ANNUAL REVIEWS

Annual Review of Plant Biology

Desiccation Tolerance: Avoiding Cellular Damage During Drying and Rehydration

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Annu. Rev. Plant Biol. 2020. 71:435–60

First published as a Review in Advance on February 10, 2020

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

https://doi.org/10.1146/annurev-arplant-071219-105542

Keywords

desiccation, desiccation tolerance, resurrection plants, cellular protection, senescence, mechanical stress, metabolic stress, reactive oxygen species

Abstract

Desiccation of plants is often lethal but is tolerated by the majority of seeds and by vegetative tissues of only a small number of land plants. Desiccation tolerance is an ancient trait, lost from vegetative tissues following the appearance of tracheids but reappearing in several lineages when selection pressures favored its evolution. Cells of all desiccation-tolerant plants and seeds must possess a core set of mechanisms to protect them from desiccation- and rehydration-induced damage. This review explores how desiccation generates cell damage and how tolerant cells assuage the complex array of mechanical, structural, metabolic, and chemical stresses and survive.

Likewise, the stress of rehydration requires appropriate mitigating cellular responses. We also explore what comparative genomics, both structural and responsive, have added to our understanding of cellular protection mechanisms induced by desiccation, and how vegetative desiccation tolerance circumvents destructive, stress-induced cell senescence.

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INTRODUCTION

Desiccation tolerance is an ancient and almost ubiquitous trait within land plants. In most, it is limited to their spores or seeds, but in a relatively small number of species, it is present in all vegetative tissues. Within the 383,671 known vascular plant species (80), only about 330 (0.086%) are known to possess vegetative desiccation tolerance (104). The number of nonvascular plants that exhibit this trait is unknown because only a few of the estimated 20,240 species have been assessed. Wood (138) reported that 210 (approximately 1%) bryophytes (158 mosses, 51 liverworts, and one hornwort) are desiccation tolerant, which is likely a significant underestimate given the plasticity of this trait in bryophytes (see below). Vascular plants exhibiting vegetative desiccation tolerance are often termed resurrection plants because they seemingly revive from death when rewatered; this term is rarely used for nonvascular desiccation-tolerant plants.

Desiccation tolerance appeared very early in the evolution of terrestrial life. It is a common feature of cyanobacteria (102), lichens (algal symbionts) (72), and green algae (59) and was likely present in the ancestor to all land plants (97). Vegetative desiccation tolerance in the nonvascular clades was lost as land plants evolved vascular systems (i.e., vascular plants evolved from a nontolerant vascular ancestor) (97, 103). This trait reappeared in the lycophytes, ferns, and at least 13 angiosperm lineages (44) (**Figure 1**). Desiccation tolerance per se was retained throughout land plant evolution, expressed in spores and later in seeds; it is hypothesized that vegetative desiccation tolerance in vascular plants evolved from a reprogramming of the genetic networks controlling desiccation tolerance in their propagules (28, 97, 131). Delineation between the ancestral mechanism(s) of vegetative desiccation tolerance in nonvascular plants and the derived mechanism(s) of vegetative desiccation tolerance in vascular plants, along with differences in their ability to survive rapid desiccation, has led to the designation of modified desiccation tolerance for vascular plant species (96).

Bewley (15, p. 196) defined desiccation tolerance as the ability to "revive from the air-dry state (the air being of low relative humidity)," or more precisely, the ability to revive from equilibration

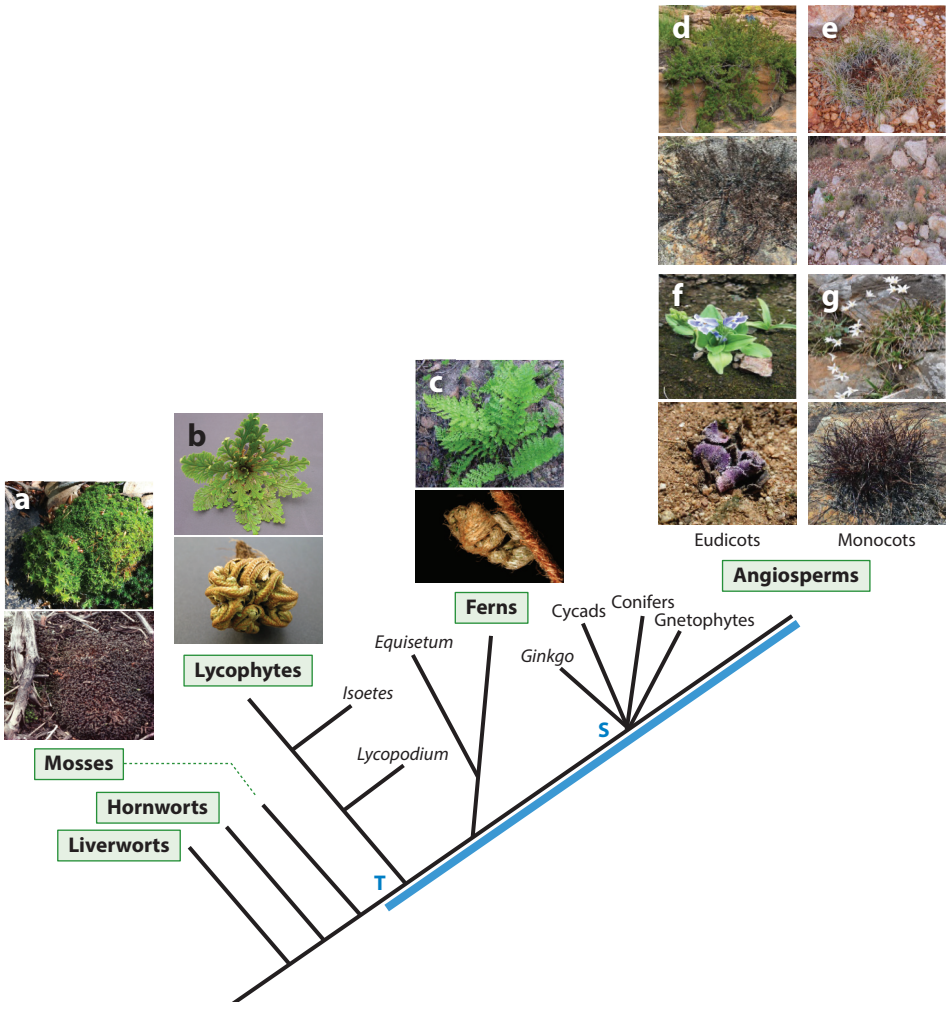

Figure 1

Desiccation-tolerant species along the ancestral lineage. Full turgor (*top*) and dried (*bottom*) plant pairs are shown. (*a*) *Syntrichia ruralis*, (*b*) *Selaginella lepidophylla*, (*c*) *Anemia caffrorum*, (*d*) *Myrothamnus flabellifolia*, (*e*) *Eragrostis nindensis*, (*f*) *Craterostigma pumilum*, and (*g*) *Xerophyta schlechteri*. The blue line indicates loss of vegetative desiccation tolerance in the ancestral lineage from the beginning of the evolution of the Tracheophyta (**T**) and relocation to orthodox seeds (**S**). Photographs in panels *a* and *b* provided by Brent Mishler and Abou Yobi, respectively. All other images are from the personal collection of Jill M. Farrant.

of the plant (tissue or cell) water potential with the predominantly low water potential of the air. Desiccation involves severe to complete water loss and should not be confused with milder water loss (dehydration) that leads to a water deficit-stressed condition. The latter occurs during drought, when the water potential of a plant never decreases to that of the surrounding air. Nor is desiccation tolerance the same as drought tolerance, which is the ability to accommodate suboptimal water availability and primarily involves mechanisms to avoid dehydration (including alterations to the life cycle to prevent growth during times of low water availability) or that allow plants to tolerate limited dehydration (125). It is unclear if vegetative desiccation- and

drought-tolerant plants employ similar mechanisms during their early response to water loss, in conjunction with mechanisms that the former employ to ultimately survive desiccation (see below).

Megapascal (MPa): a unit of pressure equivalent to 10^6 pascals; in this context, it is a measure of the potential energy of water

Relative water content (RWC): expressed as a percentage; RWC = (fresh weight − dry weight)/(full turgor weight − dry weight) × 100

Plants are classified as exhibiting vegetative desiccation tolerance if they can survive drying to a leaf water potential of approximately −100 megapascals (MPa) (i.e., equilibration with air of 50% relative humidity at 20°C) (5). This also equates to a water content of 0.1 g H_2O/g dry weight (dwt) or 10% water content on a wet-weight basis, as used in seed testing (123). This was chosen as a standard (5) in part because at a water content of 0.1 g H_2O/g dwt, there is insufficient water to form a monolayer around macromolecules and membranes; thus, enzymatic activity ceases, and metabolism is suspended (20). This works well for the classification of vascular plants as sensitive or tolerant to vegetative desiccation; the minimum leaf water potential measured for any desiccation-sensitive vascular species is −12.1 MPa for the desert bush *Larrea divaricata* (31). It also works reasonably well for desiccation-tolerant seeds because all survive drying to 0.1 g H_2O/g dwt or less, although some are classified as intermediate in tolerance because they can survive drying to below 0.2 g H_2O/g dwt but not to 0.1 g H_2O/g dwt (16).

The −100 MPa threshold standard for vegetative desiccation tolerance is less applicable to nonvascular desiccation-tolerant plants, particularly bryophytes (primarily mosses), in which this trait exhibits extensive plasticity (119). Proctor & Pence (104) state that the majority of bryophytes survive (at least for a limited time) equilibration to only −20 to −40 MPa, but the extent of survivability or damage experienced by bryophytes depends on the environment in which they grow and how rapidly or slowly they undergo drying. Desiccation tolerance can be acquired by desiccation-sensitive species of bryophytes or lost from tolerant species under certain environmental conditions; hence, desiccation tolerance may be physiologically inducible in some species and constitutive in others (119). Vegetative desiccation tolerance in bryophytes typifies a norm of reaction in which the acquisition of desiccation tolerance is defined by external and internal environmental parameters (122). Vegetative desiccation tolerance in vascular plants is primarily an inducible trait and requires a relatively slow dehydration rate for it to become established (49, 146). Nevertheless, in vegetative desiccation-tolerant lycophytes and ferns, there is a significant constitutive component to tolerance (141), as there is in the tolerance exhibited by the resurrection angiosperms *Craterostigma plantagineum* (4) and *Boea hygrometrica* (148). Resurrection plants can also be hardened or dehardened and can exhibit stress memory (79); thus, vegetative desiccation tolerance in, and loss from, tolerant plants exhibits plasticity. In orthodox (desiccation-tolerant) and recalcitrant (desiccation-intolerant) seeds, damaging cellular changes are related to the amount of time spent at a particular threshold water potential (134), adding a time-dependent parameter to tolerance.

Interest in vegetative desiccation tolerance has heightened recently with increasing concerns over crop productivity in the face of global climate change (44, 57, 85, 146). Thus, there is a current focus on comparative genomics to identify common components among desiccation-tolerant plants (28, 131, 139, 140) as well as sister-contrast comparisons (closely related species differing in desiccation tolerance) (95, 99, 130, 142). Comparative efforts have included attempts to identify common, and perhaps critical, stages during drying to determine key physiological or metabolic changes, resulting in the proposal that there are separate phases (time lines) during dehydration leading to the desiccated state (21, 146). However, identifying these phases depends on how dehydration is monitored and requires an understanding of the water relations of each species (especially when comparing drying rates), which is difficult and rarely achieved. Most studies use relative water content (RWC) to measure water loss and for species comparisons. However, RWC has significant limitations for comparisons across species or tissues because similar values may be unrelated to their tissue water potential (123). Walters & Koster (134) point out that measuring water loss by both water content (g H_2O/g dwt) and water potential is preferred when assessing expression of tolerance.

Bewley (15) suggested that to be desiccation tolerant a plant must (*a*) limit damage during desiccation, (*b*) maintain physiological integrity in the dry state, and (*c*) repair damage upon rehydration, in particular to regain integrity of membranes and membrane-bound organelles. These likely universal features, cellular protection and cellular recovery, are the focus of this review.

DAMAGING EFFECTS OF WATER LOSS FROM PLANT CELLS: THE NEED FOR PROTECTION

As cells lose water, they undergo mechanical and structural stresses associated with decreases in volume and increases in cytoplasmic compaction and viscosity. Cells lose turgor as they dehydrate to approximately −2 MPa (78), which is close to the limits (−1 to −2.5 MPa) of osmoregulation to prevent water loss (67) (**Figure 2**). Vegetative cells decrease in volume by 60–80% as they reach −4.5 to −6 MPa, which imposes a physical strain on the cell wall and plasma membrane as well as areas of attachment between them (133). The strain is particularly acute where the plasma membrane passes through the cell wall at the plasmodesmata; continuity of the cytosol between adjacent cells is essential (17). To accommodate the stresses involved in cell shrinkage, the cell wall and the plasma membrane fold, facilitating maintenance of the membrane and cell wall surface area, which is critical for cells to survive rehydration without rupture (133, 134).

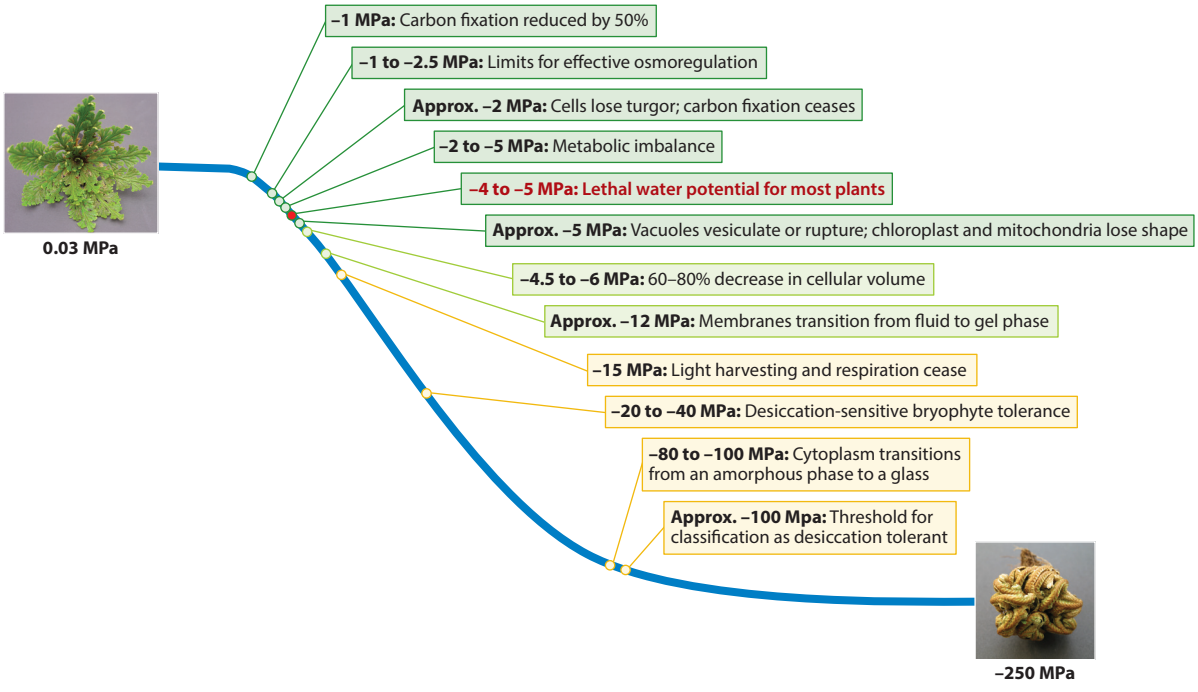

Figure 2

As water is lost from a plant cell and water potential declines, mechanical, structural, and metabolic stresses are encountered and consequences are borne. The amount of time spent at a particular water potential increases the potential for damage, and unless the cell has mechanisms to mitigate or tolerate the damage, it will succumb and die. The blue curve is the drying curve for a plant cell undergoing desiccation, and the dots are the approximate water potentials at which responses occur. The green to yellow shading of the individual text boxes indicates the approximate color of the leaves for resurrection species that are poikilochlorophyllous; homoiochlorophyllous resurrection species remain green.

Reactive oxygen species (ROS): a collective term for oxygen free radical or singlet oxygen ($^1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^\bullet$)

As water potentials are reduced to about −5 MPa in vegetative tissues, vacuoles must vesiculate to avoid rupture (134). Mitochondria and chloroplasts lose their shape and internal organization when water potentials reach −5 MPa (17); in bryophytes, they become spherical, and internal structures are dismantled (94). As cells shrink, their cytoskeleton is disrupted, and cell contents are brought into juxtaposition, resulting in novel interactions. Membrane lipid bilayers converge, and both lipids and proteins segregate into different domains; the fatty acid domains in the bilayers become more rigid (134), resulting in phase changes within the membrane and a transition from a fluid to gel phase (at about −12 MPa). This is reversible, but when regions of the bilayer become protein free, irreversible damage occurs (58). As water potentials decrease, membrane fusion and loss of cellular compartmentalization can increase, resulting in cell death.

Desiccating cells experience increases in solute concentration and changes in molecular proximity and mobility (134), and as turgor declines, the flow of carbon and nitrogen within metabolic pathways is disrupted (74). While some changes may help cells tolerate or reduce the rate of water loss, others likely have adverse consequences. While carbon fixation is negligible even at −2 MPa (75), light-harvesting reactions of photosynthesis occur at much lower water contents, and respiration can still occur in several tolerant species (and in seeds) at potentials approaching −15 MPa (135) (**Figure 2**). The continuation of respiration and light-harvesting reactions as cells desiccate leads to an accumulation of high-energy intermediates that give rise to reactive oxygen species (ROS). These interact with all cellular constituents and, in high enough concentrations, cause permanent loss of metabolic function, membrane failure, and cell death (52). Metabolic imbalances, apparently derived from differential responses to water deficits within pathways, generate cellular damage, particularly during long-term exposure to intermediate water potentials (−2 to −5 MPa), resulting in decreased seed viability (135) (**Figure 2**). Metabolites brought together during cell shrinkage are more susceptible to modification, giving rise to either unusable compounds or potentially toxic ones. Metabolite damage can occur chemically [e.g., by the Maillard reaction between the now-adjacent amino acids and reducing sugars (133)] or as enzymes are modified and become less specific during diminishing cell water content (54). Increases in production of ROS and hydrogen peroxide are also associated with rehydration in some bryophytes and lichens (82, 136).

The potential for cellular damage due to desiccation and rehydration is clearly high, so how do desiccation-tolerant plants and tissues protect their cells from these perturbations and survive? Or, more succinctly: How to dry, and not to die, that is the question.

CELLULAR PROTECTION

Desiccation-tolerant vegetative tissues reduce mechanical stresses by minimizing changes to cell volume. This may be achieved by accumulating compatible solutes in vacuoles, increased vacuolation, and wall folding (38). Extreme wall folding may be accompanied by little or no increased vacuolation, or, in contrast, there may be retention of relatively inflexible walls, with much of the cytoplasm being occupied by vacuoles. In general, wall folding is more prevalent and constitutive in less complex plants. In monocotyledonous desiccation-tolerant *Xerophyta* spp., cells have relatively inflexible walls and numerous vacuoles; in the Poales, bundle sheath cells display less wall folding and more vacuolation than mesophyll cells (37) (**Figure 3**).

The generation of smaller, more numerous vacuoles may arise by division of the original water-filled vacuoles or be formed de novo, some becoming autophagosomes, while others may become temporary storage organelles, containing by-products generated during desiccation. Vacuolar content appears to be species specific, but collectively, it contributes to mechanical stabilization, mitigates other stresses associated with desiccation, and facilitates recovery of

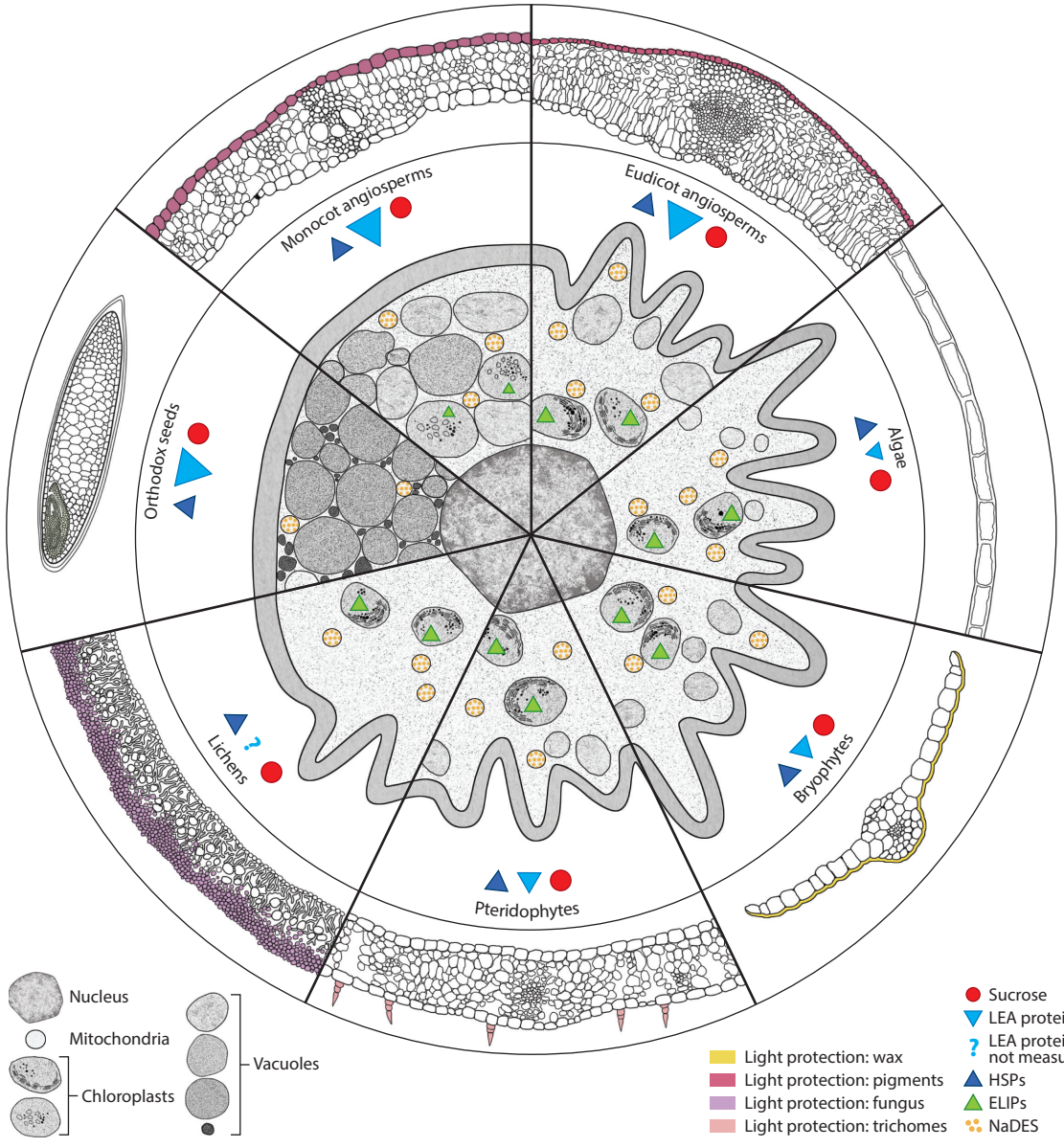

Figure 3

The evolution of cell ultrastructure and tissue anatomy of desiccation-tolerant plants. Symbols represent the relative quantitative presence of protective compounds involved in desiccation tolerance. Early light-inducible proteins (ELIPs) are confined to chloroplasts and natural deep eutectic solvent (NaDES) formation to mitochondria. Late embryogenesis abundant (LEA) proteins, heat shock proteins (HSPs), and sucrose are ubiquitous throughout the cell and are not represented. Likewise, antioxidants, amino and organic acids that occur in multiple locations, which can vary between desiccation-tolerant organisms, are not shown. Locations of several light-protective materials are shown.

Homoiochlorophylly: the ability of cells to retain their photosynthetic apparatus during desiccation

Poikilochlorophylly: the ability of cells to dismantle their photosynthetic apparatus during desiccation

Homoiochlorophyllous desiccation tolerance (HDT): a form of desiccation tolerance in which the photosynthetic apparatus is retained in the dried cell

metabolism upon rehydration. In *Myrothamnus flabellifolia*, high concentrations of the polyphenol antioxidant 3,4,5 tri-*O*-galloylquinic acid are present in vacuoles of dry cells and act as a redox buffer (88). In seeds, mechanical stabilization is predominantly achieved by accumulation of complex storage reserves within plastids, vacuoles, and the cytoplasm, and these reserves also serve as carbon and nitrogen sources for seedling establishment (16). It is likely that the vacuolar content of vegetative tissues similarly contributes carbon and nitrogen reserves for recovery.

Polysaccharide-rich cell walls and extracellular matrices were essential for survival of abiotic stresses by early land colonizers, serving to retain water and protect against rapid dehydration and high irradiance (60). Cell walls from late divergent charophycean green algae, thought to be ancestral to all land plants, contain polymers (cellulose, pectins, and hemicelluloses) and proteins [arabinogalactan proteins (AGPs) and extensins] (35) with notable similarity to those in bryophytes, pteridophytes, and angiosperms (87, 115). Wall flexibility is enabled by the nature and relative quantities of pectin (particularly homogalacturonans), hemicelluloses, AGPs, extensins, and, in eudicots, glycine-rich proteins. Their precise roles in wall folding in response to desiccation are reviewed in Shivaraj et al. (115). While degree of flexibility can be related to species-specific modifications in several of these wall components, the involvement of AGPs as proposed wall plasticizers is a common feature (87). Cell walls of desiccation-tolerant monocots that display little wall folding during dehydration (**Figure 3**) are enriched in arabinoxylans relative to their desiccation-sensitive relatives (101), suggesting that hydroxyproline-rich glycoproteins play an essential role in vegetative desiccation tolerance.

Photosynthesis is particularly prone to ROS formation. In the presence of light, loss of water progressively increases the risk of chlorophyll overexcitation, which in turn results in the formation of singlet oxygen ($^1O_2$). Desiccation-tolerant plants have evolved two contrasting strategies to maintain homeostasis between the generation and quenching of ROS during dehydration and early rehydration: homoiochlorophylly and poikilochlorophylly. The former is typified by retention of chlorophyll and maintenance and protection of the photosynthetic apparatus, with some repair required on rehydration. In the latter, chlorophyll is degraded and thylakoids are dismantled during desiccation and regenerated during early rehydration.

Homoiochlorophyllous desiccation tolerance (HDT) is an evolutionarily ancient strategy, being present in desiccation-tolerant plants in nonvascular clades, eudicots, and most C_4 monocots. These display several mechanisms to minimize light–chlorophyll interactions during desiccation, simultaneously utilizing antioxidant and protein-associated protection, which are retained until full photosynthetic competence is regained following rehydration.

The photosynthetic area exposed to light is minimized by tissue folding (37, 41). Light attenuation is further achieved by light-screening molecules, such as astaxanthin in terrestrial green algae (18), iron-complexed phenolics in the alga *Zygogonium ericetorum* (2), and anthocyanins (24, 37). In lichens, generation of air-filled spaces within the cortex during desiccation induces changes in optical properties, increasing the efficiency of light screening (118). Furthermore, light-reflecting trichomes [e.g., in the ferns *Ceterach officinarum* (41) and *Anemia caffrorum* (previously classified as *Mohria caffrorum*) (40)] and waxes [e.g., in the shrub *M. flabellifolia* (86)] on surfaces reflect light and prevent harmful bleaching of leaves and stems in the desiccated state (**Figure 3**).

HDT species maintain photochemical efficiency of photosystem II (PSII) and carbon gain (at reduced levels) at relatively low water contents (approximately 40% RWC), below which noncyclic electron flow through PSII/PSI (photophosphorylation) ceases and cyclic flow utilizing only PSI is initiated, greatly reducing ATP production. In nontracheophytes, in which desiccation is rapid, PSII activity ceases at extremely low water contents (e.g., 41, 72, 97), whereas in angiosperms, response to light by PSII is zero at about 40% RWC, which correlates with stomatal closure (149). In angiosperms, there is cessation of PSII activity during desiccation, linked to the detachment of

light-harvesting complex II from the reaction center (50, 149). PSI complexes remain intact but are fewer in number, the primary quinone receptor becomes more reduced, and the number of b6f complexes declines in the electron transport chain between PSII and PSI (149). This enables continued production of limited amounts of ATP, which is proposed to fuel specific, energy-dependent reactions necessary for cell survival during the late stages of desiccation, >30% RWC, a stress that is fatal to nontolerant species. Decoupling of PSII from PSI during desiccation occurs in the lichens (19) and the alga *Klebsormidium flaccidum* (61), and it may be common to most HDT species.

Excess energy absorbed by chlorophylls is dissipated via the xanthophyll cycle carotenoids in all plants (90). Zeaxanthin in particular accumulates in drying tissues of most HDT species (41), and in addition to nonphotochemical quenching, it is a powerful antioxidant (56). It has been implicated in the preservation of thylakoid structure (55) and in reorganization of light-harvesting complex II (66) on rehydration. Zeaxanthin and α-tocopherol, whose functions partly overlap (56), and early light-inducible proteins (ELIPs) combine to protect thylakoids and the photosystems during desiccation. Transient accumulation of chloroplast-localized stress proteins has been reported in *C. plantagineum* and postulated for other HDT species (23). While conserved chemical and enzyme antioxidants have been variously implicated in photosynthetic ROS protection (23), species-specific polyphenol antioxidants may also mitigate against such stress (37, 88, 113). Notably, Georgieva et al. (51) observed the accumulation of polyphenol-like substance(s) in the thylakoid lumen of dehydrating leaves of *Haberlea rhodopensis*.

Because the photosynthetic apparatus is maintained during drying, recovery of its activity occurs rapidly on rehydration, on the order of minutes in nontracheophytes (e.g., 41, 72, 97) and 12–48 h in tracheophytes (51). Many HDT species grow in environments with low light intensities, and time in the desiccated state is relatively short: Whatever damage occurs is limited and is easily repaired on rehydration. Some are fully exposed to high light, such as lichens, in which photoprotection by the fungal biont may minimize damage (118), and desert bryophytes, in which prolonged desiccation results in delayed recovery while repair and, in extreme cases, thylakoid regeneration are effected (145). Among the angiosperms, *Oropetium thomaeum* (13), *Sporobolus stapfianus* (42), and *Tripogon loliiformis* (68) inhabit high-light environments, and amelioration is achieved with some chlorophyll degradation, their C_4 mode of metabolism, and antioxidant protection. An exception is *M. flabellifolia*, for which up to 6 months in the desiccated state is typical; its survival has been attributed to maintenance of sufficient glutathione (73) and 3,4,5 tri-*O*-galloylquinic acid (88).

Poikilochlorophyllous desiccation tolerance (PDT) is displayed in orthodox seeds (16) and occurs in vegetative tissues of most desiccation-tolerant Velloziaceae (14), in *Borya nitida* (43), and in *Eragrostis nindensis* (132). During development, most seeds contain photoheterotrophic plastids, the photosynthetic activity of which contributes oxygen and metabolic reassimilation of CO_2 released by reserve biosynthesis (111). Cessation of this activity at the onset of maturation drying is presumed to be a prerequisite for desiccation tolerance (16).

In vegetative tissues of PDT plants, photosynthetic activity ceases at relatively high water contents (approximately 55–60% RWC) and is accompanied by total loss of chlorophyll, partial loss of carotenoids, and dismantling of thylakoid membranes (14, 132). Proteome studies of *Xerophyta schlechteri* (previously classified as *Xerophyta viscosa*) leaves indicate a considerable decline in a number of photosynthetic proteins at RWCs below 65%, including four components of PSII that are no longer detectable in the desiccated state (64). Transcriptome studies of PDT species show many transcripts associated with photosynthesis are maintained in the desiccated plant and used during early rehydration for reassembly of thylakoids and chlorophyll (27, 63). The resynthesis of the degraded proteins upon rehydration is independent of transcription in *Xerophyta humilis* (32), suggesting stable storage of transcripts in the desiccated state, similar to that in seeds (16).

Early light-inducible proteins (ELIPs): first described in greening seedlings, these are 17–20-kD chloroplast proteins suggested to bind to chlorophylls

Poikilochlorophyllous desiccation tolerance (PDT): a form of desiccation tolerance in which the photosynthetic apparatus is retained in the dried cell

The predominant photoprotection mechanism employed during dehydration and recovery in PDT plants is the elevated presence of anthocyanins, proposed to reduce light absorption by chlorophyll during its breakdown (114). Other photoprotection mechanisms such as zeaxanthin and ELIPs are not used during initial drying, but their later accumulation likely protects against photooxidation during photosynthetic apparatus reassembly (14, 130). Similarly, late accumulation in *X. schlechteri* of a chloroplast-targeted type II peroxiredoxin, which reduces peroxides, substrates to the corresponding alcohol, and water may provide protection against ROS during recovery of photosynthesis (39). As in HDT species, ubiquitous antioxidants and species-specific polyphenols may counter photosynthetic ROS production (37).

Poikilochlorophylly is a robust mechanism to avoid photosynthetic ROS damage, and PDT plants may survive for prolonged periods in the desiccated state under natural environmental conditions. In *X. humilis*, after accelerated aging, viability is retained for a minimum of 10 months in the desiccated state (28). Seeds of some species with impermeable coats can remain viable for hundreds of years (16).

In mitochondrial respiratory electron transport chains, reduction of ground state oxygen ($^3O_2$) to produce water is accompanied by formation of superoxide, hydrogen peroxide, and hydroxyl radicals. In desiccation-tolerant organisms, respiration ceases at extremely low water contents and is among the first metabolic events to resume upon rehydration (e.g., 16, 72, 132), suggesting considerable mitochondrial protection. While difficult to prove in vitro, accumulation of citrate during shutdown of the tricarboxylic acid cycle, together with elevated sucrose, proline, or trehalose, could form a natural deep eutectic solvent, a third liquid phase (other than water or lipid) in which cellular components can be effectively concentrated and proteins protected from denaturation (26). If correct, this would account for the ongoing respiratory activity at water contents below approximately 30% RWC (approximately −3 MPa) when the biophysical properties of an aqueous matrix are proposed to be typical of a syrup (133).

METABOLIC ASPECTS OF PROTECTION AND RECOVERY

Plant metabolism responds rapidly to reductions in water potential. In desiccation-sensitive species, sugars accumulate early following stress imposition, followed quickly by antioxidants and later by increases in amino acids (e.g., proline and γ-amino butyrate) (36). The sugars and amino acids act as osmolytes to slow water loss and maintain cell turgor. This also occurs in desiccation-tolerant plants, although many (if not all) already maintain elevated amounts of amino acids and sugars, as well as antioxidants, in the hydrated state. Such metabolic readiness for desiccation has been reported for the resurrection lycophyte *Selaginella lepidophylla* (143), the resurrection fern *A. caffrorum* (40), the resurrection dicots *H. rhodopensis* (48) and *Barbacenia purpurea* (121), and the resurrection C_4 grass *S. stapfianus* (95). The assumption is that their metabolic readiness slows water loss from tissues, as described for *S. stapfianus* (95), and protects cells from the initial generation of ROS because carbon fixation is hindered during the decline in water potential.

As cells lose water, the primary threat is to the integrity of both their organellar and plasma membranes. Desiccation-tolerant cells protect them in several ways: through modifications to membrane composition, through employment of efficient antioxidation mechanisms, and by nonreducing-sugar-mediated stabilization.

Membrane composition changes have been linked to maintenance of their stability during water loss from desiccation-sensitive plants and to their dehydration associated with freezing stress (124). In these, increasing membrane fluidity is regarded as being important for stress tolerance (e.g., it is enhanced when there is an increased ratio of unsaturated to saturated fatty acids). However, observations on membrane lipid saturation in desiccation-tolerant plants and tissues are

equivocal. Loss of unsaturated fatty acids from the phospholipid fraction of the plasma membrane occurs in the moss *Syntrichia ruralis* (formerly *Tortula ruralis*) during slow drying (120) and likewise in the resurrection dicot *Ramonda serbica* (106); in both species, phospholipid unsaturation recovers following rehydration. In *H. rhodopensis* and *S. stapfianus*, the degree of membrane lipid unsaturation remains unchanged during desiccation and rehydration (89, 105), but unsaturation increases in the thylakoid membranes of *Boea hygroscopica*, a resurrection dicot (93), and in leaves of *X. humilis*, a resurrection monocot (128). Although increasing or maintaining double bonds in membrane lipids is beneficial in maintaining or increasing membrane fluidity, it also incurs some risk during desiccation because such bonds are susceptible to peroxidation, resulting in oxidative damage. The need for fatty-acid-unsaturation-mediated membrane remodeling during desiccation may be a risk-benefit trait for any particular species, tissue, or cellular compartment and may be dependent on the efficiency of endogenous antioxidation mechanisms.

Water loss from desiccation-tolerant plant cells is also associated with a reduction in membrane galactolipids—in particular, loss of monogalactosyldiacylglycerol (MGDG) from chloroplast membranes, often recorded as an increase in the digalactosyldiacylglycerol (DGDG) to MGDG ratio (46, 47, 105). MGDGs are cone-shaped molecules (with a small head group) and tend to form inverted hexagonal II structures that promote membrane fusion and hence decrease membrane stability; their loss is therefore beneficial. DGDGs and other galactolipids are more cylindrical and form lamellar bilayers that proffer greater membrane stability. Also, the ratio of phosphatidylinositol to phosphatidylethanolamine increases during desiccation in extraplastidial membranes of the desiccation-tolerant algal symbiont *Asterochloris erici* (46); phosphatidylethanolamine has a conical shape that promotes deleterious membrane fusion. Increases in phosphatidylinositol and phosphatidic acid also occur in extraplastidial membranes of leaf cells of the resurrection dicot *C. plantagineum* (47), indicating that removal of, or reduction in, fusion-promoting membrane lipids is common to cellular protection mechanisms in desiccation-tolerant cells.

Desiccation-induced lipid peroxidation of membranes is a major source of damage due to decreases in their fluidity as cells become more compacted (133). Peroxidation is caused by both ROS activity and lipid-degrading enzymes such as lipoxygenase (100). Activity of lipoxygenase decreases by 70% during desiccation of the moss *S. ruralis* (120), but it remains high in seeds during desiccation and storage and in leaves of desiccating *S. lepidophylla* and *S. stapfianus* (95, 100, 142). During desiccation, the number of transcripts for lipoxygenase declines in *C. plantagineum* (110). Thus, desiccation-tolerant cells appear to either reduce their lipoxygenase activity during desiccation or recruit other mechanisms to limit or repair the damage inflicted by oxidative enzyme activity. One mechanism to protect membranes from oxidative damage is to employ tocopherols, lipid-soluble antioxidants that prevent the proliferation of lipid peroxidation (90). During desiccation, tocopherols increase in membranes of seeds (6); in leaves of *S. stapfianus* (95), *B. hygrometrica* (148), and *H. rhodopensis* (89); and in the photobionts of lichens (72). Tocopherols have an alternative protective role in desiccating leaves of *S. stapfianus*, in which there is a significant increase in lysolipids, formed by the activity of phospholipases that target lipids damaged by peroxidation (11). These lysolipids alter the fluidity and permeability of lipid bilayers and, if present in sufficient concentrations, become toxic to cells. Tocopherols target these lipid degradation products and counteract their negative effects on the fluidity and function of membranes, and they protect them from further peroxidation (11).

The involvement of increases in cellular nonreducing sugars during desiccation and their metabolism in the acquisition of cell desiccation tolerance are well documented (147). Sucrose predominantly accumulates during water loss, although how this disaccharide is derived varies; oligosaccharides such as raffinose and stachyose also accumulate, as do sugar alcohols (147). This accumulation appears to be a vital cellular protection mechanism that enables establishment of

desiccation tolerance. While the initial increases in cellular sugar content are important for osmoregulation and likely help slow water loss from cells, with increasing water loss they become important for membrane protection. Sugars are thought to act in two ways. In one, they and other small molecules are inserted between the polar head groups of membrane lipids to maintain the required spacing that ensures the integrity of the bilayers in what is termed the water replacement model (30). Alternatively, or additionally, they act as osmotic spacers that prevent membrane fusion by resisting water loss from between membranes, and, as viscosity increases during desiccation, they provide mechanical resistance to further membrane compression (71). As cell water potentials drop toward −100 MPa, sugars contribute to the transition of the cytoplasm from an amorphous, gel-like matrix to a glassy state, a process known as vitrification, which is important in establishing cellular stability under extreme stress (22); this phenomenon occurs in all desiccation-tolerant cells (**Figure 3**). Cytoplasmic glasses are complex mixtures of nonreducing sugars, proteins, organic acids, amino acids, and salts and likely vary considerably in composition from species to species and from tissue to tissue. This variability in glass composition is reflected in the variability in seed longevity between species (22). Transition of the cytoplasm into a glassy state prevents further compression of cells as desiccation progresses, which in turn relieves mechanical stresses and reduces the likelihood of membrane fusion. Glass formation also reduces diffusion rates and greatly inhibits chemical reactivity within cells, thus reducing the opportunity for cellular damage.

Late embryogenesis abundant (LEA) proteins: first described in developing cotton seeds, they are a group of highly hydrophilic and intrinsically unstructured proteins

Small heat shock proteins (sHSPs): a family of small proteins that possess chaperone activity and accumulate during heat shock and other stresses

Increases in ROS production during desiccation impact not only membranes but also many other cellular components, including proteins and nucleic acids. Therefore, desiccation-tolerant cells mount extensive and effective antioxidant defense mechanisms to scavenge ROS and limit damage to a repairable level. Desiccation-tolerant cells employ both (*a*) low-molecular-weight compounds such as reduced glutathione, ascorbate, tocopherols, raffinose, carotenoids, and polyphenols and (*b*) enzymes including catalase, superoxide dismutase (SOD), and thiol peroxidases of the peroxiredoxin and glutathione peroxidase type. The efficacy of these antioxidant defense networks and their targets during desiccation and rehydration has been well documented in a wide range of desiccation-tolerant plants, seeds, and pollen (21, 58, 146). In general, desiccation induces an increase in chemical antioxidants (e.g., glutathione and ascorbate) within cells, as well as in antioxidant enzyme activities. Regulation of their synthesis is at the transcriptional and posttranscriptional levels (142). In several desiccation-tolerant plants, the thiol-redox regulatory pathway, centered on the glutathione pathway, is the predominant antioxidation pathway (72, 95, 113). Antioxidants are also required to combat ROS production during rehydration [e.g., there is retention of elevated concentrations of tocopherols and glutathione in *S. stapfianus* (142) and of phenolics in *R. serbica* (113)].

THE PROTECTIVE ROLE OF PROTEINS IN DESICCATION TOLERANCE

Desiccation-induced proteins may act directly as protectants during desiccation and rehydration or as enzymes that catalyze the synthesis of other protective molecules such as antioxidants. Those that have received the most attention are the late embryogenesis abundant (LEA) proteins, the ELIPs, the small heat shock proteins (sHSPs), and antioxidative enzymes.

LEA proteins are a large group of hydrophilic proteins that protect cellular structure and function. Initially associated with the late stages of seed development, LEA proteins also promote desiccation tolerance in vegetative tissues (49). They function as molecular chaperones by forming a water hydration shell around molecules, a phenomenon made possible by their structure, which is highly hydrophilic, is intrinsically disordered, and becomes more structured upon dehydration (12). This aids water retention, preserving the three-dimensional structure of enzymes

and other cellular components, thus preventing denaturation (12). Lipid bilayer membranes are also protected; for example, pea mitochondrial LEA proteins interact with the negatively charged phosphate groups of dry membrane phospholipids to maintain their fluid-crystalline state, thus increasing their stability (126, 127). This protective role is not universal among LEA proteins, however, because certain homologs interact with membranes but do not increase stability (62). The interactions of LEA proteins with sugars produce a more stable cytoplasmic glass, providing further structural support during desiccation (116). LEA proteins have also been implicated in the protection of chromatin structure during desiccation and are thought to play a role in DNA repair and chromatin remodeling (12).

sHSPs are a large family of molecular chaperones, but unlike LEA proteins, which protect molecules by maintaining their hydration state, sHSPs generally stabilize and refold partially or completely unfolded proteins (76). These proteins are characterized by a greater exposure of their hydrophobic amino acids that are recognized by molecular chaperones, which then bind and prevent undesirable intermolecular interactions (108). sHSPs are highly expressed in desiccated and rehydrated tissues of resurrection plants and are assumed to prevent protein aggregation and denaturation (48).

ELIPs were initially recognized as proteins transiently expressed during greening of etiolated seedlings and ones expressed in green tissues in response to abiotic stresses (144). They were one of the first identified desiccation-induced proteins, in the resurrection plant *C. plantagineum* (3). ELIP transcripts accumulate during desiccation of many resurrection species (48, 129, 142, 144). Historically, ELIPs have been proposed to function as photoprotectants by binding to chlorophylls and stabilizing anthocyanins (1). ELIPs accumulate in the thylakoids of *C. plantagineum* during desiccation and colocalize with zeaxanthin (3), suggestive of their vital role in desiccation-tolerant HDT species.

As discussed in the previous section, ROS production during desiccation and rehydration can inflict severe damage on plant cells because ROS interactions with cellular components cause conformational changes and loss of function. Amino acids with aromatic rings and thiol groups are highly susceptible to oxidative damage; the resulting methionine sulfones, cysteine sulfinic or sulfonic acids, and carbonyl derivatives can cause irreversible loss of protein function and may result in the formation of toxic protein aggregates (29). Peroxidation of a single lipid molecule often induces a chain reaction, leading to membrane damage and leakage of cellular contents (84). Desiccation-tolerant plants use antioxidants to reduce damage due to ROS and to promote activity of cellular antioxidative enzymes (23, 34, 146). Major enzymatic antioxidant defenses include SOD, glutathione peroxidase, ascorbate peroxidase, catalases, glutathione reductase, and glutathione-*S*-transferase (65). Increased antioxidant enzyme synthesis typically occurs in response to dehydration, although which enzyme is synthesized and the extent of synthesis and activity vary with species (34, 114). In *Craterostigma wilmsii*, for instance, SOD activity increases during dehydration and rehydration, while activity peaks only during rehydration of *X. schlechteri* (114).

The rehydration proteomes of the desiccation-tolerant moss *Bryum argenteum* are characterized by a rapid accumulation of proteins associated with translation and protein synthesis, ROS scavenging, accumulation of LEA proteins and HSPs, recovery of photosynthesis, and the release of messenger RNAs masked in messenger ribonucleoprotein particles formed during desiccation (45).

GENOMIC ASPECTS OF CELLULAR PROTECTION AND RECOVERY

The concept that cellular protection and recovery are universal features of desiccation tolerance suggests that genetic changes affecting the ability of a plant to modify and improve these traits

were evolutionarily adaptive. Thus, they are expected to be conserved and traceable in the land plant phylogeny. As genomes and transcriptomes for desiccation-tolerant plants become available, the possibility of identifying genes that are vital for cellular protection against, and recovery from, desiccation is enhanced. Researchers have attempted to search for a blueprint of desiccation tolerance in the genome of a modest number of relevant, vegetative desiccation-tolerant species. Several approaches have been taken, predominantly comparative genomics of tolerant versus sensitive species [e.g., of sister groups and descendants of most recent common ancestors (7)]. Before addressing this research, the following must be noted. First, most desiccation-sensitive plants bear tolerant seeds, which implies that the genomic information for desiccation tolerance is present in these species but is expressed only in their seeds (and, in some species, pollen also). Thus, expression studies (e.g., transcriptomics) are pivotal to complement the genomic data. Second, during desiccation, sensitive species start to die below an approximate RWC of 55%, whereas tolerant species do not. Consequently, below this RWC, living tissues are being compared with dead tissues, which is obviously erroneous. But until such RWCs are reached, it is likely that gene expression is mainly related to a plant's inherent response to drought that is displayed in both tolerant and sensitive species.

Most omics studies of desiccation tolerance have focused on the important common responses related to cellular protection. These are present already in some of the earliest tolerant organisms, including algae, particularly the streptophyte green algae such as the widely studied *Klebsormidium* spp. (60), and in the bryophyte *Physcomitrella patens* (107) and the lycophyte *Selaginella tamariscina* (140). Arguably, during the ancestral transition to a terrestrial environment, these early land plants first adapted to moderately moist habitats near water, followed by a gradual transition to dry lands (97). These habitats were likely characterized by highly variable environments, and common genetic components useful for adaptation to several abiotic stresses are to be expected.

Genomic comparisons of vegetative desiccation-tolerant plants show that there is little commonality in the number of conserved orthologous genes (**Table 1**) or other genomic similarities such as ploidy, repetitive DNA, or percentage GC content (7, 57). This may be because of the small number of desiccation-tolerant plant genomes available, as well as a lack of knowledge of the genomic signature of the seed desiccation tolerance present in most sensitive species. At present, there does not appear to be an easily identifiable genomic blueprint for desiccation tolerance.

A genomic comparison of the highly syntenic and colinear vegetative desiccation-tolerant grasses *E. nindensis* and *O. thomaeum* and the desiccation-sensitive grass *Eragrostis tef* allowed a distinction to be drawn between the generic drought response displayed by all three species and the desiccation response of the two tolerant species (99). The majority of the genes expressed in their seeds exhibited similar expression patterns to those in the leaves of both desiccation-tolerant and desiccation-sensitive species during dehydration, including those purported to be involved in cellular protection. The overlap between the drought and desiccation responses led to a more nuanced explanation for the involvement of seed-specific genes in vegetative desiccation tolerance (99). However, seed maturation drying entails a gradual decrease in water content, including the range relevant to the drought response; thus, a common drought response of seeds and vegetative tissues seems likely.

Several comparisons of the genomes of desiccation-tolerant plants have focused on specific gene families related to cellular protection, of which the *ELIP* and LEA families have been studied in detail (8, 129). *ELIP* gene families are present in all land plants but are significantly greater in number in desiccation-tolerant plants (**Table 1**). The lowest number (9 families) occurs in the PDT species *X. schlechteri*, in which transcription occurs after chlorophyll and thylakoid loss and is likely related to protection during regeneration of the photosynthetic apparatus (129). By contrast,

Table 1 GC% and number of ELIP and LEA genes in the sequenced genomes of plants from various clades with desiccation-related phenotypes

Species	Clade	Phenotype	GC%	ELIP genes	LEA genes	Reference
Arabidopsis thaliana	Eudicots	DS	36	2	81	8
Boea hygrometrica	Eudicots	DT (homoiochlorophyllous)	42.3	17	67	139
Eragrostis nindensis	Monocots	DT (poikilochlorophyllous)	46.0	27	84	99
Eragrostis tef	Monocots	DS	45.2	5	51	99
Lindernia brevidens	Eudicots	DT (homoiochlorophyllous)	39.2	26	77	129
Lindernia subracemosa	Eudicots	DS	39	4	82	129
Oropetium thomaeum	Monocots	DT (homoiochlorophyllous)	45.3	17	102	131
Physcomitrella patens	Bryophyta	DT (homoiochlorophyllous)	45.9	17	50	107
Selaginella moellendorffii	Lycopodiophyta	DS	44	2	36	140
Selaginella tamariscina	Lycopodiophyta	DT (homoiochlorophyllous)	37.4	74	40	140
Syntrichia caninervis	Bryophyta	DT (homoiochlorophyllous)	41.3	35	56	A.T. Silva, B. Gao, & M.J. Oliver, unpublished data
Xerophyta schlechteri	Monocots	DT (poikilochlorophyllous)	36.5	9	126	27

Abbreviations: DS, desiccation sensitive; DT, desiccation tolerant; ELIP, early light-inducible protein; GC%, percentage GC content; LEA, late embryogenesis abundant.

HDT phenotypes, which retain the photosynthetic apparatus during desiccation, likely require considerable ELIP protection throughout dehydration and recovery of full metabolic competence.

The increase in the number of *LEA* gene families in desiccation-tolerant species is complex because LEA proteins are associated with tolerance of multiple abiotic stresses. A clear example of the involvement of *LEA* genes, and hence their encoded proteins in desiccation tolerance, has been provided by a comparison of the genomes of the anhydrobiotic (desiccation-tolerant) midge *Polypedilum vanderplanki* and the congeneric, desiccation-sensitive *Polypedilum nubifer* (53). The *P. vanderplanki* genome includes 27 *LEA* genes, which are all expressed during dehydration, but there are none in the *P. nubifer* genome.

An analysis of 60 genomes, from green algae to angiosperms, revealed that the number of *LEA* genes across species is highly variable, ranging from 1 in the freshwater alga *Chlamydomonas reinhardtii* to 180 in the tree *Malus domestica* (8). The presence of genes for ancient LEA proteins in algal genomes suggests that the evolution of preexisting *LEA* families (including for LEA 5 and seed maturation proteins [SMPs]) and the formation of new *LEA* gene families have facilitated the colonization of land (8). The later expansion and diversification of *LEA* families in embryophytes are likely concurrent with the evolution of more specialized cells, tissues, and organs (e.g., in seeds capable of withstanding severe water loss).

Abscisic acid (ABA): a plant hormone

ABA-responsive element: a gene promoter element that directs transcription factor binding initiated by ABA for regulation of transcription

Gene expression studies have recently been summarized for desiccation-tolerant angiosperms (57) and seeds (33). There are fewer such studies on desiccation-tolerant bryophytes (45) and green algae (59). Comparisons of transcriptomes in tolerant species during de- and rehydration have revealed a common set of genes and their orthologs that are associated with cellular protection and recovery (e.g., LEA proteins, dehydrins, HSPs, ELIPs, and enzymes involved in carbohydrate and antioxidative metabolism).

The occurrence of 27 *ELIP* genes in the genome of poikilochlorophyllous *E. nindensis* (**Table 1**) is unexpected because this number is very similar to that in HDT species. ELIP transcripts increase in abundance in *E. nindensis* early during dehydration, with 23 of the 27 transcripts accumulating during dehydration and being maintained at elevated levels during rehydration (99). It is suggested that protection of the photosynthetic apparatus is aided by ELIPs during the slow recovery of photosynthesis upon rehydration (99). This touches on the hardly investigated time lapse between transcription and translation. Are *ELIP* genes expressed during dehydration in preparation for quick translation upon rehydration? Expression of some 15 *LEA* genes in developing seeds of *Medicago truncatula* precedes their translation by 10–20 days. This delay points to posttranscriptional regulation of LEA protein abundance (77). Interestingly, this abundance coincides with the acquisition of seed longevity, which occurs well after the establishment of desiccation tolerance. It has been argued that gene expression in seeds is in anticipation of the next developmental phase, when translation into active proteins occurs (117).

Like leaves, germinating seeds respond to several abiotic stresses by induction of ELIP expression (109). This suggests that ELIPs are not specific to dehydration stress but, in leaves, are necessary to avoid photooxidative damage during both disassembly and reassembly of the photosynthetic apparatus.

The increased transcript abundance of LEA proteins and ELIPs during seed dehydration and in species with desiccation-tolerant vegetative tissues is, at least partly, in anticipation of subsequent rehydration. In bryophytes (97) and seeds (112), transcripts are sequestered, or protected, in messenger ribonucleoprotein particles so as to be immediately available upon rehydration.

The most highly abundant transcripts in dry seeds are likely remnants from seed maturation, during which dormancy and desiccation tolerance are induced. About 500 of the most abundant genes in dry *Arabidopsis* seeds are enriched in abscisic acid (ABA)-responsive elements within 1 kb upstream of their translational start site (92). ABA is a key regulator of dormancy and desiccation tolerance, and abundance of the ABA-responsive element–containing transcripts decreases as ABA concentration decreases during early rehydration (imbibition) (112). It is unknown if desiccation-tolerant plants use similar posttranscriptional and translational control mechanisms.

Analyses of (genome-wide) gene expression in desiccation-tolerant vegetative tissues have confirmed increased transcript abundance of supposedly seed-specific genes and proteins (57). For example, the sequenced genome of *X. schlechteri* has been used to compare the transcriptomes related to desiccation tolerance of the mature plant with those of ABA-induced desiccation tolerance of desiccation-sensitive young seedlings (27). Extensive coexpression analysis revealed clusters of genes that are considered to be seed specific, including most members of the ABA-dependent ABSCISIC ACID INSENSITIVE 3 (ABI3) regulon (83). The plant-specific B3 domain–containing transcription factor ABI3 controls a network of 98 target genes committed to seed development and maturation, during which desiccation tolerance and dormancy are acquired.

Like ABI3, the ABA-dependent bZIP transcription factor ABI5 is strongly associated with seed maturation, during which it regulates transcription of *LEA* genes and the acquisition of longevity (150). ABI5 may regulate expression of the *LEA_4* family. The ABI5 motif is prominent in the promoter regions of this gene family that, consequently, may be an important factor in the cellular protection and longevity of desiccated *X. schlechteri* plants (27).

The acquisition of desiccation tolerance has long been associated with ABA and its downstream signaling pathway that controls gene expression. ABA is also a vital regulator of seed maturation (acquisition of desiccation tolerance and dormancy), germination, and stomatal closure. Arguably, a common denominator of these ABA targets is the suppression of metabolic activity, thus providing protection from desiccation-related damage. For example, ABA accumulation during stress in the unicellular red alga *Cyanidioschyzon merolae* inhibits G1/S transition in the cell cycle (70); this may be an ancient mechanism by which ABA suppresses growth. In the moss *P. patens*, ABA-induced desiccation tolerance is mediated by *ABI3*, and mutation of this gene results in the loss of this tolerance (69). In *Arabidopsis* seeds, the *abi3* mutant, but not the *abi3–6* allele, is desiccation tolerant (98).

SUPPRESSION OF SENESCENCE AND CELL DEATH BY DESICCATION-TOLERANT PLANTS

Drought induces senescence in desiccation-sensitive plants as part of a survival strategy that reduces transpiration and recycles nutrients to the youngest leaves, fruits, and reproductive structures (25, 91). Drought can also cause imbalances in energy metabolism that prematurely induce senescence (9, 23). In contrast, desiccation-tolerant plants can suppress stress-induced senescence, particularly in younger tissues (e.g., leaves of desiccation-tolerant plants *S. stapfianus*, *T. loliiformis*, and *X. schlechteri* do not senesce, and they recover completely from drying). In contrast, older regions of more mature leaves (or, in *S. stapfianus*, the whole leaf) do not recover, resulting in tip burning due to cell death (81). This difference in ability to recover between the younger and older regions suggests that age plays a role in dictating a cell's fate upon desiccation. The failure to recover is due either to an inability of older cells to respond adequately to drying or to their less efficient maintenance and repair systems.

Desiccation-tolerant plants avoid perturbations that induce senescence. They are transcriptionally and metabolically primed for severe water loss even when in the hydrated state (79, 95). For example, *S. stapfianus* accumulates significantly more osmolytes and nitrogen metabolites and lower concentrations of metabolites associated with energy metabolism than its desiccation-sensitive sister species *S. pyramidalis* (95). Therefore, there is no requirement for the induction of senescence pathways to preserve water and nutrients, and rather than requiring the activation of such pathways to redistribute resources during drying, desiccation-tolerant plants can focus their limited energy resources toward cell protection and recovery.

Desiccation-tolerant plants use at least two strategies to remobilize nitrogen and offset senescence more efficiently than do sensitive plants: (*a*) degeneration of chlorophyll and (*b*) autophagy. Degradation of the photosynthetic machinery provides PDT plants with a significant nitrogen source, thus reducing their need for induction of senescence. HDT plants must use alternative strategies for remobilizing nitrogen. One source is the preexisting cell proteome, and when energy levels are low, nitrogen can be released therefrom by autophagy. This is a genetically controlled homeostatic process that degrades and recycles either in bulk or selectively from cytoplasmic material present in vesicles termed autophagosomes. In *T. loliiformis*, the nonreducing sugar trehalose triggers autophagy via induction of sucrose nonfermenting-related kinase 1 (SnRK1) as the plant desiccates (137). Energy status plays a role in the regulation of senescence pathways. In *T. loliiformis*, there is a distinct difference in the autophagy response between desiccating shoots and roots, because the latter maintain a high energy status throughout dehydration by acting as a sink and suppress senescence without activating autophagic pathways (10). The induction of autophagy to suppress senescence is consistent with what occurs in *T. loliiformis* and *B. hygrometrica* (9, 148). More details on the complexities of autophagy in cellular protection and recovery are presented in **Figure 4**.

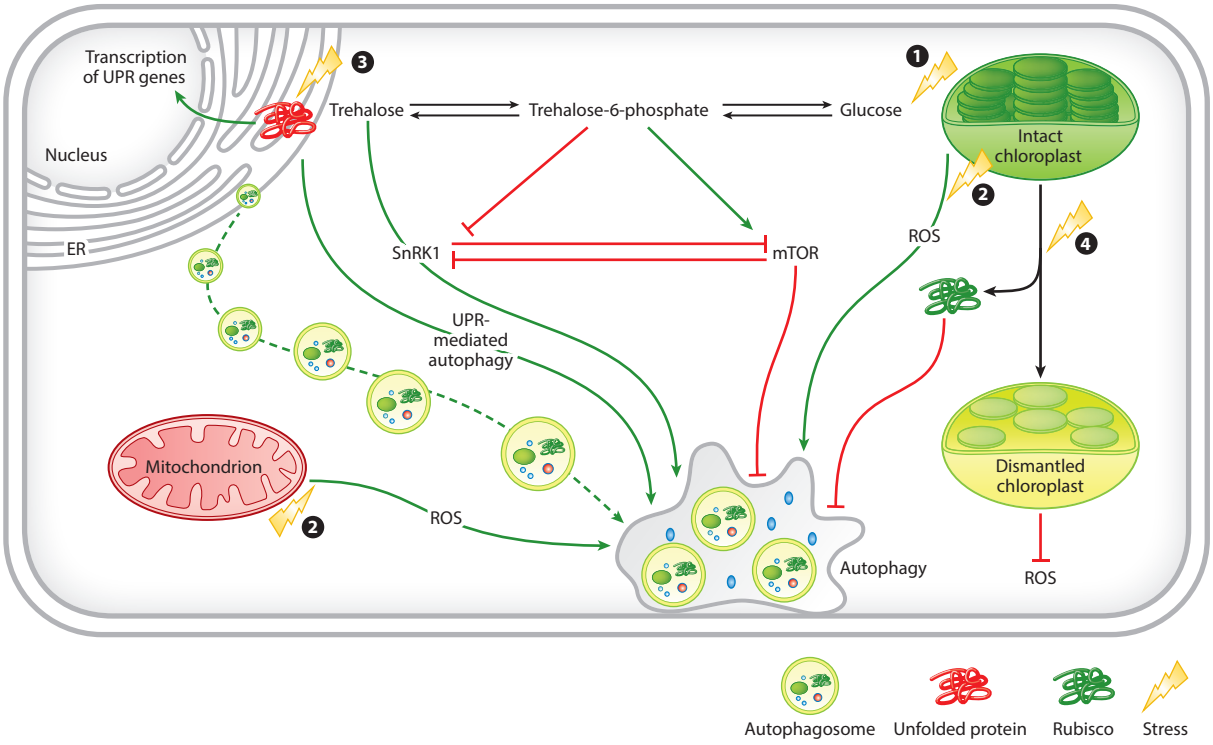

Figure 4

Cellular protection during, and recovery from, desiccation. Reduced photosynthesis during desiccation causes several stresses: ❶ lower glucose production, ❷ increases in reactive oxygen species (ROS) production in chloroplasts and mitochondria, ❸ endoplasmic reticulum (ER) perturbations and the accumulation of unfolded proteins, and ❹ dismantling of chloroplasts. ❶ Cells detect energy status via accumulation of trehalose-6-phosphate (T-6-P). Lowered glucose synthesis during drying results in high trehalose and low T-6-P, indicating an energy deficit. This activates sucrose nonfermenting-related kinase 1 (SnRK1) and catabolic pathways including autophagy, which cleanse the cell of damaged proteins, protecting it from apoptotic cell death. Conversely, increased energy status upon rehydration increases T-6-P, suppresses SnRK1, and induces activity of modified target of rapamycin (mTOR), promoting anabolic pathways. ❷ Water deficit results in overexcitation of chlorophyll and singlet oxygen ($^1O_2$) production. Additionally, inefficient electron transport in stressed mitochondria results in ROS production. These cause damage by membrane peroxidation (not shown) and protein damage, leading to induction of autophagy. ❸ The ER is extremely stress sensitive; unfolded protein accumulation therein leads to programmed cell death. In response, cells induce a cytoprotective program, the unfolded protein response (UPR). This reduces cellular stress via several pathways, in sequential order: (*a*) retrosignaling to the nucleus to increase foldase and chaperone transcription, (*b*) activation of the 26S proteasome to degrade proteins (not shown), and (*c*) autophagic pathways, if the above are insufficient, to move unfolded proteins from the ER into autophagic vesicles. Poikilochlorophyllous desiccation-tolerant (PDT) plants dismantle chloroplasts and release chlorophyll to lower photosynthesis and ROS production. This also releases a large nitrogen store (Rubisco), suppressing autophagy. Homoiochlorophyllous desiccation-tolerant (HDT) plants protect and repair most of their chlorophyll during desiccation and rehydration. Rubisco remains intact; other constituents of the proteome are degraded during desiccation, with autophagy activated to remove damaged cellular components.

The complexity of desiccation tolerance is evident from the narrative presented here, but progress is being made in understanding the physiological and molecular mechanisms that limit cellular damage during desiccation and in elucidating the essential repair mechanisms on rehydration. However, much remains to be learned, and to what extent these cellular changes are common to the diverse species and propagules that are desiccation tolerant offers fruitful territory for future research. With progressive changes to our environment resulting from global climate change, there will be increasing pressures on food security, in particular due to the increased aridity of

many regions of our planet. Therefore, it is becoming vitally important to understand more about how plants survive severe water deficits and to apply this knowledge to generate more tolerant and robust crops by conventional breeding and the application of emerging biotechnologies.

SUMMARY POINTS

1. During drying and rehydration of desiccation-tolerant plants and seeds, a complex array of structural, metabolic, chemical, mechanical, and molecular changes occur to prevent potentially lethal cellular damage.
2. Disruption of photosynthesis and respiration during desiccation and rehydration generates reactive oxygen species (ROS) that are the target of several protection mechanisms in tolerant cells.
3. During desiccation, some tolerant (resurrection) species degrade chlorophyll and disassemble chloroplasts, thereby avoiding the production of ROS (poikilochlorophylly). Species that retain chloroplasts (homoiochlorophylly) minimize light-induced reactions.
4. Metabolic responses to desiccation provide protection from both mechanical and chemical stresses (e.g., production of sugars that play an important role in cytoplasmic glass formation).
5. Specific proteins such as late embryogenesis abundant (LEA) proteins and heat shock proteins (HSPs) play an important role in cellular protection during desiccation, stabilizing cellular constituents and enhancing the properties of the cytoplasmic glass.
6. No obvious genomic blueprint has been identified in species that are desiccation tolerant, although there is strong commonality in the expression of several protein types associated with cellular protection and recovery.
7. Genes and proteins considered to be seed specific are also expressed in vegetative tissues of desiccation-tolerant plants upon dehydration.
8. Energy imbalance due to stress can trigger senescence, but cells of desiccation-tolerant plants suppress its onset by remobilizing nitrogen to balance their energy status.

DISCLOSURE STATEMENT

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture (USDA). USDA is an equal opportunity provider and employer. The authors are not aware of any other affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors wish to thank Keren Cooper of the University of Cape Town Department of Molecular and Cell Biology for the graphical development of **Figure 3**, Brent Mishler for the *S. ruralis* images, Abou Yobi for the *S. lepidophylla* images, and Marilou Oliver for her assistance in collating the bibliography. M.J.O. acknowledges partial support by the National Science Foundation through Collaborative Research: Dimensions grant number 1638972 and grant number IOS-1444448 (as

Co-PI; PI: Robert Sharp). J.M.F. acknowledges the Department of Science and Technology and the National Research Foundation of South Africa for the provision of her Research Chair in Plant Desiccation Tolerance for Food Security (grant number 98406).

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