Solute Leakage Resulting from Leaf Desiccation

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ABSTRACT

The leakage of solutes from foliar tissue is utilized as a dynamic measure of apparent changes in membrane integrity in response to desiccation. It is found that rehydrating leaf discs of cowpea (*Vigna sinensis* [L.] Endl.) show increasing leakiness in proportion to the extent of prior desiccation, whereas *Selaginella lepidophylla* Spring., a resurrection plant, does not. The elevated leakage rate of cowpea after desiccation recovers with time, and the passage of time in the stressed condition results in reduced subsequent leakiness. These characteristics are interpreted as suggesting that the leakage of solute reflects the condition of cellular membranes, and that desiccation stress leads to lesions in the membranes. The kinetics of solute leakage is suggested as a simple means of following changes in membrane lesions and associated features of membrane repair and hardening.

A desiccation experience can result in numerous physiological symptoms which are consistent with the concept that membrane damage is a basic lesion resulting from water stress. Among the symptoms of membrane damage are a lessening of the Hill reaction of chloroplasts (4), a lowering of the quantum efficiency of photosynthesis (5), a lowering of the respiratory rates of mitochondria (9), an increase in leakage of solutes from leaf tissues (7), and a hastening of senescence (5). From studies of the performance of mitochondria, Miller *et al.* (13) have suggested that one component of physiological damage due to water stress is evidenced as damage to membranes. We have employed measurements of the dynamics of leakage of solutes from leaves as a device for analyzing the desiccation damage to leaf membranes.

MATERIALS AND METHODS

Plants of cowpea (*Vigna sinensis* [L.] Endl. cv. California Blackeye) obtained from Burpee Seed Co. (1977) were grown in the greenhouse to 4 to 8 weeks of age; 7-week-old plants were the standard experimental material. Discs 1-cm in diameter were cut from fully expanded first trifoliate leaves and floated on distilled H_2O for at least 0.5 h, after which the initial fresh weight was measured. For leakage determinations, five discs were transferred to a 15 × 125 mm test tube containing 5 ml H_2O. This leaf sample represents about 0.1 g fresh weight or 0.004 g dry weight, and a total content of 1.6 to 2.4 total A_{280} equivalence. A stir bar in the bottom of the tube circulated the water around the discs which were held submerged by a small piece of screening. Solute leakage was estimated as the A_{280} of the water (16) either by continuous reading or at 2-min intervals using a Bausch and Lomb Spectronic 700 spectrophotometer.

A resurrection fern, *Selaginella lepidophylla* Spring. (which can withstand being dried out into a coiled ball, and is sold in novelty stores as a plant curiosity), was rehydrated and potted in a mixture of peat/sand/Perlite, 1/1/1. After several months, the youngest fronds were harvested. For leakage experiments, three 1-cm long pieces were added to 4 ml H_2O following the procedure used for cowpea.

Drying of the leaf material was accomplished in several ways. Routinely, leaf discs or frond pieces were air-dried for 30 to 190 min in screen-bottomed weighing dishes under fluorescent lights. To assess the effects of a long-term drought experience, discs were air-dried to 55% fresh weight and wrapped in Saran wrap to maintain that moisture content for various lengths of time. Noticeable wilting of cowpea leaf discs occurred after drying to less than 75% of fresh weight; recovery of turgor occurred after desiccation to as low as about 40% of fresh weight.

Water potential for fresh and air-dried samples of cowpea and *Selaginella* were determined using a dew point hygrometer (Wescor Inc., Logan, Utah; Model HR-33T Dew Point Microvoltmeter with C-52 chamber) following the methods of Nelsen *et al.* (14). Calibration was accomplished using Whatman No. 1 filter paper discs dipped in NaCl solutions. For cowpea, leaf discs were cut with a No. 7 cork borer; for *Selaginella*, 6-mm terminal segments were used.

RESULTS

Using the A at 280 nm as a measure of the leakage of solutes from the leaf tissues immersed in water, one can compare the apparent relative leakiness from tissues that have been desiccated to various levels. Such an experiment for leaf discs of cowpea (a plant which is relatively susceptible to desiccation) and for frond pieces of *Selaginella* (a plant with notable resistance to desiccation) is shown in Figure 1. When cowpea leaf discs had been dried to various degrees (90, 76, or 52% of original fresh weight), the subsequent leakage characteristics obtained from the discs in water show a period of relatively rapid loss of solutes lasting for about 4 or 5 min, and after that a relatively steady rate of leakage (Fig. 1A). Both the slope of that leakage curve and the total amounts of solutes leaked out over a 20-min period indicate that, after greater amounts of desiccation, there is an increased amount of leakage. On the other hand, *Selaginella* was found to suffer no perceptable increase in leakage even when desiccation had advanced to 40% of initial fresh weight (Fig. 1B).

The relation of the extent of leakage and the extent of desiccation damage is further illustrated in Figure 2. When the total amount of leakage after 20 min is plotted against the extent of prior desiccation, it is evident that the cowpea leaf discs lose increasing amounts of solutes with greater extents of desiccation, whereas the *Selaginella* tissue does not.

In the course of these experiments, it was found that differences in leakage rates were obtained as leaves of differing ages were used. A comparison of leaf discs desiccated to 55% of fresh weight and undesiccated discs of three different leaf ages are shown in Figure 3. In this case, the leakage is expressed as a percentage of total content of A_{280}-absorbing materials, since the total A_{280} content increases with the age of the leaf. It can be seen that the extent of leakage increases with leaf age, and that the desiccation experience results in substantially greater solute leakage from
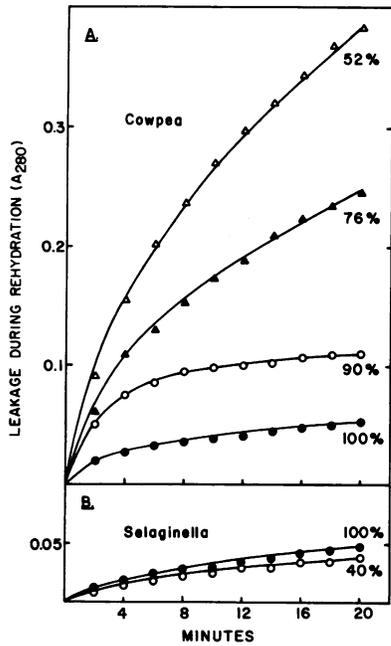
FIG. 1. The leakage of solutes from leaf discs of cowpea and foliar pieces of *Selaginella* after desiccation to various levels below initial fresh weight (100%).
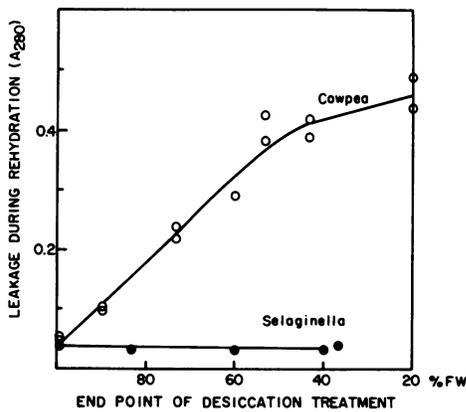
FIG. 2. The extent of leakage during rehydration of cowpea leaf discs and *Selaginella* foliar pieces as a function of the extent of prior desiccation. Total leakage after 20 min.

FIG. 3. Solute leakage from cowpea leaf discs after a desiccation stress (to 55% of fresh weight) or unstressed, as a function of leaf age. Leakage is expressed as percentage of total A_{280} absorbing materials leaking out in 20 min.
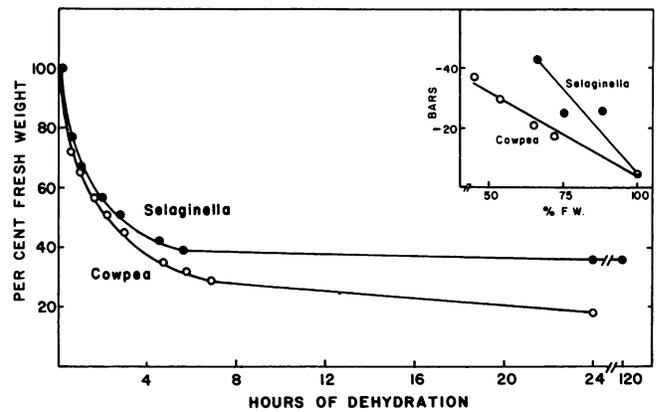
FIG. 4. The time course of dehydration of cowpea leaf discs and *Selaginella* associated changes in water potential (Ψ) (insert).
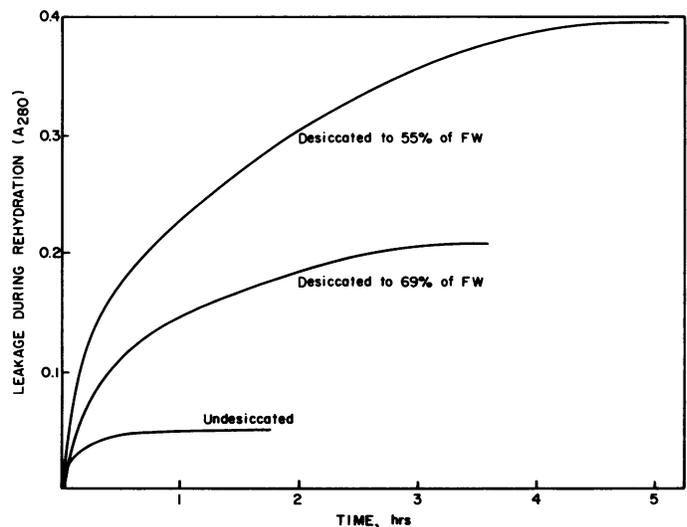
FIG. 5. The time course of leakage during hydration of cowpea leaf discs after desiccation to 69 and 55% of initial fresh weight.

older leaves.

A further difference between cowpea and *Selaginella* is that the cowpea shows a markedly lesser decrease in water potential with desiccation (Fig. 4). Thus, a Ψ value of −40 bars is achieved by cowpea after desiccation to less than 50% of its initial hydrated weight, whereas *Selaginella* reaches a Ψ value of −40 bars when desiccated to only about 75% of its hydrated weight.

Assuming that the higher levels of leakage represent higher levels of damage to the membrane systems of the leaf, examination of leakage rates in longer periods of time should reveal something of the possible time course for repair of the lesions to the membranes. Such a long-term experiment is shown in Figure 5, in which it is seen that leaf discs which had not been given a desiccation treatment reached an equilibrium with the medium such that there was no further net leakage after about 1 h. Leaf discs that had been desiccated to 69% of fresh weight before the leakage test reached such an equilibrium after about 3 h, and discs desiccated to 55% reached equilibrium after about 4.5 h.

When leaf discs had been desiccated to 55% of fully hydrated

weight and held in this state for various periods of time up to 24 h, the subsequent extent of leakage obtained over a 20-min period was greatly reduced by the preceding period of stress (Fig. 6). The extent of leakiness fell most rapidly following the first 4 h of desiccation; this decline in leakiness might be interpreted as indicating that the initial period of stress was a period of hardening which led to a lessening of subsequent leakage when the leaf tissues were returned to water.

DISCUSSION

The experiments reported here provide several types of evidence that the leakage from leaf or frond pieces can provide a measure of the extent of desiccation damage. The extent of leakage increases in proportion to the extent of desiccation in a sensitive species such as cowpea; the leakage does not increase with desiccation of a tolerant species such as *Selaginella*. The elevated leakage rate after desiccation of cowpea can be seen to be restored to the rate of an unstressed sample in time, and the passage of time in the stressed condition leads to a reduction in the amount of leakage during subsequent rehydration, which are respectively suggestive of repair and hardening of the desiccated tissue.

The idea that lesions in membranes were a crucial part of desiccation damage was first explicitly stated by Miller *et al.* (13) and Bell *et al.* (1), on the basis of depressed respiratory effectiveness of mitochondria taken from leaves after desiccation. In comparing desiccation tolerant mosses and lichens, Gupta (7) noted that the deteriorative effects of desiccation on photosynthetic capabilities were paralleled by increases in the leakage of solutes from the tissues. Bewley (2) has noted a similar relationship between desiccation damage and solute leakiness from moss tissues. It remained for Shcherbakova and Kacperska-Palacz (17) to attempt to utilize the degree of leakage of solutes from whole tissues as a measure of desiccation damage; their experiments on excised rape hypocotyls revealed a quantitative increase in leakage associated with desiccation damage. Our experiments have established that cowpea leaf tissues show increases in leakiness in proportion to the extent of desiccation; and in contrast, the resurrection plant, *Selaginella*, which can withstand enormous amounts of desiccation, is not so altered. Leakage rates were measured by Shcherbakova and Kacperska-Palacz over a period of 60 h, whereas in our experiments reliable rate differences were measured in 20 min.

Evidence for a hardening of rape shoots as a consequence of a
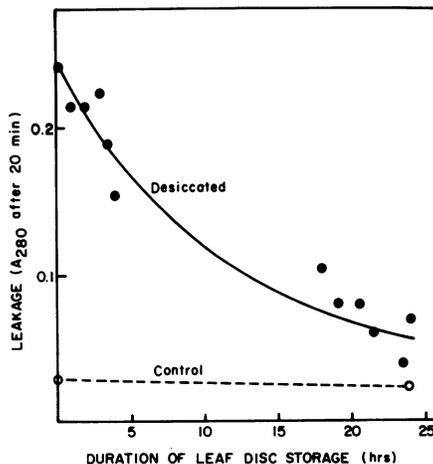
FIG. 6. The extent of leakage during rehydration of cowpea discs as a function of the duration of time held in a partially desiccated state (65% of initial fresh weight). Control discs were held in a water-saturated condition in a separate experiment. Leakage recorded as total A_{280} after 20 min.

desiccation experience was reported by Shcherbakova and Kacperska-Palacz (17). Our evidence of a reduced leakiness in cowpea leaf discs after being held in a partially desiccated state is consistent with the same interpretation, *i.e.* that the desiccation experience leads to a lessening of membrane damage in subsequent time.

Increased leakage of solutes following stress was first observed as a component of chilling injury (11), and since that time enhancement of leakage has been correlated with injury in response to high temperatures (12), freezing injury (18), drought stress (3), and chilling injury during seed imbibition (6, 10).

The time course of leakage of solutes was described by Simon (19) for the imbibition of dry seeds. He suggested that the rapid initial leakage rate occurring during the first few minutes of imbibition could be interpreted as a leakage occurring before the cells had become wet enough to have reorganized their membranes. In Figure 1 we see leakage kinetics which are similar to those for imbibing seeds, with a rapid leakage during the first 4 min followed by a more linear rate, even when there had been little or no tissue dehydration. Though numbers of workers have used Simon's interpretation of the leakage curves (6, 8, 15), such an interpretation should be made with caution since similar curves are obtained for nondehydrated leaves. The two phases of such leakage curves would better be interpreted as consequences of the geometry of the tissue, the solutes in the free space, and the degree of leakiness of the component membranes.

The possible nature of the lesions produced in membranes by desiccation stress is not clear. Such lesions are apparently not restricted to the plasmalemma, but occur in chloroplasts (5) and mitochondria (1) as well. Wiest and Steponkus (20) have suggested that a major component of damage to membranes in freezing injury may be associated with the expansion of the cell with the reentry of water, reaching a lethal state when it exceeds the capability of the plasmalemma for expansion (21). In our experiments, desiccation of cowpea leaves to less than about 75% of initial wet weight results in wilting, and the reentry of water can be assumed to impose some expansion on the plasmalemma, which may then contribute to the leaky condition. The rehydration of leaves may be associated with an extensive expansion of cell volume such as occurs during imbibition of seeds (15), and the development of lesions in the membrane system may be associated with the demands for membrane expansion upon water entry in both cases.

It is commonly observed that water stress exerts a preferential effect on older leaves; the leakage of solutes (Fig. 4) suggests a similar preferential damage to older leaves. The greater leakiness of older leaves caused by desiccation has also been reported for wheat (3) and may well be causally related to the preferential loss of older leaves following drought.

Collectively, these experiments indicate that the leakage of solutes from tissues can be used as a dynamic measure of the damage to membranes incurred by stress experiences.

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