An Overview of the Current Understanding of Desiccation Tolerance in the Vegetative Tissues of Higher Plants

MONIQUE MORSE, MOHAMED S. RAFUDEEN AND JILL M. FARRANT

Department of Molecular and Cell Biology, University of Cape Town, Rondebosch, South Africa

I. Introduction ................................................................. 320
II. Global Stresses Caused by Desiccation and Associated Protective Mechanisms .............................. 321
A. Mechanical Stress and Mechanisms used to Minimize such Damage .................................. 321
B. Metabolic Stresses and Associated Protection Mechanisms ........................................ 324
III. Proteomics ........................................................................ 330
A. Whole Proteomic Approaches ...................................................................... 331
B. Subcellular Proteomics Approaches ............................................................................ 334
IV. Metabolomics ........................................................................... 337
V. Concluding remarks ................................................................................. 337
References ................................................................................................. 338

ABSTRACT

In this chapter, we review the current understanding of desiccation tolerance in the vegetative tissues of resurrection plants. We present an overview of the stresses associated with desiccation and the physiological and biochemical protection reported to result in amelioration of these stresses and discuss the contribution of the genomics era in furthering our understanding of these protection systems in the

1Corresponding author: E-mail: jill.farrant@uct.ac.za
attainment of desiccation tolerance. We discuss the advances made in proteomics and give a brief overview of recent contributions in the field of metabolomics that have contributed to the understanding of desiccation tolerance.

I. INTRODUCTION

The phenomenon of desiccation tolerance (DT) is found throughout the microbial, fungal, animal and plant kingdoms (Alpert, 2006; Farrant, 2007; Ricci and Caprioli, 2005) and is the ability of an organism to survive the loss of most (> 95%) of its cellular water for extended periods and to recover full metabolic competence upon rehydration (Farrant et al., 2007). The commonly held definition of DT is the ability to survive drying to the air-dry state at relative humidities ≤ 65%, this usually bringing the absolute water content of the tissue to or below 0.1 g H₂O g⁻¹ dry mass (g g⁻¹) and corresponding to a water potential of ≤ −100 MPa (Vertucci and Farrant, 1995; Walters et al., 2005). In the plant kingdom, it is relatively common in reproductive tissues such as spores, seeds and pollen (Berjak et al., 2007) and in vegetative tissues of non-tracheophytes, such as bryophytes and lichens (Kappen and Valladares, 1999; Oliver et al., 2000). However, DT in vegetative tissue is rare in pteridophytes and angiosperms and non-existent in extant gymnosperms (Alpert and Oliver, 2002; Farrant, 2007; Gaff, 1989). The mechanisms of vegetative DT differ between the lower and higher orders. In the former, desiccation occurs very rapidly, and protection prior to drying is minimal and constitutive. Survival is thought to be based largely on rehydration-induced repair processes (Alpert and Oliver, 2002; Oliver et al., 1998). In angiosperms, while some repair is probably inevitable, considerable and complex protection mechanisms are laid down during drying (Bartels, 2005; Blomstedt et al., 2010; Farrant, 2007; Gaff, 1989; Moore et al., 2009), and increasingly, it is becoming evident that many of the protection systems instituted in vegetative tissues of these plants, commonly called ‘resurrection plants’, are similar to those described for DT (orthodox) seeds (Illing et al., 2005; Leprince and Buitink, 2010). While the acquisition of DT in seeds is part of a maturation programme in seed development, it is possible that resurrection plants enlist expression of these genes within vegetative tissues to survive desiccation. Understanding of the regulatory processes of how this is achieved in vegetative tissues of resurrection plants may well ultimately enable induction of an appropriate selection of these genes in crops for drought tolerance.

In this chapter, we will review the current understanding of vegetative DT in resurrection plants, and where appropriate draw parallels with DT described in orthodox seeds. In the first part of this work, we will present an
overview of the stresses associated with desiccation and the physiological and biochemical protection reported to date to result in some amelioration of these stresses. We will also discuss the contribution of the genomics era in furthering our understanding of these protection systems and its role in discovery of other putative protectants and the mechanisms of control of these in the acquisition of DT. The second part of this work will focus on the post-genomic era, specifically the advances made in proteomics towards understanding DT in the vegetative tissues of higher plants. Finally, we will conclude with the recent advances in metabolomics studies in resurrection plants to date.

II. GLOBAL STRESSES CAUSED BY DESICCATION AND ASSOCIATED PROTECTIVE MECHANISMS

In plant tissues, the role of water is complex and varied. It fills intra- and intercellular spaces providing turgor pressure and structural support, termed mechanical stabilization (Iljin, 1957; Levitt, 1980). It is involved in metabolism as both a reactant and a product of many processes, and it is the medium in which the intracellular milieu is suspended. By providing hydrophobic and hydrophilic interactions, it determines conformation of macromolecules and membranes and controls and maintains intracellular distances between them (Buitink et al., 2002; Hoekstra et al., 2001; Vertucci and Farrant, 1995; Walters et al., 2002). Even slight water loss can cause disruption of the mechanical and metabolic stability, and resurrection plants appear to have unique mechanisms to minimize such disruption in the face of near total water loss. While there are some obvious differences among the various angiosperm resurrection plants in their mechanisms of protection against such stresses (reviewed below and also in Farrant, 2007; Vicré et al., 2003), there are considerable similarities in putative protection mechanisms among them and, as indicated above, orthodox seeds.

A. MECHANICAL STRESS AND MECHANISMS USED TO MINIMIZE SUCH DAMAGE

During desiccation, cells lose most of the protoplasmic ‘free or bulk’ water, and only the ‘bound water’—water associated with the cell matrix—is available for cell survival. This loss of water causes mechanical stress as decreased cell volume places tension on the plasmalemma as it shrinks from plasmadesmatal attachments to the cell wall, the ultimate rupture of which allows entry of extracellular hydrolases and cell death. In many species, wall
collapse occurs which is equally lethal (Walters et al., 2002). Angiosperm resurrection plants are able to survive these changes by active induction of protection mechanisms that allow avoidance of plasmalemma rupture and wall collapse. This is achieved by active and reversible wall folding and/or replacement of water in the vacuoles with compatible solutes (reviewed in Farrant, 2007).

Desiccation-induced cell wall folding is essential for structural preservation of tissue (Webb and Arnott, 1982), and the extent and manner of folding is species-specific and dependent upon the chemical composition and molecular architecture of the cell wall. While the overall wall composition of the resurrection species is similar to other related desiccation-sensitive species, the resurrection species appear to utilize inherent wall characteristics, with only slight modifications during drying, to achieve stable and reversible conformational changes (Farrant et al., 2007). In Craterostigma wilmsii, where wall folding is almost exclusively used as a form of mechanical stabilization, the mechanism of folding appears to involve more complex structural and biochemical changes (Vicré et al., 1999, 2004b). On drying, there is a reduction in glucose and an increase in galactose substitutions to the xyloglucans (XG) and it has been proposed that cleavage, or partial cleavage of the long-chained XG units into shorter, more flexible ones, allows for wall folding. During the final stages of drying, an increase in wall-associated Ca$^{2+}$ occurs, and as this ion plays an important role in cross-linking wall polymers, such as acid pectins, it has been proposed that this serves to stabilize walls in the dry state and, more importantly, prevent mechanical stress of rehydration. C. wilmsii is a small plant, and rehydration is rapid and is initially mainly apoplastic (Sherwin and Farrant, 1996). If walls hydrate and unfold before cell volume is regained, plasmalemma tearing and further subcellular damage could occur (reviewed in Vicré et al., 2003, 2004a). In species where wall folding is accompanied by vacuole filling as a mechanism of mechanical stabilization, such as in Myrothamnus flabellifolia and Eragrostis nindensis, there are no notable biochemical changes on drying, but these species have constitutively high proportions of arabinose, associated with pectins in the former (Moore et al., 2006) and xyloglucans in the latter (Plancot et al., 2009). Interestingly, the desiccation-sensitive Eragrostis tef, while having similar chemical wall constituents as E. nindensis, has significantly lower arabinose content. As arabinose polymers are highly mobile and allow wall flexibility (Foster et al., 1996; Renard and Jarvis, 1999) and have a high water absorbing capacity (Belton, 1997; Goldberg et al., 1989) which would be important for rehydration, we have proposed that such constitutively high levels allow constant preparedness for dehydration/rehydration in these resurrection plants (Moore et al., 2009).
In addition to these biochemical and ultrastructural studies, transcriptomic studies on at least three species of resurrection plant have reported on genes potentially involved in wall changes upon desiccation and recovery. Genes encoding glycine-rich proteins (GRP) have been shown to be upregulated upon drying in *Sporobolus stapfianus* (Neale et al., 2000) and *Boea hygrometrica* (Wang et al., 2008). These include a GRP and proline-rich protein (SDG137c) and a small GRP (SDG137c) in the former species, while a GRP (BhGRP1) was upregulated in the latter species. A BhGRP1-GFP fusion protein was located to cell walls of *B. hygrometrica* and the desiccation-sensitive control species *Nicotiana benthamiana* (Wang et al., 2008). While the function of these genes was not elaborated upon by the authors of those papers, GRPs can facilitate structural flexibility in developing walls of most plants, and their synthesis is stimulated under stress conditions (Mousavi and Hotta, 2005). It is thus possible that upregulation of such proteins in resurrection plants enables wall folding upon dehydration and unfolding upon rehydration. Three α-expansin genes (*CplExp1/2/3*) have been implicated in desiccation-associated wall changes of *Craterostigma plantagineum* (Jones and McQueen-Mason, 2004). All three genes were upregulated upon drying, but upon rehydration of this species, *CplExp3* expression remained largely unchanged, while expression of *CplExp1* increased as the leaf regained full turgor (Jones and McQueen-Mason, 2004). Expansin proteins are proposed to increase wall elasticity and thus may facilitate wall folding observed to occur in *Craterostigma* spp. during desiccation (Vicré et al., 1999).

Replacement of water in vacuoles within dry tissues of resurrection plants was first suggested based on ultrastructural observations that vacuoles continued to take up a large proportion of the cytoplasmic space despite the fact that there was no longer bulk water available in tissues, the remaining water being purely structure associated (Farrant, 2000; Farrant et al., 2007; Moore et al., 2007a,b; van der Willigen et al., 2001). The content of vacuoles from desiccated leaves of *Eragrostis nindensis* was analyzed after non-aqueous extraction and was shown to contain proline, sucrose and protein in equal proportions (van der Willigen et al., 2004a,b). Vacuoles from both hydrated and dry leaves of *Myrothamnus flabellifolia* contain 3,4,5-tri-O-galloylquinic acid, but this chemical increases to entirely fill the vacuoles in dry leaves (Moore et al., 2005b, 2007a,b). Use of high-throughput GC–MS to analyse metabolome changes in *Mohria caffrorum* showed 10- and 12-fold increases in glycerol and monohexadecanoglycerol, respectively, during drying, and as these chemicals are believed to be cyto-toxic in large quantities (Fahy, 1986), we proposed that they accumulated in vacuoles within the dry leaves (Farrant et al., 2009). Studies using...
metabolomic protocols are currently underway in which vacuolar content of a variety of resurrection plants is being analyzed.

B. METABOLIC STRESSES AND ASSOCIATED PROTECTION MECHANISMS

Loss of water interferes with metabolic processes and, at the extreme level, results in loss of membrane structure and causes metabolic destabilization within the cell. There is increased aggregation of essential macromolecules which results in the disintegration of organelles due to increased macromolecule concentration (Hoekstra et al., 2001; Vertucci and Farrant, 1995). A commonly noted consequence of water deficit-related disruption of metabolic processes is the increasing formation of reactive oxygen species (ROS) (Berjak, 2006; Hendry, 1993; Kranner et al., 2006; Smirnoff, 1993; Walters et al., 2002). Overproduction of ROS causes damage to macromolecules and subcellular components by reacting with proteins and lipids.

In all plants, ROS form as a natural consequence of metabolic processes involving electron transport (Apel and Hirt, 2004; Bailly, 2004; Halliwell and Gutteridge, 1999). Thus, mitochondria and chloroplasts are major sites of ROS production. Photosynthesis, in particular, is very sensitive to water deficit. Electron leakage during photosynthetic electron transport and the formation of singlet oxygen are significantly increased when cells of photosynthetic tissues suffer water loss, and this has frequently been cited as a primary cause of damage and resultant plant death in most species (Kranner and Birtić, 2005; Seel et al., 1992a,b; Smirnoff, 1993). Under mild deficit stress, ROS are effectively quenched by what are termed ‘classical’ (Kranner and Birtić, 2005) or ‘housekeeping’ antioxidants (Illing et al., 2005), so called because they are present in all plants and are crucial to maintenance of cellular homeostasis under day-to-day conditions and in protection against a myriad of abiotic and biotic stresses (for an overview, see Elstner and Osswald, 1994). However, with more severe water loss, such antioxidants are themselves compromised, and ROS damage is exacerbated (Farrant et al., 2007; Kranner et al., 2006). Even in the desiccated state when metabolic activity has ceased, ROS can still be generated through auto-oxidation processes, for example, of lipids, and damage to cellular macromolecules continues to occur as a result of Maillard reactions (Bailly, 2004; Wettlaufer and Leopold, 1991). Resurrection plants appear to protect against the potential damage of desiccation-induced ROS production by: (1) minimizing the formation of photosynthesis-associated ROS; (2) more effective use of housekeeping antioxidants and (3) production of novel antioxidants in response to drying. These will be reviewed below.
1. Regulation of photosynthesis-associated ROS production

The extent of ROS production is effectively minimized by downregulation of photosynthesis relatively early in the dehydration time course, usually between 80% and 65% relative water content (RWC) depending on the species (Farrant, 2000; Farrant et al., 2003; Illing et al., 2005; Sherwin and Farrant, 1998; Tuba et al., 1998; van der Willigen, et al., 2001). Downregulation of photosynthesis is achieved by one of two mechanisms: termed poikilochlorophyll and homoiochlorophyll (Farrant, 2000; Gaff, 1989; Sherwin and Farrant, 1998; Smirnoff, 1993; Tuba et al., 1998).

Poikilochlorophyllous types, many of which are monocots, break down chlorophyll and dismantle thylakoid membranes during dehydration (Farrant, 2000; Hambler, 1961; Tuba et al., 1993a,b, 1998). Breakdown of photosystem II (PSII), which is responsible for the water-splitting, oxygen evolving and thus oxidizing reactions of photosynthesis, is a highly effective strategy to minimize damaging levels of ROS formation, and indeed it has been shown that poikilochlorophyllous species are able to retain viability in the dry state for far longer than homoiochlorophyllous ones (Proctor and Tuba, 2002; Tuba et al., 1998). However, as the photosynthetic apparatus has to be reassembled on rehydration, recovery time is generally longer in these species (Farrant et al., 2003; Sherwin and Farrant, 1996). Molecular studies have shown that partial recovery of photosystem II function is independent of transcription and that some transcripts such as PsbA and PsbD are stably stored in the dry state (Collett et al., 2003; Dace et al., 1998), while others are transcribed de novo on rehydration with water being the primary cue for induction of transcription although presence of light is ultimately essential for synthesis of chlorophyll-binding proteins and assembly of the grana (Ingle et al., 2008).

Homoiochlorophyllous species retain most of their chlorophyll (the amount retained depending on the light levels under which the plants are dried) and thylakoid membranes in the dry state but use various mechanisms to prevent the light-chlorophyll interactions that might cause ROS production during drying and rehydration (Farrant, 2000; Farrant et al., 2003, 2009; Sherwin and Farrant, 1998). This is achieved by leaf folding and shading of inner leaves (e.g. the Craterostigma spp.) or adaxial surfaces (e.g. M. flabellifolius, M. caffrorum) from light. Surfaces that remain exposed to light have reflective hairs and/or waxes, and there is accumulation of anthocyanin and xanthophylls pigments and polyphenols all of which act as ‘screens’ reflecting back photosynthetically active light, masking chlorophyll and acting as antioxidants (Farrant, 2000; Farrant et al., 2003, 2009; Georgieva et al., 2007, 2009; Moore et al., 2007a,b; Sherwin and Farrant, 1998; Smirnoff, 1993).
2. Antioxidant systems
As outlined above, resurrection plants use housekeeping antioxidants to minimize ROS damage during water loss but appear to have the additional capacity to maintain function of their such antioxidant capacity in the desiccated state or quickly resynthesize antioxidants upon rehydration (Bresler, 2010; Farrant et al., 2007; Kranner and Birtić, 2005; Pukacka and Ratajczak, 2007). They also use ‘novel’ antioxidants in that they are newly described as having antioxidant properties and/or have not to date been associated with vegetative tissues but reported to occur only in seeds (Moore et al., 2005a,b; Mowla et al., 2002; Mulako et al., 2008).

Housekeeping antioxidants are essential for maintenance of redox homeostasis by scavenging excess ROS under normal and mildly stressful conditions. These include the water-soluble glutathione (g-glutamyl-cysteinyl glycine; GSH) and ascorbic acid (Asc) (Noctor and Foyer, 1998), the lipid soluble tocopherols and $\beta$-carotene (Munne-Bosch and Alegre, 2002) and enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (AP) and other peroxidases, mono- and dehydroascorbate reductases and glutathione reductase (GR). There have been numerous reports on the activities of such antioxidants during drying and recovery in various resurrection plants. From this literature, there appears to be considerable variation between DT species with respect to the extent of upregulation of the various housekeeping antioxidants, and the water contents during a dehydration/rehydration time course that the observed changes occur (e.g., reviewed in Farrant, 2000; Farrant et al., 2003; Illing et al., 2005). It is difficult to know whether this variation is real, as reports in the literature are controversial. The conditions under which plants are dried vary, frequently the water content to which the tissues are dried is not presented and/or the activity on rehydration is not recorded, or the manner of quantification differs. Further, use of antioxidant concentrations alone has limitations, as they often show a Gaussian response to stress (Kranner et al., 2006) making interpretation of a single measurement ambiguous. However, what appears to be a distinguishing feature of the functioning of these antioxidants in resurrection plants is the ability to maintain their antioxidant potential in the dry state such that the same antioxidants can be utilized during the early stages of rehydration thus protecting against the ROS stress associated with reconstitution of full metabolism (reviewed in Farrant, 2007; Moore et al., 2009). Farrant et al. (2007) have shown that the enzymes AP, GR, CAT and SOD retain the ability (in vitro assays) to detoxify ROS even at RWC of $<10\%$, suggesting that there is some protection of these proteins that prevents their denaturation and maintains the native state in dry conditions. This was not the case in DS species and it
has been proposed that this ability in resurrection plants is a unique DT mechanism (Farrant et al., 2007; Illing et al., 2005).

The Smirnoff–Wheeler pathway of ascorbate synthesis in plants has recently been established by the discovery of VTC2 as being the gene responsible for the first committed step to ASC synthesis (Linster and Clarke, 2008; Linster et al., 2007). Bresler (2010) has shown that transcription of VTC2 in the resurrection plant Xerophyta viscosa is upregulated when the plants are dried below 60% RWC, and that mRNA levels remain high in the desiccated plant and during early stages of rehydration. Ascorbate levels in roots and leaves of this plant follow the same trend (Kamies et al., 2010), and we propose that elevated ascorbate levels are maintained during drying and early rehydration by both a combination of de novo synthesis and regeneration of ascorbate by AP, which itself retains the ability to remain active (Farrant et al., 2007; Kamies et al., 2010). This compares well with data by Suarez-Rodriguez et al. (2010) who show the AP transcript to be more abundant in the desiccated leaves of C. plantagineum with increasing levels in rehydrated leaves.

In addition to the protection afforded by housekeeping antioxidants, resurrection plants have the ability to induce, de novo, antioxidants such as 1- and 2-cys-peroxiredoxins, glyoxylase I family proteins, zinc metallothioneine, metallothionine-like antioxidants and several members of the aldehyde dehydrogenases in response to desiccation (Blomstedt et al., 1998; Chen et al., 2002; Collett et al., 2004; Farrant et al., 2007; Illing et al., 2005; Kirch et al., 2001a,b; Mowla et al., 2002; Mulako et al., 2008; Velasco et al., 1994). While these have been reported to be important in the acquisition of DT of orthodox seeds, they are never found to be upregulated in the DS vegetative tissues of such plants (Aalen, 1999; Stacy et al., 1999). Increasingly, transcriptome and proteome studies are reporting the upregulation of genes and proteins annotated as potential antioxidants in response to drying in resurrection plants (Collett et al., 2004; Deng et al., 2006; Ingle et al., 2007; Jiang et al., 2007; Le et al., 2007; Suarez-Rodriguez et al., 2010).

Polyphenols are widely believed to have antioxidant potential (Kahkonen et al., 1999; Smirnoff, 1993; Wang et al., 1996) and may well play such a role in resurrection plants. In a relatively comprehensive study on polyphenols in M. flabellifolia, it has been shown that there is extensive accumulation (up to 50% of the leaf dry weight) of 3,4,5-tri-O-galloylquinic acid upon desiccation and that this compound acts as a potent antioxidant in vitro (Moore et al., 2005a,b). Although this polyphenol is predominantly located in the vacuole and cell wall, it has been proposed that these reservoirs act to absorb electrons from the cytoplasmically located antioxidants and act in a redox buffering capacity (Moore et al., 2007a,b). Support for the antioxidant role of polyphenols and phenolic antioxidant enzymes has been gained from
studies on *Ramonda serbica*, in which polyphenol content and activity of polyphenol oxidase (PPO) were shown to be enhanced on drying (Sgherri *et al.*, 2004; Veljovic-Jovanovic *et al.*, 2008).

3. *Stabilization of the subcellular milieu*

Upon water loss to 10% RWC, the hydrophobic effect of water that is essential for the maintenance of macromolecular and membrane structure is lost and irreversible subcellular denaturation occurs. Theories on mechanisms whereby this is achieved are thought to be due to the ability to substitute water with molecules that form hydrogen bonds that are able to stabilize macromolecular interactions in their native configuration (Crowe *et al.*, 1986, 1987, 1998). In addition to water replacement, stabilization of the subcellular *milieu* is thought to be brought about by vitrification of the cytoplasm by the same candidates achieving macromolecular stabilization (Hoekstra *et al.*, 2001; Leopold, 1986; Leopold *et al.*, 1994; Vertucci and Farrant, 1995; Walters, 1998). The candidates for such replacement/stabilization reactions are given as (a) sugars, particularly sucrose together with oligosaccharides (reviewed in Berjak, 2006; Illing *et al.*, 2005; Scott, 2000); (b) proteins, particularly late embryogenesis abundant (LEA) proteins (reviewed by Illing *et al.*, 2005; Mtwisha *et al.*, 2006) and (c) small heat shock proteins (Almoguera and Jordano, 1992; Mtwisha *et al.*, 2006; Wehmeyer *et al.*, 1996).

Physiological and biochemical studies have gone some way in showing the importance of sugars, and to some extent, LEA proteins in attainment of DT, but understanding of the role and contribution of proteins and metabolites is still in its infancy.

a. *Sugars.* In virtually all resurrection plants studied to date, there is accumulation of sucrose during drying (Bartels and Salamini, 2001; Bianchi *et al.*, 1991; Farrant, 2007; Ghasempour *et al.*, 1998; Illing *et al.*, 2005; Norwood *et al.*, 2000, 2003; Peters *et al.*, 2007; Whittaker *et al.*, 2001, 2004). Sucrose is also universally accumulated in orthodox seeds (Amuti and Pollard, 1977; Berjak, 2006; Koster and Leopold, 1988; Pammenter and Berjak, 1999; Vertucci and Farrant, 1995), suggesting that sucrose plays an important role in DT in general. In both systems, the accumulation of raffinose family oligosaccharides (RFOs), particularly raffinose and stachyose, also occurs (see e.g., Blackman *et al.*, 1992; Ghasempour *et al.*, 1998; Horbowicz and Obendorf, 1994; Koster and Leopold, 1988; Leprince *et al.*, 1990; Obendorf, 1997; Peters *et al.*, 2007), and it has been suggested that together with sucrose, they play an important role in formation of an intracellular glass phase (vitrification) in dry tissues. Vitrification is thought
to limit the damaging effects of ROS through the slowing down of chemical reaction rates and molecular diffusion in the cytoplasm and prevent damaging compaction of macromolecules and organelles (Berjak et al., 2007; Hoekstra, 2005; Vertucci and Farrant, 1995; Walters et al., 2002).

Transcriptomic studies have contributed towards identification of candidates that might contribute to sugar accumulation and their roles in stabilization of the cellular *milieu*. Sucrose and galactinol synthase transcripts were found to be upregulated during early dehydration in leaves of *C. plantagineum* (Suarez-Rodriguez et al., 2010), and Collett et al. (2004) have reported upregulation of cDNAs annotated as enzymes that synthesize osmoprotectants such as aldose reductase and galactinol synthase during desiccation of *Xerophyta humilis*.

**b. LEA proteins.** As the name suggests, LEA proteins were first identified due to their abundant (4% of total cellular protein, Roberts et al., 1993) accumulation during the late stages of seed development coincident with the onset of DT (Baker et al., 1995; Blackman et al., 1992, 1995; Galau et al., 1986; Manfre et al., 2006; Russouw et al., 1995 *inter alia*). They have been reported to occur in vegetative tissues in response to various abiotic stresses such as cold, drought, salt, osmotic stress (Bray, 1993; Ditzer et al., 2001) and recently desiccation stress (Collett et al., 2004; Ingle et al., 2007). A common feature of LEA proteins is that they are extremely hydrophilic and are soluble at high temperatures. They do not possess any apparent catalytic activity or structural domains, and most of them lack cysteine and tryptophan residues (Close, 1996). As they are largely unfolded in the hydrated state, it is experimentally difficult to assign structure and determine potential function. Thus far, predicted functions of LEA proteins include the unwinding or repair of DNA, forming cytoskeletal filaments to counteract the physical stresses imposed by desiccation, acting as molecular chaperones, stabilization of membrane (Wise and Tunnicliffe, 2004, 2007), maintenance of hydration shells of proteins (Bartels, 2005) to prevent protein aggregation (Chakrabortee et al., 2007; Goyal et al., 2005) and together with sugars facilitate subcellular vitrification (reviewed in Berjak et al., 2007).

Transcriptome studies on DT organisms have shown that LEA genes are among the most differentially expressed and highly upregulated genes in DT organisms (Leprince and Buitink, 2010). In resurrection plant studies, Le et al. (2007) reported an LEA transcript from a total of four cDNA clones (specifically expressed in *S. stapfianus* desiccation-tolerant leaf tissue) upregulated on drying in *S. stapfianus*, and Collett et al. (2004) reported 16 cDNA clones, from a total of 55 upregulated genes, that were annotated as LEA proteins. A study in which Group 4 LEA genes from the resurrection plant
B. hygrometrica were overexpressed in transgenic tobacco plants showed increased drought tolerance and an increase in peroxidase and SOD activity when compared to wild-type tobacco plants (Liu et al., 2009). The increased antioxidant activities were also associated with enhanced stability of photosynthesis-related proteins and membranes in the transgenic plants following drought stress (Liu et al., 2009). Overexpression of a dehydrin or group 3 LEA gene in tobacco and Arabidopsis resulted in an increase in production of osmolytes such as proline, polyamines and sugars and significant increases in growth rates under stress conditions (Figueras et al., 2004; Roychoudhury et al., 2007). These data suggest that the accumulation of LEA proteins can, in addition to the stabilization roles suggested above, have an indirect effect on the accumulation of other protective molecules, either altering osmotic adjustment or by the induction of signalling pathways.

III. PROTEOMICS

While a number of studies have investigated the transcriptome of resurrection plants (Blomstedt et al., 1998; Collett et al., 2004; Le et al., 2007) during dehydration and rehydration, there have been few studies corresponding to the proteome of resurrection plants. Transcriptome studies offer insight into gene expression profiles, but a major advantage of proteomics over transcriptomics is that it focuses on the actively translated portion of the genome. The importance of post-transcriptional regulation has been demonstrated by several studies revealing a weak or moderate correlation between mRNA and protein levels, except for very abundant proteins in yeast (Gygi et al., 1999; Ideker et al., 2001). In the case of resurrection plants, a further consideration is that many mRNAs appear to be stored during drying and only translated during rehydration (Collett et al., 2003; Dace et al., 1998), thus there may be significant differences between mRNA and protein levels during dehydration.

In contrast to transcriptomics, proteomics provides a more physiologically accurate snapshot of biochemical processes by revealing the actual protein constituents performing the enzymatic, regulatory and structural functions encoded by the genome and transcriptome at a given point in time. Further, proteomics approaches provide additional information on gene regulation, especially important when mRNAs may be present but not translated, or when changes in protein level occur without any detectable change in transcript abundance due to translational or other levels of control (Gygi et al., 1999).

To date, there have been only a few studies reported on proteomes of angiosperm resurrection plants, which are typically aimed at identifying protein changes in leaf tissues during dehydration to identify proteins that might
facilitate the acquisition of DT. All these studies have utilized the approach of 2DGE with protein identification by mass spectrometry. There have been no reports on the proteomes of root tissues in any resurrection plant.

A. WHOLE PROTEOMIC APPROACHES

Ingle et al. (2007) have reported changes in the proteome of leaves of *X. viscosa* when whole plants were subject to dehydration. Among the proteins that were upregulated or produced, *de novo* proteins were involved in antioxidant metabolism, PSII stabilizers, chaperonins and RNA-binding proteins, and downregulated proteins were predominantly those involved in photosynthesis. As this plant is poikilochlorophyllous, reduced expression of such proteins is expected. Table I shows proteins induced during dehydration stress and identified by mass spectrometry. The *de novo* proteins are those proteins present at 65% and/or 35% RWC which could not be detected in soluble protein extracts from fully hydrated plants (Ingle et al., 2007).

They found that marked changes in protein expression appeared to occur in two phases; the first occurring upon drying to 65% RWC and the second more extensive change occurring when leaves were dried to 35% RWC, and those authors have proposed an ‘early’ and ‘late’ stage of preparation for induction of protection. Interestingly, it is at these water contents that changes in transcription have been noted and where marked changes in physiological and biochemical parameters occur in *Xerophyta* species. For example, Northern blot analysis shows the *de novo* appearance of *inter alia* metallothionines I and II, ferredoxin III, an RNA-binding protein, galactinol synthase and oleosin (Collett et al., 2004) and 16 LEA-like proteins (Illing et al., 2005) at or just below 63% RWC. Shutdown of photosynthesis is initiated around this water content and there is increased activity in several antioxidant enzymes (Farrant et al., 2007; Illing et al., 2005). Below 35%, RWC mechanisms that putatively facilitate preservation of structural integrity, for example, accumulation of sucrose, are evident (Farrant et al., 2007). Proteins associated with drying and rehydration of detached leaves of the dicotyledonous homoiochlorophyllous resurrection plant *B. hygrometrica* have been identified by Jiang et al. (2007). While use of detached leaves might preclude observation of changes that are a consequence of root-derived signalling, such leaves recovered from desiccation and *in situ* stabilization obviously occur. The rapid drying associated with use of detached leaves possibly also precluded identification of late stage (ca. 35% RWC) changes in protein abundance reported by Ingle et al. (2008), as leaves were sampled at 79%, 6.7% and 2.4% RWC only. The authors report that of the differentially expressed proteins, 19% showed decreased abundance, 35%
<table>
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<th>Response of protein</th>
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<th>pI/MW</th>
<th>Accession number</th>
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<tr>
<td>Induced within 0.5 h</td>
<td>79.3</td>
<td>ABC Transporter ATPase (<em>Chlamydia trachomatis</em>)</td>
<td>5.2/62</td>
<td>NP_220204</td>
</tr>
<tr>
<td>Induced within 0.5 h</td>
<td>79.3</td>
<td>Glutathione peroxidase-like protein from barley</td>
<td>5.1/17</td>
<td>AJ238744</td>
</tr>
<tr>
<td>Induced within 0.5 h</td>
<td>79.3</td>
<td>Polyphenol oxidase precursor from tomato</td>
<td>5.6/57</td>
<td>gi</td>
</tr>
<tr>
<td>Induced at 8–48 h</td>
<td>6.7 and 2.4</td>
<td>Rubisco large subunit (<em>Pinguicula caerulea</em>)</td>
<td>5.3/20</td>
<td>P28440</td>
</tr>
<tr>
<td>Induced at 8–48 h</td>
<td>6.7 and 2.4</td>
<td>QHK7H18, oxygen-evolving complex of photosystem II from sunflower</td>
<td>6/24</td>
<td>CF079724</td>
</tr>
<tr>
<td>Induced at 8–48 h</td>
<td>6.7 and 2.4</td>
<td>Vacuolar H$^+$ATPase A subunit (<em>Citrus unshiu</em>)</td>
<td>5.6/70</td>
<td>AB036926</td>
</tr>
<tr>
<td>Induced at 8–48 h</td>
<td>6.7 and 2.4</td>
<td>Unknown protein (<em>Arabidopsis thaliana</em>)</td>
<td>6.1/44</td>
<td>NP_567979</td>
</tr>
<tr>
<td>Induced at 8–48 h</td>
<td>6.7 and 2.4</td>
<td>ATGSTU6 (glutathione S-transferase 24) (<em>A. thaliana</em>)</td>
<td>6/29</td>
<td>gi</td>
</tr>
<tr>
<td><strong>Xerophyta viscosa (whole)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decreased abundance</td>
<td>65 and 35</td>
<td>PSII stability factor HCF136 (<em>Oryza sativa</em>)</td>
<td>5.2/40</td>
<td>BAD62115</td>
</tr>
<tr>
<td>Decreased abundance</td>
<td>35</td>
<td>PsbO (<em>A. thaliana</em>)</td>
<td>5.6/30</td>
<td>CAA36675</td>
</tr>
<tr>
<td>Decreased abundance</td>
<td>35</td>
<td>PsbP (<em>Xerophyta humilis</em>)</td>
<td>7.0/26</td>
<td>AAN77240</td>
</tr>
<tr>
<td>Decreased abundance</td>
<td>35</td>
<td>Transketolase (<em>Solamun tuberosum</em>)</td>
<td>5.4/78</td>
<td>CAA90427</td>
</tr>
<tr>
<td>Decreased abundance</td>
<td>35</td>
<td>F-ATPase (α subunit) (<em>Ranunculus macranthus</em>)</td>
<td>5.2/59</td>
<td>AAZ03784</td>
</tr>
<tr>
<td>Decreased abundance</td>
<td>35</td>
<td>Glu:glyoxylate aminotransferase I (<em>A. thaliana</em>)</td>
<td>6.5/52</td>
<td>AAN62332</td>
</tr>
<tr>
<td>Decreased abundance</td>
<td>35</td>
<td>Ascorbate peroxidase (<em>A. thaliana</em>)</td>
<td>5.0/32</td>
<td>CAA66925</td>
</tr>
<tr>
<td>Increased abundance</td>
<td>65 and 35</td>
<td>Chloroplast FtsH protease (<em>A. thaliana</em>)</td>
<td>5.2/72</td>
<td>CAA68141</td>
</tr>
<tr>
<td>Increased abundance</td>
<td>65 and 35</td>
<td>GDP-mannose-3',5'-epimerase (<em>O. sativa</em>)</td>
<td>6.2/47</td>
<td>Q2R1V8</td>
</tr>
<tr>
<td>Increased abundance</td>
<td>65 and 35</td>
<td>Protein phosphatase type 2C (<em>A. thaliana</em>)</td>
<td>6.0/33</td>
<td>CAB79642</td>
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<tr>
<td>Increased abundance</td>
<td>35</td>
<td>Alcohol dehydrogenase (<em>Citrus × paradise</em>)</td>
<td>6.4/46</td>
<td>AAY86033</td>
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<td>Increased abundance</td>
<td>35</td>
<td>VDAC1.1 (<em>Lotus corniculatus</em>)</td>
<td>6.5/28</td>
<td>AAQ87019</td>
</tr>
<tr>
<td>Increased abundance</td>
<td>35</td>
<td>2-Cys peroxiredoxin (<em>O. sativa</em>)</td>
<td>5.0/26</td>
<td>CAJ01693</td>
</tr>
<tr>
<td><strong>De novo proteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65 and 35</td>
<td>14 dnaK-type molecular chaperone (<em>O. sativa</em>)</td>
<td>5.3/75</td>
<td>NP_001048274</td>
<td></td>
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<tr>
<td>65 and 35</td>
<td>Phosphopryvuate hydratase (<em>Zea mays</em>)</td>
<td>6.0/50</td>
<td>P26301</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>RNA-binding protein (<em>Daucus carota</em>)</td>
<td>6.3/76</td>
<td>AAK30205</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Desiccation-related protein (<em>Craterostigma plantagineum</em>)</td>
<td>5.0/33</td>
<td>AAA63616</td>
<td></td>
</tr>
</tbody>
</table>
were transiently induced during dehydration and 5% were upregulated during rehydration. Among the upregulated proteins identified, at least three are associated with antioxidant metabolism, two with photosynthesis and two with energy metabolism (Table I). The relatively small number of proteins that were upregulated specifically during the rehydration phase supports the hypothesis that most changes in the gene expression occur during the dehydration phase in desiccation tolerant higher plants (Phillips et al., 2002). Some of the proteins identified by Ingle et al. (2007) and Jiang et al. (2007) are discussed briefly below.

1. Photosynthesis-related genes

Ingle et al. (2007) showed that the abundance of five chloroplast proteins involved in photosynthesis was significantly decreased at 35% RWC: psbO and psbP, two components of the luminal oxygen-evolving complex (OEC) of PSII, the PSII stability factor HCF136, the α-subunit of the F-ATPase and the Calvin cycle enzyme transketolase (Table I). Of these, only HCF136 (a thylakoid luminal protein required for PSII stability and assembly) was also significantly lower at 65% RWC. The reduced levels of HCF136 protein in X. viscosa during drying could be one of the components regulating shutdown of photosynthesis possibly by reducing the rate of stable PSII formation. The decrease in psbO and psbP protein levels in X. viscosa during drying is most likely a consequence of the dismantling of thylakoids observed to occur during the late stages of drying in poikilochlorophyllous resurrection plants (Farrant, 2000; Farrant et al., 2003). Collett et al. (2003, 2004) observed that psbP and psbO mRNA levels also decline in the related species X. humilis at RWCs below 50%. This suggests that at least some of the observed decrease in protein levels is likely caused by downregulation of gene expression.

In contrast to the observations of photosynthesis-related proteins in X. viscosa (Ingle et al., 2007), one of the chloroplast-located proteins (identified as a precursor of the OEC of PSII) by Jiang et al. (2007) was found to accumulate in dehydrated and partially dehydrated B. hygrometrica tissue. This observation that OEC PSII accumulates in dehydrated tissue (Jiang et al., 2007) contrasts with the report by Collett et al. (2004, 2005) who reported that many of the genes related to photosynthesis (including the genes encoding for OEC fragments and PSII reaction centre proteins) were downregulated in X. humilis. While B. hygrometrica is homochlorophyllous and X. humilis is poikilochlorophyllous, this difference in expression patterns of the photosynthesis-related genes in these two resurrection plants may reflect the different approaches these plants employ to minimize the damage of excess light energy during dehydration, or it may imply that in some cases, the steady state mRNA levels do not accurately reflect the
changes observed at the protein level (highlighting the necessity of proteomic approaches; (Jiang et al., 2007).

2. Energy metabolism
Jiang et al. (2007) found two ATPases induced during dehydration. An ATP-binding cassette (ABC) transporter ATPase was induced during early dehydration (after 0.5 h at RWC of 79%), and protein levels increased when leaves were dried to < 7%. High expression levels were maintained during rehydration. The expression pattern observed suggests that the protein might play a role in providing energy for protective and repair reactions in both dehydration and rehydration phases. The second ATPase, a vacuolar H\(^+\)ATPase A subunit, was apparent only at low water contents, but as there was no measure of protein levels at intermediate water contents in this study, it could have been upregulated at an earlier stage. This protein could well be involved in regulating water replacement in vacuoles, so providing a mechanical stabilization role (Farrant, 2007). Similar genes have been identified in desiccated leaf tissue from S. stapfianus and X. viscosa (Ingle et al., 2007).

3. Detoxification and protection
All the studies identified proteins with potential for protection against oxidative damage during drying. These include AP (X. viscosa), glutathione peroxidase and glutathione S-transferase (B. hygrometrica). These data correlate with enhanced ascorbate and glutathione levels in dehydrated leaves of these and other resurrection plants (Farrant et al., 2007; Jiang et al., 2007; Kranner et al., 2006). An increase in PPO protein levels and enzyme activity was observed in drying leaf tissues of B. hygrometrica (Jiang et al., 2007). It has been proposed that PPO may play an indispensable role in chloroplast function with a possible involvement in a Mehler-like reaction detoxifying oxygen species (Sherman et al., 1995), while polyphenols possess ideal structural chemistry for free radical scavenging activities (Rice-Evans et al., 1997).

PPO levels were shown to be enhanced on drying in R. serbica (Sgherri, et al., 2004; Veljovic-Jovanovic et al., 2008), and the polyphenol 3,4,5-tri-O-galloylquinic acid is present in large quantities in desiccated leaves of M. flabellifolius (Moore et al., 2005a,b); these compounds having demonstrable antioxidant properties, possibly also acting in a redox buffering capacity.

B. SUBCELLULAR PROTEOMICS APPROACHES

Whole proteomics approaches such as undertaken by Ingle et al. (2007) and Jiang et al. (2007), while useful for an overall survey of changes occurring in a particular tissue, have the disadvantage of not being able to identify changes
associated with particular organelles or cytoplasmic domains. Studying the proteomes of such sub-compartments allows for more specific localization of proteins which can in turn be related to function with respect to DT. Further, the reduction in protein complexity and increased technical resolution (Dreger, 2003; Jiang et al., 2005; Jung et al., 2000; Pandey et al., 2008) allows for identification of low abundance proteins such as signal or regulatory proteins (Bae et al., 2003; Molloy et al., 1998; Pandey et al., 2006, 2008). To this end, we have provided the first detailed report on the upregulated proteins in the nucleus of a resurrection plant in response to dehydration stress at 35% RWC (Abdalla et al., 2010). Whole plants were dehydrated, and nuclei were extracted from leaf tissues. Eighteen proteins (Table II) were found to be significantly upregulated on drying to this water content of which four proteins involved in gene regulation and four associated with translation were identified. There were two proteins with molecular chaperone type activities and one with a role in energy metabolism and seven with no assigned function. Three of these were identified as having the same protein identity (Abdalla et al., 2010). This is not unusual, as in 2D electrophoresis, the same protein identity can be obtained for different spots due to protein degradation, protein isoforms, heterodimer formation or the proteins in question may belong to an extensive protein family (Colvis and Garland, 2002; Pandey et al., 2008). This drawback, together associated with working

### TABLE II

**Dehydration-Induced Nuclear Proteins from X. viscosa**

<table>
<thead>
<tr>
<th>Homologue/putative identification</th>
<th>Accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-LTR retro element reverse transcriptase</td>
<td>Q9LGM1</td>
</tr>
<tr>
<td>Novel</td>
<td>Not available</td>
</tr>
<tr>
<td>GAG-pol</td>
<td>Q93Y69</td>
</tr>
<tr>
<td>Orf100f protein</td>
<td>Q9ASH2</td>
</tr>
<tr>
<td>Novel</td>
<td>Not available</td>
</tr>
<tr>
<td>Novel</td>
<td>Not available</td>
</tr>
<tr>
<td>Novel</td>
<td>Not available</td>
</tr>
<tr>
<td>Unknown protein F2P9.19</td>
<td>Not available</td>
</tr>
<tr>
<td>Chaperonin</td>
<td>A96767</td>
</tr>
<tr>
<td>EF-Tu precursor</td>
<td>S21567</td>
</tr>
<tr>
<td>EF-Tu precursor</td>
<td>S21567</td>
</tr>
<tr>
<td>Chaperonin</td>
<td>E86388</td>
</tr>
<tr>
<td>ATP synthase α chain</td>
<td>ATP.ARATH</td>
</tr>
<tr>
<td>Intron maturase</td>
<td>Q9BAA0</td>
</tr>
<tr>
<td>Novel</td>
<td>Not available</td>
</tr>
<tr>
<td>Zinc-finger helicase</td>
<td>Q8LHZ4</td>
</tr>
<tr>
<td>Ribosomal protein L28</td>
<td>D84580</td>
</tr>
<tr>
<td>EF-Tu precursor</td>
<td>S21567</td>
</tr>
</tbody>
</table>
on non-model organisms for which the genome has not yet been sequenced, places limitations on correct identification of proteins and thus denies detailed interpretation of the role of many proteins in the acquisition of DT. However, given that the nucleus is an important regulator of many subcellular activities, including gene expression, manufacture and transport of regulatory factors and in stress response signalling (Fink et al., 2008; Komatsu and Tanaka, 2005; Moriguchi et al., 2005; Repetto et al., 2008), this chapter, albeit with giving only a small number of positively identified protein changes, at least confirms that the X. viscosa nucleus responds to dehydration stress and that DT is controlled by multiple genes within the plant nucleus.

While these studies on the proteome changes associated with drying of resurrection plants have confirmed the importance of protection mechanisms such as minimization of photosynthetically produced ROS and associated antioxidant systems and have given some insight to other potential pathways that might afford protection against extreme water deficit, they also have highlighted some of the problems associated with use of this technology in relation to the study of DT. An overarching one is the limitations of current databases with respect to identifying proteins from plant species that are phylogenetically distant from model plants. While this is true for all plants in which the genome has not yet been sequenced, it is possible that, as vegetative DT is such a rare phenomenon, that at least some of the genes and/or regulatory aspects of these genes are unique to resurrection plants and will never be identified, given the current status of the databases. It is important that the genome of at least one monocot and one dicot be sequenced. The 2D SDS-PAGE approach used in the studies discussed above has limitations, as it allows the detection and analysis of only a subset of relatively abundant and soluble proteins. To increase the number of proteins that can be analyzed, fractionation of the proteome into subcellular fractions could be employed (as done by Abdalla et al., 2010) or investigators could use the gel-free iTRAQ MS system. The iTRAQ MS system has the additional benefit that hydrophobic proteins lost during isoelectric focusing (IEF) can be studied (Suzuki et al., 2006). Finally, studies on the proteomes of roots in response to desiccation must be initiated.

The profiling of the protein constituents together with performing correlative enzymatic, metabolic and physiological assays at given RWCs is designed to complement transcriptome studies in resurrection plants (Jiang et al., 2007). The studies discussed above have reported the differential expression of proteins and the programmed regulation of protein expression in response to dehydration (Abdalla et al., 2010; Ingle et al., 2007; Jiang et al., 2007) and rehydration (Jiang et al., 2007) to be consistent with the accompanying findings at the structural and metabolic levels in resurrection plants studied to date.
IV. METABOLOMICS

Metabolic profiling, correlated with changes in transcriptome and proteome expression, is an important tool used in identifying the early compounds that signal the perception of stress (Shulaev et al., 2008). The approaches currently used in plant metabolomics research include metabolic fingerprinting, where only signatures associated with a stress are identified, metabolic profiling for identification of global metabolites associated with a sample, and targeted analysis used to determine the precise concentration of a limited number of known metabolites (Fiehn, 2002; Halket et al., 2005; Shulaev, 2006; Shulaev et al., 2008). As outlined above, biochemical and physiological studies have shown that resurrection plants produce a number of metabolites such as sugars, amino acids, water and lipid soluble antioxidants, anthocyanins and many small signalling molecules in response to dehydration stress (reviewed in Farrant, 2007; Moore et al., 2009). However, with the exception of a limited profiling study on the resurrection fern M. caffrorum (Farrant et al., 2009), there has as yet no bona fide metabolomic studies reported on angiosperm resurrection plants.

Bioinformatics tools have allowed for the in silico metabolic profiling of C. plantagineum (Suarez-Rodriguez et al., 2010). This study characterized the transcriptomes of C. plantagineum leaves at four stages of dehydration and rehydration using deep sequencing, and data was compared with previously reported transcript profiles of orthodox seeds and pollen as well as with transcript profiles of desiccation-sensitive plants. The comparisons indicated that vegetative DT may be the result of differential regulation of pre-existing, non-vegetative DT mechanisms, and that most water-stress related genes are shared by tolerant and non-tolerant species but that changes in their expression patterns ultimately provide tolerant plants with more effective protective mechanisms. Gene ontology enrichment identified metabolic pathways which may be important for DT. These included those involved in the biosynthesis of ubiquinone and other terpenoids; phenylpropanoid, geranylgeranyl diphosphate II, photosynthetic carbon fixation and methane metabolism (Suarez-Rodriguez et al., 2010).

V. CONCLUDING REMARKS

There is no doubt that a combination of transcriptomics, proteomics and metabolomics approaches will provide us greater insight into how plants respond to dehydration stress. There is a need to do such studies at the subcellular as well as the whole plant level to gain a comprehensive
understanding of DT. Insights gained from such a systems-biology approach will ultimately allow informed biotechnological approaches for the production of drought tolerant crops.

REFERENCES


