Effect of Removal of the Root Tip on the Development of Enhanced Rb\(^+\) Absorption by Corn Roots

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ABSTRACT

Samples of primary root tissue of corn (Zea mays L.) were aged either in CaSO\(_4\) solution or in humid air, after which they were immersed for 10 minutes in a solution containing 0.1 mM \(^{86}\)RbCl. Aging in solution, but not in humid air, enhanced the subsequent rate of Rb\(^+\) absorption. Excision of roots before aging was followed by greater enhancement than when excision followed aging. The time course of aging of 1-cm segments from different portions of the root showed decreasing response with increasing distance from the root cap. The aging response of apical segments (5-15 mm from the root cap) could be detected within 10 minutes and usually reached a maximum within 2 hours. Rb\(^+\) absorption by apical segments (5-15 mm) aged without the tip (0-5 mm) was more than double that by apical segments whose tips were left attached until the end of the aging period. When apical segments without the tip were aged for 2 hours in the CaSO\(_4\) solution, in which seedlings had previously been grown for 24 hours, the rate of absorption was only 63% of samples aged in fresh solution. When apical segments were aged for 2 hours in fresh solution containing excised tips floating free in the solution, the rate of Rb\(^+\) absorption was 20% less than in samples aged in solution containing no excised tips. The data presented in this study are interpreted to indicate that a water-soluble metabolite, originating in the root tip and translocated basipetally, inhibits Rb accumulation.

Variations in rates of mineral uptake along intact plant roots have long been known (2, 5, 10). It has also been established that different species of plants may show different longitudinal patterns of uptake, corn differing from some other plants in having a short zone of very low accumulation just proximal to the meristem (1, 2). More recent studies using excised corn roots have shown that the capacity of root segments for ion accumulation can be enhanced by aging the segments before exposure to the absorption solution. This enhancement is greatest near the root apex (7). Therefore it might be expected that aged roots would have a different longitudinal pattern of accumulation than nonaged roots, especially in the region of elongation. Whether or not the enhancement is related to excision from the remainder of the plant is the subject of this paper.

MATERIALS AND METHODS

Primary roots of corn seedlings (Zea mays L., DeKalb 805A) were used. Solution-grown roots, cultured as described in an- other paper (8), were used in all experiments. Tray-grown roots were also utilized in one experiment (shown in Fig. 7). For tray- grown roots, seeds were surface-sterilized in 15% Clorox for 5 min, rinsed 10 times in distilled H\(_2\)O, and planted embryo down on several layers of white paper towels in glass trays. The towels were saturated with 0.2 mM CaSO\(_4\) and the trays covered with transparent food wrap which was pierced with small holes to permit gas exchange. The trays were incubated in the dark at 28 C and left undisturbed until used for experiments. All roots, solution-grown and tray-grown, were used at 4 days of age.

The 15 root segments comprising each sample were cut into cold (3 C) 0.5 mM CaSO\(_4\), blotted immediately, weighed, and placed in a fiberglass bag to which a piece of cotton thread was attached for handling. Each sample was then aged in 4 liters of 0.5 mM CaSO\(_4\) for a predetermined length of time. The temperature of the solution was maintained at 30 C, and aeration was vigorous. This was the standard procedure for aging. In a few instances, noted later, samples were aged in humid air by suspending them over distilled H\(_2\)O in a sealed 2-liter flask held at 30 C. After aging, each sample was immediately submerged in 400 ml of an aerated absorption solution consisting of 0.1 mM RbCl plus 0.5 mM CaCl\(_2\). Enough \(^{86}\)Rb was added to the solution to give approximately 10,000 cpm \(\mu\)mole\(^{-1}\) of Rb\(^+\). After 10 min in this solution, absorption was terminated by rinsing the tissue in three changes of cold (3 C) exchange solution consisting of 0.5 mM CaCl\(_2\) plus 5 mM KCl. The samples were then submerged for 30 min in an identical cold, aerated solution to remove all exchangeable ions.

After exchange, each sample was rinsed three times in distilled H\(_2\)O, placed in a nickel planchet, and ashed at 500 C. The ashes were dissolved in H\(_2\)O and dried on a warm hot plate. Samples were assayed for radioactivity, using a low-background counting system.

Each experiment was performed at least two times, yielding similar data. However, the data presented in this paper are the results of single experiments.

EXPERIMENTS AND RESULTS

Role of Excision in Enhancement. In the first experiment entire roots of intact seedlings and also 3-cm excised segments (taken 5-35 mm from the root cap) were transferred to fresh CaSO\(_4\) solution for aging, after which 3-cm segments were also excised from the group of intact roots, and all segments transferred to RbCl. Both groups of samples showed a continuous increase in Rb\(^+\) uptake with increased time in the aging solution (Fig. 1). The response was substantially greater in those root segments which were excised before aging than in those which were aged intact and then excised prior to salt absorption. The capacity for Rb\(^+\) absorption by excised roots increased over 13-fold during the 6-hr aging period, whereas that of intact roots increased by about 8-fold. Thus excision does not cause enhancement, but it does amplify it.

Longitudinal Gradient of Enhancement. The influence of cell
maturation on the time course of enhancement was examined, using 1-cm segments taken 5 to 15, 35 to 45, and 65 to 75 mm from the root tip. The results (Fig. 2) show that the potential for enhancement is greatest near the apex and that it declines with increasing distance from the apex. Thus in nonaged segments the most rapid Rb absorption is by the oldest segments (compare the various lines of Fig. 2 at time zero), but after aging of excised segments the most rapid Rb⁺ absorption is by the apical segments (compare the lines after 1 hr of aging). On the basis of these results, all the following experiments utilized only the apical segments (5–15 mm) which showed the greatest response to aging.

Role of Submersion in Enhancement. The enhancement response of apical segments increased with time in solution (Fig. 2). In order to determine whether the response was due to time per se, or to submersion, the following experiment was carried out. In one series, root segments were aged for varying periods of time in 2-liter flasks, suspended over (but not in contact with) distilled H₂O. In a second series, samples were submerged in CaSO₄ under the standard conditions used in other experiments. Also, one sample was aged in humid air for 2 hr and then transferred to CaSO₄ solution for 1 hr prior to submersion in the experimental RbCl solution. The results (Fig. 3) show that virtually no enhancement occurred in humid air, whereas the submerged samples showed a typical enhancement curve. The sample transferred from humid air to solution for 1 additional hr of aging showed an abrupt rise which was nearly parallel to the initial rise shown by those samples submerged from the beginning. The tissue in humid air lost less than 1% of the fresh weight and therefore the lack of enhancement response could not be attributed to desiccation. Oxygen was not limiting since the O₂ consumed by the root segments was calculated to be less than 1% of the O₂ in the flask.

![Figure 1](image1.png)

**Fig. 1.** Rb⁺ absorption by 3-cm excised corn root segments subsequent to being aged for various times as excised segments or intact roots. Each point is the average of 15 segments.

![Figure 2](image2.png)

**Fig. 2.** Time course of increase in Rb⁺ absorption rate by 1-cm excised segments from different portions of corn roots. Each point is the average of 15 segments.

![Figure 3](image3.png)

**Fig. 3.** Time course of increase in Rb⁺ absorption by 1-cm excised segments aged either in solution or in humid air. Each point is the average of 15 segments.

Influence of Other Portions of Seedling on Enhancement. The marked difference in enhancement between the tipless excised segments and intact roots (Fig. 1) indicates that excision of the experimental segment is in some way important in the response. This was further examined in an experiment in which the time course of enhancement of the 5- to 15-mm portion of the root was followed in four series of samples, the various series differing only in the time at which each of the excisions (at the 5-mm location or the 15-mm location) was made relative to the beginning and end of the washing period. In the first series, completely excised segments (5–15 mm) were placed in the aging solution, after which they were transferred to RbCl solution. In the second series roots which had the apical 5 mm removed, but which were still attached to the seedling, were placed in the aging solution; after aging, these roots were cut at the 15-mm location, and the segments were placed in the RbCl solution. In the third series, excised segments consisting of the terminal 15 mm of the root were placed in aging solution; the tips (0–5 mm) of these roots were excised at the end of the aging period, and the 5- to 15-mm segments were transferred to the RbCl solution. In the fourth series, which served as controls, seedlings remained intact in the culture solution in which they had grown for an additional time period corresponding to the aging times of the roots in the other series, after which both excisions (at the 5 mm and the 15-mm locations) were made and the segments transferred to RbCl solution. The results (Fig. 4) show that the enhancement within the 5- to 15-mm location of the root is about the same whether or not that segment is attached to the top portion of the root and shoot during the aging period. When the 5- to 15-mm portion of the root is aged with the apical 5 mm attached, enhancement is greatly diminished. This indicates that the presence of an intact root tip in some way reduces enhancement in the cells just proximal to the tip.

Effects of Various Modifications of Aging Solution on Enhancement. The lack of enhancement shown by roots with the tip attached and the occurrence of a slight washing response in intact roots transferred to fresh solution both suggest that some water-soluble metabolite which prevents enhancement originates in the root tip from which it moves basipetally. This was the basis for two additional experiments. In one experiment root segments were either: (a) given no aging treatment; (b) aged 2 hr with tips attached, after which the tips were removed and the segments transferred to RbCl; (c) aged for 2 hr in a "used" culture solution consisting of CaSO₄ (the solute in standard aging solution) plus whatever solutes had leached from the roots which had previously been growing in the solution; (d) aged for 2 hr in a standard CaSO₄ solution which contained many loose excised tips (0–5 mm); or (e) aged 2 hr in the standard way. Each treatment was replicated and the results are shown in Figure 5.
root tissue of both solution-grown and tray-grown seedlings showed the same rate of absorption (Fig. 7). However, the solution-grown roots showed a shorter lag period (usually about 10 min) than tray-grown roots (20 min or longer). Also the final absorption rates attained by solution-grown roots were substantially greater than those of tray-grown roots.

**DISCUSSION**

The time-dependent enhancement of ion absorption by root tissue (sometimes called “washing response” [6, 7]) is also regarded as one example of the “aging response” which has recently become the subject of widespread attention (13). Although the precise causes of enhancement are not yet known, it has been shown to require aerobic conditions and warm temperatures and to be inhibited by azide (7), all of which implicate metabolic involvement. On the other hand, neither growth nor respiratory increase is necessary for this enhancement, which distinguishes it from the aging response shown by some storage tissues. Another significant difference is the lag period which lasts only a few minutes in corn roots (Fig. 7) as contrasted with the lag periods of many hours typical of storage tissues (12). The work presented here shows that the root tip hinders enhancement in intact corn roots.

The potential for enhancement in solution-grown seedlings is greatest in the younger portion of the root near the apex (Fig. 2). Excised segments from progressively more basal portions of the root show less and less enhancement, indicating that with increasing age the capacity for this response may be lost (Fig. 2). Also, the excised portions of the root which are capable of the

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**Fig. 4.** Rates of Rb⁺ absorption by 1-cm excised apical segments of roots following various periods of aging. In the first series, segments were completely excised before aging. In the second series, segments were attached to the upper portion of the seedling but not to the root tip during aging. In the third series, segments were attached to the root tip but not to the upper portion of the seedling during aging. In the fourth series (controls), segments were not aged; the seedlings remained intact in the culture solution for additional time periods corresponding to the aging times shown. Excisions were made just before transfer into RbCl solution at the 15 mm location in the second series, and at the 5 mm location in the third series. Each point is the average of 15 segments.

**Fig. 5.** Effects of aging in the presence of excised root tips or in "used" culture solution. Uptake of Rb⁺ by unaged controls, segments aged with tip attached, and by segments aged in fresh solution containing no loose tips are given for comparison. Each bar is the average of two samples containing 15 segments each.

As before, aging with the tips attached subsequently gave Rb uptake which was slightly greater than in nonaged segments, but far below that of the controls. Root segments aged in used culture solution, or in fresh aging solution containing loose excised tips, subsequently absorbed Rb at lower rates than controls. The effect of inhibition by used culture solution was examined in an additional experiment (Fig. 6) in which some root segments were aged in the used culture solution and others in a used culture solution which had been reduced to 0.5 its original volume under vacuum. The concentrated culture solution produced almost exactly twice the inhibition as did the nonconcentrated culture solution.

**Influence of Method of Seedling Culture on Development of Enhanced Rb Uptake.** The degree of enhancement observed in these experiments (up to 14-fold) is far greater than any previously reported values for root segments (7), although less than reported values for decorticated steles (6). One experimental variation, the method of seedling culture, was checked in the following experiment in which comparison was made between roots from solution-grown and tray-grown seedlings. Nonaged
greatest enhancement are the very portions which show the lowest rates of absorption initially (before aging), and which also correspond to the portions of intact roots which show the lowest accumulation rates (2).

The role of excision in enhancement has frequently been suggested, but heretofore without conclusive data. Leonard and Hanson (7) noted that excision is not necessary for the response of tray-grown roots, a result which we confirm here with respect to solution-grown roots (Fig. 1). Although they found that the uptake of phosphate on a tissue weight basis was not influenced by the number of pieces into which a given longitudinal section of root was cut, our data do suggest an inverse relationship between the length of the root during aging and the degree of enhancement obtained (compare intact roots with 3-cm segments in Fig. 1, and with 1 cm-segments in Fig. 2). Possibly this difference is partly due to differences between phosphate and Rb absorption. As the results show, the most critical experimental manipulation is the timing of the single excision between the root tip and the experimental segment (i.e., at 5 mm). This fact can be demonstrated only when the two excisions are separated in time. Segments aged with the tip attached show little enhancement, whereas those which are aged with the tip removed show enhancement whether or not the experimental segment remains attached to the upper part of the plant during the aging period.

Canning and Kramer (2) had found that in intact corn roots salt absorption in the region near 5 mm from the apex was very low compared to the rest of the root, which can be explained by the presence of the attached root tip in their intact roots. We believe it is noteworthy that this portion of the root is not only the one which shows the greatest enhancement, but is also the one nearest to the root apex. Our results also provide an explanation for the differences between the aging response obtained by Yu and Kramer (15, 16) and those obtained by Laties and Budd (6). Whereas Laties and Budd used tissues from the terminal 2 cm of the root, i.e., those which have the greatest potential for the enhancement, Yu and Kramer (15, 16) used root tissue from which the terminal "1 or 2 cm" had been discarded. Our results show that the potential for enhancement drops rapidly with increasing distance from the root apex. Our results also suggest an explanation for the lack of an aging response which Leonard and Hanson noted when the terminal 5 mm of root was washed.

In previous demonstrations of enhancement of corn root tissue (6, 7), the root segments were immersed during the enhancement period. The question can therefore be raised as to whether it is time per se or immersion which is necessary for the response. The results in Figure 3 show that submersion is required for the response to occur and that aging in air caused little enhancement. This is supported by the results when a sample "aged" in air is subsequently transferred to the aging solution. The immediate rise in uptake parallels the initial slope shown by those samples which had been submerged from the beginning. Essentially no enhancement occurred until the samples were immersed. We interpret this as evidence against a volatile compound which has been suggested (6). Also, this indicates that the term "washing response" used by Laties (6) and by Leonard and Hanson (7) is a far more appropriate term than "aging response."

The reduction of enhancement by the root tip and the necessity that the root segments be submerged for enhancement to develop led us to believe that some water-soluble compound, originating in the root tip, was preventing enhanced uptake in tissues just proximal to the tip. To test this hypothesis further, experiments were performed utilizing modified wash solutions. When root tips were placed in the washing solution along with the experimental segments, the washing response was reduced. Since the free-floating root tips comprised less than 1/4000 of the total volume of the washing solution, any compound coming out of them was diluted correspondingly. Under these circumstances, the 20% reduction of enhancement as compared to controls is regarded as substantial. When root segments were washed in solutions which previously had been used to culture seedlings, enhancement was reduced, and it was further reduced when a concentrated culture solution was used. Since the Ca2+ concentration of culture solutions was less than 0.5 as much as that of standard aging solutions, the decreased enhancement in the concentrated growth solution was not due to increased salt concentration, but to the doubled concentration of some other soluble material which had leached out of the young roots previously grown in the solution. Also, the quantitative differences obtained between solution-grown and tray-grown roots are presumed to be due to the faster growth rates which occur in solution culture, and to the constant leaching effect of solution culture on the roots.

The hypothesis that the root tip is the source of some water soluble compound that prevents the enhancement of Rb absorption is supported by other data. On the basis of changes which they noted in electrophoretic properties of root tissue following excision, Pitman et al. (9) suggested that the terminal meristem is the source of some compound, possibly a hormone, which influences these changes. More recently (14) even the root cap has been identified as the source of substances influencing elongation in corn roots. In addition to the various hormones which are widely acknowledged to be present, a large number of additional compounds have been identified as originating in root tips (3). Leonard and Hanson (7) showed that both IAA and kinetin are capable of reducing the enhancement of phosphate absorption caused by washing. More recently, Shaner et al. (11) have demonstrated that abscisic acid both inhibits the growth of corn roots and also decreases the accumulation of K+ and phosphate. Since auxins, cytokinins, and ABA are all known to occur in roots, any one of them might be involved in the enhancement process.

The physiological role of the hypothetical substance is unknown. Among the possibilities are: (a) loss of K+ during washing which tends to enhance subsequent uptake of either K+ or Rb+; (b) a "tightening" of cellular membranes so that ions actually taken up will be held rather than lost (4); (c) activation of carrier molecules which already exist, but which do not function at maximal rates until some substance interfering with transport has been removed from the tissue.

We interpret the data presented here as indicating that some substance originates in the root tips, moves basipetally, and hinders ion accumulation by the tissues just proximal to the meristem. This substance is water-soluble, but not volatile. The rapidity of the washing response of excised segments as compared to that of intact roots suggests that the substance is transferred in the stele from which it can be lost rapidly if root segments are excised and submerged. Both the relatively slow washing response of intact roots and the inhibiting effect which used culture has on the development of enhancement can be explained by a comparatively slow loss of this inhibiting substance by lateral diffusion out of intact roots.

LITERATURE CITED
CORN ROOT TIP AND ENHANCED Rb ABSORPTION


