Partial dehydration and ABA induce tolerance to desiccation-induced ion leakage in the moss *Atrichum androgynum*

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A simple ion leakage assay was used to test if specific hardening treatments can increase desiccation tolerance in the moss *Atrichum androgynum*. Reducing the relative water content (RWC) of apical stem segments to *c*. 0.6 for 3 d, followed by storing the material fully hydrated for 1 d typically reduced the proportion of intracellular K^+ lost following desiccation by between 15 and 20% compared with controls kept moist. Reducing the RWC of the plants further during hardening by using air of lower humidities during hardening did not further increase tolerance. An optimal increase in tolerance occurred after 3 d of hardening; no further increase in tolerance occurred by extending the hardening period to 5 d. While heat shock had no effect, treating the moss with abscisic acid (ABA) for 1 h followed by storage for 3 d in the light increased resistance to ion leakage to the same extent as partial dehydration. The ABA-induced increase in tolerance was much less if plants were pre-treated in the dark, and could be abolished by simultaneously treating the moss with the protein synthesis inhibitor cyclohexamide. Results indicated that *A. androgynum* possesses inducible tolerance mechanisms that reduce desiccation-induced damage.

Keywords: moss, desiccation, ion leakage, Abscisic acid.

Introduction

While the leaves of most plants wilt when their water potentials reach around -1.5 MPa and then die when their water potentials drop below -15 MPa, many moss species can survive tissue water potentials of less than -150 MPa (Gaff 1997). Considerable variation in desiccation tolerance between species and populations of mosses has been reported (see Oliver *et al.* [1993] for review). While much effort has been expended attempting to explain these differences, the physiological and biochemical factors responsible for tolerance remain unclear (Bewley 1995; Oliver 1996; Oliver & Bewley 1997; Oliver & Wood 1997; Oliver *et al.* 1997; Oliver *et al.* 1998). Oliver divided desiccation tolerance mechanisms into those that protect cellular integrity while bryophytes are dry, and those that repair dehydration induced damage. Most of the experiments carried out by these workers have used the extremely desiccation tolerant moss *Tortula ruralis*. In the vegetative tissues of many poikilohydric plants, and also in seeds, desiccation tolerance is associated with the synthesis of a group of proteins termed dehydrins (Close 1996). The precise roles of most of these dehydrins are largely unknown. *T. ruralis* possesses two major dehydrins. Rather surprisingly, unlike most other systems investigated, these two dehydrins are present in hydrated vegetative tissue, and do not appear to increase or decrease during rapid or slow drying (Bewley *et al.* 1993). Instead, during rehydration the synthesis of another group of proteins the 'hydrins' is inhibited, while the synthesis of a third group, the 'rehydrins' is initiated (Oliver *et al.* 1998). Thus, *T. ruralis* appears to possess a constitutive protection system and a rehydration-induced recovery mechanism.

It is unknown whether desiccation tolerance mechanisms similar to those found in *T. ruralis* operate in bryophyte species more sensitive to desiccation. Recently, Beckett and Hoddinott (1997) used a simple ion leakage assay to test desiccation tolerance in a single population of the moss *Atrichum androgyoum*. Almost all the K^+ associated with moss cells occurs in a soluble form in the cytoplasm (Brown & Buck 1979). In the ion leakage assay, K^+ loss is measured immediately after rehydration following desiccation over silica gel. Beckett and Hoddinott (1997)

showed that large seasonal changes in K^+ leakage following desiccation occurred in *A. androgynum*. Mosses lost much less K^+ during the dry winter months than the moist summer months. This preliminary finding suggested that (unlike *T. ruralis*) *A. androgynum* has an inducible protection-based tolerance mechanism.

The large seasonal variations in desiccation tolerance displayed by *A. androgynum* suggested that this species may be useful in further, more detailed, experiments on the physiological and biochemical basis of inducible protection-based tolerance. However, rather than study seasonal changes in desiccation tolerance, it is experimentally more convenient to pretreat plants under controlled conditions to 'harden' them to subsequent stresses. Determining conditions that can induce tolerance will allow comparisons to be made between the physiology of hardened and untreated plants. The aim of the present investigation was to test the ability of various pretreatments to increase the desiccation tolerance of the moss *Atrichum androgynum*.

Pretreatments considered likely to induce desiccation tolerance included partial dehydration, heat shock and exogenous applications of the hormone ABA. Under field conditions, partial dehydration often precedes desiccation stress, and occasionally plants will receive a heat shock before they dry out. Furthermore, Hellewege *et al.* (1994,1996) and Werner *et al.* (1991) found that exogenous applications of ABA can increase desiccation tolerance in liverworts and the protonema of the moss *Funaria* respectively. These observations suggest that ABA is involved in signal transduction pathways that increase desiccation tolerance. Results presented here show that partial dehydration and treatment with abscisic acid can indeed increase desiccation tolerance, and confirm that *A. androgynum* is a good species to investigate tolerance mechanisms in bryophytes.

Materials and Methods

Plant material

The moss *Atrichum androgynum* (C. Müll.) A. Jaeger combines the upright habit of *Polytrichum* with the fragile textured leaves of *Mnium*. Material was collected during the moist summer months

from the understorey of the Doreen Clarke Nature Reserve, Hilton, KwaZulu-Natal Province, Republic of South Africa (24°39' South and 30°17' East). This nature reserve forms a small pocket of afromontane forest in the mist belt region of KwaZulu-Natal (Low & Rebelo 1996). Once collected, the moss was stored on wet filter paper for 2 d at 20°C and a light intensity of 75 μmol photons m^{-2} s^{-1} under continuous fluorescent light. Apical 2 cm segments were then cut, and the material divided into replicates of 8 segments. Each replicate typically had a fresh mass of c. 130 mg and a dry mass of c. 40 mg.

Effect of partial dehydration at different RWCs on desiccation tolerance

Hardening was carried out by placing each replicate of segments in a 2 × 10 cm glass specimen bottle in a container at 100% relative humidity (RH) (bottles suspended above distilled water) for 1 d. Segments were then left at 100% RH or transferred to 78% RH (achieved using a saturated solution of KCl), 53% RH (achieved using a saturated solution of $Mg(NO_3)_2$) and 0% RH (achieved using silica gel). Plants were kept as above for a further 1 d. All segments were then transferred to a relative humidity of 100% for 1 d, then rehydrated by shaking for 0.5 h in 10 ml deionized distilled water. Plants were then stored for 1 d on wet filter paper. Figure 1 illustrates the changes in the RWC of the moss that these treatments caused. Susceptibility of plants to ion leakage was determined using a simplification of the method of Brown and Buck (1979). Segments were desiccated by transferring them to a relative humidity of 0% for 1 d. Figure 2 illustrates typical desiccation rates. For comparison, these rates correspond to the 'slow' desiccation of Dhindsa (1991). K^+ loss caused by sudden rewetting was measured by placing the samples in 10 ml of deionized distilled water and shaking them for 0.5 h. Extracellular K^+ was then displaced by incubating the moss in 10 ml of 20 mM $NiCl_2$ for 0.5 h. After a further wash in 10 ml of deionized distilled water for 0.5 h, plants were dried to constant mass at 80°C, weighed, and intracellular K^+ displaced by incubation in 10 ml of 1M HNO_3 for 1 h. All samples were spiked with 1000 μg g^{-1} Cs^+, and K^+ determined by atomic absorption spectrophotometry in an air/acetylene flame. Although Oliver et $al.$ (1993) do not recommend using ion leakage as a measure of desiccation-induced damage in extremely tolerant bryophytes, $Atrichum$ is a sensitive genus (Brown & Buck 1979).
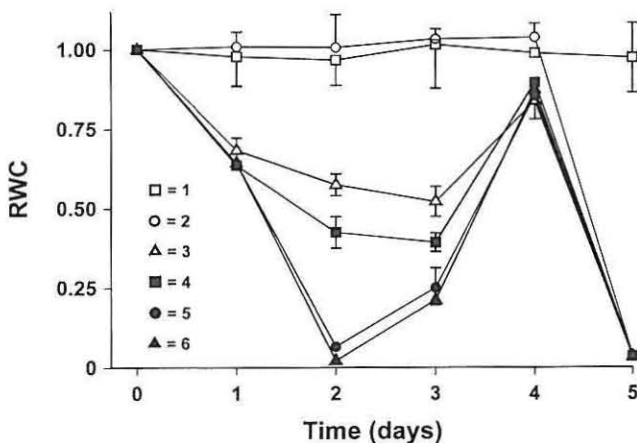

Figure 1   Relative water content of $Atrichum$ $androgynum$ stored at a range of air relative humidities, rehydrated then desiccated. 1 (■) Plants kept moist, not desiccated; 2 (○) plants kept moist; 3 (▲) hardened at a RH of 100%; 4 (□) hardened at a RH of 78%; 5 (●) hardened at a RH of 52%; 6 (△) hardened at a RH of)%. See Materials and Methods for more details, n = 5. In this and subsequent figures, error bars denote the standard deviation.
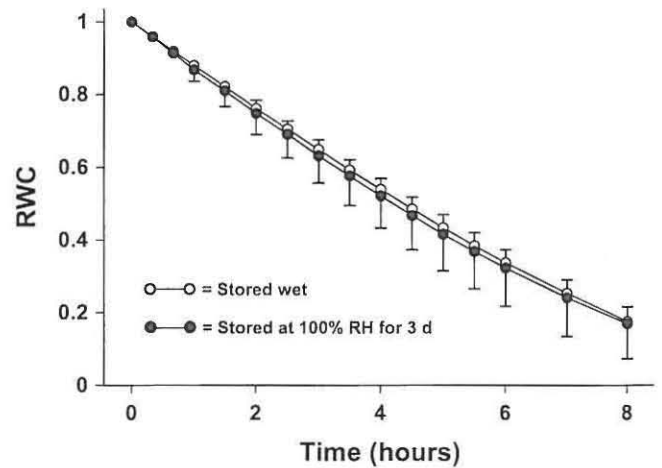

Figure 2   The rate of desiccation of $Atrichum$ $androgynum$ placed at 0% RH for material stored moist for 4 d (○) or hardened at a RWC of 0.6 for 3 d then stored moist for 1 d (●), n = 5.

Effect of partial dehydration for various times on desiccation tolerance

Mosses were hardened at 100% RH as above for 1, 3 and 5 d. Following hardening, plants were shaken for 0.5 h in 10 ml of deionized distilled water, stored for 1 d on wet filter paper, then desiccated and rehydrated as above. As controls, unhardened plants were desiccated at the start of the experiment and at the same times as the material receiving hardening treatments.

Effect of heat shock and ABA pretreatments on desiccation tolerance

Mosses were given a heat shock in the light at 35°C or 40°C for 1, 2, and 3 h, stored for 24 h at 20°C, then desiccated and rehydrated as above. In experiments with ABA, abscisic acid (cis, trans) was obtained from Sigma, dissolved in a drop of 1 M NaOH and the pH of the resulting solution adjusted to 5.6 with HCl. In the first experiment, mosses were shaken in 10 ml of deionized distilled water or 10, 100 and 1000 μM ABA for 1 h. They were then stored for 3 d at a light intensity of 75 μmol photons m^{-2} s^{-1} under continuous fluorescent light, then desiccated and rehydrated as above. In the second experiment, mosses were shaken in 10 ml of deionized distilled water or 100 μM ABA for 1 h in the light, then stored in the light as above or in the dark at 20°C for 3 d, then desiccated and rehydrated as above. In the third experiment, plants were shaken in 10 ml of deionized distilled water or 100 μM ABA for 1 h with or without 200 μM cyclohexamide (Sigma). They were then stored in the light as above for 3 d, then desiccated and rehydrated as above.

Results

Effect of partial dehydration at different RWCs on desiccation tolerance

Control plants were kept continually moist, while hardened plants were placed at 100% RH for 1 d, achieving RWCs of c. 0.7. Segments stored at a RH of 100% were then left at 100% RH or transferred to RHs of 78%, 53%, 0% for 1 d, achieving relative water contents of 0.57, 0.44, 0.07 and 0.02 respectively (Figure 1, Table 1). Segments stored at RHs of 78%, 53% or 0% were then transferred back to a RH of 100% for 1 d, increasing their RWCs. All plants were then fully hydrated by incubation in deionized distilled water. After storage hydrated for another 1 d, plants were desiccated. Control and hardened material had similar drying rates (Figure 2). Plants were suddenly rewetted, and the cellular location of K^+ determined as described in Materials and Methods. Table 1 presents full details of the cellular location of the K^+. In plants kept continually moist, nearly 100% of the K^+

Table 1 Cellular location of K$^+$ in *Atrichum androgynum* following the hardening at a range of air relative humidities, storage hydrated for 1 d, desiccation for 1 d at 0% relative humidity, then sudden rehydration. See Materials and Methods for more details. In this and subsequent tables, figures are given ± one standard deviation, n = 5 or 6

Harvesting treatment	Resulting RWC during hardening	Rehydration solution (inter-cellular) (%)	Nickel solution (extracellular) (%)	Post-nickel solution wash (extracellular)(%)	Acid solution (intracellular) (%)	Total thallus K$^+$ concentration (μmol g^{-1})	Mean reduction in K$^+$ loss caused by pre-treatment (%)
1. Plants kept moist, not desiccated	0.97 ± 0.08	0 ± 0	0 ± 0	0 ± 0	100 ± 0	264 ± 21	-
2. Plants kept moist	1.01 ± 0.10	38 ± 3	10 ± 0	1 ± 0	50 ± 3	277 ± 19	-
3. Hardened at RH of 100%	0.57 ± 0.03	22 ± 7	9 ± 2	1 ± 0	68 ± 9	258 ± 22	17.5
4. Hardened at RH of 78%	0.42 ± 0.05	22 ± 3	9 ± 1	1 ± 0	69 ± 4	254 ± 20	18.3
5.Hardened at RH of 52%	0.07 ± 0.00	26 ± 5	10 ± 2	1 ± 0	63 ± 6	255 ± 10	13.2
6. Hardened at RH of 0%	0.02 ± 0.00	25 ± 7	10 ± 1	1 ± 0	64 ± 8	245 ± 11	13.9

associated with the cells was intracellular. Rehydrating desiccated unhardened mosses caused the loss of *c.* 50% of the intercellular K$^+$. All hardening pretreatments caused plants to lose between 14 and 18% less K$^+$ than plants maintained continually moist. In all treatments *c.* 10% of the total K$^+$ content of the plants became bound to the cell wall. Therefore, the estimate of total K$^+$ loss following desiccation (and so the estimate of sensitivity to desiccation) was defined as the sum of the proportion of K$^+$ lost to the rehydration solution and the K$^+$ that became bound to the cell wall.

Effect of partial dehydration for various times on desiccation tolerance

Plants were stored at 100% RH for 1, 3 and 5 d, achieving RWCs of 0.68, 0.64 and 0.48 respectively (Table 2). Following rehydration and storage for 1 d, desiccation tolerance was measured. Tolerance to K$^+$ loss was not significantly increased after 1 d of hardening, but did increase after 3 d; increasing the hardening time to 5 d did not further increase tolerance.

Table 2 Effect of mild dehydration for varying periods of time on K$^+$ leakage following desiccation and sudden rehydration in the moss *Atrichum androgynum*

Pretreatment time (days)	0	1	3	5
RWC of hardened moss	-	0.68 ± 0.06	0.59 ± 0.07	0.48 ± 0.06
% Total K$^+$ lost following rehydration (control)	71 ± 3	77 ± 8	72 ± 7	82 ± 7
% Total K$^+$ lost following rehydration (dehydration pre-treatment)	-	72 ± 4	49 ± 2	58 ± 3
Mean reduction of K$^+$ loss caused by pretreatment	-	4.1	23.9	23.5

Effect of heat shock and ABA pretreatments on desiccation tolerance

Pretreating plants with a heat shock at 35°C for 1, 2, or 3 h followed by storage for 1 d had no effect on desiccation tolerance (Table 3). A heat shock of 40°C tended to increase the sensitivity of the plants to desiccation, but this increase was not significant. Pretreatment of the moss with ABA followed by storage for 3 d increased resistance to ion leakage to a similar extent as partial dehydration (Figure 3). The ABA-induced increase in tolerance was much less if plants were stored in the dark (Table 4). Treatment of plants simultaneously with ABA and the protein synthesis inhibitor cyclohexamide almost completely abolished the effect of ABA on desiccation tolerance (Table 5).

Discussion

The work presented here tested the ability of various pretreatments to increase tolerance to ion leakage following desiccation in the moss *A. androgynum*. Results clearly showed that reducing the RWC of the plants to *c.* 0.6 for a few days significantly increased tolerance, even following storage fully hydrated for 1 d after hardening. The benefits of hardening decreased during moist storage following hardening, but mosses still displayed some increase in tolerance one week following the hardening treatment. In one experiment, after storage for one week moist, K$^+$ loss from unhardened plants was 73.0 ± 2.8% compared with 65.8 ± 2.1% from hardened mosses. Exogenous application of ABA fully substituted for partial dehydration, strongly suggesting that ABA is involved in signal transduction pathways that increase tolerance to ion leakage. Care is needed when testing the sensitivity of a bryophyte to desiccation using the ion leakage assay introduced by Brown and Buck (1979). The hydration state of a sample before measurement can clearly influence estimates of tolerance obtained, even if plants are maintained fully hydrated for several days before testing.

Oliver *et al.* (1998) divided desiccation tolerant plants into firstly those that survive if drying is slow enough to induce mechanisms that will 'protect' the plants during desiccation or facilitate recovery during rehydration, and secondly those that tolerate even rapid drying. *T. ruralis*, which can tolerate rapid drying, appears to have both constitutive protection mechanisms, and those that 'repair' damage during rehydration. Which kind of tolerance does *A. androgynum* display? The ability of various

pretreatments to harden plants to subsequent stress as displayed by *A. androgynum* is consistent with inducible rather than constitutive tolerance mechanisms. It is tempting to speculate that partial dehydration and ABA increase tolerance by increasing the ability of the plants to reduce damage during desiccation ('protection' mechanisms). However, Oliver *et al.* (1998) suggested that even in *T. ruralis* repeated wetting and drying cycles can increase tolerance by sequestering 'recovery' mRNA transcripts in mRNA particles. Presumably, proteins synthesized from the transcripts during rehydration speed up the recovery process. Future experiments will test whether the induction of tolerance involves protection- or repair-based mechanisms by applying protein synthesis inhibitors during rehydration. Irrespective of how induction occurs, the main finding of the present study is that *A. androgynum* possesses inducible desiccation tolerance mechanisms in addition to any that are constitutive.

At present, it is very difficult to speculate on the likely distribution of inducible or constitutive desiccation tolerance mechanisms in other bryophytes. The advantage of inducible systems is that, unlike constitutive mechanisms, they do not divert energy away from growth and reproduction. The disadvantage is that a sudden, severe drought may not allow sufficient time for the induction of tolerance, and thus plants may not survive. Limited data available from liverworts (Hellwege *et al.* 1994, 1996) and protonema of the moss *Funaria* (Werner *et al.* 1991) suggests that these also possess inducible protection-based tolerance mechanisms. It seems likely that inducible tolerance mechanisms will be selected for in environments that are usually moist, and plants only occasionally (and probably slowly) desiccated, and also those in which plants are desiccated on a predictable seasonal basis. The latter typify the habitat occupied by the moss *A. androgynum* used in the present study (Beckett & Hoddinott 1997). Severely stressed microhabitats, e.g., the bare rock faces occupied by the moss *T. ruralis*, seem likely to select species with constitutive tolerance.

The precise signal triggering hardening in *A. androgynum* is unknown, but reducing the RWC of the moss to *c.* 0.6 clearly induced tolerance. According to the pressure-volume isotherm for this species from the same locality presented by Beckett and Hoddinott (1997), this corresponds to a water potential of *c.* -1 MPa. Interestingly, at this water potential plants still possessed some turgor, and water loss had not inhibited photosynthesis (data not shown). Possible signals include turgor loss or a critical increase in the concentration of a cytoplasmic solute (Bray 1997). For comparison, in *T. ruralis* the synthesis of hydrins is inhibited upon rehydration of gametophytes dried to 50% of their fresh weight, while rehydrin synthesis only occurs following rehydration of gametophytes dried to between 50 and 20% of their fresh weight (Oliver *et al.* 1997). Drought stress can induce the same genes as heat shock in higher plants (Viswanathan &
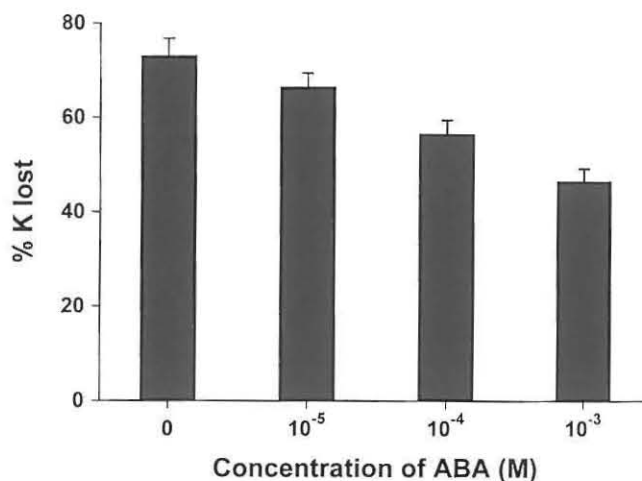

Figure 3 The effect of pretreatment with various concentrations of ABA for 1 h and storage either in the light or in the dark for 3 d on K⁺ leakage following desiccation and sudden rehydration in *Atrichum androgynum*, n = 6.

Khanna-Chopra 1996). In *A. androgynum*, mild to severe heat shock did not affect tolerance to ion leakage (Table 3). Interestingly, Alamillo *et al.* (1995) found that in the resurrection angiosperm *Craterostigma* proteins normally induced by heat shock in other species were constitutively present. Possibly the same applies for *A. androgynum*. *A. androgynum* may be a good species for studying signals that trigger hardening to desiccation stress.

The loss of *c.* 40% of the water from the mosses during the hardening treatment of 100% RH was initially surprising. Plants were placed under lights in bottles in a sealed container suspended above deionzed distilled water. However, it seems likely that the temperature of the moss segments was a few degrees higher than the air in the container, creating a gradient in water potential from the moss to the air. Using appropriate concentrations of polyethylene glycol 6000 (PEG), an attempt was made to test if exposure of *A. androgynum* to RWCs higher than 0.6 can induce tolerance to ion leakage. However, treatment with PEG did not increase resistance to ion leakage, even using concentrations and treatment times that created RWCs identical to those that hardened plants exposed to the air (data not shown). The reasons for this remain unclear, as PEG should not have been toxic to plants in the short term. However, PEG solutions are viscous and greatly slow the diffusion of oxygen to plant parts suspended in them (Pammenter, personal communication). If the hardening process requires energy, e.g., for significant protein synthesis, insufficient oxygen may have been available to support respiration. As a result, the effect of hardening plants at RWCs of greater than 0.6 has not yet been tested.

The precise way that hardening increases desiccation tolerance in *A. androgynum* is presently uncertain. In mosses, rapid water loss causes more damage than slow water loss (Krochko *et al.* 1979; Dhindsa 1987). Theoretically, hardening could increase tolerance by altering the physical properties of the moss in a way that reduces the rate of desiccation. However, reduced rates of water loss cannot explain the increase in tolerance following hardening, as measurements showed that hardened material lost water at the same rates as material maintained continually moist (Figure 2). In addition, the reversal of ABA-induced tolerance by cyclohexamide (Table 5) indicates that the induction of tolerance needs protein synthesis. This observation suggests that hardening does not simply change the physical properties of the moss.

The ability of ABA to substitute for partial dehydration

Table 3 The effect of a heat shock treatment followed by storage moist for 1 d on K⁺ leakage following desiccation and sudden rehydration in the moss *Atrichum androgynum*

Pretreatment time	None (Control	1 h	2 h	3 h
% Total K⁺ lost following rehydration (treatment at 35°C)	48 ± 4	48 ± 7	50 ± 8	46 ± 6
% Total K⁺ lost following rehydration (treatment at 40°C)	-	48 ± 7	54 ± 6	57 ± 7

Table 4 The effect of pretreatment with 100 µm ABA in the light and storage either in the light or the dark for 3 d on K⁺ leakage following desiccation and sudden rehydration n the moss *Atrichum androgynum*

Pretreatment	Light (Control	Dark	Light + ABA	Dark + ABA
% Total K⁺ lost following rehydration	67 ± 9	69 ± 5	45 ± 2	58 ± 3
Mean reduction in K⁺ loss caused by pretreatment	-	-1.9	22.7	9.5

provides good evidence for the involvement of ABA in signal transduction pathways that can increase tolerance to ion leakage (Table 4). Workers using growth or photosynthesis as measures of desiccation tolerance reported a similar effect of exogenous applications of ABA in liverworts and the protonema of the moss *Funaria* (Hellwege *et al.* 1994, 1996; Werner *et al.* 1991). Interestingly, recent data suggest that ABA may not play a role in desiccation tolerance in lichens (Dietz & Hartung 1998). In the studies of Hellewege and co-workers, and also those higher plants (Bray 1997), ABA induced some but not all of the genes induced by drought, but in the present study ABA could fully substitute for the dehydration pretreatment (Figure 3). ABA was much less effective at inducing desiccation tolerance if plants were treated in the dark (Table 4). Similar results were obtained by Alamillo and Bartels (1996) for the resurrection angiosperm *Craterostigma*. Reasons for the interaction between light and ABA remain unclear. Recent evidence suggests that both phytochrome and ABA control the transcription of the NPR1 gene in the angiosperm *Lemna gibba* (Weatherwax *et al.* 1998). Interestingly, the gene product from NRP1 is a dehydrin-like protein. Cyclohexamide prevented the ABA-induced increase in desiccation tolerance, strongly suggesting that ABA induces tolerance mechanisms that require protein synthesis (Table 5). Werner *et al.* (1991) obtained similar results using a growth assay of tolerance with protonema of the moss *Funaria*. Presumably then, the hardening treatments induce ABA synthesis, which in turn induces protein synthesis, or at least the accumulation of mRNA transcripts from which proteins can be rapidly synthesized during rehydration.

Very little information is available on which proteins ABA induces, even in higher plants (Bray 1997). In two liverworts, Hellewege *et al.* (1994, 1996) found that ABA induced the synthesis of dehydrin-like proteins with homology to proteins synthesised during seed development. The functions of dehydrins currently remain speculative (Close 1996), although it seems likely that they can inhibit the coagulation of a range of

macromolecules, thereby preserving structural integrity. Other proteins synthesized during hardening may be involved in the removal of harmful free radicals known to accumulate during desiccation stress (Christov & Bakardjieva 1998; Seel et al. 1992; see also reviews of Kranner & Beckett [1998] and Smirnoff [1993]). Dhindsa (1991) found that slow drying of *T. ruralis* increased the activity of enzymes associated with glutathione (GSH) metabolism. GSH can remove harmful free radicals formed during desiccation, and glutathione reductase allows the rapid reduction during rehydration of oxidized glutathione (GSSG). GSSG is a potent inhibitor of protein synthesis that accumulates during desiccation as a consequence of free-radical removal (Kranner & Beckett 1998). Possibly, ABA increases tolerance by increasing the activity of these enzymes. However, as noted in Materials and Methods, the drying rates used in the present study correspond to the 'slow' rates of Dhindsa (1991). Thus it seems more likely that ABA increases tolerance by means of mechanisms in addition to any based on GSH cycling. Future progress in understanding mechanisms of desiccation tolerance in bryophytes will come by elucidating the adaptive responses of the gene products produced by ABA and hardening treatments like those described in the present investigation. In *A. androgynum* defining these hardening treatments independently of drying rates is possible, making it a suitable species to carry out further experiments on desiccation tolerance mechanisms in bryophytes.

Acknowledgments

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Table 5 The effect of pretreatment with 100 µm ABA with or without 200 µM cyclohexamide in the light for 3 d on K⁺ leakage following desiccation and sudden rehydration n the moss *Atrichum androgynum*

Pretreatment	None (Control	Cyclohex-amide	ABA	ABA + cyclohexa-mide
% Total K⁺ lost following rehydration	75 ± 3	80 ± 5	56 ± 5	78 ± 3
Mean reduction in K⁺ loss caused by pretreatment	-	-4.3	19.1	-3.0

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