Characterization of Solute Efflux from Dehydration Injured Soybean (Glycine max L. Merr) Seeds

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ABSTRACT
Soybean (Glycine max L. Merr) seeds lose their tolerance of dehydration between 6 and 36 hours of imbibition. Soybean axes and cotyledons were excised 6 hours (tolerant of dehydration) and 36 hours (susceptible) after commencing imbibition and subsequently dehydrated to 10% moisture. Kinetics of the efflux of potassium, phosphate, amino acid, sugar, protein, and total electrolytes were compared in the four treatments during rehydration. Only slight differences were observed in the kinetics of solute efflux between the two cotyledon treatments dehydrated at 6 and 36 hours suggesting that the cotyledons may retain their tolerance of dehydration at this stage of germination. Several symptoms of injury were observed in the axes in which the axes dehydrated at 36 hours. An increase in the initial leakage of solutes during rehydration, as quantified by the y-intercept of the linear regression line for solute efflux between 2 and 8 hours suggests an increased incidence of cell rupture. An increase in the rate of solute efflux (slope of regression line between 2 and 8 hours) from fully rehydrated axes was observed in comparison to axes dehydrated at 6 hours. The Arhenius activation energy for potassium, phosphate, and amino acid efflux decreased and for protein remained unchanged. Both observations indicate an increase in membrane permeability in dehydration-injured tissue. Increasing the H+ concentration of the external solution increased K+ efflux from both control and dehydrated/rehydrated samples, increased sugar efflux from axes at 6 hours imbibition but decreased sugar efflux from axes at 36 hours imbibition, indicating changes in membrane properties during germination. The dehydration treatment did not alter the pattern of the pH response of axes dehydrated at 6 or 36 hours but did increase the quantity of potassium and sugar efflux from dehydrated injured axes. These results are interpreted as indicating that dehydration of soybean axes at 36 hours of imbibition increased both the incidence of cell rupture during rehydration and altered membrane permeability of the rehydrated tissue.

A seed’s tolerance of dehydration is lost at a specific stage in germination (11). For example, soybean seeds can be imbibed for 6 h, and dehydrated to 10% moisture without loss of seed viability or vigor. If the seed is imbibed for 36 h, at which time the radicle is beginning to emerge from the seed coat, and then dehydrated to 10% moisture, seed viability is lost (17). The ability of plant tissues to tolerate dehydration is thought to reflect the inherent protoplasmic properties of these tissues (2) and in seeds, the protoplasmic properties which impart tolerance are presumably lost as the seed germinates. The loss of tolerance has been asso-

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MATERIALS AND METHODS
Soybean (Glycine max L. Merr cv Maple Arrow) seeds were imbibed and dehydrated as previously described (17).

Kinetic Analysis of Solute Efflux. To quantify the rate of solute efflux from dehydration injured axes and cotyledon tissue during reimbibition, ten axes or six cotyledons, which had been previously dehydrated to 10% moisture at 6 or 36 h of imbibition, were soaked in 10 ml distilled H2O. The incubating solution was...
Table 1. Initial Leakage of Solutes from Soybean Axes and Cotyledons during Rehydration after Dehydration
Treatment at 6 or 36 Hours of Imbibition

Values represent the y-intercept of the linear regression line calculated using the data between 2 and 8 h and are expressed per 100 mg seed.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Axis</th>
<th>Cotyledon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 h</td>
<td>36 h</td>
</tr>
<tr>
<td>Potassium, μmol</td>
<td>2.31 (20.4)*</td>
<td>9.08 (47.3)</td>
</tr>
<tr>
<td>Phosphate, μmol</td>
<td>0.25 (1.1)*</td>
<td>1.75 (6.4)</td>
</tr>
<tr>
<td>Amino acid, μmol</td>
<td>2.0 (6.6)*</td>
<td>8.7 (18.7)</td>
</tr>
<tr>
<td>Sugar, μg</td>
<td>34 (0.2)*</td>
<td>225 (1.3)</td>
</tr>
<tr>
<td>Protein, μg</td>
<td>551 (1.6)*</td>
<td>1396 (3.7)</td>
</tr>
<tr>
<td>Conductivity</td>
<td>62 (16)*</td>
<td>215 (40)</td>
</tr>
</tbody>
</table>

* Values in parenthesis are expressed as per cent total homogenate concentration.

Table 2. Rate of Solute Efflux from Soybean Axes and Cotyledons during Rehydration after Dehydration
Treatment at 6 or 36 h of Imbibition

Values represent the slope of the linear regression line between 2 and 8 h and are expressed per 100 mg seed h⁻¹.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Axis</th>
<th>Cotyledon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 h</td>
<td>36 h</td>
</tr>
<tr>
<td>Potassium, μmol</td>
<td>0.75 (6)*</td>
<td>3.04 (15)</td>
</tr>
<tr>
<td>Phosphate, μmol</td>
<td>0.006 (0.03)*</td>
<td>0.79 (3)</td>
</tr>
<tr>
<td>Amino acid, μmol</td>
<td>0.26 (0.1)*</td>
<td>1.56 (3.4)</td>
</tr>
<tr>
<td>Sugar, μg</td>
<td>605 (3.3)*</td>
<td>1085 (5.8)</td>
</tr>
<tr>
<td>Protein, μg</td>
<td>161 (0.5)*</td>
<td>398 (1.0)</td>
</tr>
<tr>
<td>Conductivity</td>
<td>8.8 (2.3)*</td>
<td>23.5 (4.4)</td>
</tr>
</tbody>
</table>

* Values in parenthesis are per cent homogenate concentration leaked h⁻¹.

RESULTS

Kinetic Analysis of Solute Efflux. The rate of leakage of all cytoplasmic solutes followed a similar time profile to that previously observed in many seed systems (18). A rapid initial leakage gradually declined to a slower but constant rate of solute efflux by 2 h and continued at the same rate until 8 h. Water uptake is also completed after 2 h soaking of these tissues in distilled H₂O (17). Two parameters, y-intercept and slope, can be calculated from a linear regression analysis of the time profile between 2 and 8 h.

Assay Procedure. Conductivity of the leachate was measured using a Barnstead conductivity bridge. K⁺ was quantified with an Orion specific ion electrode. Phosphate was determined as Pi by the method of Fiske and SubbaRow as outlined in Dittmer and Wells (7). Amino acids were quantified with ninhydrin using leucine as a standard (15). Proteins were measured by the method of Lowry et al. (13), using BSA as a standard. Sugars were quantified according to the method of Dubois et al. (8) using glucose as a standard.
SOLUTE EFFLUX FROM SOYBEAN SEEDS

![Figure 1: Arrhenius plots of the efflux of total electrolytes (conductivity) from soybean axes (○, □) and cotyledons (□, □) dehydrated at 6 (○, □) or 36 h (□, □) of imbibition. Ten axes or six cotyledons were reimbibed in 10 ml distilled H2O for 2 h and then transferred to distilled H2O preincubated at specified temperatures. The conductivity of the imbibing solution was measured after a 4-h incubation.

![Figure 2: Le leakage of potassium (○) and sugar (□) from soybean axes in response to external pH. A, Axes excised from soybean seeds at 6 h imbibition. B, Axes excised from soybean seeds at 6 h imbibition, dehydrated to 10% moisture, and reimbibed in distilled H2O for 2 h. C, Axes excised from soybean seeds at 36 h imbibition. D, Axes excised from soybean seeds at 36 h imbibition, dehydrated to 10% moisture, and reimbibed in distilled H2O for 2 h. All treatments were soaked for 4 h in 25 ml of 40 mm Mes-Hepes buffer. The imbibition solution was analyzed for K⁺ and sugar at 1 and 4 h and values represent the change in solute concentration between 1 and 4 h. Vertical bars represent least significant differences (P ≤ 0.05) among pH treatments.

Table III. Calculated Arrhenius Activation Energies for Solute Efflux from Soybean Axes and Cotyledons Dehydrated at 6 or 36 Hours of Imbibition and Rehydrated for 2 Hours before Temperature Treatment.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Axis</th>
<th>cotyledon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 h</td>
<td>36 h</td>
</tr>
<tr>
<td>Potassium</td>
<td>11.3*</td>
<td>5.7</td>
</tr>
<tr>
<td>Phosphate</td>
<td>11.0*</td>
<td>8.4</td>
</tr>
<tr>
<td>Amino acid</td>
<td>7.4*</td>
<td>4.3</td>
</tr>
<tr>
<td>Protein</td>
<td>4.9</td>
<td>5.3</td>
</tr>
<tr>
<td>Conductivity</td>
<td>13.6*</td>
<td>10.5</td>
</tr>
</tbody>
</table>

*NS, significantly different or not significantly different according to t test comparison at P ≤ 0.05.

However, a 132-fold increase was observed in the rate of Pi efflux. Expressing the rate of leakage as a percentage of total available solute h⁻¹ did not alter the above pattern (Table II). There were no significant differences between 6- and 36-h cotyledons for the rates of leakage of any of the investigated solutes.

Activation Energy of Solute Efflux. As temperature of the incubation medium increased, the rate of solute efflux from both axes and cotyledons increased as log K = b−a·1/T, where K is rate of efflux, T is the absolute temperature, and a and b are constants. At temperatures between 10 and 25°C, axes dehydrated at 36 h imbibition leaked significantly more total electrolytes than those dehydrated at 6 h (Fig. 1). At 30 and 35°C, however, there was no significant difference in conductivity between the tissues. In the case of the cotyledons, conductivity was not significantly different between 6 and 36 h imbibed and dehydrated samples at any of the temperatures tested. Similar patterns were observed for all solutes.

From the regression analysis of leakage against temperature, Eₐ can be calculated from the slope of the line. Eₐ represents the energy required to move the solute from the tissue into the external solution presumably across diffusion limiting membranes. Consequently, these values provide a relative indication of membrane permeability (12). Eₐ was significantly (P ≤ 0.05) reduced for K⁺, Pi, amino acid, and total electrolytes in axes dehydrated after 36 h (Table III). Eₐ for protein leakage was not significantly reduced.

Although attempts were made to obtain Arrhenius plots of sugar efflux, the data did not fall on a straight line and therefore Eₐ could not be calculated. Sugar transport is believed to be involved in a cotransport system with H⁺ and K⁺ (1). An interaction between K⁺ and sugar efflux may have complicated the temperature profile.

No significant (P ≤ 0.05) differences in Eₐ for any of the investigated solutes, or for total conductivity were observed between cotyledons that had been imbibed, then dehydrated at either 6 or 36 h (Table III).

Effect of External pH on the Efflux of K⁺ and Sugars. The effect of pH on K⁺ and sugar efflux from fully hydrated soybean axes was measured after various treatments. Axes were excised from seeds which were either (a) imbibed for 6 h; (b) imbibed for 6 h, dehydrated, and rehydrated for 2 h; (c) imbibed for 36 h; (d)
imibed for 36 h, dehydrated, and reimbibed for 2 h.

The efflux of $K^+$ and sugar from all samples responded significantly to changes in external pH (Fig. 2). $K^+$ efflux increased with increasing $H^+$ concentration in the external solution in all samples, with maximal rates of leakage occurring at pH 5.5. The efflux of sugars responded in a more complex manner to changes in external pH. In the 6-h axes, sugar efflux increased with $K^+$ efflux at low pH (Fig. 2, A and B), but in the 36-h axes sugar efflux decreased as $K^+$ efflux and $H^+$ concentration increased (Fig. 2, C and D). This reciprocal response was observed in both the nonstressed (control) and the dehydrated-rehydrated axes and thus appears to be a response to changes during germination. The dehydration stress did not alter the qualitative response to external pH but it did increase the quantity of both sugar and $K^+$ leaked from the axes dehydrated at 36 h.

DISCUSSION

Cellular rupture is common in imbibing seeds, especially if the tests has been removed (9) as it has been in these experiments. It is assumed that solutes from ruptured cells are rapidly leached from seeds, while those from intact cells, which must cross diffusion-limiting membranes, appear more slowly in the imbibing solution. It may therefore be possible to estimate the quantity of solutes originating from each of these sites using linear regression analysis of the time profile of solute efflux (14, 17). The $y$-intercept of the linear period after full hydration approximates, on a relative basis, the quantity of solutes leaked from extracellular sites and ruptured cells, whereas the slope of the regression line approximates the rate of solute efflux across a diffusion-limiting membrane. On this basis, cell rupture appeared to be more prevalent in the axes dehydrated at 36 h of imbibition than in those dehydrated at 6 h and was more pronounced in the axes than in the cotyledons. Thus, an increased incidence of cell rupture was associated with the inability of the axis to resume elongation and with other symptoms of dehydration injury. Nonetheless, this association may not reflect a cause-effect relationship because treatments which reduce cell rupture do not reduce the other symptoms of dehydration injury (17).

Because of the increase in cell rupture as a result of dehydration treatment and because of the complications associated with measuring solute efflux from rehydrating tissues, the experiments designed to estimate change in membrane permeability used fully rehydrated axes. The solutes which leaked from axes between 2 and 8 h of rehydration do not appear to originate from ruptured cells for the following reasons. (a) The rate of phosphate efflux from fully rehydrated axes was selectively increased compared to $K^+$, sugar, and protein efflux (Table II). (b) The $E_a$ for protein efflux remained unchanged after dehydration (Table III). (c) The $E_a$ for total electrolytes, phosphate, and $K^+$ (Table III), though decreased, remained above that indicated by free diffusion (3). (d) $K^+$ and sugar efflux from injured, nonviable axes (those dehydrated at 36 h of imbibition) responded in opposite fashions to changes in external pH (Fig. 2D). Therefore, the increased rates of solute efflux from dehydration injured axes indicate that changes in membrane permeability have been induced by the dehydration treatment.

Leopold (12) has previously reported $E_a$ for leakage of total electrolytes from live and dead soybean cotyledons to be 7.3 and 7.6 kcal/mol, respectively, substantially less than was observed here. In this study, leakage was measured between 10 and 40 min of imbibition. According to our previous data (17), water uptake could still be occurring during this time period, which may account for the discrepancy. The observed $E_a$ values for leakage of $K^+$, $P_i$, and conductivity from dehydration-injured tissues were higher than what would be expected for free diffusion of small molecules (3). Consequently, the cellular membranes in the dehydration-damaged seeds still appear to provide a diffusion barrier to the movement of cytoplasmic solutes. However, the $E_a$ data suggest that this permeability barrier has been significantly reduced by the dehydration treatment in the axes but not in the cotyledons.

The significant effect of pH on $K^+$ and sugar efflux from soybean axes implies that the proton cotransport systems on the plasmalemma are functioning and that these transport systems alter the rate of solute efflux. In plant cells, $K^+$ and $H^+$ are believed to be transported across the plasmalemma by a counter exchange system which maintains the electrical potential across the membrane but which concentrates $K^+$ inside the cell and acidifies the cell wall (1). Sucrose is cotransported with $H^+$ into the cell down the $H^+$ electrochemical gradient and $K^+$ is either pumped or diffuses out of the cell until osmotic and charge equilibria are reached (1). In axes imibed for 36 h, increased external $H^+$ concentration caused an increase in the rate of $K^+$ efflux and simultaneously reduced the rate of sugar efflux, which is consistent with this model of transport across the plasmalemma. In axes imibed for 6 h, this response was not observed. Apparently, changes occur in the plasmalemma during germination which alter the response of sugar efflux to external pH. Whether these changes are related to the loss of dehydration tolerance remains to be established.

In summary, the tolerance of soybean seeds to dehydration was lost during germination. Dehydration of seeds which have initiated cell elongation prevented further elongation (17), increased the incidence of cell rupture during rehydration, and increased membrane permeability. Dehydration stress may have increased permeability by either increasing the rate of passive diffusion across the phospholipid bilayer or alternatively decreasing the rate of active uptake. An increase in passive diffusion would occur if the integrity of the lipid bilayer was altered. Alternatively, a decrease in active uptake would occur if the activity or the efficiency of the transport systems had been altered.

LITERATURE CITED


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