Accumulation of Calcium in Exudate of Individual Barley Roots[1, 2]

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Metabolic absorption of Ca by plant roots has been reported to be extremely slow in comparison to the alkali cations and Mg (5, 7, 14, 15, 16). In certain root tissues, metabolic absorption of Ca was difficult to detect if it occurred at all (1, 9, 14, 18). Since the intact plants of most species are known to take up a large amount of Ca, these results appeared to support the hypothesis of solute uptake by bulk flow of the solution (1, 3, 14).

In contrast to the above findings, Handley and Overstreet (8) reported a sizeable metabolic absorption of Ca by maize root segments 1.8 to 11.8 mm behind the root tip. A small, but nonetheless significant, metabolic absorption of Ca by bulk excised wheat roots has been reported by Johnson and Jackson (12). In addition, Lopushinsky (13) reported that Ca was accumulated against a concentration gradient in the exudate of decapitated tomato plants, indicating that a metabolic process was responsible for the movement of Ca. Evans (6) demonstrated that polar transport of Ca in corn roots was under metabolic control.

In an attempt to reconcile these differences, the absorption of Ca by excised barley roots has been examined further. In the present study, the accumulation of Ca in the exudate of individual excised barley roots has been compared with the Ca absorption by bulk excised roots. The roots used in this study were essentially the same kind of material as that which showed little or no net metabolic absorption of Ca previously reported for excised roots in bulk (14).

Materials and Methods

Experiments were conducted using excised roots of barley (*Hordeum vulgare* L. var. Hannchen, 1961 crop). The method of growing root material was that described by Jacobson et al. (10) with only minor modifications. The temperature during the growth period was 22° ± 2°, and the root material was harvested on the sixth day after germination. For the individual root studies, plants were selected and the roots excised just below the seed. The roots were washed several times in distilled water, and the individual roots were selected for the collection of exudate. Roots were cut to a uniform length of 5 cm behind the root tip and included both primary and adventitious roots. The ratio of adventitious to primary roots for these barley seedlings was about 4 : 1. The individual roots at this stage of development were relatively straight and had no branching.

Collection tubes with a constriction and a reservoir formed in the bore were prepared from thick-walled capillary tubing. The constriction tapered gradually from the original bore diameter to approximately one-half of the original. The cut end of an individual root was inserted into a collection tube until a satisfactory seal was obtained between the root and the wall at some point in the constriction. A plastic rack was placed over a tray containing the test solution, and the collection tube with protruding root was inserted into a hole in the rack. Individual roots were placed into the test solution at timed intervals, and the position of the collection tube was adjusted so that the root was immersed in the solution up to about 1 cm from the cut end. A small gap was maintained between the bottom end of the collection tube and the surface of the bathing solution to prevent capillary rise of the solution into the tube below the root seal. Approximately 80 % (4 cm) of the root length was in contact with the bathing solution. The arrangement of this apparatus is shown schematically in figure 1.

The bathing solution for the different exudate experiments consisted of various concentrations of $CaCl_2$ labeled with Ca^{45}. The specific activity was determined by chemical analysis and counting of an aliquot of the solution. Four liters of solution were used in a plastic tray, and the whole arrangement was covered to reduce evaporation. The solution was aerated during the experiment, and the pH was adjusted periodically to 5 with 1 N HCl. The temperature was 22° ± 2° for the various experiments.

The exudate from the individual roots was allowed to rise in the collection tubes during the course of an experiment. At the end of the experiment, the collection tubes were removed from the rack, and the roots were taken from the tube and weighed individually. Successive weighings of single roots dipped in H_2O between determinations showed a variation in root weight no greater than ± 0.1 mg out of an average root weight of about 5 mg.

After the root was removed, the collection tube containing the exudate was wiped dry on the outside and weighed. The weight of exudate was obtained by subtracting the tare weight of the collection tube determined prior to the experiment. After weighing, the exudate was quantitatively washed from the collection tube into a stainless steel, 2.5 cm diameter,

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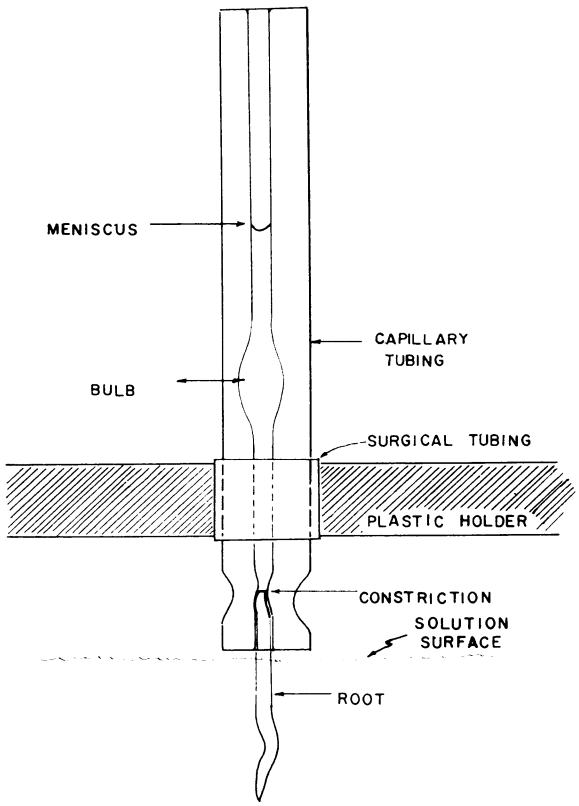

FIG. 1. Drawing of apparatus used for collection of exudate from individual excised roots. Dimensions of the collection tube are: length, 7.5 cm; bore diameter, 0.5 mm; outside diameter, 0.5 cm.

cupped planchet. The planchets were dried and assayed for radioactivity with a thin window flow counter.

The amount of labeled Ca in the exudate was calculated from the radioactive assay data and the specific activity of the ambient solution. The resulting data were placed on a per kg of root basis using the measured individual root weight. Dividing by the length of the absorption period in hours resulted in the accumulation rate expressed in meq/kg per hour.

The volume of exudate was computed from the determined exudate weight and the exudate density. Although the exudate density was not determined exactly, it was estimated to fall within 0.998 ± 0.003 g/cc at the temperature of these experiments.

The concentration of labeled Ca in the exudate was computed from the volume of exudate and the amount of labeled Ca in the exudate and was expressed on a meq/liter basis. The accumulation ratio was then obtained by dividing the calculated exudate concentration by the ambient concentration.

At the same time the individual root exudate experiment was done at 5 meq/liter, another experiment was conducted to determine the net uptake of Ca by bulk excised roots. Roots for this experiment were excised just below the seed, cut into about 2 cm lengths and washed several times in distilled water. The roots were centrifuged at 65 × *g* for 5 minutes to remove excess adhering water. Seven g samples of roots were placed into 7 liters of 5 meq of $CaCl_2$ per liter. The solutions were aerated continuously, and the pH was maintained at 5 by periodic adjustment with HCl. The uptake periods ranged up to 24 hours. At the end of each time period, the roots were collected by pouring the contents of the bottle over a nylon screen. While still on the screen, the roots were washed by pouring 3 liters of distilled H_2O over them in approximately 10 seconds. The roots were dried in an oven at 70°. After drying, the roots were digested in nitric-perchloric acid, filtered and diluted to 50 ml. A 10 ml aliquot of the digest was titrated with cyclohexanediamine tetraacetate to the calcein endpoint for Ca (4). Uptake data were expressed in meq per kg of fresh roots and represent the net change in total Ca content during the uptake period.

Results

The results of the exudate experiments are summarized in table I. The individual experiments were done at different times over a period of several months; therefore, comparisons between concentrations cannot be made precisely. However it is interesting to note certain trends. For instance, the average volume of exudate increased with increasing ambient concentration up to 1.0 meq per liter and then declined. The average concentration of labeled Ca in the exudate generally increased with the ambient concentration. The greatest accumulation in the exudate against a concentration gradient occurred at an ambient concentration of 0.1 meq per liter. At this concentration, the exudate averaged over 27 times more concentrated than the ambient. The maximum accumulation ratio for any individual root in the entire study was 58 and occurred at this same ambient concentration. At an ambient concentration of 25 meq per liter, the exudate concentration averaged only about half that of the ambient. All ambient concentrations except this one showed a definite accumulation of labeled Ca in the exudate against a concentration gradient.

The average rate of accumulation in the exudate, expressed in meq per kg of roots per hour, is shown in the last column of table I. The rate increased with increasing ambient concentration but appeared to level off at the higher concentrations.

The results of the conventional bulk excised root experiment are shown in table II. This experiment was conducted at the same time with the same batch of root material as the individual root experiment at 5 meq/liter, and the data therefore can be directly compared. The results are similar to those reported previously for excised barley roots (14). There is an initial rise in the Ca content which can

Table I. *Accumulation of Calcium in the Exudate of Individual Excised Roots*

Ambient conc (meq/liter)	No. of roots	Collection time (hr)	Avg exudate vol (μl)	Exudate conc (meq/liter)			Avg accumulation ratio*	Avg rate of accumulation (meq/kg per hr)
				min	max	avg		
0.01	6	27.5	3.4	0.05	0.07	0.06	6	0.001
0.05	11	22.5	8.1	0.15	0.88	0.41	8.2	0.02
0.1	6	27.5	6.0	1.0	5.8	2.7	27	0.09
0.5	11	23.0	11.6	1.6	6.4	4.0	8.1	0.34
1.0	17	23.0	14.4	2.0	5.6	3.2	3.2	0.35
5.0	6	24.0	9.2	5.8	11	8.1	1.6	0.35
25.0	6	23.8	3.0	6.2	19	13	0.5	0.44

* Accumulation ratio $= \frac{\text{exudate concentration}}{\text{ambient concentration}}$.

Table II. *Net Calcium Uptake by Immersed Excised Roots*

The concentration of $CaCl_2$ was 5.0 meq/liter and the pH was maintained at 5.

Time (hr)	Net uptake (meq/kg)
0.5	1.7
1.0	2.0
3.0	2.0
8.0	2.0
24.0	2.2

be all accounted for as nonmetabolic uptake (11). This phase is complete within 1 hour and amounts to an apparent free space of about 40 % and would include both the Donnan and water free space. From 1 hour through 24 hours there was an increase of only 0.2 meq/kg. Assuming that this amount represented metabolic absorption, the maximum absorption rate was less than 0.01 meq/kg per hour. The average rate of accumulation in the exudate from individual roots at this same ambient concentration was 0.35 meq/kg per hour.

Discussion

The accumulation of labeled Ca in the exudate against a concentration gradient is good evidence of the metabolic absorption of Ca (13). No attempt was made to determine the state of the Ca in the exudate. However, if the Ca existed in the exudate as an ion, the movement of Ca from the ambient solution into the conducting elements would have been against a free energy gradient and would have required the expenditure of metabolic energy. The requirement for metabolic energy to accumulate Ca holds even if it were assumed that the Ca may have moved into the xylem in response to an electrochemical gradient set up by the active accumulation of Cl in the exudate. If the Ca in the exudate were not as the free ion but were instead bound to some organic molecule, the binding agent would have been of metabolic origin. The evidence is good, however, that Ca does exist as an ion in the conducting elements (2, 13).

The data presented here for the Ca concentration and the rate of Ca accumulation in the exudate represent low values. Any isotopic exchange which occurred as the Ca^{45} moved through the root to the conducting elements would result in a decrease in the specific activity (2, 14); therefore, the actual Ca concentration in the exudate could be somewhat higher than shown. The amount of Ca still in the conducting elements at the end of the experiment could not be determined and would not be included in the calculations. In addition, only about 80 % of the root was immersed in the bathing solution, yet the entire root was used for the weight determination. Each of these considerations point to low values for the computed accumulation rate, but in no way do they invalidate the conclusions drawn from this study.

In conventional excised root experiments, the entire root is immersed, and any accumulation of Ca in the exudate would not be detected. Ca which was accumulated in this way would be forced out the cut ends of the roots back into the external solution. The failure of these barley roots to show a substantial change in the net Ca content when immersed in a Ca solution suggests that the bulk of the cells probably were not actively involved in Ca absorption. A group of specialized cells in the root such as the endodermis could be responsible for the metabolic absorption and subsequent release of Ca to the conducting elements. The endodermis is the logical anatomical feature although a segment of the root such as that reported by Handley and Overstreet (8) for maize could be responsible. The endodermal cells would likely be involved in the final release of Ca to the xylem whether the accumulation occurred over the entire root length or through a restricted segment. Evans (6) concluded that the polar transport of Ca in maize roots occurred in the xylem and was preceded by an absorption step. It was suggested that polar transport of Ca may be linked to root pressure. If this is the case, then the endodermis would likely play an important role.

The transfer of Ca into the conducting elements

by an active process is also suggested by the results of Barber and Koontz (1) and Wallace (18). These workers found that dinitrophenol (DNP) inhibited transfer of Ca to the shoot but had little effect on the net uptake of Ca by the roots themselves. On the basis of their results with DNP, Barber and Koontz concluded that there was a barrier in the root which would prevent free movement of ions into the xylem by a passive process. On the basis of free space measurements, Jacobson et al. (11) came to the same conclusion and suggested that the endodermis was that barrier. The present study supports this view in that accumulation of Ca in the exudate against a substantial gradient could not occur unless there was a barrier to prevent back diffusion. Using microautoradiographic techniques, Weigl and Lüttge (19) clearly showed metabolic uptake of sulfate into the xylem elements of maize roots and stressed the importance of the endodermis in this process.

The conflicting views on the role of metabolism and transpiration on ion uptake by intact plants have been reviewed by Russell and Barber (17). One view describes ion uptake as entirely a metabolic process and attributes the effect of transpiration to a stimulation in active secretion into the xylem. The opposing view holds that a portion of the ion uptake can be related to transpiration directly as bulk flow of solution through a continuous free space system. The largely nonmetabolic nature of net Ca uptake by bulk excised barley roots appeared to support the hypothesis of solute uptake by bulk flow of solution (1, 3, 14). With an active accumulation of Ca in the exudate of this type of root material, it is not necessary to postulate a bulk flow of ions in order to account for the uptake of Ca by the intact plant. The metabolic accumulation into the xylem would appear to be sufficient to supply the intact plant's needs.

Summary

The accumulation of Ca in the exudate from individual excised barley roots (*Hordeum vulgare* L. var. Hannchen) was examined. Ca was accumulated in the xylem against a concentration gradient over a wide range of ambient concentrations. The concentration of Ca in the exudate ranged up to 58 times that of the ambient. The observed accumulation against a concentration gradient was taken as evidence of a metabolically mediated process. The rate of accumulation of Ca in the exudate of individual roots was compared to the rate of net uptake of Ca by excised roots. At 5 meq per liter ambient concentration, the accumulation of Ca in the exudate was 35 times more rapid than the absorption of Ca by the bulk roots. It was concluded that the majority of the root cells were not active in Ca absorption and that a group of cells such as the endodermis was responsible for Ca accumulation into the xylem. It was suggested that the endodermis constituted a barrier to the free movement of ions across the root and that Ca moved metabolically across this barrier.

Literature Cited

1. Barber, D. A. and H. V. Koontz. 1963. Uptake of dinitrophenol and its effect on transpiration and calcium accumulation in barley seedlings. Plant Physiol. 38: 60–65.
2. Biddulph, O., F. S. Nakayama, and R. Cory. 1961. Transpiration stream and ascension of calcium. Plant Physiol. 36: 429–36.
3. Branton, D. and L. Jacobson. 1962. Iron transport in pea plants. Plant Physiol. 37: 539–45.
4. Carlson, R. M. and C. M. Johnson. 1961. Chelometric titration of calcium and magnesium in plant tissue. Method for elimination of interfering ions. J. Agri. Food Chem. 9: 460–63.
5. Epstein, E. and J. E. Leggett. 1954. The absorption of alkaline earth cations by barley roots: kinetics and mechanism. Am. J. Botany 41: 785–91.
6. Evans, C. E. III. 1964. Polar transport of calcium in the primary root of *Zea mays*. Science 144: 174–77.
7. Handley, R. and R. Overstreet. 1955. Respiration and salt absorption by excised barley roots. Plant Physiol. 30: 418–26.
8. Handley, R. and R. Overstreet. 1961. Uptake of calcium and chlorine in roots of *Zea mays*. Plant Physiol. 36: 766–69.
9. Hylmö, B. 1953. Transpiration and ion absorption. Physiol. Plantarum 6: 333–405.
10. Jacobson, L., R. Overstreet, H. M. King, and R. Handley. 1950. A study of potassium absorption by barley roots. Plant Physiol. 25: 639–47.
11. Jacobson, L., R. J. Hannapel, and D. P. Moore. 1958. Nonmetabolic uptake of ions by barley roots. Plant Physiol. 33: 278–82.
12. Johnson, R. E. and W. A. Jackson. 1964. Calcium uptake and transport by wheat seedlings as affected by aluminum. Soil Sci. Soc. Am. Proc. 28: 381–86.
13. Lopushinsky, W. 1964. Calcium transport in tomato roots. Nature 201: 518–19.
14. Moore, D. P., L. Jacobson, and R. Overstreet. 1961. Uptake of calcium by excised barley roots. Plant Physiol. 36: 53–57.
15. Moore, D. P., R. Overstreet, and L. Jacobson. 1961. Uptake of magnesium and its interaction with calcium in excised barley roots. Plant Physiol. 36: 290–95.
16. 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.
17. Russell, R. S. and D. A. Barber. 1960. The relationship between salt uptake and the absorption of water by intact plants. Ann. Rev. Plant Physiol. 11: 127–40.
18. Wallace, A. 1963. Solute uptake by intact plants. Arthur Wallace, Los Angeles. p 142–46.
19. Weigl, J. and U. Lüttge. 1962. Mikroautoradiographische Untersuchungen über die Aufnahme von $^{35}SO_4$ – durch Wurzeln von *Zea mays* L. die Funktion der primären Endodermis. Planta 59: 15–28.