Phloem Mobility of Magnesium

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ABSTRACT

Magnesium-28 was applied to specific leaves of bean (Phaseolus vulgaris) and barley (Hordeum vulgare) plants. After 24 hours, as much as 7% of the absorbed Mg was exported from the treated bean leaves and 11% was transported basipetally from the treated zone of the barley leaves. Transport of Mg did not occur past a heat-killed section of the treated leaf, thereby indicating that translocation took place via the phloem. Mg movement in the phloem was also evident in autoradiograms of bean stem segments in which the xylem was separated from the phloem by a thin sheet of plastic.

During the past 3 decades radioisotopes have been used to observe the behavior of mineral nutrients in plants, and, therefore, the phloem mobilities of many of the major mineral nutrients are known (2). Magnesium is an exception. The development of magnesium deficiency symptoms, which are frequently first evident in mature leaves, has been the basis of the hypothesis that magnesium is mobile in plants (10). However, this argument is often confounded by the fact that magnesium is more readily leached from mature than from younger foliage (15, 17). In studies (13, 14) in which plants or portions of plants were transferred to cultures devoid of magnesium, it was found that subsequent growth did not deplete the magnesium content of the leaves that were mature prior to transfer. Therefore, it was concluded that magnesium is not mobile in plants, although these conclusions may be invalid since equal amounts of magnesium may have entered and left the leaves. Avoiding the problems inherent in the previous studies, others (4) used 28Mg as a tracer and concluded that magnesium is not mobile in the phloem of young bean plants. Two techniques were employed in the present study to evaluate the mobility of magnesium in the phloem; both revealed that magnesium is far more mobile in the phloem than calcium with which it has been associated.

MATERIALS AND METHODS

Bean (Phaseolus vulgaris L., var. Black Valentine) and barley (Hordeum vulgare, ab 1376) plants were cultured hydroponically under controlled environmental conditions (1). A 20 mM MgCl₂ treatment solution tagged with ²⁸Mg⁺, free of excess sodium, chloride, and aluminum ions, was prepared by adding dilute NaOH to the stock ²⁸Mg solution to precipitate the Al as a hydroxide. After centrifugation and decantation, additional dilute NaOH was added to the supernatant to precipitate the magnesium as an hydroxide, which was then washed with several rinses of cold 0.3 mM NaOH to remove the NaCl. Enough dilute HCl was added to dissolve the magnesium hydroxide pellet and to bring the treatment solution to pH 5.5. Prior to making the treatment solution to volume, Tween-80, a wetting agent, was added so that the treatment solution contained 0.05% (v/v). A gamma ray spectrum and half-life determination of the treatment solution were made to insure the presence of radiochemically pure ²⁸Mg. ²⁸Mg has a half-life of 21.3 hr and upon decay emits beta particles of 1.84 Mev or 0.45 Mev along with various gamma rays.

Translocation of Foliar-applied ²⁸Mg. One primary leaf of 9-day-old and 16-day-old bean plants each received 0.5 ml of the treatment solution which contained 25 μC of ²⁸Mg. The tracer was deposited on the leaf surface in 10-μl droplets with a micro pipette, the droplets being evenly distributed on the leaf blade. Twenty-one-day-old barley plants were treated by applying 0.75 μC of the same ²⁸Mg treatment solution to 1-cm bands across the second leaves, one-third of the way from the ligules of the leaves. Parallel strips of lanolin, 1 cm apart, were placed across the leaves to prevent surface migration of the tracer. Prior to applying the tracer to the leaves of some plants, the petioles of the bean plants to be treated and the portions of barley leaves just basipetal to the treatment zones were killed by heat. These leaves retained their turgor throughout the experiment, indicating that the xylem elements were not constricted by the heat treatment. The relative humidity of the environment was maintained at 70% during the course of the experiment to aid foliar absorption of the tracer. The plants were harvested after 24 hr of exposure to the tracer, and the treated bean leaves and the treated portions of the barley leaves were removed and rinsed three times with 10 ml of 1 mM HCl, which was found to wash off adequately the residual tracer on the surfaces of the leaves. Autoradiograms were made by exposing x-ray films to the plants, with treated portions detached to prevent spurious transport of the ²⁸Mg, in a freezer chest at -12°C for 3 days. Other plants were sectioned, dried at 77°C, and digested with a nitric-perchloric acid procedure (18). The ²⁸Mg activity in the digests was assayed by counting Cerenkov radiation with a liquid scintillation spectrometer. All count rates were corrected for decay.

²⁸Mg Movement in Bean Stem Segments. Sheets of plastic were inserted between the bark (containing the phloem) and the wood (containing the xylem) along 5-cm portions of the hypocotyls of 16-day-old bean plants. Then the 7-cm portion of each hypocotyl, with the 5-cm segment in the middle, was wrapped with a thin sheet of plastic to prevent tracer in the nutrient solution from contaminating the hypocotyls. Three hours after surgery the plants were transferred to 1-liter jars containing the usual nutrient solution with 10 μC of ²⁸Mg added. The plants were harvested after 24 hr of exposure to the tracer, and the hypocotyls were removed and severed transversely 1 cm above and 1 cm below the upper and lower bounds of the plastic barriers between the bark and the wood. The strips of bark were removed from the hypocotyl segments, and they and the strips of wood were bisected longitudinally. Autoradiograms were made by

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exposing x-ray film to the stem segments in a freezer chest at −12 C for 3 days.

RESULTS

The general pattern of $^{28}$Mg exported from the primary leaf of a 9-day-old bean plant is illustrated in the autoradiogram in Figure 1; the high concentrations of label in the shoot and root apical regions suggest that export took place in the phloem. The untreated primary leaf is barely visible in the autoradiogram. This pattern of labeling was also prevalent in autoradiograms of the older bean and barley plants. The $^{28}$Mg concentrations in the foliage of both bean and barley plants varied inversely with leaf age. Moreover, it is difficult to believe that the high concentrations of tracer in the root tips resulted indirectly from the absorption of $^{28}$Mg from the culture solution because of leaching from the roots.

The possibility that the export of the tracer from the treated bean leaves may have taken place in the xylem (6-8) can be discounted since autoradiograms illustrated that no $^{28}$Mg was exported from bean leaves with petioles killed prior to the application of tracer. Autoradiograms of barley leaves indicated that no tracer moved basipetally past the heat-killed zones (Fig. 2). The $^{28}$Mg activities in digests of bean plants with heat-killed petioles, exclusive of the treated leaves, were not significantly above background, confirming the previous observations (Table I).

Foliar-applied magnesium was readily absorbed by and exported from young and old leaves (Table I). The older leaves absorbed less magnesium than the younger leaves but exported a greater percentage of the magnesium absorbed. These results require further confirmation owing to the variability in foliar absorption. This pattern is typical of the behavior of other mineral nutrients considered to be mobile in the phloem (2, 11). Although magnesium appears to be more mobile in barley than in bean plants, it must be emphasized that the label in the treated barley leaf basipetal to the treated zone is included in the percentage of absorbed $^{28}$Mg which was exported.

The autoradiograms of hypocotyl segments of a $^{28}$Mg-treated (roots) bean plant are compared with those of a plant treated in a similar fashion with $^{44}$Ca in Figure 3. Both tracers moved up in the xylem (wood sections) and were transferred laterally to the phloem (bark sections) in the regions where the plastic did not interfere. However, the 5-cm sheet of plastic between the phloem and xylem prevented the lateral transfer in the midportions of the hypocotyl segments. Consequently, the $^{28}$Mg in the midportion of the bark section is a result of phloem transport, whereas, in contrast, $^{44}$Ca was transported only to a very slight degree in the phloem.
Table I. 14Mg Absorption and Export from Primary Leaves of Bean and Basipetal 18Mg Transport from the Treated Zone of Barley Leaves

<table>
<thead>
<tr>
<th>Plant</th>
<th>Plant Age</th>
<th>No. of Replicates</th>
<th>14Mg Absorbed</th>
<th>14Mg Exported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bean</td>
<td>9</td>
<td>5</td>
<td>63 ± 12</td>
<td>4.6 ± 1.0</td>
</tr>
<tr>
<td>Bean²</td>
<td>9</td>
<td>5</td>
<td>75 ± 7</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Bean</td>
<td>16</td>
<td>4</td>
<td>47 ± 23</td>
<td>7.2 ± 1.3</td>
</tr>
<tr>
<td>Barley</td>
<td>21</td>
<td>3</td>
<td>93 ± 4</td>
<td>11.8 ± 1.9</td>
</tr>
</tbody>
</table>

1 Average ± standard deviation.
2 Petioles of leaves to be treated were killed prior to treatment with 18Mg.

DISCUSSION

The study of phloem mobility of magnesium by observing the development of deficiency symptoms or by recording the behavior of magnesium in plants or portions of plants transferred to cultures devoid of magnesium has inherent problems. Likewise, the use of radiotracers to determine the mobility of magnesium in the phloem may yield invalid results. The application of a radioactive material in forms that are toxic to the foliage, because of acidity, concentration, and contaminants, could result in erroneous observations. Care was taken in this study to avoid this problem. In addition, the quantity of the material applied to foliage may distort the normal foliar concentration to the extent that subsequent observations are meaningless. The foliar application of tracer in the present study increased the magnesium content of the 9-day-old bean leaf by approximately 11%, while the magnesium content of the 16-day-old leaf was increased about 6%. Furthermore, the movement of magnesium in the phloem, which was separated from the xylem by a barrier, supports the validity of the foliar export data, since, in this instance, the foliage was not treated directly.

The results of this study are not in accord with those of a similar study (4). However, it is very likely that Bukovac et al. (4) were unable to observe the movement of 14Mg from the treated leaves because of the combined effects of the quantity of 14Mg applied to the foliage, the percentage of the applied 14Mg which was absorbed, the 28Mg decay which took place during the experiment, the nature of the sampling procedure, and the nature of the 14Mg assay procedure. Certainly, if they had applied more 14Mg to the bean leaves, they would have found magnesium to be mobile in the phloem.

A comparison of the concentrations of different mineral nutrients in phloem sap may be an indication of their mobility in the phloem. Assays of phloem exudate from yucca inflorescences suggest that magnesium is more mobile than calcium in the phloem (16). However, these exudates may have included xylem sap and particulate phloem contaminants. Ehrhardt (9) determined the mineral composition of aphid (Megoura vicina, Buckt.) honeydew and found that the concentration of magnesium was 20-fold that of calcium, which supports the present findings.

Data on the rate of export of different radionuclides from treated Phaseolus vulgaris leaves are presented in Table II. Monovalent cations are extremely phloem-mobile. Phosphate, sulfate, and chloride are readily transported in the phloem, while calcium appears to be virtually immobile. Using the percentage of an absorbed nuclide which is exported as an index of phloem mobility, the phloem mobility of magnesium, according to the present study, more closely resembles that of chloride or sulfate than that of calcium with which it has been compared (4).

Although phloem mobility is a prerequisite for internal redistribution of a plant mineral nutrient, it may not limit the degree to which a mineral is translocated. A considerable portion of plant magnesium may be fixed in immobile fractions (12), which has undoubtedly contributed to the confusion concerning its mobility in the phloem and merits further consideration now that magnesium is known to be phloem-mobile.

LITERATURE CITED