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Characterization of Solute Efflux from Dehydration Injured Soybean (*Glycine max* L. Merr) Seeds[1]

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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 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 Arrhenius 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 dehydration 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 associated with the initiation of cell elongation and hydration of vacuoles (11), onset of DNA replication and RNA transcription (4), and changes in cellular membranes (14). Although the loss of dehydration tolerance is coincident with the initiation of cell elongation in many systems (11), the relationship does not appear to be causal. Soybean axes which have been prevented from elongating by treatment with a -6 bar solution of PEG become sensitive to dehydration even though cell enlargement did not occur (17). Similarily, soybean axes imbibed in cycloheximide to inhibit protein synthesis do not elongate but become sensitive to dehydration (17). Consequently, the changes in cell volume that occur during germination are not consistently related to the loss of dehydration tolerance. Instead, the loss of tolerance may be associated with biochemical or biophysical changes in cell membranes during germination. To define these changes precisely, it is first necessary to define the nature of the metabolic lesion induced by dehydration.

The effect of water loss on the structure of membranes is not well defined. Ultrastructural studies have indicated that substantial alterations of membrane structure occur in dehydration-injured seeds (5, 6). Freeze-fracture studies on the plasmalemma of the phycobiont *Trebouxia* have demonstrated changes in membrane structure and distribution of intramembranous particles after prolonged drying of the thallus (16). Dehydration injury in soybean seeds occurs only in the axis, not in the cotyledons, and only after the seed has been dehydrated to less than 20% moisture (17). The maintenance of the lipoprotein associations in membranes requires 20 to 30% hydration (10). Low angle x-ray diffraction has not confirmed Simon's (18) proposal that seed phospholipids are involved in a lamellar-hexagonal phase transition at 20% hydration (14). It remains unclear how the seed membranes change in structure during drying and also how the seed tolerates this stress at one stage in germination but is sensitive to it at another. A typical symptom of dehydration injury in seeds is an increase in the quantity of electrolytes and other cytoplasmic solutes that can be leached from the tissue during rehydration (14, 17). Solutes may leak from injured tissue more rapidly because the plasmalemma and/or tonoplast has been mechanically ruptured by the dehydration treatment or because the permeability of an intact membrane has been altered. To make this distinction, three experimental approaches were used in this study which varied time, temperature, and pH, respectively, and measured their effects on the efflux of various solutes.

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 H_2O. The incubating solution was

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Table I. *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.

Solute	Axis		Cotyledon		
	6 h	36 h	6 h		36 h
Potassium, μmol	2.31 (20.4)[a,b]	9.08 (47.3)	1.02 (5.7)	NS	1.36 (8.8)
Phosphate, μmol	0.25 (1.1)[b]	1.75 (6.4)	0.09 (1.9)[b]		0.21 (3.3)
Amino acid, μmol	2.0 (6.6)[b]	8.7 (18.7)	0.3 (1.4)	NS	0.3 (1.2)
Sugar, μg	34 (0.2)[b]	235 (1.3)	68 (0.4)[b]		149 (1.1)
Protein, μg	551 (1.6)[b]	1396 (3.7)	256 (0.7)	NS	343 (0.8)
Conductivity, μmhos	62 (16)[b]	215 (40)	26 (8)	NS	27 (8.0)

[a] Values in parenthesis are expressed as per cent total homogenate concentration.
[b] NS, significantly different or not significantly different according to *t* test comparison at $P \leq 0.05$.

Table II. *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}.

Solute	Axis		Cotyledons		
	6 h	36 h	6 h		36 h
Potassium, μmol	0.75 (6)[a,b]	3.04 (15)	0.54 (3)	NS	0.96 (6)
Phosphate, μmol	0.006 (0.03)[b]	0.79 (3)	0.007 (0.15)	NS	0.05 (1)
Amino acid, μmol	0.26 (0.1)[b]	1.56 (3.4)	0.12 (0.5)	NS	0.72 (2.3)
Sugar, μg	605 (3.3)[b]	1085 (5.8)	434 (2.7)	NS	267 (1.9)
Protein, μg	161 (0.5)[b]	398 (1.0)	38 (0.1)	NS	87 (0.2)
Conductivity, μmhos	8.8 (2.3)[b]	23.5 (4.4)	3.3 (1.0)	NS	4.3 (1.2)

[a] Values in parenthesis are per cent homogenate concentration leaked h^{-1}.
[b] NS, significantly different or not significantly different according to *t* test comparison of slopes at $P \leq 0.05$.

decanted at 1-h intervals and analyzed for K^+, Pi, amino acid, sugar, and protein. The experiment was replicated four times. Regression analysis of the linear phase of the time profile from 2 to 8 h was performed.

Activation Energy of Solute Efflux. To calculate Arrhenius activation energies (E_a) for solute efflux, leakage was measured from 10 to 35°C at 5°C intervals. Ten axes or six cotyledons were removed from seeds imbibed for either 6 or 36 h, dryed back to 10% moisture, and reimbibed in 10 ml distilled H_2O for 2 h at room temperature, which is sufficient time to fully rehydrate the tissue (17). The fully hydrated tissue was rinsed in distilled H_2O, transferred into 10 ml distilled H_2O that was preincubated to a designated temperature, and incubated for 4 h. There were four replicates. The incubating solution was decanted and assayed for conductivity, K^+, Pi, amino acid, sugar, and protein.

Effect of External pH on the Efflux of K^+ and Sugars. Seeds were imbibed for 6 and 36 h and axes were dehydrated as in previous experiments. Each group of ten dehydrated axes were reimbibed in 10 ml distilled H_2O for 2 h rehydration and then transferred into 25 ml buffer solution (40 mm Mes-Hepes solution) at pH 5.5, 6.0, 6.5, 7.0, 7.5, or 8.0, at room temperature. Efflux of K^+ to the external pH solution was measured after 1 and 4 h incubation. Aliquots removed simultaneously were analyzed for sugar content. A similar experiment was conducted using axes from seeds imbibed for 6 and 36 h, but without dehydrating them. The experiment was replicated four times and analyzed as a split plot design with time of dehydration as main plot and pH as subplot.

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.

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 H_2O (17). Two parameters, *y*-intercept and slope, can be calculated from a linear regression analysis of the time profile between 2 and 8 h. The *y*-intercept of K^+, Pi, amino acids, sugars, protein, and conductivity was significantly ($P \leq 0.05$) higher for the 36-h axis tissue than 6-h axis tissue (Table I). Potassium had the highest initial leakage, with 9 μmol/100 mg (47% of the homogenate concentration) being rapidly leached from 36-h axes. Only 235 μg/100 mg (1% of the homogenate content) of water-soluble sugars were leached from the same tissue. Expressing the leakages relative to the solute content of a tissue homogenate did not alter the significance of the increase in leakage for any of the solutes listed in Table I. The slope of the linear regression line indicated that all solutes leaked more rapidly from axes that were dehydrated at 36 h of imbibition than from those dehydrated at 6 h of imbibition (Table II). The rates of efflux for individual solutes from damaged tissue were not uniformly increased compared to nondamaged tissue. For example, the rate of efflux of sugar, protein, and conductivity increased 1.8-, 2.5-, and 2.6-fold, respectively. K^+ efflux increased by 4-fold and amino acid increased by 6-fold.

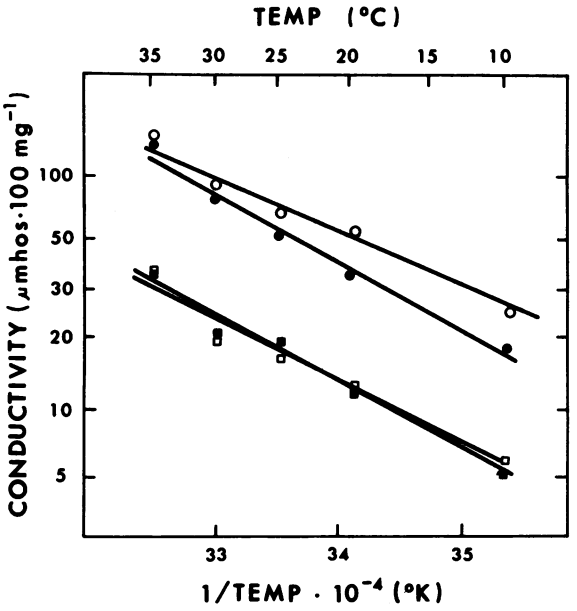

FIG. 1. Arrhenius plots of the efflux of total electrolytes (conductivity) from soybean axes (○, ●) and cotyledons (□, ■) dehydrated at 6 (●, ■) or 36 h (○, □) of imbibition. Ten axes or six cotyledons were reimbibed in 10 ml distilled H_2O for 2 h and then transferred to distilled H_2O preincubated at specified temperatures. The conductivity of the imbibing solution was measured after a 4-h incubation.

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*

Solute	Axis			Cotyledon		
	6 h		36 h	6 h		36 h
	kcal/mol					
Potassium	11.3[a]		5.7	11.0	NS	11.7
Phosphate	11.0[a]		8.4	13.0	NS	10.5
Amino acid	7.4[a]		4.3	3.3	NS	2.6
Protein	4.9	NS	5.3	8.2	NS	9.2
Conductivity	13.6[a]		10.5	11.3	NS	11.1

[a] NS, significantly different or not significantly different according to *t* test comparison at $P \leq 0.05$.

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 $\log K = b - a \cdot 1/T$, 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 did 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, E_a can be calculated from the slope of the line. E_a 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). E_a was significantly ($P \leq 0.05$) reduced for K^+, Pi, amino acid, and total electrolytes in axes dehydrated after 36 h (Table III). E_a for protein leakage was not significantly reduced.

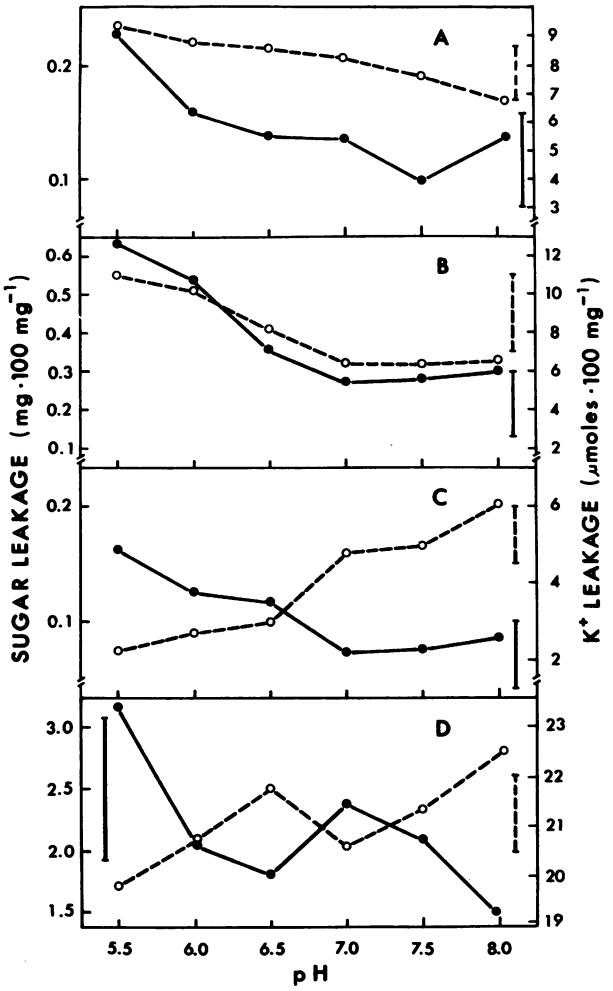

FIG. 2. Leakage of potassium (●) and sugar (○) from soybean axes in response to external pH. A, Axes excised from soybean seeds at 6 h imbibition. B, Axes excised from soybean seeds at 6 h imbibition, dehydrated to 10% moisture, and reimbibed in distilled H_2O for 2 h. C, Axes excised from soybean seeds at 36 h imbibition. D, Axes excised from soybean seeds at 36 h imbibition, dehydrated to 10% moisture, and reimbibed in distilled H_2O for 2 h. All treatments were soaked for 4 h in 25 ml of 40 mM Mes-Hepes buffer. The imbibing 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 ($P \leq 0.05$) among pH treatments.

Although attempts were made to obtain Arrhenius plots of sugar efflux, the data did not fall on a straight line and therefore E_a 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 ($P \leq 0.05$) differences in E_a 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 reimbibed for 2 h; (c) imbibed for 36 h; (d)

imbibed 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 testa 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 analyses 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. Nontheless, 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^+, Pi, 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 imbibed 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 imbibed 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.

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