agronomy

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Plant Desiccation Tolerance and its Regulation in the Foliage of Resurrection "Flowering-Plant" Species

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Abstract: The majority of flowering-plant species can survive complete air-dryness in their seed and/or pollen. Relatively few species ('resurrection plants') express this desiccation tolerance in their foliage. Knowledge of the regulation of desiccation tolerance in resurrection plant foliage is reviewed. Elucidation of the regulatory mechanism in resurrection grasses may lead to identification of genes that can improve stress tolerance and yield of major crop species. Well-hydrated leaves of resurrection plants are desiccation-sensitive and the leaves become desiccation tolerant as they are drying. Such drought-induction of desiccation tolerance involves changes in gene-expression causing extensive changes in the complement of proteins and the transition to a highly-stable quiescent state lasting months to years. These changes in gene-expression are regulated by several interacting phytohormones, of which drought-induced abscisic acid (ABA) is particularly important in some species. Treatment with only ABA induces desiccation tolerance in vegetative tissue of *Borya constricta* Churchill. and *Craterostigma plantagineum* Hochstetter. but not in the resurrection grass *Sporobolus stapfianus* Gandoger. Suppression of drought-induced senescence is also important for survival of drying. Further research is needed on the triggering of the induction of desiccation tolerance, on the transition between phases of protein synthesis and on the role of the phytohormone, strigolactone and other potential xylem-messengers during drying and rehydration.

Keywords: abscisic acid (ABA); acetate; CLAVATA3/Embryo-surrounding region-related 25 (CLE25) peptide; desiccation-tolerant foliage; gene expression; phytohormones; protein complement; resurrection grass; *Sporobolus stapfianus*; strigolactone

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1. Introduction

The ability of leaves to survive dehydration below 5% relative water content is found in a very small proportion of angiosperm species. In most such 'resurrection' species, desiccation tolerance is induced in them in the early stage of drying by the drought stress itself [1]. The occurrence of desiccation tolerance in the foliage of 39 species of grasses (Table 1) is of particular interest in view of the importance of the family Poaceae for agriculture and grazing. (See https://www.youtube.com/watch?v=tGg-_tOiUvA for a summary, including time-lapse video, of resurrection plants reviving).

Table 1. Grass species (Poaceae) with desiccation-tolerant foliage. The number of desiccation tolerant species is given in relation to the total number of species in each genus. Additional details on tolerance limits are noted for each species. Data from [2–9].

Genus & Species No. of Desiccation Tolerant Species/of Total spp. in Genus	Notes on Tolerance Limits		Genus & Species No. of Desiccation Tolerant Species/of Total spp. in Genus	Notes on Tolerance Limits	
Brachyachne 1/10			*Oropetium* 3/4		
B. patentifolia	0% RH	>15 cm	*O. capense*	0% RH	
Eragrostiella 3/5			*O. roxburghianum*	0% RH	
E. bifaria var. *bifaria*	15% RH	G	*O. thomaeum*	0% RH	
E. brachyphylla	0% RH,		*Poa* 1/~500		
E. nardoides	11% RH		*Poa bulbosa*	5% RH	Bs V
Eragrostis 4/350			*Sporobolus* 7/160		
Er. hispida	0–5% RH	Bs, >15 cm	*S. atrovirens*	<4% RWC	
Er. invalida	0% RH	Bs, >30 cm	*S. blakei*	0% RH	Bs
Er. nindensis	0–2% RH	G, >15 cm	*S. elongatus*	<10% RWC	Bs > 30 cm
Er. paradoxa	0% RH	>15 cm	*S. festivus*	0% RH	>15 cm
Micraira 7/14			*S. lampranthus*	0% RH	Bs
M. adamsii	0–2% RH		*S. pellucidus*	0–5% RH	>15 cm
M. lazaridis	-		*S. stapfianus*	2% RH	>15 cm
M. multinervia	>5% RH		*Tripogon* 10/30		
M. spinifera	<7.5% RWC		*T. capillatus*	0% RH	>15 cm
M. subulifolia	5% RH		*T. curvatus*	0% RH	
M. tenuis	<8% RWC		*T. filiformis*	2% RH	>25 cm
M. viscidula	<7.5% RWC		*T. jaquemontii*	0% RH	G
Microchloa 3/4			*T. lisboae*	0% RH	>25 cm
Mi. caffra	0–5% RH	>15 cm	*T. loliiformis*	0% RH	G
Mi. indica	0% RH		*T. major*	0% RH	Bs
Mi. kunthii	0% RH		*T. minimus*	0–2% RH	
			T. polyanthus	2% RH	>15 cm
			T. spicatus	0% RH	

Abbreviations: 'PDT' is leaf Protoplasmic Drought Tolerance = 'RH' the lowest relative humidity of air survived at equilibrium or 'RWC' the lowest relative water content survived; '>numerical value' e.g., >15 = vegetative foliage 15 cm or more tall; 'Bs' = only the basal 1cm of leaf is desiccation tolerant; 'G' = plants recorded as grazed by animals; V = vivaporous bulbils are desiccation-tolerant.

The mechanisms for implementing desiccation tolerance (constitutive stability, protection, and repair) have often been reviewed [10–15]. In addition, stability of extraplastidic lipids is achieved in the resurrection gesneriad *Paraisometrum mileense* W.T. Wang by maintaining low levels of phospholipase D throughout drying and rehydration [16]. This review focuses on the regulation of drought-induced desiccation tolerance, with particular focus on the resurrection grass *S. stapfianus*.

Angiosperm species with desiccation-tolerant foliage emerged in largely unrelated families and they do not form a sequence from early-evolved families to late-evolved families. Desiccation-tolerant foliage has not been observed in the large early-evolved angiosperm families, such as Ranunculaceae and Proteaceae, nor in the earlier groups, the cycads and other gymnosperms. Desiccation-tolerant foliage in the angiosperms did not begin in the foliage of early spermatophytes and then progress through continuous lines of related angiosperm families. In the large grass genera *Eragrostis* and *Sporobolus* only a small proportion of the species are resurrection grasses. Desiccation-tolerant foliage appears to have originated late in the evolution of these genera and in the family [10].

2. Single Basic Mechanism of Desiccation Tolerance in Seed, Pollen and Resurrection Foliage

The small proportion of species with desiccation-tolerant foliage in the angiosperms contrasts markedly with the 95% of angiosperm species that have desiccation-tolerant seed and the 87% of spermatophyte species that possess desiccation-tolerant pollen [10]. Every angiosperm species in which both seed and pollen have been examined has either desiccation-tolerant seed or desiccation-tolerant pollen. It appears that almost all angiosperm species contain the genetic information for desiccation tolerance, even though it is expressed in the foliage of only a few species. The properties associated with desiccation tolerance are similar in foliage, embryos and pollen [10,17]. This supports the simplest working hypothesis that a single functional module for the mechanism of desiccation tolerance is expressed in the seed and pollen of most angiosperms, and in the foliage of only resurrection

species. That is, the appropriate genes are commonly expressed in the reproductive tissues that in each generation are exposed to drying during aerial dispersal-whereas in most angiosperm species, well-developed mechanisms of drought avoidance protect foliage from dehydrating, largely obviating selective pressure for expression in foliage of the seed/pollen mechanism of desiccation tolerance.

3. Predisposing Factors for Evolution of Angiosperm Resurrection Species

Particular angiosperm families appear to have a predisposition for the evolution of foliage-desiccation tolerance. Most resurrection species, for example, occur in the Gesneriaceae, Scrophulariaceae, Velloziaceae and the Poaceae. Some predisposing factors (A–D below) may stem from the importance of shallow soils (often on rock outcrops) in the ecology and evolution of desiccation-tolerant plants. (A) Small seed size (found in all the numerous resurrection angiosperm species inspected by the authors) improves long distance dispersal between rock outcrops. Small assimilate seed-reserves limit the initial depth of root systems, and so plants on shallow soils are liable to drought stress, promoting evolution of desiccation tolerance. (B) Plants have sufficient drought avoidance to colonize shallow soils and to allow sufficient time during drying for drought-induction of desiccation tolerance. (C) For plants with short-stature (linked with small seed size), evolving desiccation tolerance mechanisms may protect them from competition from tall desiccation-sensitive species that succumb to drought on shallow soils. (D) High plasticity in genetic-expression increases scope for drought-driven selection of genes regulating seed/pollen desiccation tolerance to be expressed in the foliage. There is evidence from desiccation-tolerant rotifers that desiccation-prone habitats increase inter-chromosomal transfer of DNA (intra- and inter-species) [18]. Intraspecies proliferation of dehydration-inducible retroelements, such as *CDT-1* transcripts in *C. plantagineum* [19], and conceivably, viral transfer of transposons between species might increase genetic plasticity.

4. Desiccation Tolerance in Plant Taxa That Preceded the Angiosperms

Desiccation tolerance is found in all the extant plant taxa that reflect steps in the evolution of the angiosperms. Although no gymnosperm species is known to have desiccation-tolerant leaves, desiccation-tolerant seed and/or pollen have been reported e.g., in the 'orthodox seed' of the early evolved gymnosperm taxa (cycads, *Ginkgo*), and in the conifers, *Gnetum*, *Ephedra* and *Welwitschia* [20]. The pollen of several conifer species in *Pinus* and *Picea* are also desiccation-tolerant [21,22]. The earlier-evolved taxa, lycopods, ferns, bryophytes and green-algae, all have numerous species with vegetative cells and/or spores that are desiccation-tolerant [10].

This distribution pattern suggests that desiccation tolerance was widespread in terrestrial cryptogams with little or no drought avoidance. As survival became less dependent on desiccation tolerance, more complex plant structures and reproductive mechanisms evolved. As further evolution produced stomata, leaf cuticle, water-conducting xylem, cambium and root systems, the effectiveness of drought-avoidance mechanisms increased and desiccation tolerance was needed mainly in the aerially-spread reproductive structures. Thus, the genes producing desiccation tolerance in seed and pollen were carried forward to the angiosperms, but were expressed in the foliage of only the few resurrection plants [10]. In many resurrection angiosperm species, leaf desiccation tolerance is confined to immature leaves and the meristematic base of monocot leaves (Table 2). Evolution of desiccation tolerant leaves appears to commence in young leaves and progresses with further evolution to mature leaves (but, in the authors' experience, never to senescent leaf tissue), i.e., the expression of genes involved in the regulation of desiccation tolerance is dependent on the developmental stage of the plant.

Table 2. Species with desiccation tolerance confined to immature leaf tissue. Data from the references cited in Table 1 and [23].

DICOTYLEDONS	Tissue Type
Gesneriaceae	
Conandron ramondioides	Young leaf to 1 cm size
Opithandra primuloides	To 2nd youngest leaf (1 cm long)
Stylidiaceae	
Stylidium sp.	Buds only
MONOCOTYLEDONS	
Anthericaceae	
Borya scirpioides	High in 3 youngest leaves
Cyperaceae	
Carex physodes	Bs = ~1 cm at base of the leaf lamina, including the leaf meristem
Carex pachystilis	Bs
Cyperus bellis	Bs
Fimbristylis dichotoma	Bs
Kyllinga alba Nees	Bs
Kyllinga alata	Bs
Kyllinga nervosa	Bs
Mariscus capensis	Bs
Poaceae: see Table 1	

The main organelles of plant cells are considered to derive from the 'capture' of endosymbiotic organisms into the host cell. It is thought that chloroplasts are derived from cyanobacteria [24], mitochondria from bacteria, and that the eukaryote nucleus has origins in the early archaea, with some inflow from bacteria [25,26]. Each of these endosymbionts may have contributed via the derivative organelles to the desiccation tolerance of plants, since desiccation-tolerant species occur in extant archaea (e.g., *Methanosarcina barkeri*, [27]), bacteria [28,29] and cyanobacteria (see [10]).

5. Regulation of the Induction of Desiccation Tolerance in Angiosperms

Several phytohormones are implicated in the regulation of terrestrial-plant desiccation tolerance (specific hormones are discussed in later sections below). Drought-induction alters transport of some hormones, which are carried in the xylem from the roots to the shoot and may act to signal that the soil is losing moisture content [30]. In detached leaves of eight desiccation-tolerant angiosperm species tested, drought stress did not induce desiccation tolerance suggesting that transported hormone signals may be acting in tolerance-induction in intact plants [31]. Desiccation tolerance, however, appeared to be constitutive in *Myrothamnus flabellifolia* Welw [31]. Xylem transport is disrupted relatively early during drought stress when the xylem sap cavitates (at water potentials of −0.7 to −3 MPa; [32,33]). Cavitation would curtail xylem-traffic of phytohormones, which are synthesized in the roots-ABA, acetate, CLAVATA3/EMBRYO-surrounding region-related 25 (CLE25) peptide, cytokinins, auxin, strigolactone, and gibberellins. Recent experiments utilizing *Arabidopsis* suggest that soil water stress elicits a rapid hydraulic response in the shoot that stimulates ABA biosynthesis in the leaves. Hence, the shoot ABA response does not require translocation of ABA from the water stressed roots [34]. Droughted leaves of the resurrection grass *S. stapfianus* synthesize ABA when they are detached, but ABA contents are higher in leaves that remain attached to droughted plants [35]. Wall associated kinases (WAKs) are transmembrane serine-threonine protein kinases, that have been proposed to act as mechanoreceptors which may link hydraulic signals to physiological responses. Induction of desiccation tolerance first becomes evident at ~60% RWC in *S. stapfianus* (Table 3), a water content sufficiently low to produce large changes in the hydraulic pressure on the cell contents. A glycine rich protein (GRP) that may interact with WAK kinase is specifically expressed in desiccation tolerant tissue of *S. stapfianus* [36]. A similar GRP from *C. plantagineum* has been shown to interact with CpWAK1 [37]. These proteins may be a mechanism for linking hydraulic signals to the desiccation tolerance program.

Further water loss decreases transport in the phloem [38], compromising bipolar mass-flow transport of phytohormones in the phloem, including of ABA, azelaic acid, jasmonate, and salicylate [39]. Beyond that point, reactive oxygen species (ROS) movement in the phloem apoplast is feasible, as are also electrical pulses along the phloem cell membrane pathways [39]. Later in drying, vapours of ethylene and of jasmonate may, in theory, still exert effects away from the organs which synthesize them.

Table 3. Beginning of induction of desiccation tolerance in leaves on drying *S. stapfianus* plants. Leaves, detached from plants at different stages of drying, were equilibrated to air of 76% Relative Humidity, then rehydrated 24 h in water and their survival scored as the % of their leaf area that survived. Data based on [35].

Leaf RWC (%)	94	82	66	61	58	49	48	46
Survival % of total leaf area	6	2	0	48	67	59	85	85

The complexity of phytohormones and their transportation raises a potential for development of phased regulation of desiccation tolerance during algal-to-angiosperm evolution. The presence of phases in induction of desiccation tolerance is evidenced by the changes in the complement of leaf proteins observed during drought-induced desiccation tolerance in the resurrection grass *S. stapfianus* [40]. The proteome is influenced by alterations in synthesis of phytohormones, especially ABA, as drought stress intensifies. Ismail et al. [41] emphasised the importance of the timing of successive phases of the induction of plant stress-tolerance. A phase immediately preceding the quiescent desiccated state has been distinguished from the desiccated state by differences in molecular responses in angiosperms [42].

5.1. ABA Induction Pathway for Desiccation Tolerance

Borya constricta is a poikilochlorophyllous resurrection plant (i.e., the foliage loses all chlorophyll during dehydration). Drought-induction of chlorophyll-loss is tightly linked with the induction of desiccation tolerance. The breakdown of chlorophyll in both homoio- and poikilo-chlorophyllous resurrection plants, is by the same Pheophorbide α Oxygenase (PAO)/phyllobilin pathway found in senescing non-resurrection plants and in maturing embryos [43]. Both chlorophyll loss and desiccation tolerance are induced concurrently in detached *Borya* leaves by 2 days of drought stress (in 96% RH air) or by ABA treatment of fully hydrated leaves [1]. Increases in endogenous levels of ABA (up to 14-fold) accompany the drought-induction of desiccation tolerance and yellowing in *Borya* leaves [44]. This suggests that drought-induced accumulation of ABA mediates both responses to drought in intact *Borya* plants. In intact drying *Borya* plants, non-senescent leaves yellow and survive the dehydration, but senescing leaves do not survive even though they also become yellow. ABA-induced chlorophyll-loss in drying immature to mature leaves is linked with desiccation tolerance but it is uncoupled from the process of senescence, which drying normally induces in immature/mature leaves [45].

In the homoiochlorophyllous resurrection plant *M. flabellifolia* (i.e., the foliage retains all chlorophyll during dehydration), 38% of freshly detached leaves survive drying in air of ~0% RH but their final ABA content is not high. Equilibration of detached leaves to 96% RH air raises survival to 80% while endogenous ABA accumulates up to 12-fold at 24 h equilibration [44]. In all these treatments, chlorophyll was retained and ABA appears to be uncoupled from degradation of chlorophyll. On the other hand, senescent leaves lost chlorophyll during drying. In the homoiochlorophyllous resurrection grass *S. stapfianus*, endogenous ABA accumulates late during drying but exogenous ABA produces little improvement in protoplasmic drought tolerance [35,46]. ABA responses in plants are influenced by interconversion of free ABA to an inactive 'bound' glycosylated ABA that is found in the xylem of drought stressed plants [47,48].

The leaves of desiccation-tolerant plant *C. plantagineum* lose about half their chlorophyll as they dry, i.e., it is intermediate between the homoio- and poikilochlorophyllous behaviour [49]. Exogenous ABA induces desiccation tolerance in well-hydrated callus of this species [50]. Several ABA-inducible proteins accumulate in *C. plantagineum* and *S. stapfianus* leaves as they dry, including protective late embryogenesis abundant (LEA) proteins, which become abundant during dehydration in resurrection plants in general and in the embryos of non-resurrection plants [51]. A hydrophilic protein LLA23, which has some similarity to LEA proteins, is induced in pollen by drying or ABA [52]. The *Lilium longiflorum* Thunberg protein, LLA23 may have a regulatory role in mediating stress responsive ABA signalling [53].

These findings indicate that ABA plays a considerable role in mediating the induction of desiccation tolerance in both chlorophyll -losing and -retaining resurrection foliage, in seed and presumably in pollen. That a single exogenous substance, ABA, induces desiccation tolerance in hydrated *B. constricta* leaves, suggests that ABA is a major regulatory factor upstream in a transduction pathway controlling the expression of a large number of genes. ABA is one of several potential signals to foliage that soil water is depleted, leading to long-term drought stress rather than short-term stress of foliage due to transient aerial conditions. Drought-induced ABA transported to the foliage triggers a range of drought resistance mechanisms [48]. It controls the expression of several drought-induced genes in *S. stapfianus* also; but in that species, factors other than ABA appear to be limiting and controlling the transduction pathway for desiccation tolerance [35]. During drought-induction of desiccation tolerance, the action of ABA is uncoupled from drought-induction of senescence. The importance of inhibition of senescence for desiccation tolerance has been raised by [45].

More is known of the ABA induction pathway in seed than in resurrection plants. Lack of endogenous ABA compromises induction of desiccation tolerance in maturing seed and the application of exogenous ABA may induce desiccation tolerance. In the *Arabidopsis* mutants, ABA deficient (*aba*) and ABA insensitive (*abI3*) and the double mutant (*aba* × *abI3*), the seed are slow to desiccate, remain green and lose viability upon drying [54]. Desiccation tolerance was not displayed in seed when the plant was both insensitive to ABA and deficient in ABA [54]. Double mutants lack ABA, acquire only low levels of storage proteins and are deficient in many low molecular weight polypeptides (soluble and bound), some of which are heat stable [54]. A major 100 kDa glycoprotein also shows a decrease in size associated with the *abI3* mutation.

5.2. Transcription Factors and the ABA Induction Pathway for Desiccation Tolerance

Two important ABA receptors are PYR/PYL/RCAR phosphatase 2C-ABA complex in the cytosol and the ABA binding protein, ABAR-ABA complex in the bounding membranes of chloroplasts [55]. If ABA perception through PYR/PYL/RCAR is impaired in *Arabidopsis*, vegetative growth and seed production is reduced and the ABA-insensitive characteristics appear even though other ABA perception mechanisms still function [56]. The large subunit (CHLH) of magnesium-protoporphyrin IX chelatase acts as an ABA receptor in chloroplasts. CHLH catalyzes the ATP-dependent incorporation of Mg into protoporphyrin IX in the biosynthesis of chlorophyll and it also functions in a plastid-to-nucleus signalling pathway [57]. It is possible that Mg-chelatase may be involved in the breakdown of chlorophyll in drying poikilochlorophyllous resurrection plants and in catalysing the resynthesis of chlorophyll in their yellow, rehydrated leaves. The PEBP (phosphatidylethanolamine-binding protein) family is involved in ABA signalling in germinating seeds and in the biogenesis and fluidity of membranes. A putative member of the PEBP family of proteins increased 9-fold in abundance in drying plants of the desiccation-tolerant moss *Physcomitrella patens* (Hedw.) Bruch & Schimp [58].

Comparison of the transcriptomes of tissues of *Medicago truncatula* Gaertner undergoing a transition to desiccation tolerance (in drying seeds and in germinating seeds treated with polyethylene glycol −1.7 MPa osmoticum) showed changed abundance of numerous transcripts for transcription factors (TF), including two crucial TFs, *MtABI3* and *MtABI5* [14]. ABA responsive elements (ABRE)

were present in the promoter regions of those genes exhibiting altered transcript levels—as also was found in comparisons with transcriptomes from dry desiccation-tolerant tissue in a diversity of other species [59]. *Medicago truncatula* mutation studies revealed that *MtABI5* was necessary for osmotic stress to re-establish desiccation tolerance in germinating seed but not during drying in maturing seed. *MtABI5* and *MtABI3* are each upstream of large numbers of genes leading to desiccation tolerance (~14% and ~30% respectively of the genes with changed expression). The ABI3- and ABI5-pathways are considered to be complementary to and interacting with each other. ABI5 acts in the upregulation of two genes (*EM1* and *EM6)* that encode LEA protectants [60]. ABA-responsive genes downstream of MtABI3 include genes for heat stress proteins (HSPs), genes involved in protein-repair, in microtubule-processes and encoding LEAs [14]. LEA proteins appear to be ubiquitous in the tissue of numerous desiccation tolerant life forms [61]. Comparative studies of dehydrated desiccation-tolerant species indicated that the LEA-5 (with higher hydrophobic amino-acid residues than other LEA groups) plays a crucial role in the induction of desiccation tolerance in drying tissue [59]. Increases in abundance of *LEA-14* transcripts were correlated with desiccation tolerance in all species compared by Costa et al. [59].

WRKY transcription factors (TFs) bind to the W box, TTGAC(C/T), in promoters of specific genes to activate their expression [62]. The WRKY TFs occur in developing seed and are key agents in the ABA regulatory pathways that respond to environmental stresses, senescence, dormancy and germination of seeds. Various WRKY TFs act in different pathway 'hubs' downstream from the reception of ABA by ABAR and by PYR/PYL/RCAR [55]. In *Arabidopsis*, before drought stress AtWRK40 represses expression of *ABI5*. ABA binding to receptors removes this repression of ABI5, leading to activation of *AtWRKY63*, whose encoded protein in turn activates ABA-response genes, for instance *RD29A* and *COR47*.

In the foliage of the resurrection gesneriad, *Boea hygrometrica* (Bunge) R.Br., BhWRKY1, induced by drought stress and by ABA, binds to the promoter of the galactinol synthase gene *BhGolS1* [63]. Overexpression of WRKY in rice induced expression of galactinol synthase and raffinose synthase genes and also improved drought tolerance [62]. Raffinose may play a protectant role in drying resurrection plants [64]. WRKY and galactinol synthase genes are possibly involved in an ABA-signalling pathway during the induction of desiccation tolerance in ABA-responsive resurrection plants, such as *B. constricta* [1]. ABA may aid the formation of protective sugars, especially sucrose, that assist ABA induction of desiccation tolerance in maturing *Arabidopsis* seed [54].

When desiccation tolerance was re-established in germinating seeds of *Arabidopsis thaliana* (L.) Heynh. by treatment with polyethylene glycol (−1.7 MPa), the most-upregulated genes in the resulting transcriptomes contained the promoter sequences (ACGT), which is often associated with ABA activity. In the majority of cases this nucleotide sequence was present in 11 different sequence ABRE motifs (<u>AB</u>A <u>r</u>esponsive <u>e</u>lements) [65]. Five additional transcription factor sites (with a dehydration-responsive element (DRE)-motif and MYCATERD1, RY-repeat promoter motifs) may indicate influences of non-ABA-avenues of transcriptional control. ABA-regulated and non-ABA signalling pathways interact and converge in the induction of desiccation tolerance [66,67]. Many genes in *Arabidopsis* are regulated in an ABA-dependent and ABA-independent manner, e.g., *RD29A* [68]. In ABA-independent responses to dehydration and cold, the DRE 9bp element is an essential *cis*-acting element to the regulation of *RD29A* [68]. The Clp protease regulatory subunit encoding gene, *ERD1* (early responsive to dehydration 1) responds to dehydration and salinity stress before the accumulation of ABA, which suggests that regulators other than ABA also control dehydration stress responses of *Arabidopsis* [69,70].

6. Potential Regulators of Desiccation Tolerance Other Than ABA

In the following sections the information on drought stress is complemented with data on other stresses. There is considerable overlap in tolerances to drying, salinity, low temperature and heat (e.g., [71]), an overlap corroborated by recent studies of the responses of gene expression to both

abiotic and biotic stresses, e.g., [55,72–74]. Desiccation tolerance was induced in *Brassica napus* L. microspore-derived embryos by stress other than drought, for example heat shock (10 min) and cold shock for 2 days to the point where desiccated embryos survived 3 weeks in the dry state, before rehydration allowed renewed growth [75].

6.1. Brassinosteroids (BR), Jasmonates (JA) and Strigolactones (SL)

The first evidence that the brassinosteroid, brassinolide (BR), and methyl jasmonate (MeJa) participate in the induction of desiccation tolerance in angiosperm foliage showed that, out of 11 phytohormones and growth substances tested, exogenous BR and MeJa each gave the greatest enhancement of the protoplasmic drought tolerance of *S. stapfianus* mesophyll cells [46]. Electophoretic partitions (2D-PAGE) of soluble proteins from BR- or MeJa-treated plants registered 10–14 proteins with increased abundance and 3–4 with decreases. Two proteins were both BR- and MeJa-responsive. Evidence of BR synthesis was reported for drying foliage of the resurrection grass *S. stapfianus* [76]. Rice plants transgenically modified to synthesize elevated cytokinins under drought stress augmented the expression of brassinosteroid-related genes but diminished the expression of jasmonate-related genes [77].

Genes encoding enzymes involved in jasmonate (JA)-biosynthesis were upregulated in droughted non-resurrection plants in the Solanaceae and in salt-stressed barley plants [72,78]. In barley, JA-inducible genes were also upregulated by salinity, suggesting action of endogenous JA formed during salt-stress [78]. Pre-treatment with JA lessened salt-induced inhibition of photosynthesis in barley plants [78]. In rice (*Oryza sativa* L.), BR induces ABA synthesis and the BR pathway interacts with ABA, with strigolactone and with gibberellins in relation to plant stress reactions, growth and development [79]. As levels of BR increase, a maximum effect is attained but the effect declines with higher BR levels.

Whilst there have been no comprehensive studies on the involvement of strigolactones (SL) in induction of desiccation tolerance, recent reports indicate SL play important roles in responses to drought [80–84]. Analyses of impaired SL biosynthesis and signalling mutants in *Arabidopsis* suggest that SL has a role in the positive regulation of dehydration stress via both ABA-dependent and ABA-independent mechanisms [80]. The SL mutants exhibited hypersensitivity to drought and salt stress and slower ABA-induced stomatal closure. Strigolactone biosynthetic genes *AtMAX3* and *AtMAX4* are induced by both ABA and dehydration in wildtype (WT) plants, and the drought tolerance of both mutant and WT plants is enhanced by application exogenous SL. While SL biosynthesis genes are upregulated by drought in *Arabidopsis* leaves they are repressed in the drought-stressed roots of *Lotus japonicus* L. and tomato [82,84]. A comparative transcriptome analysis between WT and the *Atmax2* SL-signalling mutant under both water-stress and well-hydrated conditions found several interesting differences in the expression profiles of ABA regulatory and transporter proteins, cytokinin-catabolizing CKX genes, as well as genes associated with flavonoid biosynthesis and photosynthetic activity [80]. Somewhat similar results utilizing *max2* SL-signalling mutant were reported by Bu et al. [81], although they hypothesized that the drought-sensitivity of the mutant may result from the *max2* F-box mutation interacting with other hormonal pathways. The direct role of the SL compound in drought resistance of *Arabidopsis* as reported by Ha et al. [80] was confirmed in *L. japonicus* by Lui et al. [82] who found that SL was down-regulated in roots early during osmotic stress and that the SL decrease may be required to allow ABA production. Visentin et al. [83] suggested that a reduction in SL production and its acropetal transport from tomato roots may trigger a stress response in the shoots that does not rely on ABA transport from the roots, but may lead to increased localized SL production in the shoot which could induce stomatal closure and suppress shoot branching.

6.2. Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS) are produced under unstressed conditions from aberrant electron flow related to ATP-producing metabolism. ROS levels accumulate above normal levels

as electron flows are perturbed under a wide range of environmental stresses including drought [85]. ROS signaling is widespread in plants [85]. As an early chemical result of stress, ROS accumulation is well placed to be a first or early signalling-trigger for adaptive responses to the stress [86], involving both systems against injury by ROS (catalase, peroxidase, dismutase, glutathione, ascorbate, isoprenoids) and also ROS-signalling toward induction of stress tolerance. Early ROS accumulation in resurrection plants would need to be high enough to trigger tolerance-induction but not so high as to produce cell injury.

In the desiccation-tolerant dicot *Haberlea rhodopensis* Friv. expression of catalase genes fell to ~40% during plant drying and recovered to initial values on plant rehydration. The 40% catalase expression level remains important since inhibition of catalase activity rendered the plants partially sensitive to dehydration [87]. During drying *H. rhodopensis* increased its total content of phenols, especially of the antioxidant myconoside, but the plant's total antioxidant capacity fell to 70% of the level in the hydrated control [88]. In *B. hygrometrica* (a desiccation-tolerant species) levels of ROS were low in dry viable plants but high in plants killed by rapid drying. Contents of the antioxidant tocopherol and the expression of three enzymes controlling tocopherol biosynthesis were much higher in dry viable plants than in the rapidly-dried nonviable plants [89]. The accumulated tocopherol presumably helps limit oxidative damage in dry viable plants and during early stages of subsequent rehydration.

The desiccation-tolerant poikilochlorophyllous monocot *Xerophyta humilis* (Baker) Dur. & Schinz released, at moderate drought stress (~80% RWC), the volatile isoprenoid, isoprene, which protects chloroplasts against ozone and ROS [90]. The antioxidant role of volatile isoprenoids in protecting membranes was restricted to early drought stress and was replaced by the action of non-volatile isoprenoids later in dehydration of *X. humilis*.

Low ROS accumulation and high antioxidant ability are crucial to plant endurance of combinations of abiotic stresses [74]. Combined drought and heat stresses can cause 4-times as much agricultural loss in the USA than drought alone [74]. Cytosolic ascorbate peroxidase 1 (APX1) is needed for *Arabidopsis* to tolerate combined drought and heat stresses [91]. In *Arabidopsis*, drought and other stresses, including heat stress and viral pathogens (singly and in combination) greatly down-regulated a transcript *Rap2.9*, a putative transcriptional repressor. Under non-stress conditions *Rap2.9* may suppress expression of stress-adaptive genes, whereas down-regulation of *Rap2.9* under drought or other stresses would allow expression of genes inductive of tolerance [92]. Conversely a transcript for a G-box binding factor 3 (*GBF3*), implicated in ABA-signaling was markedly upregulated.

The ROS transduction pathway is considered to involve oxidative inactivation of specific proteins (by carbonylation or disulfide formation) and activation of mitogen-activated protein MAP kinases and calcium channels [85]. Genes encoding enzymes for cysteine biosynthesis and thence redox-sensitive signalling molecules in abiotically stressed plants promote survival in abiotically-stressed plants [93]. The capacity of the antioxidant defence system determines continued functioning (supporting survival) or progressive photo-oxidation (leading to injury) [94]. Nucleoside diphosphate kinase 1, associated with ROS signalling, was markedly higher in dry and in rehydrated plants than in hydrated control samples in the moss *Fontinalis antipyretica* Hedwig [95].

The enzyme ascorbate peroxidase (APX6) maintains low levels of ROS until, under environmental stress, ROS overwhelms the capacity of the APX system. Increasing ROS stimulates synthesis of auxin and of ABA, which inhibit germination [86]. ROS can accumulate, under temperature and salt stress, in the phloem apoplast where it mediates a rapid signalling pathway that is independent of other signalling pathways [39]. Ismail et al. [41] propose that during salinity stress, cell uptake of Na^+ and Ca^{++} within minutes, stimulates both accumulation of ROS and rapid removal of Na^+ and Ca^{++} (by vacuolar uptake and extracellular secretion). Removal of the ions curtails the Ca signal. The amplitude of the Ca signal and the speed of its attenuation determine which of two subsequent parallel pathways predominates. Rapid attenuation of the Ca signal favors ROS signalling, its stimulation of ABA signalling leading to increased stress tolerance. Slow Ca signal attenuation diverts ROS signalling to produce an excessive activation of the JA pathway which leads to oxidative damage to membranes.

In the resurrection grass *S. stapfianus*, JA was found to have a beneficial rather than deleterious effect on protoplasmic drought tolerance [46].

ROS, ABA and JA all appear to be involved in the induction of desiccation tolerance. The processes described by Ismail et al. [41] for induction of salt-tolerance may well be operating in resurrection plants during induction of desiccation tolerance as plants progress through drying, but further research is needed in resurrection plants to elucidate this possibility. It is conceivable that since ROS can be transported in phloem, ROS levels rise in the foliage apoplast as part of a signal translocated from drought-stressed roots in drying soil. The primary triggering role that Ismail et al. [41] ascribed to uptake of apoplastic Na^+ in signal transduction in salt stress/tolerance is less likely in resurrection angiosperms, which usually grow in habitats with low soil Na^+ (at high altitudes and distant from coasts). Immense turgor pressures strain the plasmalemmas of plant cells (commonly up to 2 to 3 MPa) in the early stages of drought stress until leaves wilt. Altered inflow of calcium via mechano-sensitive calcium channels [41] may conceivably result from changes in pressures on membranes and trigger signalling for induction of desiccation tolerance.

Strigolactones and the recently-reported messenger agents, acetate [96] and CLE25 peptide [97] have yet to be examined as possible root-to-shoot signals in the induction of desiccation tolerance: CLE25 synthesized in droughted *Arabidopsis* roots is transmitted in the vascular system to leaves where it acts with BARELY ANY MERISTEM (BAM) receptors to influence ABA-synthesis and thereby produces closure of stomata [97]. In *Arabidopsis* plants, drought stimulated the synthesis of acetic acid, while 10–20 mM acetic acid applied to the roots lowered the pH of xylem sap and ultimately promoted the drought resistance of the plants. A similar promotion of drought resistance was found in plants of maize, rice, rapeseed and wheat [96].

7. Sucrose and Induction of Desiccation Tolerance

When the aquatic moss *F. antipyretica*, was dried slowly in 50% relative humidity to reach 25% RWC, the plants survived for one month and an increase in the synthesis of proteins associated with sucrose production was observed [95]. Pretreatment of cell suspensions from *S. stapfianus* leaves with high concentrations of sucrose for 1 h enhanced the protoplasmic drought tolerance (PDT) markedly from equilibration to <50% RH compared with 86–87% RH for controls. In plants pretreated with separate solutions of glucose, fructose, trehalose or raffinose, the PDT remained at the control levels [98]. The cells absorbed sucrose rapidly, doubling their osmotic pressure, whereas the four other sugars produced no detectable change in osmotic pressure. Sucrose absorption into suspended *S. stapfianus* cells was suppressed by PCMBS (*p*-chloromercuribenzenesulfonic acid) an inhibitor of the proton-sucrose symport of the plasmalemma [99].

The improvement induced by sucrose in the PDT of *S. stapfianus* cell suspensions (~36%RH) was much greater than that induced by pretreatment with methyl jasmonate/jasmonic acid, or brassinolide or ABA (3%, 3% and 1% RH respectively; [46]). The need for high concentrations of pretreatment with sucrose and the rapid absorption of sucrose suggest that in *S. stapfianus* the stimulation of PDT may be a direct protective effect of the sucrose and/or be a regulatory role by high intracellular sucrose concentrations. These data indicate that sucrose-loading into young leaves of *S. stapfianus* is important for induction of desiccation tolerance. In intact drying resurrection plants, considerable sucrose would be supplied by remobilization of carbohydrates from senescing leaves via the phloem.

The SUT4 subfamily of sucrose proton co-transporters and sucrose facilitators displays broad physiological involvements and directions of regulation which vary between species-variation that in part, is linked with whether the path of sucrose to the phloem is via the apoplast or via the symplast [100,101]. Moreover, the promoter of the *SUT4* homolog in *Arabidopsis* contains stress-responsive regions whereas the homolog in rice has mainly hormone-related elements [102]. The situation in these two regards is not known in the resurrection angiosperms. The widely separated taxa containing resurrection angiosperms may well differ in the mechanism of sucrose transfer.

Mechanisms of regulation by sugars in non-resurrection plants are reviewed by Smeekens and Hellmann [103]. Target of Rapamycin (TOR) kinase complexes are stimulated by glucose and repress synthesis of sucrose and starch [104]. In *S. stapfianus* glucose accumulation occurs early during drought stress, while starch and sucrose contents are decreasing [64], consistent with TOR kinase associated sugar accumulation. However, low levels of glucose and high levels of sucrose are seen in later stages of desiccation where drought stress is severe [64]. Sucrose accumulation and transport is for the most part associated with the sucrose-related kinase SnRK1 (addressed in Section 8).

8. The Role of Trehalose-6-Phospate in Desiccation Tolerance

While trehalose is the major protective sugar that accumulates in some desiccation-tolerant species, such as nematodes and tardigrades, sucrose is the predominant protective sugar accumulating in drying resurrection plants, with trehalose and other sugars accumulating at lower levels [64]. The exceptions to this are *Selaginella* spp. that also accumulate trehalose to high concentrations when dehydrated, however, there is no strong link to desiccation tolerance as other desiccation-sensitive members of *Selaginella* spp. also accumulate high levels of trehalose [105]. *Craterostigma plantagineum* and *Lindernia brevidens* Skan. also accumulate sugars in response to desiccation [42,106]. Fully hydrated leaves of *C. plantagineum* contain high levels of the unusual sugar 2-octulose which upon dehydration is converted to sucrose [107]. This interconversion of 2-octulose to sucrose is conserved in the close relative, *L. brevidens* [106].

Several studies have demonstrated that exogenously applied, and genetically enhanced trehalose content results in a higher capacity for withstanding drought [108,109], however, the direct mechanism whereby trehalose fuels this response remains to be elucidated. Trehalose biosynthesis involves the enzymes trehalose-6-phosphate (T6P) synthase (TPS) and T6P phosphatase (TPP). TPS generates T6P from derivatives of sucrose metabolism and other pathways, followed by the action of TPP, which removes the phosphate generating trehalose. T6P and the genes controlling T6P synthesis and conversion to trehalose have been implicated in the regulation of photosynthetic ability, starch synthesis and breakdown, glycolysis-linked hexokinase activity, and embryo maturation [110]. The role of trehalose as a minor sugar is not clear, however trehalose is thought to accumulate at low levels due to the rapid degradation of trehalose into two glucose units by trehalase [111].

Water deficit results in the rapid inhibition of growth yet photosynthesis can continue and result in increases in cellular carbohydrate contents [112]. Key carbon storage molecules, such as sucrose, are generated through photosynthesis and can be stored or transported to where they are required within the plant [113]. T6P has been implicated as an indication of cellular sucrose status where T6P levels are directly proportional to sucrose levels, termed the T6P: sucrose nexus [114]. Importantly, T6P has been shown to inhibit the sucrose homeostasis enzyme SnF-Related Kinase 1 (SnRK1) [115]. Put simply, SnRK1 inhibited by T6P directs the "feast" response, whereas uninhibited SnRK1 directs a "famine" response. T6P-inhibited SnRK1 will up-regulate carbohydrate storage, prime gene expression for growth, growth recovery and signals satiety, whereas uninhibited SnRK1 will signal starvation, catabolism and will slow growth. Indeed, the T6P:SnRK1 relationship is considered a major player in directing carbohydrates between source and sink tissues, and can be a key factor in the survival of environmental stress [116–119]. This has been addressed in *Arabidopsis*, where accumulated T6P under sink-limited stress conditions inhibits SnRK1 activity, and thus allows synthesis and growth to resume rapidly once the growth limiting stress conditions were alleviated [118]. A similar acceleration of growth is observed in *S. stapfianus* that has been through a dehydration cycle [36].

Evidence of T6P affecting changes in carbohydrate storage and desiccation-related processes has been demonstrated in both transgenic *Arabidopsis* and maize. *Arabidopsis* with increased T6P content stimulated rapid starch synthesis (requiring SnRK1) following redox-activation of adenosine-5′-diphosphoglucose (ADP-glucose) pyrophosphorylase (AGPase) [120,121]. This rapid increase in starch mirrors that which is seen in the rehydration of most resurrection plant species following the loss of starch in the earlier stages of drought stress [122,123]. Conversely, a decrease

in T6P during embryo development in *Arabidopsis*, demonstrated in an *AtTPS1* mutant, prevents the accumulation of storage molecules during the latter stage of seed development [124]. In addition, there is evidence that trehalose phosphate/trehalose interconversion acts as a system regulating the vascular flow of sucrose to *Zea mays* L. inflorescences and protects florets against drought injury [119]. It is possible that this system, catalysed by TPP, may assist the phloem-transportation of sucrose from remobilized carbohydrate in old leaves on drying resurrection plants to young leaves, so assisting the survival of young plant-parts.

Furthermore, T6P has also been associated with ABA signaling, where low levels of T6P correlated to hypersensitivity to ABA in early stages of embryo development [125]. The induction of the desiccation tolerance pathways in some resurrection plants will occur as relative water content falls below 60% (Table 3), whereas in non-resurrection plants drought-related ABA processes, such as stomatal closure, will occur earlier during water deficit [125]. It seems likely that T6P driven ABA signaling, where high T6P levels presumably instigates ABA hyposensitivity, would be required for the prevention of innate drought-related responses prior to the activation of the ABA-dependent desiccation-tolerance pathway [126].

The trehalose biosynthesis pathway potentially has a significant role in the establishment of intracellular glasses that form within desiccated plant tissues in a process called vitrification. During this process, significant increases in sucrose, in addition to increases in raffinose, trehalose and other sugars come together to form intracellular glass [127]. Sugar accumulation has been observed during drought stress in transgenic tobacco expressing the *E. coli* TPS gene [128] providing indirect evidence of the potential involvement of the trehalose biosynthesis pathway in some aspects of vitrification observed in desiccation tolerance. Trehalose has a high glass transition temperature, and forms strong hydrogen bonds with water [129]. Furthermore trehalose is thought to provide stabilisation to proteins, and prevents membrane fusion [130]. This may potentially help in the formation of a scaffold during vitrification, which forms the building blocks for T6P-driven sucrose accumulation during the desiccation tolerance network.

While the above mentioned are important during the dehydration cycle, the rehydration cycle is equally as important, however, sparsely covered in scientific literature. Mentioned above, transcripts, proteins and energy-related compounds are essential in desiccation tolerance and as metabolism slows as resurrection plants enter the quiescent state, these sensitive compounds need to be stored. In *Tortula ruralis* (Hedw.) Gaertn., Meyer & Scherb. mRNPs (micro RiboNuceloProteins) accumulate during water loss, and are possibly involved in mRNA storage and protection [131]. This discovery offers a potential mechanism of rapid protein synthesis post-rehydration.

9. Protein-Synthesis in Resurrection Plants under Drought Stress

As resurrection plants dry, protein synthesis is necessary for the production of the specific proteins associated with implementation of desiccation tolerance and for the specific proteins regulating implementation. The major changes in the protein complement during induction of desiccation tolerance imply changes in gene expression and turnover of degraded protein. In resurrection plants protein synthesis continues to surprisingly low water contents. In the resurrection monocot *Xerophyta villosa* Th.Dur. & Schinz. protein synthesis rates increase markedly below 60% RWC before declining again at 30% RWC (Table 4). In *B. hygrometrica*, increased abundance of transcripts related to chaperones, to ubiquitination and to unfolded-protein-stress signaling in dry desiccation-tolerant and desiccation-sensitive plants, indicated unfolded-protein-stress occurred in both cases [89]. Transcripts related to autophagy accumulated in the drying desiccation tolerant plants. Specific staining for autophagosomes in these plants remained at levels seen in the hydrated controls, whereas this staining decreased in drought injured plants [89]. Autophagy may be contributing to switching cells from a growth-related state to a quiescent/dormant dry state.

Table 4. Protein synthesis indicated by polyribosome content in leaves of the resurrection plant *Xerophyta villosa* on intact plants drying to a viable airdry condition. Data based on [132].

RWC (%)	96	90–75	75–60	45–40	30	20	4
% RNA as Polysomes	~30	~25	~45	~55	~32	~1	0

Transcripts encoding biosynthetic enzymes generally decreased and those encoding catabolic enzymes generally increased in *B. hygrometrica* plants surviving dehydration [89]. Increased hydrolytic breakdown of cell wall constituents may raise the plasticity of the cell walls. Allowing folding of the cell walls in the resurrection plant during drying reduces the risk of mechanical injury to tissues and may augment growth following subsequent rehydration of plants.

Numerous proteins change in abundance in all the studies of drying resurrection angiosperms and in desiccation-tolerant (orthodox) seed. Transcriptome and proteome studies of desiccation tolerant plants compare the initial hydrated tissue with air-dry phase [58,76,87,95]. All the studies found high numbers of transcripts or proteins with large increases or decreases in their abundance during drying. Specific transcripts with >50% change in abundances during acquisition of desiccation tolerance in drying *Arabidopsis* seed numbered almost 7000 [12]: 74% of the 7000 transcripts were specific to drying seed. A major shift clearly occurred from a growth/development metabolism before drying to a desiccation-tolerance metabolism during drying.

As the homoiochlorophyllous resurrection grass *S. stapfianus* dries, the proteomic changes vary with the different RWC levels before and during the appearance of desiccation tolerance [40,51,133,134]. In the poikilochlorophyllous resurrection monocot, *B. constricta*, complete chlorophyll-destruction in drying leaves is tightly linked with the induction of desiccation tolerance [135]. Following 1D-PAGE partitions of soluble protein extracts from *B. constricta* leaves, the protein-pattern for yellow-green leaves undergoing induction of desiccation tolerance resembled the pattern for green leaves killed by dehydration (strong emphasis of a peak of mid-molecular-weight proteins in both cases). Whereas the protein-pattern for leaves that had become fully yellow and desiccation-tolerant, showed restoration of a three-peak pattern similar to that for fully hydrated control leaves. It appears that in drought-hardening *B. constricta*, an early phase moves toward injury and a subsequent phase moves toward reinstatement of a 'hydrated pattern' as a basis for successful recovery in subsequent rehydration. In drying plants of *X. villosa* an early phase of declining protein synthesis is followed by a phase where protein synthesis rises above levels in fully-hydrated control plants, and then a phase of decline to no protein synthesis at ~20% RWC [132].

Transcript for a protein initiation factor *eIF1* became abundant in drying *S. stapfianus* plants at moderate stress (60–79% RWC, comparable to the phase of increasing polysome content in drying *X. villosa*) and was most abundant in airdry leaves [51]. Thus eIF1 content may limit the rate of protein synthesis during early induction of desiccation tolerance but eIF1 is in good supply for protein synthesis early in rehydrating leaves. The desiccation-tolerant moss *F. antipyretica* had low abundance of RNA-binding proteins, elongation factor 1-α and translation initiation factor eIF-4A in dry plants; the level of the former protein remained low on rehydration and recovery whereas the eIF-4A protein accumulated to almost twice the control value [95].

Desiccation-tolerant plants maintain their ATP content as they dry, while in several resurrection species the ATP content in the air-dry state exceeded levels in hydrated plants [136] indicating higher ATP synthesis than ATP usage. Drying desiccation-tolerant angiosperms show accumulation of ATP synthases which would support the ATP levels in drying and rehydrating plants (e.g., *S. stapfianus*, [76]; *H. rhodopensis*, [87]). Slow-dried moss *F. antipyretica* accumulated one form of ATP synthase during drying but a second form declined in abundance [87]. Abundance of a putative ATP synthase almost doubled during drying in the desiccation-tolerant moss *P. patens* [58]. ATP contents do not appear to limit protein synthesis in drying desiccation-tolerant plants.

Processes in Seed and Pollen during Desiccation

There have been many studies of seed- and pollen-specific gene expression relating to the process establishing desiccation tolerance as either the seed or pollen matures [137], but it has been difficult to untangle the expression of genes directly involved in desiccation tolerance from those related to other aspects of seed and pollen development. Molecular studies in *Arabidopsis* have shown that mutations in the transcription factors ABSCISIC ACID INSENSITIVE 3 (ABI3), LEAFY COTYLEDON2 (LEC2) and FUSCA3 (FUS3) are lethal at the late stages of seed maturity. These transcription factors contain the B3 domain, a highly conserved DNA binding region specific to higher plants [138]. FUSCA3 for example, has a role in regulating ABA and gibberellic acid (GA) levels and is involved in controlling seed dormancy and preventing germination under adverse conditions [138]. Additional transcription factors identified which may have a role, such as DREBs (dehydration responsive element binding) and TaNAC69, are stress-related and expressed in pollen/anthers during development [139].

Costa et al. [59] compared published transcriptomes of plant species following induction of desiccation tolerance (leaves of the resurrection plants *C. plantagineum* and *S. stapfianus*, germinating seeds of *M.* treated with ABA or osmoticum, and the lichen *Cladonia rangiferina* (L.) Weber ex F.H.Wigg). Of 12 changes in specific transcript abundance with induction of desiccation that were consistent across all five species, only two showed elevated abundance. These, include the gene transcripts for the genes *LEA-14* (AT1G01470) and NAD (P)-linked oxidoreductase superfamily protein (AT5G01670) that have putative roles in protein-protection and counter-oxidation, respectively. Comparison of transcripts for all datasets with the genome sequence data for *Arabidopsis*, yielded a core of 260 *Arabidopsis* orthologs, of which "77 increased and 184 decreased in abundance". The categories of those that accumulated were broad in coverage, and included, lipid storage, multicellular organismal processes, seed development, reproduction and response to temperature, whilst those decreasing in abundance related to carbohydrate metabolism and biosynthesis of phenylpropanoids. Data on regulatory motifs in the *cis*-acting promoter elements in the "77-increased-abundance" core genes highlighted genes responsive to ABA (ABRE binding site motif), light regulation, stress (DRE core motif), circadian rhythm (EveningElement promoter motif) and gibberellin (GADOWNAT), whilst promoter motifs for the "184-declined-abundance" core genes also included the ABA related ABRE binding site. Once again an important role of ABA *inter alia* is evident.

Studies of mutant lines by Costa et al. [59] apply to the full period of induction of desiccation tolerance. Analysis of T-DNA mutant *Arabidopsis* lines for 13 genes chosen from the 260 DT set, revealed mutations in three genes (*LEA 5-1*, *PCK1*, and *RCI2H*) which resulted in reduced seedling ability to re-induce desiccation tolerance. In further studies of the T-DNA mutant lines, dormancy was increased by disruption in 5 genes (AT3G10020, *LEA7*, *PCK1*, *RCI2H* and *SAG29*) and seed longevity was lowered in the *PCK1* line.

10. Identification of Genes Involved in Desiccation Tolerance

Protection against the damaging effects of desiccation is essential to enable the vegetative parts of resurrection species to remain viable and intact in their typically dry, high light and otherwise hostile environments, often for extended periods of time [11,140]. Most of the initial reports dealing with the molecular genetics of desiccation tolerance involved the use of a range of differential cDNA screening procedures to identify genes with altered (mainly increasing, though some decreasing) transcript abundance in RNA extracted from leaf tissues at various stages in the dehydration process. Although the number of desiccation-related genes identified in such studies was relatively small, their characterization did provide valuable insights into the molecular processes that underpin the capacity of plants to survive desiccation. These included upregulation of genes encoding enzymes likely to reduce negative effects of reactive oxygen species, glycine-rich components of cell walls and membrane intrinsic aquaporin proteins that facilitate transport of water molecules across cellular membranes in response to moderate drought stress [51,133,134,141–150].

Dramatic increases in transcript abundance of LEA-type dehydrin proteins were also observed in many of these studies. This broad group of hydrophilins is generally regarded as providing a broadly protective role against drought and related stress-induced denaturation of cellular proteins [151–153]. Furthermore, in *C. plantagineum* changes in lipid metabolism have been associated with the establishment of desiccation tolerance. The removal of MGDG (MonoGalactosylDiacylGlycerol) a conic-shaped non-bilayer-forming chloroplast lipid was strongly associated with desiccation tolerance. A fraction of MGDG is converted to oligogalactolipids in the chloroplast envelope, which are then hydrolysed and sent to the ER (Endoplasmic Reticulum), these are then used for the synthesis of phospholipids. Remaining MGDG may be used for the synthesis of phosphatidylinositol (PI), a key lipid involved in signalling and membrane trafficking. PI only accumulated during the dehydration of desiccation-tolerant species [154].

As methodology and cost effectiveness of DNA analysis has steadily improved over recent years, involving larger scale transcriptomic studies and entire genomic analysis of monocotyledenous and dicotyledonous species from different regions of the globe, so there has been a steady increase in the number and range of gene transcripts that appear to be involved in the onset of desiccation tolerance [59,87,101,155–161]. Although there are differences in the specific molecular responses of different types resurrection plants, probably reflecting lineage-specific adaptations to widely variable geographic areas and localised responses to environmental stresses, together analysis of the predicted proteins encoded by many of these genes does point to them playing a role in protection of cellular components from the otherwise destructive effects of water loss and oxidative damage upon cellular biochemical processes and structural integrity of cell walls and entire tissues.

Interestingly, in general, these 'omic' studies are also supportive of the earlier hypotheses that vegetative desiccation tolerance has independently evolved in different taxa via reactivation of pathways normally restricted to seed maturation in plants (e.g., [137,155,159,162]. A major challenge over the next few years will undoubtedly be to understand the nature of regulatory genes, and their activation, which control the expression of these 'protective protein' genes at appropriate stages of the desiccation process. Some promising results have been reported here (e.g., [73,163–165]) but much remains to be discovered in this area of investigation.

11. Direct DNA Manipulation of Desiccation-Related Genes

Effective gene transfer procedures in many plant species have enabled DNA disruption, RNAi-mediated or viral-induced gene silencing protocols to be developed for researchers wishing to study the role of any given gene in a developmental or biochemical process (e.g., [166] and references therein). Transformation procedures have been reported for some resurrection species, such as the well-studied dicotyledenous species *C. plantagineum* [167] and also *L. brevidens* [168]. The latter species is a more recently described resurrection plant, also from within the family Linderniaceae, and interestingly is found naturally in the montane rainforests of Tanzania and Kenya [106]. However, reliable DNA transformation protocols for other desiccation-tolerant plants have not been reported and development of such methods would be extremely beneficial to the subject area, particularly involving desiccation-tolerant monocotyledonous species. Such procedures may also allow CRISPR/cas9-mediated genome editing technology to be employed in direct functional genomics studies to investigate the role of particular genes in conferring desiccation tolerance in such species, as has recently been demonstrated with other genes in monocotyledonous crops [169,170]. Another powerful functional genetics methodology, which does not necessitate the development of DNA transformation technology, involves targeting induced local lesions in genomes (TILLING) [171,172]. This approach has been successfully used to identify mutants in monocotyledonous crops that possess disruptions in specific genes [172–174]. An alternative approach which has proven valuable and which may be of direct interest to agricultural crops, is the ectopic expression of genes from resurrection plants in otherwise desiccation-sensitive species where reproducible transformation protocols do exist.

One of the first reports of successful ectopic expression of drought activated genes from the resurrection plant *C. plantagineum* involved transgenic tobacco [175]. Here three genes, encoding a serine and lysine-rich repeat motifs protein, a LEA-type protein and a novel sequence, were introduced under control of a modified version of the powerful *Cauliflower mosaic virus 35S* (CaMV35S) promoter. Although the introduced proteins were detected, these transgenic plants did not appear to show enhanced tolerance to drought stress [175]. However, ectopic expression of *LEA-4* group genes from the resurrection plant *B. hygrometrica* did confer elevated dehydration tolerance in transgenic tobacco [176]. In separate studies, ectopic expression of the dehydration inducible *XvSAP1* gene from *Xerophyta viscosa* Baker in tobacco increased osmotic-, salinity- and high-temperature-stress tolerance in transgenic plants compared to controls. The *XvSAP1* gene encodes a highly hydrophobic and likely membrane-bound protein with significant identity to a cold-induced dehydrin-like gene from wheat [145,146]. Also using *X. viscosa* as a source of stress-related genetic information, it was observed that ectopic expression of aldose reductase (*ALDRXV4*) conferred enhanced drought and also salinity tolerance on transgenic tobacco plants [177], most likely due to enhanced scavenging of methylglyoxal and reduction in the level of abiotic stress-induced membrane damage. Over expression of a MYB-type transcription factor gene (*CpMYB10*) from *C. plantagineum* in *Arabidopis* increased drought (and salt) tolerance of transgenic plants, apparently via elevated sensitivity to endogenous ABA levels [164]. Constitutive expression of the dehydration-induced family 1 uridine diphosphate (UDP)-glycosyltransferse (UGT) gene *SDG8i*, from *S. stapfianus* in *Arabidopsis* led to altered growth characteristics in transgenic plants, which possessed significantly enhanced drought tolerance compared to non-transgenic plants [73]. The underlying reasons for the increased drought tolerance here may be related to altered hormonal metabolism in transformants, which, in turn, may have activated or enhanced endogenous protection mechanisms dealing with abiotic stress in general [73]. Interestingly here, overexpression of a related *Arabidopsis* family 1 UDP-glycosyltransferase gene *UGT87A2*, in *Arabidopsis*, yielded transgenic plants also exhibiting enhanced tolerance to drought and other abiotic stresses. Analysis of these transgenic plants showed increased expression of a number of stress-related genes compared to control *Arabidopsis* plants [178]. Focussing on crop species of direct relevance to global food security, ectopic expression of a drought induced mRNA Splicing-Activated Endoplasmic Reticulum Stress Regulator gene (*BhbZIP60*) from *B. hygrometrica* in rice [160] and over-expression of an osmotin gene (*TIOsm*) from the resurrection grass *Tripogon loliiformis* F. Muell, also in rice, produced transgenic plants which displayed improved tolerance to drought compared to their non-transgenic counterparts [179].

As the 21st century unfolds, plant breeders, farmers and the wider public will need to consider the role of biotechnological approaches to combat deleterious effects of drought stress which are predicted to steadily increase in the face of ongoing climatic change, diminishing available arable land and increasing global population pressures [180–182]. The few examples noted above, albeit largely laboratory based and obviously quite limited in scope, are encouraging for future applications of resurrection plant molecular genetics and genomic studies to agricultural production and environmental sustainability.

12. Stress-Induction of Senescence in Mature and Premature Leaves

Griffiths et al. [45] postulate that drought-invoked senescence is a critical cause of cell degeneration and death in the pre-mature/mature leaves of non-resurrection plants and that suppression of such drought-induced senescence is crucial for resurrection plants to survive desiccation. The immature and mature leaves of homoiochlorophyllous resurrection plants undergo little change in organelle microstructure during drying [123,183]. In drying poikilochlorophyllous resurrection plants, the leaves lose chloroplast grana and thylakoids transform into vesicles [122], i.e., the resulting plastids resemble the proplastids of meristematic cells. This indicates that drought-induced post-mitotic senescence is uncoupled from processes that would lead to irreversible cell injury in resurrection plants.

The desiccation-tolerance-correlated gene *SDG8i* (from the resurrection grass *S. stapfianus*) encodes an enzyme UDP glycosyltransferase, which glycosylates an analogue of strigolactone (SL) [45,73].

SL is a phytohormone that in non-resurrection species inhibits growth of buds, promotes reallocation of resources from senescing leaves, promotes growth of interfascicular cambium and alters root architecture [184]. Some of these effects are mediated by interactions of SL with auxin and cytokinin. Glycosylation of indole-3-acetic acid (IAA) and ABA is known to produce pools of the reversibly deactivated hormones [185]. This suggests that glycosylation of SL may deactivate SL. Transcripts of *SDG8i* accumulate during induction of desiccation tolerance in young leaves of *S. stapfianus*, possibly leading to glycosylation of SL and preventing SL-stimulation of senescence in young leaves as they dry and rehydrate [45]. *SDG8i* transcripts remain high in dry foliage [134]. Deactivation of the SL during drying would also allow the rapid growth observed in desiccation-tolerant shoots of *S. stapfianus* following their subsequent rehydration [36].

Exogenous SL increases the overall drought resistance of plants, including by stimulating root growth and stomatal responsiveness [80,184]. SL biosynthesis from carotenoid is regulated by genes in the MAX (<u>m</u>ore <u>ax</u>illary growth) gene family [186]. Whereas SL inhibits bud growth, cytokinins promote it. The actions of both hormones target the gene for a transcription factor BRC1 that leads to suppression of bud growth [187]. Comparison of the transcriptomes of desiccation tolerant tissues in diverse plant species, led Costa et al. [59] to emphasize the overlap of gene expression changes related to desiccation tolerance with those related to dormancy. It is noteworthy then that SL appears to be implicated in bud-dormancy and possibly desiccation tolerance. The SL signalling pathway involves the protein ORE9/MAX2, which promotes senescence [188,189] whereas expression of *SDG8i* UGT inhibits senescence, possibly by altering ORE9/MAX activity [45].

Some breakdown products and reactive oxidants that accumulate during senescence may accelerate senescence [190]. Products of lipid catabolism include precursors for synthesis of jasmonic acid, a senescence-promoting hormone [191]. Exogenous methyl jasmonate and brassinolide improve the protoplasmic drought tolerance of non-senescent leaf cells from hydrated *S. stapfianus* plants [46]. Sugars accumulate from starch hydrolysis during senescence and during drought stress. Transgenic studies using the promoter of the senescence-associated gene *SAG12* (an *Arabidopsis* gene encoding a cysteine proteinase), fused to a gene catalysing cytokinin synthesis, linked overproduction of cytokinin with the early onset of senescence and high hexose contents [192,193]. Sugar accumulation is general during drought stress in both resurrection and non-resurrection plants. Drying resurrection plants appear to have either a mechanism for preventing high-sugar-induced senescence or from uncoupling senescence from production of cell injury.

Senescence involves differential expression of a large number of genes (many already expressed to some degree in non-senescent tissue) in monocot and dicot species [194,195]. Roles include hydrolysis of macromolecules, remobilization of nutrients, protection against oxidation and against pathogen [192, 196–198].

13. Epigenetic Modifications

Epigenetic regulation has also been linked with differentiation and development in plants as well as with the responses of plants to their environment, including reproduction, pathogen invasion and cold stress [199–201]. Differentiation (organ-type), development (juvenility, maturity or senescence) and environment (drought stress) together determine the ability of resurrection plant organs to become desiccation tolerant [10]. Epigenetic regulation thus appears to lie at the intersection of these three factors and so presents as a likely over-arching mode of regulation of the expression of desiccation tolerance. The major epigenetic chromosomal 'tags' controlling availability for expression are DNA methylation, histone modifications and RNA mediated regulation (small interfering (si) RNA, micro (mi) RNA and long non-coding (lnc) RNA) [202]. Epigenetic regulation allows rapid and flexible responses to both short- and long-term stresses, resulting in increased plant plasticity in response to environmental conditions. In recent years there have been extensive reviews of epigenetic regulation of gene expression in plants and the reader is referred to these [202–206]. Here we focus on the potential role of epigenetics in the evolution of extreme desiccation tolerance.

There are only a few published reports on the identification of epigenetic mechanisms associated with desiccation tolerance in resurrection plants. In *C. plantagineum* an ABA-inducible retrotransposon, *CDT-1*, has been shown to control the synthesis of a 21 bp double stranded siRNA that has some sequence similarity to miR159 [19,207]. miR159 has been implicated in both drought and ABA responses in plants [208]. To show that the *CDT-1* siRNA was involved in gene regulation, *C. plantagineum* protoplasts were transfected with the siRNA, resulting in increased expression of desiccation-associated genes compared to control protoplasts, indicating a role for this siRNA in the induction of the desiccation tolerance pathway [19]. siRNA and miRNA play an important role in stress signalling networks and are induced by abiotic and biotic stresses in many plant species [209]. The *Craterostigma* siRNA may act as a signal of stress, passing through plasmodesmata, and may initiate desiccation tolerance pathways, though the specific target sequence of *CDT-1* is unknown [19]. A screen of a *C. plantagineum* genomic library showed that the *CDT-1* retrotransposon is present at several loci but flanked by different sequences. This suggests that through transposition of *CDT-1*, there has been an increase in the synthesis of siRNA and as the stress increases greater amounts of transcripts are induced, which leads to an increase in the transcription of desiccation tolerance-related genes. Further screens of *C. plantagineum* identified an additional mutant, *CDT-2*, which showed similarities to *CDT-1* [210]. *CDT-2* also leads to increased expression of desiccation associated genes and results in callus that is desiccation tolerant without application of ABA. This suggests the involvement of the *CDT-1/2* gene family in ABA signal transduction. A similar retrotransposon to *CDT-1/2* has also been identified in *B. hygrometrica* [211]. Within the 49 kb LTR-retrotransposon a smaller element of ~2 kb was identified and named *Osmotic and Alkaline Resistance 1* (*OAR1*). Transformation of *Arabidopsis* with *AOR1* conferred tolerance to both osmotic and alkaline stress. When stressed, short transcripts originating from *OAR1* increased in both *B. hygrometrica* and the transformed *Arabidopsis.* In addition, stressing *B. hygrometrica* resulted in an increase in the *OAR1* copy number [211]. With the advances in molecular techniques the genomes of three resurrection plants have been sequenced in recent years; *B. hygrometrica* [161], *Oropetium thomaeum* (L.f.) Trin. [158] and *X. viscosa* [155]. The proportion of transposable elements (TE) in each genome varied considerably, with 18% in *X. viscosa*, compared to 43% in *B. hygrometrica* and 75% in *O. thomaeum.* This suggests that the level of TEs in a genome is not directly linked to desiccation tolerance, but they appear to play important roles, possibly in the evolution of desiccation tolerance in these unique plants.

Histone modifications have been identified as important for gene regulation in response to abiotic stress in non-resurrection species. The promoter of a *LEA* gene from *C. plantagineum* was used in a yeast one hybrid screen to isolate binding proteins resulting in the identification of three histone H3 variants [212]. Characterizations of these proteins suggest that they may be involved in gene regulation via histone modifications, but more studies are required to confirm this. Histone variants replace core histones in the nucleosome resulting in structural and functional variations. Proteome analysis of *S. stapfianus* leaf tissue identified a protein with similarity to SNF2P that doubled in abundance in drying leaves [76]. SNF2 proteins are nucleus-located ATP-dependent helicases that are involved in chromatin remodelling and have also been implicated in conserving domains for specific histone variants, thereby altering chromosome accessibility and hence gene expression [213].

A similar regulatory role to that of miRNA is exerted by RNA-binding proteins that modify mRNA activity and turnover of mRNA [214]. More than 1000 RNA-binding proteins have been identified from eukaryotes, but as yet none have been examined in relation to desiccation tolerance to the authors' knowledge. The rice 'alba' protein ('<u>a</u>cetylation <u>l</u>owers <u>b</u>inding <u>a</u>ffinity'), which binds to nuclear DNA and responds to drought stress and to oxidative stress, could be implicated in epigenetic regulation of stress responses [215]. This protein appears to have ancient origins since a related alba-like protein binds with DNA and RNA in the Archaea [215].

It is hypothesised that the mechanisms that operate in seed and pollen, resulting in desiccation tolerance, were re-activated in the vegetative tissue of resurrection plants [155,159,162]. To date, several genes involved in seed maturation have been linked to desiccation tolerance in resurrection

plants. However, support for this theory and the involvement of epigenetic regulation in desiccation tolerance would come from the identification in resurrection plants of epigenetic mechanisms characterised in seeds. Epigenetic involvement in seed desiccation tolerance is suggested by the identification of DME-family glycosylase genes in orthodox desiccation-tolerant seed of barley (*HvDME)* and *Arabidopsis* (e.g., *AtDME)* [216]. *HvDME* transcripts accumulated in maturing seed and drought-stressed vegetative organs. Differences in methylation levels of *HvDME* genes in two barley cultivars were indicative of species-related epigenetic distinctions. Other modifications include histone acetylation, which has been shown to mediate ABA sensitivity during seed germination [217], whilst the specific histone deacetylase 6 (HDA6) affects the expression of some of the genes induced during acclimation to freezing [218]. The expression of the *phas* gene, encoding the seed storage protein phaseolin, is induced during seed embryogenesis but is silent in vegetative tissue. Analysis of the promoter of *phas* indicated that the chromatin structure of the gene represses expression in leaf tissue. However, during embryogenesis the ABA insensitive 3 (ABI3)-like factor (*PvALF*, a putative activator), in conjunction with ABA, disrupts the chromatin structure leading to *phas* expression [219]. DNA-methylating/demethylating enzymes ('DNMT' DNA methyltransferases/DNA glycoylases) or histone-acetylating/deacetylating enzymes ('HAT' histone acetyl transferases/'HDAC' histone deacetylases) are not evident in proteome-investigations of dehydrating desiccation-tolerant plants [58,76,87,95], suggesting their abundances may be too low to be readily detected.

Epigenetic modifications can be reset during mitosis/meiosis but it has also been shown that these changes can be passed to subsequent generations and provide a mechanism for short-term stress memory (acclimation) as well as long-term stress (adaptation) [220]. Priming of *Arabidopsis* seedlings with mild salinity, followed by a period of non-saline growth conditions, increased the salt resistance and drought resistance of the plants to later salt-stress. This stress priming was accompanied by tissue-specific epigenomic changes [220]. Changes in profiles of four histones across the genome were particularly evident with tri-methylated lysine in histone 3 (H3K27me3), which persisted during seedling growth in normal non-saline conditions [220]. The histone modification, H3K4me3 also appears to function as an epigenetic mark of stress memory [201].

14. Conclusions

We suggest the following tentative hypothesis for the regulation of desiccation tolerance in the flowering-plants, the group that provides the great majority of our food. Since the information on responses to drought-stress by species with desiccation tolerant-foliage is sparse compared to the complex situation, findings from non-resurrection species and for other environmental stresses have also been considered to fill gaps in our information.

Fully hydrated flowering-plants, including resurrection plants, are generally desiccation-sensitive; desiccation-tolerance is induced in resurrection plants by drought stress as the plant is drying. In most resurrection plants, induction of desiccation-tolerance in the foliage depends on the foliage being attached to the plant, which indicates that hormonal messages from the drought-stressed root system are necessary for induction. We propose the following phases in the induction of desiccation tolerance in resurrection plants (Figure 1). The phases should not be regarded as discrete compartments since plant responses to drought are a network of progressive interacting processes. Phases overlap and intergrade in the field. For example, Phase 1 processes overlap in time and also in drought stress levels with Phase 2 processes in field plants as diurnal fluctuations in foliage water potential occur throughout the drying. Foliage is stressed during the day, resulting in temporary wilting, but becomes well hydrated during the night. After the soil water potential falls below −1.5 MPa the foliage becomes permanently wilted. These overlapping phases last several days overall, depending on the resurrection species and on environmental circumstances. The values given below are not meant to delimit the phases but only to give a rough idea of their duration and RWC at some points.
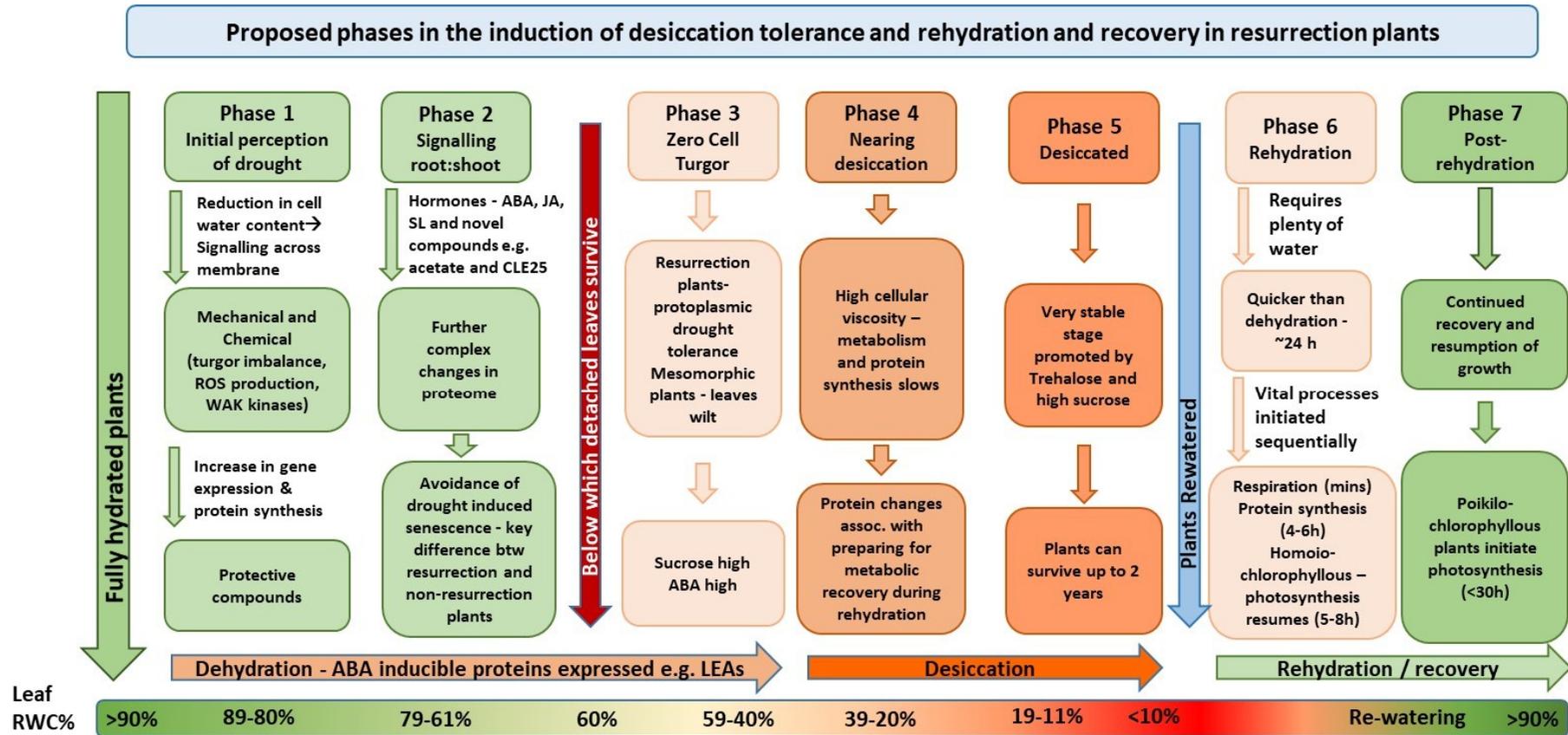
Figure 1. Summary and flow chart detailing the hypothesis for the regulation of desiccation tolerance in flowering-plants, with a focus on *S. stapfianus*.

Phase 1 Processes: Initial Perception of Drought Stress: Commencing with fully hydrated cells, small reductions in cell water content produce large reductions (often 10 to 30 times atmospheric pressure) in the wall pressure on the cell contents and the cell membrane becomes less stretched. WAK kinases spanning across the cell membrane from the wall to the cytosol register the change in pressure and initiate intracellular signals in the cell. Concurrently, increasing concentration of the cell sap augments the formation of ROS that initiate signal pathways to both restrict further ROS production and to trigger mechanisms contributing to desiccation tolerance. Gupta et al. [221] present a schema at a cell and organelle level for stress-induced triggering and signal transduction.

Phase 2 Processes: Signalling: Drying of soil around even only a part of a root system generally improves the drought resistance of the shoot in flowering-plant species; a mix of hormones rather than a single hormone seem to be involved. Important potential plant-hormones for this messaging in resurrection plants are ABA (in those species accumulating ABA under mild stress), jasmonate and strigolactone and also hormones not yet shown for resurrection plants, acetate (generated in drought-stressed roots from glycolysis products) and CLE25 polypeptide (drought-enhanced synthesis; CLE25 stimulates ABA synthesis in leaves). At the leaf-cell surfaces, hormones combine with specific receptor molecules at the cell membrane or are taken into the cell, where they initiate their specific transduction pathways, which interact together and with other pathways in the leaf cells (see hormone-interaction schema for drying resurrection plants in [45]). Complex changes in the protein complement begin at mild drought stress and continue as stress intensifies. Ismail et al. [41] consider that stress-signalling should be regarded as a message-pulse passing along the signal-pathway, and they stress the importance of timing and crosstalk between the various signalling-pathways. Starch grains disappear as they are hydrolysed into sugars; trehalose may influence starch breakdown and phloem relocation of sugars. Leaves in which senescence has already been triggered, senesce rapidly as they dehydrate and their remobilized minerals, amino acids and sucrose are translocated to younger leaves. In resurrection plants, younger leaves are not subject to drought-induced senescence during appropriate drying. This avoidance or suppression of drought-induced senescence has been proposed as a crucial distinction between resurrection and non-resurrection plants [45]. Much of the mechanisms for cell survival may be in place by the end of Phase 2 in resurrection plants, since leaves detached at mid-phase-3 survive full drying.

Phase 3 Processes: Zero Cell Turgor: When cell water is too low to stretch the cell wall, cell turgor becomes zero and the cell sap solutes concentrate more markedly as cell water potential declines further. Mesomorphic leaves wilt at about this point, after which vascular transport ceases and with it inter-organ signalling stops. Each cell is independently generating desiccation tolerance as drying continues. Glucose and fructose contents decline and sucrose begins to accumulate as drying progresses. Sucrose protects proteins and lipoprotein membranes from degrading. Sucrose signalling presumably commences in this phase. ABA content rises markedly. ABA is a major controller directly and indirectly of metabolism: in the resurrection monocot *B. constricta* exogenous ABA induces desiccation tolerance in detached hydrated leaves. The ABA signalling pathway interacts with a number of other pathways. A high proportion of major proteins expressed in Phases 1, 2 & 3 are ABA-inducible. ABA-induced protective LEA proteins particularly attain high levels. Protein synthesis increases: increases in the abundance of initiation factor eIF1 may have a key role in stimulating protein synthesis [51,76,222]. Numerous changes occur in the proteome. As drying continues, water contents approach the critical levels at which detached (desiccation-sensitive) leaves would be injured and die (roughly 30–40% RWC). The desiccation tolerance inducing process must be well advanced before these low water levels are reached and surpassed in resurrection plant foliage.

Phase 4 Processes: Nearing Desiccation: In this phase, termed by Zhang and Bartels [42] the 'Dehydration' stage (~25% RWC and below), protein synthesis slows. Changes in the proteome at this stage are thought to prepare for metabolic recovery during rehydration, to allow restoration of 'normal' metabolism in fully rehydrated plants and eventual resumption of growth. At very low water contents

metabolism slows (as metabolite diffusion slows in the increasing viscosity of sucrose-rich cell sap) and appears to cease in the desiccated plants.

Phase 5: Desiccated State: The desiccated state (most commonly 5–8% RWC) is very stable, a situation promoted by trehalose and high sucrose concentrations. In some species dry resurrection plants survive 6 months in the field, but most resurrection species recover if they are rehydrated after up to 2 years storage in the desiccated condition (Figure 1).

Phase 6 Processes: Rehydrating: In good contact with water, resurrection plants usually rehydrate in a shorter time (usually <24 h) than they dehydrate (usually 2 to 6 days after their soil dries). At 20 °C and above, root pressure may assist the rise of water from the roots to the foliage and so hasten leaf rehydration. Vital processes commence at staggered times: respiration recommences within minutes; protein synthesis commences after 4–6 h. In homoiochlorophyllous resurrection plants photosynthesis commences at 5–8 h after watering [31,44,223].

Phase 7 Processes: Post-rehydration: On full rehydration, some vital processes may continue increasing as recovery proceeds. Poikilochlorophyllous resurrection plants are yellow when first fully rehydrated; they commence synthesizing chlorophyll when illuminated; photosynthesis may begin in 30 h and growth recommences [31].

There is a dearth of information on the regulation of the ordered recovery that takes place in Phase 6 and Phase 7.

The extensive alterations in the proteome during the phases 1–5 and the progression from a growth-based state to a quiescent/survival state lead us to the view that changes in epigenetic regulation are involved in the control of some inductive gene expression in most phases. Further studies into epigenetic mechanisms and the genes involved may lead to a greater understanding of extreme desiccation tolerance and allow manipulation of important crop plants to improve yield under adverse environmental conditions.

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