

CHAPTER 7-7

WATER RELATIONS: BIOCHEMICAL ADAPTATIONS TO DRYING



Figure 1. *Grimmia affinis* drying on a rock. Photo by Michael Lüth.

Protection from Oxidation

Just what is it that varies within the bryophytes that dry out, become metabolically inactive, and then revive? What physiological mechanism protects, or fails to protect them? How can photosynthesis achieve its maximum rate within 30 seconds upon receiving rain or dew in some desiccated species (Anderson 1980)? Proctor (1990) and Alpert (2000) suggest that in drought-hardening the cell must protect itself from oxidative damage, as well as loss of configuration of macromolecules, and this protection depends on the intensity and duration of desiccation.

Minibayeva and Beckett (2001) noted that drought-sensitive bryophytes can release an oxidative burst in response to rehydration. Such oxidative bursts can help to limit the spread of invading pathogens because of oxidation toxicity, as well as inducing expression of defense-related genes. Cells with damaged membranes from desiccation would be vulnerable to invasion by such microorganisms. These bursts were best developed in the hornwort and two thalloid liverworts they tested. A similar oxygen burst was, however, almost absent in all the mosses tested as well as a leafy liverwort and desiccation-tolerant lichens.

This absence lends support to the hypothesis that mosses can protect themselves from the damage such oxidative bursts can cause during rehydration. Shiono *et al.* (2000) found that in testing the liverwort *Marchantia paleacea* var. *diptera*, the moss *Barbula unguiculata*, and the hornwort *Anthoceros punctatus*, the liverwort differed from the other two in its isozyme patterns for **superoxide dismutase**. This enzyme is known for its ability to maintain safe levels of the highly reactive oxides that are produced during cell stress, including effects of desiccation. Minibayeva and Beckett (2001) concluded that patterns of oxide production are correlated with the moisture status of the habitat. Those species with high basal rates of oxide production grow in moist microhabitats, have a moderately high thallus water content, have high K^+ contents, and have well developed oxidative bursts. Species with such oxidative bursts also lose a high proportion of their intracellular K^+ (55-98% in liverworts and hornworts) upon rehydration. Mosses and the one leafy liverwort were all collected from wet habitats and all produced oxides at low rates. Furthermore, some

bryophytes produce high quantities of oxides when they are not stressed, and some bryophytes produce them at extremely high rates. For example, *Anthoceros natalensis* exceeds 1000 $\mu\text{mol g}^{-1}$ dry mass h^{-1} , whereas excised tracheophyte roots produce only about 1% of that amount (Minibayeva *et al.* 1998). The data thus far do not present a consistent pattern that permits us to interpret the role of oxidative bursts or superoxide dismutase in protecting bryophyte cells that undergo desiccation.

Mayaba *et al.* (2002) later found that *Atrichum androgynum* from the Afromontane understory displayed an oxidative burst of hydrogen peroxide (H_2O_2), not superoxides, during rehydration, with maximum rates during the first 15 minutes. The moss even produced peroxide during times when dehydration was insufficient to cause K^+ leakage. Using polyethylene glycol to induce desiccation caused the moss to produce significant amounts of H_2O_2 . Mayaba and coworkers suggested that peroxidases might be responsible for the production of H_2O_2 . They determined that ABA (abscisic acid), well known as a stress response hormone in tracheophytes, and light influenced the rate of production of peroxide.

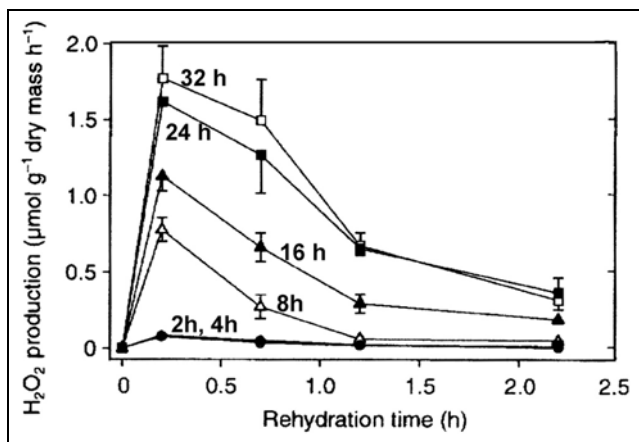


Figure 2. Peroxide (H_2O_2) production during rehydration following various dehydration periods (indicated on each line) in *Atrichum androgynum* from KwaZulu-Natal Province, Republic of South Africa, during summer. Vertical bars indicate standard deviation; $n=5$. Redrawn from Mayaba *et al.* (2002).

This system would have several advantages. Peroxidases oxidize phenolics to quinones and generate peroxide (H_2O_2). Peroxide, a well-known antibacterial agent for cleaning cuts and wounds, can itself help to kill invading organisms. Furthermore, peroxide releases free radicals that increase polymerization of phenolics into lignin-like substances. In tracheophytes, these substances are known to reinforce the cell wall and contain the pathogens. They may have similar roles in bryophytes.

Marchantia polymorpha contains a peroxidase that has been characterized as a glycoprotein that is different from any known tracheophyte peroxidase (Hirata *et al.* 2000). Hirata and coworkers demonstrated that it is able to perform oxidative polymerization of lunularin, the liverwort counterpart of ABA.

Other known constituents also influence the activity of peroxidases. Seel and coworkers (1992a) examined the effects of desiccation on **superoxide dismutase** (enzyme that destroys highly reactive superoxides by converting them into peroxide and O_2) activity in *Syntrichia ruralis*

var. *arenicola* (= *Tortula ruraliformis*), a desiccation-tolerant moss, and *Dicranella palustris*, a flush moss with limited desiccation tolerance. Activity of this enzyme is known to enhance membrane integrity (Dhindsa & Matowe 1981; Dhindsa *et al.* 1981; Gong *et al.* 1997). They found that *Syntrichia ruralis* var. *arenicola* had higher superoxide dismutase activity in both the hydrated and desiccated states than did *D. palustris*. But effects on the activities of peroxidase or ascorbic peroxidase did not seem to be related to hydration state. Nevertheless, both species became depleted of the anti-oxidant ascorbic acid when desiccated. From these experiments, Seel and coworkers deduced that anti-oxidants may be more important than removal of chloroplastic peroxide in endowing desiccation tolerance. Using different methods, Seel and coworkers (1992b) found a greater lipid peroxidation in *D. palustris* than in *S. ruralis* var. *arenicola* following desiccation. Calcium also seems to play a role by increasing superoxide dismutase activity, thus enhancing membrane integrity (Gong *et al.* 1997).

ABA Role

Using immunoassay, Hartung and coworkers (1987, 1994) demonstrated the presence of ABA in all Bryopsida, Anthocerotophyta, and Marchantiopsida tested. They were able to extract more ABA from *Anthoceros* grown under slightly drier areas than from those in wetter areas. Furthermore, they have shown that the sporophyte of *Anthoceros laevis* produces it in response to stress and that the sporophyte guard cells close in response to ABA, much as in tracheophytes. One of the unusual abilities of ABA is to cause the conversion of the aquatic forms of *Riccia fluitans* and *Ricciocarpos natans* into their terrestrial forms (Hellwege *et al.* 1992; Hartung *et al.* 1994). This conversion results in plants with greater volume, hence a smaller surface area to volume ratio, making them somewhat less vulnerable to desiccation.

Liverworts use lunularic acid where other plants use ABA as a dormancy hormone and, apparently, to help prepare them for drying (Schwabe 1990). When subjected to long days, their drought resistance increases (Figure 3), as does their lunularic acid content.

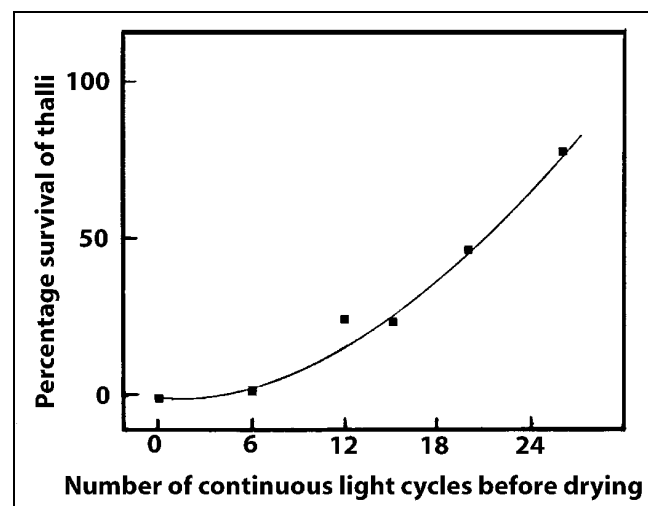


Figure 3. Effect of long-day (continuous) light on induction of drought resistance, resulting in drought survival in *Lumularia cruciata*. Based on Schwabe (1990).

Although the presence of lunularic acid seems to be universal in liverworts, and has functions like those of ABA, liverworts seem to be fully responsive to ABA. Pence (1998) found that ABA was necessary for the cryopreservation of some liverworts such as *Riccia fluitans* and *Marchantia polymorpha*, preventing desiccation damage, but it had little effect on *Plagiochila*. Burch and Wilkinson (2002) used ABA and sucrose to increase the success of cryopreservation of *Ditrichum cornubicum* protonemata. We also know that application of ABA increases the desiccation tolerance of the mesophytic moss *Atrichum undulatum* (Beckett *et al.* 2000).

But how does this relate to preventing the oxidative damage? Beckett and coworkers (2000) suggested that ABA pretreatment may act by reducing the energy transfer between light-harvesting chlorophyll II and photosystem II. This could harden the moss to desiccation stress by reducing the production of reactive oxygen at the site of photosystem II. Experiments indicated that photosystem II photosynthesis recovered faster in the pre-treated plants.

ABA may play another role as well. One of the most serious consequences of desiccation is loss of membrane integrity, causing membranes to become leaky (Bewley 1979). Beckett (1999) found that application of ABA could reduce the loss of K^+ from *Atrichum androgynum* in much the same manner as partial dehydration treatment prior to desiccation. The response was similar to that obtained by reducing the relative water content to 0.6 for three days, which reduced the K^+ loss by 15-20%. This seemed to be the ideal combination because using less humid air or more time did not decrease the K^+ loss further. This species, and probably most, experiences **drought hardening** (process of increasing resistance drought) as the dry season progresses, as indicated by the loss of 80% of its intracellular K^+ at the beginning of the dry season, but less than 25% by the end of that season (Beckett & Hodginott 1997).

Membrane Chemistry

Since membrane damage is a common response to desiccation stress, Guschina *et al.* (2002) examined lipid composition of membranes in *Atrichum androgynum* during desiccation in an effort to understand the role of ABA. Drought stress caused changes in the phosphoglyceride composition of the membranes. Reduction of thylakoid lipids, resulting in chlorophyll damage, caused a loss in photosynthesis as a result of desiccation, as already demonstrated in tracheophytes. Guschina *et al.* found that application of ABA reduced the extent of these membrane lipid changes.

Some plants may take advantage of the leakage to rid cells of protectants used during dehydration. Working with canopy liverworts in the tropical rainforest of Guadeloupe, Coxson and coworkers (1992) found that for *Frullania atrata*, exposure to simulated wetting/drying resulted in production of substantial glucose, erythritol, glycerol, and sucrose. They suggest that whereas these sugars may help this liverwort survive severe desiccation, the liverwort subsequently releases them into throughfall upon rewetting.

Robinson *et al.* (2000) suggest that sugars may indeed help some mosses survive desiccation. They found stachyose, an oligosaccharide known for its role in desiccation tolerance of seeds, in *Bryum pseudotriquetrum*

(Figure 4), but not in *Ceratodon purpureus* (Figure 4; most tolerant) or *Schistidium antarcticum* (least tolerant). This appears to be another example showing that not all bryophytes have the same adaptations to desiccation.



Figure 4. **Upper:** *Bryum pseudotriquetrum*. **Lower:** *Ceratodon purpureus*. Photos by Janice Glime.



Figure 5. Drought-intolerant *Schistidium antarcticum* on McQuarie Island. Photo by Rod Seppelt.

Shoot Tips

Some moss shoot tips may have a rehydration potential not afforded the rest of the plant. In *Polytrichum formosum*, desiccation in the shoot tips induces the rapid resorption of starch grains in plastids of the meristematic cells without any major **thylakoid** disorganization (Hallet *et al.* 1987). In the adult leaves, however, the starch grains are preserved. Upon rehydration, the plastid ultrastructure of the apex is entirely restored and new starch inclusions appear in less than 4 hours.

The Genes

While the physiologists are attempting to find substances that affect desiccation tolerance and recovery rates, the geneticists are attempting to identify genes and the biochemical pathways they affect. Chen and coworkers (2002), working with the desiccation-tolerant model system in *Syntrichia ruralis*, found a new polypeptide, known as ALDH21A1, that is less than 30% identical to known ALDH proteins. Data suggest that this new aldehyde dehydrogenase plays an important role in the detoxification of aldehydes generated in response to desiccation and may represent a unique stress tolerance mechanism among eukaryotes. Could it be this aldehyde dehydrogenase or ABA that explains why Hamerlynck and coworkers (2002) found *Syntrichia ruralis* to be **homoiochlorous** (maintaining constant chlorophyll concentration) in its response to desiccation? Growing in the sun endowed these plants with a greater desiccation tolerance than that experienced by shade-grown plants.

To fit these pieces together requires a great deal of speculation because our knowledge is still too meager. However, let us look at what we know about these pieces and see if we can develop a hypothetical story (Figure 6).

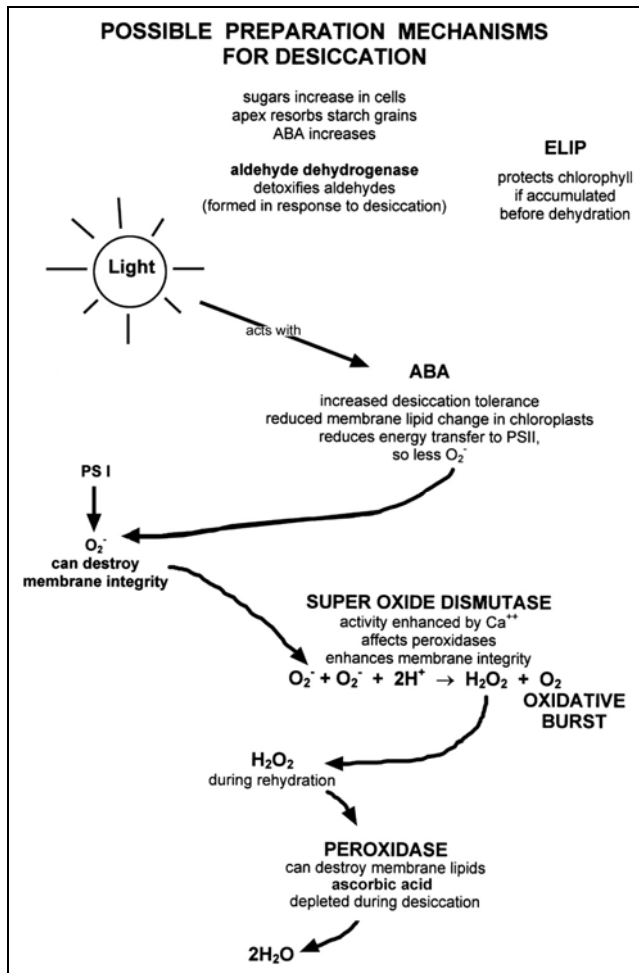


Figure 6. Speculation on possible relationships of the observations that have been made on pre-desiccation events and possibly related rehydration events in desiccation-tolerant bryophytes.

Summary

ABA increases the stress tolerance of bryophytes and is known to turn on the promoters of **stress tolerance genes**. Hence, it is important in controlling transcription. That is consistent with the conclusions of several authors who have determined that drought tolerance in bryophytes evokes control of gene transcription. We also know that **peroxidases** destroy H_2O_2 (**peroxide**), which is harmful to plants. We know that H_2O_2 is responsible for lipid damage of membranes and that lipid peroxidation and increased membrane permeability correlate with the decrease of **superoxide dismutase** (Dhindsa *et al.* 1981). And we know that superoxide dismutase controls **oxygen toxicity** by converting the superoxide radical to less dangerous forms (Michael Potter of Andrew McCammon's group at the University of California, San Diego). Since *Syntrichia ruralis* var. *arenicola* has a higher concentration of superoxide dismutase than the less desiccation-tolerant *Dicranella palustris*, we can then hypothesize that the superoxide dismutase is an important contributor to drought tolerance in bryophytes. Perhaps it is one of the 74 proteins produced in response to desiccation stress.

Acknowledgments

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