Plant Stress Physiology

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Desiccation Tolerance

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Abstract
Desiccation tolerance is the ability to survive loss of 90% of cellular water or dehydration to tissue water concentrations of ≤0.1 g H_2O g^{-1} dry mass. It is relatively common in reproductive structures such as seeds (termed orthodox), but is rare in vegetative tissues, occurring in some 350 species of higher plants (termed 'resurrection plants'). In this chapter we present an overview of the stresses associated with desiccation and review the current mechanisms proposed to explain how orthodox seeds and resurrection plants tolerate such water loss. Physiological, biochemical and molecular processes involved in protection from mechanical stress, oxidative damage and metabolic disruptions are discussed and similarities between seeds and resurrection plants are drawn. Protective mechanisms unique to vegetative tissues are presented and differences among species are discussed. We propose that the developmentally regulated programme of acquisition of desiccation tolerance in seeds is utilized in the acquisition of tolerance in vegetative tissues of resurrection plants, possibly in response to environmentally regulated rather than developmental cues.

11.1 Introduction
The now commonly held definition of desiccation tolerance is the ability of an organ or organism to survive loss of more than 90% of its cellular water (corresponding to a tissue water concentration of or below 0.1 g H_2O g^{-1} dry mass (DM) and a water potential of ≤-100 MPa) for extended periods and to recover full metabolic competence upon rehydration (Vertucci and Farrant 1995; Walters et al., 2002; Berjak et al., 2007; Farrant 2007). It differs from what is often termed 'drought tolerance', which is essentially the ability to survive periods of limited water availability (and thus lower water potentials of ≤-1 MPa) either by maintenance of high internal water concentrations (technically drought resistant) or tolerance of the loss of a small amount of water (up to 20%) for short periods only (Iljin, 1957; Walters et al., 2002; Moore et al., 2009). The distinction between drought and desiccation tolerance lies in the nature and extent of protection mechanisms implemented by the plant. In the former these include mechanisms that enable sequestration of water and/or brief periods of survival in the face of some loss of water. In the latter these include mechanisms that enable organisms to survive without water, i.e. anhydrobiosis.

In the plant kingdom desiccation tolerance is relatively common in reproductive tissues such as spores, seeds and pollen (Berjak et al., 2007; Leprince and Buitink 2007) and in vegetative tissues of non-tracheophytes, such as bryophytes and lichens (Kappen and Valadares, 1999;
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Oliver et al., 2000; Beckett and Minibayeva, 2007; Proctor et al., 2007). However, desiccation tolerance in vegetative tissue is rare in pteridophytes and angiosperms and non-existent in extant gymnosperms (Gaff, 1989; Alpert and Oliver, 2002; Farrant, 2007). The mechanisms of vegetative desiccation tolerance differ between the lower and higher orders. In the former, desiccation occurs very rapidly and protection prior to drying is minimal and constitutive. Survival is thought to be based largely on rehydration-induced repair processes (Oliver et al., 1998; Alpert and Oliver, 2002). In tracheophytes, while some repair is probably inevitable, considerable and complex protection mechanisms are laid down during drying (Gaff, 1989; Vicrè et al., 2004a; Farrant, 2007; Farrant et al., 2007; Oliver, 2007; Moore et al., 2009; Blomstedt et al., 2010). Because of the relative importance of angiosperms to our agricultural practices, considerable research has been conducted on mechanisms of desiccation tolerance in seeds (termed orthodox) and more recently on vegetative tissues of the few species (commonly called resurrection plants) that exhibit such characteristics. The ability of orthodox seeds to survive desiccation has been exploited by humans to store them for prolonged periods for economic and conservation purposes. Understanding of metabolic processes associated with desiccation tolerance is thus confounded by metabolism associated with development. Furthermore the timing of onset and loss of desiccation tolerance can differ among seed tissues, which are rarely studied separately. Experiments in which developmental aspects were separated from desiccation tolerance acquisition in seeds of the genomic model organism Medicago truncatula (by allowing germination to the point of radicle protrusion and re-establishing desiccation tolerance by subjecting them to a mild osmotic stress (ca. -1.5MPa)) have added considerable 'omic' data on what genes and proteins might be important in the attainment of desiccation tolerance (Buitink et al., 2003, 2006; Boudet et al., 2006).

Although overlapping metabolic processes are not a hindrance in the study of resurrection plants, other drawbacks have limited our attainment as yet of a clear and comprehensive understanding of desiccation tolerance. Due to constraints associated with collection of plants in and transport from remote areas, as well as the difficulty experienced in the propagation of the plants (by transplanting, tissue culture or seed), there are frequently limits to the number of biological replicates for research studies. In addition, insufficient attention is often paid to light intensities, day length and temperatures under which plants are subject to dehydration and rehydration events and time of day in which samples are taken (overlooking possibly the effect of circadian rhythms), making comparisons among species and even within a species difficult. Disparities in drying rate among plants and in similar tissues of the same plants (including old versus young leaves) can cause further difficulties in sampling, resulting in

11.2 Challenges Associated with the Study of Desiccation Tolerance

In seeds, desiccation tolerance is acquired during the mid to late stages of maturation, as reserve deposition occurs and prior to the onset of maturation drying and is lost during germination usually coincident with radical elongation. Understanding of metabolic processes associated with desiccation tolerance is thus confounded by metabolism associated with development. Furthermore the timing of onset and loss of desiccation tolerance can differ among seed tissues, which are rarely studied separately. Experiments in which developmental aspects were separated from desiccation tolerance acquisition in seeds of the genomic model organism Medicago truncatula (by allowing germination to the point of radicle protrusion and re-establishing desiccation tolerance by subjecting them to a mild osmotic stress (ca. -1.5MPa)) have added considerable 'omic' data on what genes and proteins might be important in the attainment of desiccation tolerance (Buitink et al., 2003, 2006; Boudet et al., 2006).

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large variations in water content and metabolic parameters tested. Furthermore, once water loss is initiated it can occur very rapidly (Farrant, 2007), usually over several hours, making sampling at regular water content intervals at the same time of day (to account for daily metabolic cycles) problematic. In some cases in order to obtain more even drying rates, researchers excise leaves from a hydrated plant and dry these. Such experiments, however, remove the effect of roots on establishment of desiccation tolerance in leaves. Indeed in some species, such as *Sporobolus stapfianus*, leaves do not acquire desiccation tolerance unless some drying has occurred while still attached to the roots (Gaff and Loveys, 1992; Whittaker et al., 2004). There is currently a paucity of information on the attainment of desiccation tolerance in roots and their contribution to tolerance of the whole plant due to difficulties associated with root studies. Sampling of roots compromises the plant for subsequent measurements in a drying course and adhering soil particles with associated microbes cannot be easily removed since washing of roots changes their water content.

It is important to be aware of the above limitations in data interpretation. That having been said, we do have a fair understanding of what enables desiccation tolerance in seeds and vegetative tissues of angiosperm resurrection plants. In this chapter, we have attempted to understand better the similarities in the desiccation response in seeds and vegetative tissues.

### 11.3 Stresses Associated with Water Loss

In understanding desiccation tolerance it is important to recognize the effect of loss of water on physical and metabolic processes. In the light of that we can assess how seeds and resurrection plants are able to prevent, slow down and/or repair the deleterious reactions induced by the removal of water. Figure 11.1 shows the sequence of stresses as well as the cellular and metabolic responses in seeds and plants during desiccation tolerance attainment associated with five hydration levels. These levels have been proposed as a tool to analyse the processes involved in desiccation tolerance, however boundaries are not completely distinct.

Since water has multiple and various roles in supporting life, it is not surprising that there are numerous stresses associated with its loss. It has a structural role providing mechanical stabilization at the cellular level by filling intracellular spaces resulting in turgor pressure (Iljin, 1957; Levitt, 1980). At the molecular level water provides hydrophobic and hydrophilic associations and controls intermolecular distances that determine the conformation of macromolecules and their partitioning within organelles. Tissues are fully hydrated at the start of hydration and loss of this water results in loss of turgor (Levitt, 1980). Further loss of water within level IV results in cell shrinkage and mechanical stress in which tension is placed on the plasmalemma as it shrinks from plasmodesmatal attachments to the cell wall. The ultimate rupture of the plasmalemma allows entry of extracellular hydrolases and cell death. In many species wall collapse occurs, which is equally lethal (Walters et al., 2002). As water deficit gets progressively worse, loss of the aqueous medium results in membrane appression, demixing and lipid bilayer transitions, protein degradation and destabilization of macromolecules and membrane structures (Vertucci and Farrant, 1995; Walters et al., 2002, 2005).

Water also plays a role in controlling metabolism as it is a reactant and product of many reactions. It provides the fluid matrix that allows diffusion of substances to reactive sites. Its loss profoundly affects the nature of biochemical reactions and thus metabolism. This happens progressively, with free radical production and unregulated metabolism being initiated, followed by anaerobic respiration, catabolic activity, alcohol emission and Maillard reactions, with free radical production via autooxidation and emission of carbonyls occurring at low water contents (Vertucci and Farrant, 1995; Walters et al., 2002, 2005). The properties of water also change with its progressive loss from
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Figure 11.1. Changes in hydration levels in seeds and resurrection plants with associated physical and metabolic damage reported to occur on water loss (modified from Vertucci and Farrant, 1995 and Berjak et al., 2007). Responses of seeds and plants to these stresses are shown, with those common to seeds and plants indicated by white arrows and those specific to resurrection plants by black arrows. The decline in water concentration (g H₂O g⁻¹ FW) as a function of relative water content (RWC) for the resurrection plants Craterostigma wilmsii (CW), Xerophyta humilis (XH) and Myrothamnus flabellifolia (MF) are shown. The trend in changes in water concentrations are similar to those proposed for seeds (Berjak et al., 2007).

Seed tissues (Vertucci, 1990; Walters et al., 2002). Level V water behaves as it would in a dilute solution but the aqueous matrix becomes more viscous having the properties of a syrup (level IV), rubber (level III) and glasses (levels II and I) (Walters et al., 2002 and references therein). The stability of the latter may be critical to survival of desiccation
and the length of time an organism can remain in the desiccated state (Walters et al., 2005).

Figure 11.1 shows the various hydration levels that have been proposed for seed tissues (Vertucci and Farrant, 1995; Berjak et al., 2007) but there has been little research in plants on the properties of water at the various water contents/potentials. To date, papers mentioning these aspects in resurrection plants still refer to the information available for seeds. The changes in water concentration (g H₂O g⁻¹ DW) as a function of relative water content (RWC; water concentration relative to that at full turgor; the measure in which most studies on resurrection plants are reported) during drying of resurrection plants shows a similar trend to that proposed to occur in seeds (Berjak et al., 2007), although initial water loss is more rapid in plants, and it is likely that the properties of water in tissues of plants are similar to those of seeds.

11.4 Protection Against Mechanical Stress

11.4.1 Vacuole filling and water replacement

Although osmotic adjustment (to prevent departure of water from the cells/vacuoles) during the initial phase of water loss occurs in most plants (including those that are desiccation sensitive), this is insufficient to prevent the strain related to severe water loss and desiccation.

In seed tissues, progressive storage reserve accumulation within vacuoles and cytoplasm fills the cells with dry matter minimizing cell shrinkage and plasmalemma withdrawal (Figs 11.2a, b). For most orthodox seeds the timing of maximum dry matter accumulation and acquisition of maximum desiccation tolerance are coincident and, conversely, the loss of desiccation tolerance upon germination is associated with mobilization of these reserves and increased vacuolation within seedling tissues (reviewed in Vertucci and Farrant, 1995; Berjak et al., 2007; Leprince and Buitink, 2010). Re-establishment of desiccation tolerance in germinating radicles of M. truncatula resulted in the up-regulation of genes involved in sucrose and seed storage reserve production (Buitink et al., 2006), indicating the importance of such metabolism in desiccation tolerance, most likely in minimizing mechanical stress associated with severe water loss. Seeds that do not acquire desiccation tolerance during maturation or even post shedding are termed recalcitrant. Most of these do accumulate storage reserves in a similar manner to orthodox seeds, these being required as an energy source for germination, and this presumably can minimize mechanical stresses associated with desiccation. It has been noted that there is a range in degree of reserve accumulation in seeds of such species and that particularly the extent of vacuolation is correlated with the amount of water lost tolerated before viability is lost (Berjak et al., 1989; Farrant et al., 1989; Kermode, 1990; Vertucci and Farrant, 1995).

The mechanical stabilization of cells by means of reserve accumulation in seeds shares similarities to the strategy of angiosperm resurrection plants, where water is replaced in cells with compatible solutes (Fig. 11.2i, c–e). Replacement of water in vacuoles within dry tissues of resurrection plants was first suggested based on ultrastructural observations that vacuoles continued to take up a large proportion of the cytoplasmic space despite there being no bulk water available in tissues, the remaining water being purely structurally associated (Farrant, 2000; van der Willigen et al., 2001; Farrant et al., 2007; Moore et al., 2007a, b). Given the accumulation of proteins and solutes observed in numerous studies in resurrection plant tissues, it seemed likely that these vacuoles were being filled with non-aqueous media. The content of vacuoles from desiccated leaves of Eragrostis nindensis was analysed after non-aqueous extraction and was shown to contain proline, sucrose and protein in equal proportions (van der Willigen et al., 2004). Vacuoles from both hydrated and dry leaves of Myrothamnus flabellifolia contain 3, 4, 5 tri-O-galloylquinic
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The up-regulation of genes and seed storage reserve metabolism in desiccation-tolerant seeds likely minimizes stress associated with severe drought. Most orthodox seeds, these storage reserves in a form that can be used as an energy source for germination. It is believed that these reserves can minimize the stress associated with desiccation in seeds, indicating that such metabolism in desiccation-tolerant seeds is not acquired during maturation or even after dormancy.

The stabilization of cells by dehydration (Fig. 11.2i, f) continues to be no bulk water remaining in the plant tissues, as water is replaced by non-aqueous solutes (Fig. 11.2ii, f). Given that these solutes are believed to be cytotoxic in large quantities (Fahy, 1986) and since these chemicals are believed to be accumulated in vacuoles within the dry leaves (Farrant et al., 2009).

11.4.2 Cell wall folding

For some angiosperm resurrection plants, mechanical stabilization is further enhanced by considerable and reversible wall folding (something that is unique to vegetative desiccation tolerance tissue) (Fig. 11.2i, f). The extent of wall folding varies among species however. In Craterostigma wilmsii, where this is almost exclusively used as a form of mechanical stabilization, the mechanism of folding appears to involve both structural and biochemical changes that are reversed on rehydration (Vicré et al., 1999, 2004). On drying there is a reduction in glucose and an increase in galactose substitutions to the xyloglucan chains and it has been proposed that cleavage, or partial cleavage of the long-chained xyloglucan units into shorter, more flexible ones, allows for wall folding. Further water loss results in an increase in wall-associated Ca$^{2+}$ and since this ion plays an important role in cross-linking wall polymers, such as acid pectins, it has been proposed that this serves to stabilize walls in the dry state and, more importantly, prevent mechanical stress of rehydration. Craterostigma wilmsii rehydrates rapidly due to its small size and initial movement of water is mainly apoplastic.
If walls hydrate and unfold before cell volume is regained, plasmalemma tearing and further subcellular damage could occur (reviewed in Viser et al., 2003, 2004a). In species such as *M. flabellifolia* and *E. nindensis*, in which some wall folding occurs (vacuole filling being extensive and presumably the predominant means of mechanical stabilization), there appear to be no notable biochemical wall changes upon drying. The leaf cell walls of these plants have constitutively high proportions of arabinose, associated with pectins in the former (Moore et al., 2006) and xyloglucans in the latter (Plancot et al., 2003). Interestingly, the desiccation sensitive *Eragrostis tef*, while having similar chemical wall constituents to *E. nindensis*, has significantly lower levels of xyloglucan-associated arabinose. In the resurrection fern *M. caffrorum*, which displays seasonal desiccation tolerance, being desiccation tolerant in the dry but desiccation sensitive in the wet season (Farrant et al., 2009), the walls of the tolerant fronds have a higher proportion of arabinose polymers than the sensitive form (Reynolds, 2008). Since arabinose polymers are highly mobile, allow wall flexibility (Foster et al., 1996; Renard and Jarvis, 1999) and have a high water-absorbing capacity (Goldberg et al., 1989; Belton, 1997), which would be important for rehydration, we have proposed that such constitutively high levels allow constant preparedness for dehydration-rehydration in these resurrection plants (Moore et al., 2009).

### 11.5 Protection Against Metabolic Stress

#### 11.5.1 Reactive oxygen species formation and prevention of oxidative stress

Various metabolic stresses are also initiated with loss of water and at very low water contents become lethal for desiccation sensitive tissues. These include formation of reactive oxygen species (ROS), which form as a natural consequence of metabolic processes involving electron transport (Halliwell and Gutteridge, 1999; Apel and Hirt, 2004; Bailly, 2004). Thus mitochondria and chloroplasts are major sites of ROS production (Fig. 11.3). Photosynthesis, in particular, is very sensitive to water deficit. Electron leakage during photosynthetic electron transport and the formation of singlet oxygen are significantly increased when cells of photosynthetic tissues suffer water loss and this has frequently been cited as a primary cause of damage and resultant plant death in most species (Seel et al., 1992; Smirnoff, 1993; Kraner and Birtić, 2005).

While many seeds contain photoheterotrophic plastids, the photosynthetic activity of which contributes oxygen and metabolic re-assimilation of CO₂, released by reserve biosynthesis (Rolletschek et al., 2004, 2005; Ruuska et al., 2004), this activity is switched off at the onset of maturation drying (Fait et al., 2006) probably during the loss of type V and IV water. Whether this is the consequence of drying, or a prerequisite for desiccation tolerance (by minimizing the amount of photosynthetically produced ROS) is not clear. Angiosperm resurrection plants down-regulate photosynthesis at water contents of between 80 and 65% RWC depending on the species, minimizing photosynthetically-produced ROS (Sherwin and Farrant, 1998; Tuba et al., 1998; Farrant, 2000; van der Willigen et al., 2001; Farrant et al., 2003; Illing et al., 2005). Down-regulation of photosynthesis is achieved by one of two mechanisms: termed poikilochlorophyllous and homiochlorophyllous (Gaff, 1989; Smirnoff, 1993; Sherwin and Farrant, 1998; Tuba et al., 1998; Farrant, 2000).

Poikilochlorophyllous types, many of which are monocots, break down chlorophyll and dismantle thylakoid membranes during dehydration (Tuba et al., 1993a, b, 1998; Farrant, 2000). Breakdown of photosystem II (PS II), which is responsible for the water-splitting, oxygen evolving and thus oxidizing reactions of photosynthesis, is a highly effective strategy to minimize damaging levels of ROS formation and indeed it has been shown that poikilochlorophyllous
species are able to retain viability in the dry state for far longer than homoiochlorophyllous ones (Tuba et al., 1998; Proctor and Tuba, 2002). The drawback of this strategy is that re-assembly of the photosynthetic apparatus on rehydration requires coordinated transcription and de novo translation (Dace et al., 1998; Collett et al., 2003; Ingle et al., 2008) and poikilochlorophyllous plants require longer periods after rehydration to resume normal growth and development. Homoiochlorophyllous species retain most of their chlorophyll (the amount retained depending on the light levels under which the plants are dried) and thylakoid membranes remain intact in the dry state, but utilize various mechanisms to prevent ROS production from photo-activated chlorophyll uncoupled from metabolic dissipation mechanisms (Sherwin and Farrant, 1998; Farrant, 2000; Farrant et al., 2003, 2009). This is achieved by leaf folding and shading of inner leaves (e.g. the Craterostigma spp.) or adaxial surfaces (e.g. M. flabellifolia, M. caffrorum) from light. Surfaces that remain exposed to light may have reflective hairs and/or waxes and there is an accumulation of anthocyanin, xanthophylls, pigments and polyphenols all of which act as ‘sunscreens’ reflecting back photosynthetically active light, masking chlorophyll and acting as antioxidants (Smirnoff, 1993; Sherwin and Farrant, 1998; Farrant, 2000; Farrant et al., 2003, 2009; Georgieva et al., 2007, 2009; Moore et al., 2007a, b).
11.5.2 Antioxidant protection against reactive oxygen species

While these mechanisms minimize ROS production associated with photosynthesis, ROS production associated with other metabolic processes is exacerbated with acute water loss. This is initially accompanied by increased production and/or up-regulation of activities of what are termed ‘classical’ (Kranner and Birtić, 2005) or ‘housekeeping’ antioxidants (Illing et al., 2005), so called because they are present in all plants and are crucial to maintenance of cellular homeostasis under day-to-day conditions and in protection against a myriad of abiotic and biotic stresses (for an overview, see Elstner and Osswald, 1994). The difference between desiccation-tolerant and -sensitive tissues is that the former are able to maintain their antioxidant potential in the dry state such that these same antioxidants can be utilized during the early stages of rehydration thus protecting against the ROS associated with reconstitution of full metabolism. In desiccation-sensitive tissues such antioxidants are compromised by drying below critical water contents (Illing et al., 2005; Kranner et al., 2006; Farrant et al., 2007). Furthermore, desiccation-tolerant tissues utilize antioxidants not reported in desiccation-sensitive tissues. Such extensive protection is probably required to effectively cope with ROS production via Maillard and auto-oxidation reactions (Wettlaufer and Leopold, 1991; Bailly, 2004).

Classical/housekeeping antioxidants include the water-soluble glutathione (g-glutamyl-cysteinylglycine; GSH) and ascorbic acid (Asc) (Noctor and Foyer, 1998), the lipid soluble tocopherols and β-carotene (Munne-Bosch and Alegre, 2002) and enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (AP) and other peroxidases, mono- and dehydroascorbate reductases and glutathione reductase (GR) (Elstner and Osswald, 1994; Bailly, 2004; Kraner and Birtić, 2005). Typically, studies investigating their role in desiccation tolerance follow concentrations of particular antioxidant pools and/or activities of the antioxidant enzymes. Maintenance of antioxidant potential involves regeneration and/or de novo synthesis of antioxidants and activities of enzymes involved in these processes are also monitored. There is interaction between the various antioxidant systems (Foyer and Halliwell, 1976; Bailly, 2004; Kranner and Birtić, 2005) and thus the amount or activity of any one antioxidant can be influenced by others. Furthermore, the changes in activity of various antioxidants can fluctuate in space (between tissues and within sub-compartments within cells) and time, as water is lost and desiccation tolerance is attained. Due to this complexity, changes in antioxidant status should be monitored at regular intervals during drying in order to identify trends and fluctuations in concentrations and enzyme activities that might be important to the attainment of desiccation tolerance. Such rigorous studies are rarely undertaken and consequently from reports in the literature there appears to be considerable variation among desiccation-tolerant seeds and resurrection plants with respect to the nature and extent of up-regulation of the various housekeeping antioxidants, and the water contents during a dehydration/rehydration time course at which the observed changes occur (reviewed e.g. in Farrant, 2000; Farrant et al., 2003; Illing et al., 2005). However, what does appear to be consistent in desiccation-tolerant tissues is that the antioxidant enzymes AP, GR, CAT and SOD retain the ability (in vitro assays) to detoxify ROS even at RWC of <10%, suggesting that there is some protection of these proteins that prevents their denaturation and maintains the native state in dry conditions. This was not the case in desiccation-sensitive species and it has been proposed that it is this ability in desiccation-tolerant systems that is a unique mechanism of desiccation tolerance (Illing et al., 2005; Farrant, 2007). Kranner et al. (2006) have proposed that glutathione is key to survival of desiccation-tolerant systems and it has been suggested that this ability in desiccation-tolerant systems is a key to desiccation-tolerant systems. It was shown that the half-cell redox potential \( E_{\text{GSSG/GSH}} \) can be used as a marker for plant stress and values of \( \geq -160 \) mV correlate with loss of viability. Since ROS production continues in dry tissues
Many polyphenols are believed to have antioxidant properties, zinc metallothioneine, metallothioneine-like antioxidants, oxidoreductases and several members of the de novo antioxidase response to desiccation (Velasco et al., 1994; Blomstedt et al., 1998; Kirch et al., 2001; Chen et al., 2002; Mowla et al., 2002; Collett et al., 2004; Illing et al., 2005; Ingle et al., 2007; Mulako et al., 2008; Walford, 2008). Some of these are reported to occur in orthodox seeds (Aalen, 1999; Stacy et al., 1999; Boudet et al., 2006; Buitink et al., 2006; Mulako et al., 2008; Walford, 2008; Shin et al., 2009) and transcripts of 1-cys-peroxiredoxin, aldehyde dehydrogenase and aldehyde reductase were again accumulated during the re-induction of desiccation tolerance in M. truncatula seeds (Buitink et al., 2006; Leprince and Buitink, 2010). These do not appear to be up-regulated in the vegetative tissues of desiccation-sensitive plants (Aalen, 1999; Mulako et al., 2008) and we believe they play an important role in attainment of desiccation tolerance. In resurrection plants their production is clearly desiccation induced. Transcription of 1-cys-peroxiredoxin and metallothioneine-like antioxidants occurs between 60 and 50% RWC and translation at water contents below this, and both transcripts and proteins disappear rapidly during dehydration either prior to or upon reaching full turgor (Mowla et al., 2002; Collett et al., 2004; Mulako et al., 2008). We propose that such antioxidants serve to scavenge ROS and neutralize toxic metabolic intermediates that occur as a consequence of unregulated metabolism associated with severe water deficit.

Many polyphenols are believed to have antioxidant properties (Smirnoff, 1993; Wang et al., 1996; Kahkonen et al., 1999) and may well play a role in antioxidant protection in resurrection plants. In seeds there have been few studies reported on the role of polyphenols as antioxidants, possibly because they have been bred out of species that are most studied. In a comprehensive study on polyphenols in M. flabellifolia it has been demonstrated that dry leaves contain high levels (up to 50% of the leaf dry weight) of 3,4,5-tri-O-galloylquinic acid, which acts as a potent antioxidant (Moore et al., 2003; Smirnoff, 1993; Moore et al., 2003).
et al., 2005). Although this polyphenol is predominantly located in the vacuole it has been proposed to act as an antioxidant reservoir linked to the cytoplasmic antioxidants and functioning as a redox buffer (Moore et al., 2007b). A survey conducted on seven other resurrection plants showed that total polyphenol content was lower than in M. flabellifolia, and varied among the species (Farrant et al., 2007). However, the relative antioxidant potential as determined by FRAP (ferric reducing/antioxidant power) and DPPH (2,2-diphenyl-1-picrylhydrazyl) antioxidant activity assays was higher in the resurrection plants than in related desiccation-sensitive species. In Ramonda serbica, polyphenols and the activity of polyphenol oxidase increases during desiccation (Sgherri et al., 2004; Veljovic-Jovanovic et al., 2008). A proteome study of Boea hygrometrica showed that proteins relating to phenolics metabolism are up-regulated on drying in leaves of this species (Jiang et al., 2007). The contribution of such antioxidants to desiccation tolerance in resurrection plants might be small but necessary, in combination with other antioxidants, to best facilitate protection of photosynthetic vegetative tissues from ROS stress associated with desiccation.

11.5.3 Water replacement and glass formation

Progressive loss of water (level IV and below) results in metabolic stress related to cytoplasmic crowding. The cytoplasm becomes increasingly viscous, proteins begin to denature and membrane fusion occurs (Vertucci and Farrant, 1995). It has been proposed that desiccation-tolerant organisms counteract cytoplasmic crowding by replacing water with compatible solutes capable of substituting for the hydrogen bonds lost due to dehydration (Fig. 11.4). This water replacement hypothesis presupposes that these molecules are able to stabilize macromolecules in their native configuration during desiccation (Crowe et al., 1986, 1989). Additional stabilization of the subcellular milieu is believed to be achieved via cytosolic vitrification (Vertucci and Farrant, 1995; Hoekstra et al., 2001; Walters et al., 2002). Solutes believed responsible for replacement and stabilization include: (i) sucrose and oligosaccharides (reviewed in Berjak et al., 2007; Farrant, 2007); and (ii) proteins, particularly late embryogenesis abundant (LEA) proteins (reviewed in Hoekstra et al., 2001; Illing et al., 2005; Mtwisha et al., 2006; Berjak et al., 2007) and small heat shock proteins (Almoguera and Jordano, 1992; Alamillo et al., 1995; Wehmeyer et al., 1996; Mtwisha et al., 2006).

11.5.4 Sucrose and oligosaccharides

The accumulation of non-reducing sugars and certain oligosaccharides in both seed development (reviewed in Vertucci and Farrant, 1995; Berjak et al., 2007) and during dehydration of resurrection plant tissues (Illing et al., 2005; reviewed in Farrant, 2007) has been correlated with the acquisition of desiccation tolerance.

Sucrose increases in response to desiccation in all angiosperm resurrection plants studied to date, with a large proportion accumulating after the cessation of photosynthesis, below a leaf RWC of 60% (Farrant, 2007). It has therefore been proposed that this results from an alteration in carbon partitioning and not as a product of carbon assimilation (Illing et al., 2007). The source of carbon has been investigated in some resurrection species and has been proposed to partially originate from conversion of reserve carbohydrates, for example octulose (Norwood et al., 2000), and starch (Whittaker et al., 2007). Although to a lesser extent than sucrose, oligosaccharides, particularly raffinose and to some degree stachyose, also accumulate in resurrection plants during drying. However, due to the variation in the quantity accumulated, it is thought that oligosaccharides, together with other compatible solutes function interactively in protection against desiccation (reviewed in Farrant, 2007).
Seeds show a universal increase in sucrose during development and this has again been linked with desiccation tolerance. However, as mentioned previously, it is difficult to separate accumulation due to normal developmental metabolism from that associated with desiccation tolerance, particularly as it is required as a substrate for germination in all seeds, including desiccation-sensitive (recalcitrant) seeds (Berjak et al., 2007). Though it has been widely held that the primary source of sucrose in developing seeds is transport from the parent plant, recent studies on model systems that attempted to separate development from desiccation tolerance acquisition indicated that sucrose accumulation results from metabolic switches within the seed itself. Transcriptome and metabolite profiling studies have indicated that starch and lipids are the main reserves mobilized in *M. truncatula* (Buitink et al., 2006) and metabolite profiling in *Arabidopsis thaliana* suggest lipid is mobilized (Fait et al., 2006) for sucrose production. Bogdan and Zagdańska (2009) also recorded significant changes in enzyme activity involved in...
sucrose synthesis and hydrolysis in response to dehydration in wheat seedlings, confirming the involvement of sugar metabolism in the regulation of dehydration tolerance. Total sugar content and sucrose levels were found to be highly variable with no simple correlation to desiccation tolerance in a study on seed embryos in a variety of species, representing three seed storage categories: orthodox, intermediate and recalcitrant (Steadman et al., 1996). Similarly, raffinose contents show contradicting results (Still et al., 1994; Bochicchio et al., 1997; Black et al., 1999) and its role in glass formation (discussed below) is debated (Buitink et al., 2000). Instead, sugar composition in seeds, specifically the ratio of sucrose to raffinose, has been implicated as the critical aspect in the acquisition of desiccation tolerance (Steadman et al., 1996). This hypothesis has been corroborated by several studies. Figure 11.5 shows a summary of the data from these studies in addition to the ratios for hydrated and dry tissue in a variety of resurrection plants. In seeds, despite some variability, a value of sucrose-to-raffinose family oligosaccharide (RFO) was proposed in the order of 7:1 for several species of orthodox seeds. Recalcitrant seeds were recorded as having a 12:1 ratio (Horbowicz and Obendorf, 1994; Lin and Huang, 1994; Steadman et al., 1996). A decreased ratio in orthodox seeds compared to recalcitrant seeds can clearly be seen in Fig. 11.5. Although the clustering is not as evident for hydrated tissue of desiccation-tolerant plants, there is a definite trend towards a lower ratio in dry tissue. If the change in ratio for individual desiccation-tolerant plants is examined (Fig. 11.6) however, it is apparent that though sucrose, raffinose and stachyose increase, most show a decrease in sucrose-to-RFO from hydrated to dry tissue.

Despite the acknowledged importance in protection against desiccation tolerance and the evident benefit of the presence of a reserve carbon source for rehydration and germination, the exact role of sugars in desiccation tolerance has yet to be fully elucidated. We have already discussed the osmoprotectant and stabilization functions above. Allied to this is the hypothesis that sugars can facilitate the formation of a glass (vitrification), which acts to further stabilize the subcellular milieu (Vertucci and Farrant, 1995). Although sucrose is thought to be the main glass-forming component, raffinose has been suggested as important
in preventing sucrose crystallization. It has also been proposed that the formation of non-reducing sugars indirectly acts to remove monosaccharides, which are involved in the ROS-forming Maillard-type reactions (reviewed in Farrant, 2007). This is supported by observations of declining monosaccharide levels during maturation and drying in seeds (Vertucci and Farrant, 1995; Steadman et al., 1996) and resurrection plants, respectively (Farrant, 2007). More recently, studies have suggested additional roles for sugars. Sucrose and sucrosyl oligosaccharides (including raffinose family oligosaccharides (RFOs)) have been linked to the protection against oxidative stress, acting as antioxidants in a ROS-scavenging capacity (Van den Ende and Valluru, 2009). Also, by means of sugar-sensing, soluble sugars are thought to contribute to the regulation of gene expression involved in plant growth and metabolism. These sugars act as signals in pathways associated with stress tolerance to moderate cellular metabolism, growth and plant development responses under oxidative stresses (reviewed in Rosa et al., 2009).

### 11.5.5 Late embryogenesis abundant proteins

As the name suggests, late embryogenesis abundant (LEA) proteins were first identified due to their abundant (4% of total cellular protein, Roberts et al., 1993) accumulation during the late stages of seed development coincident with the onset of desiccation tolerance (Galau et al., 1986; Blackman et al., 1992, 1995; Baker et al., 1995; Russouw et al., 1995; Manfre et al., 2006 inter alia). They have been subsequently found in many bacteria (Garay-Arroyo et al., 2000; Cytryn et al., 2007), nematodes and tardigrades (Goyal et al., 2005; Tunncliffe and Wise, 2007) and are widely present in the plant kingdom, both
in seeds and vegetative tissues (reviewed inter alia in Cuming, 1999; Berjak et al., 2007; Moore et al., 2009; Leprince and Buitink, 2010). While their expression appears to be correlated with abiotic stresses such as water deficit, osmotic and cold stresses (all of which do affect subcellular water status) (Wise and Tunnacliffe, 2004; Illing et al., 1999; Berjak et al., 2007; Chakrabortee et al., 2006; Mtwisha et al., 2006; Wise and Tunnacliffe, 2004; Bartels, 2005). Over the years, use of different nomenclatures in reporting presence of LEAs has confused rather than facilitated our understanding of the roles of the different LEAs in abiotic stress tolerance. In Table 11.1 we have listed the various classifications used in studies reporting on the presence of such proteins in desiccation-tolerant systems. The data presented are probably only a small selection of LEAs that might be involved in desiccation tolerance. Many others may play a role in early acquisition of desiccation tolerance but these are overlooked due to lack of studies investigating their presence at a range of water contents.

LEA genes are among the most differentially expressed and highly up-regulated as shown in recent transcriptomics studies on orthodox seeds and resurrection plants and this is supported by proteomic studies where these have been performed (Collett et al., 2004; Illing et al., 2005; Boudet et al., 2006; Buitink et al., 2006; Farrant, 2007; Liu et al., 2009; Leprince and Buitink, 2010).

Publicly available Arabidopsis genome and microarray data have shown that 74% of LEAs are expressed during seed development. Fifteen of the 35 LEAs analysed were seed specific and a further six LEAs were expressed at their maximum during seed development when desiccation tolerance is acquired but were also expressed in response to other abiotic stresses (Illing et al., 2005; Table 11.1). Both transcriptome and proteome studies on M. truncatula have shown a number of LEA-like proteins in mature seeds of which expression is up-regulated when desiccation is re-induced during germination (Boudet et al., 2006; Buitink et al., 2006; Table 11.1). At least two, a group 1 LEA (Em6; PM25; PMF04927) and a group 5 LEA (PM25; PMF04927) were confirmed to be associated with desiccation tolerance, the remainder being proposed to be associated with osmotic stress and drought tolerance since their induction in this and other species appears to occur at higher hydration levels of >0.8 g H2O g−1 FW (Black et al., 1999; Reyes et al., 2005; Boudet et al., 2006). It is likely that these are indeed required for desiccation tolerance but are needed at earlier stages of water loss. In the resurrection plant X. humilis, 16 of 55 genes shown to be up-regulated during desiccation were annotated as LEAs (Collett et al., 2004; Table 11.1). This microarray study tested only 424 cDNAs randomly selected from an 11 K normalized library made from leaf and root tissues of the plant and this, together with the observation that the transcripts only became evident once the water content fell below 50% RWC, with transcripts disappearing early during the rehydration process (Illing et al., 2005) indicates the potential importance of these in acquisition of desiccation tolerance in this species. Further microarray profiling in which genes from desiccated roots, leaves and seeds of X. humilis were compared showed considerable enrichment of LEA family proteins in all of these tissues of which three, a group 1 LEA (At2g40170), a group 6 LEA (At3g22490) and an LEA10 (At1g04560) were among the Arabidopsis seed-specific genes and one, an
### Table 11.1. Classifications of late embryogenesis abundant (LEA) proteins thought to be related to desiccation tolerance.

<table>
<thead>
<tr>
<th>LEA group/Pfam domain</th>
<th>Interpro superfamily</th>
<th>Locus ID/gene-bank accession</th>
<th>Description/annotated gene name</th>
<th>Water content at which induced</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seeds</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Arabidopsis thaliana</em> seed specific</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 PF00477</td>
<td>IPR000389</td>
<td>At2g40170</td>
<td>LEA 1</td>
<td>n/a</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>1 PF00477</td>
<td>IPR000389</td>
<td>At3g51810</td>
<td>LEA 1</td>
<td>n/a</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>2 PF00257</td>
<td>IPR000167</td>
<td>At2g21490</td>
<td>LEA 2</td>
<td>n/a</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>2 PF00257</td>
<td>IPR000167</td>
<td>At3g50980</td>
<td>LEA 2</td>
<td>n/a</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>2 PF00257</td>
<td>IPR000167</td>
<td>At3g39130</td>
<td>LEA 2</td>
<td>n/a</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>3 PF02987</td>
<td>IPR004238</td>
<td>At2g36840</td>
<td>LEA 3</td>
<td>n/a</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>3 PF02987</td>
<td>IPR004238</td>
<td>At3g15670</td>
<td>LEA 3</td>
<td>n/a</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>3 PF02987</td>
<td>IPR004238</td>
<td>At4g38600</td>
<td>LEA 3</td>
<td>n/a</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>4 PF03760</td>
<td>IPR005513</td>
<td>At2g35300</td>
<td>LEA 4</td>
<td>n/a</td>
<td>Illing et al. (2005); Walford (2008)</td>
</tr>
<tr>
<td>6 PF04927</td>
<td>IPR007011</td>
<td>At3g22490</td>
<td>LEA 6</td>
<td>n/a</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>6 PF04927</td>
<td>IPR007011</td>
<td>At3g22500</td>
<td>LEA 6</td>
<td>n/a</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>9 PD68804</td>
<td>IPR008390</td>
<td>At2g41280</td>
<td>LEA 9</td>
<td>n/a</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>10 PF05512</td>
<td>IPR008390</td>
<td>At1g04560</td>
<td>LEA 10</td>
<td>n/a</td>
<td>Illing et al. (2005); Walford (2008)</td>
</tr>
<tr>
<td>Maximum expression during acquisition of DT but expression in other abiotic stresses too</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 PF00257</td>
<td>IPR000167</td>
<td>At5g66400</td>
<td>LEA 2</td>
<td>n/a</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>3 PF02987</td>
<td>IPR004238</td>
<td>At1g52690</td>
<td>LEA 3</td>
<td>n/a</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>3 PF02987</td>
<td>IPR004238</td>
<td>At3g02480</td>
<td>LEA 3</td>
<td>n/a</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>3 PF02987</td>
<td>IPR004238</td>
<td>At3g17520</td>
<td>LEA 3</td>
<td>n/a</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>3 PF02987</td>
<td>IPR004238</td>
<td>At4g13230</td>
<td>LEA 3</td>
<td>n/a</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>4 PF03760</td>
<td>IPR005513</td>
<td>At5g06760</td>
<td>LEA 4</td>
<td>n/a</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>Medicago truncatula</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 PF00447</td>
<td>IPR000389</td>
<td>TC96799</td>
<td>Em6, homology to At2g40170</td>
<td>≤ 0.3 g/g</td>
<td>Boudet et al. (2006); Buitink et al. (2006)</td>
</tr>
<tr>
<td>2 PF00257</td>
<td>IPR000167</td>
<td>TC100921</td>
<td>DHN3, dehydrin-like (<em>M. sativa</em>)</td>
<td>≤ 0.8 g/g</td>
<td>Boudet et al. (2006); Buitink et al. (2006)</td>
</tr>
<tr>
<td>3 PF02987</td>
<td>IPR004238</td>
<td>TC102224</td>
<td>homology to Sbp66 (<em>soybean protein</em>); <em>P. sativum</em></td>
<td>≤ 0.8 g/g</td>
<td>Boudet et al. (2006)</td>
</tr>
</tbody>
</table>

Continued
Table 11.1. Continued.

<table>
<thead>
<tr>
<th>LEA group</th>
<th>PFam domain</th>
<th>Interpro superfamily</th>
<th>Locus ID/ gene-bank accession</th>
<th>Description/annotated gene name</th>
<th>Water content at which induced</th>
<th>Reference</th>
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<tr>
<td>3</td>
<td>PF02987</td>
<td>IPR004238</td>
<td>TC95538</td>
<td>MP2 (G. max)</td>
<td>≤ 0.8 g/g</td>
<td>Boudet et al. (2006)</td>
</tr>
<tr>
<td>3</td>
<td>PF02987</td>
<td>IPR004238</td>
<td>TC96265</td>
<td>PM18; seed maturation protein (G. max)</td>
<td>≤ 0.8 g/g</td>
<td>Boudet et al. (2006)</td>
</tr>
<tr>
<td>5</td>
<td>PF04927</td>
<td>CA917414</td>
<td></td>
<td>PM25; seed maturation protein (G. max)</td>
<td>≤ 0.3 g/g</td>
<td>Boudet et al. (2006)</td>
</tr>
</tbody>
</table>

**Xerophyta humilis LEAs**

- Xerophyta humilis LEAs present in vegetative tissue only: regular type
- LEAs present in seed and vegetative tissue: bold type

<table>
<thead>
<tr>
<th>LEA group</th>
<th>PFam domain</th>
<th>Interpro superfamily</th>
<th>Locus ID/ gene-bank accession</th>
<th>Description/annotated gene name</th>
<th>Water content at which induced</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PF00477</td>
<td>IPR000389</td>
<td>At2g40170</td>
<td>LEA1, seed specific in A. thaliana</td>
<td>n/a but present at 10% RWC</td>
<td>Walford (2008)</td>
</tr>
<tr>
<td>2</td>
<td>PF00257</td>
<td>IPR000167</td>
<td>CK906358</td>
<td>No Blastx homologue identified</td>
<td>&lt;50% RWC</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>2</td>
<td>PF00257</td>
<td>IPR000167</td>
<td>CK988413</td>
<td>44 kDa dehydrin-like (C. sericea)</td>
<td>&lt;50% RWC</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>2</td>
<td>PF00257</td>
<td>IPR000167</td>
<td>CK906432</td>
<td>Embryonic abundant protein, radish</td>
<td>&lt;50% RWC</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>2</td>
<td>PF00257</td>
<td>IPR000167</td>
<td>CK906386</td>
<td>DHN3; dehydrin-like (M. sativa)</td>
<td>&lt;50% RWC</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>3</td>
<td>PF02987</td>
<td>IPR004238</td>
<td>CK906406</td>
<td>LEA-like protein (L. longiflorum)</td>
<td>&lt;50% RWC</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>3</td>
<td>PF02987</td>
<td>IPR004238</td>
<td>CK906427; At5g44310</td>
<td>Late embryogenesis abundant protein-like (A. thaliana)</td>
<td>&lt;50% RWC</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>3</td>
<td>PF02987</td>
<td>IPR004238</td>
<td>CK906404</td>
<td>LEA protein 76; rape</td>
<td>&lt;50% RWC</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>3</td>
<td>PF02987</td>
<td>IPR004238</td>
<td>CK906402</td>
<td>LEA1 protein (Triticum aestivum)</td>
<td>&lt;50% RWC</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>3</td>
<td>PF02987</td>
<td>IPR004238</td>
<td>CK906398</td>
<td>LEA protein (B. inermis)</td>
<td>&lt;50% RWC</td>
<td>Illing et al. (2005)</td>
</tr>
<tr>
<td>3</td>
<td>PF02987</td>
<td>IPR004238</td>
<td>At1g52690</td>
<td>LEA3; maximum expression in A. thaliana seed but also expressed in response to other abiotic stresses</td>
<td>n/a but present at 10% RWC</td>
<td>Walford (2008)</td>
</tr>
</tbody>
</table>
Late embryogenesis abundant protein-like (A. thaliana) LEA protein 76; rape LEA1 protein (Triticum aestivum) LEA protein (8. inermis) LEA3; maximum expression in A. thaliana seed but also expressed in response to other abiotic stresses

Putative seed maturation protein (O. sativa) PM26, seed maturation protein (Glycine max); seed specific in A. thaliana

LEA homologue; tomato LEA LEA7

LEA protein Lea14-A; upland cotton LEA protein with hydrophobic domain (G. max) Hydrophobic LEA-like protein (O. sativa) Putative plasma membrane associated protein (O. sativa) LEA10, seed specific in A. thaliana

Desiccation-related protein (C. plantagineum) Desiccation-related protein (C. plantagineum) Desiccation-related protein (C. plantagineum) Desiccation-related protein (C. plantagineum) Desiccation-related protein (C. plantagineum)

n/a but present at 10% RWC n/a but present at 10% RWC n/a but present at 10% RWC n/a but present at 10% RWC n/a but present at 10% RWC

<50% RWC <50% RWC <50% RWC <50% RWC n/a but present at 10% RWC


Continued
Table 11.1. Continued.

<table>
<thead>
<tr>
<th>LEA group Pfam domain</th>
<th>Interpro superfamily</th>
<th>Locus ID/ gene-bank accession</th>
<th>Description/annotated gene name</th>
<th>Water content at which induced</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 PF03168</td>
<td>IPR004864</td>
<td>P22241</td>
<td>Desiccation-related protein (C. plantagineum) XVT6; dehydrin-like</td>
<td>n/a</td>
<td>Piatkowski et al. (1990)</td>
</tr>
<tr>
<td>2 PF00257</td>
<td>IPR000167</td>
<td>AAP22171</td>
<td>XVT8; LEA</td>
<td>42% RWC</td>
<td>Mundree and Farrant (2000)</td>
</tr>
<tr>
<td>2 PF00257</td>
<td>IPR000167</td>
<td>No gene bank accession</td>
<td>XVT8; LEA</td>
<td>n/a</td>
<td>Ndima et al. (2001)</td>
</tr>
<tr>
<td>Xerophyta viscosa</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 PF00257</td>
<td>IPR000167</td>
<td>EMBL:Y10778</td>
<td>Dehydrin DEA-like protein; wheat</td>
<td>23–27% RWC</td>
<td>Blomstedt et al. (1998)</td>
</tr>
<tr>
<td>3 PF02987</td>
<td>IPR004238</td>
<td>Y10779</td>
<td></td>
<td>57% RWC</td>
<td>Blomstedt et al. (1998)</td>
</tr>
<tr>
<td>3 PF02987</td>
<td>IPR004238</td>
<td>sdg2I AM261429</td>
<td></td>
<td>&lt;60% RWC</td>
<td>Le et al. (1998)</td>
</tr>
<tr>
<td>Sporobolus stapianus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g/g, g H₂O g⁻¹ DW; RWC, relative water content.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| 8 PF03168             | IPR004864            | P22241                        | Desiccation-related protein (C. plantagineum) XVT6; dehydrin-like | n/a                           | Piatkowski et al. (1990) |
| 2 PF00257             | IPR000167            | AAP22171                      | XVT8; LEA                      | 42% RWC                       | Mundree and Farrant (2000) |
| 2 PF00257             | IPR000167            | No gene bank accession        | XVT8; LEA                      | n/a                           | Ndima et al. (2001)      |

Xerophyta viscosa

| 2 PF00257             | IPR000167            | EMBL:Y10778                   | Dehydrin DEA-like protein; wheat | 23–27% RWC                    | Blomstedt et al. (1998) |
| 3 PF02987             | IPR004238            | Y10779                        |                                | 57% RWC                       | Blomstedt et al. (1998) |
| 3 PF02987             | IPR004238            | sdg2I AM261429                |                                | <60% RWC                      | Le et al. (1998)        |

g/g, g H₂O g⁻¹ DW; RWC, relative water content.
LEA3 (At1g52690) is highly up-regulated during desiccation tolerance in seeds of this species (Walford, 2008). A further four group 3 LEAs (At2g42560, At3g3040, At4g15910, At4g21020), a group 4 LEA (At1g75100), a group 7 LEA (At3g53770) and a group 8 LEA (At1g01470) were found to be up-regulated in desiccated vegetative and seed tissues of X. humilis (Table 11.1) although these did not fall into the Arabidopsis seed-specific LEAs identified by Illing et al. (2005). Transcriptome studies on other resurrection plants have all reported the presence of LEA genes associated with desiccation (Table 11.1; Piatkowski et al., 1990; Blomstedt et al., 1998; Mundree and Farrant, 2000; Neale et al., 2000; Le et al., 2007; Blomstedt et al., 2010). These studies have ranged in the numbers of genes tested, and so final numbers of LEAs and the groups to which they belong vary. However, in all studies they form a high percentage of the genes tested with LEAs from groups 2 and 3 predominating.

As mentioned above, we still have no full understanding of what these specific LEAs might do to facilitate desiccation tolerance. The fact that multiple LEAs are induced during acquisition of desiccation tolerance in seeds and resurrection plants would make us assume that there are multiple roles, these acting in conjunction with other protectants to ensure desiccation tolerance. For example, it has been demonstrated that LEA proteins can act synergistically with sugars to prevent protein aggregation during desiccation (Goyal et al., 2005) and/or to replace water during desiccation and thus maintain the hydration shell of proteins and other molecules and/or to form intercellular glasses that stabilize the subcellular milieu in the desiccated state (Berjak, 2006; Berjak et al., 2007).

11.5.6 Heat shock proteins

There is increasing evidence for the role of heat shock proteins (HSPs) in cellular protection during desiccation. The small HSPs (sHSPs, 15–42 kDa) are the most prominent HSPs in plants (Waters et al., 1996). They accumulate in the maturing seeds of many plant species prior to desiccation and persist in the dry state indicating a role in the acquisition of desiccation tolerance (Vierling, 1991; Coca et al., 1994; Wehmeyer et al., 1996; Kernode and Finch-Savage, 2002). Seeds of M. truncatula are reported to have two HSP genes (HSP 83 and HSP 18.2) that accumulate during the acquisition of desiccation tolerance, both during maturation and after re-establishment of desiccation tolerance in germinating radicles (Buitink et al., 2006). In resurrection plants they have been reported to be up-regulated during drying in Craterostigma plantagineum and X. humilis (Alamillo et al., 1995; Walford, 2008). In C. plantagineum, sHSP are constitutively present in the vegetative tissues but levels increase in response to water stress and heat shock. Additionally, exogenous application of the stress hormone, abscisic acid (ABA), induces the expression of sHSPs and the acquisition of desiccation tolerance in previously desiccation-sensitive C. plantagineum callus (Alamillo et al., 1995). In a microarray analysis in which mRNA transcripts from hydrated leaves and roots were compared with those in desiccated leaves, roots and seeds of X. humilis, we identified two sHSPs (At1g535340; At3g57340) that were up-regulated in all the dry tissues. In a study of the heat stable proteome of leaves of this species, two of the 33 proteins that appeared de novo on desiccation were HSPs, one a sHSP (HSP26.7b, a chloroplast low molecular weight HSP) and the other was annotated as HSP70 (Liu et al., 2009). Characterization of the nucleus proteome of X. viscosa showed that there was considerable up-regulation of a 17.5 kDa sHSP upon dehydration (Abdalla et al., 2010) and a member of the HSP90 family (Grp94) was found to be induced by desiccation and heat stress in leaves of this species (Walford et al., 2004). To date there is little experimental evidence that points to a specific role for HSPs in desiccation tolerance but they are thought to offer a general protective role in the dry state based on their chaperone-like activity. In this regard they
can act to minimize inappropriate interactions among molecules and so enable maintenance of protein structure in the dry state and may also facilitate appropriate refolding upon rehydration (Alpert and Oliver, 2002; Mtwisha et al., 2006). While there are few studies focused on the water contents at which these proteins are up-regulated and/or induced, it is likely that they protect in all phases of water loss from type IV and below.

11.6 Conclusion

This review has shown that there are considerable similarities in the mechanisms of desiccation tolerance in seeds and resurrection plants. These include \textit{inter alia}, vacuole filling, antioxidant production, synthesis of sucrose and RFOs such as raffinose and stachyose and production of protective proteins such as LEAs and sHSPs. However, some of the mechanisms are also unique to plants, such as wall folding, leaf folding, pigment production and photosynthetic down-regulation. We propose that the developmentally regulated programme of acquisition of desiccation tolerance in seeds is utilized in the acquisition of tolerance in vegetative tissues of resurrection plants, possibly in response to environmentally regulated rather than developmental cues. Additional plant-specific mechanisms, such as those associated with desiccation-induced photosynthetic stresses, have been acquired in these plants. The differences among them are likely to be due to independent evolution among different lineages, desiccation tolerance in angiosperms having been reported to have appeared in at least ten independent phylogenetic lineages (Oliver, 2007).

In order to gain deeper insight into the mechanism of desiccation tolerance, and the similarities among seeds and resurrection plants, it is important that future studies should:

- Determine the physical properties of water at different water concentrations and water potentials in resurrection plants.
- Be conducted under reproducible conditions of light, relative humidity and temperature and which should be standardized for each resurrection plant species at conditions as close as possible to that occurring in the natural environment.
- Changes in water content and associated physiological/biochemical/molecular measurements should be carried out at more frequent intervals to capture as best possible all the changes occurring during a drying and rehydration time course.
- Trials on seeds should be done on individual tissues, or just the embryonic axis, and measurements should include monitoring of parameters at the start of or just prior to the onset of desiccation tolerance and at its completion.

References


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