



# Specific metabolic and cellular mechanisms of the vegetative desiccation tolerance in resurrection plants for adaptation to extreme dryness

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## Abstract

**Main conclusion** Substantial advancements have been made in our comprehension of vegetative desiccation tolerance in resurrection plants, and further research is still warranted to elucidate the mechanisms governing distinct cellular adaptations.

**Abstract** Resurrection plants are commonly referred to as a small group of extremophile vascular plants that exhibit vegetative desiccation tolerance (VDT), meaning that their vegetative tissues can survive extreme drought stress (> 90% water loss) and subsequently recover rapidly upon rehydration. In contrast to most vascular plants, which typically employ water-saving strategies to resist partial water loss and optimize water absorption and utilization to a limited extent under moderate drought stress, ultimately succumbing to cell death when confronted with severe and extreme drought conditions, resurrection plants have evolved unique mechanisms of VDT, enabling them to maintain viability even in the absence of water for extended periods, permitting them to rejuvenate without harm upon water contact. Understanding the mechanisms associated with VDT in resurrection plants holds the promise of expanding our understanding of how plants adapt to exceedingly arid environments, a phenomenon increasingly prevalent due to global warming. This review offers an updated and comprehensive overview of recent advances in VDT within resurrection plants, with particular emphasis on elucidating the metabolic and cellular adaptations during desiccation, including the intricate processes of cell wall folding and the prevention of cell death. Furthermore, this review highlights existing unanswered questions in the field, suggests potential avenues for further research to gain deeper insights into the remarkable VDT adaptations observed in resurrection plants, and highlights the potential application of VDT-derived techniques in crop breeding to enhance tolerance to extreme drought stress.

**Keywords** Anti-cell death · Autophagy · Cell wall folding · Chloroplast · Extremophile · Mitochondria

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## Introduction

Given the increasing frequency of extreme meteorological events driven by global warming, many countries recognize the urgent need to address their threat to food security and sustainable agriculture. Drought stress, often exacerbated by high temperatures, is a major environmental factor limiting plant growth and productivity. Extreme and flash droughts can lead to seedling and organ primordia mortality, pollination and seed set failure, reduced nutrient assimilation, grain filling, and ultimately low crop yields (Walker and Loon 2023). However, identifying effective genes or genetic modules to enhance crop drought tolerance by improving cell protection and survival under drought stress is a significant challenge, and these genetic resources are unlikely to be found in conventional crops or model plants unable to withstand extreme drought stress.

Extremophiles have attracted considerable interest within the biotech industry due to their remarkable resilience in harsh environmental conditions. A prime example is the groundbreaking discovery of the highly heat-stable *Taq* DNA ligase in the thermophilic bacterium *Thermus aquaticus*, which catalyzed a revolutionary advancement in molecular biology (Kahashi et al. 1984). Thus, the study of extremophiles holds the potential to shed light on our understanding of extreme drought stress tolerance and the identification of genes or genetic modules essential for enhancing cellular desiccation tolerance in crops. While extremophile plants are relatively rare, they have been identified as thriving in extremely arid habitats. Some examples include certain algae, lichens, bryophytes (such as mosses, liverworts, and hornworts), and a specific subset of vascular plants known as “resurrection plants” (Bouléh et al. 2020). This review provides an updated account of the progress made in understanding vegetative desiccation tolerance (VDT) within resurrection plants, with particular emphasis on elucidating the specific metabolic and cellular mechanisms

that enable resurrection plants to withstand desiccation. Furthermore, the review outlines perspectives on future directions for comprehending and harnessing VDT to breed crops better adapted to extremely dry conditions.

Water is an indispensable and vital element for all living organisms. In general, when water stress occurs, the relative water content (RWC) in plant vegetative tissues decreases in response to the severity of drought stress, transitioning from fully hydrated (RWC = 80–100%) to mildly dehydrated (RWC = 70–80%), moderately dehydrated (RWC = 50–70%), severely dehydrated (RWC = 30–50%), or desiccated (RWC < 30%) (Zhang and Bartels 2018). While most subsistence and cash crops exhibit mild-to-moderate drought tolerance, they are highly sensitive to severe and prolonged drought conditions due to their limited capacity for survival and recovery in the face of extreme aridity. In contrast, resurrection plants possess a remarkable ability known as vegetative desiccation tolerance (VDT), enabling them to withstand water loss exceeding 90% and reduce their absolute water content to approximately 0.1 g water g<sup>-1</sup> dry weight for extended periods, even years. Upon the availability of water, resurrection plants can fully restore their physiological and metabolic activities, resuming normal growth and reproduction. Approximately 330 vascular resurrection plant species have been identified on all continents except Antarctica, including both angiosperms and ferns, but not any gymnosperm (Oliver et al. 2020). The majority of these species are native to arid climates in tropical and subtropical regions, typically characterized by dry and hot conditions, such as southern and eastern Africa, South America, and western Australia (Oliver et al. 2020). Notably, certain Gesneriaceae family species are found in the north temperate zone, where temperatures can plummet below –10 °C, which indicates that these plants have remarkable abilities to tolerate both dry conditions and low temperatures (Fernández-Marín et al. 2020; Mihailova et al. 2023; Mitra et al. 2013). Resurrection plants, such as *Boea hygrometrica* (also known as *Dorcoceras hygrometricum*) in their natural habitat (Fig. 1), often thrive on rocky outcrops,



**Fig. 1** *Boea hygrometrica* plant in its native habitat (Phoenix Mountain, Beijing, 116°20'E, 40°00'N), experiencing an annual desiccation-resurrection cycle. Photographs taken by Xiaoqiang Liu and Yuanyuan Wang on the dates indicated below each photograph

experience multiple cycles of dehydration and rehydration, as well as high temperatures on the rocky surface. However, an intriguing exception is *Lindernia brevidens*, which thrives in a high-humidity habitat within the tropical rainforests of Africa (Phillips et al. 2008).

Most vascular plants cope with drought by minimizing water evaporation and promoting root growth to increase the water absorbance and improve the efficiency of water utilization, an approach to avoid cellular water loss and hydraulic failure (Johnson et al. 2018). Severe drought stress disrupts plants' water uptake, nutrient transport, and physiological and metabolic activities, leading to the accumulation of toxic byproducts, secondary oxidative stress, and damage to cell membranes and organelles as cellular water content gradually decreases to lethal levels. Surviving desiccation is a prerequisite for the subsequent recovery of resurrection plants upon rehydration. Resurrection plant cells have to employ unique or additional protective mechanisms to mitigate and resist impairments caused by cellular desiccation to bring them to a repairable level at a required time, thus achieving a balance between growth and environmental adaptation. The common view is that when the relative water content (RWC) of resurrection plants drops to 20–40%, transcriptional reprogramming occurs. This activation of genes is responsible for maintaining cellular and organelle structural integrity, suspending specific biochemical and physiological activities, inactivating protein complexes and enzymes, and initiating repair processes.

Although the VDT traits are commonly found across different species, it is important to note that the underlying mechanisms are diverse and species specific (Alejo-Jacuinde et al. 2020). For instance, even the rate of water loss has varying effects on the ability of resurrection species to recover after complete desiccation. Concerning the inducible traits related to desiccation tolerance in vascular plants, slow drying has been established as the most suitable method for simulating drought stress (Oliver et al. 2020). This choice is based on the observation that certain resurrection species, such as *Xerophyta humilis* and *Myrothamnus flabellifolius*, lose their ability to revive after rapid drying (Farrant et al. 1999). In contrast, *Craterostigma wilmsii* can survive rapid drying due to its capacity to promptly activate a set of protective mechanisms that ensure plant survival, although the observed cell damages are more prominent compared to the slow drying procedures (Farrant et al. 1999). These cellular damages are subsequently repaired during the rehydration process through the accumulation of different transcripts and proteins. It is worth noting that rapidly dried plants cannot revive if transcription or translation processes are blocked (Cooper and Farrant 2002). Another example is *Boea hygrometrica*, which loses viability when rapidly dried, but when subjected to slow drying (acclimated), it recovers and

becomes capable of enduring air-drying, ultimately resur-recting upon rehydration (Zhu et al. 2015). Intriguingly, this acclimation-primed air-drying tolerance can be retained for approximately 3 months but is lost thereafter (Sun et al. 2021). This observation explains why plants collected from their native habitats typically exhibit air-drying tolerance (Mitra et al. 2013). Furthermore, not only can whole plants but also detached leaves and cut leaf discs from mature plants collected in their native habitats survive desicca-tion and fully recover upon rehydration (Fig. 2), similar to *Paraisometrum mileense* (Li et al. 2014). However, VDT does not seem to depend on the presence of specialized mor-phological structures for water transport and conservation. For instance, a recent study on the epiphytic fern *Pleopeltis polypodioides* found that fronds could endure desiccation, regardless of whether fronds were connected to their rhi-zome (Prats and Brodersen 2021). Taken together, these studies demonstrate that desiccation tolerance primarily relies on cellular-level protection and regulation, independ-ent of root–shoot communication or functional vessel water transport. Given these discoveries, resurrection plants have emerged as valuable models for uncovering mechanisms of cellular desiccation protection and molecular modules that could be harnessed for developing crop plants with increased tolerance to extreme drought stress.

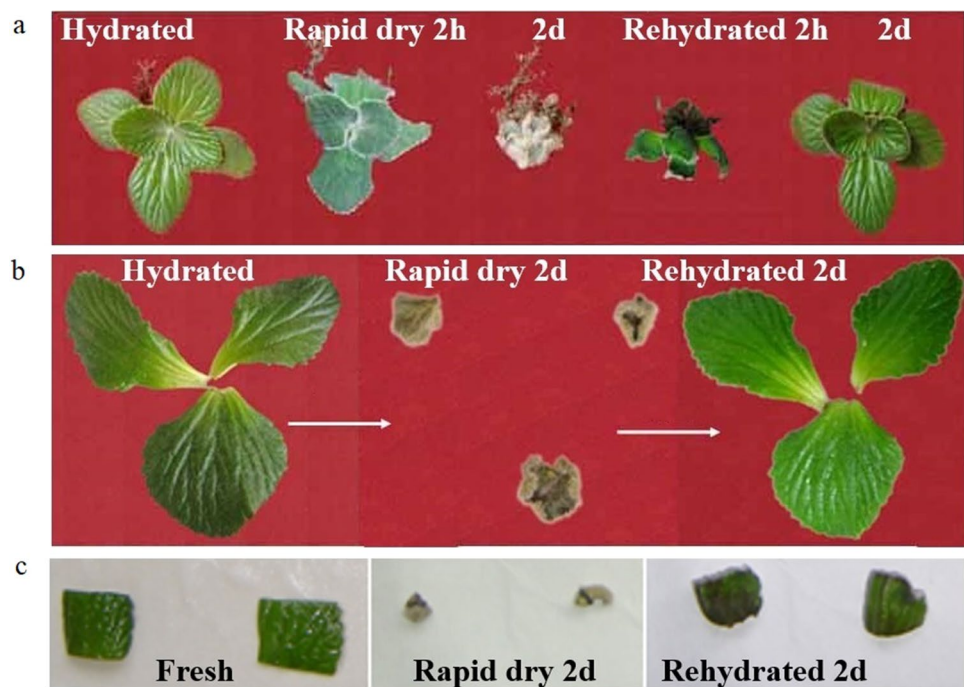
## VDT mechanisms in resurrection plants in the multi-omics era

Over the past century, researchers have achieved significant progress in four main facets of desiccation tolerance within resurrection plants: first, the discovery and categorization of these plants along with their natural habitats; secondly, the elucidation of the conditions necessary for revival after desiccation, encompassing factors such as the degree of water loss, duration of drought, and cycles of dehydration and rehydration; thirdly, the comprehensive characterization of vital morphological adjustments, physiological and metabolic adaptations, and cellular and subcellular alterations occurring during dehydration and subsequent rehydration cycles; and fourthly, the identification of genes and proteins linked to safeguarding plant tissues from desiccation, with notable examples including *Craterostigma plantagineum*, *Haberlea rhodopensis*, *Boea hygrometrica*, *Xerophyta viscosa*, and *Sporobolus stapfianus* (Gechev et al. 2013; Mitra et al. 2013; Yobi et al. 2017; Costa et al. 2017; Xu et al. 2021), thereby contributing to our understanding of desiccation tolerance in these remarkable plants.

In the early twenty-first century, advancements in tech-nology, computer science, and the availability of molecular biological data led to a significant expansion of the four fundamental ideas in biology: the cell as the fundamental



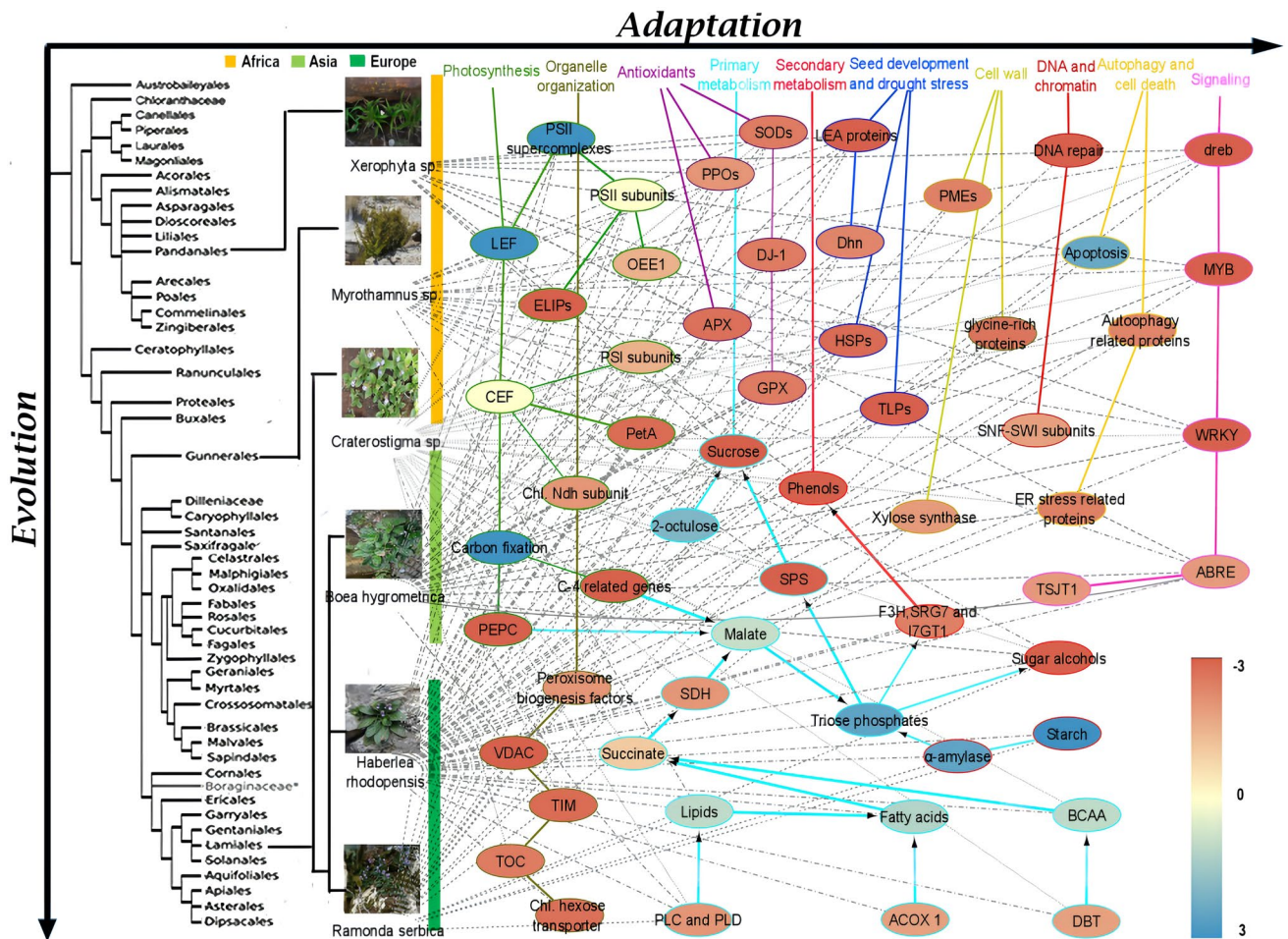
**Fig. 2** Vegetative desiccation tolerance demonstrated in *Boea hygrometrica*. **A** A mature plant was removed from the pot and exposed to drought conditions in the air for 2 days before being re-watered. **B** Leaves were detached from mature plants in pots, subjected to drought in the air for 2 days, and then re-watered. **C** Leaf discs were collected from detached leaves of mature plants in pots, exposed to drought in the air for 2 days, and then re-watered



structural and functional unit of life, the gene as the mechanism of heredity, evolution, and life as a chemical process. This expansion gave rise to what has been termed the “fifth great idea” in biology, which characterizes life as a multi-scale dynamic complex system explained through logical and informational processes and structures (Nurse 2003). The development of ‘omics’ technologies has enabled the simultaneous measurement of multiple variables, facilitating the construction of biochemical networks by integrating data from transcripts, proteins, and metabolites, allowing for pattern recognition and the identification of sets of correlated biomarkers associated with distinct physiological processes (Fiehn and Weckwerth 2003; Weckwerth et al. 2004). By employing various high-throughput screening methods, researchers have made significant discoveries concerning changes in photosynthetic electron transport, transcripts, proteins and metabolites in response to dehydration in various resurrection plants (Jiang et al. 2007; Moyankova et al. 2014; Ma et al. 2015; Zhu et al. 2015; Mladenov et al. 2015; Liu et al. 2018a; Sun et al. 2018; Xu et al. 2021; Lyall et al. 2020; Vidović et al. 2022; Mladenov et al. 2022). In general, findings from the above studies have revealed numerous changes at various levels, encompassing the genomic, molecular, physiological, cellular, and metabolic aspects, which are also observed in non-vegetative desiccation-tolerant plants. For instance, transcripts, proteins, and metabolites related to photosynthesis, antioxidant production, primary and secondary metabolism, seed development, cell signaling, DNA methylations, and chromatin modifications exhibit significant alterations in resurrection plants across different

taxonomic families and geographic regions (Fig. 3). The signal transduction network triggered by drought, involving key components like abscisic acid (ABA) and reactive oxygen species (ROS), plays a crucial role in modulating signal pathways, osmolyte accumulation, energy supply, and ROS scavenging in resurrection plants, and these mechanisms closely parallel those observed in non-vegetative desiccation-tolerant plants (Zhang and Bartels 2018; Oliver et al. 2020; Gechev et al. 2021). However, during a comparative transcriptome analysis between the desiccation-tolerant species *Selaginella sellowii* and *Selaginella lepidophylla*, in contrast to their desiccation-sensitive relative *Selaginella denticulata*, significant differences were observed not only between the tolerant and sensitive species during water loss, but also in the shared and contrasting transcriptional profiles in response to desiccation between the two tolerant species. In addition, a species-specific response related to cell wall modification and N-metabolism was observed in *Selaginella lepidophylla* (Alejo-Jacuinde et al. 2020). Collectively, these findings suggest that *Selaginella sellowii* and *Selaginella lepidophylla* have evolved distinct strategies to acquire desiccation tolerance, despite their phylogenetic relatedness. Interestingly, similar and specific transcriptomic and metabolomic responses to desiccation in *Boea hygrometrica* and *Haberlea rhodopensis*, two Gesneriaceae resurrection plants, have also been reported, indicating the diverse approaches and independent evolution of desiccation tolerance in this botanical family (Liu et al. 2019a).

Moreover, the increasing availability of fully sequenced plant genomes in recent years has facilitated the



**Fig. 3** Representation of drought-induced changes in different cellular processes as a result of evolutionary adaptation (arrows in the outer frame) of several resurrection plants from different geographical regions. The confirmed available transcriptomic, proteomics, metabolomics, and biophysical data for each model plant in the evolutionary tree of angiosperms (Williams et al. 2013) is represented by colored nodes linked to the categories of biological functions listed at the top. The dashed lines indicate the species in which each gene/protein/metabolite was reported. For primary metabolism, unidirectional edges with arrows shows the flow of metabolites in the condensed topology according to the networks from the KEGG database containing sucrose accumulation; starch degradation and synthesis; carbon fixation; lipid, fatty acid, and branched amino acid degradation; glycolysis/gluconeogenesis; and the TCA cycle. Each node represents

transcripts, proteins, metabolites, or biophysical processes and is labeled with the corresponding gene/protein or metabolite names or biophysical processes. The node color indicates the log<sub>2</sub> fold change (FC) of means between fresh and dry plants, with the range shown in the color bar. The enriched cellular processes, including photosynthesis, organelle organization, antioxidants, primary and secondary metabolism, seed development, cell wall modification, DNA repair and chromatin remodeling, autophagy and cell death, and cell signaling, are represented by different colors and functionally connected based on gene ontology (GO) analysis, with edge and node border colors indicating specific processes. The geographical region for each plant denoted by a color code

identification of regulatory sites within extensive "genetic circuits" in plants. Consequently, the integration of genomic and postgenomic data allows for the comparison of evolutionary strategies and responses to environmental conditions across diverse living systems, as well as the identification of quantitative trait loci that can be harnessed for targeted genome engineering aimed at enhancing drought tolerance in crops (Zhang et al. 2018a). However, it is worth noting that genome sequencing has emerged as a prominent frontier and

focal point in the study of resurrection plants since the publication of the first resurrection plant genome assembly (Xiao et al. 2015). This endeavor poses significant challenges as most resurrection plants exhibit characteristics such as high heterozygosity, polyploidy and substantial content of repetitive DNA, all of which substantially increase the complexity and difficulty of sequence assembly, particularly when utilizing next-generation short-read sequencing techniques. Third-generation long-read sequencing technologies, such

as PacBio SMRT sequencing and Nanopore, along with improved scaffolding methods like Hi-C, have greatly facilitated the creation of high-quality, chromosome-level genome assemblies for resurrection plants. To date, whole-genome sequencing has been completed for ten vascular resurrection plant species (Table 1), with four of them having published Hi-C sequencing data (VanBuren et al. 2018a, b; Gao et al. 2021; Vanburen et al. 2023). It is worth noting that transposable elements (TEs) constitute a significant proportion of the genomes in several species, including *Oropetium thomaeum* (43%), *Selaginella tamariscina* (60.58%), and *Boea hygrometrica* (75.16%) (Xiao et al. 2015; VanBuren et al. 2015; Xu et al. 2018). Pilot studies in *Craterostigma plantagineum* and *Boea hygrometrica* have suggested that these TEs play a role in regulating desiccation tolerance through the encoding of small RNAs (Hilbricht et al. 2008; Zhao et al. 2014). Recently, it has been reported that changes in the global methylation state of the *Boea hygrometrica* genome lead to conformational changes in chromatin structure and epigenetic regulation of vegetative desiccation tolerance (VDT) (Sun et al. 2021). However, comprehensive studies on chromatin accessibility and state changes in resurrection plants during drought have not yet been published.

Genome comparisons between closely related desiccation-tolerant (*Lindernia brevidens*) and -sensitive (*Lindernia subracemosa*) species from the Scrophulariaceae family have been instrumental in identifying evolutionary changes linked to desiccation tolerance. Notably, significant gene duplication events have been observed in *Lindernia brevidens*, particularly in early light-induced

proteins. In addition, the presence of seed-specific and abscisic acid-associated *cis*-regulatory elements associated with the expression of LEA proteins has been detected in desiccation-tolerant plants (VanBuren et al. 2018b). However, direct evidence establishing the indispensable role of the expansion of these gene families in the establishment of VDT remains somewhat limited. Conversely, the sequencing of the genome of the resurrection plant *Xerophyta viscosa* has revealed the existence of clusters of desiccation-associated genes (CoDAGs), defined as genomic regions housing compact clusters of genes associated with desiccation, which exhibit differential expression upon desiccation (Costa et al. 2017). Such comparative genome studies and the integration of genomic resources, which involve the identification of clusters and syntenic blocks as loci (i.e., ESTs, genes, SNPs, and other loci) on a single linkage group or chromosome, contribute significantly to the acceleration of sequence generation and other biological studies. Furthermore, they represent valuable tools for identifying markers in target regions (Liu D et al., 2018) as these markers can subsequently be transferred to crops through various transgenic and transgene-free techniques to enhance their drought tolerance in response to the changing environmental conditions.

**Table 1** Properties of the sequenced resurrection species genomes

	Species	Genome size (Mb)	Assembly size (Mb)	Chromosomes	GC content	DNA transposon content	References
<b>Lycophyte</b>	<i>Selaginella lepidophylla</i>	109	122.5	2n=20	–	24.61%	VanBuren et al. (2018c)
	<i>Selaginella tamariscina</i>	150	300.73	2n=20	37.44%	60.58%	Xu et al. (2018)
<b>Dicot</b>	<i>Boea hygrometrica</i>	1690	1540	2n=18	42.30%	75.16%	Xiao et al. (2015)
	<i>Craterostigma plantagineum</i>	1030	1716.72	2x=8n=112	40.30%	35%	Vanburen et al. (2023)
	<i>Lindernia brevidens</i>	270	263.2	2n=2x=28	39.20%	34%	VanBuren et al. (2018a)
<b>Monocot</b>	<i>Acanthochlamys bracteata</i>	204.24	197.97	2n=40	35.06%	28.63%	Gao et al. (2021)
	<i>Eragrostis nindensis</i>	1000	986	2n=4x=40	46.60%	–	Pardo et al. (2020)
	<i>Oropetium thomaeum</i>	245	236	2n=2x=18	34.86%	43%	VanBuren et al. (2018b)
	<i>Sporobolus stapfianus</i>	1354	1080	–	44.00%	39.70%	Chávez et al., (2022)
	<i>Xerophyta viscosa</i>	295.5	295.5	8x=48	36.51%	18.00%	Costa et al. (2017)

– No data available



## The metabolites essential for VDT acquisition in resurrection plants

In recent years, extensive research efforts have been focused on documenting metabolic changes triggered by drought or desiccation in a broader array of monocot and dicot resurrection plants (Sun et al. 2018), which have provided fresh insights into the importance of sugar metabolism, including sucrose, trehalose and the raffinose family of oligosaccharides, during the dehydration process. Sugars are recognized as pivotal agents serving roles in osmoprotection, the formation of glassy fluids, and as energy sources. Notably, the dynamic fluctuations of an unusual carbohydrate, C8 ketose-sugar 2-octulose, which is abundant in fresh leaves of *Craterostigma plantagineum* suggests its role as a sucrose precursor poised for conversion before severe water loss occurs (Xu et al. 2021). Furthermore, these studies showed associations between amino acids, organic acids, antioxidants and specific metabolites, such as phenolic compounds in Linderniaceae, and the degree of VDT (Passon et al. 2021). Conversely, these investigations have also revealed the intricate and diverse dynamics of the metabolome in resurrection plants in response to dehydration, as comprehensively reviewed by Zhang et al. (2016) and Dace et al. (2023).

Research efforts focused on identifying VDT-specific metabolic changes by comparing various sister-group species or tissues of the same plant across different DT levels and seasons (Martinelli et al. 2007; Farrant et al. 2009; Oliver et al. 2011; Yobi et al. 2012). These investigations have provided insights into a constitutive model of metabolic profiles in VDT species/tissues, contrasted with a comparatively limited metabolic responses to dehydration stress in desiccation-sensitive species/tissues. To mitigate potential variations arising from species differences, leaf maturity, and seasonal or environmental factors during comparison, we conducted an untargeted global metabolomic analysis using gas chromatography–mass spectrometry on leaves from *Boea hygrometrica* plants that were cultivated and subjected to treatments under parallel conditions but exhibited distinct desiccation tolerance to air drying. One of the advantages exploited in this study is the ability of *Boea hygrometrica* plants grown in a greenhouse to withstand soil dryness, leading to a gradual decrease in RWC over several days (referred to as slow desiccation). However, these plants are unable to tolerate air dry conditions, which result in a rapid reduction in RWC within a few hours unless they have been previously primed by a cycle of slow soil drying followed by rehydration (slow desiccation). By comparing these plants, we have not only identified metabolites that exhibited changes in

response to desiccation but also observed distinct metabolic profiles between acclimated (air dry-tolerant) and non-acclimated (air dry-sensitive) plants (Sun et al. 2018). Overall, we identified 23 metabolites that were significantly increased and 30 that were significantly decreased in response to slow desiccation. Furthermore, in regards to acclimated plants, 11 metabolites were found to be increased and 16 were decreased in response to air drying, while in non-acclimated plants, 15 metabolites were found to be increased and 21 were decreased. When examining these desiccation-regulated metabolites with those found in various resurrection plant species, 47 metabolites that were common to 2 or more species were identified. Among these, fructose-6-phosphate showed a decrease after dehydration in both *Craterostigma plantagineum* and *Haberlea rhodopensis*. In addition, four sugars (maltose, myo-inositol, raffinose, and stachyose), four amino acids (arginine, isoleucine, phenylalanine and tryptophan), two organic acids (benzoic acid and fumarate), 4-aminobutyric acid (GABA),  $\alpha$ -tocopherol (Vitamin E), and choline were found to be increased after dehydration in two or more species (Table S1). These metabolites deserve more attention for elucidating the processes associated with VDT. Conversely, the remaining 33 metabolites were found to be induced by drought in certain species but repressed in others, including glucose and sucrose. This finding again underscores the varied mechanisms of desiccation tolerance exhibited by resurrection plants. Furthermore, in our analysis comparing metabolites distinct between acclimated (air dry-tolerant) and non-acclimated (air dry-sensitive) *Boea hygrometrica* plants, 25 known metabolites and 51 compounds with unknown structures as putative biomarkers were identified to possess significant contributions to the rapid acquisition of tolerance (Sun et al. 2018). Among these known metabolites whose amount are lower in acclimated *Boea hygrometrica* plants compared to non-acclimated plants, galactose, asparagine (Asn), aspartic acid (Asp), glutamine (Gln) and serine (Ser) were also found to be lower in the well-hydrated samples of *Selaginella lepidophylla* compared to its desiccation-sensitive relative *Selaginella tamariscina* (Table 2). These comparisons provide independent evidence supporting the hypothesis that a preset metabolome "ready for desiccation" is a prerequisite for resurrection plants to survive desiccation and is likely species-dependent, as previously proposed by Yobi et al. (2012; 2013). Furthermore, the preset metabolome "ready for desiccation" can be induced during slow drying and acquired after drought acclimation in primed plants such as *Boea hygrometrica*. Interestingly, among the 23 metabolites triggered by slow desiccation in *Boea hygrometrica*, only 2 are commonly induced by both slow desiccation and air drying in both acclimated and non-acclimated plants. Similarly, only three metabolites

**Table 2** Metabolomic biomarkers contributed to the discrimination between drought acclimated and non-acclimated *Boea hygrometrica* plants and comparison with several other resurrection plants and non-resurrection relative species

Compounds	<i>Boea hygrometrica</i>	<i>Sporobolus stapfianus</i>	<i>Sporobolus stapfianus</i> / <i>S. pyramidalis</i>	<i>Selaginella lepidophylla</i>	<i>Selaginella lepidophylla</i> / <i>S. tamariscina</i>
Maltose	↑★	D1 ↑			
Turanose	↑★		H ↓		
Pentanedioic acid	↑★				
Tryptophan	↑★	D1-D5 ↑			
alpha-Tocopherol	↑★	D4, D5 ↑	H, D ↓		
Butane	↑				
Heptacosane	↑★				
Heptadecane	↑				
Phenylethanolamine	↑★				
Tetradecyne	↑★				
Furanone	↓				
Galactose	↓	D1 ↑			H, D ↓
Mannose	↓★				
2-Butenedioic acid	↓★				
2,3-Dimethyl-3-hydroxyglutaric acid	↓★				
Ribonic acid	↓				
Asparagine	↓		H, D ↑		H, D ↓
Aspartic Acid	↓★	D2 ↓	H ↑	D2 ↑	H ↓
Glutamine	↓	D5 ↑	H, D ↑	D2 ↑	H, D ↓
Proline	↓★	D1-D5 ↑			
Serine	↓		H, D ↑		H, D ↓
Threonine	↓	D5 ↑	H, D ↑		
Butanal	↓				
Ethylamine	↓				
Octadecyne	↓				
Octadiene	↓				
Phosphoric acid	↓				
Detection methods	GC-MS	LC-MS, GC-MS	LC-MS, GC-MS	LC-MS, GC-MS	LC-MS, GC-MS
Biomarkers					
Treatments	between drought acclimated and non-acclimated plants	D1-D5: 60%, 40%, and 20% RWC and desiccated	Comparison between species. H: fully hydrated; D: 60% RWC	GSSG, glutathione oxidized	Comparison between species. H: 100% RWC; D: 50% RWC
Statistical significance	PLS-DA ( Pcorr  ≥ 0.8 and VIP value ≥ 1)	Welch two-sample t test and FDR (P < 0.05, q < 0.10)	Welch two-sample t test and FDR (P < 0.05, q < 0.10)	Welch two-sample t test and FDR (P < 0.05, q < 0.10)	Welch two-sample t test and FDR (P < 0.05, q < 0.10)
References	Sun et al., 2018	Oliver et al., 2011		Yobi et al., 2013	Yobi et al., 2012



**Table 2** (continued)

The arrows indicate changes in individual metabolites, with increases shown in red and decreases in blue, in response to the described treatments for each species, as reported in various studies. The stars identify metabolites that were identified to be primed by slow desiccation to preset the metabolome "ready for desiccation", thereby contributing to vegetative desiccation tolerance (VDT)

induced by air drying were found to be shared between acclimated and non-acclimated plants, including the two shared by all types of desiccation. These findings demonstrate distinct metabolic profiles in response to different desiccation stresses (slow desiccation or air drying) and among plants in different conditions (acclimated or non-acclimated), which may directly or indirectly determine the survival of the plants after stress. Notably, the levels of 12 of the 23 slow desiccation-induced metabolites remain high after rehydration and exhibit no further change (with the exception of methoxy acetophenone, which further increases) when exposed to air drying in acclimated plants, defining them as "primed metabolites" (Table 2, Table S1). In contrast, the majority of these "primed metabolites", including heptacosane, 1-tetradecyne, phenylethanolamine,  $\alpha$ -tocopherol, xylopyranose, pentanedioic acid, are not significantly induced by air drying in non-acclimated plants. These findings confirm the existence of an primed or inducible preset metabolome "ready for desiccation" that likely contributes to vegetative desiccation tolerance (VDT). However, the key metabolites that are crucial for establishing the desiccation-protective status are largely unknown and are being actively investigated.

## The cellular modulation for VDT in resurrection plants

### Signaling modulates cell wall folding in resurrection plants in response to extreme dryness

In most plants subjected to severe drought stress, the structural integrity of their tissues, as well as the shape and size of their cells, remain intact even as the cells lose water and the cytoplasm contracts, owing to the rigidity of their cell walls. However, massive water loss may increase mechanical tension that builds between the rigid cell wall and the contracting cytoplasm, leading to plasma membrane damage and cytoplasmic content leakage. This is evident through the sharp increase in electrolyte leakage, which results in plasma membrane damage and cell disintegration in non-VDT tissues under extreme drought stress but not in resurrection plants (Deng et al. 2003). Conversely, electron microscopic observations have revealed distinct characteristics in desiccated tissues of resurrection plants. Specifically, these tissues experience significant cell shrinkage, where cell sizes decrease to less than half of their original size while maintaining

their integral cellular structure. Notably, the cell walls in these desiccated tissues were found to be significantly twisted (Wang et al. 2009; Zhu et al. 2015). Subsequent investigations have unveiled an ordered and reversible process of cell wall folding and curling in resurrection plants, as well as in other desiccation-tolerant organisms and organs, during both dehydration and rehydration. This process enables the cell wall to adapt its shape, making contact with the plasma membrane, which contracts during dehydration and expands during rehydration at specific locations, which tends to alleviate harmful mechanical tension and prevent lethal structural damage (Vicré et al. 1999; 2004; Moore et al. 2006; Mihailova et al. 2018). Biochemical determinations have demonstrated changes in cell wall components in various Southern African resurrection plant species, suggesting a common mechanism involving the "plasticization" of cell walls, which emphasizes the significance of arabinose-rich polymers, including pectin-arabinans, arabinogalactan proteins and arabinoxylans, in providing the required flexibility during the dehydration and rehydration processes in these plants (Moore et al. 2008; 2013). Transcriptomic and proteomic studies have supported these findings, revealing several apoplastic protein-coding genes among statistically significant and differentially abundant transcripts (SDATs) or dehydration-induced differentially expressed genes (DEGs). These genes include those encoding cell wall loosening enzymes, such as endo-beta-mannanase, beta-mannan endohydrolase, beta-D-glucan exohydrolase, glucan endo-1,3-beta-glucosidase, feruloyl esterase, and glycosyltransferases in *Sporobolus stapfianus* (Yobi et al. 2017). In addition, proteins associated with cell wall reinforcement, such as Expansin in *Craterostigma plantagineum*, have been identified (Jones and McQueen-Mason 2004), and proteins related to apoplastic signal sensing and transduction, such as apoplastic glycine-rich proteins in *Boea hygrometrica* and *Craterostigma plantagineum*, have been implicated in these processes (Wang et al. 2009; Giarola et al. 2016; Guerriero et al. 2021; Tang et al. 2022). A recent integrative multi-omic study on *Ramonda serbica* has provided additional insights into desiccation-induced cell wall remodeling, particularly related to germin-like proteins (GLP)-derived  $H_2O_2/HO^{\cdot}$  activity and pectin demethylesterification (Vidović et al. 2022).

Research on *Craterostigma plantagineum* has provided valuable experimental evidence in deciphering signals derived from pectin de-methylesterification, revealing that

homogalacturonan, a predominant pectic polysaccharide, undergoes reduced methylesterification during desiccation. In addition, CpGRP1, a dehydration-induced apoplastic glycine-rich protein (GRP), has been shown to interact with both de-methylesterified and methylesterified pectin with varying affinities, allowing CpGRP1 to sense changes in pectin composition during dehydration and rehydration (Jung et al. 2019). Furthermore, CpGRP1 can interact with a cell wall-associated protein kinase called CpWAK1 (Giarola et al. 2016). WAKs can form aggregates with a high affinity for pectin in the cell wall, adopting the "egg box" conformation. This interaction is modulated by CpGRP1 during the early stages of dehydration and is influenced by the apoplastic pH (Chen et al. 2021). Consequently, it is proposed that CpWAK1 collaborates with CpGRP1 to decode cell wall signals, potentially triggering cell wall remodeling processes during dehydration (Chen et al. 2021). In a prior review by the same authors, various other types of possible cell wall signals, including pectin-derived oligosaccharides, calcium ions, and other small molecules in resurrection plants during dehydration, were also discussed (Chen et al. 2020).

The interaction between WAK1 and AtGRP3 was initially reported in *Arabidopsis* (Park et al. 2001). Interestingly, while none of the *AtGRPs* were reported to be induced by drought stress, drought-induced GRPs have been identified in rice and *Boea hygrometrica* (Wang et al. 2009; Shim et al. 2021). Moreover, the overexpression of *OsGRP3*, *AtGRP2*, or *AtGRP7* has been shown to significantly enhance rice drought tolerance (Shim et al. 2021; Yang et al. 2014). Further research is warranted to elucidate whether the CpGRP1-CpWAK1 complex is responsible for specific desiccation tolerance signals and whether additional signal components are associated with the dynamic changes in the cell wall during desiccation in resurrection plants.

Recent genome-wide analysis of the *CrRLK1L* gene family in *Boea hygrometrica* has unveiled intriguing patterns of expansion and contraction. Notably, this gene family has experienced significant expansion, particularly within the FER clade. In addition, a novel *Boea hygrometrica*-specific clade has been observed, while the MEDOS and CVY clades are notably absent. These observations suggest a species-specific role for CrRLK1L receptors in the context of desiccation tolerance. Within the FER subfamily, BhFER1 is significantly upregulated during desiccation in *Boea hygrometrica*, indicating the potential involvement of BhFER1 in the intricate signaling network operating within plasma membranes and cell walls during desiccation.

## Organelle preservation in resurrection plants in response to extreme dryness

Mitochondria and chloroplasts play pivotal roles in energy production and primary metabolic processes throughout plant development and in response to stress. When subjected to drought stress, these organelles become sources of increased reactive oxygen species (ROS) production, leading to secondary oxidative stress as their respective chain reactions are disrupted. In resurrection plants, both photosynthesis and respiration rates decline as dehydration progresses, regardless of whether they are poikilochlorophyllous or homoiochlorophyllous (Challabathula et al. 2016; Oliver et al. 2020). Moreover, even in the homoiochlorophyllous resurrection plant *Haberlea rhodopensis*, some chloroplasts undergo grana destacking, and a portion of the mitochondria become disorganized and reduced during dehydration (Mladenov et al. 2022).

Nevertheless, numerous microscopic studies have revealed well-preserved cell structures with intact organelles, such as chloroplasts and mitochondria, in the leaves of several resurrection plants, including *Haberlea rhodopensis* (Mladenov et al. 2022). Although the underlying mechanism of this preservation remains unknown, it holds significant importance for cell viability after desiccation and subsequent rapid recovery, which is supported by findings from recent studies. In *Haberlea rhodopensis*, despite a twofold decrease in the rate of electron transport chain-linked respiration during desiccation, the alternative oxidase increases, ensuring the functionality of mitochondria and preserving mitochondrial components in an active state, thereby allowing for almost immediate activation of respiration to full capacity upon rehydration (Ivanova et al. 2022). The abundance of protein transcripts involved in mitochondrial biogenesis persists at high levels in desiccated leaves and remains consistent during rehydration, alongside fully assembled oxidative phosphorylation machinery. Furthermore, alternative respiratory components and mitochondrial stress-responsive elements were observed to be most prevalent in desiccated tissues, correlating with the elevated alternative oxidase activity (Ivanova et al. 2022). This study presents novel evidence for specialized mechanisms that facilitate mitochondrial integrity during desiccation in resurrection vascular plants.

Furthermore, studies have reported an accumulation of tricarboxylic acid cycle (TCA) intermediates and oxidative phosphorylation (OXPHOS) machinery in *Craterostigma plantagineum*, which align with the high abundance of proteins and transcripts related to mitochondrial biogenesis and function in desiccated leaves (Ivanova et al. 2022; Liu et al. 2018b). In addition, the protein levels of mitochondrial outer membrane porins, such as voltage-dependent anion

channels (VDACs), were found to be increased in dry leaves of *Haberlea rhodopensis*, and mitochondrial protein-import components (TIM17 and TIM23) exhibited elevated levels in *Craterostigma plantagineum* (Mladenov et al. 2022; Xu et al. 2021). These porins are crucial for the exchange of ions and molecules and play a significant role in regulating organelle metabolism and stress responses. Notably, VDACs have been proposed as conserved components of mitochondria-mediated apoptotic pathways in both plant and animal systems, serving a dual function in facilitating outer mitochondrial membrane-specific Bax recruitment and concurrent Bax inhibition through retrotranslocation (Godbole et al. 2003; Lauterwasser et al. 2016). These studies have contributed to our understanding of the mechanisms behind organelle preservation and repair during desiccation and rehydration in resurrection plants.

During desiccation, photosynthetic activity becomes undetectable in all studied resurrection plants, which occurs due to limited CO<sub>2</sub> availability caused by drought-induced stomatal closure, leading to an imbalance between absorbed light energy and the energy required for CO<sub>2</sub> fixation (Challabathula et al. 2016). This imbalance results in the production of ROS at photosystem (PS) II and PSI, causing irreparable damage to photosynthetic membranes, chloroplastic and cytosolic proteins, and other intracellular components. Interestingly, resurrection plants exhibit a reversible imbalance between light-dependent and non-light-dependent reactions in chloroplasts upon water loss, and this damage can be repaired upon rehydration (Challabathula et al. 2016; Oliver et al. 2020). There has been remarkable progress in research on the mechanisms protecting the photosynthetic apparatus, with this section focusing solely on the latest advancements and some underemphasized aspects in previous summaries.

Resurrection plants employ a primary response to counter chloroplast-generated ROS, which involves the folding or curling of their leaves. This adaptive action reduces light absorption by minimizing the exposed surface area of the adaxial (upper) leaf surfaces. As drought stress intensifies, leaves exhibit varying structural adjustments, including random curling in *Craterostigma plantagineum* and *Craterostigma wilmsii*, folding in *Myrothamnus flabellifolius* (Sherwin and Farrant 1998), and inward shrinking and rolling with the hairy back exposed in *Boea hygrometrica* (Deng et al. 2003). These morphological transformations typically occur within 2–24 h after exposure to arid conditions and are reversed upon water exposure (Fig. 2). Rafsanjani et al. (2015) studied the unique conformational changes in the outer and inner stems of *Selaginella lepidophylla* caused by dehydration. They discovered that the outer stems bend into circular rings within a relatively short period of desiccation, exhibiting classical bilayer curling with an approximately constant curvature. In contrast, the inner stems curl slowly

into spirals due to a hydro-actuated strain gradient along their length, facilitated by the introduction of a class of rod-like hygromorphs with planar spiraling capacity. The distinct curling pattern is related to the asymmetric cell density between the abaxial and adaxial sides of the stem in the outer stems and the asymmetric lignification in the cortical tissue towards the abaxial side of the inner stem (Rafsanjani et al. 2015). These leaf movements effectively minimize light absorption and consequently reduce chloroplast-derived ROS production during desiccation. On the other hand, desiccation-triggered leaf folding or curling clearly results in low light or dark condition to the inner layer cells. Interestingly, dehydration can induce zeaxanthin accumulation in darkness in desiccation-tolerant species from all major clades, including mosses, liverworts, ferns and angiosperms, as recently reviewed by Fernández-Marín et al. (2021). The desiccation-induced synthesis of zeaxanthin in darkness may contribute to the protection of photosynthetic machinery in various ways in resurrection plants. It not only aids in the thermal dissipation of excess light energy through nonphotochemical quenching (NPQ), thus preventing lipid damage under oxidative stress, but also maintains thylakoid membrane stability during desiccation. In addition, it accelerates the recovery of photosynthesis during rehydration (Fernández-Marín et al. 2021). In addition to xanthophyll cycle pigments, resurrection plants also regulate the levels of antioxidants such as ascorbate, glutathione and  $\alpha$ -tocopherol, as well as ROS scavenge enzymes, early light-inducible proteins (ELIPs), and late embryogenesis abundant (LEA) proteins, during desiccation (Challabathula et al. 2016; Mladenov et al. 2022). Furthermore, a series of coordinated processes progressively downregulate photosynthesis, as revealed in *Craterostigma pumilum*, including: (1) a gradual decrease in PSII complex density in grana and stroma lamellar membrane domains; (2) diminishing levels of PSII proteins; (3) the attenuation of photosynthetic linear electron flux (LEF) alongside a gradual increase in cyclic electron transport (CEF) at the early stages of dehydration; and (4) the shutdown of PSII photochemical activity, accompanied by the detachment of peripheral antenna complexes (LHCII), coinciding with sucrose accumulation and the emergence of inverted hexagonal lipid phases within membranes in fully desiccated leaves (Charuvi et al. 2015, 2019; Zia et al. 2016). This intricate and precise process, involving the inactivation and macromolecular reorganization of photosystems, reroutes electrons into alternative sinks and deactivates key protein complexes responsible for energy transduction to minimize ROS formation during dehydration while preserving the ability to reactivate metabolic processes upon water availability.

Resurrection plants have long been known to possess the remarkable ability to regulate photosynthesis either

on or off, alternately using poikilochlorophyllous or homoiochlorophyllous strategies (Challabathula et al. 2016). However, recent molecular and biochemical findings suggest that both protective and degradative mechanisms contribute to desiccation tolerance in homoiochlorophyllous resurrection plants, such as *Boea hygrometrica* and *Craterostigma pumilum* (Charuvi et al. 2015, 2019; Zia et al. 2016; Tan et al. 2017). Plant age plays a crucial role in determining whether a “more photoprotective mechanism” or a “more degradative mechanism” is activated in response to drought stress. Young plants demonstrated a “more photoprotective mechanism” by retaining more D1 proteins, thereby enhancing their high-energy quenching capacity during dehydration and facilitating faster recovery during rehydration (Oung et al. 2022). In contrast, older plants exhibit a pre-existing higher qE level, and employ a degradation-based strategy during dehydration, aligned with an overall senescence phenotype, exemplified by a significant reduction in chlorophyll content and photosynthetic proteins (subunits of the cyt b6f complex and the D1 proteins), thylakoid membrane damage, and a notable increase in plastoglobules (PGs) (Oung et al. 2022).

*Haberlea rhodopensis*, like other resurrection plants, exhibits protective protein accumulation and degradation of photosynthetic proteins within chloroplasts. It undergoes alterations in the balance between linear electron flux (LEF) and cyclic electron flux (CEF) and experiences changes in the organization of supercomplexes within the photosynthetic machinery, which are accompanied by transcriptional upregulation of chloroplast NADPH-dehydrogenase-subunit-like proteins and increased phosphorylation of the light-harvesting complex subunit LHCP (CAB40), as well as a consistently abundant dehydrin (Dhn2) (Mladenov et al. 2015, 2022; Liu et al. 2018a, b). Biochemical evidence supports the occurrence of the dehydration-induced translocation of cytoplasmic dehydrins to chloroplasts in response to desiccation, which is regulated by protein phosphorylation and is validated by a more than two-fold increase in the abundance of phosphorylated Dhn2 in the thylakoid fraction compared to its level in fully hydrated plants (Mladenov et al. 2022). In *Craterostigma pumilum* and *Haberlea rhodopensis*, both ‘Rubisco-containing bodies’ (RCBs), a structure shuttle chloroplast stroma components for degradation in the vacuole via autophagy, and a high number of plastoglobules (PGs), a reservoir for thylakoid lipids and/or proteins during membrane degradation are characteristic features of the dehydrated state, together with the observation of grana destacking and damaged chloroplasts (Charuvi et al. 2019; Mladenov et al. 2022). These observations further confirm the presence of the “more degradative mechanism” for recycling or removing damaged organelles in response to desiccation in resurrection plants.

## Avoidance of drought-induced cell death in resurrection plants under extreme dryness

While drought-induced programmed cell death has been documented in numerous plant species, such as wheat and Arabidopsis plants (Duan et al. 2010; Hameed et al. 2013), resurrection plants, by definition, must ensure the preservation of cell viability under extreme drought conditions and during subsequent rehydration. In line with this requirement, recent findings have indicated that apoptotic-like cell death can be triggered by stachyose in drought-sensitive tobacco cells (*Nicotiana benthamiana*) but not in *Tripogon loliiformis* (Okemo et al. 2021). Clearly, understanding the mechanisms used by resurrection plants to evade drought-induced cell death holds paramount importance and significance for achieving vegetative desiccation tolerance in these remarkable organisms.

Cell death frequently occurs in response to stress-induced ROS production and energy shortages resulting from the inactivation of photosynthesis and respiration. Drought stress triggers ROS accumulation, leading to cellular component damage, disruption of cellular redox balance, protein and lipid peroxidation, endoplasmic reticulum (ER) stress, and the generation of signals that activate caspases in mitochondria, chloroplasts, and other cellular locations (Ye et al. 2021). Both the unfolded protein response (UPR) and the ER-associated degradation pathway (ERAD) are activated to alleviate ER stress in tissues exposed to drought stress, whether in desiccation-tolerant or sensitive plants (Zhu et al. 2015; Guerriero et al. 2021). UPR reduces protein synthesis and accumulation while enhancing protein-folding capabilities. ERAD transports properly folded protein substrates out of the ER via retro-translocation and targets them for degradation through the ubiquitin–proteasome system. However, prolonged stress can lead to an overload of these processes, resulting in cellular dysfunction and eventual cell death (Guerriero et al. 2021). In contrast, desiccation-tolerant *Boea hygrometrica* leaves exhibit a significant reduction in protein ubiquitination, which benefits the presence of protective proteins such as BhLEA2 (Lin et al. 2019; Liu et al. 2009a). This finding suggests a negative association between protein ubiquitination and desiccation tolerance, potentially involving B3 domain transcription factors, and also implies the necessity for other cellular mechanisms to degrade misfolded proteins that inevitably accumulate during dehydration in resurrection plants. Furthermore, as previously mentioned, the increase in mitochondrial outer membrane protein VDACs and the presence of Rubisco-containing bodies (RCBs) and damaged chloroplasts in dry *Haberlea rhodopensis* underscore the importance of preventing mitochondrial and chloroplast-mediated cell death in resurrection plants.



Autophagy is a cellular process responsible for the degradation of denatured and misfolded proteins and damaged organelles and the elimination of apoptotic signals. It plays a crucial role in maintaining cellular homeostasis under both normal and abnormal conditions, including biotic and abiotic stresses. Autophagy accomplishes this by recycling nutrients, breaking down harmful cellular components, and removing damaged proteins and organelles (Liu et al. 2009b). Important components involved in drought-induced autophagy have been identified in plants. For instance, in tomato plants, the heat shock factor HsfA1a activates the transcription of ATG genes, inducing autophagy to enhance plant drought tolerance (Wang et al. 2015). In *Arabidopsis*, COST1 (constitutively stressed 1), a plant-specific negative regulator of autophagy, is degraded through the 26S proteasome pathway under drought stress to activate autophagy and modulate the trade-off between plant growth and stress tolerance (Bao et al. 2020). However, the role of autophagy can vary between pro-survival and pro-death depending on factors such as plant species, cell type, and specific stimuli. Unfortunately, under severe drought stress, autophagy can lead to cell death in these plants (Duan et al. 2010; Li et al. 2019).

Conversely, in resurrection plants, the progression of cell death is prevented, as evidenced by cell viability staining and the complete recovery of plants upon rehydration, which occurs despite the increase in autophagy features and the expression of autophagy-related genes (ATGs) under severe drought stress, as observed in *Tripogon loliiiformis*, *Boea hygrometrica* and *Haberlea rhodopensis* (Williams et al. 2015; Zhu et al. 2015; Liu et al. 2018b; Mladenov et al. 2022). In *Tripogon loliiiformis*, findings from transcriptome analysis, gas chromatography, mass spectrometry, immunoblotting and confocal microscopy have directly linked the accumulation of the non-reducing sugar trehalose to the initiation of autophagy and the deceleration of ongoing cell death in leaves (Williams et al. 2015; Asami et al. 2019). The authors propose that dehydration-induced trehalose triggers the induction of SnRK1, which is usually suppressed by mTOR when energy is sufficient. SnRK1-mediated autophagy promotes desiccation tolerance by removing cellular toxins, suppressing the preset progression of cell death, and recycling nutrients to delay the onset of senescence (Williams et al. 2015). Further research is required to investigate whether similar mechanisms involving small molecule metabolite-triggered autophagy exist in other resurrection plants during dehydration.

In *Haberlea rhodopensis*, the activation of autophagy in dry leaves has been linked to the reduction of metacaspase-4 (AMC4), a positive regulator in ROS-mediated abiotic stress-induced programmed cell death (Mladenov et al. 2022). Similarly, in *Boea hygrometrica*, drought-induced autophagy has been associated with ROS and antioxidants,

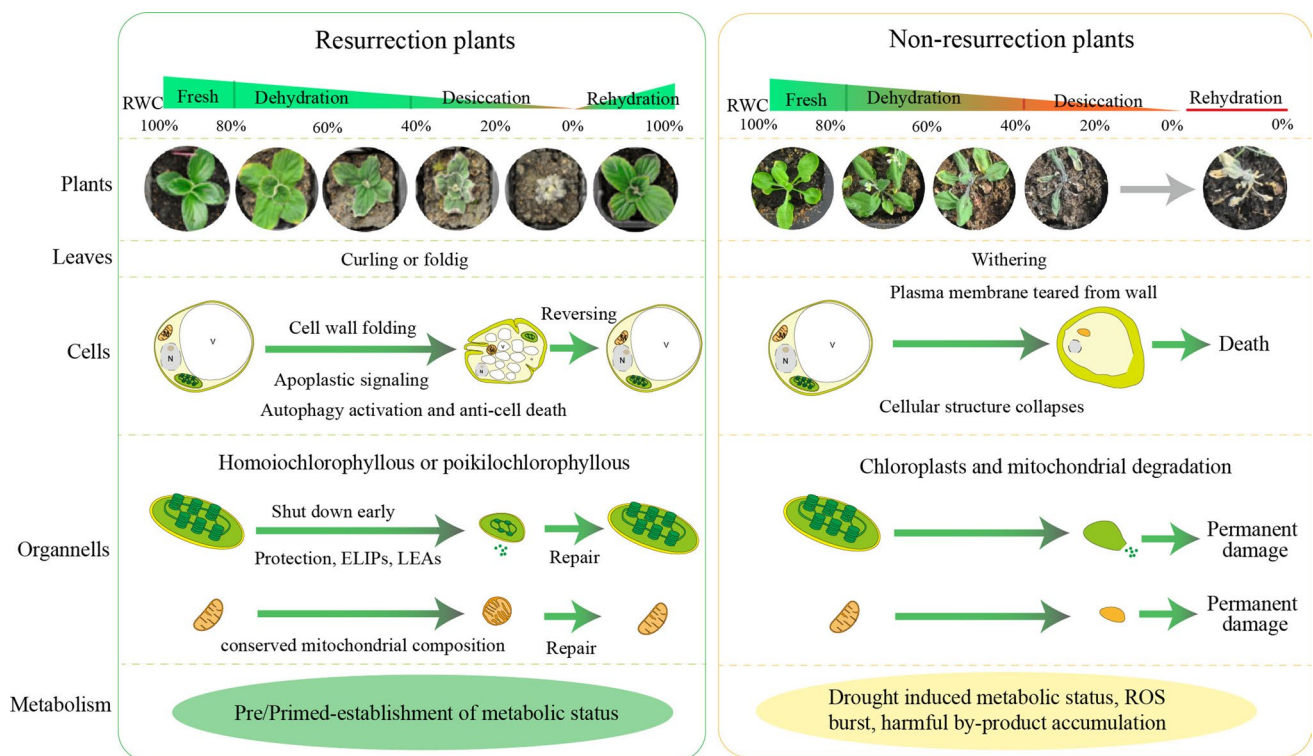
such as the accumulation of  $\alpha$ -tocopherol during desiccation and in drought acclimation-primed plants that are tolerant to air drying (Zhu et al. 2015). However, the pro-survival role of autophagy in desiccation tolerance remains ambiguous due to the lack of consistent genetic evidence. Since efficient gene editing tools for resurrection plants are still in development, further research is needed to gain a deep understanding of the underlying mechanisms of vegetative desiccation tolerance and the mechanism via which resurrection plants avert the programmed cell death pathway under extreme drought stress conditions.

## Conclusions and perspectives

This review summarizes the recent advances in the metabolic and intracellular adaptive aspects of VDT in resurrection plants, which have not been discussed in other reviews to attract more attention to the signaling pathways and mechanisms responsible for activating or maintaining the physiological and biochemical characteristics of cell compartments, organelles and proteins essential for sustaining viability and preparedness for rehydration. Figure 4 illustrates a workflow comparing the distinctive cellular mechanisms of VDT in resurrection plants with the typical cellular responses to drought stress in desiccation-sensitive plants.

Compared to desiccation-tolerant mosses and ferns, resurrection plants share a closer evolutionary relationship with angiosperm crops and have garnered attention as potential genetic donors for drought-tolerant molecular modules that could be harnessed in genetic engineering and gene editing for developing climate-resilient crops, encompassing both monocotyledonous and dicotyledonous species, particularly in arid environments (Zhang and Bartels 2018; Farrant and Hilhorst 2022). Moreover, among the Gesneriaceae family, which is typically associated with tropical and subtropical regions, only resurrection species such as *Haberlea rhodopensis*, *Ramonda serbica*, *Ramonda myconi* and *Boea hygrometrica* have been observed in temperate regions of Europe and Asia that experience cold winters, suggesting that desiccation tolerance may confer an advantage in adapting to the combined stresses of cold and freezing conditions (Mitra et al. 2013; Fernández-Marín et al. 2020; Mihailova et al. 2020, 2022; Fig. 1). The development of cross-stress tolerance is also a valuable trait in these plants for improving crop stress tolerance.

However, in order to utilize the potential of resurrection plants and their valuable mechanisms of VDT and cross-stress resilience for crop biotechnology, several outstanding questions necessitate further investigation. For instance, the origins of desiccation tolerance remain a subject of inquiry: is it an innate trait or acquired through adaptation? In addition, the significant diversity and distinctiveness of



**Fig. 4** A schematic representation of the unique adaptations and mechanisms employed by resurrection plants to withstand extreme drought, as compared to non-resurrection plants. The distinct responses of resurrection (exemplified by *Boea hygrometrica*) and non-resurrection (exemplified by *Arabidopsis thaliana*) plants to changes in leaf relative water content (RWC) resulting from drought stress are illustrated. The arrows indicate the direction of water loss and the resulting changes in leaf RWC in both resurrection and non-resurrection plants. The dashed lines represent the different levels of responses of the two types of plants to drought stress. As water loss occurs, resurrection plants gradually exhibit leaf wilting, shrinking, and curling, while non-resurrection plants display signs of withering and metabolic dysfunction. Drought-induced cellular processes, such

as cell shrinkage and cell wall folding, trigger apoplastic signaling in resurrection plants, leading to the activation of protective and repair mechanisms including autophagy, photosynthetic shutdown, alternative oxidase upregulation in mitochondria, and the establishment of a pre-set or primed metabolic state. These mechanisms contribute to the maintenance of intact cell membranes, well-structured organelles, and miniaturized vacuoles in resurrection plants. In contrast, non-resurrection plants experience plasma membrane disruption and collapsed cellular structures, leading to cell death and drought sensitivity. This model provides a comprehensive overview of the desiccation tolerance mechanisms in resurrection plants, highlighting their remarkable ability to survive extreme dryness. *V* vacuole, *M* mitochondrion, *C* chloroplast, *N* nucleus

tolerant traits and mechanisms across individual resurrection plants prompt questions regarding their underlying genetic basis. While it is likely that desiccation tolerance involves the action of multiple genes, there may be core genes, gene modules, proteins or metabolites that have uniquely evolved to govern pivotal steps in achieving desiccation tolerance in resurrection plants. Furthermore, are there any genomic signatures or hallmarks of desiccation tolerance evolution preserved in the genomes of resurrection plants? Could the development of VDT be linked to the specific environmental conditions in the regions where resurrection plants have thrived? Is there a potential connection between the cellular adaptations for VDT and the preset metabolome "ready for desiccation"? Are there even specific metabolites that are unique to certain species and play a role in desiccation tolerance?

According to the widely accepted opinion, the VDT in vascular resurrection plants evolves from the reprogramming of genetic networks controlling desiccation tolerance in their reproductive structures such as seeds (Oliver et al. 2020). The activation of "seed-specific" desiccation protection mechanisms (sucrose accumulation and expression of LEAs) in association with ABA signaling pathways (ABI3-ABI5 regulators and LAFL regulatory network) in the vegetative tissues of resurrection plants has been supported by the identification of a large number of differentially expressed genes that are commonly found in maturing seeds and desiccating leaves (Costa et al. 2017; Van Buren et al. 2017). In addition, observations in species like *Boea hygrometrica* and *Craterostigma plantagineum*, which can enhance their desiccation tolerance through drought acclimation similar to seed priming, further support this hypothesis (Zhu et al. 2015; Liu et al. 2019a,

b). However, it is worth noting that some studies have presented opposing observations (Lyll et al. 2020; Pardo et al. 2020). Despite these insights, attempts to confer VDT to non-resurrection seed plants through ABA pretreatment or drought priming have proven unsuccessful. Conversely, certain species, such as young seedlings of *Xerophyta viscosa*, *Marchantia polymorpha* and callus of *Craterostigma plantagineum*, which are initially sensitive to desiccation, can acquire desiccation tolerance through exogenous ABA application or overexpression of specific genes like MpPYL1-GFP (Chandler et al. 1997; Costa et al. 2017; Jahan et al. 2019). Based on these findings, it can be hypothesized that the nature of VDT in resurrection plants is, to some extent, contingent on the species and environmental conditions. This variability may be partially attributed to species-specific variations in cell wall components, drought-induced signaling metabolites, and key regulatory factors governing processes such as cell wall folding, organelle preservation, and anti-cell death mechanisms, as discussed in this review.

Comparative genome analysis among closely related species holds the potential to shed light on the evolutionary trajectory of resurrection plants and identify key genetic signatures linked to the acquisition of VDT. Simultaneously, the development of an efficient and user-friendly transgenic system for generating ample genetically modified materials is an urgent requirement for model resurrection plants with sequenced genomes. The lack of such a system has been a significant hurdle in exploring the roles of pivotal regulators and genetic modules governing desiccation tolerance through forward and reverse genetic approaches.

In conclusion, substantial progress has been made in recent years in elucidating the intricate cellular mechanisms associated with VDT in resurrection plants. Nevertheless, further scientific advancements are needed to address questions pertaining to the signaling and regulation of specific cellular adaptations, notably cell wall folding, organelle preservation, and the promotion of survival through autophagy, which may hold great promise not only in enhancing our comprehension of VDT in resurrection plants but also in leveraging their genetic resources and VDT-related genetic information for crop breeding. These would bolster crop resilience and tolerance to the challenges posed by extreme drought stress, which is of critical significance in the context of evolving climate patterns and agricultural sustainability.

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**Data availability** Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

## Declarations

**Conflict of interest** The authors have no conflicts of interest to declare relevant to the content of this article.

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