Review

Vegetative desiccation tolerance: Is it a goldmine for bioengineering crops?

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ABSTRACT

In desiccation-tolerant plants vegetative organs can dry to about 4–13% relative water content, while desiccation-sensitive plants die when their relative water content drops below 20–50%. Desiccation-tolerant plants have different stress-adaptation strategies that provides good basis for their catalogization. Thus, these plants may be subdivided into homoiochlorophyllous and poikilo-chlorophyllous types according to the status of their photosynthetic apparatus upon dehydration. During dehydration homoiochlorophyllous species retain their photosynthetic apparatus and chlorophylls in a readily recoverable form, whereas in poikilo-chlorophyllous species desiccation results in the loss of chlorophyll, which must be resynthesized following rehydration. Desiccation-tolerant plants can be subdivided also on the basis of differences in the molecular mechanism of desiccation tolerance. Fully desiccation-tolerant plants are able to withstand rapid drying and possess constitutive tolerance, while modified desiccation-tolerant species are able to survive slow drying and possess inducible tolerance. Mechanisms proposed to explain the ability of vegetative desiccation-tolerant plants to survive desiccation include sucrose and trehalose accumulation, accumulation of stress proteins, increased folding ability of cell wall structures and accumulation of membrane stabilizing polyphenols and antioxidants. However, any of the above mechanisms may not have the same effect in desiccation-sensitive plants, such as crop plants. The utility of using genes from the desiccation-tolerant plants lies in the prospect of finding novel ways to maintain productivity of crop plants following periods of drought by either broadening the limits of cell maintenance to encompass lower water potentials and/or repairing damage as it occurs allowing for broadening of the sensitivity range. The question is how it can be achieved technically, because the weakest point within the whole high-throughput technologies is the limited capacity of conventional genetic transformation techniques. However, despite the limitations, conventional genetic transformation is still an unavoidable part of the functional genomics and molecular breeding programmes. Since resurrection plants show extreme sensitivity and vulnerability in tissue culture, lessons learned from their genetic transformation can be extended over other recalcitrant species.

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1. Introduction

1.1. Fully desiccation-tolerant plants versus modified desiccation-tolerant plants

A pyrrhic victory is a victory so costly that it is almost equivalent to a defeat. It derives from the battle won by King Pyrus of Epirus over the Romans at Asculum in 279 BC. Noting the heavy losses his own side had taken, he is reported to have said: “One more such victory and I am lost.” This could be one's first conclusions after reading the review entitled “Constraints of tolerance: why are desiccation-tolerant organisms so small or rare?” [1] Although the initial evolution of vegetative desiccation tolerance was a crucial step required for the colonization of the land by primitive plants, it came at a cost [2]. In desiccation-tolerant plants vegetative organs can dry to about 4–13% relative water content, while desiccation-sensitive plants die when their relative water content drops below 20–50%. Probably because of the extra energy-requiring protection and repair mechanisms, the interrelated features of metabolic rates, biomass production and competitive abilities are usually lower in desiccation-tolerant plants as compared to desiccation-sensitive plants. The most serious consequence of this conclusion is that a fully desiccation-tolerant crop plant would have little agricultural value. However, this should not deflect us from asking two important questions: what can we learn from desiccation-tolerant plants, and to what extent will we be able to utilize this knowledge for bioengineering increased transient drought tolerance in crop plants? For that we need to select essential components of desiccation tolerance that are readily transferable to non-tolerant systems. There are already examples where outcomes of targeted studies in desiccation-tolerant plants are going to be directly utilized to genetically engineer crop plants [3]. For example, as a part of an ongoing project, some desiccation tolerance-related genes of Xerophyta viscosa are to be expressed in maize. It is not known whether the transfer of protective proteins, osmolyte sugars, signal metabolites or antioxidants from desiccation-tolerant species will result in crop plants with increased abiotic stress tolerance.

A vast majority of plant tissues are sensitive to dehydration. The tissue is damaged and will ultimately die once the water content of the tissue falls below a certain percentage. Ironically most plants possess at least one stage of their life cycle where at least some tissues or cells can survive severe dehydration. For many plant species this is limited to seeds, pollen or in dormant buds. During maturation, these organs lose most of their water and enter a dormant state and then can remain apparently inactive for long periods. For example seeds can lie in this state for centuries, seeds of sacred lotus (Nelumbo nucifera): 75% germinated after 1300...
years of storage [4]. However, most plants lack this ability in organs such as leaves. Desiccation-tolerant plants are able to do this. In this small group of plants the mature leaves, roots and shoots can lose up to 95% of their water. This results in a shrivelled dried plant that is actually still alive. The loss of more than the half water content is enough to kill the tissue of most plants. Desiccation-tolerant, or in other words poikilohydric plants, possess mechanisms to protect them in the dried state. Higher desiccation-tolerant plants are also referred to as resurrection plants, because they can be resurrected by rehydration. Desiccation tolerance is also common in many algae and among microorganisms, such as rotifers, nematodes and tardigrades and in Crustacea of seasonal water bodies. It is thus a widely expressed potentiality of living organisms.

Desiccation tolerance occurs throughout the plant kingdom. It is commonplace among lichens, relatively common in mosses and is found sporadically among vascular plants from a range of families. Desiccation tolerance of the vegetative tissues in vascular plants has been demonstrated in some 350 species, making up less than 0.2% of the total flora [5] but the list is constantly being extended. Desiccation tolerance is thus thinly and unevenly, scattered amongst vascular plants. Virtually all vascular plants have desiccation-tolerant spores (including pollen) or seeds, so the potential for desiccation tolerance is probably universal.

The majority of vegetative desiccation-tolerant plants are found in the less complex clades that constitute the algae, lichens, and mosses. These plants are also called fully desiccation-tolerant plants (Fig. 1), because they withstand the total loss of free protoplasmic water [6]. The internal water content of these plants rapidly equilibrates to the water potential of the environment, as they possess very few of morphological or physiological adaptations for the retention of water. As a result of this, many lichens, algae, and desert bryophytes have rapid drying rates, i.e., reaching air dryness within an hour. Desiccation of such plants can even be achieved in a few minutes in a lyophilizer, which indicates that an inducible protection mechanism is not necessary for survival in this group of lower desiccation-tolerant plants [2]. This strengthens the hypothesis that the primitive mechanism of tolerance probably involves a low intensity, but constitutively functioning protection mechanism that is coupled with active cellular repair [2]. A larger and more complex group of vegetative desiccation-tolerant plants are named modified desiccation-tolerant plants. The vascular desiccation-tolerant plant species belong to this group [7]. They dry more slowly by an array of morphological and physiological mechanisms that retard the rate of water loss to the extent required to establish tolerance. Available evidence concerning desiccation tolerance of modified desiccation-tolerant plants strongly suggests that they utilize preventive mechanisms that rely heavily on inducible cellular protection systems [8,9].

1.2. Homoiochlorophyll and poikilochlorophyll: two alternatives in the modified desiccation tolerance

Desiccation-tolerant plants may be subdivided also into homoiochlorophyllous and poikilochlorophyllous types (Fig. 1). During desiccation homoiochlorophyllous species retain their photosynthetic apparatus and chlorophylls in a readily recoverable form, whereas in poikilochlorophyllous species desiccation results in the loss of chlorophyll, which must be resynthesized following rehydration [10]. Much has been published on the photosynthetic responses of homoiochlorophyllous desiccation-tolerant plants, especially on the cryptogamic plants [7]. A great deal of research has been conducted on the tolerance limits of homoiochlorophyllous desiccation-tolerant cryptogams and phanerogams [7,11]. However, until our recent studies [7,10,12–15] little was known about the ecology, ecophysiology and distribution and abundance of poikilochlorophyllous desiccation-tolerant plants.

Poikilochlorophyll, the phenomenon of loss of chlorophyll during desiccation was first described in Carex physodes from central Asia [16]; then it was reintroduced [17], and was regarded as a special phenomenon in certain desiccation-tolerant monocotyledonous plants [18]. In contrast, the rate of chlorophyll loss in the homoiochlorophyllous desiccation-tolerant plants varies from species to species, and it is influenced by environmental factors, however the rate of chlorophyll loss in photosynthetically active plants does not exceed the critical physiological amount. In other words, homoiochlorophyllous desiccation-tolerant plants cannot survive the complete or even the physiologically dangerous partial loss of their chlorophyll content. Poikilochlorophyll evolved as a different evolutionarily strategy [10,14,19]. It is based on the dismantling of internal chloroplast structure by an ordered deconstruction process during drying, and its resynthesis upon rehydration by an ordered reconstruction process [13]. These processes can thus be thought of not only as being superimposed on an existing cellular protection mechanism of vegetative desiccation tolerance [19] but also as a distinct new class of desiccation-tolerance strategy [7,14]. The selective advantage of poikilochlorophyll, in minimising photo-oxidative damage and not having to maintain an intact photosynthetic system through long, inactive periods of desiccation, presumably outweighs the disadvantage of slow recovery and the energy costs of reconstruc- tion. Taxonomically poikilochlorophyllous desiccation tolerance appears to be restricted to the monocots [18]. Poikilochlorophyll is currently known in eight genera of four families (Cyperaceae, Liliaceae/Anthericaceae, Poaceae and Velloziaceae). Most occupy the almost soil-less rocky outcrops known as inselbergs, in strongly seasonal tropical and sub-tropical climates [5]; the best studied physiologically are the African Xerophyta scabrida, X. viscissa and Xerophyta humilis and the Australian Borya nitida [7].

The homoiochlorophyllous desiccation-tolerant and poikilo- chlorophyllous desiccation-tolerant strategies solve the same ecological problem, but cover a broad temporal range of adaptation [10]. The homoiochlorophyllous desiccation-tolerant ferns and angiosperms are generally adapted to longer drying–wetting cycles than mosses and lichens, but to more rapid alternations of wet and dry periods than the poikilochlorophyllous desiccation-tolerant monocot species [10,11,20,21], though some can survive desiccation for very long periods of time. The poikilochlorophyllous desiccation-tolerance strategy has evolved in habitats where the plants remain in the desiccated state for 8–10 months. Under these conditions it is evidently more advantageous to dismantle the whole photosynthetic apparatus and reconstitute it after rehydration. Of course there is variation within each category and the categories overlap in their ecological adaptation, and two or more may coexist in one habitat, e.g. on inselbergs [22]. Both ends of this ecological spectrum have particular points of interest. There is probably a trade-off between the ‘cost’ of protection and repair to the photosynthetic apparatus if this is kept in a quickly recoverable state during prolonged periods of desiccation, and the ‘cost’ of reconstituting the photosynthetic apparatus de novo [10].

1.3. Evolutionary aspects

The early evolution of vegetative desiccation tolerance is thought to be a critical step in the colonization of the land by primitive plants [2]. As plant species evolved, vegetative desicca- tion tolerance was lost as increased growth rates, competitive ability, structural and morphological complexity involving mechanisms that conserve water within the plant became valuable
traits for plants. Genes that had evolved for cellular protection and repair were recruited for different but related processes such as response to water stress and the desiccation tolerance of reproductive organs like seeds, pollens, dormant buds and protecorms. However, their expression in vegetative tissues is rare, and must have re-evolved independently in Selaginella, in ferns, and at least eight times in angiosperms [19]. The only major class of vascular plants that does not have a representative species that has desiccation-tolerant vegetative tissues is the gymnosperms (a taxonomic group consisting of the phylogenetically distinct cycads, conifers, and genetophytes), a fact may signify a minimum size limitation for desiccation tolerance, which members of this group exceed. We continue this speculation to assert that modified desiccation tolerance typical of angiosperms is not a developmental continuation of the more primitive full desiccation tolerance, but it evolved from that programmed into seed development. However, to answer the question, why was it an evolutionary advantage to re-introduce vegetative desiccation tolerance, instead of surviving unfavourable conditions as a seed, still remains an enigma. Moreover, we know that the natural habitats of vegetative desiccation-tolerant plants are often deficient in mineral and organic nutrients [22]. This could suggest that the resurrection cycle requires fewer nutrients from the environment than that of completing the whole developmental process from a seed. The other hypothesis is that vegetative desiccation tolerance evolved from the response of desiccation-sensitive plants to abiotic stresses such as cold, salt and drought [23]. Desiccation-sensitive plants use an interconnected signaling network to activate a common repertoire of responses to abiotic stress [24]. These responses appear to overlap with those described for desiccation-tolerant plants during extreme water loss as they include accumulation of compatible osmolytes, and the upregulation of antioxidants and antioxidant enzymes [24]. Small-scale microarray analysis of X. humilis, an indigenous Southern African resurrection plant, has revealed that dehydration-upregulated cDNAs included known stress-responsive genes encoding metallothioneins, galactinol synthases, an aldose reductase and a glyoxalase [25]. A large number of genes encoding late embryonic abundant proteins, dehydrins and desiccation-related proteins were also identified, suggesting that proteins that provide mechanical and antioxidant protection against water loss dominate the mRNA population in desiccated X. humilis leaf tissue [25]. Under this scenario, it was predicted that there should be significant overlap between genes that are introduced in response to abiotic stresses and desiccation tolerance [24]. This coincides with the conclusion of those who suggest that desiccation tolerance in vegetative tissues in Craterostigma plantagineum is probably not due to structural genes that are unique to resurrection plants, but that relevant genes are also present in the genome of non-tolerant plants [26]. The difference between tolerant and non-tolerant plants probably resides in gene expression patterns, meaning that desiccation-tolerant plant-specific regulation of a common set of genes is probably responsible for vegetative desiccation tolerance. However, there are notable reports on large-scale partial sequencing of randomly selected cDNA clones or expressed sequence tags (ESTs) in different desiccation-tolerant plants that has led to a contrasting hypothesis. A complementary DNA library was constructed from the dehydrated microphyll fronds of the resurrection plant Selaginella lepidophylla and used to generate an EST database. ESTs were obtained for 1046 clones representing 874 unique transcripts. Putative functions were assigned to 653 of these clones after comparison with protein databases, whereas 212 sequences were significantly similar to known sequences whose functions are unclear and 181 sequences having no similarity to known sequences [27]. Small-scale microarray analysis of Tortura ruralis, a bryophyte model for genomic level investigations, showed that only 29% of the desiccation-upregulated cDNAs had significant similarity to previously identified nucleotide and/or peptide sequences [28]. In an earlier report cold-plaque screening was used to analyze 200 cDNA clones from C. plantagineum leaves that had been either dried for 1 h or totally dried down [29]. One half of the sequences showed no significant similarity to those in public databases. A comparison of these reports suggests that considerable progress was achieved in exploring gene functions, but the high proportion of unknown sequences and sequences without known function still makes it difficult to establish provisional pathways.

Desiccation-tolerant plants are important constituents in many ecosystems from the tropics to the polar arctic region. For example, lichens and mosses play a major role in temperate semidesert grasslands, in cool and hot deserts, and importantly in tundra and polar/arctic vegetation. These plants dominate under unfavourable climate conditions, where the normal homoiohydric plants maintain much of their biomass below the soil surface, succumb to stresses and/or are unable to establish themselves. Large pools of nutrients and carbon accumulate in desiccation-tolerant plants in these extreme ecosystems, and therefore significant aspects of ecosystem function depend on their physiological response, production and turnover pattern. Thus, any analysis of terrestrial vegetation warrants investigation of the desiccation-tolerant plants. But the desiccation-tolerant plants do have a direct practical significance, also: the understanding of mechanisms of desiccation tolerance will be useful in the future to modify species by the powerful technique of genetic engineering, to develop crops that are tolerant of the harmful effects of drought.

Desiccation tolerance is qualitatively different from drought tolerance as ordinarily understood in vascular-plant physiology; indeed desiccation tolerance could be seen as a drought-adaptation mechanism. Desiccation-tolerant plants are not simply an odd sideline from mainstream homoiohyd, [7]. They are an adaptive optimum in particular ecological situations, and (as ‘normal’ vascular plants) can be only understood fully in the context of a wide and multidimensional field of physiological and ecological possibilities. Most vascular desiccation-tolerant plants function as normal (‘homoiohydric’) vascular plants until water becomes limiting. Like winter annuals and desert ephemerals (and other mesophytes), they then dry out within a few hours or days. The difference is that instead of dying and re-establishing from seed, their fall-back is to survive in a desiccated, but still viable vegetative state.

Wild species have been chosen as crops because of their high productivity, and bred to make them more so. Because crops are in general desiccation-sensitive, they must either be watered or grown where there is no drought. Aridity thus remains the greatest enemy of agriculture. In addition, there is a necessary functional conflict between high productivity and desiccation tolerance.

Although there are other protective processes, three major interacting biochemical mechanisms for recovery from desiccation-induced injury are emphasized in this review. Firstly, it was proposed that non-reducing sugars facilitate tolerance to desiccation by protecting membranes and proteins [22,30–32] and by inducing vitrification [32]. Secondly, another mechanism is based on the capability to scavenge desiccation-induced free radicals. Desiccation tolerance has been correlated with an increased alarm status of both the non-enzymic (reduced glutathione, ascorbic acid, tocopherols) and enzymic components of the antioxidant defense system [21,33,34]. The third mechanism includes proteins such as the late embryogenesis abundant (LEA) proteins [20,26] and LEA-related proteins such as dehydrins and rehydrins [2,38].
2. Sugars serve as osmoprotectants, components of the biological glass, and mobilizable energy sources during stress

A fundamental component of desiccation tolerance appears to be sugars [22,35,36]. In all of the modified desiccation-tolerant plants studied to date, drying induces a major change in carbohydrate metabolism, which may be directly related to desiccation tolerance. Sucrose is the only free sugar available for cellular protection in fully desiccation-tolerant mosses, including Tortula ruraliformis and T. rurals [37,38]. The amount of this sugar in T. rurals gametophytic cells is approximately 10% dry weight, which is sufficient to offer membrane protection during drying, at least in vitro. Moreover, neither drying nor rehydration in the dark or light, results in a change in sucrose concentration on a dry weight basis, suggesting that it is important for cells to maintain sufficient amounts of sucrose [37]. The lack of a dehydration-specific increase in soluble sugars during drying appears to be a common feature of fully desiccation-tolerant mosses that maintain high sucrose content constitutively [38]. In contrast to fully desiccation-tolerant plants, modified desiccation-tolerant plants accumulate high concentrations of sucrose during the dehydration phase (Fig. 2) in all species when this has been studied [39–47]. In tandem with specific proteins [48], sucrose is among the sugars that probably stabilize drying cells both by direct interactions with macromolecules and membranes and by reversibly immobilizing the cytoplasm to become an extremely slow-flowing glass-like (vitrified) liquid [47]. Many isolated enzymes, dried in the presence of sucrose, remain stable in the dried state [32]. Parallel work on isolated membrane vesicles also supports the view that sucrose preserves the integrity of the lipid bilayer during dehydration [31]. Sucrose accumulation occurs relatively late in the dehydration time course, initiated usually at relative water content below 60%, in some the majority occurs at 20%. The consensus is that the accumulation of sugar is one of the last preparatory steps in the cell protection pathway when the cell is fully committed to a period of quiescence.

To understand the biochemical basis for the stress-specific switch in sugar metabolism, the expression of genes encoding sugar-metabolizing enzymes was investigated in a higher plant model C. plantagineum. This plant possesses a set of genes encoding isoenzymes of sucrose synthase [49] and sucrose phosphate synthase [42]. A characteristic rise in transcript levels of class-I sucrose synthase genes was observed in response to dehydration or abscisic acid (ABA) treatment, which indicates an increased glycolytic demand. This coincides with the finding that the transcript levels of cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) also rapidly increase in abundance during dehydration or ABA treatment [50]. The increase in class-I sucrose synthase and GAPDH mRNA levels directly correlates with higher protein and enzyme contents. As both sucrose synthase and GAPDH are glycolytic enzymes, these results imply that enhanced rates of glycolysis are one of the immediate cellular responses to water deficit. Besides these enzymes, the transketolase–transaldolase functional enzyme complex might contribute to the conversion of the eight carbon carbohydrate, 2-octulose, to sucrose in C. plantagineum [51] and, alternatively, it might enhance pentose-to-hexose flux providing more hexose for glycolysis. This may be a mechanism by which the plant cell prepares for a demand of ATP and NADH during recovery [49]. Sucrose phosphate synthase behaves differently. The activity of this enzyme increases in C. plantagineum leaves together with sucrose accumulation during dehydration [42]. However, during the periods when the highest extractable sucrose phosphate synthase activity was found, the transcript levels were at their lowest, suggesting additional regulatory mechanisms. The increased activity of sucrose phosphate synthase may reflect the activation state of the enzyme rather than just the amount of protein [42]. This coincides with the recent consensus that sucrose phosphate synthase is a subject of strong allosteric regulation [52]. The actual activity of an allosterically regulated enzyme by positive and negative effectors mirrors cellular homeostasis and thus, allows continuous physiological adjustment.

The most potent negative effector of sucrose phosphate synthase is the signal metabolite fructose-2,6-bisphosphate (Fru-2,6-P2), which is an essential regulator of sugar metabolism in fungi, plants and animals [53]. Plants use Fru-2,6-P2 to switch between direct fuel use and storage, which is somewhat comparable to the role of the compound in animals and humans [54]. In plants Fru-2,6-P2 contributes both to coordination of sucrose synthesis with the rate of CO2 fixation [52] and indirectly to the control of assimilate partitioning between sucrose and starch [55]. Fru-2,6-P2 allosterically inhibits cytosolic fructose-1,6-bisphosphatase (cytFBPase), which catalyzes an irreversible regulatory step in the sucrose synthesis pathway. A decrease in the level of Fru-2,6-P2 increases the flux towards sucrose by releasing cytFBPase from an inhibited state, which in turn, stimulates sucrose phosphate synthase, another key enzyme in the sucrose synthetic pathway [52]. At the same time, an increase in the level of Fru-2,6-P2 increases the 3PGA:Pi ratio in plastids that
leads to a rise in ADP-glucose pyrophosphorylase (AGPase) activity and consequently to an elevated flux towards starch [55,56]. Thus, manipulation of the endogenous levels of Fru-2,6-P₂ provides a testing system for molecular and metabolic processes that are based on photosynthetic partitioning. The upregulation of Fru-2,6-P₂ synthesis suppresses sucrose accumulation in photosynthesizing leaves during the light period. Sucrose synthesis is enhanced following downregulation of Fru-2,6-P₂ synthesis. This approach has already been utilized to examine Crassulacean acid metabolism (CAM) in Kalanchoë daigremontiana [57]. We created transgenic C. plantagineum plants with elevated levels of Fru-2,6-P₂ (O. Toldi, P. Scott, unpublished results) with the view of suppressing sucrose accumulation during dehydration. All strong expressors of the mammalian 6-phosphofructo-2-kinase gene (6-PF-2-K), which synthesizes Fru-2,6-P₂, were unable to ‘resurrect’ during rehydration. At the same time, wild type plants and transgenic controls, carrying empty vectors, started the recovery process when irrigation was continued (O. Toldi, P. Scott, unpublished results). This demonstrates well that sucrose accumulation is an indispensable mechanism within the vegetative desiccation tolerance at least for modified desiccation-tolerant species. However, when Fru-2,6-P₂ levels were downregulated by the introduction of its Fru-2,6-P₂-ase-mediated catabolism, plants became even more sensitive to drought. The high sucrose content throughout the diurnal cycle gave rise to an increased turgor pressure in leaves, stomatal conductance, therefore, remained also high during the dehydration phase, resulting in an uncontrolled transpiration. Thus, manipulation of sucrose metabolism in the view of engineering crops for drought tolerance does not seem to be a viable approach. Most likely that it would interfere with osmoregulation, metabolism and the biomass production.

However, there are other components of the photosynthetic carbohydrate metabolism with a more realistic agronomic potential. Raffinose family oligosaccharides have long been suggested to act as antistress agents in both generative and vegetative tissues [3,58,59]. They are the most widely distributed non-structural carbohydrates in the plant kingdom, occurring in a wide variety of species [60]. As non-reducing carbohydrates they are good storage compounds, being able to accumulate in large quantities without affecting primary metabolic processes [3]. Research in seeds [61,62] and root systems [63,64] has revealed strong correlations between accumulation of raffinose family oligosaccharides, primarily raffinose, stachyose, and verbascose, and desiccation tolerance. We propose that the functional identification of galactinol synthase (XvGoS3), catalyzing the first committed step towards raffinose family oligosaccharides from X. viscosa [3], opens the possibility to introduce a new protective trait into crop plants.

These contrasting stories about the different agronomic potentials of sucrose and raffinose family oligosaccharides shed light on a third important target of molecular breeding efforts. The non-reducing disaccharide trehalose also serves as a protectant against a variety of stresses in different organisms [65] and significantly contributes to vegetative desiccation tolerance in plants [8,66,67]. Trehalose is among the most chemically unreactive sugars and its strong stability is a result of very low energy (1 kcal mol⁻¹) of the glycoside oxygen bond joining the two hexose rings [68]. Thus, trehalose certainly will not interfere with primary metabolism and the energy homeostasis. Nevertheless, results from the introduction of genes encoding trehalose-synthesizing enzymes to certain crop plants are contradictory. In some instances, transgenic plants had increased tolerance to abiotic stressors that were associated with sustained plant growth [69]. Three different approaches resulted in improved drought tolerance without causing growth defects. First a double construct carrying the Saccharomyces cerevisiae trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase genes driven by a constitutive promoter (AtRbcS1A) were introduced into tobacco [70]. In the second approach, a trehalose-6-phosphate synthase gene was driven by AtRAB18 [70] and rd29A [71] stress-inducible promoters. The third strategy was the use of a constitutive AtRbcS1A promoter together with a transit peptide in front of the coding sequence of the S. cerevisiae trehalose-6-phosphate synthase, which directed the enzyme to the chloroplast [70]. However, although the majority of transgenic plants were more or less drought tolerant, they were stunted with different morphological abnormalities [72]. One of the reasons for this can be the use of strong constitutive promoters instead of stress-inducible ones [70,71]. However, it cannot be generalized, because drought-specific expression of the S. cerevisiae trehalose-6-phosphate synthase gene in potato also resulted in drought tolerance, but slow growing, retarded plants [73]. Recent microarray data on plants modified in the trehalose biosynthesis pathways indicate that many of the trehalose-inducible genes are associated with jasmonic acid and ethylene-dependent stress signaling pathways [74,75]. The overexpressed Arabidopsis trehalose-6-phosphate synthase influences sugar sensing and photosynthetic partitioning [76,77]. Whatever the basis for these observations, trehalose is an important component of the sugar-signaling pathway in higher plants [78]. Engineering trehalose metabolism with the aim of producing crops more tolerant to abiotic stressors may result in severe interference with sugar and other stress signaling pathways.

3. Proteins closely associated with desiccation tolerance

Some proteins specific to desiccation also have a major role in cellular protection and recovery in vascular plants [79]. It is important to determine the roles of these proteins attempting to understand the mechanism by which modified desiccation-tolerant plants establish tolerance [2]. Several of the cDNA clones isolated from the desiccation-tolerant Craterostigma tissues are putatively related to late-embryogenesis abundant proteins (LEAs), proteins responsive-to-ABA, and dehydrins [9,20]. These are all implicated in cellular protection during seed desiccation and water stress [80,81]. Similarly, several cDNAs representing drying-induced transcripts in Sporobolus stapfianus also code for proteins related to LEAs [82]. However, LEA genes have been cloned not only from desiccation tolerant ones but also from many plant species. At least six different groups of LEA proteins have been identified on the basis of expression pattern and sequence [83]. As their name suggests, they are produced in abundance during late embryonic development, comprising up to 4% of cellular protein [84] with maximum expression post-abscession during desiccation [85]. Their expression is coincident with the acquisition of desiccation tolerance in seeds and pollen, as well as desiccation-tolerant plants. Many LEA proteins are also induced by cold, osmotic stress or exogenous abscisic acid, or can even be expressed constitutively [86]. They have been variously proposed to protect cellular structures from the effects of water loss by acting as a hydration buffer, by sequestering ions, by direct protection of other proteins or membranes, or by renaturing unfolded proteins [80,83]. These suggested functions have been made on a largely intuitive basis and are supported by relatively little evidence [86]. However, some effect on stress tolerance seems apparent because LEA proteins from tomato, wheat and barley confer increased tolerance to osmotic or freezing stresses when introduced into yeast [87–90]. Barley LEA protein improved tolerance to water deficit in transgenic rice [91] and wheat [92]. Furthermore, an algal LEA protein diminished freezing damage to lactate dehydrogenase in
vitro [93]. Chimeric LEA genes have already been extensively utilized in molecular breeding programmes [91,92]. However, their real contribution to drought and desiccation tolerance can be evaluated only when stress-tolerant crops carrying LEA genes will be commercially available for users in the realistic hope of profitable production. The integration of LEA-mediated stress responses will be necessary to identify of regulatory elements with synchronizing role. This could be complemented by a one-by-one functional analysis of LEA genes to identify the ones most specific to desiccation tolerance. LEA proteins can also be processed into small polypeptides that have activity in preventing protein aggregation upon drought stress [48]. An additional option that LEA genes provide is that their promoter elements can be used for the transcriptional modulation of other resistance genes allowing stress specific expression [94].

LEA proteins are not the only proteins important in the acquisition of dehydration or desiccation tolerance. There is growing evidence that small heat-shock proteins play a key role perhaps by virtue of their reported chaperone-like activity. LEA proteins can prevent protein aggregation [48,95], which could mean they have similar properties to other chaperonins and that at the very least it would indicate that chaperonins might be a good target for a biotechnology approach to dehydration tolerance.

4. Abscisic acid-based stress signaling in vegetative desiccation tolerance: whether specific or follows the drought-sensitive scheme?

Abscisic acid is thought to play a co-ordinating role in the activation of tolerance genes in response to desiccation [2]. Exposure of C. plantagineum plants and callus to exogenous abscisic acid induces genes that are otherwise activated by dehydration [9,26]. Callus of this plant is not inherently desiccation tolerant, but becomes so if treated for 4 days with abscisic acid before drying. Numerous new proteins, typical of abiotic stress, are synthesized when abscisic acid is applied to non-stressed tissues [96]. For example, a novel stress-induced antioxidant has been identified in X. villosa [97] and a new gene, encoding XvSAP1 protein, which is inducible by exogenous abscisic acid and thought to play a role in membrane stabilization during water deficit [98]. The XvSap1 protein displays greater than 55% overall identity with the TaCOR413 and is consequently clustered with the COR413 proteins. XvSap1 is regulated by environmental stress and abscisic acid that it is able to increase desiccation and salt tolerance when expressed in Arabidopsis thaliana [99,100]. qPCR analysis indicated that XvSap1 mRNA was upregulated at 60% relative water content. Thereafter, expression decreased but increased again at 15% relative water content. This would suggest that XvSap1 is involved in the initial and late stages of desiccation protection. Genes responsive to abscisic acid usually contain abscisic acid-responsive elements consisting of the (C/T)ACGTGCG consensus sequence and are transactivated by bZIP transcription factors [101]. Three classes of transcription factors have been characterized in C. plantagineum: a heat-shock transcription factor [29], two members of the homoeodomain Leu zipper family [102], and three MYB genes [103]. Transgenic Arabidopsis lines overexpressing the heterologous Craterostigma MYB transcription factor gene CpMYB10 were drought stress-tolerant, glucose-insensitive and abscisic acid-hypersensitive [104]. Although stress tolerance can be increased by manipulation of abscisic acid-transduced stress responses, it seems that undesired interference with other signaling processes is highly probable.

In addition, several desiccation-induced genes, expressed early in the drying process, are not inducible by abscisic acid. This raises the possibility that other signaling pathways are also involved in desiccation tolerance in Craterostigma [105]. In other words, stress-related gene expression responses utilize multiple signaling pathways [106]. Other desiccation-tolerant plants also have independent signaling cascades [107,108]. Abscisic acid has not been found in the desiccation-tolerant moss, T. ruralis [2]. However, fluoroscopic evidence was delivered that abscisic acid helps induce desiccation tolerance in the afromontane moss Atrichum undulatum [109]. Abscisic acid also acts as a signal for increased non-photothermal quenching during water loss, decreasing the production of harmful singlet oxygen [109].

Abscisic acid has a wide range of effects, therefore will elicit many processes not specific to desiccation tolerance. Abscisic acid is a cell cycle inhibitor [110], regulates stomatal movement [111], suppresses growth [112] accelerates accumulation of storage compounds [113], induces development of storage organs in vitro [114,115], initiates dormancy and water loss [116] in most of the plant tissues where it was examined. It is used exogenously for maturation of somatic embryos [117], to induce differentiation of protocorms and embryogenic structures in dedifferentiated cell cultures [118], as well as to harden plant tissues prior to low temperature storage [119]. Looking at the problem just superficially, it seems that abscisic acid does the same things in vegetative desiccation-tolerant plants as it does in non-tolerant plants. However, integrated redirection of complex molecular events and metabolic processes might require such a multifaceted signal like abscisic acid. Cell cycling and growth must be slowed down to survive dehydration, while accumulation of energy storing compounds and building units of essential metabolites and macromolecules must be accelerated. It is hard to find a satisfactory consensus in relation to the complex role of abscisic acid in the regulation of desiccation tolerance. These preliminary explanations are descriptive and based mostly on opinions and not hard evidence. Our belief is that abscisic acid integrates the signal transduction processes during dehydration phase that make the survival of vegetative desiccation possible both on cellular and whole plant levels. This would mean that the regulatory role of abscisic acid both spatially and temporally is limited to certain steps where integrated actions are required during the dehydration. If it is so, is there another regulator that replaces abscisic acid during the rehydration phase? Do the recovery of normal functions, acceleration of cell cycling and growth certainly requires different signals?

5. Glutathione might synchronize different stress-avoiding processes during desiccation

Chloroplasts are particularly sensitive to oxidative damage during the dehydration phase [11,34]. In addition to switching on physiological stress-avoiding mechanisms, angiosperm desiccation-tolerant plants employ physical means to prevent free-radical formation. As we discussed previously chlorophyll is degraded and thylakoid membranes are dismantled during drying in poikilochlorophyllous desiccation-tolerant plants [120]. There are indications that the degradation of vast amounts of chloroplast membranes provides carbon for the dehydration-specific protection processes in this group of desiccation-tolerant plants through the re-activated peroxisomal glyoxylate cycle (O. Toldi, et al., unpublished results). Homoiochlorophyllous species retain chlorophyll [10], but use various mechanisms to prevent light-chlorophyll interaction while plants are dry [121]. However, the active photosynthesis [122,123] and the high concentration of chlorophyll during desiccation is a source for the production of potentially harmful reactive oxygen species [34]. As the complete recovery of photosynthetic ability has central impact on the resurrection from the dehydrated state, effective protection of the
thylakoid membranes and important photosynthesis-associated enzymes are indispensable in homoioclorophyllous desiccation-tolerant plants. To counteract the toxicity of reactive oxygen, a multicomponent antioxidative defense system, including both non-enzymic and enzymic constituents, are present in plant cells. There is one abundant representative of this defense system, the tripeptide thiol glutathione, which is involved in the oxidative stress responses in nearly all desiccation-tolerant plants where it was examined. Transcriptome [20] and proteome level examinations [124] revealed that genes encoding enzymes involved in glutathione metabolism are among the genes most sensitive to dehydration. As a result, the dehydration process could be characterized by significantly increased glutathione levels in the desiccation-tolerant *Myrothamnus flabellifolia* [34] and *Boea hygrometrica* [124]. Desiccation of *S. stapfianus* results in increased glutathione reductase activity [125], reaching a maximum midway during rehydration in *Craterostigma wilmsii* [21]. The total glutathione content does not vary, but the glutathione status, i.e. the proportion of its reduced (GSH) and oxidized (GSSG) forms, does in three species of desiccation-tolerant lichens as representatives of fully desiccation-tolerant lower plants [126]. Glutathione, as an important redox buffer, that protects many cellular components [127] and the thiol status of proteins against oxidative stress [128]. Glutathione can also detoxify hydrogen peroxide by participating in the ascorbate/glutathione cycle or in the reaction catalyzed by glutathione peroxidase [129]. Hydrogen peroxide is especially toxic in the chloroplast, because even at low concentrations it inhibits the Calvin-cycle enzymes possessing exposed sulphhydryl groups such as NADP⁺-dependent glyceraldehyde-3-phosphate dehydrogenase and the plastidic form of fructose-1,6-bisphosphatase, hence reducing CO₂ assimilation [130]. This might provide an explanation why glutathione is so important in the recovery of photosynthesis during the rehydration phase. Although its specific role in vegetative desiccation has not yet been established, it appears more than coincidental that glutathione and genes encoding enzymes of its metabolism are so frequently implicated by molecular and biochemical analyses. Exogenously applied glutathione suppresses tissue necrosis and hyperhydricity in two out of three successfully tissue cultured and transformed desiccation-tolerant plants (Fig. 3) [131–133]. We suppose that glutathione not only acts as an antioxidant during the subsequent dehydration–rehydration cycles, but it also can synchronize different recovery processes, just as abscisic acid integrates protection processes during the dehydration phase. Let us consider the arguments for it. Numerous physiological functions are attributed to glutathione in plants [134]. Glutathione is synthesized through two sequential ATP-dependent reactions that yield γ-glutamylcysteine from L-glutamate and L-cysteine, followed by the formation of glutathione by addition of glycine to the C-terminal end of γ-glutamylcysteine [135]. Besides being an indicator of oxidative stress [136], glutathione is the exclusive storage and transport-form of the reduced sulphur regulating...
interorgan sulphur allocation [137]. Glutathione has also been shown to act as a regulator of gene expression [138], is the precursor of phytochelatins, which bind heavy metals [139], and is a substrate for the glutathione S-transferases, which catalyze the conjugation of glutathione with potentially dangerous xenobiotics such as herbicides [140]. How can the aforementioned functional abilities help a plant to survive desiccation? First, glutathione synthesis depends on the adenylate status of the cells (proportion of ATP versus ADP plus AMP), therefore it is likely that glutathione levels serve to sense energy provision. Second, glutathione is also an indicator of carbon and nitrogen metabolism, because glutamate availability results in kinetic limitations to its synthesis [141]. The third parameter which is mirrored in the actual level of glutathione is the efficiency of photosynthesis, because glutathione synthesis requires photosynthetic glycolytic. This means that glutathione levels can be upregulated when the photosynthetic activity is high [135]. Photosynthesis results from the oxygenase reaction catalyzed by ribulose-1,5-bisphosphate carboxylase/oxygenase [142]. During this metabolic process, CO₂ and NH₃ are produced and ATP and reducing equivalents are consumed, thus making photosynthesis a wasteful process. However, precisely because of this inefficiency, photosynthesis could serve as an energy sink preventing the over-reduction of the photosynthetic electron transport chain and photo inhibition, especially under stress conditions that lead to reduced rates of photosynthetic CO₂ assimilation [142]. Fourth, glutathione has a capacity to neutralize not only reactive oxygen species, but also other metabolic byproducts that might accumulate during dehydration that otherwise could result in tissue toxicosis upon rehydration.

Taken together, although most of the above arguments are just speculations, glutathione has all the potential to be involved in the synchronization of repair processes allowing recovery from desiccation in tolerant plants. Through the actual levels of glutathione the nutritional and energy status of the cells can be sensed together with the efficiency of CO₂ fixation. Besides the sensory role, glutathione participates in detoxification processes directly, preventing the accumulation of toxic metabolic byproducts associated with the dehydration process such as, for example, PUFA hydroperoxides. Our belief is that engineering glutathione metabolism could lead to the creation of crop plants that are more tolerant to dehydration–rehydration cycles.

6. Genetic transformation of desiccation-tolerant plants

Databanks only provide information about putative function of genes on the basis of sequence similarities. *In vivo*-proofs must be obtained after examining transgenic plants possessing upregulated or downregulated expression of the genes associated with desiccation tolerance [143]. For this purpose, *in vitro* plant regeneration protocols had been established in resurrection plants such as *C. plantagineum* [131,144], *R. myconi* [132], *H. rhodopensis* [145] and *Lindernia brevidens* [94] of which *Craterostigma*, *Ramonda* and *Lindernia* have been successfully transformed [94,131,133,144].

Vegetative desiccation-tolerant plants possess an extreme sensitivity to tissue culture-level manipulations during the establishment of their plant regeneration and genetic transformation systems. These tissues all displayed frequent evidence of necrosis, development of hyperhydrated leaves and secretion of polyphenols into culture media under suboptimal conditions. Therefore, so-called ‘low stress’ transformation technologies were established for nearly all resurrection plants [146] involving the development of non-lethal selection strategies and the application of modified tissue culture media supplemented with decreased amounts of macro- and microelements, and different antioxidant agents.

An additional increase in the transformation frequency was obtained when the colonization of the wounded surfaces by the transforming *Agrobacterium* cells was enhanced by biochemical pre-induction of the *vir* genes. This included the application of low pH liquid medium consisting of acetosyringone, aldose-type sugar source and organic nitrogen during the infection phase. These modifications together with the appropriate tissue culture system resulted in reliable methods for genetic transformation of resurrection plants. This knowledge is now ready to be extended over other recalcitrant species.

7. Conclusion and future aspects

Genes with potential role in triggering desiccation tolerance can be identified by transcriptome and proteome analysis, and metabolic processes that are involved in the functioning stress responses can be encompassed by metabolic profiling in an ideal world. We propose that performing such complete analyses of resurrection plants both technically and intellectually is still a complicated task. However, partial profiling of the transcriptome and proteome has already resulted in a provisional hierarchy among stress avoiding mechanisms and thus elucidating candidate genes for molecular breeding programmes (Table 1).

Although sucrose plays an indispensable role in establishing vegetative desiccation tolerance, manipulation of its metabolism in the view of engineering crops for drought tolerance does not seem to be a viable approach. Both upregulation and down-regulation of its synthesis could mean a serious threat to the fine balance in osmotic adjustment, energy metabolism, growth and development as has been demonstrated. Enhancing trehalose synthesis in transgenic plants does not seem to interfere with the primary metabolism as does sucrose, but with jasmonic acid/ethylene-signaling. This can result in false signals that can perturb plant responses in general.

In contrast to sucrose and trehalose, there are other components of the photosynthetic carbohydrate metabolism with a

<table>
<thead>
<tr>
<th>Protection agent</th>
<th>Metabolism</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
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<tbody>
<tr>
<td>Sucrose</td>
<td>Carbohydrate</td>
<td>Carbon and energy source, osmoprotectant</td>
<td>Possible interference with growth and osmoregulation</td>
</tr>
<tr>
<td>Trehalose</td>
<td>Carbohydrate</td>
<td>Osmoprotectant, signal metabolite</td>
<td>Possible interference with sugar sensing and photosynthetic performance</td>
</tr>
<tr>
<td>RFOs</td>
<td>Carbohydrate</td>
<td>Osmoprotectant, antistress agent, metabolically inactive, synergistic effects</td>
<td>None</td>
</tr>
<tr>
<td>LEAs</td>
<td>Protein</td>
<td>Hydration buffer, protein and membrane protectant</td>
<td>The most specific and most determinative LEAs are still missing</td>
</tr>
<tr>
<td>Glutathione</td>
<td>Tripeptide</td>
<td>Antioxidant, integrated sensor, signal metabolite, synergistic effects</td>
<td>None</td>
</tr>
<tr>
<td>ABA</td>
<td>Mevalonate</td>
<td>Growth regulator, signal metabolite</td>
<td>Possible interference with plant development and growth, contrasting effects</td>
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greater agronomic potential. Large quantities of raffinose family oligosaccharides accumulate without affecting primary metabolic processes and signaling cascades. Over-expression of genes in crop plants with determinative role in the metabolism of raffinose family oligosaccharides seems a good choice for future practical applications. For example, a dehydration-specific aldose reductase implicated in sorbitol synthesis [147] and galactenol synthase (XvGoLs), catalysing the first step in the biosynthesis of raffinose family oligosaccharides [3] could be among the first desiccation associated genes with potential to be applied in molecular breeding programmes.

LEA genes, which are believed to play a crucial role in providing tolerance to different abiotic stressors, have already been quite extensively utilized in molecular breeding programmes. However, their practical contribution has not been tested under real field situations. Further studies are necessary to identify the most effective types of LEA genes. In the meantime, the utilization of their promoter regions opens the possibility to drive the expression of different antistress genes stress-specifically.

Glutathione has primarily been evaluated as a potent detoxifying agent. We are certain that glutathione is much more than that. It is potentially involved in the synchronization of recovery processes allowing resurrection from the desiccation state in tolerant plants. Besides being a sensor and antioxidant, glutathione pleiotropically influences signaling processes similarly to abscisic acid. Its different functions can act synergistically while the different functions of abscisic acid have both negative and positive impact on the productivity. On this basis, our belief is that stress-specific engineering glutathione metabolism could lead to the creation of crop plants that are more tolerant to dehydration-rehydration cycles.

Now there are signaling and metabolic processes to modify and there are genes to achieve these modifications. The question is how, because the weakest point within the whole high-throughput technology is the limited capacity of conventional genetic transformation techniques. Therefore, the development of alternative in planta transformation appears promising, at least in the case of some model plants [148] and is currently available for both monocotyledonous and dicotyledonous plants. The advantage of this technique is that the time consuming and laborious in vitro tissue culture procedures can be replaced by immersing plants at a suitable developmental stage directly into Agrobacterium suspension cultures, which is followed by screening T1 generation for transgenic individuals. This method only works in a few species. Until in planta transformation techniques are available, the conventional ways of genetic transformation through in vitro tissue culture systems are still vital in terms of functional genetics [143]. However, stable transformants are not always needed for analysing gene function. Large-scale transient gene expression assays, based on inoculation of plants by viral vectors, can be also performed at both the protoplast and the whole plant levels [149,150]. This method could also mean a reasonable alternative to conventional transformation approaches and thus can accelerate cataloging genes associated with vegetative desiccation tolerance.

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