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Bryophytes as experimental models for the study of environmental stress tolerance: *Tortula ruralis* and desiccation-tolerance in mosses

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Abstract

The development of a complete understanding of how plants interact with the environment at the cellular level is a crucial step in advancing our ability to unravel the complexities of plant ecology particularly with regard to the role that many of the less complex plants (i.e., algae, lichens, and bryophytes) play in plant communities and in establishing areas for colonization by their more complex brothers. One of the main barriers to the advancement of this area of plant biology has been the paucity of simple and appropriate experimental models that would enable the researcher to biochemically and genetically dissect the response of less complex plants to environmental stress. A number of bryophytes model systems have been developed and they have been powerful experimental tools for the elucidation of complex biological processes in plants. Recently there has been a resurgent interest in bryophytes as models systems due to the discovery and development of homologous recombination technologies in the moss *Physcomitrella patens* (Hedw.) Brach & Schimp. In this report we introduce the desiccation-tolerant moss *Tortula ruralis* (Hedw.) Gaert., Meyer, and Scherb, as a model for stress tolerance mechanisms that offers a great deal of promise for advancing our efforts to understand how plants respond to and survive the severest of stressful environments. *T. ruralis*, a species native to Northern and Western North America, has been the most intensely studied of all bryophytes with respect to its physiological, biochemical, and cellular responses, to the severest of water stresses, desiccation. It is our hope that the research conducted using this bryophyte will lay the foundation for not only the ecology of bryophytes and other less complex plants but also for the role of desiccation-tolerance in the evolution of land plants and the determination of mechanisms by which plant cells can withstand environmental insults. We will focus the discussion on the research we and others have conducted in an effort to understand the ability of *T. ruralis* to withstand the complete loss of free water from the protoplasm of its cells.

Introduction

The development of a complete understanding of how plants interact with the environment at the cellular level is a crucial step in advancing our ability to unravel the complexities of plant ecology. This is especially true if one is interested in the role that many of the less complex plants, such as the algae, lichens, and bryophytes, play in plant communities and in establishing areas for colonization by their more complex brothers. Unfortunately we are a long way from achieving this goal. One of the main barriers to the advancement of this area of plant biology has been the paucity of simple and appropriate experimental models that would enable the researcher to biochemically and genetically dissect the response of less complex plants to environmental stress. However, recently there has been a resurgence of interest in bryophytes as models systems for a myriad of cellular and molecular level processes spawned from the discovery and development of homologous recombination technologies in the moss *Physcomitrella patens* (Hedw.) Brach & Schimp (see Wood et al. 2000 for review).

In this report we would like to introduce a bryophytic model for stress tolerance mechanisms that offers a great deal of promise for advancing our efforts to understand how plants respond to and survive the severest of stressful environments. The bryophyte that we introduce is the desiccation-tolerant moss *Tortula ruralis* (Hedw.) Gaert., Meyer, and Scherb (synonymous with *Syntrichia ruralis* (Hedw.) Weber & Mohr). *T. ruralis* is a species native to Northern and Western North America given the common name of Star Moss (in Europe it is known as the Hairy Screw Moss) and derives its Latin name from its twisted peristome but the name also aptly describes its habit of curling its leaves around the central stalk in a tortuous movement as it dries. This moss has been the most intensely studied of all bryophytes with respect to its physiological, biochemical, and cellular responses, to the severest of vegetative water stresses, desiccation (see reviews by Bewley 1979; Oliver & Bewley 1997; Smirnoff, 1992, 1993). It is our hope that the research conducted using this bryophyte will lay the foundation for not only the ecology of bryophytes and other less complex plants but also for the role of desiccation-tolerance in the evolution of land plants and the determination of mechanisms by which plant cells can withstand environmental insults. We will focus the discussion on the research we and others have conducted in an effort to understand the ability of *T. ruralis* to withstand the complete loss of free water, i.e., that water that can be removed, without resorting to elevated temperatures, from the protoplasm of its cells.

Desiccation-tolerance

Desiccation-tolerance is the ability of cells to revive from the air-dried state (Bewley 1979). Most plants are capable of producing desiccation-tolerant tissues, in particular seeds and pollen, but few can survive the drying of their vegetative structures. Even though such plants are few in number, individual species represent most major classes (see companion article in this volume by Oliver et al.). Plants that represent the more complex groups of plants, 60–70 species of ferns and fern allies and at least 60 species of angiosperms (Bewley & Krochko 1982), require that water loss be a slow process in order to induce and establish mechanisms for tolerance of desiccation, and have been categorized as modified desiccation-tolerant plants (Oliver & Bewley 1997; Oliver et al. 2000). Such plants have morphological and physiological mechanisms that retard the rate of water loss to the extent required to establish tolerance. Many of the desiccation-tolerant plants that are found in the phylogenetically basal clades that constitute the algae, bryophytes and lichens exhibit a type of desiccation-tolerance, believed to be primitive (see Oliver et al. 2000), that allows for the survival of even rapid rates of drying. These plants have very little in the way of water-retaining morphological or physiological characteristics and thus the rate of water loss depends upon the water status of the immediate environment. As a result of this, many lichens, algae, and arid-land bryophytes experience drying rates that are extreme, i.e., where their tissues may pass from full turgor to air dryness within an hour (or less). Such rates would prove lethal to desiccation-tolerant ferns or angiosperms.

Bewley (1979) postulated three criteria which a plant or plant structure must meet in order to survive desiccation. It must: (1) limit the damage incurred to a repairable level, (2) maintain its physiological integrity in the dried state (perhaps for extended periods of time), and (3) mobilize repair mechanisms upon rehydration which effect restitution of damage suffered during desiccation (and upon the inrush of water back into the cells). These criteria can be simplified into two basic components by which desiccation-tolerance can be achieved; the protection of cellular integrity and the repair of desiccation- (or rehydration-) induced cellular damage, as described by Bewley & Oliver (1992). Plants, in all probability, employ mechanisms that encompass both components but to varying degrees.

Work with desiccation-tolerant seeds and modified desiccation-tolerant angiosperms leads one to the conclusion that these tissues and plants utilize mechanisms that rely heavily on inducible (either developmentally programmed for seeds or environmentally induced in vegetative tissues) cellular protection systems (for reviews see Bartels & Nelson 1994; Bartels et al. 1993; Bewley et al. 1993; Bewley & Oliver 1992; Burke 1986; Close et al. 1993; Crowe et al. 1992; Dure 1993; Gaff 1989; Leopold et al. 1992; Oliver & Bewley 1997). The mechanisms of tolerance that are used by seeds and modified desiccation-tolerant plants appear to involve two major components, sugars and proteins, both of which are postulated to be involved in protection of cellular integrity during the drying phases (Bewley et al. 1993; Crowe et al. 1992; Dure 1993; Leopold et al. 1992; Oliver & Bewley 1997). The induction and establishment of such protective components is thought to be why modified-desiccation tolerant plants do not survive rapid water loss. It thus

appears that the speed of desiccation may have important consequences for the type of mechanism by which plants achieve vegetative desiccation-tolerance.

Plants that experience rapid desiccation rates have to rely on mechanisms that are either constitutive to the hydrated state, quickly induced during the first phases of water loss, or induced when the stress is relieved by the re-addition of water (or perhaps a combination of all three). Such mechanisms may be reduced or absent in plants that cannot withstand rapid drying and thus novel insights into plant stress responses and perhaps basic cellular repair mechanisms may be gained by the study of these tolerant species. Indeed, as we will demonstrate, desiccation-tolerant plants that withstand rapid rates of drying, in particular the moss *T. ruralis* (Hedw.) Gaert., Meyer, and Scherb, indicate that such plants employ a level of constitutive protection in conjunction with a rehydration induced recovery system to establish tolerance (Bewley & Oliver 1992, Bewley et al. 1993; Oliver 1996; Oliver & Bewley 1997).

Structural responses

An important question in the study of desiccation-tolerance is when or if cellular damage occurs? The answer to this question has important consequences not only to the ecology of a particular species but also for the determination of which mechanism of tolerance a plant employs. Each species may be different so it is important to establish when and if cellular damage occurs and to what extent for each type of plant.

The question of how damage occurs during desiccation has received some attention and has been directed towards an understanding of desiccation-associated oxidative damage and its prevention (see McKersie 1991; Smirnoff 1993 for review). Oxidative damage during desiccation results in several types of cellular damage, the major categories are: oxidation of protein sulfhydryl groups leading to denaturation, pigment loss and photosystem damage (especially in the light), and lipid peroxidation and free fatty acid accumulation in membranes (McKersie 1991; Smirnoff 1993). Protective mechanisms are of two types which are synergistic (Smirnoff 1993). The first involves mechanisms that are implicated in lowering the amount of free radicals during desiccation. This includes enzymes such as peroxidase, catalase and super oxide dismutase (SOD) along with antioxidant compounds such as ascorbic acid, a-tocopherol, carotenoids and glutathione (GSH) The second involves mechanisms that regenerate these antioxidants (glutathione reductase, ascorbate peroxidase and mono and dehydroreductases).

Oxidative inactivation of sulfhydryl-containing enzymes as a result of desiccation has been reported in several desiccation-tolerant mosses (Stewart & Lee 1972). This damage apparently continues during storage of the tissues in the dried state and can be alleviated by incubation with GSH (Stewart & Lee 1972). Slow desiccation of *Tortula* results in the oxidation of approximately 30% of the GSH pool to oxidized glutathione (GSSG) (Dhindsa 1987), indicating a decreased ability of the moss to withstand oxidative injury. Interestingly, desiccation itself does not result in an increase in GSH oxidation since GSSG does not increase when the moss is dried rapidly (Dhindsa 1987), but it does increase upon rehydration of rapidly dried moss. Desiccation of the tolerant moss *T. ruraliformis* does not result in a loss of GSH (Seel et al. 1992a). How these conflicting findings relate to an overall mechanism of desiccation-tolerance is enigmatic but may simply reflect varying capabilities among desiccation-tolerant species to buffer oxidative damage in this way. In *T. ruraliformis* (Seel et al. 1992a) ascorbate decreases during drying; in these plants maintenance of high amounts of GSH may be more important in protection than it is in *T. ruralis*. *T. ruraliformis* also maintains an appreciable α-tocopherol content during drying, but this is depleted in a desiccation-intolerant species, *Dicranella palustris*, (Dicks.) Crundw.; again this indicates that other antioxidants can be more important than ascorbic acid or GSH (Seel et al. 1992a).

In all plant tissues studied to date, light increases the amount of desiccation-induced damage as a result of oxidation (Smirnoff 1993). Oxidative damage incurred during drying of *T. ruraliformis* and *D. palustris* increases if the plants are irradiated under high light conditions (1100–1200 μmol m^{-2} s^{-1}). Neither photosynthetic pigment content, nor the ability of *T. ruraliformis* to recover was affected, but there was a reduction in pigments in the sensitive species, *D. palustris* (Seels et al. 1992a).

Lipid peroxidation also occurs during desiccation of vegetative tissues in bryophytes. By measurement of malondialdehyde, an indicator of lipid peroxidation, Seel et al. (1992b) demonstrated that the relatively desiccation-intolerant moss *D. palustris* exhibited increased lipid peroxidation following desiccation, while the tolerant species *T. ruraliformis* did

not. Interestingly, the extent of lipid peroxidation as a whole, whether in hydrated or desiccated gametophytes, was five- to six-fold higher in the intolerant species. This may be indicative of an inherent protection against lipid peroxidation in tolerant species. Stewart & Bewley (1982) recorded a decrease in lipoxygenase activity (a lipid peroxidation enzyme) during desiccation in *T. ruralis*, again indicating a protective mechanism inherent in desiccation-tolerant species.

One of the prime methods for assessing damage has been the use of light and electron microscopy. Although there is not a wealth of information concerning the effects of water loss on cellular integrity it does appear that desiccation-tolerant plants do not exhibit any major differences in the type of damage incurred from that seen in desiccated non-tolerant tissues or in non-tolerant tissues exposed to sub-lethal water stress (Bewley & Krochko 1982; Oliver & Bewley 1984a). Nevertheless it is important to ascertain when in a wet/dry/wet cycle cellular damage takes place in order to gain an insight into the overall strategy employed as a mechanism for desiccation-tolerance, i.e., where in the spectrum from protection to repair does the mechanism lie.

Many studies into the structure of dried vegetative tissues have been conducted using gametophytic tissues of desiccation-tolerant bryophytes, in particular mosses (see Oliver & Bewley 1984a for review). Non-ultrastructural observations using Nomarski optics, for which fixation is not required, demonstrate that *Tortula* species undergo extensive plasmolysis upon desiccation. In *T. ruralis* protoplasm condenses at the proximal and distal ends of the cell leaving the central portions of the cell empty (Tucker et al. 1975). The regions of condensed protoplasm are connected by cytoplasmic bridges that extend along the abaxial, adaxial and lateral sides of the dehydrated cells. The chloroplasts in these cells are smaller and more rounded than those in hydrated cells and the nucleus appears unaffected by drying. Not all desiccation-tolerant mosses exhibit this type of plasmolysis. Drying cells of *Barbula torquata* Tayl. and *Triquetrella papillata* (Hook. *f.* and Wils.) Broth. to 50 to 70% of their original volume at desiccation apparently excludes the entry of air through the cell walls. This results in the protoplasm of these cells occupying the total area enclosed by the cell walls (Moore et al. 1982). These studies demonstrate that drying elicits major changes in cell structure but do not directly demonstrate cellular damage. Such information is best gained from more detailed ultrastructural studies. The results from early studies of fixed dried cells of desiccation-tolerant mosses suggest that desiccation elicits shrinkage of organelles, coupled with a breakdown of internal membrane structures such as thylakoids and cristae, and of cytoplasmic membranes such as the endoplasmic reticulum (reviewed by Oliver & Bewley 1984a). However, there is a caveat to the conclusions drawn from electronmicrographs of fixed dried plant cells. Fixation procedures, for however brief a time, always result in some rehydration which in all probability results in damage to the dried tissues. Thus it is difficult to assess to what extent drying per se has on cellular integrity. Recently, Platt et al. (1994) attacked this problem using freeze-fracture techniques, which avoids rehydration, to establish the condition of plasma- and organellar membranes in dried leaf cells of *T. ruralis* and *Selaginella lepidophylla* Hook. & Grev. (a modified desiccation-tolerant pteridophyte). The cell membranes of both species in the dried state remain as intact bilayers containing normally dispersed intramembranous particles. The structural organization of the organelles is also maintained in both species, both thylakoid and cristae membranes appear intact, and no areas of disrupted bilayer organization were detected in any of the cell membranes. Thus it appears that for *Tortula* and *Selaginella* membrane disruption does not occur during drying but during subsequent rehydration. Similar conclusions have been drawn from the use of freeze-fracture to investigate membrane structure in dried seeds (Thompson & Platt-Aloia 1982; Bliss et al. 1984; Platt-Aloia et al. 1986). It is therefore possible that this may be a general rule for dry plant tissues and if so it could be inferred that protection mechanisms, whether induced or constitutive, are very successful in maintaining membrane structures during the removal of water from plant cells.

Regardless of the ability to protect membranes during drying, all desiccation-tolerant vegetative plants and tissues exhibit cellular damage immediately upon rehydration, either as a result of the inrush of water or as a consequence of the drying process, or both. That the drying process does influence cellular damage is suggested by the observation that rapidly (within an hour) dried *T. ruralis* leaf cells suffer a greater degree of disruption upon rehydration than do the same cells dried slowly (Schonbeck & Bewley 1981; Oliver & Bewley 1984b). Thus although drying does not result in visible injury to membranes there is some component of the drying process that affects the capability of the membranes to withstand the rigors of rehydration.

The most evident symptom of cellular damage in all desiccation-tolerant plant tissues is the leakage of cellular ions into the surrounding water during and immediately following rehydration. Such leakage has been used as a measure of the extent of one aspect of cellular disruption, i.e., membrane damage, resulting from the desiccation/rehydration event (see Bewley 1979; Bewley & Krochko 1982; Gaff 1980; Simon 1978; Simon & Mills 1983, for reviews). In desiccation-tolerant bryophytes most of the leakage of ions occurs in the first five minutes of re-exposure to water and the amount of ions leaked is dependent upon the speed at which drying occurred prior to rehydration (Bewley & Krochko 1982, Oliver & Bewley 1984a, Oliver et al., 1993) Again this indicates that the drying phase has an impact on cellular damage manifested upon rehydration; perhaps time is required even in these desiccation-tolerant plants for full protective measures to be implemented. In the minutes following the early outrush of ions desiccation-tolerant bryophytes reabsorb the cellular nutrients lost to the surrounding water, which is indicative of a rapid return of the leaf-cell membranes to their normal selectively permeable state. Such a recovery does not occur in rehydrating cells of desiccation-intolerant species (Oliver & Bewley 1984a).

As water enters the dried leaf cells of *T. ruralis* the condensed cytoplasm rapidly expands to fill the empty cell cavity formed by plasmolysis (Tucker et al. 1975) Within minutes after rehydration chloroplasts are swollen and globular in shape and their outer membranes are folded and separated from the thylakoids, which themselves are no longer compacted (Tucker et al. 1975, Bewley & Pacey 1978). The extent of thylakoid disruption is dependent upon the prior speed of desiccation; the more rapid the drying rate the more disruption occurs. Mitochondria also swell and exhibit disruption of the internal membrane structures (cristae), but the appearance of this organelle upon rehydration is not affected by the rate of desiccation. Similar results have been reported for other desiccation-tolerant moss species (Noailles 1978; Swanson et al. 1976; Moore et al. 1982). In all cases organelles regain normal structure within 24 h of the readdition of water. Rehydrated cells of dried gametophytes of the desiccation-sensitive moss *Cratoneuron filicinum* (Hedw.) Spruce., exhibit identical structural abnormalities to those seen in *T. ruralis* but in this case the cells never regain a normal appearance and die (Bewley & Pacey 1978; Krochko et al. 1978). These studies are testament to the fact that cells of desiccation-tolerant bryophytes are not immune to the rigors of drying and rehydration. It is their ability to recover from such damage that makes them unique and useful as model plants for the study of stress-induced cellular processes.

Gene expression responses

As gametophytic tissues of mosses dry, whether or not they are desiccation-tolerant, the ability for the cells to conduct protein synthesis rapidly declines (Bewley 1972, 1973b; Henckel et al. 1977; Siebert et al. 1976; and M. J. Oliver unpublished data for *T. caninervis* and *T. norvegica*). In *T. ruralis* loss of protein synthetic capacity during desiccation is manifested in a loss of polysomes, the active protein synthetic machinery. The loss of polysomes results from the run-off of ribosomes from mRNAs coupled with a breakdown in the ability to form new initiation complexes (see Bewley 1979; Bewley & Oliver 1992 for reviews). Rapid desiccation of *T. ruralis*, however, leads to the retention of 50% of the polysomes in the dried state, indicative that water loss alone is not the cause of the detachment of ribosomes from mRNAs. In this case, it is thought that water loss is so fast that mRNAs are trapped on polysomes before run-off is completed. The inhibition of protein synthesis during desiccation has also been linked to levels of oxidized glutathione, which accumulate during slow drying, in *T. ruralis* (see above – Dhindsa 1987, 1990). Although a direct cause and effect between the redox state of glutathione and protein synthesis has not been demonstrated the correlation is good. The rapid loss of polysomes during drying (under 'natural' drying rates) and the apparent sensitivity of the initiation step of protein synthesis to protoplasmic drying leads us to the conclusion that the induction of synthesis of 'protective' proteins during drying is highly unlikely. This is borne out by the observation that no new mRNAs are recruited into the protein synthetic complex even during slow drying (Oliver 1991). The fact that the moss survives rapid desiccation indicates that an *inducible* protection mechanism is not necessary for survival. In addition, if protective proteins (or other compounds) are an important part of the desiccation tolerance of this species they must be present at all times, i.e., *constitutively* expressed.

Protein and RNA synthesis recover rapidly upon rehydration of desiccation-tolerant mosses (Bewley 1973a,b; Gwozdz et al. 1974; Henckel et al. 1977; Oliver & Bewley 1984b; Oliver et al. 1993; Siebert

et al. 1976). In all cases the rate of recovery to control levels is faster the slower the rate of prior desiccation (Gwozdz et al. 1974; Oliver & Bewley 1984b). The slower rate of recovery for protein synthesis upon rehydration of rapid-dried moss occurs even though polysomes are retained in the dried state. This is thought to be indicative of the greater degree of cellular damage in these gametophytes. In *T. ruralis*, ribosomes and ribosomal RNAs are stable during desiccation and the conserved pools of these components quickly embark on the formation of new polysomes upon rehydration (Gwozdz & Bewley 1975; Tucker & Bewley 1976; Oliver & Bewley 1984b,c). Messenger RNAs are also stable to desiccation, more so in slow-dried moss than in rapid-dried moss (see below), and are rapidly utilized in protein synthesis upon rehydration (Oliver & Bewley 1984c,d). Upon rehydration of the dried gametophytes there is a turnover of mRNAs stored in the dried state accompanied by a replenishment of the message pool as a result of *de novo* synthesis (Oliver & Bewley 1984d). The rate of mRNA synthesis is faster if there is greater cellular disruption as a result of rapid desiccation. This may be in response to the greater loss of mRNA during rapid drying or it could reflect the activation of a repair mechanism to overcome the greater degree of disruption in these cells. The turnover rate of conserved mRNAs upon rehydration is unaffected by the desiccation event. However, the proportion of conserved mRNA in polysomal fractions of rehydrated gametophytes, whether dried slowly or rapidly, soon declines such that within 2h of rehydration little is present (Oliver and Bewley, 1984d): most of the mRNA within the activated protein synthetic complex at this time is newly synthesized.

As discussed earlier, the rapid loss of polysomes during desiccation coupled with a desiccation-induced failure in initiation of message recruitment argues against the synthesis of novel proteins during drying (indeed it is unlikely that any protein synthesis continues for any length of time after water begins to leave the cells). Direct evidence for this comes from the lack of the synthesis of novel proteins from polysomal complexes isolated from drying gametophytes (Oliver 1991). In response to rehydration, however, protein synthesis rapidly recovers and the pattern of proteins synthesized is extensively different from that seen under the non-stressed condition (Oliver & Bewley 1984d; Oliver 1991). This change in the pattern of protein synthesis occurs without a qualitative change in the pool of mRNA available for translation (Oliver & Bewley 1984d; Scott & Oliver 1994). This suggests that the transcriptional responses to effect the synthesis of stress related proteins that are common in stressed plants (Sachs & Ho 1986) and in modified desiccation-tolerant angiosperms (Ingrams & Bartels 1998; Oliver & Bewley 1997) are not operating in the response of the moss to desiccation. The inference from this study is that the alteration in gene expression associated with rehydration is mediated mainly by an alteration in translational controls, at the level of mRNA selection, and that if transcription is important it serves to replenish the pools of pre-existing messages. Such a scenario is attractive since the moss has to respond quickly to rapid changes in the water status of its environment; the speed of the response would be greater if effected at the level of translation.

In a detailed study of the change in gene expression upon rehydration, Oliver (1991) demonstrated that during the first two hours the rates of synthesis of almost 80% of the proteins is either increased or decreased significantly from their rates of synthesis observed under continuously hydrated conditions. Of particular note are the proteins whose synthesis is dramatically affected. These include 25 proteins whose synthesis is terminated, or substantially decreased (greater than five-fold), and 74 proteins whose synthesis is initiated, or substantially increased (greater than five-fold). These proteins have been designated hydrins and rehydrins respectively. These are functional terms and do not refer to any common sequence motif or structural property nor imply a common enzymatic function. The altered synthesis of these two groups of proteins is not co-ordinately regulated (Oliver 1991). Rehydrin synthesis is initiated or stimulated upon rehydration of gametophytes that had been previously dried to between 50 and 20% of their original fresh weight, while the synthesis of hydrins is steadily inhibited as gametophytes are subjected to greater degrees of water loss prior to rehydration. Their synthesis is almost completely inhibited in rehydrated tissues that were previously dried to 50% of their original fresh weight. These findings indicate that the synthesis of rehydrins is fully activated only after a threshold of prior water loss is reached. If this is so then, at least for *T. ruralis*, the activation of a repair or recovery mechanism requires that a certain level of stress is experienced and to accommodate this there may be a stress level 'sensing' component to the response.

Hydrins and rehydrins also differ in the time required to return to normal levels of synthesis during extended periods of hydration. The synthesis of all

hydrins returns to control levels between 2 and 4 h following rehydration. The reduction in synthesis of rehydrins to control levels depends upon each individual rehydrin, however. Some rehydrins are only synthesized for less than 2 h, while others are still being synthesized at elevated levels 10 to 12 h after rehydration. A full return to control levels of synthesis for all proteins is evident after 24 h. From this study it is possible to classify rehydrins as either early recovery proteins or rehydrins that are required for extended periods during recovery of the moss from desiccation. This observed diversity of rehydrin gene expression is consistent with the idea that *T. ruralis* utilizes a cellular repair based strategy of tolerance. One would expect that once the initial potentially lethal damage is repaired in the first hours of rehydration (see earlier section on leakage and ultrastructural damage) the longer term repair and recovery of organelles and associated metabolism would take place. If this notion is correct then one would predict the early rehydrins to be involved in such processes as the repair of the damage to the plasmalemma (to stop the loss of the cytoplasmic contents) and those whose synthesis is protracted to be involved in the longer term recovery of organellar function and structure. At this time this is simply a working hypothesis and its proof or disproof will await the identification and characterization of individual rehydrins.

In order to more closely follow rehydrin mRNA pools and gain an insight into both the identity of rehydrins and what translational controls were affected during desiccation and rehydration, Scott & Oliver (1994) isolated 18 cDNAs that correspond to transcripts preferentially translated during rehydration of dried *T. ruralis* gametophytes. Consistent with the earlier findings that the mRNA pools do not change qualitatively during desiccation and rehydration rehydrin transcripts are not unique to rehydration. All 18 rehydrin cDNAs represent mRNAs present in the gametophytes prior to desiccation but they are present in greater amounts in the polysomes of rehydrated gametophytes compared to those from undesiccated moss. This latter finding supports the hypothesis that it is an alteration in translational controls that drives the induced change in gene expression. Again, it is also suggestive that the alteration in control is at the level of differential selection and/or recruitment by the translational machinery from a qualitatively constant mRNA pool (Oliver 1991).

Utilizing both hydrin and rehydrin cDNAs as probes, it was determined that during slow drying rehydrin cDNAs accumulate, even though they cannot be translated at this time, and that this accumulated RNA can be fractionated into the 100 K $\times$ *g* pellet of cellular extracts (Oliver 1996; Oliver & Wood 1997). The 100 K $\times$ *g* pellet (polysomal fraction) contains most of the cytosolic translational machinery (such as RNA-protein complexes, ribosomes, polysomes, etc.) as well as other cellular constituents which have sufficient mass to pellet through a 1.5 M sucrose pad at 100,000 x g over 90 min. Hydrin cDNAs do not exhibit this behavior (Oliver & Wood 1997; MJ Oliver, unpublished data). This accumulation of transcripts in the 100 K $\times$ *g* pellet during drying occurs when gametophyte polysomes have been fully depleted and protein synthetic activity is completely inhibited (Bewley & Oliver 1992; Oliver 1996; Oliver & Bewley 1997). This suggests the formation of protein/mRNA complexes, termed messenger ribonucleoprotein particles (mRNPs), which form large enough structures to sediment in the polysomal fraction during centrifugation. The presence of such particles is a common feature of quiescent eukaryotic cells (see Bag 1988 for review). mRNPs are cytoplasmic entities (nuclear forms are termed hnRNPs) and are classified into two classes; stored mRNPs (e.g., in an untranslated form) or as polysomal mRNPs (e.g., a polyribosomal-associated form) (Minich & Ovchinnikov 1992). The presence of stored, untranslated mRNAs is common in animal embryo development, first reported by Spirin (1964), and has been extensively studied in *Xenopus* embryos (reviewed in Davidson 1986). In plants, there is considerable evidence for the presence of stored mRNAs in the mature, dry seeds of higher plants (Silverstein 1973; Pramanik et al. 1992).

To demonstrate that mRNPs do form during slow drying of *T. ruralis*, Wood and Oliver (1999) utilized a rehydrin cDNA , Tr288 (see below), to fractionate, characterize and isolate the structure within which rehydrin mRNAs are stored in slow-dried gametophytes. Sucrose density gradient fractionation of cellular extracts (in which cellular components sediment down the density gradient at a velocity determined by their density) of slow-dried moss revealed that the rehydin co-sedimented with a particle slightly lighter than 40S ribosomal sub-units. The position of Tr288 transcript within the gradient was determined by RT-PCR. If the polysomal pellet was isolated under low salt conditions the Tr288 transcript is detected in much lower fractions indicating a population of particles of much greater average size (Wood & Oliver, 1999). In both sets of samples there are no polysomes present (see

earlier discussion) and the presence of these larger particles is not discernable by simple optical density measurements. A more detailed analysis utilizing cesium chloride equilibrium density gradients (within which cellular components separate according to their bouyant density) demonstrated that transcript for the rehydrin Tr288 can be detected in structures that have a density between 1.44 and 1.6 g cm^{-3}. Such intermediate densities are consistent with the presence of mRNPs and suggest a tight RNA to protein interaction (Bag 1988). Wood & Oliver (1999) also demonstrated that Tr288 transcript is only associated with such particles during the drying process. It is important to note that the formation of mRNPs in *T. ruralis* during drying requires a sufficient period of time since there is no accumulation of rehydrin mRNAs if water loss is rapid. Once the presence of rehydrin transcripts in mRNP particles had been demonstrated, Wood and Oliver (1999) attempted to isolate them. Using oligo-dT chromatography and FPLC they were able to isolate desiccation-specific particles of various sizes ranging from 1275 kDa to 65 kDa approximately. The reason for different size classes of rehydrin mRNPs is not known at this time and at present the identity of the protein components of these particles is unknown.

The formation of mRNPs in response to water loss and their possible roles in mRNA storage and protection has important consequences for the study of vegetative desiccation-tolerance and perhaps stress responses of plants in general. The ability to store components during a stress event that are needed for recovery offers a newer dimension to the concept of damage control and the possibility for a more rapid return to growth than does the relatively slower activation and transcription of specific stress or stress-recovery response genes. It is distinctly possible that even in plants where gene activation is the common response to water loss that certain transcripts required for the recovery process are stored in mRNPs during drying. At the least, the demonstration that the moss *T. ruralis* makes use of stored mRNAs during the rehydration process and beyond should indicate that not all transcripts that are made in response to a stress event are required for immediate use but may be synthesized and stored for when the stress is over and recovery is set in motion.

Rehydrins

In order to gain and idea of what processes are involved in the response of *T. ruralis* to desiccation and rehydration it is important to determine the identity of those genes represented by the rehydrin class of transcripts. Such a goal is not only important with regard to understanding desiccation-tolerance but may shed light on how plants in general respond to stress at the level of gene activation. All of the 18 rehydrin cDNAs, isolated by Scott & Oliver (1994), have been sequenced (Oliver et al. 1997; Wood et al. 1999). Only three exhibit significant sequence homology to known genes in the Genbank databases. Tr155 has a strong sequence similarity to an alkyl hydroperoxidase linked to seed dormancy in barley (Aalen et al. 1994) and *Arabidopsis* embryos (Haslekas et al. 1998), and in rehydrated but dormant *Bromus secalinas* seeds (Goldmark et al. 1992). The indication is that this gene is important in the antioxidant pathways that protect cells from the effects of reactive oxygen species formed during desiccation and perhaps upon rehydration. Tr213 exhibits a high degree of similarity to polyubiquitins from several plant sources. This latter clone is indicative of an increased need for protein turnover during the recovery from desiccation. The final clone to exhibit similarity to known genes is Tr 288 the cDNA used in our earlier studies into translational control of gene expression. Tr288 has a dehydrin-like K box sequence at the carboxy terminus of the predicted protein but exhibits little similarity to known dehydrins other than similarities in its predicted secondary structure. This finding is somewhat enigmatic as dehydrins have long been thought to provide protection to cellular components during the drying process (see Close et al. 1993 and Dure 1993 for reviews) and Tr288 has been classified as a rehydrin as its synthesis appears to be specific to the rehydration event.

In addition to our rehydrin analyses we have also established a small expressed sequence tag (EST) database for *T. ruralis* (Wood et al. 1999) from a cDNA library constructed from slow-dried gametophyte polysomal RNA (in an attempt to target sequences sequestered in mRNPs, see above). The analysis of randomly selected cDNA clones or ESTs (so named because they represent only genes expressed at a particular time or under a particular circumstance) has been an important technique for the discovery of new genes (Boguski 1995). The eventual aim of EST sequencing is to generate a sequence database of all rehydrin transcripts stored in the polysomal

Table 1. Inventory of *Tortula ruralis* ESTs with significant similarity to genes from other organisms.

Clone	Accession #	Putative identity/homolog	Clone	Accession #	Putative identity/homolog
RNP1	AI305036	HSC70 BIP mRNA	RNP24	AI305059	Nucleotide-binding protein
RNP4	AI305038	Ribosomal protein L15	RNP27	AI305062	Ribosomal protein L19
RNP10	AI305045	V-type ATPase	RNP29a	AI305064	Desiccation-related protein
RNP11	AI305046	NADH-oxidoreductase	RNP33	AI305068	High MW antigen
RNP20	AI305056	Ribosomal protein S14	RNP44	AI305079	Glutathione-S-transferase
RNP35	AI305070	NAD-dependent fdh	RNP55	AI305090	Extensin
RNP37	AI305072	Ribosomal protein S16	RNP57	AI305092	ERD10 (dehydrin)
RNP47	AI305082	Ribosomal protein L23	RNP63	AI305098	Phenylalanine hydroxylase
RNP49	AI305084	Histone H3 protein	RNP73	AI305068	Catalase
RNP51	AI305086	Chlorophyll a/b apoprotein	RNP74	AI305069	RHD3
RNP54	AI305089	Rieske iron-sulfur protein	RNP78	AI304973	Core protein
RNP59	AI305094	S-phase-specific protein	RNP80a	AI304975	Desiccation-stress protein
RNP66	AI305101	Ribosomal protein L22	RNP83	AI304978	Aminopeptidase
RNP68	AI305103	Lipoxygenase	RNP94	AI304989	HVA-1
RNP70	AI305105	*psaG* gene, PSI	RNP124	AI305019	Group 3 LEA-like
RNP71	AI305016	Chlorophyll-binding protein	RNP123	AI305018	GapD homologue
RNP72	AI304967	Orthophosphate dikinase	RNP128	AI305023	Ribosomal protein S8
RNP82a	AI304977	10kDa polypeptide, PSII	RNP130	AI305025	ADP-gluc phosophorylase
RNP86	AI304981	Cinnamic acid hydroxylase	RNP155b	U40818	RAB 24
RNP114	AI305009	Ribosomal protein L30	RNP213c	AI313704	Polyubiquitin
RNP119	AI305014	Group 3 LEA			

The putative functional identity of each gene was established by sequencing $>$ 375 bp of the 3′ end of each cDNA (Wood et al. 1999). Genes presented exhibited significant similarity at the nucleotide (left column) or amino acid level (right column).
[a]Two copies of this clone have been identified.
[b]Also known as Tr155.
[c]Also known as Tr213, see Scott and Oliver (1994).

fraction of slow-dried gametophytes. At present we have generated and partially sequenced 152 ESTs only 30% of which showed significant similarity to previously identified nucleic acid and/or polypeptide sequences (see Table 1 for an inventory). Interestingly, several ESTs showed significant similarity to unidentified desiccation-tolerance genes isolated from the desiccation-tolerant angiosperm *Craterostigma plantagineum* Hochst. As for the *Craterostigma* EST study (Bockel et al. 1998), the *Tortula* ESTs indicated the involvement of several cellular processes in the response to desiccation. However, further studies are needed before their importance in desiccation-tolerance mechanisms can be elucidated.

Homologous recombination and future directions

An important tool in the quest to determine which genes are important for desiccation-tolerance in bryophytes, and indeed all desiccation-tolerant plants, is the ability to remove and replace genes that have been identified as responding to desiccation and/or rehydration. To accomplish gene replacement or removal one must have the ability to introduce DNA into the target cell (transformation) and to insert the exogenous DNA sequence into the corresponding homologous genomic sequence, termed homologous recombination. In angiosperms transformation protocols abound but the ability to target the exogenous DNA has proved extremely difficult, and when it occurs it is an inefficient process that cannot be effectively exploited (Reski 1998). In bryophytes, however, transformation methods have only recently been developed but homologous recombination appears to occur at a relatively high frequency (Schaefer & Zyrd, 1997; Hofmann et al. 1999; Reski 1998, 1999) making them ideal candidates for the development of model systems for identifying gene function. As of yet only *Physcomitrella patens*, a moss from mesic habitats that is a model for plant developmental processes, has been developed as an experimental tool for homolo-

gous recombination (see Reski 1988, 1999 for review). We are presently involved in developing these techniques for *T. ruralis* to extend its capabilities as a model for plant stress studies.

Research to the present, that described above and others that centers on the effects of desiccation on the photosynthetic apparatus (see other articles in this series) clearly indicate the value of the moss *Tortula ruralis* as a model plant in helping to understand, and to add new dimensions to, our understanding of how plants respond to the extremes of water stress. The value of *Tortula* as a model plant will increase in the future as we develop the molecular-genetic tools for the manipulation of its genome. In general, the haploid genomes of mosses provide an advantage over more complex plant models, especially in such endeavors as gene tagging, directed mutagenesis and antisense expression. In addition the new methods designed to look at the expression characteristics of large numbers of genes, such as DNA microarray analysis, will also impact the role of bryophytes in the study of plant processes.

As is evident from the above narrative we have taken, with no apology, a strong reductionistic approach to understanding desiccation-tolerance as exhibited by the moss *T. ruralis*. Perhaps it is now time to take what we have discovered and learned and apply it to some of the larger questions that have yet to be studied. In particular such ecological questions as to how a particular mechanism of desiccation tolerance impacts habitat selection, succession and the role that desiccation-tolerant plants play in the ecology of deserts and extreme habitats. Some inroads are being made in these areas and we would like to direct your attention to the other reports in this issue. We are, however, turning our attention to the questions that relate to the evolution of desiccation-tolerance by establishing a phylogenetic approach based upon the genes that we have and will establish as being central to this phenotype (see Oliver et al. 2000).

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References

Aalen, R. B., Opsahl-Ferstad, H. G., Linnestad, C. & Olsen, O. A. 1994. Transcripts encoding an oleosin and a dormancy-related protein are present in both the aleurone layer and in the embryo of developing barley (*Hordeum vulgare* L.). Plant J. 5:385-396.

Bag, J. 1988. Messenger ribonucleoprotein complexes and translational control of gene expression, Mol. Gen. (Life Sci. Adv.). 7: 117-123.

Bartels, D., Alexander, R., Schneider, K., Elster, R., Velasco, R., Alamillo, J., Bianchi, G., Nelson, D., & Salamini, F. 1993. Desiccation-related gene products analyzed in a resurrection plant and in barley embryos. Pp. 119–127. In: Close, T. J. & Bray, E. A. (eds), Plant responses to cellular dehydration during environmental stress. Current Topics in Plant Physiology: American Society Plant Physiology. Series Vol. 10.

Bartels, D. R. & Nelson, D. 1994. Approaches to improve stress tolerance using molecular genetics. Plant Cell Environ 17: 659–667.

Bewley, J. D. 1972. The conservation of polyribosomes in the moss *Tortula ruralis* during total desiccation. J. Exp. Bot. 2: 692–698.

Bewley, J. D. 1973a. Polyribosomes conserved during desiccation of the moss *Tortula ruralis* are active. Plant Physiol. 51: 285–288.

Bewley, J. D. 1973b. Desiccation and protein synthesis in the moss *Tortula ruralis*. Can. J. Bot. 51: 203–206.

Bewley, J. D. 1979. Physiological aspects of desiccation-tolerance. Ann. Rev. Plant Physiol. 30: 195–238.

Bewley, J. D. & Krochko, J. E. 1982. Desiccation-tolerance. Pp. 325–378. In: Lange, O. L., Nobel, P. S., Osmond, C. B., & Ziegler, H. (eds), Encyclopedia of plant physiology. Vol 12B, Physiological Ecology II. Springer-Verlag, Berlin.

Bewley, J. D. & Oliver, M. J. 1992. Desiccation-tolerance in vegetative plant tissues and seeds: Protein synthesis in relation to desiccation and a potential role for protection and repair mechanisms. Pp. 141–160. In: Osmond, C. B. & Somero, G. (eds), Water and life: A comparative analysis of water relationships at the organismic, cellular and molecular levels. Springer-Verlag, Berlin.

Bewley, J. D. & Pacey, J. 1978, Desiccation-induced ultrastructural changes in drought-sensitive and drought-tolerant plants. Pp. 53–73. In: Crowe, J. H. & Clegg, J. S. (eds), Dry biological systems. Academic Press, London, New York.

Bewley, J. D., Reynolds, T. L., & Oliver, M. J. 1993. Evolving Strategies in the adaptation to desiccation. Pp. 193–201. In: Close, T. J. & Bray, E. A. (eds), Plant responses to cellular dehydration during environmental stress. Current Topics in Plant Physiology: American Society Plant Physiology, Series Vol. 10.

Bliss, R. D, Platt-Aloia, K. A., & Thomson, W. W. 1984. Changes in plasmalemma organization in cowpea radicle during imbibition in water and NaCl solutions. Plant Cell Environ. 7: 601–606.

Bockel, C., Salamini, F., Bartels, D. 1998. Isolation and characterization of genes expressed during early events of the dehydration process in the resurrection plant *Craterostigma plantagineum*. J. Plant Physiol. 152: 158–166.

Boguski, M. S. 1995. The turning point in genome research. Trends Biochem. Sci. 20: 295–296.

Burke, M. J. 1986. The glassy state and survival of anhydrous biological systems. Pp. 358-363. In: Leopold, A. C. (ed.), Membranes, metabolism and dry organisms. Cornell University Press, Ithaca, NY.

Close, T. J., Fenton, R. D., Yang, A., Asghar, R., DeMason, D. A., Crone, D. E., Meyer, N. C. & Moonan, F. 1993. Dehydrin: The protein. Pp. 104–118. In: Close, T. J. & Bray, E. A. (eds.), Plant responses to cellular dehydration during environmental stress. Current Topics in Plant Physiology: American Society Plant Physiology, Series Vol. 10.

Crowe, J. H., Hoekstra, F. A., & Crowe, L. M. 1992. Anhydrobiosis. Ann. Rev. Physiol. 54: 579–599.

Dhindsa, R. 1987. Glutathione status and protein synthesis during drought and subsequent rehydration of Tortula ruralis. Plant Physiol. 83: 816–819.

Dhindsa, R. 1991. Drought stress, enzymes of glutathione metabolism, oxidation injury, and protein synthesis in Tortula ruralis. Plant Physiol. 95: 648–651.

Dure, L. III. 1993. A repeating 11-mer amino acid motif and plant desiccation. Plant J. 3: 363–369.

Gaff, D, 1980, Protoplasmic tolerance to extreme water stress. Pp. 207–230. In: Turner, N. C. & Kramer, P. J. (eds), Adaptation of plants to water and high temperature stress, Wiley Interscience, New York.

Gaff, D. 1989. Responses of desiccation-tolerant 'resurrection' plants to water stress. pp. 255-268. In: Krebb, K. H., Richter, H., Hinkley, T. M. (eds.), Structural and functional responses to environmental stresses. SPB Academic Publishers, The Hague, The Netherlands.

Goldmark, P. J., Curry, J., Morris, C. F., & Walker-Simmons, M. K. 1992. Cloning and expression of an embryo-specific mRNA upregulated in hydrated dormant seeds. Plant Molec. Biol. 19: 433-441.

Gwozdz, E. A. & Bewley, J. D. 1975 Plant desiccation and protein synthesis: An in vitro system from dry and hydrated mosses using endogenous and synthetic messenger RNA. Plant Physiol. 55: 340–345.

Gwozdz, E. A., Bewley, J. D. & Tucker, E. B. 1974. Studies on protein synthesis in *Tortula ruralis*: polyribosome reformation following desiccation. J. Exp. Bot. 25: 599–608.

Haslekas, C., Stacy, R. A. P., Nygaard, V., Culianez Macia, F. A. & Aalen, R. B. 1998 The expression of a peroxiredoxin antioxidant gene, AtPer1, in *Arabidopsis thaliana* is seed specific and related to dormancy. Plant Molec. Biol. 36: 833–845.

Henckel, R. A., Statrova, N. A. & Shaposnikova, S. V. 1977. Protein synthesis in poikiloxerophyte and wheat embryos during the initial period of swelling. Soviet Plant Physiol. 14: 754–762.

Hofmann A. H., Codon, A. C., Ivascu, C., Russo, V. E. A., Knight, C., Cove, D., Schaefer, D., Chakhparonian, M. & Zryd, J. P. 1999. A specific member of the Cab multigene family can be efficiently targeted and disrupted in the moss *Physcomitrella patens*. Molec. General Genetics 261: 92–99.

Ingram, J. & Bartels, D. 1996. The molecular basis of dehydration tolerance in plants. Ann. Rev. Plant Physiol. Plant Molec. Biol. 47: 377–403.

Krochko, J. E., Bewley, J. D. & Pacey, J. 1978 The effects of rapid and very slow speeds of drying on the ultrastructure and metabolism of the desiccation-sensitive moss *Cratoneuron filicinum*, J. Exp. Bot. 29: 905–917.

Leopold, A. C., Bruni, F. & Williams, R. J. 1992. Water in dry organisms. pp. 161-169. In: Somero, G. N., Osmond, C. B., & Bolis, C. L. (eds), Water and life. Comparative analysis of water relationships at the organismic, cellular and molecular levels. Springer-Verlag, Berlin.

McKersie, B. 1991. The role of oxygen free radicals in mediating freezing and desiccation stress in plants. Pp. 107–118. In: Pell, E. and Staffen, K. (eds), Active oxygen and oxidative stress and plant metabolism. Current Topics in Plant Physiology. American Society of Plant Physiologists Series, Vol. 8, Rockville, Maryland

Minich, W. B. & Ovchinnikov, L. P. 1992. Role of cytoplasmic mRNP proteins in translation. Biochimie 74: 477-483.

Moore, C. J., Luft, S. E. & Hallum, N. D. 1982. Fine structure and physiology of the desiccation-tolerant mosses, *Barbula torquata* and *Triquetrella papillata* (Mook. F. and Wils.) Broth., during desiccation and rehydration. Bot. Gazatte 143: 358–367.

Noailles, M. C. 1978. Etude ultrastructurale de la recuperation hydrique apres une periode de secheresse chez une *Hypnobryale*: *Pleurzium sherberi* (Willd.) Mitt.. Ann. Sci. Nature Bot. 19: 249–265.

Oliver, M. J. 1991. Influence of protoplasmic water loss on the control of protein synthesis in the desiccation-tolerant moss *Tortula ruralis*: Ramifications for a repair-based mechanism of desiccation-tolerance. J. Plant Physiol. 97: 1501–1511.

Oliver, M. J. 1996. Desiccation-tolerance in plant cells. A mini review. Physiol. Plant. 97: 779–787.

Oliver, M. J. & Bewley, J. D. 1984a. Desiccation and ultrastructure in bryophytes. Adv. Bryol. 2: 91–131.

Oliver, M. J. & Bewley, J. D. 1984b. Plant desiccation and protein synthesis: IV. RNA synthesis, stability, and recruitment of RNA into protein synthesis upon rehydration of the desiccation-tolerant moss *Tortula ruralis*. Plant Physiol. 74: 21–25.

Oliver, M. J. & Bewley, J. D. 1984c. Plant desiccation and protein synthesis: V. Stability of poly(A)- and poly(A)+ RNA during desiccation and their synthesis upon rehydration in the desiccation-tolerant moss *Tortula ruralis* and the intolerant moss *Crateneuron filicinum*. Plant Physiol. 74: 917–922.

Oliver M. J. & Bewley, J. D. 1984d. Plant desiccation and protein synthesis: VI. Changes in protein synthesis elicited by desiccation of the moss *Tortula ruralis* are effected at the translational level. Plant Physiol. 74: 923–927.

Oliver, M. J. & Bewley, J. D. 1997. Desiccation-tolerance of plant tissues: A mechanistic overview. Hortic. Rev. 18: 171–214.

Oliver, M. J., Mishler, B. & Quisenberry, J. E. 1993. Comparative measures of desiccation-tolerance in the *Tortula ruralis* complex. I. Variation in damage control and repair. American J. Bot. 80: 127–136.

Oliver, M. J. & Wood, A. J. 1997. Desiccation-tolerance in mosses. Pp. 1–26. In: Koval, T. M. (ed.), Stress induced processes in higher eukaryotic cells. Plenum, New York.

Oliver, M. J., Wood, A. J. & O'Mahony, P. 1997. How some plants recover from vegetative desiccation: A repair based strategy. Acta Physiol. Plant. 19: 419–425.

Platt-Aloia, K. A., Lord, E. M., Demason, D. A. & Thomson, W. W. 1986. Freeze-fracture observations on membranes of dry and hydrated pollen from Collomia, Phoenix and Zea. Planta 168: 291–298.

Platt, K. A., Oliver, M. J., & Thomson, W. W. 1994. Membranes and organelles of dehydrated *Selaginella* and *Tortula* retain their normal configuration and structural integrity: freeze fracture evidence. Protoplasma 178: 57–65.

Pramanik, S. K., Krochko, J. E., & Bewley, J. D. 1992. Distribution of cytosolic mRNAs between polysomal and ribonucleoprotein complex fractions in alfalfa embryos. Plant Physiol. 99: 1590–1596.

Reski, R. 1998. *Physcomitrella* and *Arabidopsis* – the David and Goliath of reverse genetics. Trends Plant Sci. 3: 209–210.

Reski, R. 1999. Molecular genetics of *Physcomitrella*. Planta 208: 301–309.

Sachs, M. & Ho, T. H. D. 1986. Alteration of gene expression during environmental stress. Annual Rev. Plant Physiol. 37: 363–376.

Schaefer D. & Zryd, J. P. 1997. Efficient gene targeting in the moss *Physcomitrella patens*. Plant J. 11: 1195–1206.

Schonbeck, M. W. & Bewley, J. D. 1981. Responses of the moss *Tortula ruralis* to desiccation treatments. II. Variations in desiccation tolerance. Can. J. Bot. 59: 2707–2712.

Scott, H. B. II, & Oliver, M. J. 1994. Accumulation and polysomal recruitment of transcripts in response to desiccation and rehydration of the moss *Tortula ruralis*. J. Exp. Bot. 45: 577–583.

Seel, W. E., Hendry, G. A. F. & Lee, J. E. 1992a. Effects of desiccation on some activated oxygen processing enzymes and anti-oxidants in mosses. J. Exp. Bot. 43: 1031–1037.

Seel, W. E., Hendry, G. A. F. & Lee, J. E. 1992b. The combined effects of desiccation and irradiance on mosses from xeric and hydric habitats. J. Exp. Bot. 43: 1023–1030.

Siebert, G., Loris, J., Zollner, B., Frenzel, B., & Zahn, R. K. 1976. The conservation of poly (A) containing RNA during the dormant state of the moss *Polytrichum commune*. Nucleic Acid Res. 3: 1997–2003.

Silverstein, E. 1973. Subribosomal ribonucleoprotein particles of developing wheat embryo. Biochemistry 12: 951–958.

Simon, E. W, 1978, Membranes in dry and imbibing seeds. Pp. 2-5–224. In: Crowe, J. H. & Clegg, J. S. (eds), Dry biological systems. Academic Press, New York.

Simon, E. W. & Mills, L. K. 1983. Imbibition, leakage, and membranes. Pp. 9–27. In: Nozzolillo, C., Lee, P. J. & Loewus, F. A. (eds), Mobilization of reserves in germination, Plenum Publishing Corp., New York.

Smirnoff, N. 1992. The carbohydrates of bryophytes in relation to desiccation-tolerance. J. Bryol. 17: 185–191.

Smirnoff, N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. Tansley Review No 52. New Phytologist 125: 27–58.

Spirin, A. S., Belitsina, N. V. & Ajtkhozhin, M. A. 1964. Messenger RNA in early embryogenesis. Zh. Obshch. Biol. 25: 321–338.

Stewart, G. R., & Lee, J. E. 1972. Desiccation-injury in mosses. II. The effect of moisture stress on enzyme levels. New Phytol. 71: 461–466.

Swanson, E. S., Anderson, N. H., Gellerman, J. L. & Schlenk, H. 1976. Ultrastructure and lipid composition of mosses. Bryologist 79: 339–349.

Thomson, W. W. & Platt-Aloia, K. A. 1982. Ultrastructure and membrane permeability in cowpea seeds. Plant Cell Environ. 5: 367–373.

Tucker, E. B., Costerton, J. W., & Bewley, J. D. 1975. The ultrastructure of the moss *Tortula ruralis* on recovery from desiccation. Canadian J. Bot. 53: 94–101.

Tucker, E. B. & Bewley, J. D. 1976. Plant desiccation and protein synthesis. III. Stability of cytoplasmic RNA during dehydration and its synthesis on rehydration of the moss *Tortula ruralis*. Plant Physiol. 57: 564–567.

Wood, A. J., Duff, R. J., & Oliver, M. J. 1999. Expressed Sequence Tags (ESTs) from desiccated *Tortula ruralis* identify a large number of novel plant genes. Plant Cell Physiol. 40: 361–368.

Wood, A. J. & Oliver, M. J. 1999. Translational control in plant stress: Formation of messenger ribonucleoprotein complexes (mRNPs) in *Tortula ruralis* in response to desiccation. Plant J. 18(4): 359–370.

Wood, A. J., Oliver, M. J. & Cove, D. J. 2000. Frontiers in bryological & lichenological research. I. Bryophytes as model systems. The Bryologist (in press).