Programming desiccation-tolerance: from plants to seeds to resurrection plants
Jill M Farrant\textsuperscript{1} and John P Moore\textsuperscript{2}

Desiccation-tolerance (DT) evolved as the key solution to survival on land by the early algal ancestors of terrestrial plants. This ‘first’ DT involved utilizing rapidly mobilisable repair mechanisms and is still found today in mosses, such as Tortula ruralis, and ferns, such as Mohria caffrorum. The first seed plants lost vegetative DT while investing their seeds with tolerance mechanisms improving their survival in unfavourable environments. The mechanisms of DT in seeds are strongly connected to their developmentally regulated maturation programs. We propose that angiosperm resurrection plants acquired tolerance by re-activating their innate DT mechanisms in their vegetative tissues. Here we review the current hypotheses regarding the genetic evidence for the evolution of DT in resurrection plants. We also present strong evidence showing the activation of seed specific genetic elements in the vegetative tissues of resurrection plants.

Addresses
\textsuperscript{1} Department of Molecular and Cell Biology, University of Cape Town, Private Bag, Rondebosch, 7701, South Africa  
\textsuperscript{2} Institute for Wine Biotechnology, Stellenbosch University, Private Bag X1, Matieland, 7682, South Africa

Corresponding author: Farrant, Jill M (Jill.Farrant@uct.ac.za)

Current Opinion in Plant Biology 2011, 14:340–345
This review comes from a themed issue on Physiology and metabolism
Edited by Ute Krämer and Anna Amtmann
Available online 19th April 2011
1369-5266/$ – see front matter
Published by Elsevier Ltd.

DOI 10.1016/j.pbi.2011.03.018

Introduction – the invasion of dry land
Early land plants evolved from aquatic algal ancestors millions of years ago [1]. One of the first and most formidable obstacles to the successful adaptation to terrestrial environments is desiccation [1]. Rapid drying owing to heat, sunlight or wind can cause desiccation to occur in sensitive vegetative tissues within minutes of exposure. These desiccation-induced stresses are visible at all intertidal shore zones where algae are exposed to rapid changes in water availability throughout a normal day. Chlorophytic algae, specifically the Choleochetales group, which gave rise to Charophyceaean species were the precursors to the Bryophyte and Tracheophyte lineages of terrestrial plants and must have been the first groups to acquire desiccation tolerance [2]. Bryophytes widely evolved the ‘gametophytic’ solution to desiccation tolerance and these vegetative tissues are termed ‘fully’ desiccation-tolerant as they can withstand very rapid drying, on the order of seconds to minutes [3]. The main mechanisms responsible for the DT of mosses are believed to be related to cellular repair strategies upon re-hydration. The lack of vascular tissues and sophisticated tissue patterning ensures a rapid and uniform drying rate upon desiccation in mosses [4]. The molecular processes related to DT in mosses have been most intensively studied in Tortula ruralis (Figure 1A). One of the key adaptations in Tortula ruralis is the packaging of desiccation-related mRNA transcripts into mRNP complexes with polysomes so that upon dehydration these transcripts would become immediately available for the effective repair of desiccation-associated damage [5]. Pteridophytes have been suggested to show a mixed form of DT; intermediate between ‘full’ and ‘modified’ DT [2]. A recent study has shown that DT is widespread among fern gametophytes and related to habitat preference [6]. The simple morphology of the fern gametophytes governs the water-holding capacity of the thallus and probably the DT [6]. Hence it is likely that moss and fern gametophyte DT is of a similar, if not identical, nature. In the case of some ferns the sporophyte is stages, as well as the spores, are desiccation-tolerant, the nature of which is largely similar to that of the gametophytic tissue. In these cases the desiccation mechanisms of DT reported to occur in angiosperms (which have ‘modified’ DT) are present [7,8,9], confirming an intermediate status of DT. In the case of the fern Mohria caffrorum (Figure 1C) the DT of the sporophyte is seasonal, being DT in the dry and desiccation sensitive (DS) in the wet season [9] and it would be interesting to know if genetic programming of the gametophyte is responsible for this switching process.

Physiological and metabolic processes associated with DT
The changes in hydration levels and cellular stresses associated with water loss as proposed for seeds [10,11] are shown in Figure 2. While there has been no research on water properties in resurrection plants, our research has shown they experience similar stresses [reviewed in \textsuperscript{12,13}] and the changes in water content and metabolic responses to desiccation are similar to those proposed for seeds (Figure 2. [11]). Initial water loss (type V water) is accompanied by osmotic adjustment to prevent turgor loss. With loss of type IV water (<0.7 g H\(_2\text{O}\)/g DW\(^{-1}\)) mechanical stress associated with decreasing cell volume [14,15] occurs and in seeds this is minimized by progressive

\[ \text{0.7 gH}_2\text{O/g DW} \]
accumulation of storage reserves within vacuoles and cytoplasm preventing plasmalemma withdrawal and wall collapse [reviewed in [10,11]]. In resurrection plants, while complex reserves (protein and lipid bodies and starch) do not accumulate per se, mechanical stabilization is alleviated by replacement of water in vacuoles with compatible solutes and reversible changes to cell wall architecture [9,12,16,17]. Progressive water loss below 0.45 gH2O g DW−1 (type III water and below) results in membrane appression and ultimate destabilization, and increasing metabolic perturbations associated with free radical production, alcohol and carbonyl emission inter alia (Figure 2). Damage associated with cytoplasmic compaction resulting in membrane fusion, and protein and membrane denaturation is believed to be prevented by the replacement of water with solutes capable of substituting for the hydrogen bonds lost owing to dehydration [21–23]. Additional stabilization of the subcellular milieu is believed to be achieved via cytosolic vitrification [10,23,24]. Solutes responsible for replacement and stabilization include: 1) sucrose and oligosaccharides [reviewed in [11,12]] and 2) proteins, particularly Late Embryogenesis Abundant (LEA) proteins (reviewed in [11,18,23,25]) and small heat shock proteins (sHSP) [25–28]. Physiological and biochemical studies on resurrection plants have shown that all of these changes accompany desiccation in resurrection plants (Figure 2 [reviewed [12,13]]).

Genetic programs underlying seed DT
The genetic programs responsible for DT in (orthodox) seeds must have arisen from the gametophytic and/or sporophytic tissues of mosses and ferns, the bryophyte and/or pteridophyte ancestors of seed plants [2]. Not all seeds are desiccation tolerant and a number of species produce seeds, termed recalcitrant, that are desiccation sensitive. Such species occur in environments in which seeds are released into conditions that are immediately conducive to germination. It has been proposed that as a consequence of evolution within such environments genes for seed DT in such species either have been lost or are permanently repressed [29]. Comparative studies between recalcitrant and orthodox seeds are required to establish the reason for differences in DT.

A number of transcriptomic and proteomic studies have been undertaken in order to investigate the genetic program utilized by orthodox seeds upon acquisition of DT during their development [19*,20,30,31]. One of the most comprehensive transcriptomic studies in seeds has been performed on Medicago trunculata [19*]. Transcript profiling of mature dry seeds have revealed highly abundant transcripts encoding, among others, LEAs, sHSPs and peroxiredoxins. Highly expressed functional protein categories included transcripts involved in signal transduction, development, transcriptional regulation, carbohydrate metabolism, storage protein synthesis, lipid metabolism, ‘abiotic stress response’ and photosynthesis. Proteomics analysis has confirmed that many of the proteins encoded by these transcripts are highly abundant in seeds [20,30,31]. In order to differentiate between genes associated with developmental processes and those associated with acquisition of DT, osmotic stress was reinduced in radicles of germinating M. trunculata seedlings by incubation in polyethylene glycol (PEG) and transcripts upregulated during this process were analyzed [19*]. The expression of 16086 M. trunculata genes was followed of which 1300 genes were differentially expressed during PEG treatment. These were grouped into several clusters based on the temporal patterning of
the data. Early clustered genes encoded proteins required for initial protection, including many of the LEAs and several genes encoding stress and defence proteins, whereas late clusters consisted of genes involved in sucrose synthesis, storage protein production and downregulation of metabolism. The importance of sucrose was confirmed as a fold increase occurred with reestablishment of DT [19]. A functional proteomics strategy for analyzing *M. trunculata* seeds was also undertaken on the heat stable proteome extracted [20]. Comparative analysis of desiccation-tolerant versus - sensitive imbibed seeds identified 15 proteins showing
Programming desiccation-tolerance: from plants to seeds to resurrection plants

Farrant and Moore

seed-specific expression, including 6 LEA proteins. We propose that it was this genetic programming in seeds, probably consisting mainly of the early-stage genes involved in protection that were not de-activated during germination and seedling growth that gave rise to the numerous lineages of resurrection plants during angiosperm evolution and radiation.

The evolution of resurrection plants

All modified-desiccation tolerant plants are seed plants and therefore seeds are likely to be the source of genetic programming for the evolution of all angiosperm resurrection plants [2]. Different desiccation-tolerant resurrection plant lineages exist and therefore, acquisition of ‘seed’ DT must have occurred multiple times during angiosperm evolution. Owing to the lack of a fully sequenced resurrection plant genome transcriptomic studies have been limited to the analysis of expressed sequence tags (EST) [17]. Microarray and small-scale sequence cDNA analysis has been performed on a number of species, including, Tortula ruralis [5], Selaginella lepidophylla [32], Xerophyta humilis [33], Myrothamnus flabellifolia [34], Sporobolus stapfianus [35] and Craterostigma plantagineum [36]. Figure 1 shows all these species in the hydrated state. The most comprehensive transcriptomic analysis of a resurrection plant has recently been performed for C. plantagineum using pyro-sequencing technology [37**]. Four cDNA libraries were constructed from fully hydrated, 48 h dehydrated, 15 day dehydrated and 24 h rehydrated leaf tissue of C. plantagineum. After sequence assembly over 15,000 UniProt identities were obtained, the highest coverage to date for any resurrection plant. The 500 most variable transcripts, across all experimental samples, were partition clustered and subjected to functional enrichment analysis. Gene ontology (GO) categories enriched within the six expression clusters included responses to abiotic stimuli such as ABA, stress response pathways, oxidative processes, antioxidant responses to oxidative metabolism, cellular polysaccharide metabolism and cell wall organization, and photosynthesis and cytoskeletal organization. This study confirmed the results of many previous molecular studies of DT in this species and others; re-enforcing the importance of LEAs, sugars, antioxidants and cell wall genes encoding expansins and xyloglucan endotransglucosylases during desiccation [12,13,38]. One interesting observation from this data is the many chromosome scaffold genes are responsive to desiccation and one hypothesis is that C. plantagineum has evolved proteins aiding the recruitment of transcripts to histone complexes during desiccation and thus utilizes a similar strategy of mRNP production to that of Tortula ruralis [37**]. However we know that the DT of T. ruralis is a constitutive repair strategy that is metabolically expensive. By contrast, DT in C. plantagineum probably evolved from a developmental-seed program that was ‘re-activated’ to respond to environmental cues. A molecular ‘signature of seeds’ in resurrection plants, however, has not yet been convincingly demonstrated [18]. One molecular study, currently unpublished, has produced data that strongly supports the seed origin of the DT genetic program in angiosperm resurrection plants [39]. It was reasoned that one approach to prove the seed origin was to compare gene expression of desiccated vegetative tissue with mature dry seed material sourced from a resurrection plant. A control parallel experiment involving use of a desiccation-sensitive plant in order to compare water stressed vegetative tissue with its corresponding mature seed transcriptome was included. In addition to profiling for potential new pathways, this approach could identify pathways common to desiccated vegetative tissue and mature dry seeds. This experiment was carried out using Xerophyta humilis, a resurrection plant (Figure 1D), and Arabidopsis thaliana, the desiccation sensitive control, using cDNA and Microarray technology [39]. Of the X. humilis genes analyzed, 46% were found to be differentially expressed between seed and desiccated vegetative tissue. Cluster analysis and multivariate techniques revealed that the transcriptomes of desiccated root, desiccated leaf and seed tissue were very similar to each other in X. humilis. This is in contrast to A. thaliana where there is no clear overlap between gene expression clusters of stressed vegetative tissue and seed tissue, indicating that the response to water stress is tissue specific in A. thaliana. Of particular interest was the identification of a common set of genes in X. humilis, encoding LEAs, HSPs, peroxiredoxins and storage proteins, that were expressed in roots, leaves and seeds of desiccated X. humilis, but are seed-specific in A. thaliana (according to TAIR annotation). The overall conclusions from this study are that desiccation tolerant angiosperms, such as X. humilis and C. plantagineum, utilize a seed-specific developmental program that is ‘re-activated’ in vegetative tissues to protect against desiccation.

Common patterns of desiccation-tolerance and outstanding questions for future research

In light of the considerable similarities in mechanisms of DT in seeds and vegetative tissues of resurrection plants (reviewed above) it is likely that angiosperms acquired the initial DT programming from seeds but had to co-opt or adapt to a whole plant situation by developing additional specific mechanisms, for example, to deal with desiccation-associated photosynthetic damage and mechanical stress associated with plasmolysis and cytorhesis (Figure 2). Understanding the interplay between seed and angiosperm DT mechanisms will inform our approach to utilize knowledge obtained from resurrection plants to improve agricultural crop species. Outstanding questions still in need of answering include:

1. Is early land plant DT of monophyletic origin?
2. Do all orthodox seeds possess the same types of genetic DT mechanisms?
3. Do different angiosperm resurrection plant lineages possess different genetic tolerance mechanisms and can these be traced to their seed progenitors?
4. How many different DT genetic programs evolved?
5. How are seed genes activated in vegetative tissues?

Answering these questions, and others, will help to elucidate the scientific mechanisms behind the remarkable DT abilities of seeds and resurrection plants.

Conflict of interest statement
The authors are not aware of any biases or conflicts of interest that might be perceived as affecting the objectivity of this review.

Acknowledgements
We thank Keren Cooper and Hanlie Nell for invaluable assistance in compiling Figure 2.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest


A good recent review of physiological processes involved in desiccation tolerance in seeds and resurrection plant species.


A good summary review on the key mechanisms proposed for imparting desiccation-tolerance in a variety of resurrection plant species.


This paper provides the first systematic transcriptomic study on the desiccation tolerance maturation process in drying seeds. Important insights into key candidate gene families involved are indicated.


37. Suarez Rodriguez MC, Edgardi D, Hussain SS, Alquezar D, Rasmussen M, Gilbert T, Nielsen BH, Bartels D, Mundy J: Transcriptomes of the desiccation-tolerant resurrection plant Craterostigma plantagineum. Plant J 2010, 63:212-228. The most comprehensive transcriptomic study to date on a resurrection plant. This excellent study confirms many previous molecular investigations on this species and also highlights a number of new genes, and possible mechanisms that are key for desiccation-tolerance in Craterostigma plantagineum.


39. Walford S: Activation of seed-specific genes in leaves and roots of the desiccation tolerant plant, Xerophyta humilis. PhD Thesis. University of Cape Town, South Africa; 2008. Currently the most convincing study performed linking seed desiccation-tolerance genes with desiccation-induced vegetative tissue gene expression in a resurrection plant. The thesis was Supervised by Nicola Illing (Department of Molecular and Cell Biology, University of Cape Town, South Africa) and Co-Supervised by Katherine J. Denby (Warwick Life Sciences, University of Warwick, UK) and Jill M. Farrant (author of this article).