

The evolution of desiccation tolerance in angiosperm plants: a rare yet common phenomenon

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Abstract. In a minute proportion of angiosperm species, rehydrating foliage can revive from airdryness or even from equilibration with air of ~0% RH. Such desiccation tolerance is known from vegetative cells of some species of algae and of major groups close to the evolutionary path of the angiosperms. It is also found in the reproductive structures of some algae, moss spores and probably the aerial spores of other terrestrial cryptogamic taxa. The occurrence of desiccation tolerance in the seed plants is overwhelmingly in the aerial reproductive structures; the pollen and seed embryos. Spatially and temporally, pollen and embryos are close ontogenetic derivatives of the angiosperm microspores and megaspores respectively. This suggests that the desiccation tolerance of pollen and embryos derives from the desiccation tolerance of the spores of antecedent taxa and that the basic pollen/embryo mechanism of desiccation tolerance has eventually become expressed also in the vegetative tissue of certain angiosperm species whose drought avoidance is inadequate in microhabitats that suffer extremely xeric episodes. The protective compounds and processes that contribute to desiccation tolerance in angiosperms are found in the modern groups related to the evolutionary path leading to the angiosperms and are also present in the algae and in the cyanobacteria. The mechanism of desiccation tolerance in the angiosperms thus appears to have its origins in algal ancestors and possibly in the endosymbiotic cyanobacteria-related progenitor of chloroplasts and the bacteria-related progenitor of mitochondria. The mechanism may involve the regulation and timing of the accumulation of protective compounds and of other contributing substances and processes.

Additional keywords: abscisic acid, *Borya*, *Craterostigma*, gene expression, modular evolution, proteome, protoplasmic drought tolerance, *Sporobolus*, *Tortula*, *Xerophyta*.

Received 28 October 2012, accepted 10 January 2013, published online 22 February 2013

Introduction

In the 8000 or so years that crops have been cultivated, drought has been a major scourge. The failure and death of a crop reinforced the general observation that poorly hydrated plants do not grow and that dry plants are dead plants. The angiosperm (flowering) plants that we depend on totally for our food are overwhelmingly sensitive to desiccation. Early in the 20th century, reports appeared of rare angiosperm species with a spectacular ability to recover from dryness (Dinter 1919; Table 1). Kurt Dinter, a plant collector in south-west Africa, observed the dry season small bushes of *Myrothamnus flabellifolia* Welw. that were desiccated, with hard dark brown shrunken leaves folded against the dehydrated stem. The foliage was dry enough to snap and rub into a powder between his fingers. Completely dead to all appearances, but after a single day of rain the leaves and plants became green healthy plants — not a mere semblance of recovery but a real return to active life, as the plants resumed normal growth. *Myrothamnus flabellifolia* well deserves its vernacular name, the ‘resurrection bush’.

Salient features of this phenomenon of desiccation tolerance are the extreme degree of dryness tolerated, the rapidity of

rehydration and recovery, the multiple cycles of dehydration and rehydration endured, the stability of the dry tissue and its longevity in the dry state.

Actively growing plants have relative water contents (RWC) of ~85–100%. Crop plants die as water content falls from 59 to 30% RWC (Höfler *et al.* 1941). Air-dry desiccation-tolerant plants in the field are literally as dry as straw; their water contents are only 5–13% RWC and they survive even further drying to ~1% RWC in the laboratory (Gaff 1977).

Desiccation-tolerant angiosperm plants retain most of their water, until the soil moisture has been exhausted; the water content of desiccation-tolerant plant then falls rapidly until the plant reaches air-dryness in 2–4 days (Gaff 1977). The 1 cm plants of *Chamaeigigas intrepidus* Dinter ex Heil dry extremely rapidly, ~1.8 h after the soil dries. Rehydration is usually more rapid than dehydration. Again *Chamaeigigas intrepidus* is exceptional, rehydrating fully after only 1.5 h in water (Gaff and Giess 1986). A rainfall of 10 mm is sufficient for full rehydration of the air-dry desiccation-tolerant angiosperm plants (Gaff 1977). Less rain may produce partial rehydration, usually of the lower leaves whereas the upper leaves remain airdry. Full rehydration

Table 1. The angiosperm families that contain species with desiccation-tolerant vegetative tissue and the number of genera in each family with one or more desiccation-tolerant species

Family	Number of genera with desiccation-tolerant species	Number of all genera in the family (prone to taxonomic revisions)
<i>Dicotyledoneae</i>		
Cactaceae	1	100
Gesneriaceae	9	133
Lamiaceae	1	212
Myrothamnaceae	1 ^A	1
Ranunculaceae	2	51
Scrophulariaceae	4	292
Stylidiaceae	1	5
Tamaricaceae	1	4
<i>Monocotyledoneae</i>		
Anthericaceae	1	29
Cyperaceae	7	102
Philydraceae	1	3
Poaceae	9	657
Velloziaceae	6 ^B	10

^AThe two species in the genus and in the family have desiccation-tolerant foliage.

^BAll tested species in the family have desiccation-tolerant foliage.

often occurs within 24 h of a rainfall in the field, but may take longer depending on the particular species or if the temperature is low.

Desiccation-tolerant angiosperm plants may traverse several cycles of dehydration and rehydration in a year, especially after isolated rainfalls at the beginning and the end of the dry season in the subtropics, e.g. 17 cycles in a year in the perennial *Chamaeigigas intrepidus* (Gaff and Giess 1986). There appears to be no limit on the number of such cycles they can endure, provided there are periodic opportunities for photosynthesis. Many desiccation-tolerant species are long-lived perennials: the desiccation-tolerant velloziad *Xerophyta equisetoides* Baker with stems 2.6 m tall would exceed 200 years, based on the height of 40-year-old greenhouse-plants at Monash University. Dead leaf bases form a velloziad trunk that supports a tuft of live leaves – approaching an epiphytic state (Raven and Andrews 2010).

The air-dry state is both static and surprisingly stable. As plants dry, growth stops and metabolism slows until it ceases or nearly so; the plant is in a state of suspended animation or ‘anabiosis’. The walls of dry cells are often folded, indicative of a plasticity that is thought to avoid mechanical damage to the drying wall, the protoplast and the contact between them (Farrant 2007). The plasticity would also assist the rapid growth seen in rehydrated resurrection grasses (Blomstedt *et al.* 2010). The airdry leaves tolerate temperatures approaching 60°C and subfreezing temperatures of –80°C. The time-span over which air-dry leaves remain viable is impressive. *Myrothamnus flabellifolia* plants survive 6–10 months, depending on the ecotype (Farrant and Kruger 2001). Most angiosperm species examined survive air-dryness for ~2 years and leaves of *Borya constricta* Churchill recovered when rehydrated after 5 years of storage air-dry (Gaff 1981). The longevity of anabiotic tissue varies greatly with the

humidity of the storage air, the storage temperature, the species and the plant organ. Air-dry desiccation-tolerant leaves stored at high humidity may lose their viability within a few days, whereas storage at 20–40% RH, leading to tissue water potentials of approximately –215 MPa to –123.6 MPa, generally allows the longest survival times (Gaff 1980).

In the past 50 years, the number of angiosperm plant species that have been reported to possess desiccation-tolerant vegetative tissue has risen from 10 species to 135 species. Desiccation tolerance has been quantified in 83 of these species in terms of the lowest water potential survived (Table 2) and in a further 33 species in terms of the lowest relative water content survived.

The evolution of an ecological assemblage

Desiccation-tolerant ‘resurrection plants’ commonly grow on shallow soils (often only 1 cm) overlying rock slabs or collecting in depressions on rock outcrops (Gaff 1977; Porembski and Barthlott 2000). Their desiccation tolerance allows them to persist during the periodic dehydration to which such shallow soils are prone. In this situation, the low growing resurrection plants colonise the bare soils and by trapping further soil and detritus allow taller desiccation-tolerant plant species to follow them. Eventually taller desiccation-sensitive species overshadow and out-compete the desiccation-tolerant plants. Granitic outcrops and inselbergs in particular offer numerous soil pans suitable for desiccation-tolerant colonisers. Whereas desiccation-tolerant species exploit a much wider range of habitats beyond rock outcrops, the shallow soils in rock pans may be central to the origin of desiccation-tolerant angiosperms from non-tolerant ancestors and a major influence on their further evolution.

The evolution of a cuticle resisting water loss of intercellular spaces and stomata regulating water vapor loss and of a xylem, which enables long-range transport of water from moist soil to foliage, led in the angiosperms to a general evolutionary thrust towards improved drought-avoidance mechanisms that can be very effective preventing injuriously low plant water contents developing (i.e. homoiohydric). Most desiccation-tolerant angiosperm species have one or more mechanisms retarding transpiration: for example, xeromorphy, inrolling curling or folding leaves, stomata that are confined to grooves, densely-hairy leaf surfaces and ephemeralism. These mechanisms of drought avoidance and evasion are functionally compatible with desiccation tolerance. Desiccation-sensitive species with well-developed avoidance/evasion mechanisms can colonise shallow soils. Any inadequacy of the avoidance mechanisms during dry spells subjects the species to selective pressure for improved protoplasmic drought tolerance (PDT), leading to the extreme case – desiccation tolerance – a definite advantage for pioneer species.

Selection on the rock pan site influences the evolution of associated characteristics of desiccation-tolerant species. The periodic dryness of shallow rock-pan soils prevents tall-growing desiccation-sensitive species from establishing and limits the growth of drought-resistant non-resurrection species. Selection pressure on the desiccation-tolerant plants, for height and dry-mass productivity therefore is weakened; selection focuses on plant survival, reproduction and dispersal. The

Table 2. The distribution of protoplasmic drought tolerance (PDT) values of foliage of terrestrial plants

PDT is given as the minimum water potential survived by leaves in vapour-equilibrium with air of set relative humidity, RH, (MPa estimated from RH at 20°C). Values are the number of species within the range as a percentage of the number of studied species. *N* is the number of species in each data set. Data for liverworts are calculated from Höfler (1943), for mosses from Abel (1956), and for other taxa see Gaff (1987)

PDT water potential (MPa)	PDT% RH (equilibrium)	Liverworts	Mosses non-hardened	Same moss species hardened	Desiccation-tolerant ferns	Desiccation-tolerant fern-allies	Desiccation-tolerant dicots	Desiccation-tolerant chlorophyll-retainers	Desiccation-tolerant monocots chlorophyll-losers	Monocots desiccation-tolerant leaf-base	Spermatophyte pollen
-14 to 0	90–100	25	37	23	–	–	–	–	–	–	6
-30 to -16	80–89	27	16	–	–	–	–	–	–	–	4
-48 to -32	70–79	13	3	–	–	–	–	–	–	–	1
-68 to -50	60–69	9	13	–	–	–	–	–	–	–	1
-94 to -71	50–59	9	3	3	–	–	–	–	–	–	1
-123 to -96	40–49	4	–	–	–	–	–	–	–	–	–
-163 to -127	30–39	5	–	–	7	–	–	–	–	–	5
-217 to -167	20–29	2	–	–	7	–	–	–	–	–	18
-310 to -224	10–19	4	3	3	23	13	15	7	7	–	14
-404 to -325	5–9	2	19	45	7	–	–	7	7	42	49 ^B
-600 ^A to -434	0–4	4	3	26	56	87	73	86	87	58	–
<i>N</i>		56	62	62	43	8	26	14	31	12	142

^AValue over activated silica gel.

^BFor a range 0–9% RH.

majority of desiccation-tolerant angiosperm species have short plants, mostly 1–15 cm, with short internodes (Gaff 1972). *Myrothamnus* spp. (50–100 cm tall) and species in the Velloziaceae (up to 1–2 m) are notable exceptions. Desiccation-tolerant dicot plants are frequently rosettes and most non-velloziad monocot plants have numerous shoots forming a tight clump (a caespitose habit). In both growth habits the shoot meristems are on or under the soil partially protected from direct sunlight and from wildfires. As a result, resurrection plants in burnt areas shoot again after rain, often rapidly. A thick tightly-packed mass of persistent leaf bases surround the aerial stems and apical meristems of velloziads; fire lightly chars only the surface of this pseudostem, leaving the true stem and its meristems unharmed.

In many resurrection grass species, phenotypic shortness of the vegetative shoots has become largely genetically determined; a few however respond dramatically to transfer from shallow soils to deep soils, e.g. the desiccation-tolerant grasses *Microchloa caffra* Nees and *Eragrostis invalida* Pilger grow 10–50 cm taller respectively. Rock pans also limit the space available to desiccation-tolerant plants. Root systems of most desiccation-tolerant angiosperms form dense much-branched masses that bind the limited soil resource firmly against erosion. Root systems of plants transferred to deep soil grow extensively.

Many desiccation-tolerant angiosperm plants spread across a rock pan by vegetative reproduction as well as from seed. The numerous seed released from the infructescences of desiccation-tolerant angiosperms are small to minute and so are well adapted for long-distance dispersal by wind and water and on animals to remote rock outcrops.

Whilst predominant environments for desiccation-tolerant angiosperms can be discerned, the plants are found over most of the range available – tropics to polar zones, altitudes of 0–5000 m, on rocks to in deep soils on trees to swamps and stony soil to clay (D F Gaff, unpubl. data).

Distribution of desiccation-tolerant foliage in the angiosperms

Taxonomic

Desiccation-tolerant vegetative tissue occurs in 13 mainly unrelated angiosperm families: implying it has arisen separately at least 13 times during angiosperm evolution. The phenomenon is poorly represented in the families that are in the more direct lineages from the ancestral angiosperms where it occurs only in the corms and tubers of two species in the dicot family Ranunculaceae and in the foliage of only *Borya* in the monocot Anthericaceae (or Liliaceae *sensu lato*; Table 1). In the family Velloziaceae, every tested species is desiccation tolerant, suggesting an early appearance in this large monocot family. The largest number of genera containing desiccation-tolerant species are found in two families Poaceae and Cyperaceae, in which the desiccation-tolerant species make up only a small proportion of their genus and these genera only a small fraction of the family. The late appearance of the phenomenon in apparently early-evolved families and its early appearance and representation mainly in some later-evolved families suggests a relatively 'mid-stream' rather than an early appearance of desiccation tolerant vegetative tissue in the angiosperms. In regard to

phylogeny, information given later in this paper indicates that desiccation tolerance occurs right across the Angiosperm families in general: of the thousands of species examined, all species without exception have desiccation-tolerant seed or pollen. That is the total phylogeny of the angiosperms possesses desiccation tolerance. Nevertheless, desiccation tolerance is expressed in the foliage of only a few angiosperm families that are not in a linear phylogenetic sequence one to the other. These families appear to be predisposed to express desiccation tolerance – but usually only in a small proportion of the species in the family. At an ecological level, a factor in this predisposition may be as simple as the production of numerous minute seed that can readily be dispersed over long distances to suitable shallow-soil sites. The natures of other factors that may contribute to the predisposition at a physiological or molecular level are as yet not clear.

Quantitative: protoplasmic drought tolerance of angiosperms

Desiccation tolerance is the extremity of protoplasmic drought tolerance (PDT) being the lowest water potential of the cell protoplast that the protoplast can survive. The PDT of angiosperm plants lie in the range from -22 to -2.7 MPa (derived from the 85–98% RH values reported by Levitt *et al.* 1960), except for $\sim 0.01\%$ of the angiosperm species whose PDT are all -162 MPa or less.

Desiccation tolerance is well developed in angiosperm resurrection plants: the proportions of dicot and monocot resurrection plants with the most extreme PDT values (below -400 MPa) range from 58 to 87% in the four angiosperm categories in Table 2. In the angiosperm data, no PDT values are found in the intermediate range of PDTs (-123 to -30 MPa), which lies above the PDT of resurrection plants and below the PDT of nonresurrection plants.

The situation in *Borya* is of interest here. Leaves of *Borya constricta* are not constitutively desiccation-tolerant. The mechanism for desiccation tolerance is activated when drying leaves are in the water potential range from -14 to -2.7 MPa for 2 days (Gaff and Churchill 1976). Desiccation tolerant vegetative tissue has not been found in related genera or families. In *Borya scirpoides* Lindley desiccation tolerance is restricted to the 4 mm base of the first three immature leaves (Table 3). In a series of other *Borya* species desiccation tolerance spreads to the full length of young leaves and to increasingly more mature leaves (but as in all resurrection plants, senescing tissues do not become desiccation tolerant). In all *Borya* species the PDT values are in the extreme range of -404 MPa and below, even in the most restricted case *B. scirpoides*. There is no evidence of leaf PDT evolution through a range of intermediate PDT values.

It appears that in the *Borya* spp. desiccation tolerance in foliage has evolved *de novo* by the expression of a fully-developed desiccation tolerance mechanism first in immature meristematic leaf tissue then evolving in other species in progressively more mature tissue.

The facultative induction of desiccation tolerance during drought stress seen in *Borya constricta* seems to occur in the few poikilohydrous angiosperm species examined to date, in all but one of which fully hydrated leaves are desiccation sensitive when detached (Gaff 1980). In the single exception, *Myrothamnus flabellifolia*, desiccation tolerance is constitutive, since leaves detached while hydrated survive subsequent airdrying. Drought-induced desiccation tolerance is not confined to angiosperm resurrection species. Such an induction is evident in 49% of moss species, compared with 26% of moss species with constitutive desiccation tolerance (Table 2). Constitutive desiccation tolerance has been studied intensively in the moss *Tortula ruralis* (Bewley 1979; Oliver 2007).

Table 3. Desiccation-tolerant foliage in *Borya* species, with leaf protoplasmic drought tolerance (PDT), the lowest RH at equilibrium survived by the foliage

Species	PDT (% RH)	PDT water potential	For leaves no. (youngest visible leaf = 1)		Comments
<i>Borya scirpoides</i> Lindley	2% RH	-528	1-3	4 mm base of leaf ^{A, B}	Summer deciduous
<i>Borya laciniata</i> Churchill	2	-528	1-3	A, D	Summer deciduous
<i>Borya longiscapa</i> Churchill	2, 30	-528 – -162	1-2, 5-20	A	Not deciduous
<i>Borya sphaerocephala</i> R.Br.	2, 5	-528 – -404	1-5, 6-11	A, D	Not deciduous
<i>Borya nitida</i> Churchill <i>sensu stricto</i>	2, 11	-528 – -298	1-10, 11-14	A, D	Not deciduous
<i>Borya mirabilis</i> Churchill	2, 5	-528 – -404	1-12, 13-20	A, D	Not deciduous
<i>Borya septentrionalis</i> F.Muell.	5	-404	Full size leaves	B	Not deciduous
<i>Borya subulata</i> C.A.Gardner	0	< -600	Full size leaves	A, F	Not deciduous
<i>Borya constricta</i> Churchill	0, 2	< -600 – -528	Full size to leaf 18	C, D	Not deciduous
<i>Borya jabirabella</i> Churchill	(<6% RWC)	Not determined	Full size	B, E	Collectn Latz7750 and Dunlop4430
<i>Borya inopinata</i> P.I.Forster and E.J.Thompson	Not determined	–	Full size?	–	Forster and Thompson 1997;

^APDT values are original here (DF Gaff) determined by vapour equilibration and from synthesis of chlorophyll of dry yellow leaves when rehydrated (Gaff and Churchill 1976).

^BGaff and Latz (1978).

^CGaff and Churchill (1976).

^DPlants kindly supplied by DM Churchill, ^Eby CR Dunlop and P Latz, ^Fby R Tudor.

Desiccation tolerance in other angiosperm organs

A few angiosperm species have vegetative organs that perennate in a desiccated state, e.g. corms of *Limosella grandiflora* Benth in D.C., *Philydrella pygmaea* (R.Br.) Caruel and *Stylidium petiolare* Sond, rhizomes of *Anenome coronaria* L. and tubers of *Ranunculus asiaticus* L. (Pate and Dixon 1982; Antipov and Romanyak 1983; Dixon *et al.* 1983; Gaff and Giess 1986). Desiccation tolerance in the angiosperms occurs most commonly in the reproductive organs, the seed embryos and the pollen. At least 15 species have embryos with the extreme PDT values of ~0% RH or -600 MPa (including the grasses *Avena sativa* L., *Hordeum vulgare* L., *Triticum durum* Desf., *Triticum spelta* L., *Zea mays* L., De Saussure 1827), Seed PDT values range up to -2.7 MPa (Gaff 1980), however, in most of species, seeds are considered 'orthodox', i.e. seed are desiccation-tolerant with long-term viability (95% of species, Table 4). Of spermatophyte species ~4% have 'recalcitrant' seed, i.e. their seed are desiccation-sensitive with only a brief period of viability, whereas only ~1% are 'intermediate'. Pollen too is desiccation-tolerant in most spermatophyte species (~87% of spp. with PDT < -124 MPa, Table 2); 33% of these have PDT of approximately -600 MPa and ~57% have PDT < -310 MPa. 'Intermediates' (PDT = -93 to -30 MPa) constitute ~3.5% of all species. Desiccation-sensitive pollen occurs in ~10% of all species, including the important cereals barley, *Hordeum vulgare* L., and rye, *Secale cereale* L.

In 61 species, we have data on the PDT levels of both seed and pollen (Table 5). No species with recalcitrant or intermediate seed had desiccation-sensitive pollen; desiccation sensitive pollen is

Table 4. The numbers of *Spermatophyta* species with desiccation-tolerant or with desiccation-sensitive seed and the proportions of the total number categorised in the list of Liu *et al.* (2008)

Uncertain cases "?" and "p" are not included

Seed type	No. of species listed in category	Category as a % of the total species
Recalcitrant seed (desiccation-sensitive)	281	4.0%
Intermediate seed (mid-range PDT, not desiccation-tolerant)	72	1.0%
Orthodox seed (desiccation-tolerant)	6703	95.0%

found only in species with desiccation-tolerant seed whereas the majority of the orthodox-seed species have desiccation-tolerant pollen. If these data are representative of angiosperms and gymnosperms in general, then virtually all seed-plant species have the genetic information for desiccation tolerance, which is expressed in their seed embryo or the pollen but in the foliage of relatively few species (resurrection plants). It is only partially expressed in the 'intermediate' seed and pollen of a small proportion of species.

Modules in evolution

An underlying aspect of biological evolution is that once a particularly successful module has evolved, with further

Table 5. Protoplasmic drought tolerance of pollen, as % RH units and estimated water potential (in brackets) at equilibrium, determined from data in references as indicated

Species are categorised according to the sensitivity or tolerance of the seed embryos to desiccation (Liu *et al.* 2008)

Species	Pollen PDT	
<i>Desiccation-sensitive (recalcitrant) seed</i>		
<i>Aesculus hippocastanum</i> L.	60% RH (−69 MPa)	Pruzsinsky (1960)
<i>Nymphaea alba</i> L.	2 (−528)	Pruzsinsky (1960)
<i>Persea americana</i> Mill.	0 (<−600)	Visser (1955)
<i>Quercus coccinea</i> Wengen. recalcitrant? seed	15 (−256)	Visser (1955)
<i>Seed with intermediate PDT</i>		
<i>Salix caprea</i> L.	2 (−528)	Pruzsinsky (1960)
<i>Carica papaya</i> L. intermediate? Seed	10 (−311)	Visser (1955)
<i>Carica quercifolia</i> Benth. and Hook.f. intermediate?	<10 (<−311)	Visser (1955)
<i>Desiccation-tolerant (orthodox) seed</i>		
<i>with desiccation-sensitive pollen (80–100% RH)</i>		
<i>Nicotiana glutinosa</i> L.	~100 (0)	Pruzsinsky (1960)
<i>Triglochin maritima</i> L.	99 (−1.4)	Pruzsinsky (1960)
<i>Plantago maritima</i> L.	95 (−6.9)	Pruzsinsky (1960)
<i>Plantago media</i> ; <i>Hordeum vulgare</i> L.	90 (−14)	Pruzsinsky (1960); Visser (1955)
<i>Secale cereale</i> L.; <i>Triticum vulgare</i> L.	humid	Visser (1955)
<i>with ‘intermediate’ pollen (>50–80% RH)</i>		
<i>Cinchona ledgeriana</i> Moench	>50 (>−94)	Visser (1955)
<i>Prunus persica</i> (L.)Batsch seed orthodox?	50 (−94)	Visser (1955)
<i>with desiccation-tolerant pollen (<50–0% RH)</i>		
45 Species of gymnosperms, monocots and dicots	0–36 (Dry to −138)	Pruzsinsky (1960); Visser (1955); Holman and Brubaker (1926)

evolution the module is replicated to form a multi-modular individual and later in evolution the various modules differentiate into a diversity of forms and functions. For example, unicellular algae, multicellular algae with undifferentiated cells, multicellular algae with some cells photosynthetic and other cells reproductive.

A single module of physiological-cum-biochemical assemblage may also evolve and differentiate to suit a variety of functions, e.g. chloroplasts with C_3 photosynthesis, chloroplasts associated with CAM, and chloroplasts differentiated in sheath and mesophyll cells for C_4 photosynthesis. The simplest working hypothesis of desiccation tolerance in angiosperms is that a single mechanism of desiccation tolerance evolved for the survival of reproductive units shed from the plant and consequently subject to air-drying (spores, seed and pollen). For vegetative angiosperm tissue also to show 'resurrection' behaviour, the mechanism controlling the coordinated gene-expression for the implementation of fully-developed desiccation tolerance, must itself undergo further evolution to develop specific expression in non-reproductive cells.

We propose that evolution extended expression of the desiccation tolerance module from the seed/pollen first to the young meristematic vegetative tissue, later to the immature leaf tissue, and lastly to the mature leaf tissue as exemplified in the *Borya* spp. (Table 3). The desiccation tolerance is fully developed even in the *Borya* species where only immature leaf tissue revives (PDT of -528 MPa). This supports the view that a fully developed seed/pollen desiccation tolerance mechanism is being invoked. This hypothesis, an extension of the suggestion that angiosperm vegetative desiccation tolerance derived the developmentally programmed seed tolerance mechanism, was first alluded to by Bewley (as reported by Bewley and Oliver 1992; and later discussed by Oliver *et al.* 2000, 2005; and more recently by Farrant and Moore 2011), and implies a common basic mechanism of desiccation tolerance from early in (or probably well before) the evolution of the angiosperms. Divergences in the mechanism of desiccation tolerance would be expected to arise between foliage, pollen and seed as evolution proceeded. Such divergences in organ specificity of the mechanism are likely to involve the regulatory elements rather than the elements implementing desiccation tolerance.

Several features are shared by desiccation tolerance in pollen, in seed and in resurrection-angiosperm foliage. The airdry cells are very stable. They tolerate temperatures below -70°C and high temperatures. They may remain viable for weeks to years, depending on the ambient conditions, the organ and the species. The dry cells contain substances opposing damage by free-radicals, carotenoids, catalase and peroxidase. Physically protective substances in the dry cells, particularly sucrose and LEA proteins, are thought to make a large contribution to desiccation tolerance. A large number of protein species are conserved in the dry tissues. Polysomes are not present in the dry cells, but ribosomes and mRNA (possibly as messenger ribonucleoprotein complexes) are present and so are available to support protein synthesis during rehydration. Mitochondria persist in dry cells and respiration recovers in the first 30–60 min of rehydration. Dry viable cells are injured by storage in air of $>50\%$ RH (-94 MPa), in which case the higher the humidity is, the more rapidly cells are damaged. In those desiccation-

tolerant tissues which are prone to injury during storage in air of $<50\%$ RH, the lower the RH in the $<50\%$ RH range then the greater is the injury. Rehydration of dry anabiotic cells is swift (1–48 h).

Divergent features also exist between dry desiccation tolerant organs. Dry pollen contains starch, but there is little or no starch in resurrection plant foliage and embryos. Whereas dry resurrection plant leaves contain minor amounts of the protectant disaccharide trehalose, none are detectable in seed and pollen. Nitrogen content is low in embryos ($\sim 0.1 \text{ mg g}^{-1}$ FW) compared with pollen and resurrection plant foliage ($3\text{--}4 \text{ mg g}^{-1}$ FW). Protein synthesis in pollen reaches maximum rates at 1 h of rehydration, embryos at ~ 4 h rehydration and resurrection plant foliage at ~ 16 h. Longevity in the dry state covers 0.5 days to 3 years for pollen (Visser 1955), 0.5–5 years for resurrection plant leaves and <1 to approximately 1900 years for seed (1900 for date seed *Phoenix dactylifera* L. at Masada, Israel, Sallon *et al.* 2008). The ecological need for longevity varies greatly from hours to days for pollen to reach a stigma, or 5–10 months of the subtropical dry season endured by dry resurrection foliage or years to centuries for seed to outlast climatic catastrophes or suppression by ecological succession. The surprising longevity of some pollens, compared with an ecological need of hours or days for pollen to reach a stigma, is explicable if (i) ecological selection for a long-lasting seed-bank sets the maximum dry-state longevity for the basic desiccation tolerance mechanism, and (ii) with lower selective pressure acting for dry-state longevity in foliage and pollen than in seeds, the expression of the potential longevity tends to decline at differing evolutionary rates in foliage and in pollen and in different species.

Desiccation-tolerance in other photosynthetic organisms

The major physiological and morphological features of the evolutionary antecedents of the angiosperms are exemplified by a series of extant plant groups. Tracing phylogenetically from the fern-allies, ferns, towards the mosses and liverworts, and algae, we pass from plants with mechanisms for controlling water loss (cuticles and stomata) and for replacing lost water (xylem and roots or rhizoids) to plants with little or no drought avoidance mechanisms and whose water potential fluctuate markedly with ambient water potentials ('poikilohydrous' plants). Poikilohydrous plants have much greater exposure to selection for desiccation tolerance than do drought-avoiding angiosperm species.

In the fern-allies desiccation-tolerance is met in the shoots of several species of *Selaginella* and in the corms in several *Isoetes* species. All of the investigated desiccation-tolerant *Selaginella* species have foliage PDT below -311 MPa. The PDT values of foliage of desiccation-tolerant fern species range more widely, from <-600 to -127 MPa, with 56% of desiccation-tolerant species in the -434 MPa and lower range. The proportion of desiccation-tolerant species (per total number of species in the taxon) is ~ 10 times greater in the ferns than in the angiosperms (Gaff 1972). In addition, recent work has determined that desiccation tolerance, with PDT values between -94.6 and -220 MPa, is more widely distributed in the gametophytes of ferns (Watkins *et al.* 2007).

The PDT distribution of mosses is broadly bimodal with low representation in the mid PDT range (Table 2). Desiccation-tolerant moss species are common — 25% of species have PDT values below -220 MPa. A preliminary drought stress (equilibration to -5.5 MPa) improves the PDT in moss species and (as in the monocot *Borya constricta*) it induces desiccation tolerance in 49 of the tested species (Table 2). The distribution of thallus PDT among the species of liverworts (Hepaticae) is a continuum (as if desiccation tolerance were newly evolving in the taxon) with 10% of species having a PDT below -220 MPa. The PDT of seven species improved during dry months, suggesting a degree of facultative alteration in the PDT in these cases (Clausen 1952).

Among the algae, several species in the Phaeophyta (brown algae), Rhodophyta (red algae) and Bacillariophyta (diatoms) have desiccation tolerant vegetative tissue (Table 6). Desiccation-tolerant species are especially well represented in the Chlorophyta (green algae) whose habitats spread across marine, freshwater and terrestrial environments (Table 6). Desiccation tolerance occurs in vegetative cells of 25 species, in the reproductive cells of 19 species and in both cysts and resting cells in 29 species.

Desiccation tolerance was reported in Table 7 in 59 species of present day Cyanobacteria (blue-green bacteria), specifically in the vegetative cells in 12 species (eight genera). Evidence from DNA sequencing indicates that the chloroplasts of algae and of the other plant groups are derived from endosymbiotic cyanobacteria-related cells captured by the host and incorporated during evolution into eukaryote cells (Gould *et al.* 2008). It is conceivable then that the cyanobacterioid endosymbiont may have carried the genetic information for desiccation tolerance across with it into the evolving composite host-cum-symbiont cell. The same reasoning may apply also to the mitochondria derived from bacterioid endosymbionts. Neither of these possibilities necessarily precludes pre-existing desiccation tolerance in the host eukaryotoid organisms, which also were probably small and poikilohydrous.

In all the groups (fern-allies to hepatics to algae) spores rather of seeds are the major reproductive and dispersive structures. In species where long-distance dispersal depends on spore longevity, the aerial spores of terrestrial plants need to be desiccation tolerant. Desiccation-tolerant spores have been reported from pteridophytes (e.g. 33 fern species with nonchlorophyllous spores and five fern species with

chlorophyllous spores; Pence 2000) and from the distantly related sphenophyte *Equisetum hyemale* L. (spore PDT approximately -525 MPa, Lebkuecher 1997). Desiccation-tolerant spores are common in the mosses dispersed across oceans (Van Zanten 1978). Among the algae, 20 species are known to possess desiccation-tolerant reproductive cells (Table 6). We note that desiccation-tolerant oospores are produced by all eight of the species studied in the aquatic genus *Chara* (*Ch. canescens* Desvaux and L.Deslongschamps, *Ch. contraria* A.Braun and Kützing, *Ch. evoluta* Allen, *Ch. globularis* Thuill., *Ch. rusbyana* Howe, *Ch. sejuncta* A.Braun, *Ch. zeylanica* Klein; Davis 1972). The chlorophyte Order Charales is close to the progenitors of the bryophytes and of the vascular plants (McCourt *et al.* 2004). Beyond the algae, at least 10 species of cyanobacteria have desiccation-tolerant akinetes (Table 7).

The molecular biology of desiccation tolerance

As desiccation-tolerant angiosperms dry, they accumulate compounds that can physically protect cell membranes and protein against the disorganising effects of high ion concentrations and dehydration. Marked accumulation of sucrose and lesser accumulations of other known protectants (e.g. raffinose and trehalose) are general in the desiccation-tolerant angiosperms (Ghasempour *et al.* 1998b). The main protectant, sucrose, in desiccation-tolerant angiosperm plants, is constitutively at a high content in the desiccation-tolerant moss *Tortula ruralis* (Bewley 1979). A wide variety of osmoregulant solutes, including sucrose, are produced in algae and cyanobacteria in response to environmental stress (Grant *et al.* 1976; Reed *et al.* 1984).

Trehalose appeared to be the main protective sugar accumulated in the desiccation tolerant fern-allies *Selaginella lepidophylla* (Hook. and Grev.) Spring and *Selaginella sartorii* Hieron. (Iturriaga *et al.* 2000). However, recent work has demonstrated that trehalose also accumulates to higher levels in the desiccation sensitive species *Selaginella moellendorffii* than it does in *Selaginella lepidophylla* under the same conditions (Yobi *et al.* 2012). Thus it appears that trehalose accumulation is not necessarily required for desiccation tolerance and indeed in the same study it became clear that sucrose, as in other resurrection species, is the main protectant.

Table 6. Algae with desiccation-tolerant cells, according to the data collated by Davis (1972)

Only those species for which the desiccation tolerant cell type was specified are included. Species for which drying was not clearly sufficient for air-dryness (i.e. drying was <24 h) are excluded

Taxon	Desiccation-tolerant vegetative cells	Desiccation-tolerant reproductive cells	Desiccation-tolerant cysts and resting cells
Chlorophyta (Green algae):72 genera: 119 spp. ^A	17 genera: 25 spp.	11 genera: 19spp.	26 genera: 29 spp.
Phaeophyta (Brown algae):2 genera: 4 spp. ^A	2 genera: 4 spp.	—	—
Rhodophyta (Red algae):4 genera: 4 spp. ^A	4 genera: 4 spp.	—	—
Bacillariophyta (Diatoms):8 genera: 28 spp. ^A	1 genus: 1 sp.	—	—
Other algal groups:15 genera: 21 spp. ^A	1 genus: 1 sp.	1 genus: 1 sp.	9 genera: 9 spp.
Totals	25 genera: 35 spp.	12 genera: 20 spp.	35 genera: 38 spp.

^ATotal desiccation tolerant whether or not the cell type was specified.

Table 7. Cyanobacteria with desiccation-tolerant cells, from the data collated by Davis (1972) (59 desiccation-tolerant species in 22 genera) Only those species for which the desiccation tolerant cell type was specified and where drying was clearly sufficient for air-dryness are listed here

Vegetative cells desiccation tolerant:	Akinetes desiccation tolerant:
<i>Calothrix scopulorum</i> young cells	<i>Anabaenopsis circularis</i>
<i>Cylindrospermum macrosporum</i> ^A	<i>Calothrix scopulorum</i>
<i>Lyngbya limnetica</i>	<i>Cylindrospermum licheniforme</i>
<i>L. versicolor</i>	<i>Cyl. macrosporum</i> ^A
<i>Nostoc ellipsosporum</i>	<i>Cyl. musicola</i>
<i>N. muscorum</i> ^A	<i>Nostoc muscorum</i> ^A
<i>Oscillaria antliaria</i>	<i>N. rivulare</i>
<i>Oscillatoria tenuis</i> var. <i>tergestina</i>	<i>Scytonema mirabile</i>
<i>Phormidium gelatinosum</i>	<i>Tolypothrix distorta</i>
<i>P. luridum</i>	<i>Tol. tenuis</i>
<i>P. rubroterricola</i>	–
<i>Scytonema mirabile</i>	–
Total: 12 spp. in 8 genera	10 spp. in 6 genera

^ABoth vegetative cells and akinetes are desiccation-tolerant in species marked.

Protection by sucrose is augmented by its interaction with LEA-proteins that also accumulate in response to drought stress and in maturing seeds. The effectiveness of LEA-protein may be increased by its greater tertiary structure at the high solute concentrations in airdry tissue (Goyal *et al.* 2005a, 2005b). A LEA-like protein in *Craterostigma plantagineum* binds to two enzymes, stabilising their activity after repeated cycles of dehydration (Petersen *et al.* 2012). Wider roles than stabilisation of protein have been suggested for LEA4 proteins, including increased drought avoidance, membrane permeability and antioxidant enzyme activity (Liu *et al.* 2009). Concurrent action of the various LEA proteins may be needed for desiccation tolerance (Oliver 2007). LEA proteins accumulate late in the maturation of orthodox seed when the embryo is becoming desiccation tolerant. They also accumulate in other desiccation-tolerant organs, pollen and resurrection plant foliage, as they dry and in drought-stressed desiccation-sensitive angiosperms. LEA-like proteins are also found in some bacteria and in nematodes (Browne *et al.* 2002). In contrast to the induced adaptive desiccation of angiosperm resurrection species, the constitutive desiccation tolerance of *Tortula ruralis* does not involve accumulation of LEA during dehydration but is based on a constant high sucrose content and on synthesis of rehydrin-proteins encoded by RNA transcribed during dehydration and conserved in the dry moss plants (Oliver and Bewley 1997) and presumably other macromolecules essential for revival are also conserved. The constitutive levels of LEA proteins in this moss have not been determined.

Dry resurrection plants retain protective mechanisms against chemical injury by stress-related free-radicals, e.g. quenching by carotenoids and the antioxidant action of glutathione-ascorbate cycle enzymes and catalase (Gaff *et al.* 2009; Oliver *et al.* 2011b; Gechev *et al.* 2012; Dinakar *et al.* 2012). Rises in the contents of tocopherol, putrescine and agmatine, lipid anti-oxidants, may safeguard membrane integrity during drying of *Sporobolus*

stapfianus (Oliver *et al.* 2011a). Reactive oxidants accumulate less as desiccation tolerance is induced in slow drying plants of the moss *Fontinalis antipyretica* L. ex Hedw., compared with plants damaged by rapid drying (Cruz de Carvalho *et al.* 2012). Antioxidant mechanisms are probably general in the eukaryotes. Antioxidants may trace back to the cyanobacterioid endosymbiont progenitor of the plant chloroplast. In the cyanobacterium *Spirulina platensis* (Gomont) Geitler high antioxidant activity stems from its contents of carotenoids, α -tocopherol and phenolic compounds (Abd El-Baky *et al.* 2009).

Dehydration-induced pigment binding ELIP proteins may protect plastids against injury from high-light induced free-radical generation and ultraviolet radiation (Bartels *et al.* 1992) and their induction is a common feature during dehydration of resurrection plants (Gechev *et al.* 2013; Dinakar *et al.* 2012). These proteins are thought to play a role in non-photochemical quenching of light energy and the protection and repair of photosystems during desiccation and appear to be an active participant in the drying induced accumulation of protective pigments in resurrection plants (Dinakar *et al.* 2012). ELIP proteins evolved early in the evolution of photosynthetic organisms and their ancestry and probable role in protection from light induced oxidative stress can be traced back to the cyanobacteria (Hedddad and Adamska 2002).

In contrast with cryptogamic desiccation-tolerant species, many desiccation-tolerant vascular plants have physical barriers such as light-scattering epidermal hairs or scales and drought-induced folding or rolling-up of foliage that shade the mesophyll and meristems. These adaptations diminish the formation of radiation-induced free-radicals, as also do epidermal pigments in numerous dry angiosperm resurrection plants (Dinakar *et al.* 2012) and an absence of chlorophyll in many dry monocot examples (poikilochlorophylly) (Gaff and Hallam 1974).

Strikingly higher contents of asparagine, glutamine and allantoin in fully hydrated leaves of *Sporobolus stapfianus* than in *Sporobolus pyramidalis* P.Beauv. suggests the importance of spatial and metabolic mobility of a reserve of nitrogen in the desiccation-tolerant species (Oliver *et al.* 2011a). Their contents declined at intermediate RWCs in *Sporobolus stapfianus*. A later resurgence at 20% RWC in asparagine and glutamine levels may reflect detoxification of ammonia (produced below 30% RWC in this resurrection grass, Martinelli *et al.* 2007) as well as providing a reserve of organic nitrogen compounds for metabolic recovery during rehydration. Among early-evolved organisms, some species of bacteria have long been noted for a versatile metabolism of inorganic and organic nitrogenous molecules.

The link between nitrogen metabolism, antioxidant pathways, and desiccation tolerance is highlighted by the significant accumulation of several gamma glutamyl amino acids (GGAA) as leaves of *Sporobolus stapfianus* dry below 30% RWC (Oliver *et al.* 2011a). These compounds are postulated to be involved in the replenishment of 5-oxoproline in the glutathione biosynthetic pathway but their exact role in desiccation tolerance is not known. That the accumulation of these compounds has significance in desiccation tolerance and its evolutionary journey is implied by the fact that these compounds also accumulate in the later stages of drying of *Selaginella*

lepidophylla (Oliver *et al.* 2011a; Yobi *et al.* 2012) and the moss *Tortula ruralis* (Oliver *et al.* 2011a).

Chaperonins (some of which are stress-inducible) including some heat shock proteins (HSP) that associate with and protect other protein species against denaturation are found from the algae to angiosperm resurrection plants *Boea hygrometrica* F. Muell. and *Sporobolus stapfianus* (M Oliver, P Payton, unpubl. data). A lowered abundance of a large HSP (70 kDa) inhibitor of the synthesis of small HSPs probably allows HSP18 to accumulate (Kim and Schoffl 2002; Oliver *et al.* 2011b). Small HSPs are associated with induction of desiccation tolerance in plants of *Craterostigma plantagineum* Hochst. and in seeds (Alamillo *et al.* 1995; Wehmeyer and Vierling 2000). Overexpression of a sunflower-seed transcription factor HaHSFA9 in transgenic tobacco seedlings resulted both in ectopic expression of a seed-specific small HSP and in an increase in seedling survival of severe drought stress (−40MPa) that were not accompanied by increased levels of the protective sugars and LEA (Prieto-Dapena *et al.* 2008; Cushman and Oliver 2011).

Aquaporins act as channels for cross-membrane transfer of small molecules, including water and gas molecules. They occur in both resurrection plants and nonresurrection plants and are distributed widely in the plant and in animal kingdoms (Bartels *et al.* 2007; Danielson and Johanson 2008). Genes encoding them are highly expressed in drying resurrection plants, in which the encoded proteins may be important in hastening the intracellular movement of water and of respiratory gases (Neale *et al.* 2000).

Anabiotic leaves of angiosperms retain high contents of ATP, reflecting an excess production compared with usage during drying and constituting an energy reserve to support the resumption of cell metabolism early in rehydration (Gaff and Ziegler 1989). Although photosynthesis may be restricted at 30% RWC in *Sporobolus stapfianus* by stomatal closure and reduced abundance of the Rubisco large subunit, synthesis of ATP and NADPH is supported by increased abundance of cytoplasmic and plastidic enzymes of glycolysis, a process well established in algae and microorganisms (Oliver *et al.* 2011b). Lowered abundance of malate dehydrogenase may reduce metabolism of pyruvate in the TCA respiratory cycle (Oliver *et al.* 2011b).

Desiccation-tolerant *Craterostigma plantagineum* and *Sporobolus stapfianus*, as their water content falls, display major changes in the complement of proteins that reflect protein turnover even at very low water contents. Many seemingly novel proteins appear, several of which correlate well with the induction of desiccation tolerance. The main group of specific transcripts whose abundance increases during drying encode proteins putatively are involved in membrane protection or membrane function (Bartels *et al.* 1990; Gaff *et al.* 2009). Other 'more-abundant' transcripts encoded proteins that are implicated in protein turnover or have regulatory roles. Prominent in this regard is the protein initiation factor eIF1, whose abundance increases with moderate stress to reach a maximum at air-dryness (Neale *et al.* 2000; Oliver *et al.* 2011a, 2011b).

The phytohormone abscisic acid (ABA) induced desiccation tolerance in hydrated leaves of *Borya constricta* and in callus of

Craterostigma plantagineum (Gaff and Loveys 1984; Bartels *et al.* 1990). ABA also activated strong expression of genes for some of the above transcripts, e.g. genes encoding the protective LEA group 2 (dehydrin) and LEA group 3 proteins and encoding the membrane-associated proteins aquaporin and a pore-like protein (Bartels *et al.* 1990; Gaff *et al.* 2009). Synthesis of LEA proteins appears to be universally inducible in plants by ABA. Immunological evidence indicates that abscisic acid is widely distributed in bryophytes and can evoke drought resistance in some mosses (Hartung *et al.* 1987; Werner *et al.* 1991). ABA is released under salt stress from the alga *Dunaliella* sp. and the cyanobacterium *Trichormus variabilis* (Kützing) Komárek and Anagnostidis (Tietz *et al.* 1989; Zahradníčková *et al.* 1991).

More information on the mechanisms of desiccation tolerance in cyanobacteria and in plants is presented in detail in the wide-ranging chapters by Lüttge *et al.* (2011).

Large comprehensive transcriptome profiling studies of drying and rehydration tissues have been reported for two dicot resurrection plants, *Craterostigma plantagineum* (Rodriguez *et al.* 2010) and *Haberlea rhodopensis* (Gechev *et al.* 2013), in an attempt to build a more comprehensive assessment of the cellular protection aspects of the molecular mechanisms of tolerance in these plants (reviewed by Gechev *et al.* 2012). These studies, in general, support the proteomic and metabolomic aspects of the desiccation responses as discussed earlier but they also point to novel aspects that offer new insights into the cellular protection mechanisms and the processes that are important for successful recovery upon rehydration. Of particular note are the transcription factor and signalling associated gene transcripts that respond to desiccation and rehydration. The genes these transcripts represent are targeted as the underlying genetic components that control the massive reprogramming of cellular activity that determines desiccation tolerance (Gechev *et al.* 2012). The larger transcriptome studies also support earlier more targeted gene expression profiling studies with the resurrection monocot *Xerophyta humilis* that offered the first transcriptomic evidence for the hypothesis that vegetative desiccation tolerance in the resurrection angiosperms derived from the genetic program that is operational in the seed (Illing *et al.* 2005). Recent studies centred on the dissection of the desiccation tolerance genetic program from that of seed development and germination in seed based systems also support this notion (Buitink *et al.* 2006; Maia *et al.* 2011).

Mechanisms involved in desiccation tolerance arose early in plant evolution

The major metabolites and proteins that are associated with desiccation tolerance are wide-spread in desiccation-sensitive as well as desiccation-tolerant angiosperms and throughout the plant kingdom, as are the tolerance-associated processes of ATP-production, protein turnover, DNA-repair and removal of free-radicals. Since the above substances and processes occur in both desiccation-sensitive and desiccation-tolerant species and tissues, their role in implementing desiccation tolerance presumably involves factors such as their concentration, their cellular location, their interactions and the timing of their accumulation or diminution in content. The metabolic assemblage

implementing desiccation tolerance in angiosperms is subject to intense natural selection in the dehydrated seed or pollen of each generation, consequently much of the implementation mechanism is likely to be highly conserved. Just as different types of switches can be chosen to open and close an electrical circuit, it is conceivable that the mechanism which regulates the implementation of desiccation tolerance may vary from one plant taxon to another as the happenstances of discrete evolutionary events separated in geological time. The observation, that foliage desiccation tolerance appears to arise independently in the several angiosperm families endowed with such tolerance, supports this notion. A wide array of systems of transduction and regulation are available in plants. Regulatory differences also may be implied by the specificity of expression of desiccation tolerance in seed, pollen, leaves, corms or tubers, and to the stage of development (in meristematic, in young and mature leaves but never in senescent tissue). The different responses of PDT to exogenous ABA in diverse families supports the view that substantial differences in the regulatory mechanism of desiccation tolerance may exist. Exogenous ABA induced full desiccation tolerance in hydrated leaves of the monocot *Borya constricta* and in the hydrated callus of *Craterostigma plantagineum* (Scrophulariaceae). ABA treatment stimulated a small improvement in the desiccation tolerance of detached shoots of the dicot *Myrothamnus flabellifolia*, but evoked only a slight improvement in the PDT (which remained in the desiccation-sensitive range) of free cells of the grass *Sporobolus stapfianus*.

In addition to the ABA-regulated induction path for desiccation tolerance in *Borya constricta*, a complementary non-ABA pathway of gene activation for DT induction was postulated in this species (Gaff 1981). In *Craterostigma plantagineum*, a short interference RNA (derived from the 'desiccation-tolerant gene' *CDT*), together with calcium- and phospholipid-based transduction pathways are implicated in a non-ABA regulatory path, that complements the major ABA desiccation-tolerance-inductive pathway (Bartels 2005; Hilbricht *et al.* 2008). A non-ABA pathway dominates the regulation of desiccation tolerance induction in *Sporobolus stapfianus* (Gaff and Loveys 1993). In the drought-tolerant *Boea crassifolia* a drought-induced *MYB* gene, *BcMYB1*, appears to be implicated in a non-ABA pathway regulating plant responses to drought stress (Shen *et al.* 2004) as do some of the transcription factors upregulated by dehydration in *Haberlea rhodopensis* (Gechev *et al.* 2013).

Environmental stress induces ABA production in several cyanobacteria and algae, but the concentrations of exogenous ABA required to elicit observable effects exceed normal physiological levels (Hartung 2010). Adaptive effects of drought induced ABA synthesis arose with the colonisation of the terrestrial environment by bryophytes and with the evolution of stomata and of water conducting tissue in early embryophytes (Raven 2002; Hartung 2010). ABA is pre-eminent in the angiosperms in the drought stimulation of many drought avoidance mechanisms and in the induction of desiccation tolerance in some resurrection angiosperms. The greatest role of ABA in resurrection angiosperms is reached in the poikilochlorophyllous monocots, in which exogenous ABA induces desiccation tolerance in fully-hydrated leaves while it induces loss of their chlorophyll (Gaff and Churchill 1976).

Hartung's (2010) review implies that, in the algae and cyanobacteria, any occurrence of drought-induced desiccation tolerance would probably involve non-ABA pathways. In the resurrection grass *Sporobolus stapfianus* jasmonate and brassinosteroids are implicated in non-ABA induction of desiccation tolerance (Ghasempour *et al.* 2001).

Plants of many drought-sensitive species die at ~30% RWC. Comparison of the leaf proteome of *Sporobolus stapfianus* plants at the critical 30% RWC with those of fully-hydrated plants disclosed altered abundances of several proteins that influence the structure and function of chromatin indicating that dehydration may affect these (Oliver *et al.* 2011b) (cf. two drought-upregulated proteins, one with a SNF2/helicase domain and a chloroplast RNA-binding protein, in desiccation-tolerant *Xerophyta humilis* (Baker) Dur and Schinz; Collett *et al.* 2004). Two such proteins in *Sporobolus stapfianus* included a protein able to bind phosphorylated protein and a protein receptor for a specific kinase that is required for the perception of brassinosteroid (BR) and the transduction of its signal (Oliver *et al.* 2011b). The latter observation is consistent with exogenous brassinosteroids' ability to improve the PDT of free cells of this species and strengthens the case for involvement of brassinosteroids in the induction of its desiccation tolerance (Ghasempour *et al.* 2001). Brassinosteroids were detected in *Sporobolus stapfianus* leaves but drought-induced increases in the content (JM Sasse, DF Gaff, unpubl. data) were not confirmed in a second test. Treatment of free cell suspensions with methyl jasmonic acid (MJA) or brassinosteroid gave identical improvements in the PDT of this species (Ghasempour *et al.* 2001). MJA and BR (applied separately) altered the expression of several genes: some MJA-induced changes in the proteome were identical with BR-induced changes, other MJA-induced changes differed from BR-induced alterations (Ghasempour *et al.* 1998). Brassinosteroids and MJA are found across the plant taxa from algae to angiosperms (Yokota *et al.* 1987; Collén *et al.* 2006).

Despite the evolving complexities of the mechanism of desiccation tolerance and its regulation, the taxonomic variation of these in the angiosperms is small enough in the Gesneriaceae that hybrids of resurrection gesneriad species in different genera are as desiccation tolerant as their parent species (Table 8).

Phases in the induction of desiccation tolerance

Even before any stress arises the resurrection grass *Sporobolus stapfianus* is constitutively better prepared to face the initial stress than is the desiccation sensitive *Sporobolus pyramidalis* (Oliver *et al.* 2011a). Metabolome comparisons between these two grass species when both were fully hydrated, found higher concentrations of osmotically-active solute (osmoregulation and protection roles) and of nitrogen metabolites (retranslocation into young tissue and reserve roles) in *Sporobolus stapfianus* than in the sensitive species (Oliver *et al.* 2011a). Similar observations have recently been reported for a sister group comparison between *Selaginella lepidophylla* and *Selaginella moellendorffii* (Yobi *et al.* 2012).

Soluble protein extracts from detached leaves of *Borya constricta* showed three distinct phases while they were

Table 8. The protoplasmic drought tolerance (PDT) of artificial hybrids of *Gesneriad* species where the parent species have desiccation-tolerant leaf tissue

Leaves, detached from wilted potted plants, were equilibrated to air of a range of humidities, then rehydrated in water for 24 h and examined for recovery of crisp full turgor and the ability of cells to accumulate neutral red. Plants were kindly supplied by A Kress, Botanischer Garten München and by Takayuli Kawahara, University of Tokyo. Hybridisations (A and B) were by O Schwarz and Van Dedem (Halda 1979)

Parent species 1	PDT of species 1	Parent species 2	PDT of species 2	Hybrid species Sp 1 × Sp 2	PDT of hybrid
A. <i>Jankaia heldreichii</i> Boiss	2% RH	<i>Ramonda myconii</i> (L.) H.Reichenbach	5–30% RH	<i>Jankaemonda vandedemii</i> Halda	2–11% RH
B. <i>Opithandra primuloides</i> (Miq.)B.L.Burt	2–30%, 0.5–1 cm young leaves	<i>Briggsia aurantiaca</i> B.L.Burt	5–30%	<i>Brigandra calliantha</i> (O.Schwarz) Jungnickel	2–11%

equilibrated to air of 96% RH to induce desiccation tolerance. Electrophoresis patterns (PAGE 1D) of the first and second phases differed markedly from each other and from the control (100% RH), whilst in the third phase (desiccation tolerant leaves) the pattern of the control was largely restored but with an increased abundance of low molecular weight proteins (probably including LEA proteins) (Daniel and Gaff 1980).

Two main phases of major proteomic changes occurred in drying *Sporobolus stapfianus* plants. In the first phase: (85–60% RWC) interchange of materials with the rest of the plant is important (hormonal signalling and import of remobilised carbohydrate, minerals and nitrogenous compounds from aging leaves) since leaves detached before 60% RWC do not become desiccation tolerant with further drying (Kuang *et al.* 1995). Metabolome studies indicate that this phase ‘instigates a metabolic shift towards the production of protective compounds’ (Oliver *et al.* 2011a) that was not seen in the desiccation sensitive *Sporobolus pyramidalis*. The antioxidant tocopherol accumulated only in *Sporobolus stapfianus* in this phase, a lipophilic compound that is important in protecting cell-membranes lipids from oxidative damage (Oliver *et al.* 2011a). Successful completion of the first phase allows the initiation of the second phase: (51–37% RWC, which is approaching the death point of desiccation-sensitive plants) a phase that proceeds in drying leaves independently of any connection with the plant. Protective sugars and LEA proteins accumulate rapidly, measures to prevent toxicity from oxidant free radicals and from ammonia are evident whilst nitrogenous compounds are remobilised ready to support recovery-metabolism in the event of subsequent leaf rehydration (Martinelli *et al.* 2007; Oliver *et al.* 2011a) – processes that are seen widely in resurrection species at these extreme water deficits (Illing *et al.* 2005; Farrant 2007; Peters *et al.* 2007).

The presence of the above phases and their complexity raise the possibility of a diversity of regulatory mechanisms (acting at different times as drying proceeds), which are the products of past evolution and are substrates for ongoing evolutionary change.

Conclusion

Our interpretation of the above information postulates that desiccation tolerance in the angiosperms had its distant evolutionary origins in poikilohydrous unicells that contributed to the formation of the eukaryotic plant cell. Desiccation tolerance was carried forward into the plant cell probably from cyanobacteria-related endosymbionts (evolving to chloroplasts), possibly also from other bacteria-related

endosymbionts (to mitochondria) and possibly also from the host polychromosome-nucleate cells. The molecular species implementing desiccation tolerance in the angiosperm are largely present in the cyanobacteria and in the algae.

Desiccation tolerance is expressed in both reproductive and vegetative cells of many species of algae, bryophytes, ferns and fern allies. The seed or pollen are desiccation tolerant in almost all angiosperms, probably an extension into the angiosperm reproductive structures of the desiccation tolerance of the spores of non-seed-bearing ancestral taxa. We propose that desiccation tolerance of seed/pollen has become expressed in the vegetative angiosperm tissue of resurrection angiosperms.

Constitutive desiccation tolerance is probably appropriate for the rapid drying of poikilohydrous unicells, but it is found also in some bryophytes and angiosperms. An early evolutionary appearance of facultative adaptive desiccation tolerance in vegetative tissue is seen in some bryophytes and such facultative tolerance persists in the angiosperms — whose vascularisation and drought avoidance allow ample time for induction of desiccation tolerance during drying.

ABA emerges as ‘stress hormone’ in the bryophytes. In the angiosperms, ABA accumulation under drought stress stimulates many diverse mechanisms of plant drought resistance. Its role in inducing desiccation tolerance in foliage is maximised in the poikilochlorophyllous monocot resurrection plants such as *Borya constricta*, whereas in the homoiochlorophyllous grass *Sporobolus stapfianus* a non-ABA pathway regulates the induction of desiccation tolerance, albeit with concomitant activity of several ABA-responsive genes.

Desiccation tolerance in foliage arose independently in several angiosperm families. This raises the possibility that different pathways regulate the implementation of desiccation tolerance in the diverse families. If so, genetic alterations in, for example, a cereal species to induce the expression of its seed-mechanism of desiccation tolerance in its foliage would need an understanding of the regulatory mechanism in a related resurrection grass or in the embryo of that cereal species.

Acknowledgments

The authors are grateful to Dr Janet L Gaff for helpful suggestions in the preparation of the manuscript, to Dr Rana Munns for proposing it and for her encouragement, and to our colleagues Dr Cecilia K Blomstedt, John D Hamill and Dr Alan D Neale for helpful discussion. The authors’ research given here was supported by grants from the ARC (A19230441), the Meat Research Corporation (UMON.004) and the Deutsche Forschungsgemeinschaft (MU 94/58–14810/83).

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