Dehydration Effects on Imbibitional Leakage from Desiccation-Sensitive Seeds

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ABSTRACT

Changes in electrolyte leakage and viability in response to dehydration stress were examined in two species of seeds that do not survive desiccation. Leakage from silver maple (Acer saccharinum L.) seeds increased markedly as seed moisture contents decreased from 45 to 35% (fresh weight basis) and germination decreased from 97 to 5%, coincidentally. Time course curves of imbibitional leakage from areca palm (Chrysalidocarpus lutescens [Bory] Wendel) embryos showed an increase in both initial leakage and steady-state leakage rates in response to dehydration from an original moisture content of 84 to as low as 35%. Absorbance at 530 nanometers of extracts from triphenyl tetrazolium chloride stained embryos of areca palm was used as a measure of viability. Absorbance decreased significantly in response to dehydration as embryo moisture content decreased from 80 to 30%. Collectively, the data suggest that membranes in the desiccation-sensitive seed tissues studied are damaged by dehydration below a critical moisture content, 40% in silver maple seed and 55% in areca palm embryos, and that the membrane damage contributes to loss of viability.

Seeds of many tropical and some temperate plant species do not survive dehydration (2, 6). The seeds of silver maple are an example (8). It is known that germination of desiccation-sensitive seeds declines rapidly as seed moisture content is decreased, but little is known about the mechanism or events leading to this dehydration-induced loss of viability. Studies on the mechanism of aging and deterioration in desiccation-tolerant seeds show that an increase in electrolyte leakage is associated with a loss of germination capacity (3, 15). Because an increase in electrolyte leakage reflects an increase in membrane permeability to solutes, deterioration of cellular membranes has been implicated as a causal mechanism of deterioration and senescence in seeds (15, 20).

Time course curves of leakage from dehydrated seeds show initial rapid leakage rates associated with the amibution of water (14, 17). It has been proposed that this phase of rapid leakage represents a period of membrane reorganization associated with rehydration, and that subsequent linear leakage rates represent steady-state diffusion of solutes through organized membranes (16). It has also been suggested that water uptake by desiccation-tolerant seeds reinstates the original structure of the cellular membranes, whereas the membranes of desiccation-sensitive seeds that have been dehydrated are unable to reform completely (12).

If dehydration stress disrupts membrane integrity in desiccation-sensitive seeds, then changes in leakage rates and increases in the amount of solutes leaked may be detectable in response to dehydration, and these changes should be associated with loss of viability. Our results show that increased imbibitional leakage is associated with dehydration stress in two species of desiccation-sensitive seeds and that there is a close correlation between increased leakage and loss of viability.

MATERIALS AND METHODS

Seed moisture contents are expressed on a fresh weight basis with dry weight determined after 24 h drying at 103°C according to standardized methods (7).

Silver Maple. Mature fruits were collected from a silver maple (Acer saccharinum L.) tree in Fort Collins, CO on 6 June 1980. The moisture content of seeds extracted from samaras was 48% at the time of collection. Fruits were stored in sealed glass vials at 5°C until use. Seeds extracted from samaras were dehydrated at 35°C. Seeds were removed from the dehydration treatment at 2-3-h intervals. Dehydration times ranged from 2 to 20 h. After dehydration, seeds were again sealed in glass vials and held at 5°C for 48 to 72 h. Ten seeds were then removed from each group to determine seed moisture contents which ranged from 46% with no dehydration treatment to as low as 21% with 20 h of dehydration. Germination tests (30°C, dark) were conducted with 100 seeds at each moisture content on filter paper moistened with distilled H2O in Petri plates (19). Germination (radical emergence) was determined 7 days after imbibition.

Electrolyte leakage was measured from whole seeds with intact testae, whole seeds with testae removed, and from halved seeds. Ten seeds at each moisture content were placed in 10 ml distilled H2O for 24 h at 25°C after which the initial conductance (Ci) of the leachate was measured with a conductivity meter. Seeds were then exposed to 80°C for 30 min to lyse cellular membranes and returned to 25°C for 30 min before measuring the total conductance (Ct). Percent electrolyte leakage was expressed as (Ct/Ci) x 100. All electrolyte leakage experiments on silver maple seeds were repeated at least twice.

Areca Palm. Mature fruits were collected from an areca palm (Chrysalidocarpus lutescens [Bory] Wendel) in Oahu, Hawaii on 11 March 1981 and shipped to the National Seed Storage Laboratory. Embryo and endosperm moisture contents of the seed on receipt were 84 and 62%, respectively, and germination (30°C, dark) was 85%. Seeds removed from fruits were stored in sealed glass vials at 10°C. Seeds were dehydrated by exposure to ambient laboratory conditions (22°C) for 1, 2, 3, and 4 days. After dehydration seeds were held at 10°C in sealed glass vials for 48 to 72 h. Moisture contents of embryos excised from seeds ranged from 84% with no dehydration treatment to 53% with 4 days of dehydration.

Electrolyte leakage experiments were conducted on embryos excised from areca palm seeds which had been equilibrated to

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various moisture contents as previously described. Triplicate samples of 10 embryos at each moisture content were immersed in 2 ml distilled H$_2$O and the conductance of the leachate measured over time according to methods previously described. TTC$^2$ reduction test, similar to that described elsewhere (18), was used to measure viability of areca palm embryos in relation to moisture content. Triplicate samples of 10 excised embryos were dehydrated at room temperature to 55 and 30% moisture content and held in sealed vials at 10°C for 48 h before measuring TTC reduction as follows: embryos were imbibed in distilled water at 22°C for 1 h and then incubated at 32°C for 3 h in a solution of 1% (w/v) TTC in a phosphate buffer (pH 7). Samples were heated in 2 ml 95% ethanol at 60°C for 2 h to extract the reduced formazan. A at 530 nm of the cooled extracts was used to quantify the level of TTC reduction and compared to that of non-dehydrated embryos and heat (80°C) killed controls.

RESULTS

Silver Maple. Representative curves of electrolyte leakage from silver maple seeds show that dehydration had a profound effect on leakage (Fig. 1A). Leakage from whole seeds with testae removed prior to imbibition increased markedly at seed moisture contents between 45 and 35%. Leakage after 24 h from whole seeds with intact testae did not increase appreciably in response to dehydration. Previous studies have shown that the testae of some seeds may restrict leakage (4, 16). When the whole seeds were removed from the leachate, cut in half, and returned to the leachate for an additional 24 h, a marked increase in leakage also occurred between 45 and 35% moisture (Fig. 1A). The midpoint in the transition from minimum to maximum leakage from whole seeds without testae and from halved seeds occurred at 41 and 37% moisture contents, respectively. Germination of silver maple seeds decreased from 97 to 5% between 45 and 35% moisture content (Fig. 1B). Germination also declined from 96 to 9%

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3 Abbreviation: TTC, triphenyl tetrazolium chloride.

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![Graph](image-url)  
**Fig. 1.** Electrolyte leakage and germination of silver maple seeds in response to dehydration. A, imbibitional leakage after 24 h at 25°C from whole seeds with intact testae (○), halved seeds (□), and whole seeds with testae removed (□). B, germination of seeds with intact testae.

![Graph](image-url)  
**Fig. 2.** Electrolyte leakage from areca palm embryos in response to dehydration. A, time course curves of imbibitional leakage from embryos at 84 (○), 80 (□), 70 (△), 61 (▲), and 53% (△) moisture contents. B, leakage at 2 h in relation to embryo moisture content. Vertical bars are ± SE for three observations.

<table>
<thead>
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<th>Embryo Moisture</th>
<th>Initial Leakage</th>
<th>Leakage Rate between 45 and 120 min</th>
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<tr>
<td>% fresh weight</td>
<td>%</td>
<td>/h</td>
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between 45 and 35% moisture content when seeds were dehydrated at room temperature (data not shown) rather than at 35°C. Thus, there was a close correlation between the seed moisture content at which leakage increased rapidly and at which germination declined rapidly.

**Areca Palm.** Electrolyte leakage from areca palm embryos increased in response to dehydration in a manner similar to that observed in silver maple seed, except that the increase occurred at a higher moisture content and over a wider range of dehydration stress (Figs. 1A and 2B). Time course curves of leakage during imbibition revealed that rates of leakage from areca palm embryos were non-linear during the initial 45 min of imbibition (Fig. 2A). A linear or steady phase of leakage followed. The net leakage during the initial nonlinear phase was estimated by extrapolating the slope of the linear portion of the curve to the ordinate (14). Initial net leakage and subsequent steady-state leakage rates from embryos increased 7-fold in response to dehydration from 84 to 53% (Table I). Leakage after 2 h imbibition from embryos dehydrated to 61 and 53% moisture contents was significantly ($P < 0.05$) greater than leakage from non-dehydrated embryos at 84% moisture content (Fig. 2B).

Embryos from nondehydrated areca palm seeds, which had an 84% germination level, had a correspondingly high level of TTC reduction (Table II). Viability of areca palm embryos, as determined by TTC reduction, decreased in response to increasing dehydration stress. A at 530 nm of extracts from embryos decreased significantly ($P < 0.05$) as embryo moisture content decreased from 80 to 30%. A at 530 nm of extracts from embryos dehydrated to 30% moisture content was not significantly different from that of embryos exposed to 80°C. The results of leakage and viability tests on areca palm embryos, therefore, agree with those on silver maple seeds; as dehydration stress increased, leakage increased significantly and viability decreased concomitantly.

**DISCUSSION**

Electrolyte leakage from tissues can be used to indicate the effectiveness of membranes as barriers to solute diffusion (1, 10, 16). Whereas relatively low levels of leakage indicate cellular membranes are semipermeable, high levels of leakage indicate damage to membranes. The results of our experiments indicate that the cellular membranes of the two desiccation-sensitive seeds were severely damaged by excessive dehydration. Relative to leakage after seeds were exposed to 80°C to lyse cellular membranes, 80 to 100% of the cellular electrolytes leaked from silver maple seeds dehydrated below 35% moisture content after 24 h of imbibition. Less than 20% of the cellular electrolytes leaked from nondehydrated seeds at 45% moisture content during the same time. Areca palm embryos dehydrated to 53% moisture content leaked nearly 70% of their cellular electrolytes after only 2 h of imbibition. In contrast, only 10% leaked from nondehydrated areca palm embryos at 84% moisture content during the same time. Thus, the effect of excessive dehydration on silver maple seed and areca palm embryo tissues was manifested as a nearly complete removal of the diffusion barrier to cellular electrolytes.

Our experiments were conducted on tissues from detached seeds sensitive to excessive dehydration at maturity and have shown a close correlation between increased leakage and loss of viability in response to dehydration stress. We have not determined if similar physiological changes occur in immature and/or attached seeds. Previous investigations on changes in membrane integrity of seeds in response to dehydration stress have been conducted on seeds that tolerate excessive dehydration at maturity. McKersie and Stinson (12) have shown that desiccation-tolerant seeds which have been imbibed and subsequently dehydrated to their original weight maintained viability and leaked relatively low levels of electrolytes upon imbibition if the dehydration treatment was imposed very early in the initial germination process. However, if the dehydration treatment was delayed the same seeds were sensitive to desiccation; leakage increased and germination declined. Birdsfoot trefoil seeds undergo a transition from a desiccation-tolerant to a desiccation-sensitive state after 24 h of imbibition (13). Furthermore, McKersie and Tomes (13) showed that once desiccation-tolerant seeds had entered the desiccation-sensitive state subsequent dehydration decreased germination and increased leakage, coincidentally, only if seeds were dehydrated below 20% moisture content. We have shown that in silver maple seeds and areca palm embryos, which are desiccation-sensitive tissues at maturity, a similar increase in leakage and decrease in viability occurred below a critical moisture content; 40% in silver maple seeds and 55% in areca palm embryos. Studies on foliar tissue also provide evidence that solute leakage from dehydrated tissue serves as a measure of dehydration-induced stress damage to membranes (5, 11). Our results are complementary to these findings and consistent with the concept that membranes of desiccation-sensitive seeds are damaged by dehydration below a critical moisture content and do not become effective barriers to solute leakage during imbibition.

McKersie and Stinson (12) interpreted the increased leakage from seeds in the desiccation-sensitive state to indicate an alteration in membrane structure in response to dehydration. Simon (16) suggested that the membrane alterations in seeds at low moisture contents involved a lipid phase change from a lamellar to a hexagonal structure. However, the x-ray diffraction data of McKersie and Stinson (12) indicated a lamellar membrane structure at seed moisture contents as low as 5%. Our results do not provide direct information on the mechanism of membrane damage associated with dehydration stress. The rapid increase in leakage from areca palm embryos dehydrated to 53% moisture content (Fig. 2A) suggests that membrane damage occurred during dehydration rather than during subsequent imbibition.

Many maple species have seeds that mature in the fall of the year and require stratification before germination can occur. In contrast, silver maple seeds mature in the spring and readily germinate at maturity (19). As indicated, the extremely high leakage from silver maple seeds dehydrated below 35% moisture content suggests severe dehydration-induced damage occurred to membranes. Therefore, it does not appear likely that loss of germination capacity between 45 and 35% moisture content was simply due to a dehydration-induced dormancy. The similarity in response to dehydration of silver maple seeds at either a relatively high temperature (35°C) or at room temperature (22°C) suggests that loss of viability was due to dehydration stress rather than indirectly due to heat stress. Jones (8) also reported the critical moisture content of silver maple seeds near 35% independent of the temperature of dehydration between 0 and 35°C. TTC leakage and viability of seed tissue, was used to evaluate viability of areca palm embryos in response to dehydration stress. A high level of TTC reduction by nondehydrated embryos correlated with a high level of germination by nondehydrated seeds, indicating the effectiveness of the TTC reduction test on viable tissues. The negligible level of TTC...
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reduction by embryos dehydrated to 30% moisture content was nearly identical to the negligible level of TTC reduction by heat killed embryos. These results provided evidence that lack of TTC reduction was a reliable indicator of loss of viability in areca palm embryos exposed to excessive dehydration stress.

Increased leakage during imbibition is associated with removal of the testa from some seeds (4, 9) The testa may inhibit leakage and its removal may enhance leakage of solutes from previously damaged cells. Alternatively, it has been proposed that rapid imbibition which occurs in the absence of the testa causes damage to membranes and increases leakage (9). Increased leakage from silver maple seeds in response to dehydration stress occurred from seeds with disrupted or removed testae but not from seeds with intact testae (Fig. 1A). Germination of silver maple seeds with intact testae (Fig. 1B) decreased at about the same moisture content as leakage increased from seeds with disrupted or removed testae. Thus, testa removal, per se, was not necessary for the deleterious effects of dehydration stress to be manifested. We, therefore, conclude that the testa of desiccation-sensitive silver maple seed acts as a barrier to solute leakage in response to dehydration stress rather than that its removal causes cellular damage.

The results presented here are consistent with the concepts that membranes in desiccation-sensitive seeds are damaged by dehydration below a critical moisture content and do not become functional during imbibition, and that this membrane damage contributes to dehydration-induced loss of viability.

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LITERATURE CITED

2. CHIN HF, EH Roberts 1980 Recalcitrant crop seeds. Tropical Press SDN BHD, Kuala Lumpur, Malaysia