Absorption and Transport of Fe and Mn in Germinating Sorghum

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ABSTRACT

The absorption of Fe from FeSO₄, FeEDTA, and FeEDDHA (ferrous ethylenediaminedi(o-hydroxyphenylacetate)), and Mn from MnSO₄, MnEDTA, and MnEDDHA, by germinating sorghum (Sorghum vulgare Pers. var. M 35-1) was studied. The seeds were treated to absorb Fe and Mn from all the sources, and these ions moved to the scutellum, shoot, and root. EDDHA facilitated greater translocation of Fe and Mn from the seed to the shoot and root. The translocation of Fe was more towards the root than to the shoot, whereas it was the reverse in the case of Mn.

We observed that the leaves of seedlings treated with Mn were slightly chlorotic. Further studies revealed that Mn did not affect the translocation of Fe, and possibly interfered with Fe utilization in chlorophyll synthesis.

Seeds are capable of absorbing not only water but also the organic and inorganic substances present in the medium, during germination (3, 8). This capacity for absorption has provided the basis for the agronomic practice of soaking seeds in dilute salt solutions, in order to supply at least a part of the nutrient requirement, which promotes vigorous growth of the seedlings. Experiments by Traverse and Rickels (6) revealed that tomato and onion seeds are capable of accumulating sufficient Mn to meet the requirement of plants for 40 days.

Our interest has been to investigate the possibility of supplying Fe and Mn to plants by presoaking the germinating seeds in salt solutions. The present study has been directed towards an understanding of the processes of absorption and translocation of Fe and Mn, and also their mutual interactions, in germinating sorghum seeds.

MATERIALS AND METHODS

Sorghum seeds (Sorghum vulgare Pers., var. M 35-1) were surface-sterilized, germinated in aerated 0.1 mM CaSO₄ solution for 30 hr, and then treated with 0.1 mM of ⁵⁹Fe-labeled FeSO₄, FeEDTA, or FeEDDHA, for 18 hr. Likewise, another set of seeds was treated with 0.1 mM of ⁵⁹Mn-labeled MnSO₄, MnEDTA, or MnEDDHA for the same period. The seeds were transferred to cold (5 C) unlabeled salt solutions for 1 hr for desorption, and germinated in trays over aerated CaSO₄ solution (2). The seedlings were grown in the dark by covering the trays with plastic bowls. Plant samples were drawn at intervals, and separated into endosperm, scutellum, shoot, and root for radioassay.

In another experiment, the effects of Mn on the translocation of Fe, and those of Fe on the translocation of Mn, were studied. The seeds were germinated in CaSO₄ solution for 6 hr, and allowed to absorb ⁵⁹Fe or ⁵⁹Mn-labeled 0.01 mM FeSO₄ or MnSO₄ for 18 hr. The effects of Mn on ⁵⁹Fe transport were studied by subsequent treatment of the seeds in 0.1 mM of MnSO₄, MnEDTA, or MnEDDHA for 24 hr. Likewise, the effects of Fe on the transport of ⁵⁹Mn were studied by treating with 0.1 mM FeSO₄, FeEDTA, or FeEDDHA. Subsequent germination was carried out in trays as in the previous experiment. Samples were taken after 3 days for radioassay.

The seedlings treated with Mn were found to be slightly chlorotic. In order to find out if this effect of Mn could be reversed by treatment with Fe, the seeds were first soaked in 0.1 mM MnSO₄, MnEDTA, or MnEDDHA for 24 hr and then transferred to solutions containing 0.1 or 0.5 mM FeSO₄, for 6 hr. Finally the seeds were placed in trays and grown over CaSO₄ under 12 hr low intensity (80 ft-c) photoperiod. Chlorophyll a and b were extracted from the leaves on the 3rd, 6th, and 9th day and were measured colorimetrically (1).

All experiments were carried out at 22 ± 2 C and at pH of 5 ± 0.2. CaSO₄, at 0.1 mM was routinely included in all the experimental solutions, besides other chemicals. The specific radioactivity of ⁵⁹Fe and ⁵⁹Mn was approximately 0.1 μCi/μmole Fe or Mn, and the radioactivity was assayed in a gamma ray spectrometer. The treatments in all the experiments consisted of four replicates.

RESULTS

Absorption and Distribution of Fe. The distribution of absorbed Fe in the endosperm, scutellum, shoot, and root is shown in Figure 1. The amount of Fe in the endosperm is found to decline from the 3rd day to the 9th day, in all the Fe treatments. In the case of scutellum, the content decreased from the initial level on the 1st day to the 3rd day in FeSO₄, up to the 5th day in FeEDDHA, and throughout up to the 9th day in FeEDTA treatments. The Fe content of shoot and root increased slightly in FeSO₄, and FeEDDHA, and significantly in FeEDTA-treated seeds. In general, Fe content in the root nearly paralleled that in the shoot. The treatments with FeSO₄ and FeEDDHA resulted in a higher Fe content in the root than in the shoot. The amount absorbed and distributed to other parts was the highest in FeSO₄, followed by FeEDDHA and FeEDTA treatments.

Absorption and Distribution of Mn. The amount of Mn present in the endosperm and scutellum of seeds treated with MnEDDHA is higher than in those receiving MnSO₄, and MnEDTA (Fig. 2). Mn content in the endosperm decreased
with time in all treatments, while in the scutellum it increased after the 5th day in MnEDDHA, and remained constant in MnSO₄ and MnEDTA treatments. In contrast to Fe, Mn is found to be higher in the shoot than in the root and it increased with time in general. On the other hand, Mn content in the root remained more or less the same from the 3rd to the 9th day.

Effects of Mn on Fe and Fe on Mn Transport. In this experiment, the seeds were first allowed to absorb ⁵⁹Fe from a low concentration of FeSO₄ (0.01 mM), and then treated with 0.1 mM MnSO₄, MnEDTA, or MnEDDHA. The main objective of this treatment is to find out if Mn interferes with the translocation of Fe. The effect of Fe on translocation of Mn was also studied for comparison. The results show that the translocation of Fe is not reduced by treatment with Mn (Table 1). The chelates, viz., EDTA and EDDHA, decreased the total uptake of either Fe or Mn, although the amount transported to shoot and root as a per cent of total absorption was increased.

**DISCUSSION**

The results of studies on the absorption of Fe and Mn show that considerable amounts of these elements are retained in the seeds even after desorption (Figs. 1 and 2). It was evident that germinating seeds are capable of accumulating Fe and Mn from pure and chelated forms. Traverse and Riekels (6) have also reported the occurrence of absorption of Mn by onion and tomato seeds. It is not known whether the endosperm or the scutellum takes part in this uptake. Although it is seen that both the endosperm and scutellum retained considerable amounts of Fe and Mn on the 1st day, differences between the absorption of Fe and Mn were also observed. While Mn absorption by endosperm is more than that by scutellum, Fe

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**Table 1. Translocation of Fe and Mn as Affected by Post Treatment with Mn and Fe Salts**

Sorghum seeds were germinated for 6 hr and then transferred to 0.01 mM ⁵⁹FeSO₄ or ⁴⁺MnSO₄ for 18 hr. The seeds kept in ⁵⁹FeSO₄ were treated with 0.1 mM MnSO₄, MnEDTA, or MnEDDHA, for 24 hr. Seeds exposed to ⁴⁺MnSO₄ were treated with 0.1 mM FeSO₄, FeEDTA, or FeEDDHA. Samples were assayed on the 3rd day after final treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>⁴⁺Fe/⁵⁹Mn Content (nmoles/10 seedlings)</th>
<th>Transport</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endosperm + scutellum</td>
<td>Shoot + root</td>
<td></td>
</tr>
<tr>
<td>⁵⁹Fe</td>
<td>47.01 ± 3.56</td>
<td>6.93 ± 0.99</td>
<td>12.8</td>
</tr>
<tr>
<td>⁵⁹Fe + MnSO₄</td>
<td>42.14 ± 2.24</td>
<td>5.74 ± 0.30</td>
<td>11.9</td>
</tr>
<tr>
<td>⁵⁹Fe + MnEDTA</td>
<td>26.58 ± 1.56</td>
<td>4.00 ± 0.12</td>
<td>13.1</td>
</tr>
<tr>
<td>⁵⁹Fe + MnEDDHA</td>
<td>9.94 ± 0.39</td>
<td>2.14 ± 0.16</td>
<td>17.7</td>
</tr>
<tr>
<td>⁴⁺Mn</td>
<td>36.20 ± 0.89</td>
<td>26.92 ± 1.66</td>
<td>42.6</td>
</tr>
<tr>
<td>⁴⁺Mn + FeSO₄</td>
<td>30.15 ± 2.67</td>
<td>19.82 ± 1.11</td>
<td>39.7</td>
</tr>
<tr>
<td>⁴⁺Mn + FeEDTA</td>
<td>12.95 ± 0.92</td>
<td>13.30 ± 1.23</td>
<td>50.7</td>
</tr>
<tr>
<td>⁴⁺Mn + FeEDDHA</td>
<td>16.60 ± 1.89</td>
<td>18.51 ± 0.90</td>
<td>52.7</td>
</tr>
</tbody>
</table>
absorption by scutellum was more than that by the endosperm, in general.

The elements absorbed by the endosperm will be transported in the normal course to the embryo during growth. We found that Fe and Mn content increased in the scutellum, with the concomitant decrease in the endosperm. The decrease in the endosperm could be due to either a translocation to the scutellum or a loss into the culture medium. A significant rise in the Fe and Mn content of scutellum, shoot, and root suggests that the decrease in the endosperm is largely due to translocation to other parts. It is significant that a greater amount of Fe was translocated to the root, while more was transported to the shoot (Figs. 1 and 2). Furthermore, EDDHA appeared to facilitate greater translocation of Fe and Mn to shoot and root than EDTA.

In these experiments (Figs. 1 and 2), we observed that the leaves of seedlings treated with Mn showed mild chlorosis, while those receiving Fe were normal and green. Since Fe is essential for Chl synthesis, it was thought that Mn interfered with either Fe translocation, or its availability for Chl synthesis. We found that the post-treatments with Mn and Fe did not reduce the percentage of translocation of Fe and Mn (Table 1). However, the treatments with EDTA and EDDHA salts enhanced the translocation. This effect is largely due to the combining of chelates with the labeled Fe and Mn, thus giving a greater transport (9, 11). Since Mn did not interfere with Fe translocation, we presumed that it affected the utilization of Fe in Chl synthesis. The results shown in Figure 3 support this conclusion. Both Chl a and b were reduced in treatments with MnSO₄ and MnEDTA. When the seeds were further treated with FeSO₄ (0.1 and 0.5 mM) the Chl content increased. FeSO₄, at high concentrations (0.5 mM) increased the Chl more significantly in MnEDTA and MnEDDHA treatments.

Somers et al. (5) and Twyman (7) reported high levels of Mn depressed Fe absorption from nutrient solution, and thereby lowered the water-soluble Fe in the plant tissue. Fe also has been found to reduce water-soluble Mn in plants, although to a lesser degree (10). Our study shows that Fe and Mn interfere mutually in their metabolic utilization rather than in their translocation. It has been suggested that Fe chlorosis occurs as a result of excess Mn which affects Fe utilization (4). Although our studies reveal a possible interference of Mn in Fe utilization, the exact manner in which this takes place needs further investigation. Since Mn also is important for Chl synthesis, a study of the effects of different concentrations of Fe on the translocation and utilization of Mn would be quite revealing.

LITERATURE CITED