



Desiccation tolerance: From genomics to the field

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ABSTRACT

Desiccation tolerance is defined as the ability to survive the removal of all, or almost all the cellular water without irreversible damage. It confers to dried organisms the ability to survive extreme conditions of the environment and to stay alive in a suspended animation for long periods of time. The biotechnological potential of anhydrous biology has been recognized for more than 60 years. With the fast development of “omics” technologies, it is now possible to better appreciate the biotechnological promises that can be made from the understanding of desiccation tolerance. This review will discuss the impact of post-genomics tools on identifying genes or gene products, and will give a comprehensive overview of the agronomical and biotechnological applications. We propose the term desiccomics to define the approach consisting of combining “omics” approaches to address the specific issues associated with the dry state.

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1. Introduction

Desiccation tolerance constitutes the exception of the established principle that life consists of chemical interactions that take place in liquid water. Water is therefore an indispensable component to the function and structure of the cell. Desiccation tolerance is defined as the ability to survive the removal of all, or almost all the cellular water without irreversible damage. In practice, desiccation tolerance is scored when drying to equilibrium with ambient air (below 65% RH in nature, around 50–70% in the laboratory) does not kill the subject upon rehydration. Remarkably, much of what is known about the requirements for desiccation tolerance was already determined in the late 19th century. The reviews of [1] and [2] have described the fascinating story leading to the establishment of anhydrous biology. With awareness of this remarkable property, the great potential of anhydrous biology for human benefit was immediately identified [1]. With the increasing knowledge of the mechanisms and regulatory processes leading to desiccation tolerance, it is now possible to better appreciate the biotechnological promises that can and will be made. These promises rely on the biological advantages conferred by the ability to survive in the dry state: (1) desiccation provides a mean of bridging periods of environmental stress; (2) the dry state permits to stabilize the biological

entity for long periods of time (ranging from years to centuries) by suspending its metabolic activity and (3) the dry state also provides a state of tolerance to environmental extremes (temperatures ranging from -196°C to $+100^{\circ}\text{C}$, irradiation, vacuum, [2–4]). There are great expectations that the knowledge gained from unraveling the genes and regulatory pathways leading to these adaptive advantages will bring benefits such as producing crops more tolerant to abiotic stresses [5–9], tools to preserve better genetic biodiversity *ex situ* [6,10,11] or protocols to ship dried blood to battle fields [12,13]. Several authors have even considered interplanetary transfer of life as a putative outcome from anhydrous biology [4,14].

Desiccation tolerance occurs over a large range of taxa, including invertebrates, bacteria, terrestrial microalgae, fungi, yeasts, lichens, spores. In higher plants, desiccation tolerance is widespread in seeds and pollens and also occurs in the vegetative tissues of vascular plants (the so-called resurrection plants, consisting of approx. 350 species). Most of these organisms are of use to man (e.g. seeds in agriculture; brine shrimps in aquaculture; yeasts and lactic acid bacteria in the food industry), or are pathogenic to him, his domesticated animals and crops (e.g. micro-organisms, the concept of viable but not cultivable in bacteria, nematodes) [12,15].

The taxonomic diversity of anhydrobiotes suggests that the potential to evolve tolerance is ancestral and widespread [16–18]. Alpert [16] has argued that there are no phylogenetic nor geographical constraints that limit desiccation tolerance. Also, there is so far no apparent dependence of desiccation tolerance on the complexity of living forms [16]. Finally, there is increasing evidence that desiccation tolerance in vegetative tissues of resurrection plants has a polyphyletic origin and arose through the adoption of mechanisms present in reproductive propagules [17–19]. Therefore, the

Abbreviations: DW, dry weight; g/g, g H₂O/g dry weight; HSP, heat shock protein; LEA, late embryogenesis abundant; RH, relative humidity; TF, transcription factor; TPS, trehalose-6-phosphate synthase.

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Table 1

Survey of principal “-omics” studies that specifically aimed to unravel mechanisms of desiccation tolerance in microbes, fungi and plants. T, transcriptomics; P, proteomics; PP phosphoproteomics.

Species	Omics	Material and methods	Observations	References
Bacteria				
<i>Bradyrhizobium japonicum</i>	T	Microarray analysis of the whole expressed genome; comparison of control and desiccated cells (27% RH)	Upregulation and down regulation of genes encoding hypothetical proteins (27%), and transcriptional regulators (9%), to a lesser extent, upregulation of genes involved in trehalose metabolism, DNA repair, chaperone activity, energy transfer, protection from oxidative damage	[112]
<i>Rhodococcus jostii</i> RHA1 (<i>Actinomycete</i>)	T	Microarray analysis of 89% of predicted genes; comparison of control and desiccated cells (20% RH)	Upregulation of genes involved in protection from oxidative damage, DNA repair, lipid metabolism and cell envelope modification, synthesis of compatible solutes (ectoine)	[57]
Fungi				
<i>Saccharomyces cerevisiae</i>	T	Microarray analysis of 7000 ORF. Comparison between control, desiccated, rehydrated BY4743 cells together with a lyophilized commercial strain	Similar transcriptome profiles in samples dried by different means. Similarities between “stationary-phase-essential” genes and upregulated genes during drying, activation of fatty acid catabolism, gluconeogenesis and glyoxylate during desiccation; upregulation of LEA genes, down-regulation of cell wall related genes	[113]
<i>Saccharomyces cerevisiae</i>	T	Comparison of dried yeast with human HEK cells dried to 0,6 g H ₂ O/g DW exhibiting partial survival	45 out of the 664 genes common to both transcriptomes are differentially expressed in both systems: activation of the antioxidant response, repression of genes involved in cell cycle and encoding mitochondrial proteins	[12]
Moss				
<i>Tortula ruralis</i>	T	10368 EST corresponding to 5563 TC unigenes obtained from gametophytes during drying and rehydration	Identification of LEA genes; importance of repair processes	[23]
<i>Tortula ruralis</i>	T	Two cDNA libraries enriched with EST differentially sequestered in slowly-dried gametophytes and EST differentially translated during rehydration	31% unknown genes; importance of a rapid recovery of metabolism; enrichment in stress responsive genes similar to <i>X. humilis</i> , new components involving jasmonic acid signaling, proteosomal activation and alternate splicing	[114]
Lycophyte				
<i>Selaginella lepidophylla</i>	T	EST library corresponding to 864 unigenes obtained after 2 h of desiccation and compared to 1300 unigenes of <i>S. moellendorffii</i> , which is desiccation sensitive	Increased proportion of genes involved in transport, cytoskeleton, phenylpropanoid biosynthesis, LEA proteins, ELIPs, HSPs	[115]
Ressurrection plants				
<i>Sporobolus stapfianus</i>	T	144 cDNA clones corresponding to upregulated genes in dried leaves	Abundance of LEA genes, defense and detoxification genes, presence of a phosphatase PP2C	[116]
<i>Xerophyta humilis</i>	T	Normalized library (10900 clones) from dried roots and leaves containing 424 sequenced and arrayed cDNA, Transcriptome comparison before and after drying	Upregulated genes: LEA (29%), antioxidant (187%), unknown (18%), signaling (2%); downregulated genes: unknown (30%), metabolism and growth (28%), regulation (13%)	[49]
<i>Xerophyta viscosa</i>	P	Soluble proteins extracted at 3 relative water content during drying	Breakdown of photosynthesis associated proteins, de novo synthesis of chaperones, RNA- binding protein, 2-Cys peroxiredoxin, protein phosphatase 2C	[38]
<i>Boea hygrometrica</i>	P	Soluble proteins extracted from control, drying and rehydrating leaves	8 out 14 polypeptides identified linked to photosynthesis, glutathione metabolism and phenolics metabolism.	[32]
<i>Craterostigma plantagineum</i>	PP	Enriched phosphoproteins extracted in control, dried and rehydrated leaves	Identification of 20 proteins that are dephosphorylated during drying and rephosphorylated during rehydration (chlorophyll a/b binding protein, rubisco, 14-3-3 proteins, HSPs, elongation factor 1 beta 2, Eukaryotic initiation factor 5A)	[84]
Pollens				
<i>Lilium longiflorum</i>	T	cDNA library at desiccation of anthers using a suppressive subtractive hybridization between immature and mature anthers, 80 sequenced clones	33 upregulated genes with predominance for cell wall synthesis related genes, regulatory proteins (ABA regulated), transporter proteins, 18% unknown genes	[32]
Seeds				
<i>Medicago truncatula</i>	T	Microarray (covering 50% of the expressed genome) analyses of the kinetics in transcriptome changes during the induction of desiccation tolerance in germinated radicles submitted to an osmotic treatment and comparison with two stages of maturation corresponding to the acquisition of desiccation tolerance	Upregulation of C metabolism-related genes to produce sucrose early during induction of desiccation tolerance, increased antioxidant defenses and LEA proteins, regulatory genes typically expressed during abiotic/drought stresses are also upregulated during maturation (partial overlap of ABA-dependent and -independent regulatory pathways involved in both drought and desiccation tolerance. Concomitantly, a massive repression of genes belonging to occurred, including cell cycle, biogenesis, primary and energy metabolism.	[36]

Table 1 (Continued)

Species	Omics	Material and methods	Observations	References
<i>Medicago truncatula</i>	P	Comparative analysis of the heat stable proteome in desiccation tolerant and sensitive radicles	Identification of 11 LEA polypeptides specifically associated with desiccation tolerance and not drought tolerance (EM6, PM25, several isoforms of group 3, SBP65)	[35]

above observations should in theory facilitate the translation of the knowledge on how to survive in the dry state into crop improvement and enhanced utilization for human benefit.

Excellent reviews have recently been published on the molecular mechanisms and regulatory aspects of desiccation tolerance [6,11,15,20,21] and on the damages occurring during desiccation on sensitive tissues or over long periods of times [10,22]. The aim of this paper is to present an overview on the plant anhydrobiotic models used for “omics approaches” and how information gained from these approaches has led to biotechnological applications. The perspectives discuss the challenges facing “desiccomics”, which we defined as -omics technologies (genomics, transcriptomics, proteomics, metabolomics, and beyond) applied to tissues dried to moisture contents or water potentials below which drought-tolerant organisms die.

2. A survey of models and “omics” approaches to study desiccation tolerance

Both seeds and resurrection plants have been used as models to understand desiccation tolerance by “omics” approaches. Here, we describe the advantages and disadvantages of such models. An overview of the conclusions of “omics” studies are presented in Table 1. Most of these studies targeted the transcriptome of fresh or dried anhydrobiotes, and to our knowledge none was devoted to the metabolome. Prokaryotes, yeasts and lower plants are also useful models, particularly for repair mechanisms induced upon rehydration after drying. These have been described elsewhere [12,15,18,23] and will not be discussed in this review.

2.1. Plant seeds

Orthodox seeds of those species for which the genome is already sequenced and a large array of genomic tools are available approaches are the top of the list of powerful tools to unravel the mechanisms of desiccation tolerance (Table 1). More than 50 transcriptomic, proteomic and metabolomic studies have been performed to describe changes associated with seed development and germination. These studies have led to a significant increase in the knowledge of processes such as seed filling ([24–27] and references within), regulation of dormancy and germination (reviewed in [27–29]) and seed tolerance to abiotic stress [30,31]. Typically, those studies included the dry seed as one of the stages studied. Unfortunately, it is difficult to infer from these global data which of the observed changes are associated with desiccation tolerance because of the overlap of various developmental processes occurring during the acquisition of desiccation tolerance (seed filling) and during the maintenance or loss of desiccation tolerance (respectively dormancy and radicle growth during germination). This interpretation is further complicated by the fact that desiccation tolerance is gained and lost sequentially in the different parts of the developing/germinating seeds, which were not necessarily studied separately. The same lines of argument are also valid for pollens, whose transcriptome and proteome during desiccation have been studied (Table 1, [32]).

To overcome such difficulties, it is possible to design “physiological” models that uncouple desiccation tolerance from other developmental processes and combine them with “omics” approaches. The best example is that of the re-establishment of desiccation tolerance in emerged radicles of germinated seeds [33–36] by a mild osmotic potential (*ca.* –1.5 MPa). Longer radicles submitted to the same treatment do not re-acquire the tolerance to drying but are hardened against a partial desiccation, making it possible to distinguish changes associated with drought tolerance. Another advantage is the ability to follow in detail the kinetics of changes associated with the induction of desiccation tolerance [36]. In *Medicago truncatula*, this approach combined to a proteomic analysis of the heat stable proteome led to the identification of a number candidate LEA genes specifically associated with DT rather drought tolerance (such EM6 (PFAM domain PF00447) and PM25 (PF04927) [35]). At the gene expression level, common changes between transcriptomes associated with the acquisition of desiccation tolerance in developing seeds and re-establishment of tolerance in germinated radicles were an up-regulation of genes belonging to stress defense (LEA, antioxidant, secondary metabolites) and seed storage reserves and a down-regulation of genes involved in cell cycle, DNA processing and primary and energy metabolism (Table 1, [36]). Also, it was possible to discriminate between genes involved in signaling pathways regulating an early osmotic response (such as transcription factors homologous to dehydration-responsive element-binding protein, DREB) from those correlating with the actual appearance of desiccation-tolerant radicles (such as SNF4b, an activating subunit of a SnRK1 complex, [36]). In germinating cucumber, the metabolite profiling using ³¹P NMR during the osmotic induction of desiccation tolerance led to the suggestion that the remodeling and/or increased phospholipids catabolism is an adaptive response common to osmotic adjustment and desiccation tolerance but is controlled differently in desiccation tolerant and sensitive radicles [34]. Another physiological model worth investigating is that of the coleoptiles of wheat seedlings [37]. In this system, three days-old intact coleoptiles can survive drying to 0.3 g/g. However, when the coleoptile sheaths are cut open, they are rendered desiccation sensitive due to a rapid drying.

2.2. Resurrection plants

Table 1 lists the species of resurrection plants used to unravel the transcriptome and proteome associated with vegetative desiccation tolerance. The main advantages to study these plants are two-fold. The first advantage is that experimental data are largely free of complications due to overlapping developmental processes inherent in seeds. Secondly, desiccation tolerance can be studied in whole photosynthetic plants for which the physiology and morphology is more complex than seeds and lower plants. Therefore, it can be easily argued that understanding vegetative desiccation tolerance should facilitate the improvement of crops against drought [7,8]. In contrast to seeds, resurrection plants must cope with mechanical stress during leading to membrane shrinkage followed by cytorhesis and the folding in cell walls [7,8,20,38]. To avoid oxidative stress during drying, they achieve a controlled arrest of photosynthesis activities by two means: (1) dismantle-

ment of thylakoids and chlorophyll degradation or (2) leaf folding and synthesis of anthocyanins while the chlorophylls and membranes are retained [7,38]. Plants belonging to the first category are restricted to monocots so far (e.g. *Xerophyta viscosa*, *X. humilis*) and are defined as poikilochlorophyllous whereas those belonging to the second category are called homiochlorophyllous. Among them, *Myrothamnus flabellifolia* is a woody shrub that grows up to 2 m. This is an interesting species used to understand how plants have developed strategies to protect vascular tissues upon drying and allow the safe refilling of dry xylem during rehydration [39]. However, *Craterostigma plantagineum* is the best studied species. It was the first resurrection plant to be genetically transformed by *Agrobacterium tumefaciens* [8,40], which enabled the production of mutants using T-DNA activation tagging. This, together with the ability to manipulate desiccation tolerance in calli by addition of ABA, has allowed for the isolation of 3 desiccation-tolerant callus lines (*cdt-1*, *cdt-2*; *cdt-3*, [40–42]). Intriguingly, *CDT-1*, the first gene isolated from these lines is a retrotransposon that directs the synthesis of a short interfering RNA (siRNA), suggesting that a mechanism of RNA interference may play a key role in inducing desiccation tolerance in vegetative tissues [42]. *CDT-2* appears to play similar function but affect other yet undefined pathways [41]. Therefore, investigations into the role of non-protein-coding genes in desiccation tolerance are warranted.

In our opinion, three obstacles are impeding the development of large scale -omics on resurrection plants. First, there is a lack of large genomic and EST databases (Table 1) to support transcriptomic and proteomic studies in those species studied so far [20]. In the proteomic analysis of leaves of *Boea hygrometrica* [43] and *X. viscosa* [38], only 4 and 30%, respectively of the polypeptides were identified. Second, because these plants were taken from wild populations, large variations in metabolites contents (such as RFO in *X. viscosa* [44] and galloylquinic acids in *M. flabellifolia* [20]) have been observed. There is not yet a consensus as to which genotype should be used for -omics approaches or how to exploit the genetic diversity within the species. Third, in connection with this, the genome of resurrection plants is largely unknown but likely to be complex. *C. plantagineum* is polyploid ($2n = 28$, [8]) whereas *Ramonda serbica*, a rare resurrection plant from the Balkans and the Iberian peninsula is hexaploid ($2n = 6x = 144$, [45]). The genetic relationships and the significance of ploidy changes with vegetative desiccation are unknown.

3. Biotechnological applications of desiccation tolerance genes

The studies on model systems mentioned in the previous section have provided a number of clues as to which genes, gene products or metabolites might be involved in desiccation tolerance. This section presents a survey of the different functional genomics studies showing proof of concept. They are grouped based on the different mechanisms or molecules that have been identified.

3.1. Antioxidants and detoxification

In plants, oxidative stress is one of the major causes of damage as a result of various environmental stresses. Transcriptomics data (Table 1) show that many genes related to antioxidant defense are up-regulated in desiccation-tolerant tissues. However, antioxidants are ubiquitously expressed and not only in desiccation-tolerant tissues. Their expression level is modulated by an array of biotic and abiotic stresses. Indeed, genetic engineering of antioxidants levels has been shown to enhance general stress tolerance. For instance, overexpression of glutathione S-transferase with glutathione peroxidase activity in transgenic

tobacco enhanced seedling growth under a variety of stressful conditions [46]. Likewise, Mn superoxide dismutase (SOD) overexpression in alfalfa increased tolerance to winter stress in the field [47].

Thus, the question that needs to be addressed is whether there exists an additional, particular protection from oxidative damage in drying anhydrobiotes. An elegant study from Illing et al. [19] investigated whether any antioxidant genes could be identified that are expressed exclusively during the desiccation phase of seed maturation in *Arabidopsis* are also up-regulated during desiccation in the vegetative tissues of resurrection plants. The *in silico* comparison with transcriptome data related to abiotic stress revealed that 72% of the *Arabidopsis* antioxidant genes were expressed at high levels in control plants and were therefore classified as housekeeping genes. Very few genes demonstrated a gene-specific expression in relation to desiccation tolerance. One of those genes encodes 1-cys peroxiredoxin (*1-Cys-Prx*), whose transcripts accumulate during the acquisition or re-induction of desiccation tolerance in *M. truncatula* seeds [36]. Homologues of 1-cys peroxiredoxin have been identified in resurrection plants [48,49] and the moss *T. ruralis* [50], suggesting that this protein has a specific role in desiccation tolerance. Overexpression of *1-Cys-Prx* in tobacco plants did lead to an increased tolerance of non-senescent leaves against lesions induced by infiltration in 5 mM H₂O₂. However, the germination behaviour of the transgenic seeds was not affected by a cold or after 3 months of after-ripening [51]. In contrast, *Arabidopsis* seeds overexpressing the barley (*Hordeum vulgare*) 1-Cys-PRX protein were less inclined to germinate than wild-type seeds in the presence of NaCl, mannitol, and methyl viologen, suggesting that it can sense harsh environmental surroundings and play a part in the inhibition of germination under unfavorable conditions [52].

Oxidative stress occurs primarily because of the excessive accumulation of reactive oxygen species. It leads to the accumulation of toxic compounds such as aldehydes, via complex and yet undefined reactions. Although they are toxic for the cells, they also serve as intermediates in metabolism [53] and therefore their amounts must be tightly regulated. Aldehyde dehydrogenases (ALDHs) are organized in gene families and have also different metabolic function. Some play a major role in the detoxification of aldehydes generated in plants when exposed to abiotic stress [53,54]. Aldehyde/aldehyde reductase transcript levels were found to increase in several anhydrobiotic models such as *T. ruralis*, resurrection plants [49,55,56] and seeds [36]. Despite possible redundancy among ALDH genes, there is experimental evidence suggesting that ALDH plays a role in desiccation tolerance and seed vigor. This was recently examined using T-DNA insertional mutants of rice [53]. All dry seeds defective in *ALDH7* germinated but also accumulated significant amounts of malonyldialdehyde and Maillard reaction products both during maturation and open air storage. Interestingly, when they were incubated at 100% RH and 50 °C, mutant seeds died faster than wild type seeds. Overexpressing various *ALDH* genes is considered as transgenic option to improve tolerance against a wide range of stress. For example, when *Ath-ALDH3* was overexpressed in *Arabidopsis*, the transgenic seedlings displayed an improved tolerance to dehydration, NaCl, 20 mM H₂O₂, heavy metals, and the herbicide paraquat [54]. The improved tolerance of the *Ath-ALDH3*-overexpressing transgenic lines could be explained by diminished damages caused by different stressors. Improved stress tolerance was present in seedlings and adult plants, indicating that the stress tolerance is present throughout the vegetative growth period.

3.2. Non-reducing sugars

Non-reducing sugars appear to be universally present in relatively high concentrations in dry orthodox seeds and their amount

increases in response to desiccation in all resurrection plants studied to date ([20,44] and references therein). Transcriptomics data concur with these observations, except for the case of an actinomyces which accumulates ectoine [57] (Table 1). Experimental evidence accumulated over more than 20 years demonstrated that high concentrations of trehalose, sucrose or oligosaccharides could stabilize *in vitro* phospholipid membranes and proteins during drying (see [6,11,13] for recent reviews on the putative molecular mechanisms for protection). These observations have helped the pharmaceutical industry to successfully prepare dry proteins using disaccharides as stabilizing excipients, providing that the protein secondary structure is retained [13,58]. Sucrose is also used to produce freeze-dried functional proteins attached on the surface of various polymers for microarray diagnostic purposes [59]. However, sugars are not always the panacea and additional excipients are often necessary, such as surfactants, polymers or salts in order to inhibit protein aggregation [60,61]. In the pharmaceutical industry, this is mainly achieved by trial and error because of the lack of a clear understanding of the mechanisms behind the aggregation induced by drying [61]. Lessons could be learned from developing seeds regarding mechanisms to prevent protein aggregation. In maize embryos, enforced drying of immature, desiccation-sensitive embryos leads to large structural changes in protein structure. However, when these embryos have gained their desiccation tolerance, the same drying treatment does not induce any structural changes [6,62].

Building on early successes to achieve lyopreservation of test proteins and liposomes using various sugars, several groups attempted to dry and revive desiccation-sensitive mammalian cells loaded with trehalose by different means. Apart from freeze-drying human platelets [63], these attempts achieved a limited success (reviewed by [12,13,64]). Typically, survival curves as a function of water content during drying show an important loss of viability/integrity when the cells are dried below 1 g/g at best and are usually dead below 0.3 g/g. Adding other compounds such as a HSP protein (HSP) or catalase showed a slight improvement in viability and integrity in the moisture content range of 0.3 to 4 g/g [13,64]. Aside from the need to improve the experimental design in terms of cell culture and methods of drying [12], there is also a need to understand the cause of damages occurring during drying as already mentioned by [22]. Obviously, non-reducing sugars are not the sole factor involved in desiccation tolerance.

If non-reducing sugars are critical for desiccation tolerance, then suppressing their accumulation in dried anhydrobiotes should lead to desiccation sensitivity. This has recently been demonstrated for the resurrection plant *C. plantagineum* [7]. Plants were rendered desiccation sensitive when the sucrose accumulation was suppressed by over-expression of the mammalian 6-phosphofructo-2-kinase gene. Likewise, the symbiotic bacterium *Rhizobium leguminosarum* bv. *trifolii* which forms nitrogen-fixing root nodules on legume [65] were sensitive to drying when trehalose accumulation was suppressed by engineering double mutants defective in the two biosynthetic pathways [65].

Metabolic engineering to accumulate protective sugars associated with desiccation tolerance in crops has not yet led to the production of desiccation-tolerant plants. Nevertheless, attempts to engineer the plant metabolism for enhanced production trehalose has not only demonstrated the feasibility of engineering crops for increased tolerance to abiotic stress [66,67] but has also highlighted the essential function played by the trehalose metabolism in plants [68–71]. These studies are noteworthy here because they were initially based on the documentation of the effective protecting activity of trehalose against drying. Trehalose, which is only present in trace amounts in plants, is synthesized in a two-step process: the trehalose-6-phosphate synthase (TPS) converts UDP-Glc and glucose-6-phosphate into

trehalose-6-phosphate, which is then converted into trehalose by the trehalose-6-phosphatase. When one or both genes were over-expressed in various plants, pleiotropic effects were observed, including an increased tolerance to short water deficits and a range of undesired morphological and biochemical phenotypes (such as stunted growth, altered metabolism under normal conditions, glucose and ABA insensitivity, for references see [66,68,72]). It turned out that trehalose-6-P has important roles as a signalling metabolite of the sugar status [69], in the plant architecture [70], [73] and in embryo development [71]. An increased tolerance against drought, salt, freezing and heat can be achieved by overexpressing a trehalose-6-phosphate synthase/phosphate fusion gene under a stress responsive promoter, thereby overcoming the undesirable pleiotropic effects due to the accumulation of trehalose-6-phosphate [66,67]. Likewise, in common bean, the overexpression of TPS in the symbiote rather than the host plant led to an increased biomass and grain yield under constant irrigation and during 3 weeks of water deficit [74]. It appears that the neosynthesis of trehalose in overexpressors modulates sugar sensing and carbohydrate metabolism rather than providing an osmoprotectant function [66,74]. It is suggested that during the course of evolution, the TPS gene family (11 genes in *Arabidopsis*) has derived from a role against osmotic stress to more complex functions related to growth and developmental regulation [72].

3.3. Signaling and gene regulation

Identifying the *cis* and *trans* regulatory factors together with the signaling pathways leading to survival in the dry state is particularly important for such a multigenic trait and potentially rewarding in term of biotechnological applications. In this field, Bartels' group has been particularly active at characterizing the *cis*-acting elements and the regulatory factors of *C. plantagineum* genes [8,20,75–77]. The emerging picture is that of a complex combination of transcription factors (TF) (i.e. members of MYB, HD-Zip, bZIP and Zn finger family) that are also known to be involved in mediating responses to environmental stress and developmental cues, ABA and sugar signalling, as well as siRNA (see Section 2). Furthermore, according to [77] no resurrection-plant-specific motifs or module have been identified so far. Here, we briefly review as to how modulating the expression of these TF in a transgenic scenario brought interesting leads towards crop improvement. Overexpression of the dehydration-induced *CpMYB10* was studied in *Arabidopsis* [75]. Seeds of 35S-*CpMYP10* lines germinated faster in 200 mM sorbitol or 400 mM NaCl, whereas germination speed was similar in the absence of osmoticum. The clearest phenotype could be observed on adult plants. Plants over-expressing *CpMYP10* showed enhanced tolerance to salt stress (250 mM NaCl) and recovered better after withholding watering for two weeks [75]. Furthermore, it was found that overexpressors exhibited Glc-insensitive and ABA hypersensitive phenotypes. These results indicate that *CpMYB10* in *Arabidopsis* mediates stress tolerance but the alteration of ABA and Glc signalling responses by overexpressing this gene decreases its agronomic value [75]. Perhaps, a stress inducible promoter rather than a constitutive expression could eliminate the undesired phenotypes. Several drought-regulated HD-Zip genes have also been isolated from *C. plantagineum*, with some being inducible by both dehydration and exogenously applied ABA. Interestingly, one of the potential targets is the dehydration-responsive dehydrin gene, *CDeT6-19*, which is associated with drought tolerance [76]. Seeds of transgenic tobacco that ectopically express *CpHB-7* germinated earlier and seedlings developed more rapidly. Incubation in ABA or 200 mM NaCl amplified this difference with the wild type [76]. However, these beneficial effects on growth and stress tolerance are counteracted by the phenotype of adult transgenic plants. Transgenics plants exhibited a higher per-

centage of closed stomata in well-watered conditions compared to wild type and displayed a reduced sensitivity towards ABA on stomatal closure, suggesting that transpiration is affected. Therefore, further work is necessary to understand how *C. plantagineum* recruits its genes during dehydration before foreseeing biotechnological applications.

A similar strategy as described above has been used with heat shock transcription factors (Hsfs). These TFs are known for their induction of heat shock proteins. Transcriptome studies have regularly picked up HSP or chaperone genes (Table 1), reinforcing the hypothesis of a link between prevention of protein aggregation and desiccation tolerance [78,79]. In the light of these observations, the question was addressed as to whether the overexpression of heat shock factors isolated from resurrection plant *B. hygrometrica* would lead to interesting and relevant phenotypes for agronomy purposes [80]. Transgenic Arabidopsis seedlings ectopically expressing *BhHsf1* under the 35S promoter could resist exposure to 49 °C for 1 h after they acquired thermotolerance (1 h 37 °C, 3 h 22 °C) whereas all the wild type seedlings died. The bad news is that overall, the over-expression of *BhHsf1* conferred growth retardation of aerial organs in the transgenic plants resulting from the reduction of cell proliferation. Gene expression profiling showed that Hsps and stress-associated genes were induced whereas the genes related to DNA replication and mitotic cell cycle were down-regulated in *BhHsf1* over-expressing. *BhHsf1* may play dual roles in mediating the processes in heat stress tolerance and growth retardation via regulation of target genes related to stress protection and mitotic cell cycle library [80].

In *M. truncatula* seeds, a number of genes involved in gene regulation and signaling were identified in our transcriptome analysis on desiccation tolerance [36]. Only those that are either seed-specific and/or previously identified in resurrection plants are interesting candidates as they would be implicated in complete desiccation tolerance. Surprisingly, a very limited number fell in this category and most of them have been discovered and characterized in relation to abiotic stress tolerance (see [81] for a recent review). Among the few desiccation tolerance-specific regulatory genes, two are noteworthy: the homologue of the MYB TF *CpMYB10* described above and *SNF4b*, an activating subunit of the sucrose non-fermenting-related kinase (SnRK1) complex. In *M. truncatula*, this subunit seems to play a key role in regulating factors involved in the physiological quality of seeds [82]. Silencing *MtSNF4b* using a RNA interference (RNAi) approach resulted in a phenotype with reduced seed longevity. In parallel to the assembly of the SnRK1 complex, seeds of the RNAi lines showed impaired accumulation of oligosaccharides compared with control seeds. This and additional findings from Bradford and colleagues [83] suggests that *SNF4b* confers a specific and temporal function to SnRK1 complexes in the regulation of traits associated with seed quality. The findings on *SNF4b* can thus be construed as evidence that desiccation tolerance or survival in the dry state is at least partly regulated at the post-translational level. There is also evidence from a recent analysis of the phosphoproteome of *C. plantagineum* that a dehydration responsive post-transcriptional regulation might be implicated in desiccation tolerance [84]. These authors found that several proteins involved in translation and RNA-binding activity were dephosphorylated during drying and rephosphorylated during rehydration.

3.4. LEA proteins

The contribution of -omics studies in identifying and clustering genes involved in desiccation tolerance is the most evident from the LEA protein field. Numerous studies identified LEA proteins in relation to desiccation tolerance in a range of anhydrobiotes including bacteria, yeasts, mosses, seeds, resurrection plants and

nematodes (Table 1). The putative functions of LEA proteins are mostly inferred from *in vitro* studies and are described in detail in an excellent recent review [85]. Considering the confusing use of different nomenclature, we used in this review the Pfam classification to identify the genuine LEA families. Thus, we omitted those studies that report the effects of putative LEA proteins which do not possess a Pfam domain characteristic for a particular LEA family (e.g. some COR proteins).

In general, LEA genes are amongst the most differentially expressed and highly up-regulated genes in transcriptomic studies in all desiccation-tolerant organisms. This has also provided further proof that vegetative desiccation tolerance arose through the adaptation of mechanisms related to the onset of desiccation tolerance in reproductive propagules [17]. Using publicly available Arabidopsis genome and microarray data, Illing and colleagues [19] also investigated whether the gene expression of any of the LEA groups is restricted to seeds or abiotic stress by analysing the expression patterns of 35 out of 49 LEAs during the desiccation-sensitive phase in relation to abiotic stress (cold, osmotic, salt and drought stress) and during seed development, when desiccation tolerance is acquired. These authors identified two groups of LEA genes being seed specific as there was very low (if any) expression (<10% of seed expression) in roots or leaves submitted to stress. These groups encompass the well known Em proteins (D-19, PF00477, LEA_5) and the poorly characterized D-34 Seed Maturation Proteins (PF04927). Interestingly, a proteomic analysis of the heat stable proteome in germinating *M. truncatula* seeds identified members of both groups as specific to desiccation tolerance [35]. However, their role in desiccation tolerance is unclear. In *Arabidopsis*, mutant seeds with no detectable Em6 protein are still desiccation tolerant, probably as a result of redundancy with *EM1* gene [86]. The deficiency of Em6 apparently induced other pleiotropic effects on seed maturation. In contrast, mutational inactivation of a homologue of a *Brassica napus* *LEA76* gene (belonging to PF02987, D-7) greatly sensitized the desiccation tolerant bacterium *Deinococcus radiodurans* to drying at 5% RH [87]. To our knowledge, this is the only genetic evidence for a causative link between a LEA gene and desiccation tolerance. Yet, in *Arabidopsis*, At3g15670, a homologue of *LEA76* is also expressed in seedlings incubated in NaCl and mannitol, as indicated by the e-FPB browser using two different sets of microarray data. These observations underscore once more the complex characteristic of seed desiccation tolerance.

As can be seen from Table 2, the impact of overexpressing a LEA gene from all of the seven LEA groups has been analysed in relation to tolerance against abiotic stress. In general, all groups have representatives for which enhanced stress tolerance was demonstrated on a number of host species, often being related to salt or drought stress. Most studies reported enhanced growth rates and reduced wilting of the aerial parts under stress in laboratory conditions (Table 2) and in some field trials [88,89], demonstrating a real potential of LEA proteins in engineering crops more tolerant to stress. In this respect, studies reporting the molecular effects of over-expressing a dehydrin or group 3 LEA genes are noteworthy [90,91]. Concomitantly with a significant increase in growth rates under stress conditions, transgenic tobacco [90] or Arabidopsis [91] plants exhibited an increased osmolyte production such as proline, polyamines and sugars. Also, transgenic tobacco over-expressing a PF03760 LEA protein from the resurrection plant *B. hygrometrica* exhibited an increased peroxidase and superoxide dismutase activity during drought [92]. These data suggest that the accumulation of LEA proteins can have an indirect effect on the accumulation of other protective molecules, either via changing the osmotic adjustment or by the induction of signalling pathways. Whether the increased growth rate under stress (Table 2) is a result of an increased transpiration is not clear. According to [9], this issue is important from an agronomic point of view in very dry

Table 2
Survey of functional genomics studies aiming at testing the effect of overexpressing LEA genes towards crop improvement. Only those genes corresponding to a PFAM domain associated to a LEA protein were taken into account. For the sake of comparison, the earlier Dure's classification is also given (see [85] for details).

LEA gene				Species of origin	Host	Phenotypes	Ref.
Pfam	Dure	Seed specific ^a	Gene name				
PF00477 LEA.5	D-19	Yes	<i>PMA1959</i>	Wheat	Rice	Reduced ion leakage in leaves after salt stress, increased plant height and shoot biomass	[117]
PF00257 dehydrin	D-11	No	<i>PMA80</i>	Wheat	Rice	Reduced ion leakage in leaves after salt stress, increased plant height and shoot biomass	[117]
			<i>Rab17</i>	Maize	<i>Arabidopsis</i>	Higher sugar and proline content after drying, more tolerant to salinity and recovery after mannitol stress	[91]
			<i>Rab16A, Rab21^b</i>	Rice	Tobacco	Sustained growth rates under salinity, increased osmolyte production, reduced H ₂ O ₂ levels and lipid peroxidation under drought stress	[90]
			<i>pcC6-19</i>	<i>Craterostigma plantagineum</i>	Tobacco	No obvious drought-tolerant phenotype	[118]
			<i>RAB18/CORZ^c LTI29/LTI30</i>	<i>Arabidopsis</i>	<i>Arabidopsis</i>	Improved freezing tolerance, no effect on drought tolerance	[119]
PF02987 LEA.4	D-7	No	<i>CuCOR19</i>	Citrus ssp.	Tobacco	Enhancement of cold tolerance	[120]
			<i>HVA1</i>	Barley, chinese cabbage	Rice, wheat, chinese cabbage, creeping bentgrass	Increased biomass during water stress, decreased leaf wilting and membrane leakage	[89], [121–125]
			<i>pcC 3_06</i>	<i>Craterostigma plantagineum</i>	Tobacco	No obvious drought-tolerant phenotype	[118]
PF03760 LEA.1	D-113	No	<i>OsLEA3-1</i>	Rice	Rice	Decreased spikelet fertility, no effect on absolute grain yield during drought and salt stress but increased biomass	[88], [126]
			<i>BnLEA4-1</i>	Oilseed rape	<i>Arabidopsis</i> , lettuce, radish, bean	Enhanced growth ability under salt and drought stress	[127–130]
			<i>BhLEA1,2</i>	<i>Boea hygrometrica</i>	Tobacco	Higher relative water content, increased photosystem II activity (Fv/Fm), decreased electrolyte leakage and increased peroxidase and superoxide dismutase activity during drought stress	[92]
<i>PF04927 SMP</i>	<i>D-34</i>	Yes	<i>Atrab28</i>	<i>Arabidopsis</i>	<i>Arabidopsis</i>	Improved germination under salt and desiccation stress	[131]
<i>PF03168- LEA.2</i>	<i>D-95</i>	No	<i>CaLEA6</i>	Hot pepper	Chinese cabbage	Higher level of maximal photosynthetic rate of O ₂ evolution (Pmax) during drought, salt, and heat.	[132]
<i>PF03242 LEA.3</i>	<i>D-73</i>	No	<i>DQ663481^d</i>	<i>Tamarix androssowii</i>	Tobacco	Lower malonyldialdehyde content and conductivity; improved growth rate upon drought stress	[133]

(a) Seed specific expression according to [19]; (b) two names exist for this gene; (c) genes tested in combination; (d) accession number because there is no name given for this gene.

climates. In contrast, only one study [88] indicates that the over-expression of LEA genes under normal growth conditions led to side-effects such as a reduced spikelet fertility, like those found for over-expressed desiccation-related TFs (see Section 3.3 above). To further ascertain the use of LEA proteins, it will be necessary to secure data comparing the efficiency of various members of LEA genes within the same species and the efficiency of the same gene in a range of species in an array of agronomic scenarios by measuring the key process in yield formation: evapotranspiration, water use efficiency (i.e. the ratio of accumulated biomass to transpired water) and the harvest index (i.e. the ratio of harvested biomass to total biomass) [9].

Apart for agronomical purposes, LEA proteins might be useful for other biotechnological applications in relation to their capacity to prevent formation of aggregated proteins. For instance, fusion of a truncated peptide of *BNECP63* (PF02987) to target proteins is sufficient to provide a recombinant intrinsic membrane protein and hepatitis C viral protein in a soluble form or outside of inclusion bodies in *E. coli* [93]. This experiment demonstrates the use of LEA-type peptides in facilitating the production of recombinant proteins. Likewise, another member of the same PFAM family is able to reduce protein aggregate formation *in vivo* when introduced into mammalian cells expressing aggregation-prone proteins containing long polyglutamine (polyQ) or polyalanine (polyA) sequences [94]. The polyQ or polyA expansion proteins are associated with a number of human neurodegenerative diseases including Huntington's disease and oculopharyngeal muscular dystrophy.

4. Perspectives: the challenges of desiccomics

In a recent review, Walters et al. [22] have thoroughly presented the experimental evidence suggesting that the nature and extent of desiccation-induced damages occurring at molecular and cellular level occurs at different levels of stress. There is physiological evidence that there is a graduation of the responses to desiccation leading to a graduum of tolerance [21,22]. So far, transcriptome and proteome data (Table 1) are based on the view that desiccation tolerance is “an all-or-nothing” trait. This has led to the concept of a common set of genes that could make up the “desiccome”, a term coined by Potts and colleagues [12]. However, if indeed changes in gene expression or protein abundance relate to the hydration level to which tissues can survive, models should be developed to allow for a quantitative approach of desiccomics. Some examples of potential models are discussed below. It should be noted that we also need to develop functional genomics tools to validate gene function in relation to tolerance against various hydration levels. Molecular tools that allow discriminating the gene response relative to different drying levels will be an asset to improve drought tolerance.

One way to get further insights into desiccation tolerance is via seed mutants that are known to be conditionally lethal at the late stages of maturation. In *Arabidopsis*, *FUSCA3* (*FUS3*), *ABSCISIC ACID INSENSITIVE3* (*ABI3*), and *LEAFY COTYLEDON2* (*LEC2*) are interesting candidates. *ABI3*, *LEC2* and *FUS3* are B3 transcription factors interacting with each other to regulate many developmental processes such as accumulation of storage reserves and anthocyanins, precocious germination, dormancy and desiccation tolerance (for references see [26,95,96]). The severe alleles must be rescued before the loss of water during maturation kills the seeds [97,98]. Recent studies reported on mutants that exhibit a range of desiccation sensitivities, thereby providing a valuable tool for the dissection of the genetic basis of desiccation tolerance by “omics” approaches. For example, in *Arabidopsis*, seeds of the *fus3-3* mutant are desiccation sensitive whereas seeds bearing the *fus3-T* mutation are desiccation-tolerant but exhibit reduced seed longevity in open

storage [99]. In maize, the screening of 25 *vp* mutant seeds impaired in the synthesis of ABA to various degrees also showed a range of desiccation sensitivities [98].

Another interesting model to determine the genetic basis of desiccation tolerance could be seeds of *Coffea* spp., despite difficulties in obtaining such material and the long development on the mother plant. According to the species, seeds exhibit a range of desiccation sensitivities during drying and also show a decreased storability in the cold compared to warm temperatures ([100] and references therein). For example *C. pseudozanguebariae* and *C. liberica* seeds start to die when they reach a moisture content of 0.07 and 0.14 g/g, respectively. From the phenotype analysis of the F1 siblings from crosses between both species and from backcrosses with *C. liberica*, [100] could offer a plausible genetics-based explanation as to why sucrose content, a known protectant purportedly involved in desiccation tolerance, is not significantly correlated with the level of desiccation tolerance. This information is interesting considering that the issue as to whether non-reducing sugars play a minor (if any) role in the survival in the dry state is still debated [2,6,11,13]. An EST database generated from developing seeds is now available for transcriptome analyses [101].

Finally, ecological genomics is an emerging field that combines ecology and molecular approaches to study the adaptation of organisms in their natural habitats. It will be interesting to develop this approach in desiccation tolerance using the high-throughput deep sequencing techniques and other genomics developments [102,103]. An estimated 18% of tree and shrub taxa produce seeds that remain sensitive to enforced drying after shedding [104]. These seeds are called recalcitrant because they cannot be stored over sufficiently long periods (see [21] for reviews). Certain recalcitrant seeds can exhibit a wide sensitivity to drying within the same species such as *Aesculus hippocastanum* [21,105]. There are also examples, such as *Acer pseudoplatanus*, where seeds exhibit either a desiccation tolerance or a sensitivity phenotype according to their provenance because of the magnitude of the climatic effect on their development ([106] and references therein). Understanding why these seeds do not tolerate different levels of drying could be helpful to understand desiccation tolerance. This is supported by the comparative analysis of the transcriptome of human cells submitted either to a killing desiccation treatment or to a hypertonic stress [107,108]. These authors showed that despite their sensitivity to drying, these cells were able to trigger a complex stress response involving both known survival and death pathways [108]. They speculated that identifying and validating these putative decision points between survival and death could lead to the path of engineering cells or organisms more tolerant to desiccation [108]. In a similar approach, a comparison of changes between the transcriptomes of the anhydrobiotic yeast and human HEK 293 cells submitted to air drying showed commonalities and dissimilarities in the gene expression response [12] (Table 1). Similar studies have not yet been performed on recalcitrant seeds and it remains to be ascertained whether similar life-or-death decision networks exist.

Two more points deserve attention to extent our knowledge of desiccation tolerance and survival in the dry state. The first point is related to the choice of candidate genes for further functional analysis. So far, little focus has been given to the function of unknown genes expressed in relation to desiccation tolerance, which number can represent up to 30% of the desiccome [36]. In addition, only up-regulated genes have been considered so far, but studies have shown that there are many proteins or transcripts that are respectively hydrolyzed or shutdown [36,84], perhaps because their products or underlying process are incompatible with for desiccation tolerance.

The second point pertains to the use of -omics technologies to assess what is happening in the dry state. During drying, viscosity increases dramatically by several orders of magnitude between

0.3 and 0.1 g/g. Below 0.1 g/g (or 50% RH at room temperature), the cytoplasm transforms in a glassy state resulting in the stabilization of molecules. As a result, the molecular mobility is severely restricted (reviewed by [11,109]). However, molecular mobility and diffusion of small molecules (such as oxygen) can still occur in the dry state and reactions can take place, albeit at a strongly reduced rate. This explains why seeds age in the dry state and probably why dormancy is released during after-ripening. Recently, workers have observed changes in transcripts and polypeptides in dry seeds (RH between 75 and 40%) during after-ripening (reviewed by [28]). The changes in the transcriptome and proteome associated with after-ripening are very intriguing because they occur at moisture contents at which there is vanishingly small amount of ATP (energy charge between 0.2 and 0.4) and no electron transport leading to respiratory and photosynthetic activities [3] and references therein, [22,110]. The established theory on glassy states would also rule out the presence of free water pockets [10,11,22]. So far, no evidence is provided that there is a regulation of specific gene networks in the dry state. Hypotheses can be put forward to explain some of the results, such as lipid-bound enzyme activities or oxidation, known to occur at low moisture contents [111]. This calls for studies on the kinetic changes of physical and biochemical/molecular processes during storage in the dry state by combining the characterization of the glassy state and non-invasive -omics tools to assess those processes and how they lead to a physiological response during imbibition. This is yet another challenge awaiting desiccomics that needs to be tackled before the original transcriptome and proteome analyses (see [28]) are taken as proofs that metabolism occurs in the dry state. Using functional genomics, it would be equally interesting to investigate which part of the desiccome plays a role in the stability of macromolecules, physicochemical properties of the glassy state or kinetics of oxidative damage in the dry state.

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