experiments probably results from 2 regions of the root. A portion of the Ca comes from the entire radial section and another portion from near the root surface. This is seen from the calculated value of the apparent diffusion coefficient for the initial loss being larger than for longer time periods showing a negligible tortuosity. This being the case, the Ca must be localized near the epidermal layer in order to minimize movement through the tortuous pathway of the cell walls. One must keep in mind that the product Dt will remain essentially constant with changes in the internal concentration, whereas one expects variation in the D and t values. Thus, it is the relative shape of the curves which is important and not the absolute values of the constants.

Discussion

The identification of ions in a tissue by microautoradiographic studies has met only limited success (2). These investigations must be concerned with possible movement of the isotope during sample preparation and the resolution may not permit identification of an isotope with a particular cell component or in some cases even a single layer of cells. However, if we accept the results, without the possible modifying influence of sample preparation, they indicate that after 45Ca uptake by bean root, the isotope concentration is greater in the stele and epidermis than in the cortical cells. The work of Luttge and Weigl (17) was similar to, although not directly comparable to, Biddulph's (2) because the former primarily observed the undifferentiated region while the latter the more mature regions, i.e. greater than 8 mm from the root apex.

Lauchli (14) has made use of an X-ray microanalyzer to detect localization of ions in thin sections of plant material. These results indicated a greater concentration of Sr and presumably Ca in the epidermis and stele of the differentiated part of the root. Strontium was only in the dermatogen of the root tip. The findings are similar to those obtained by use of microautoradiographic technique Biddulph (1), and Luttge and Wiegl (17). However, determination of amounts in a given region of the root is not obtainable by either technique. Nevertheless, on a relative basis, the data suggest that a large portion of the divalent cation in the root is located in the epidermis.

The epidermis was considered by Sandstrom (25) to be directly involved in the selective absorption of ions. This is in agreement with the proposal that a large portion of the Ca is located in the epidermis of the soybean root. That the Ca is located in or near the epidermis is also suggested from the rapid exchange of Ca (18) and Sr (9) and the interaction of Ca with 32P uptake (15) by plant roots. The time of this Ca exchange is usually 30 minutes, but should not be equated to depth of location since a period of 2 to 3 hours may be required for the exchange reaction in baker's yeast (16). The path length of ion movement in

this single cell organism is less than 3 μ, compared to a thickness of 30 to 50 μ for a soybean epidermal cell.

Further identification of the Ca reaction with the root surface area was deduced from the reversal of K–Mg uptake ratio upon addition of Ca. The effect was evident immediately and occurred without a large change in Ca content. Any observed increase in Ca was relatively fast, while the rates and uptake levels of K increased and those of Mg decreased. If Ca or Mg penetrated the root, one would expect an inhibition of K retention at some level of internal divalent cation. Since entry of Mg was shown to induce a loss of K (figs 3 and 4) and was reversed by Ca (table I), one must deduce an interaction blocking Mg uptake at the root-solution interface or more specifically at the epidermal cells.

The movement of an ion into a root may be considered as a series of interactions occurring stepwise at succeedingly smaller and smaller cylindrical interfaces until reaching a conducting tissue such as a xylem element. Furthermore, the entry of an ion either results in the exit of another ion of similar charge in order to maintain electrostatic neutrality or entry of an ion of opposite charge (K accompanied by Cl). It is also possible that uptake of 1 ion may increase the leakage of other ions (see fig 4). The Mg content of the roots is considered to be uptake without stipulation as to involvement of an energy step. Magnesium entry is considered in this case to be a radial movement and induces leakiness in each cell layer as it proceeds toward the stele. In which case a given amount of Mg would be required to induce total K and Cl leakage. Thus, it appears possible that the entry of Mg would be related to K and Cl efflux by the effected root volume on the assumption of uniform distribution. That the rate of change of K and Cl is similar to the Mg doesn't imply stoichiometry, but instead indicates equivalency in volume distribution. From the above deductions, one then places a large part of the total Ca in the epidermal layer rather than as uniformly distributed in the root. If Ca has been equally distributed, then all cell membranes would have been immediately effected and an initial surge in K loss should have been observed as was for the Ca loss.

Additional information on the localization of the Ca and K in the soybean root with respect to the external solution was obtained by placing the roots in 0.1 N HCl for short-time periods. The HCl is considered to enter the root and disrupt cellular membranes in its radial movement to the center of the root. The loss of Ca and K, under these conditions, would be in sequential order beginning with the epidermal layer and approaching the stele. Uniform distribution of K and Ca would be indicated by a constant K/Ca in their loss. Because the initial loss of Ca was greater than K suggests that relatively more Ca is in the cell layer adjacent to the external solution—the epidermis. If the major part of the Ca as an exchangeable ion or salt associated with the soybean root is uniformly distributed in or external to the plasmalemma in the free space, then the loss of Ca to HCl solution should near completion in 30 seconds (1). Since this was not observed is evidence that: (1) the total Ca in a soybean root is not equally available to the external solution, (2) penetration of the root free space by HCl as assayed by Ca loss is not as rapid as one might assume, and (3) the rapid initial Ca loss is indicative of loss from a region proximal to the external solution.

The observed loss of Ca and K as a percentage of the initial content should be useful in evaluating the root volume containing a large fraction of the Ca. Examination of figure 4 shows that in the first 2 hours in $MgCl_2$ solution, the roots lost $\sim$ 90 % of their Ca and only 9 % of the K. After 2 hours, the amount of Cl and K decreased by 9 % every 2 hours, indicating homogeneity of the K and Cl distribution. It appears that 10 % of the Ca content is distributed in the same space as 91 % of the K because the rate of loss of this remaining Ca approaches 9 % per 2 hours as found for K and Cl. Accordingly, 90 % of the root Ca was located in the root volume occupied by 9 % of the total K. Since K appears to be uniformly distributed throughout the root cross section, it follows that 90 % of the root Ca was localized in the 9 % of the root volume nearest the external solution.

The calculated thickness of an outer cylindrical volume containing 9 % of the root volume is 14 μ based upon a uniform root diameter of 0.6 mm. This calculated thickness would place 90 % of the Ca in association with the epidermal layer of cells which have an average thickness of 30 to 50 μ. On the basis of the calculation and the assumption that Ca associated in this volume is uniformly distributed, the minimum Ca concentration is 5×10^{-2} N. Hence, it becomes more plausible that this concentration of Ca can react with the cell components at this interface to modify the ion uptake process.

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