Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Plant Biology

Review

A review of the role of metabolites in vegetative desiccation tolerance of angiosperms

Halford JW. Dace[1], Ademola E. Adetunji[2], John P. Moore[3], Jill M. Farrant[2] and Henk WM. Hilhorst[1,2]

Abstract

The survival of extreme water deficit stress by tolerant organisms requires a coordinated series of responses, including those at cellular, transcriptional, translational and metabolic levels. Small molecules play a pivotal role in creating the proper chemical environment for the preservation of cellular integrity and homeostasis during dehydration. This review surveys recent insights in the importance of primary and specialised metabolites in the response to drying of angiosperms with vegetative desiccation tolerance, i.e. the ability to survive near total loss of water. Important metabolites include sugars such as sucrose, trehalose and raffinose family of oligosaccharides, amino acids and organic acids, as well as antioxidants, representing a common core mechanism of desiccation tolerance. Additional metabolites are discussed in the context of species specificity and adaptation.

Addresses

[1] Laboratory of Plant Physiology, Wageningen University and Research, The Netherlands

[2] Department of Molecular and Cell Biology, University of Cape Town, South Africa

[3] South African Grape and Wine Research Institute, Department of Viticulture and Oenology, Stellenbosch University, South Africa

Corresponding authors: Farrant, Jill M. (jill.farrant@uct.ac.za); Hilhorst, Henk WM. (henk.hilhorst@wur.nl)

Current Opinion in Plant Biology 2023, **75:**102410

This review comes from a themed issue on **Physiology and Metabolism** 2023

Edited by **Aleksandra Skirycz** and **Alexandra Jazz Dickinson**

For a complete overview see the Issue and the Editorial

Available online xxx

https://doi.org/10.1016/j.pbi.2023.102410

Keywords

Anhydrobiotic metabolome, Antioxidants, Dehydration, Primary metabolites, Specialised metabolites, Vegetative desiccation tolerance.

Introduction

Desiccation tolerance (DT) (the ability of organisms to survive drying to a water potential of −100MPa or below and to resume growth and metabolism on rehydration) is widespread in the plant kingdom, but while common in reproductive structures, it is rare in vegetative organs. The mechanisms of vegetative DT appear to be similar to those involved in reproductive DT [1], suggesting a common genetic and evolutionary basis for plant DT in general [2,3]. However, plants with vegetative desiccation tolerance — commonly called resurrection plants — face distinct stresses under water deficit that are commonly avoided or less severe in pollen and seeds, including photo-oxidative damage in photosynthetic organs, and the mechanical tension between shrinking cytoplasm and rigid cell walls. These require adaptations that are not necessary in specialised reproductive structures.

The survival of such extreme water deficit stress involves coordinated regulation of responses at various levels of organisation, including cellular, proteomic and metabolomic, involving both primary metabolites and specialised metabolites [1,4]. The latter have also been referred to as 'secondary' metabolites, although the term 'specialised' is to be preferred as they are not necessarily of secondary biological importance. To preserve the potential for recovery on rehydration, such responses must modulate the stresses on the functional network of the plant cell, including by stabilising the intracellular milieu and quenching destructive chemical reactions [5].

In this review, we survey the state of knowledge about the anhydrobiotic metabolome in angiosperms, highlight important areas of uncertainty, and propose experimental directions that may help fill gaps in our knowledge of how metabolic shifts contribute to anhydrobiosis.

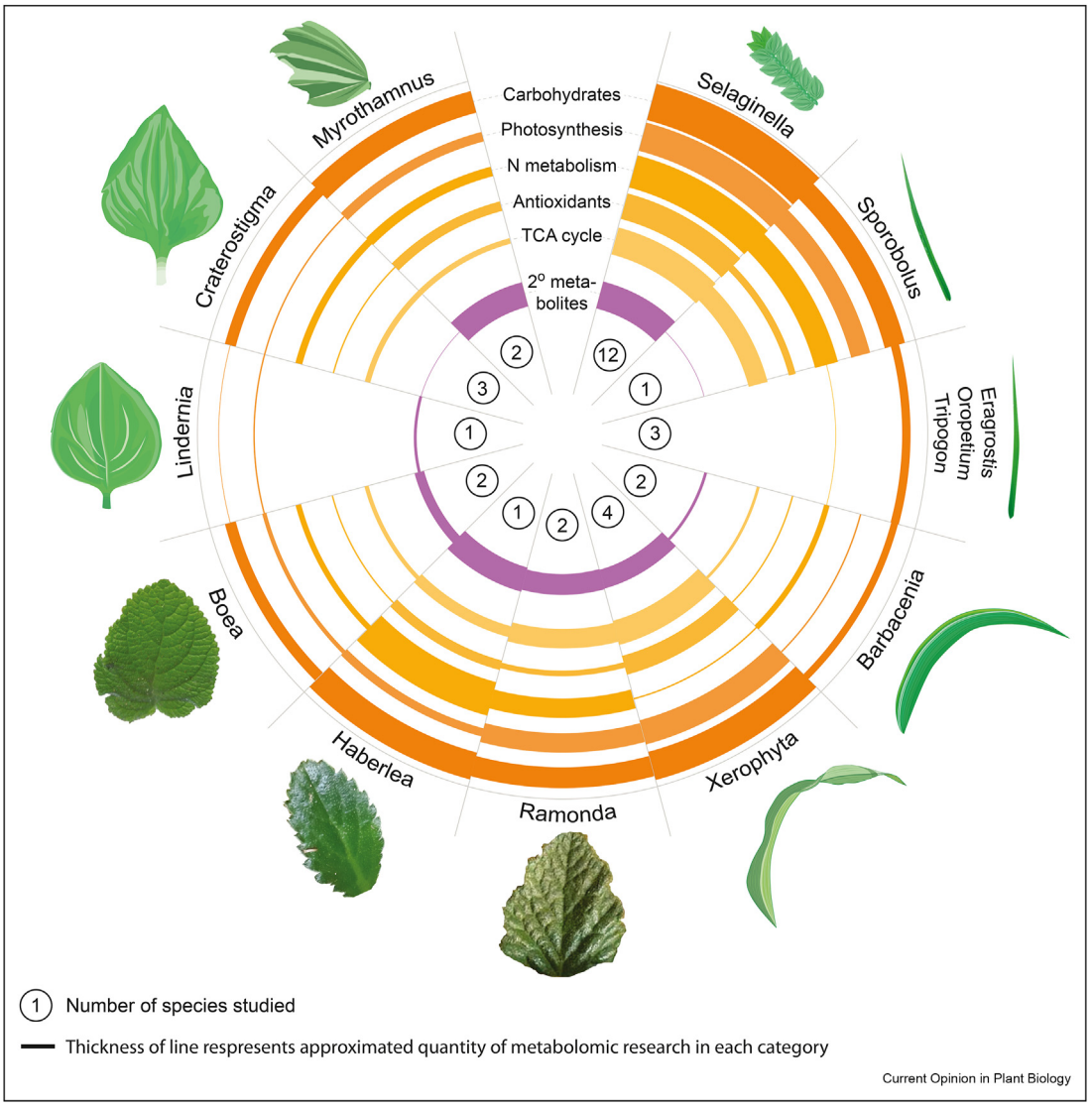
An inventory of desiccation-related metabolites

Figure 1 summarises the discussion in this section.

Primary metabolites

Primary metabolite analysis has been reported for only a small number of Angiosperm species (Figure 1). These studies primarily make use of gas chromatography−mass spectrometry (GC−MS) for identification and relative quantitation of small, Tandem Mass Spectrometry

Figure 1



Estimation of the amount of metabolite data currently available from the studies of various genera of resurrection plants. The numbers of species reported on are indicated, and leaf morphology typical of each genus shown. Primary metabolites (excluding lipids, but see Table 1), representing core mechanisms associated with anhydrobiosis, are depicted in shades of orange, while specialized metabolites are shown in purple. The thickness of the lines represents the approximate number of metabolites identified, combined from published studies.

(TMS)-derivatisable molecular species, providing valuable information about changes in abundance of numerous primary metabolites during drying and rehydration. However, the lack of absolute quantitation in most GC–MS studies, coupled with variation in the physiological states of experimental treatments, complicates their interpretation as a body of literature.

Specifically, various studies based on other -omics modalities [6] have suggested that there are three critical regulatory and metabolic shifts during drying, occurring during early (Relative Water Content (RWC), >60%), mid (30% < RWC <50%) and late (5% < RWC <25%) drying stages, respectively. Yet, existing metabolomics studies are not always well aligned to identify metabolic shifts corresponding to these regulatory/transcriptional phases. Recovery on rehydration is similarly complex, with various aspects of metabolism being restored in time frames varying from hours to a week [7] and varying widely among species. It should also be noted that it is difficult or impossible to infer changes in pathway flux from changes in the snapshot abundance of individual metabolites, even though flux may be of more biological interest.

Despite these interpretive challenges, important conclusions can be drawn about the regulation of various metabolites in relation to DT in various species.

Important metabolites associated with desiccation tolerance are listed in Table 1, according to the species and drying stage at which regulatory shifts have been observed.

Carbohydrates

Sugars appear central to the stabilisation of anhydrobiotic cells and tissues, for examples see the study by Oliver et al. [5], Peters et al. [19], Farrant et al. [24], Sherwin and Farrant [20], Gasulla et al. [21], Fait et al. [22], Costa et al. [23], Ghasempour et al. [25], Zhang et al. [26] and Djilianov et al. [27]. There appears to be consensus among previous studies that both sucrose and raffinose family oligosaccharides (RFOs) including raffinose, stachyose and verbascose are key to the desiccation response in many species [19–27]. The disaccharide trehalose has also been reported in various non-angiosperm resurrection plants [28], as well as the angiosperm *Myrothamnus flabellifolia*. The latter contains of the order of 50 mg g^{-1} dry weight of trehalose, while accumulating up to approximately 200 mg g^{-1} during drying [29]. Data from our laboratory suggest that all resurrection plants depend on either constitutively high levels of trehalose or induced increases in both sucrose and on one or more RFOs in order to acquire desiccation tolerance. Of those that depend on sucrose and RFOs, desiccation tolerance only appears possible when RFOs exceed a critical threshold of approximately 5–10% (w/w) of sucrose levels [30]. Other changes in carbohydrates include increasing levels of sugar alcohols in *Xerophyta viscosa* [7] and a decline in monosaccharides (notably glucose, fructose and galactose) in most species measured [30,17]. Less common changes include modulations in levels of lactose and xylose, which have been observed to decrease on drying in *Xerophyta schlechteri* [7]. *Craterostigma* spp, along with some other members of the Linderneaceae, exhibits unusual carbohydrate metabolism, accumulating large quantities of the rare sugar octulose during hydrated metabolism. Although the ratio of octulose to sucrose in *Craterostigma plantagineum* correlates with RWC, its precise role in relation to desiccation is not yet clear, although it may serve as a store for excess diurnal carbon and serve as a carbon source for sucrose biosynthesis during drying [31].

Amino acids and nitrogen metabolism

Free amino acid profiles display a variety of responses in various clades of resurrection plants. While the large-scale reorganisation of the cell with its shutdown of physiologically normal metabolism (including photosynthesis) in favour of protective species such as Late Enmbryogenesis Abundant (LEA) proteins might be expected to reveal itself in fluctuating amino acid profiles, amino acids have other roles in stress tolerance. Proline, for example, is involved in early drying responses as an osmoprotectant in several species, declining during further drying [32]. This is not universal; for example, it decreases in early drying but increases in late drying in *C. plantagineium* [33]. Serine, on the other hand, has been observed to deplete in abundance in a variety of species [7,17], though whether this is because it becomes harmful in the dry state or because it is a precursor to some other important resource (proteins or specialised metabolites) is not known.

In the grass *Sporobolus stapfianus*, aspartate, glutamate, cysteine glutathione disulphide and phenylalanine are all observed at elevated levels in the dry state and remain elevated on rehydration. Phenylalanine levels are particularly high and variable through drying and recovery, suggesting considerable flux through the pool of this aromatic intermediate [34].

While the diversity of amino acid profiles suggests that specific amino acids are not core requirements for surviving in the dry state, the scale of changes in these profiles suggests interesting relationships between nitrogen metabolism and the desiccation tolerant phenotype. Significant flux through the amino acid pools may be related to major changes in the proteome or to the modulation of senescence-related pathways for nitrogen conservation [35].

Tricarboxylic Acid (TCA) Cycle intermediates

It may be expected that the progressive shutdown of metabolism as the plant enters a quiescent state on drying will result in a decrease in abundance of pathway intermediates. However, it must be borne in mind that the general reduction in flux and energy status is contemporaneous with a significant shift in the overall metabolic network. This may involve carbon being introduced to and drawn from the TCA cycle at different points in the cycle, to support the differential regulation of numerous metabolic subnetworks, resulting in specific intermediates either increasing or decreasing in abundance [33]. For example, while *X. schlechteri* shows consistent decreases in the abundance of citric and malic acids during drying [8], *Xerophyta elegans* shows little change in citric acid levels and significant increases in malic acid during drying [30].

Antioxidants

Under even moderate levels of water deficit, photosynthetic organs produce reactive oxygen species (ROS) at potentially fatal levels [36,37]. At desiccation-associated RWCs, while the high peroxide production associated with photorespiration does not occur, nonenzymatic ROS production remains a risk as long as photosynthetic pigments are able to absorb light.

Modulating ROS flux and redox homeostasis in general is, therefore, an essential part of vegetative DT. Upregulation of antioxidant enzyme systems such as ascorbate

Table 1

Main metabolites reported with reference to the species and the relative water content.

Metabolites	Early response to drying (100-60%RWC)		Mid response to drying (60-40%RWC)		Late response to drying (below 40%)	
Amino acid and nitrogen metabolism	Increase	Decrease	Increase	Decrease	Increase	Decrease
5-oxoproline	*X. schlechteri* [7]					
Alanine[a]	*X. schlechteri* [7]				*X. schlechteri* [7]	
Allantoin		*S. stapfianus* [8]		*S. stapfianus* [8]	*S. stapfianus* [8]	
Arginine[a]					*B. purpurea* [9], *S. stapfianus* [10]	
Asparagine[a]	*B. purpurea* [9], *X. elegans* [11]	*S. stapfianus* [8,10]		*S. stapfianus* [8,10]	*B. purpurea* [9], *S. stapfianus* [10], *X. schlechteri* [7]	*S. stapfianus* [8]
Aspartic acid	*B. purpurea* [9]	*S. stapfianus* [10]		*S. stapfianus* [10]	*X. humilis* [12], *X. schlechteri* [7],	*S. stapfianus* [10]
Cysteine					*S. stapfianus* [8]	
Glutamic acid[a]	*B. purpurea* [9]		*X. schlechteri* [7]		*X. schlechteri* [7]	
Glutamine[a]	*B. purpurea* [9]			*S. stapfianus* [8]	*S. stapfianus* [8]	
Glutathione disulphide					*S. stapfianus* [8]	
Glycine[a]	*S. stapfianus* [8]				*B. purpurea* [9], *X. humilis* [12], *S. stapfianus* [8]	
Histidine	*B. purpurea* [9]				*B. purpurea* [9]	
Isoleucine	*S. stapfianus* [8]					
Leucine	*S. stapfianus* [8], *X. humilis* [11], *X. schlechteri* [7], *X. viscosa* [11]	*B. purpurea* [9]				
Lysine[a]	*B. purpurea* [9]		*X. schlechteri* [7]		*B. purpurea* [9]	
N-acetylproline	*S. stapfianus* [8]					
N-acetylthreonine	*S. stapfianus* [8]					
Ornithine			*X. schlechteri* [7]		*X. schlechteri* [7]	
Phenylalanine[a]					*X. humilis* [12], *X. schlechteri* [7]	
Pipecolic acid					*X. schlechteri* [7]	
Proline[a]	*B. purpurea* [9], *S. stapfianus* [8,10]	*R. serbica* [13]			*B. purpurea* [10], *R. serbica* [13], *S. stapfianus* [10] in old leaves	*S. stapfianus* [10] in young leaves
Serine[a]	*B. purpurea* [10]	*X. schlechteri* [7]			*B. purpurea* [10]	
Threonine[a]					*X. humilis* [12]	

	X. humilis [11], *X. schlechteri* [7], *X. viscosa* [11]					
Tryptophan[a]	*B. purpurea* [10], *S. stapfianus* [8], *X. schlechteri* [7]		*X. schlechteri* [7]		*B. purpurea* [10], *X. humilis* [12], *X. schlechteri* [7]	
Tyrosine[a]	*B. purpurea* [10], *S. stapfianus* [8], *X. schlechteri* [7]				*B. purpurea* [10], *X. humilis* [12], *X. schlechteri* [7]	
Valine[a]	*B. purpurea* [10], *S. stapfianus* [8]		*S. stapfianus* [10]		*B. purpurea* [10], *S. stapfianus* [10]	
γ-aminobutiric acid[a]	*B. purpurea* [10]				*B. purpurea* [10], *S. stapfianus* [10]	
γ-glutamylisoleucine						*S. stapfianus* [8]
γ-glutamylphenylalanine						*S. stapfianus* [8]
γ-glutamyltryptophan						*S. stapfianus* [8]
Nucleotide						
Adenosine diphosphate (ADP)	*X. viscosa* [14]		*S. stapfianus* [14]			*S. stapfianus* [14]
Adenosine triphosphate (ATP)	*S. stapfianus* [14], *X. viscosa* [14]		*S. stapfianus* [14]			*S. stapfianus* [14]
Guanine	*S. stapfianus* [8]					
Carbohydrates						
Allofuranose	*C. pumilum* [11]		*C. pumilum* [11]			
Arabinose[a]					*X. humilis* [12]	
Arabitol	*S. stapfianus* [8]				*X. humilis* [12]	
Fructose	*C. pumilum* [11], *M. flabellifolia* [11], *S. stapfianus* [8,14], *X. elegans* [11], *X. humilis* [11], *X. viscosa* [11]	*X. schlechteri* [7], *X. viscosa* [14]	*X. schlechteri* [7]	*S. stapfianus* [14], *X. viscosa* [14]		*M. flabellifolia* [15], *R. serbica* [13], *S. stapfianus*, *H. rhodopensis* [8,16,17], [14], *X. humilis* [12]
Fructose-12-phosphate						*X. humilis* [12]
Galactinol[a]	*C. pumilum* [11], *C. plantagineum* [18], *X. elegans* [11], *X. humilis* [11], *X. viscosa* [11]		*B. purpurea* [10], *C. pumilum* [11]		*B. purpurea* [10]	*C. plantagineum* [18], *X. humilis* [12]
Galactose	*S. stapfianus* [8]	*X. schlechteri* [7]	*X. schlechteri* [7]			
Galacturonate	*S. stapfianus* [8]					
Glucose	*C. pumilum* [11], *M. flabellifolia* [11], *S.*	*X. schlechteri* [7], *X. viscosa* [14]		*S. stapfianus* [14], *X. viscosa* [14]		*R. serbica* [13], *S. stapfianus*, *H. rhodopensis*

(continued on next page)

Table 1. (*continued*)

Metabolites	Early response to drying (100-60%RWC)		Mid response to drying (60-40%RWC)		Late response to drying (below 40%)	
Amino acid and nitrogen metabolism	Increase	Decrease	Increase	Decrease	Increase	Decrease
	stapfianus [8,14], *X. elegans* [11], *X. humilis* [11], *X. viscosa* [11]					[8,16,17], [14], *X. humilis* [18]
Glycerol[a]	*X. humilis* [11], *X. viscosa* [11]					
Lactose		*X. schlechteri* [7]				
Levoglucosan			*X. schlechteri* [7]			
Maltose	*S. stapfianus* [8]					
Maltotetraose					*S. stapfianus* [8]	
Mannitol	*B. purpurea* [10], *S. stapfianus* [8]		*B. purpurea* [10]		*X. humilis* [12]	*B. purpurea* [10]
Myo-inositol[a]	*X. humilis* [11], *X. viscosa* [11]	*X. schlechteri* [7]		*B. purpurea* [10]	*S. stapfianus* [8], *X. humilis* [11], *X. viscosa* [11]	*B. purpurea* [10], *X. viscosa* [19]
Octulose	*C. pumilum* [11], *C. plantagineum* [18]		*C. pumilum* [11]			*C. plantagineum* [18]
Raffinose[a]	*S. stapfianus* [8], *X. viscosa* [19]		*X. viscosa* [19]		*B. purpurea* [10], *C. plantagineum* [18], *C. pumilum* [11], *M. flabellifolia* [15], *S. stapfianus* [2], *X. humilis* [11], *X. viscosa* [11,19]	*R. serbica* [13]
Rhamnose	*X. schlechteri* [7]					
Sophorose	*S. stapfianus* [8]					
Sorbitol	*X. viscosa* [80]					
Stachyose	*C. plantagineum* [18], *X. viscosa* [19]		*X. viscosa* [19]		*B. purpurea* [10], *C. plantagineum* [18], *S. stapfianus* [8], *X. viscosa* [19]	
Sucrose[a]	*S. stapfianus* [8,14], *X. viscosa* [14,19]		*B. purpurea* [10], *S. stapfianus* [8,14], *X. viscosa* [14]		*B. purpurea* [10], *C. plantagineum* [18], *C. pumilum* [11], *H. rhodopensis* [8,16,17],	

				M. flabellifolia [11,15], *R. serbica* [13], *S. stapfianus* [8,14], *X. humilis* [11,12], *X. schlechteri* [7], *X. viscosa* [11,19]	
Trehalose[a]	*X. schlechteri* [7]			*B. purpurea* [10], *M. flabellifolia* [15]	
Verbascose	*X. viscosa* [19]		*X. viscosa* [19]	*B. purpurea* [10], *C. plantagineum* [18], *X. viscosa* [19]	
Xylitol				*X. humilis* [12]	
β-D-xylopyranose		*X. schlechteri* [7]			
Organic acid					
Aminocaproic acid				*X. schlechteri* [7]	
Citric acid	*X. elegans* [11]		*X. schlechteri* [7]	*X. humilis* [12], *X. schlechteri* [7]	
Fumaric acid[a]			*B. purpurea* [10]	*B. purpurea* [9]	
Glycolic acid	*X. schlechteri* [7]				
Malic acid	*X. humilis* [11], *X. viscosa* [11]				*X. humilis* [12]
Methylmalonic acid	*X. schlechteri* [7]			*X. schlechteri* [7]	
Phosphate	*X. elegans* [11], *X. humilis* [11], *X. viscosa* [11]				
Succinic acid					*X. humilis* [12]
Antioxidants/cofactors					
Ascorbate					
Ascorbate peroxidase			*C. wilmsii* [20]	*X. viscosa* [20]	*C. wilmsii* [20]
Glutathione reductase			*X. viscosa* [20]	*X. viscosa* [20]	*C. wilmsii* [20]
Superoxide dismutase			*X. viscosa* [20]	*C. wilmsii* [20], *X. viscosa* [20]	
Threonate	*S. stapfianus* [8]				
α-tocopherol	*S. stapfianus* [8]			*S. stapfianus* [8]	
B-tocopherol	*S. stapfianus* [8]			*S. stapfianus* [8]	
Δ-tocopherols				*S. stapfianus* [8]	
Lipids					
1-linoleoylglycerol	*S. stapfianus* [8]				
1-palmitoylglycerol	*S. stapfianus* [8]				
1-palmitoylglycerophosphoinositol				*S. stapfianus* [8]	
1-palmitoylglycerophospocholine				*S. stapfianus* [8]	
1-stearoylglycerophosphocholine				*S. stapfianus* [8]	
8-hydroxypalmitate	*S. stapfianus* [8]				
14-hydroxyoctanoate	*S. stapfianus* [8]				

(*continued on next page*)

Table 1. (*continued*)

Metabolites	Early response to drying (100-60%RWC)		Mid response to drying (60-40%RWC)		Late response to drying (below 40%)	
Amino acid and nitrogen metabolism	Increase	Decrease	Increase	Decrease	Increase	Decrease
Diacylglycerol		*C. plantagineum* [21]				*C. plantagineum* [21]
Digalactosyldiacylglycerol					*C. plantagineum, L. brevidens* [21]	
Linolenate		*S. stapfianus* [8]				
Monogalactosyldiacylglycerol		*C. plantagineum* [21]				*C. plantagineum, L. brevidens* [21]
Phosphatidic acid	*C. plantagineum* [21]				*C. plantagineum, L. brevidens* [21]	
Phosphatidylcholine					*C. plantagineum* [21]	*L. brevidens* [21]
Phosphatidylethanolamine					*C. plantagineum* [21]	*L. brevidens* [21]
Phosphatidylglycerol						*C. plantagineum, L. brevidens* [21]
Phosphatidylinositol					*C. plantagineum, L. brevidens* [21]	
Phosphatidylserine	*C. plantagineum* [21]				*C. plantagineum* [21]	*L. brevidens* [21]
Sulfoquinovosyldiacylglycerol					*C. plantagineum* [21]	*L. brevidens* [21]
Tetragalactosyldiacylglycerol					*C. plantagineum, L. brevidens* [21]	
Triacylglycerol	*C. plantagineum* [21]				*C. plantagineum, L. brevidens* [21]	
Trigalactosyldiacylglycerol					*C. plantagineum, L. brevidens* [21]	

[a] Primary metabolites in seeds correlating with late response to desiccation [22,23].

peroxidase and glutathione reductase has been reported during early- to mid-drying in various resurrection plants (reviewed in the study by Farrant and Hilhorst [6]). Increases in antioxidants, such as ascorbate and glutathione, and lipid-soluble antioxidants, such as tocopherols, have been observed in *Sporobolus stepfianus* [34]; carotenoids and chlorogenic acids respond to drying in *X. schlechteri* [8] and *Barbacennia purpurea* [9]; lipid-soluble tocopherols are seen in *Boea hygrometrica* [38]; other phenolic antioxidants have been observed in *Ramonda serbica* [39] and *M. flabellifolia* [40].

Photosynthesis-related compounds

The negative impact of chlorophyll on the longevity of orthodox seeds has been extensively documented [41]. Like seeds, resurrection plants have adapted mechanisms either to catabolise chlorophyll during early- to mid-drying (poikilochlorophylly) [42] or to retain chlorophyll but minimise its exposure to light in the dry state (homoiochlorophylly). In the latter case, the persistence of intact photosystems is thought to be associated with other protective mechanisms or behaviour, such as shade affinity, leaf folding or the accumulation of anthocyanins or other protective pigments, although there has been no recent systematisation of such strategies.

Specialised metabolites

Relatively little detailed research has been undertaken on the natural products chemistry of resurrection plants (Figure 1). This is largely because the annotation of specialised metabolites, whether by liquid chromatography–mass spectrometry or nuclear magnetic resonance (NMR) is expensive and time-consuming, and plants that are neither widespread physiological models nor important crops in themselves do not benefit from substantial existing peak databases.

There have been exceptions, and species that have enjoyed significant attention in this regard have yielded interesting sets of specialised metabolites, some with potential medicinal applications. *M. flabellifolia* accumulates large quantities of the polyphenol 3,4,5-tri-O-galloylquinic acid in its vacuoles [43,44]. This metabolite, which has been shown to protect liposomes against desiccation and oxidative damage, is found in greater abundance in *M. flabellifolia* populations in more arid regions [45], suggesting that it may be associated with improving the longevity of the plant in the desiccated state. *R. serbica* has been shown to accumulate phenolic acid at the onset of desiccation [39,45], while phenolic flavonoids have been identified in *B. hygrometrica* [46]. Southern African *Xerophyta* species contain chlorogenic acids and feruloyl conjugates which seem to increase on dehydration [47]. A recent survey of phenolic compounds in resurrection plants of the family Linderniaceae identified eight phenylethanoid glycosides and one flavone, luteolin hexoside pentoside [48]. In that study, the levels of verbascoside correlated with the degree of vegetative DT.

Phenolics and other aromatic metabolites (and their precursors) appear frequently to be regulated in response to water deficit in resurrection plants. As a group, they present the interesting possibility of 'dual utility', with both bittering/antifeedant functions, and as reservoirs of readily available π-electrons to quench excess ROS in a form that is compatible with the biophysical context of anhydrobiosis.

In general, significant further work is needed to fully dissect the role of phenolic compounds and other specialised metabolites in the DT of angiosperm resurrection plants.

How selected metabolites protect cells

Although there is considerable variation in the metabolic response of various resurrection plants, a number of broad themes emerge concerning the protective mechanisms of core metabolite families.

The most consistent observations concern the mass accumulation of sugars: sucrose and RFOs or trehalose. These hydroxyl-rich compounds appear necessary to stabilise macromolecules and enable the transition to stable, noncrystalline vitreous phases at very low water contents. Considerable prior work on the thermodynamics of anhydrous life forms has shown that such glasses are critical to the survival of desiccation tolerant organisms and explains a considerable amount of the difference in low-water-content survival between sensitive and tolerant tissues [49]. Even though given the enormous complexity of the intracellular matrix, the sugars alone cannot account for its biophysical stability in the dry state, they are likely to be necessary in combination with LEAs and other intrinsically disordered proteins [50].

Despite the compelling existing evidence for a predominantly glassy state, there have been hints of unexpected levels of molecular mobility and possible metabolic activities in otherwise-quiescent anhydrobiotic systems [6,33]. This leaves open the possibility that at least some components of the cellular matrix may remain liquid in the anhydrous state, possibly in the form of natural deep eutectic solvents (NADESs) [51]. These ionic liquids have been synthesised *in vitro* from various combinations of, among other compounds, citric and malic acids, choline, and various sugars including sucrose, glucose and fructose.

Several potentially NADES-forming metabolites have been observed to increase on drying in resurrection plants and have been shown *in vitro* to form compatible solvent systems for certain biological processes [52,53].

Pockets of such anhydrous liquids could be stabilised by liquid−glass phase separation systems analogous to the liquid−liquid systems already shown to be involved in other desiccation tolerant systems [54].

The second major theme that emerges from a cross-species view of desiccation-related metabolism is redox homeostasis and the control of ROS fluxes. While the classical view has been that ROS are harmful to cells and in direct tension with their survival and longevity, recent studies have shown that redox homeostasis is far more subtle and that ROS have valuable roles in signalling and state communication, suggesting that the establishment and maintenance of antioxidant pools is likely to be carefully coordinated at transcriptional and metabolic levels [43,55].

In addition to these core physiological functions, it is possible that accumulated metabolites also ameliorate challenges in the field that plants would normally respond to dynamically. For example, sucrose-filled leaves in an arid landscape may be very appealing to herbivores, making the accumulation of antifeedant compounds advantageous. And plants that accumulate high levels of compounds adapted to interact with animals are potentially interesting targets for drug discovery.

Some open questions and ways forward

The study of anhydrobiosis has countless possible benefits — from climate change mitigation to postharvest supply chains, biomedical applications such as room-temperature-shippable vaccines and potential novel drugs. Yet it appears that in recent years, while progress has surged ahead on questions of the genetic and evolutionary basis for plant DT, some key open questions about its physiochemical nature have remained open.

What we know about the thermodynamics of phase transitions in desiccation tolerant systems is based on relatively simple systems measured on a previous generation of instrumentation. High-throughput combinatorial fluid handling systems, combined with the latest calorimetry, spectroscopy and imaging instruments, could allow much finer-grained exploration of the constraints of the vitreous and NADES systems thought to be involved in stabilising plants in the dry state.

Since the effects and interactions of molecular species, small and large, depend on their localisation in time and place, valuable contributions could be made using chemical imaging technologies such as NMR micro-imaging, Matrix-associated laser desportion/ionization imaging and Fourier transform infrared microscopy [45]. Collaborations with colleagues from fields including physical chemistry and food physics could enrich our practical and theoretical toolset for tackling such questions.

Techniques for the fractionation of anhydrous materials remain limited and crude, often depending on old protocols and hazardous materials. The DT community would certainly welcome the development of more refined techniques for the subcellular fractionation of desiccated tissues.

The topic of redox homeostasis as it relates to stress remains lively, with exciting possibilities for measurement and manipulation of redox potentials in both classical model and nonmodel species.

On the phytochemistry front, there is such a paucity of data about resurrection plants, and so much reason to believe that their specialised metabolites may have additional uses, that this represents a rich vein of potential projects. We note that of the natural products isolated to date, most have been characterised by low-cost "traditional" techniques of chromatography and spectroscopy, meaning that such studies need not be confined to resource-rich laboratories or developed countries.

Author contributions

JMF was invited to write the review. **She**, **HWMH** and **HJWD** conceptualised the review, the majority of which was compiled and written by **HJWD**, with final editing by **JMF**. **AEA** provided information from studies on primary metabolites and composed Table 1 based on these data, and **JPM** provided information on studies on specialised metabolites.

Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

Data availability

No data was used for the research described in the article.

Acknowledgements

The authors thank Keren Cooper, Department of Molecular and Cell Biology, University of Cape Town, South Africa for providing Figure 1. JMF acknowledges the South African Department of Science and Innovation − National Research Foundation for her Research Chair funding. Grant number 98406.

References

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest

1. Farrant JM, Moore JP, Hilhorst HWM: **Editorial: unifying insights into the desiccation tolerance mechanisms of resurrection plants and seeds**. *Front Plant Sci* 2020, **11**:1089.

2. Gaff DF, Oliver M: **The evolution of desiccation tolerance in angiosperm plants: a rare yet common phenomenon**. *Funct Plant Biol* 2013, **40**:315−328.

3. VanBuren R, Wai CM, Pardo J, Giarola V, Ambrosini S, Song X, *et al.*: **Desiccation tolerance evolved through gene**

duplication and network rewiring in lindernia. *Plant Cell* 2018, **30**:2943–2958.

4. Dinakar C, Bartels D: **Desiccation tolerance in resurrection plants: new insights from transcriptome, proteome and metabolome analysis**. *Front Plant Sci* 2013, **4**.

5. Oliver MJ, Farrant JM, Hilhorst HWM, Mundree S, Williams B, Bewley JD. *Desiccation tolerance: avoiding cellular damage during drying and rehydration*, **71**; 2020:435–460. https://doi-org.ezproxy.library.wur.nl/101146/annurev-arplant-071219-105542.

6. • Farrant JM, Hilhorst H: **Crops for dry environments**. *Curr Opin Biotechnol* 2022, **74**:84–91.
This recent review anticipates possibilities for translating DT research to agricultural applications in the face of climate change.

7. Radermacher AL, du Toit SF, Farrant JM: **Desiccation-driven senescence in the resurrection plant Xerophyta schlechteri (Baker) N.L. Menezes: comparison of anatomical, ultrastructural, and metabolic responses between senescent and non-senescent tissues**. *Front Plant Sci* 2019, **10**:1396.

8. Oliver MJ, Guo L, Alexander DC, Ryals JA, Wone BWM, Cushman JC: **A sister group contrast using untargeted global metabolomic analysis delineates the biochemical regulation underlying desiccation tolerance in Sporobolus stapfianus**. *Plant Cell* 2011, **23**:1231–1248.

9. Suguiyama VF, Silva EA, Meirelles ST, Centeno DC, Braga MR: **Leaf metabolite profile of the Brazilian resurrection plant Barbacenia purpurea Hook. (Velloziaceae) shows two time-dependent responses during desiccation and recovering**. *Front Plant Sci* 2014, **5**:193.

10. Martinelli T, Whittaker A, Bochicchio A, Vazzana C, Suzuki A, Masclaux-Daubresse C: **Amino acid pattern and glutamate metabolism during dehydration stress in the "resurrection" plant Sporobolus stapfianus: a comparison between desiccation-sensitive and desiccation-tolerant leaves**. *J Exp Bot* 2007, **58**:3037–3046.

11. Ricco CR: *Metabolite analysis of resurrection plants*. Town: MSc thesis Wageningen University & University of Cape; 2018.

12. Dace H: ***The metabolomics of desiccation tolerance in Xerophyta humilis***. Town: University of Cape; 2014. MSc thesis.

13. Živković T, Quartacci MF, Stevanović B, Marinone F, Navari-Izzo F: **Low-molecular weight substances in the poikilohydric plant Ramonda serbica during dehydration and rehydration**. *Plant Sci* 2005, **168**:105–111.

14. Whittaker A, Bochicchio A, Vazzana C, Lindsey G, Farrant J: **Changes in leaf hexokinase activity and metabolite levels in response to drying in the desiccation-tolerant species Sporobolus stapfianus and Xerophyta viscosa**. *J Exp Bot* 2001, **52**:961–969.

15. Moore JP, Hearshaw M, Ravenscroft N, Lindsey GG, Farrant JM, Brandt WF: **Desiccation-induced ultrastructural and biochemical changes in the leaves of the resurrection plant Myrothamnus flabellifolia**. *Aust J Bot* 2007, **55**:482–491.

16. Gechev TS, Benina M, Obata T, Tohge T, Sujeeth N, Minkov I, Hille J, Temanni M-R, Marriott AS, Bergström E, *et al.*: **Molecular mechanisms of desiccation tolerance in the resurrection glacial relic Haberlea rhodopensis**. *Cell Mol Life Sci* 2013, **70**:689–709.

17. Moyankova D, Mladenov P, Berkov S, Peshev D, Georgieva D, Djilianov D: **Metabolic profiling of the resurrection plant Haberlea rhodopensis during desiccation and recovery**. *Physiol Plantarum* 2014, **152**:675–687.

18. Egert A, Eicher B, Keller F, Peters S: **Evidence for water deficit-induced mass increases of raffinose family oligosaccharides (RFOs) in the leaves of three Craterostigma resurrection plant species**. *Front Physiol* 2015, **0**:991.

19. Peters S, Mundree SG, Thomson JA, Farrant JM, Keller F: **Protection mechanisms in the resurrection plant Xerophyta viscosa (Baker): both sucrose and raffinose family oligosaccharides (RFOs) accumulate in leaves in response to water deficit**. *J Exp Bot* 2007, **58**:1947–1956.

20. Sherwin HW, Farrant JM: **Protection mechanisms against excess light in the resurrection plants Craterostigma wilmsii and Xerophyta viscosa**. *Plant Growth Regul* 1998, **24**:203–210.

21. Gasulla F, Vom Dorp K, Dombrink I, Zähringer U, Gisch N, Dörmann P, *et al.*: **The role of lipid metabolism in the acquisition of desiccation tolerance in Craterostigma plantagineum: a comparative approach**. *Plant J* 2013, **75**:726–741.

22. Fait A, Angelovici R, Less H, Ohad I, Urbanczyk-Wochniak E, Fernie AR, Galili G: **Arabidopsis seed development and germination is associated with temporally distinct metabolic switches**. *Plant Physiol* 2006, **142**:839–854.

23. Costa MCD, Cooper K, Hilhorst HWM, Farrant JM: **Orthodox seeds and resurrection plants: two of a kind?** *Plant Physiol* 2017, **175**:589–599.

24. Farrant JM, Cooper K, Hilgart A, Abdalla KO, Bentley J, Thomson JA, *et al.*: **A molecular physiological review of vegetative desiccation tolerance in the resurrection plant Xerophyta viscosa (Baker)**. *Planta* 2015, **242**:407–426.

25. Ghasempour HR, Gaff DF, Williams RPW, Gianello RD: **Contents of sugars in leaves of drying desiccation tolerant flowering plants, particularly grasses**. *Plant Growth Regul* 1998, **24**:185–191.

26. Zhang Q, Song X, Bartels D: **Enzymes and metabolites in carbohydrate metabolism of desiccation tolerant plants**. *Proteomes* 2016, **4**:40.

27. Djilianov D, Ivanov S, Moyankova D, Miteva L, Kirova E, Alexieva V, *et al.*: **Sugar ratios, glutathione redox status and phenols in the resurrection species Haberlea rhodopensis and the closely related non-resurrection species Chirita eberhardtii**. *Plant Biol* 2011, **13**:767–776.

28. Yobi A, Wone BWM, Xu W, Alexander DC, Guo L, Ryals JA, *et al.*: **Metabolomic profiling in Selaginella lepidophylla at various hydration states provides new insights into the mechanistic basis of desiccation tolerance**. *Mol Plant* 2013, **6**:369–385.

29. Bianchi G, Gamba A, Limiroli R, Pozzi N, Elster R, Salamini F, Bartels D: **The unusual sugar composition in leaves of the resurrection plant Myrothamnus flabellifolia**. *Physiol Plantarum* 1993, **87**:223–226.

30. Dace HJW, Reus R, Ricco CR, Hall R, Farrant JM, Hilhorst HWM: *A horizontal view of primary metabolomes in vegetative desiccation tolerance*. 2023, https://doi.org/10.1101/2023.02.10.528018.

31. Zhang Q, Bartels D: **Octulose: a forgotten metabolite?** *J Exp Bot* 2017, **68**:5689–5694.

32. Kishor PBK, Sreenivasulu N: **Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue?** *Plant Cell Environ* 2014, **37**:300–311.

33. • Xu X, Legay S, Sergeant K, Zorzan S, Leclercq CC, Charton S, *et al.*: **Molecular insights into plant desiccation tolerance: transcriptomics, proteomics and targeted metabolite profiling in Craterostigma plantagineum**. *Plant J* 2021, **107**:377–398.
An excellent recent example of integrative multi-omics analysis to elucidate regulatory relationships between genetic and metabolic responses to desiccation.

34. • Yobi A, Schlauch KA, Tillett RL, Yim WC, Espinoza C, Wone BWM, *et al.*: **Sporobolus stapfianus: insights into desiccation tolerance in the resurrection grasses from linking transcriptomics to metabolomics**. *BMC Plant Biol* 2017, **17**:67.
A key recent multi-omics analysis that reveals important aspects of the relationship of transcriptional with metabolic control, across a physiologically relevant range of treatments.

35. Kabbage M, Kessens R, Bartholomay L, Williams B: **The life and death of a plant cell**. *Annu Rev Plant Biol* 2015, **68**:375–404.

36. Hasanuzzaman M, Nahar K, Gill SS, Fujita M: *Climate change and plant abiotic stress tolerance*. 2013, https://doi.org/10.1002/9783527675265.ch09.

37. Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, *et al.*: **Crop production under drought and heat stress: plant**

responses and management options. *Front Plant Sci* 2017, **8**: 1147.

38. Zhu Y, Wang B, Phillips J, Zhang Z-N, Du H, Xu T, Huang L-C, Zhang X-F, Xu G-H, Li W-L, *et al.*: **Global transcriptome analysis reveals acclimation-primed processes involved in the acquisition of desiccation tolerance in Boea hygrometrica**. *Plant Cell Physiol* 2015, **56**:1429–1441.

39. Sgherri C, Stevanovic B, Navari-Izzo F: **Role of phenolics in the antioxidative status of the resurrection plant Ramonda serbica during dehydration and rehydration**. *Physiol Plantarum* 2004, **122**:478–485.

40. Moore JP, Lindsey GG, Farrant JM, Brandt WF: **An overview of the biology of the desiccation-tolerant resurrection plant Myrothamnus flabellifolia**. *Ann Bot-london* 2007, **99**:211–217.

41. Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H: *Seeds, physiology of development, germination and dormancy*. 3rd ed. 2013, https://doi.org/10.1007/978-1-4614-4693-4.

42. Christ B, Egert A, Süssenbacher I, Kräutler B, Bartels D, Peters S, Hörtensteiner S: **Chlorophyll breakdown in resurrection plants**. *Plant Cell Environ* 2014, **37**:2521–2531.

43. Moore JP, Westall KL, Ravenscroft N, Farrant JM, Lindsey GG, Brandt WF: **The predominant polyphenol in the leaves of the resurrection plant Myrothamnus flabellifolius, 3,4,5 tri-O-galloylquinic acid, protects membranes against desiccation and free radical-induced oxidation**. *Biochem J* 2005, **385**:301–308.

44. Moore JP, Farrant JM, Lindsey GG, Brandt WF: **The South African and Namibian populations of the resurrection plant Myrothamnus flabellifolius are genetically distinct and display variation in their galloylquinic acid composition**. *J Chem Ecol* 2005, **31**:2823–2834.

45. Veljovic-Jovanovic S, Kukavica B, Navari-Izzo F: **Characterization of polyphenol oxidase changes induced by desiccation of Ramonda serbica leaves**. *Physiol Plantarum* 2008, **132**:407–416.

46. Feng W-S, Li Y-J, Zheng X-K, Wang Y-Z, Su F-Y: **Chemical constituents of Boea hygrometrica**. *Chin J Nat Med* 2011, **9**: 406–409.

47. Dzobo K: *Characterization of polyphenols in leaves of four desiccation tolerant plant families*. MSc Thesis, University of Cape Town; 2005.

48. Passon M, Weber F, Jung NU, Bartels D: **Profiling of phenolic compounds in desiccation-tolerant and non-desiccation-tolerant Linderniaceae**. *Phytochem Anal* 2021, **32**: 521–529.

49. Buitink J, Leprince O: **Intracellular glasses and seed survival in the dry state**. *C R Biol* 2008, **331**:788–795.

50. • Berjak P: **Unifying perspectives of some mechanisms basic to desiccation tolerance across life forms**. *Seed Sci Res* 2006, **16**:1–15.
Despite its age, this review remains arguably the most lucidly accessible description of the cell biology and physical chemistry of vitrification in plant tissues.

51. Choi YH, Spronsen J van, Dai Y, Verberne M, Hollmann F, Arends IWCE, Witkamp GJ, Verpoorte R: **Are natural Deep eutectic solvents the missing link in understanding cellular metabolism and physiology?** *Plant Physiol* 2011, **156**: 1701–1705.

52. • du Toit SF du, Bentley J, Farrant JM: **NaDES formation in vegetative desiccation tolerance: prospects and challenges**. *Adv Bot Res* 2020, https://doi.org/10.1016/bs.abr.2020.09.007.
A very useful overview of NADES formation, containing a comprehensive table of primary metabolites and their occurrence in a wide variety of resurrection plants.

53. • Bentley J, Olsen EK, Moore JP, Farrant JM: **The phenolic profile extracted from the desiccation-tolerant medicinal shrub Myrothamnus flabellifolia using Natural Deep Eutectic Solvents varies according to the solvation conditions**. *Phytochemistry* 2020, https://doi.org/10.1016/j.phytochem.2020.112323.
This paper progresses the state of the art in anhydrobiosis by demonstrating the biocompatible solvent properties of synthetic NADES.

54. Belott C, Janis B, Menze MA: **Liquid-liquid phase separation promotes animal desiccation tolerance**. *Proc Natl Acad Sci USA* 2020, https://doi.org/10.1073/pnas.2014463117.

55. Noctor G, Mhamdi A, Foyer CH: **The roles of reactive oxygen metabolism in drought: not so cut and dried**. *Plant Physiol* 2014, **164**:1636–1648.