Plant Physiol. (1983) 72, 620–624
0032-0889/83/72/0620/05/$00.50/0

Dehydration Injury in Germinating Soybean (*Glycine max* L. Merr.) Seeds[1]

Received for publication December 2, 1982 and in revised form March 11, 1983

TISSA SENARATNA AND BRYAN D. MCKERSIE
Department of Crop Science, University of Guelph, Guelph, Ontario N1G 2W1 Canada

ABSTRACT

The sensitivity of soybean (*Glycine max* L. Merr. cv Maple Arrow) seeds to dehydration changed during germination. Seeds were tolerant of dehydration to 10% moisture if dried at 6 hours of imbibition, but were susceptible to dehydration injury if dried at 36 hours of imbibition. Dehydration injury appeared as loss of germination, slower growth rates of isolated axes, hypocotyl and root curling, and altered membrane permeability. Increased electrolyte leakage due to dehydration treatment was observed only from isolated axes but not from cotyledons, suggesting that cotyledons are more tolerant of dehydration. The transition from a dehydration-tolerant to a dehydration-susceptible state coincided with radicle elongation. However, the prevention of cell elongation by osmotic treatment in polyethylene glycol (−6 bars) or imbibition in 20 micrograms per milliliter cycloheximide did not prevent the loss of dehydration tolerance suggesting that neither cell elongation nor cytoplasmic protein synthesis was responsible for the change in sensitivity of soybean seeds to dehydration. Furthermore, the rate of dehydration or rate of rehydration did not alter the response to the dehydration stress.

Exposure to environmental stress often induces injury to cellular membrane systems in the plant cell. Increased leakage of cytoplasmic solutes follows the exposure of sensitive plants to freezing (21), chilling (11), dehydration (16, 17), air pollutants (14), and osmotic shock (9). This has led several authors to conclude that the primary site of injury is at the level of the membrane. Several models, the most notable being for chilling injury (12), have been proposed which relate environmental stress with alterations in membrane properties and eventual cell death. However, for several stresses, such as freezing and dehydration, it has not been resolved whether the increased rates of solute leakage which are characteristic of membrane injury occur as a result of the physical rupture of cells (23) or as a result of permeability or other functional changes in membranes (19, 22). Without specific knowledge of the nature of the membrane lesion induced by either freezing or dehydration stresses, the physiological or biochemical basis for the tolerance of some plants to these stresses can not be defined. The objective of this and a subsequent study (15) was to determine if the injury to the cellular membranes which occurs as a result of a lethal dehydration treatment is associated with cell rupture or with functional changes in an intact cell membrane.

Seeds provide a convenient system to study dehydration tolerance (8). After seed maturation and in the early stages of germination, seeds of a wide variety of plants can be dried to 10% moisture without loss of viability, but if they are dried prior to maturation or after radicle emergence, the seeds are not able to germinate (6, 16, 17). In this study, the changes in dehydration tolerance of the axis and cotyledons of soybean (*Glycine max* L. Merr) seeds are compared during seed germination. The effects of cell size, the rate of dehydration, and the rate of rehydration are described. In the subsequent paper, the effect of a lethal dehydration treatment on solute efflux and membrane function are detailed (15).

MATERIALS AND METHODS

Sensitivity of Intact Seeds to Dehydration. Seeds of soybean (*Glycine max* L. Merr. cv Maple Arrow) were obtained from the 1979 harvest at the Elora Research Station of the University of Guelph, Ontario. Soybean seeds were imbibed at 25°C in 9-cm Petri dishes between two filter papers moistened with 6.5 ml distilled H_2O. Seed samples were removed at 6, 12, 18, 24, and 36 h. Seeds from each imbibitional time were air dried at 25°C in an incubator to 60, 40, 20, and 10% moisture by weight. Moisture content (%) was calculated as g H_2O/g fresh weight × 100. To determine viability, 20 dehydrated seeds were transferred back to the moistened filter paper in Petri dishes and the number of germinating seeds were counted after 4 d. Seeds with further elongation of the axis were considered as germinated. To quantify electrolyte leakage, three dehydrated seeds from each treatment were reimbibed in 10 ml distilled H_2O at room temperature for 1 h, and conductivity of the imbibing solution was measured using a Barnstead conductivity bridge. The experiment was replicated four times and data analyzed for statistical significance as a split plot arrangement of a RCB[2] design with time of dehydration as main plot and moisture content as subplot.

Sensitivity of Axis Tissue to Dehydration. Seeds were imbibed as before for specified lengths of time between 3 and 36 h. Axis tissue was removed and dehydrated to 10% moisture under the same drying conditions as before. Ability of the axes to regrow was determined *in vitro* as the increase in fresh weight of 10 axes grown on a medium of 1% (w/v) agar + 2% (w/v) sucrose in Petri dishes under sterile conditions at 25°C. The experiment was replicated three times and the data analyzed as a completely randomized design.

Electrolyte Leakage. To determine the rates of electrolyte leakage from dehydration damaged and undamaged tissues, soybean seeds were imbibed for 6 and 36 h and divided into axis and cotyledon tissues. Each tissue was dried back to 10% moisture, and 10 dehydrated axes or six dehydrated cotyledons were reimbibed in 10 ml distilled H_2O. Conductivity of the imbibing solution was measured at 15, 30, 45, 60, 120, 180, 240, 300, 360, and 420 min of soaking period. Water uptake by the axes and cotyledons was monitored as the increase in fresh weight after blotting the tissue

[1] Supported by the Natural Sciences and Engineering Research Council of Canada.

[2] Abbreviations: RCB, randomized complete block; CHI, cycloheximide.

dry on filter paper. The experiment was replicated four times and analyzed statistically as a RCB design.

Prevention of Cell Elongation. Seeds were imbibed as before in Petri dishes between two filter papers moistened with 6.5 ml distilled H_2O and sampled at 6 and 36 h of imbibition. A third sample of seeds was transferred after 6 h imbibition in distilled H_2O to Petri dishes with 6.5 ml PEG solution at −6 bars water potential, and incubated for an additional 30 h (total time of imbibition was 6 + 30 h). A fourth sample of seeds was imbibed in 6.5 ml of a solution containing 20 μg/ml cycloheximide for 36 h. Subsequently, axes were removed and air dried to 10% moisture, and electrolyte leakage was measured as previously described. Viability of the seeds after drying was determined as per cent germination of intact seeds reimbibed on moist filter paper. The experiment was repeated four times and analyzed statistically as a RCB design.

Rate of Dehydration. Seeds were imbibed in distilled H_2O for 36 h as previously described. Axes were placed inside desiccators above 20, 40, or 60% $CaCl_2$ solutions, with a fourth sample air dried as in previous experiments. Water loss from the tissues was monitored as loss in fresh weight. Once the samples in a desiccator approached equilibrium with the atmosphere of a desiccator, each sample was transferred into the appropriate desiccator with the next higher concentration of $CaCl_2$, *i.e.* from the desiccator with 40% $CaCl_2$ to the desiccator with 60%; from the desiccator with 20% to 40% and then to 60%, etc. By this method, approximately linear rates of dehydration were obtained. Ultimately, all axes were air dryed to a common moisture level. Dehydrated axes were reimbibed in distilled H_2O, and electrolyte leakage measurements were made as before. Regrowth of treated axes was determined *in vitro* as before. The experiment was repeated three times and analyzed statistically as a RCB design.

Rate of Rehydration. Seeds were imbibed for 36 h, and axes were air dried to 10% moisture as before. Samples of 10 dehydrated axes were reimbibed in Petri dishes on filter paper moistened with 5, 10, 20, 40, or 50% PEG solutions. Water uptake by the tissue was monitored as increase in fresh weight. Once the axes approached equilibrium with the external solution, they were transferred to a Petri dish with PEG at the next highest water potential, *i.e.* the axis sample which started at 50% PEG solution was transferred to 40% and then to 20%, etc., to obtain approximately linear rates of rehydration. Ultimately, all axes equilibrated in the 5% solution. A sixth sample of axes was kept in a chamber saturated with water vapor (100% RH) and allowed to absorb moisture from the air for 24 h. Finally, all treatments, including controls of 6- and 36-h imbibed/dehydrated axes were reimbibed on filter papers moistened with distilled H_2O, until fully hydrated. These fully hydrated tissues were transferred into 10 ml distilled H_2O, and the rate of electrolyte leakage from the tissue was measured. At the end of the experiment, the samples were homogenized in the imbibing solution and the conductivity of the homogenate was determined to estimate the total electrolyte content of the tissue. Regrowth of treated axes was determined *in vitro* as increase in fresh weight. The experiment was repeated four times and analyzed statistically as a RCB design.

RESULTS

Sensitivity of Intact Soybean Seeds to Dehydration. The sensitivity of soybean seeds to dehydration was influenced by the stage of germination at which they were dried. Soybean seeds which were imbibed for 6 h and subsequently dehydrated did not suffer impaired germination even after dehydration to 10% moisture (Table I). This tolerance of dehydration was lost as the length of the imbibition period increased as shown by the fact that seeds imbibed for 12, 18, or 24 h exhibited reduced viability (per cent germination) after dehydration to 10% moisture. At 36 h imbibition, the radicle had emerged from the seed coat. Dehydration to

Table I. *Germination of Intact Soybean (Glycine max L. Merr.) Seeds after Dehydration at Different Stages of Germination*

Germination of nondehydrated seeds was 93 ± 3% (SE).

Moisture Content after Dehydration	Time of Imbibition before Dehydration (h)				
	6	12	18	24	36
%	% germination				
60	90 a[a]	90 a	88 a	88 a	90 a
40	90 a	90 a	90 a	90 a	90 a
20	93 a	88 a	88 a	93 a	93 a
10	90 a	60 b	65 b	65 b	0 b

[a] Values within a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

Table II. *Electrolyte Leakage from Intact Soybean (Glycine max L. Merr.) Seeds after Dehydration at Different Stages of Germination*

Values represent the conductivity of the imbibing solution after 1 h of incubation.

Moisture Content after Dehydration	Time of Imbibition before Dehydration (h)				
	6	12	18	24	36
%	μmhos/100 mg				
60	9.7 a[a]	14.0 b	9.2 a	8.3 b	6.8 b
40	10.1 a	12.0 b	9.7 a	6.3 b	6.8 b
20	10.0 a	14.0 b	9.4 a	7.9 b	7.8 b
10	9.0 a	16.1 a	10.2 a	10.3 a	21.8 a

[a] Values within a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

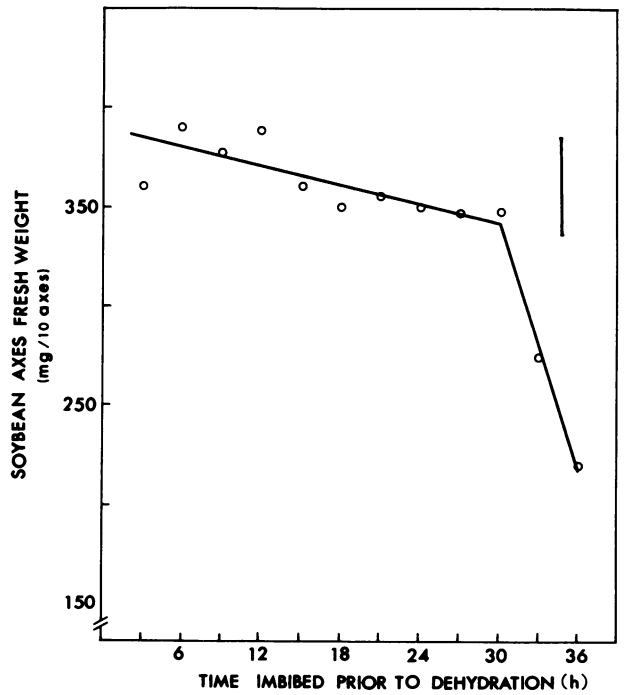

FIG. 1. Fresh weight of soybean axes on 1% (w/v) agar, 2% (w/v) sucrose media after 96 h growth. Seeds were imbibed for 3 to 36 h, the axes were removed and dried to 10% moisture. Average weight of 10 axes at 10% moisture prior to transfer to agar was 41 mg ± 0.5 (SE). Vertical bar represents LSD at $P = 0.05$; $n = 3$.

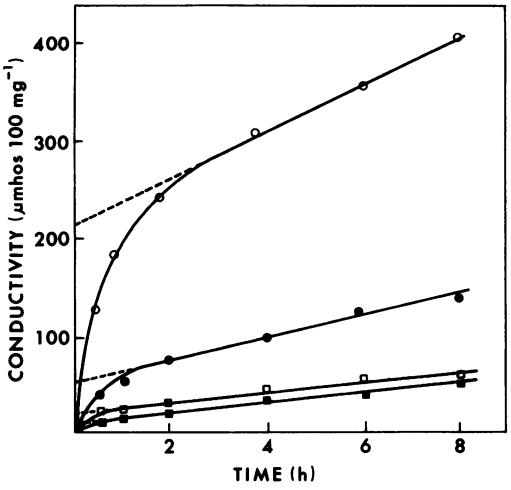

FIG. 2. Time profile of total electrolyte leakage (conductivity) during reimbibition of soybean axes (○, ●) and cotyledons (□, ■) dehydrated after 6 (●, ■) and 36 h (○, □) imbibition. Values represent the conductivity of 10 ml distilled H_2O during the imbibition of 10 axes or 6 cotyledons. Regression analysis was performed between 2 and 8 h; $n = 4$.

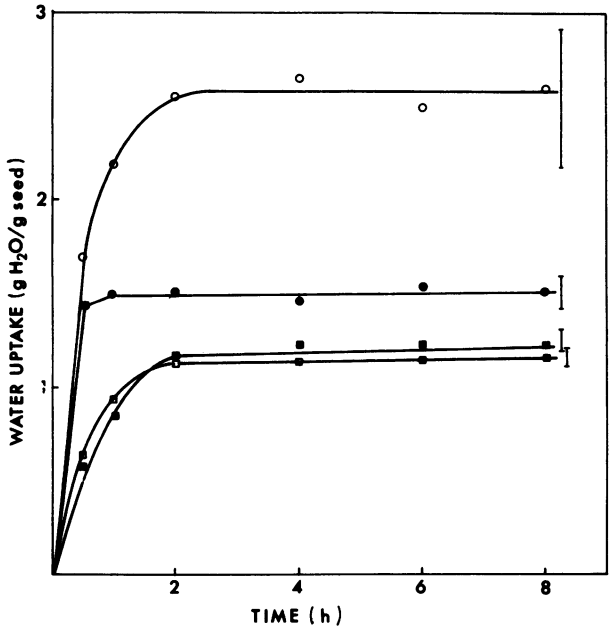

FIG. 3. Rate of water uptake during reimbibition of soybean axes (○, ●) and cotyledons (□, ■) dehydrated after 6 (●, ■) and 36 h (○, □) of imbibition. Vertical bars represent LSD at P = 0.05.

10% moisture at this stage prevented subsequent germination. The severity of the dehydration stress also influenced viability. The seeds at any stage of germination which were dehydrated to a moisture content of 20% or above did not lose their ability to germinate upon subsequent imbibition (Table I).

Seeds dehydrated at 6 h imbibition did not exhibit increased electrolyte leakage due to dehydration treatments (Table II). Dehydration to 10% moisture content increased leakage from seeds imbibed for 12, 18, 24, and 36 h with highest leakage from the seeds dehydrated to 10% moisture after 36 h of imbibition. A significant ($P \leq 0.01$) negative relationship ($r = -0.79$) exists between per cent germination (Table I) and electrolyte leakage (Table II) in intact dehydrated soybean seeds. There was greater leakage from seeds dehydrated at 12 h imbibition, averaged over all moisture contents, than at other times (Table II), but this aspect was not further investigated in this study.

Sensitivity of Soybean Axes to Dehydration. Axes which were removed from the imbibed seeds between 3 and 30 h imbibition and dehydrated to 10% moisture had similar increases in fresh weight when placed on agar medium for 96 h (Fig. 1). Damage was apparent in those axes dehydrated at 33 or 36 h imbibition as evidenced by an inability to elongate (data not shown) and reduced fresh weight (Fig. 1). Visual signs of injury, such as curled hypocotyls and roots, were evident in axes dehydrated after 12 h imbibition (data not shown) even though growth rates were not impaired. This visual damage may have been due to localized damage in the growing axis which was not sufficient to affect the increase in fresh weight. Inasmuch as dehydration did not induce visual injury, reduce growth rate, or impair germination in seeds imbibed for 6 h, but did in those imbibed for 36 h, subsequent experiments compared only these two extremes as representative of dehydration-tolerant and dehydration-sensitive states, respectively.

Electrolyte Leakage from Axes and Cotyledons. Electrolyte leakage from axes and cotyledons, which were dehydrated to 10% moisture after 6 or 36 h imbibition, followed a biphasic time profile as the tissue was rehydrated (Fig. 2). A rapid release of electrolytes during the 1st h of imbibition was followed by a slower constant rate of leakage for up to 8 h. During the first phase, some of the electrolytes are leached from extracellular sites such as the seed surface, intercellular spaces, and ruptured cells. During the second phase, the leakage appears linear and apparently the movement of electrolytes is limited by a diffusion barrier, presumably the plasma membrane, which regulates the rates at which solutes diffuse out of the tissue.

The quantity of electrolytes leaked during the first phase increased in the axes dehydrated after 36 h of imbibition relative to axes dehydrated after 6 h of imbibition, possibly reflecting an increased incidence of cellular rupture. The subsequent linear rate of leakage between 2 and 8 h was greater in the axes dehydrated after 36 h imbibition. In contrast, there was no significant difference in leakage between cotyledons dehydrated at 6 and 36 h of imbibition.

Differences in water uptake upon reimbibition of the tissue may influence the relative rate of leakage. Axes dehydrated at 36 h imbibition had greater water uptake during the first 2 h of reimbibition compared to axes dehydrated at 6 h imbibition (Fig. 3). This was probably because the elongated cells at 36 h had greater volume and possibly a higher osmotic potential. There were no significant differences in water uptake between cotyledons. After 2 h reimbibition, all the tissues were fully hydrated and there was no further water uptake during the course of the experiment. Therefore, measurements of the rate of electrolyte leakage after 2 h (Fig. 2) did not include any complications attributable to differences in water uptake. The rate of leakage after 2 h presumably indicates the release of solutes from cytoplasmic sites which must pass through the plasma membrane and, as a result, the rate of leakage between 2 and 8 h is a better indicator of membrane permeability than measurements of leakage at an earlier time.

Effect of Cell Elongation. Treatment of axes with PEG at −6 bars prevented radicle elongation at 36 h (Table III). These axes had lower fresh weights but similar dry weights after imbibition compared to those remaining in distilled H_2O, indicating that differential water uptake was induced by PEG treatment. Similarly, the axes of seeds imbibed in CHI did not elongate and had reduced fresh weights indicating that cell expansion had also been prevented by this treatment. The prevention of cell elongation with PEG did not prevent the axes from completing the transition to dehydration sensitivity. Seeds which were imbibed for 6 h, transferred to PEG until 36 h, and subsequently dehydrated, were unable to germinate when transferred back to distilled H_2O (Table III).

Table III. *Effect of PEG and CHI on Loss of Dehydration-Tolerance of Soybean (Glycine max L. Merr.) Seeds*

Time of Dehydration	Imbibing Solution	Axis Elongation	Axis Dry Wt	Axis Fresh Wt[a]	Germination	Phase 1 Leakage[b]	Rate of Leakage[c]
h			*mg/10 axes*		%	*μmhos/100 mg*	
6	Water	Absent	42.7 ± 0.6	108 ± 0.8	93 a[d]	27 c	10 b
36	Water	Present	42.4 ± 6.9	178 ± 5.1	0 b	323 a	27 a
36	PEG	Absent	42.1 ± 1.5	85 ± 1.3	0 b	84 b	26 a
36	CHI	Absent	42.4 ± 0.4	98 ± 1.9	ND[e]	57 b	25 a

[a] Fresh weight prior to dehydration treatment ± SE.

[b] Phase 1 leakage was calculated as the *y*-intercept of the linear regression line calculated from electrolyte leakage values between 2 and 7 h soaking period.

[c] Rate of leakage was calculated as the slope of the regression line between 2 and 7 h soaking period.

[d] Values within a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

[e] Not determined.

Table IV. *Effect of the Rate of Dehydration on Electrolyte Leakage and Regrowth of Soybean (Glycine max L. Merr.) Axes*

Time of Dehydration	Concn. of $CaCl_2$	Rate of Dehydration	Regrowth[a]	Phase 1 Leakage[b]	Rate of Leakage[c]
h	%	*g $H_2O \cdot g^{-1}$ dry wt $\cdot h^{-1}$*	*mg*	*μmhos/100 mg*	*μmhos $\cdot$ 100 $mg^{-1} \cdot h^{-1}$*
6	Air	0.500	560 a[d]	62 d	8 b
36	20	0.077	192 b	205 bc	22 a
36	40	0.126	183 b	189 c	21 a
36	60	0.310	188 b	253 b	24 a
36	Air	0.480	175 b	306 a	25 a

[a] Fresh weight of 10 axes after regrowth on agar + 2% sucrose media for 96 h.

[b] Phase 1 leakage was calculated as the *y*-intercept of the linear regression line between 2 and 7 h soaking periods.

[c] Rate of leakage was calculated as the slope of the same regression line.

[d] Values within a column followed by the same letter are not significantly different at $P \leq 0.05$ according to Duncan's Multiple Range Test.

Table V. *Effect of the Rate of Rehydration on Electrolyte Leakage and Regrowth of Soybean (Glycine max L. Merr.) Axes*

Time of Dehydration	Imbibing Solution	Rate of Rehydration	Regrowth[a]	Rate of Leakage[b]
h		*g $H_2O \cdot g^{-1}$ dry wt $\cdot h^{-1}$*	*mg*	*%/h*
6	Distilled H_2O	1.30	631 a[c]	1.8 b
36	Moist air	0.06	172 b	7.0 a
36	50% PEG	0.09	184 b	5.5 a
36	40% PEG	0.09	187 b	5.8 a
36	20% PEG	0.16	208 b	5.7 a
36	10% PEG	0.36	173 b	5.9 a
36	5% PEG	0.36	200 b	5.7 a
36	Distilled H_2O	1.20	187 b	7.6 a

[a] Fresh weight of 10 axes after regrowth on agar + 2% sucrose media for 96 h.

[b] Rate of leakage was calculated as the slope of the regression line between 2 and 7 h of reimbibition.

[c] Value within a column followed by the same letter are not significantly different at $P \leq 0.05$.

The time profile of electrolyte leakage from axes which were dehydrated and reimbibed in distilled H_2O followed the typical biphasic pattern shown in Figure 2. The total electrolytes leaked during phase 1 reimbibition was lowest from the axes imbibed in water for 6 h, was intermediate from those imbibed in PEG and CHI for 36 h, and was greatest from the elongated axes imbibed for 36 h in water (Table III). The rate of leakage between 2 and 7 h was not significantly ($P \leq 0.05$) different among PEG, CHI, and control axes which were imbibed for 36 h.

Rate of Dehydration. Drying soybean axes over different concentrations of $CaCl_2$ induced different rates of water loss (Table IV), but all axes were dried to 10% moisture by a final period of air drying, so that differences in the severity of dehydration did not exist among treatments. The rate of dehydration had no significant effect on the ability of the axes to regrow or on the rate of electrolyte leakage from axes during reimbibition. A slight reduction of the phase 1 leakage was observed at the slower rates of drying indicating a possible reduction in cellular rupture with slow drying.

Rate of Rehydration. By reimbibing seeds in different concentrations of PEG or in moist air, different rates of rehydration were achieved (Table V). The most rapid rate of water uptake was observed in distilled H_2O and the slowest in moist air. Increasing the concentration of PEG reduced the rate of water uptake. The slow imbibition treatments did not increase the growth of those axes dehydrated at 36 h nor did they reduce the rate of electrolyte leakage, when expressed as a percentage of total cellular electrolytes. The absolute amount of electrolytes leaked from those seeds previously imbibed in PEG was significantly lower than that from seeds imbibed in moist air if expressed as $\mu mhos \cdot 100\ mg^{-1} \cdot h^{-1}$. However, approximately 70% of the total electrolytes had been previously leached from the axes into the PEG solutions so that expressing the data on a per mg basis would be misleading.

DISCUSSION

The viability of soybean seeds after an imbibition-dehydration treatment was influenced by the severity of the dehydration treatment and the length of the germination period prior to dehydration. Loss of viability was observed only in seeds which were dried to less than 20% moisture and only in seeds which had been imbibed for 12 h or more. A threshold of 20% moisture has been reported in other species (17) but desiccation-sensitive seeds

can be injured at higher moisture levels (2). Similar changes in sensitivity to dehydration have been reported during the germination of oats (1, 17), wheat (4), corn (5, 7), birdsfoot trefoil (16, 17), and rye (20) and would appear to be a general phenomenon associated with germination.

In this study, the inverse relationship between germination and electrolyte leakage suggests that dehydration of sensitive soybean seeds may alter properties of cellular membranes. Because the alterations in membrane properties observed in dehydration-damaged soybean seeds and isolated axes are detectable immediately upon reimbibition of the tissue, membrane damage apparently occurred during the dehydration period. Because cellular metabolism would be expected to be minimal at this time, especially below 20% moisture, the dehydration treatment appears to have induced a physical disruption of the cellular membranes as a consequence of water loss and not as a consequence of altered metabolism.

It could be hypothesized that the observed change in dehydration sensitivity of the seed was due to an increase in cell size during radicle emergence. According to Meryman's (18) model of freezing injury, a cell is injured when the cell volume is reduced below a critical size. The strain on membranes as the cell dehydrates and shrinks would induce lateral compression of the membrane leading to dehydration injury. An analogous situation could exist in seeds if dehydration tolerance was lost once the cell exceeded a specific size. The observation that the change in sensitivity to dehydration temporally coincided with the initiation of cell elongation and radicle emergence supported this hypothesis. In addition, membrane injury appeared only in the elongating axis, but not in the cotyledons. On the other hand, preventing cell elongation with PEG did not prevent the loss of dehydration tolerance. The rate of electrolyte leakage from fully hydrated tissue was not significantly different between axes which had elongated and axes which were prevented from elongating by PEG or CHI. Thus, these data indicate that cell enlargement is not the primary factor which changes the sensitivity of the axes to dehydration.

Another important event occurring in the seed at this stage is protein synthesis, which is essential to maintain the supply of enzymes associated with the germination process (13). Inasmuch as the rate of leakage from the CHI-treated axis tissue was similar to that of the control, it seems that the inhibition of cytoplasmic protein synthesis does not prevent the development of dehydration sensitivity.

Preventing cell elongation with PEG or CHI did substantially reduce the quantity of electrolytes leaked during the first phase of leakage prior to complete hydration. This may reflect a decrease in cell rupture during reimbibition. Reducing the rate of dehydration also reduced electrolyte leakage during phase 1, but in neither case did the reduction in phase 1 leakage, and presumably cell rupture correspond to an increase in viability or growth rate.

Slow rates of dehydration are able to prevent injury in some mosses (3, 10), and slow rates of rehydration in PEG solution have restored viability in aged soybean axes (24). However, in dehydrated soybean axes, varying the rate of dehydration or rehydration of sensitive seeds (those imbibed for 36 h) did not prevent injury or restore viability. Imbibing in PEG did reduce the absolute quantity of electrolytes leaked when the axes were subsequently transferred to distilled H_2O, but this is because 70% of the electrolytes were leaked into the PEG solution.

In summary, these data imply that physical differences, such as cell size, rate of dehydration, and rate of rehydration, do not influence the sensitivity of soybean axes to dehydration. Therefore, an alternate model which is currently under investigation assumes that biochemical or biophysical changes in cellular membranes occur during germination, which are associated with metabolic alterations essential for cell elongation. Dehydration of the seed after a critical stage perturbs these membrane systems and irreversibly blocks this developmental process such that it cannot continue upon reimbibition.

Acknowledgments—The facilities provided by the Ontario Ministry of Agriculture and Food are gratefully acknowledged. A scholarship from the International Development Research Centre, Canada, to Tissa Senaratna is also gratefully acknowledged.

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