Adaptive Mechanisms of Desiccation Tolerance in Resurrection Plants

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Abstract

The present changing climate scenario suggests an increased aridity in many areas of the globe in coming years, so the research into plant responses to water stress has become increasingly important. On a global basis, drought, in addition to high temperature and radiation, poses the most important environmental constraint to plant survival and crop productivity.

Desiccation tolerance is not synonymous with drought tolerance. Desiccation tolerance is defined as the ability of a living structure to survive drying to equilibrium with low (<5%) RH and maintain low intracellular water concentrations (WCs), while drought tolerance is defined as survival at low environmental water availability while maintaining high internal water contents (WCs). Desiccation tolerance, which is one mechanism of drought tolerance, involves an integrated mechanism where morphological adaptations are complemented with physiological, biochemical and genetic tolerance. With the advent of genomic and proteomic tools, the knowledge of the adaptive strategies involved has been greatly improved.

Keywords

Water stress · Osmoregulation · Genomics · Proteomics · Stable isotope · Thermal imaging

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2.1 Introduction

The present changing climate scenario depicts an increased aridity in many areas of the globe (Petit et al. 1999), so the research into plant responses to water stress has become increasingly important. Globally, drought, in addition to high temperature and radiation, poses the most important environmental constraint to plant survival and crop productivity (Boyer 1982).

Desiccation tolerance is not synonymous with drought tolerance (Alpert 2005). Desiccation tolerance is defined as the ability of a living structure to survive drying to equilibrium with low (<5%) RH and maintain low intracellular water concentrations. Drought tolerance is defined as survival at low environmental water availability while maintaining high internal water contents. A drought-tolerant organism that is not desiccation tolerant will die if it loses much of its internal water, whereas a desiccation-tolerant organism will survive under similar circumstances. Thus, desiccation tolerance is one mechanism of drought tolerance.

Desiccation tolerance is a phenomenon of drying to equilibrium of an air dry state or less than 5% of cellular water content. This condition is lethal for all the living being including animals and plants. However, there are some species of animals and plants that can withstand the complete water loss. For example, among animals, desiccation tolerance is commonly found in three phyla, namely, nematodes (Wharton 2003), rotifers (Ricci 1998; Ricci and Carprioli 2005) and tardigrades (Wright et al. 1992; Wright 2001). Juvenile tolerance is also known in two more phyla: one is the encysted embryos of one crustacean genus (Clegg 2005) and the other is the larvae of the fly (Kikawada et al. 2005). However, among plants, bryophytes are best examples for desiccation tolerance (Proctor and Tuba 2002; Oliver et al. 2005), but in adult pteridophytes and angiosperms, this phenomenon is found rarely (Porembski and Barthlott 2000) but common in their spores, seeds and pollens (Dickie and Prichard 2002; Tweddle et al. 2003; Farmsworth 2004; Illing et al. 2005). Prokaryots and fungi are lesser known for desiccation tolerance, but many bacteria (Potts 1994; Guerrero et al. 1999; Billi and Potts 2002), terrestrial microalgae (Ong et al. 1992; Agrawal and Pal 2003) and lichens (Palmqvist 2000; Beckett et al. 2003; dela Kranner et al. 2003) and some yeast (Sales et al. 2000) tolerate desiccation as does at least one intertidal microalgae (Abe et al. 2001).

Vegetative organs can tolerate dryness to ca 4–13% of relative water content in desiccation-tolerant plants, while it's almost impossible for desiccation-sensitive plants to survive below 20–50% of water content. The reason might be the extra energy required for the protection and repair mechanisms, metabolic processes, biomass production and competitive abilities in desiccation-sensitive plants as compared to desiccation-tolerant plants.

Resurrection plants are known mostly for their poikilohydrous nature which means plants adjust their water content with the relative humidity in the environment. These plants remain alive in dehydrated state until water becomes available and resume all their physiological activities on rehydration. The leaves of resurrection plants shrink their volume by curling up inside or outside along the midrib or main axis. They can remain alive in a quiescent phase up to the air-dried state for months, and this state can

be compared with dormancy in seeds in several aspects. Resurrection plants revive their growth on rainfall and reproduce before other species can do so (Scott 2000). For example, seeds of sacred lotus (*Nelumbo nucifera*) germinated around 75% after 1300 years of storage (Shen-Miller et al. 2002). However, very few plants exhibit this ability of desiccation tolerance in some organs such as leaves, but desiccation-tolerant plants are capable of surviving under these circumstances.

Mosses and liverworts remain in quiescent state at air dryness and revived after 20–25 years, while mature angiosperms and pteridophytes revived after 5 years (Alpert and Oliver 2002). There were no significant DNA damage in cyanobacterium *Nostoc commune* even after 13 years of being dry, and its growth could be resumed after 55 years under herbarium storage (Shirkey et al. 2003).

There are around 330 species of resurrection plants, which include nine pteridophyte and ten angiosperm families (Proctor and Pence 2002). Resurrection plants have been found both in monocotyledonous and dicotyledonous families, but there is no report of desiccation-tolerant gymnosperms yet. The Scrophulariaceae and Myrothamnaceae families majorly contribute for dicot species, whereas the monocot plants are more represented by the families including the plants *Xerophyta viscosa*, *X. humilis*, *Sporobolus stapfianus* and *Eragrostis nindensis* (Ingram and Bartels 1996; Alpert and Oliver 2002; Moore et al. 2009; Cushman and Oliver 2011; Oliver et al. 2011a, b). Desiccation tolerance is supposed to be linked with the limitation of size, as available references of desiccation-tolerant flowering plants are limited to certain height (Bewley and Krochko 1982). The largest resurrection plant, which is known till date, is a small woody shrub *Myrothamnus flabellifolia* (Sherwin et al. 1998). Most often the known resurrection plants are herbaceous in nature.

The mechanisms of desiccation tolerance differ between the extant lower orders and the angiosperms. In the lower plants, the process of desiccation occurs very rapidly, and protection prior to drying seems to be minimal and constitutive. The strategy of survival is considered to be based mainly on rehydration-induced repair processes (Oliver et al. 1998; Alpert and Oliver 2002).

Although some damages are probably irreversible in angiosperm vegetative tissues, there appears to be some complex and considerable protection mechanisms during drying which are thought to minimise the need for extensive repair (Gaff 1989; Farrant 2000; Scott 2000; Alpert and Oliver 2002; Vicre et al. 2003, 2004; Bartels 2005; Illing et al. 2005; Farrant 2007). These mechanisms include accumulation of sucrose and other oligosaccharides (reviewed in Pammenter and Berjak 1999; Scott 2000; Farrant 2007), the production of late embryogenesis abundant (LEA) proteins (Russouw et al. 1995; Illing et al. 2005), the upregulation of "housekeeping" antioxidants and the appearance of novel antioxidants that are apparently unique to desiccation-tolerant organisms (Illing et al. 2005; Farrant 2007). All of these contribute to protecting the subcellular *milieu* (reviewed by Berjak 2006). The phytohormone abscisic acid (ABA) plays an important role in mediating the responses to environmental stresses such as desiccation, salt and low temperature (Leung and Giraudat 1998). Increased levels of ABA in response to desiccation have been reported in many physiological studies. The plant hormone ABA is supposed to accumulate on trigger by drought which ultimately recruits various

stress-associated genes that are thought to induce protective mechanisms. The characterisation of ABA-deficient and ABA-insensitive mutants from non-tolerant plants has contributed towards the understanding of the gene expression regulation in desiccation tolerance (Leung and Giraudat 1998; Bray 1997). These mutants have led to the isolation of genes involved in ABA synthesis and various components involved in desiccation signalling during stress conditions.

With the increasing problems of drought and growing population, water shortage is going to be a big issue for the upcoming future. A better understanding of plant's responses to drought will not only provide a better chance for improved management practices and breeding efforts in agriculture and but also be useful for assessing the immediate fate of natural vegetation (also crops) under climate change. In this chapter we have discussed various aspects involved in desiccation tolerance that enable resurrection plants to survive in the extreme environmental conditions.

2.2 Defintion of Water Stress and Desiccation Tolerance

Berjak (2006) emphasised that desiccation tolerance involves not only potential of extreme water loss from an organism but also to remain in this state for prolonged period. Drought tolerance is the survival under low water availability while maintaining a higher internal water content but not for prolonged period. Desiccation tolerance is the survival of organism under dehydration to an overall water content, equal to, or less than, 0.1 g (water) per gram dry mass (g g^{-1}). Anhydrobiosis is synonymous with desiccation tolerance in literature, but Berjak (2006) strictly used this term for complete water loss which is not the case of water content of around 0.1 g g^{-1}.

Desiccation tolerance is the result of a dynamic process and supposed to be mediated by several protective measures that ensure to prevent the plant system from lethal damage.

As discussed earlier, orthodox seeds are desiccation tolerant because this is the prerequisite of seeds to overcome the process of desiccation tolerance for completion of their life cycle. It is generally considered as an approach for seed survival and dissemination of a particular species.

Plants have developed two major strategies to cope with desiccation stress: stress avoidance and tolerance. Avoidance strategy involves the formation of seeds before drought conditions prevail; it is also achieved by specialised adaptations at morphological level that may be, for example, the development of specialised leaf surfaces to decrease the rate of transpiration, reduce leaf area, sunken stomata or increase root length and density to use water more efficiently. On the other hand, stress tolerance appears to be the result of synchronised execution of physiological and biochemical alterations at the cellular and molecular levels: i.e. the accumulation of various osmolytes and late embryogenesis abundant (LEA) proteins coupled with an efficient antioxidant system. Many of these mechanisms have been characterised. They have been found to exist both in desiccation-tolerant and non-tolerant plants.

2.3 Geographical Distribution and Nature of Desiccation Tolerance

Desiccation-tolerant vascular plants are geographically widely but unevenly distributed. Nonetheless there is an inclined richness of species from temperate regions towards the tropics, where most of the species occur. They are found abundantly in seasonally wet regions than constantly wet or arid regions in the tropics. Desiccation-tolerant angiosperms are mostly located in three areas of the southern hemisphere: Southern Africa, eastern South America and Western Australia (Gaff 1977, 1987; Porembski 2000). The plants from the families, Scrophulariaceae and Velloziaceae, mostly occupy the desiccation-tolerant vascular flora here, but Poaceae and Cyperaceae also have many species. The desiccation-tolerant Myrothamnaceae (comprising two species) is an endemic family present in Africa and Madagascar. There is characteristic vegetation on outcrops in this region: ferns and the genus *Streptocarpus* (Gesneriaceae) but less rich in desiccation-tolerant species. Remarkably, the latter family is represented by a few desiccation-tolerant lithophytes in Southern Europe (e.g. *Ramonda pyrenaica*). Among the ferns and fern allies, the genera *Selaginella*, *Asplenium* and *Pellaea* are dominant. On Australian inselbergs, the genus *Borya* (Boryaceae) is represented by a number of desiccation-tolerant species. Most occur in the western part of the continent. The Poaceae are also well-represented among Australian resurrection plants; *Micraira* is the most species-rich genus. It has been suggested that the Australian resurrection plants are less desiccation tolerant than their Southern African counterparts (Gaff 1981). This might indicate a shorter time period for adaptation within the genera of desiccation-tolerant species in Australia. India and Sri Lanka are well-known for the widespread occurrence of inselbergs, but there are no detailed accounts of their vegetation. Gaff and Bole (1986) reported desiccation-tolerant grasses (e.g. *Tripogon*) from shallow soils in rocky areas in India.There are no desiccation-tolerant angiosperms reported from North America, but ferns clearly dominate among the desiccation-tolerant vascular plants on North American inselbergs. There are several lichens and bryophytes which are desiccation tolerant, probably common on all continents, including Antarctica (Davey 1997).

Mostly the desiccation-tolerant species are rosette plants and woody shrubs and perennial herbs measuring up to 2 m in height, and probably succulents (Gaff 1986; Barthlott and Porembski 1996; Porembski 2000). Morphological characteristics may be similar with xerophytes (e.g. fibrous, needle-like leaves in *Borya nitida*; Gaff and Churchill 1976), mesophytes (thin and broad leaves in *Boea hygroscopica*; Gaff 1981) or even hydrophytes (aerenchyma in floating leaves of *Chamaegigas intrepidus*; Gaff and Geiss 1986). There are no desiccation-tolerant trees known till date. Sherwin et al. (1998) suggested that inability to reverse the cavitation of xylem may interdict desiccation-tolerant plants from growing more than a few metres tall.

Desiccation-tolerant lichens, mosses and bryophytes differ in the timing of resuming their all physiological activities. In general highly desiccation-tolerant lichens and bryophytes take less than 15 min to rehydrate, and within 24 h, their full photosynthetic functions recover (Alpert and Oechel 1987; Csintalan et al. 1998, 1999).

However, it takes 12 h to several days rehydration time for desiccation-tolerant ferns and angiosperms (Gaff 1997). There is one exception of aquatic species of *Craterostigma*, which can rehydrate in 1.5 h (Gaff and Giess 1986). Lichens and bryophytes readily absorb moisture and can rehydrate with dew also (Lange et al. 1994). There is no desiccation-tolerant vascular plant known to have this ability. Lichens can even absorb enough water from water vapour to regain positive CO_2 uptake (Hahn et al. 1993). That's the reason that lichens are prevalently distributed in coastal deserts where fog is a major source of moisture (Thompson and Iltis 1968; Rundel 1978).

2.4 Types of Desiccation-Tolerant Plants

2.4.1 Fully Desiccation-Tolerant vs. Modified Desiccation-Tolerant Plants

Vascular plants have shown desiccation tolerance abilities in vegetative tissues with some 350 species, which contributes less than 0.2% of the total flora (Porembski and Barthlott 2000), but this figure keeps on expanding. As all the vascular plants supposedly have desiccation-tolerant spores or seeds, it seems desiccation tolerance is probably universal. Majorly, vegetative desiccation-tolerant plants constitute two categories based on their rate of drying, namely, (1) fully desiccation-tolerant plants including the less complex clades that constitute the algae, lichens and mosses, while the other is (2) modified desiccation-tolerant plants including most of the higher angiospermic resurrection plants. Fully desiccation-tolerant plants are called so, because they can tolerate the total loss of free protoplasmic water (Oliver et al. 1998) very rapidly. These plants rapidly equilibrate their internal water content with that of the environment because they possess very few morphological and physiological adaptations for water retention. The ability of rapid drying in this group of plants also strongly suggests that a constitutive protection mechanism is necessary for survival in this group of lower desiccation-tolerant plants (Oliver et al. 2000). This sets the basis for the hypothesis that the primitive tolerance mechanism involves a constitutively protection mechanism coupled with active cellular repair (Oliver et al. 2000). Modified desiccation-tolerant plants are more complex and relatively larger group of vegetative desiccation-tolerant plants. These plants usually dry slowly followed by a series of morphological and physiological mechanisms that reduce the rate of water loss to the extent required to establish tolerance. There are several evidences available for modified desiccation-tolerant plants which strongly suggest that they utilise preventive mechanisms that majorly rely on inducible cellular protection systems (Gaff 1989).

Fig. 2.1 Hydrated (**a**, **c**, and **e**) and dry (≤5% RWC (**b**, **d**, and **f**)) monocotyledonous resurrection plants *X. viscosa* (**a** and **b**) and *X. humilis* (**c** and **d**) and the grass *E. nindensis* (**e** and **f**). Scale bars: A, B, D, E, F = 10 cm; C = 1 cm (Image Source: Farrant et al. 2007)

2.4.2 Alternatives in the Modified Desiccation Tolerance: Homoiochlorophylly and Poikilochlorophylly

Based on retention of photosynthetic apparatus, desiccation-tolerant plants may be divided into two major categories, namely, (1) homoiochlorophyllous desiccation tolerance (HDT) and (2) poikilochlorophyllous desiccation-tolerant (PDT) plants. The HDT plants retain their photosynthetic apparatus intact along with chlorophyll while drying, but on the contrary PDT plants disintegrate the chloroplast structure, thus leading to loss of chlorophyll during drying which can be recovered following rehydration (Figs. 2.1 and 2.2).

Fig. 2.2 Hydrated (**a** and **c**) and dry (≤5% RWC (**b** and **d**)) dicotylendous resurrection plants C. wilmsii (**a** and **b**) and *M. flabellifolia* (**c** and **d**). Inset to D: cross section of dry leaves *of M. flabellifolia* showing leaf curling and retention of chlorophyll in the shaded adaxial surfaces and waxy anthocyanin in the outer abaxial surfaces. Scale bars = 1 cm (Image source: Farrant et al. 2007)

PDT plants follow an ordered dismantling of internal chloroplast structure during drying after which an ordered reconstruvtive assembly takes place upon rehydration. These processes can thus be thought of as not only being superimposed on an existing cellular protection mechanism of vegetative desiccation tolerance (Oliver et al. 2000) but as a distinct new DT strategy (Tuba et al. 1994). Mostly monocot plants belong to category of PDT (Gaff 1977, 1989; Bewley and Krochko 1982). Poikilochlorophylly is currently constituted by eight genera of five families (Cyperaceae, Liliaceae, Anthericaceae, Poaceae and Velloziaceae). The best physiologically studied PDT plants are the African *Xerophyta scabrida*, *X. viscosa*, *X. humilis* and the Australian *Borya nitida* (Gaff and Churchill 1976; Hetherington and Smillie 1982; Tuba et al. 1993a, 1996; Dace et al. 1998; Farrant 2000; Cooper 2001).

Much or all of the chlorophyll are retained by most of the HDT plants during the desiccation –rehydration cycle. The chlorophyll loss is variable in dicotyledons species which depends on species and mostly influenced by several environmental factors. There were no significant changes observed in chlorophyll content on drying and rewetting cycle in two HDT plants, namely, *Haberlea rhodopensis* and *Ramonda serbica* from Southeast Europe (Markovska et al. 1994), but *Ramonda nathaliae* exhibited loss of 20% and 70% of its chlorophyll when desiccated in the glasshouse and in natural habitats, respectively (Drazic et al. 1999). *Myrothamnus flabellifolia* retains its thylakoids, but half of its chlorophyll is lost on drying (Farrant et al. 1999).

In PDT plants the breakdown of photosynthetic apparatus due to desiccation is different from the process with that of leaf senescence. The systematic disintegration of photosyntheitic apparatus seems to beam a protective mechanism rather than damage to be repaired after rehydration. If the *Borya nitida* plant, which is a PDT plant, dried with a fast speed, it didn't get the sufficient time to break down chlorophyll and loses its viability (Gaff and Churchill 1976), and the same is true for *Xerophyta humilis* (Cooper 2001).

As we have discussed earliar in PDT plants, disintegration of the thylakoid membranes, which is a strictly organised process, leads to the formation of nearly similar-sized globuli called "desiccoplasts", which, unlike chromoplasts, are capable of regreening its tissues and start the process of photosynthesise after rehydration (Tuba et al. 1993b). There occupies a granular stroma inside desiccoplasts which contains lipid-filled translucent vesicles called as plastoglobuli, also containing lipoquinones and neutral lipids. The thylakoid material is represented by osmiophilic lipid material which is found stretched in desiccoplast (Tuba et al. 1993b). Most of the mitochondrial cristae also disappear during the process of desiccation, while the remaining ones seem to decompose within 30 min after rewetting in *X. villosa*. This is exhibited by a loss of almost 50% of insoluble or structural proteins which is found to be very less observed (generally *c*. 10%) in HDT plants (Gaff and Hallam 1974).

2.5 Morphological Adaptations

2.5.1 Leaf Curling and Cell Wall Folding

There are several changes in leaf architecture during the periods of water deficit. These modulations are generally slower responses but provide great potential for survival during desiccation. For example, many resurrection plants undergo leaf rolling towards inside in such a way that the epidermal hairs on abaxial surface now face the upper surface, which turn into a grey-green or purplish colouration. If this process doesn't occur, it can cause photoinhibition and production of reactive oxygen species (ROS). Leaf curling is followed by the accumulation of anthyocyanins and other phenolic compounds that in turn protect against solar radiation (Rakic et al. 2015; Mitra et al. 2013; Figs. 2.3, 2.4 and 2.5). The process of leaf folding is reversible, and it resumes its shape after rehydration and is considered to be related to cell wall folding. Homoiochlorophyllous angiosperm resurrection plants employ

Fig. 2.3 *Ramonda serbica* plants. (**a**) Well hydrated, (**b**) desiccated (Image source: Rakíc et al. 2015)

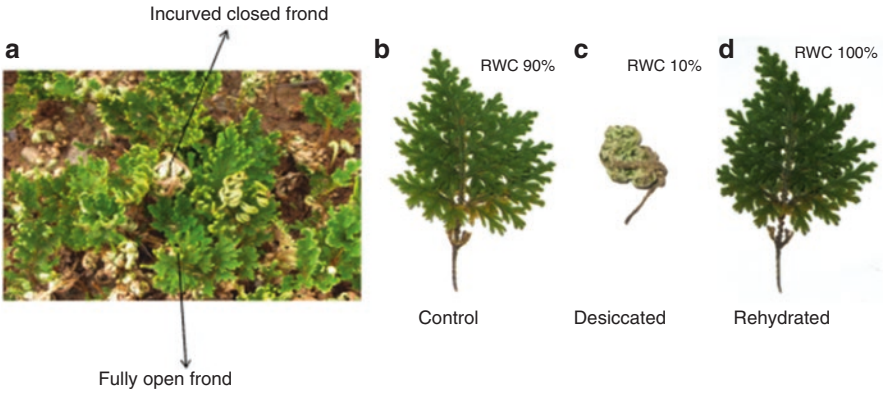

Fig. 2.4 *Selaginella bryopteris*. (**a**) In natural habitat, (**b**) fully hydrated, (**c**) desiccated and (**d**) rehydrated (Image source: Pandey et al. 2010; Deeba et al. 2009)

this strategy of chlorophyll shading and masking (Sherwin and Farrant 1998; Rakic et al. 2015; Mitra et al. 2013), and frond curling has also been noted in the DT fern *Polypodium polypodioides* (Helseth and Fischer 2005) and fern ally *Selaginella lepidophylla (*Lebkuecher and Eickmeier 1993*)*, *S. bryopteris* (Deeba et al. 2016), from the family Gesneriaceae *Ramonda serbica* (Rakic et al. 2015) and *Boea hygrometrica* (Mitra et al. 2013).

The loss of water from the cell causes conformational changes in the plant cell wall polysaccharides and proteins (Moore et al. 2008). The cell wall becomes highly flexible and gets folded during desiccation, which helps in reducing the loss due to plasmolysis (Jones and McQueen-Mason 2004). By doing so, plasma membrane is saved from damage which in turn maintains the coherence in the cell structures and the cell-to-cell communication through plasmodesmata (Neale et al. 2000; Jones and McQueen-Mason 2004). The process of cell wall folding causes shrinkage of

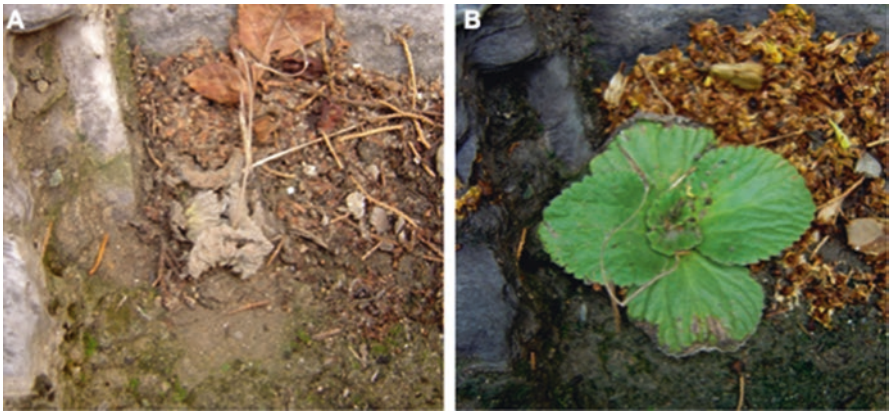

Fig. 2.5 *Boea hygrometrica*. (**a**) Desiccated state, (**b**) fully hydtated state (Image source: Mitra et al. 2013)

cells which in turn enables leaf curling and reversible rolling (Moore et al. 2008; Farrant et al. 2007). Plants also minimise the mechanical stress by increasing the number of vacuoles wherein the water in vacuoles is replaced by nonaqueous substances (Oliver et al. 2011b). Angiospermic resurrection plants undergo considerable shrinkage in their leaves and roots (Farrant et al. 2007), and the extent of shrinkage is found greater in dicots, because wall folding plays an important role in mechanical stabilisation in these plants.

The leaves of *Craterostigma plantagineum* show increased abundance of α-expansin transcript during drying and rehydration cycle which might be correlated with the modulations in wall extensibility (Jones and McQueen-Mason 2004). The wall loosening can be corrrelated well with the expansins via disruption of noncovalent bonds between polysaccharides (McQueen- Mason et al. 1995), and this provides an additional mechanism of wall folding in the *Craterostigma* species.

2.5.2 Root Adaptations

The roots are the first organ of plant to detect a drop in water availability. In these tissues the drought response is more rapid than in the leaves. In addition, shrinkage in roots is found to be very less as compared to leaves during dehydration as they are embedded in a solid soil. Major root network damage occurs if shrinkage of roots go beyond that of the soil. In *Craterostigma plantagineum*, it has been observed that secondary roots contract more than the soil, but this contraction was found to be very minor. Most of the secondary/tertiary roots survive desiccation but die rapidly after rehydration (24 h after rehydration). The primary roots, which constitute about 70% of the total root biomass in terms of mass, were found to be alive and functional because they contained functionally active enzymes (Norwood et al. 2003). Although drought stress did not affect the growth of the primary root in

higher plants, due to lateral root meristem inhibition, its growth is significantly reduced (Deak and Malamy 2005). Crop plants employ another adaptive strategy of hydrotropism to counter the stress, and it has been shown in a study that amyloplast degradation due to drought stress in the columella cells of roots tends to increase the habit of hydrotropism in plants. Architecture of the root system is being modulated by chemical signals produced by hormonal crosstalk mediated by auxin, CK, GA and ABA in response to water stress (Basu et al. 2016). Response of xylem vessels also plays a major role in desiccation tolerance in resurrection plants (Sherwin et al. 1998). Xylem blockage occurs due to the formation of small air bubbles during the scarcity of water (Sperry and Tyree 1988). In *M. fabellifolia*, it has been suggested that narrow reticulate xylem vessels, which cavitate on desiccation, refilled on rehydration through the capillarity and root pressure (Sherwin et al. 1998).

In response to drought, ABA is synthesised in roots and is released to different plant parts. The release of ABA in turn activates the various genes which are required for metabolic processes, e.g. the accumulation of sucrose from either stored carbohydrates or through altered photosynthetic carbon partitioning. Generally it is known that ABA, which is released in roots, is transported to shoots where it modulates various metabolic functions (Schulze 1986).

Although there are few studies pertaining to desiccation tolerance responses in roots of resurrection plants, some studies have been undertaken to observe the changes in carbohydrate metabolism and mobilisation of reserve food (Norwood et al. 1999, 2003). It has been observed that oligosaccharides make up the majority of carbohydrate contents of the root. Gas chromatography (GC) analysis revealed that 97% of raffinose series oligosaccharides were in the form of stachyose. On drying, there is a small decrease in the raffinose series of oligosaccharides and dramatic rise in the amount of sucrose in the roots. Little changes occurred in other carbohydrate. Neglegible quantities of 2-octulose were detected in root sample (Norwood et al. 1999). Enzymes responsible for the carbohydrate metabolism and gluconeogenesis (aldolase and GAPDH) have been found to be increased in roots during desiccation. However, glycolysis and Krebs cycle's enzymes did not show any significant increase in activity (PFP, PFK and malate dehydrogenase) suggesting a minor role of respiration during the preparation of desiccation (Norwood et al. 1999). In higher plants upon mild drought stress, enzymes related to root morphology (e.g. xyloglucan endotransglucosylase) were found to be increased, which strongly correlates with the enhanced surface area for increased water uptake. The modulations in the expression of these proteins lead to more lateral development which in turn affects the process of photosynthesis (Sengupta and Reddy 2011). This enhanced lateral root development and root hair formations were found in lines possessing a QTL, qDTY12.1, only under drought condition. Drought-responsive specific traits of such roots in these plants contributed towards higher capacity for increased grain yield under drought. Moreover, various tissues in the roots of different cultivar or species undergo varied anatomical adaptations under drought stress conditions. For example, in order to increase the retention of water in sclerenchyma layer cells, there were found to be decreased suberisation and compaction in rice under drought stress condition (Basu et al. 2016).

It has been found in a study on *S. bryopteris* undergoing dehydration followed by two rehydrations (RI and RII) that almost all the identified proteins (barring one) showed higher abundance during dehydrations and on rehydration most of them came to their normal control values (Deeba et al. 2016). Roots exhibited higher abundance of the proteins involved in the process of signalling, stress and defence, protein and nucleotide metabolism, carbohydrate and energy metabolism, storage and epigenetic control. Most of these proteins remained upregulated on first rehydration, suggesting their role in recovery phase also (Deeba et al. 2016). It has been suggested that disturbance of the root system during the drying period leads to disruption of acquisition of the desiccation-tolerant state (Gaff 1997) in *Sporobolus stapfianus* suggesting that root signal(s) may be important for desiccation tolerance. In contrast to this, some resurrection plants possess the same capacity for desiccation tolerance as that of the whole plant (Deeba et al. 2009; Mitra et al. 2013).

2.6 Physiological Adaptations

2.6.1 Stomatal Control of Gas Exchange and Water Status of Leaves

In order to avoid extensive water loss, plants prefer to close their stomatal aperture as an initial response under water-deficit conditions, thus suggesting a preventive measure from cell dehydration, xylem cavitation and death. Plants develop these responses quickly or slowly, depending on the rate of water loss, and may result from shoot and/or root dehydration (Schulze 1986; Chaves 1991). The changes in turgidity of guard cell in relation to adjacent epidermal cell influence the opening and closing of stomata which in turn is dependent on metabolic energy coming from mesophyll photosynthesis, and changes in membrane permeability as well. Stomata respond to a number of stresses at any given moment, so it is very difficult to interpret its response under any specific condition of drought stress ranging from light intensity to CO_2 concentration.

Reduction in leaf surface area via leaf curling in response to dehydration is considered as a common response in resurrection plants which ultimately leads to the reduced transpiration, thus regulating temperature and limiting the damage caused by radiation incidence (Dinakar et al. 2012). The decreased transpiration due to reduced stomatal conductance was observed in *Barbacenia purpurea* (*Pleurostima purpurea*) upon water deficit (Suguiyama et al. 2014). It has been observed that a metabolite, fumarate, acts as stomatal controller which avoids water loss and thus controls gas exchange. Therefore, stomatal closure can be correlated well with increased fumarate content during winter season thus suggesting diminished transpiration and photosynthetic rate in *B. purpurea* (Aidar et al. 2010).

A net CO_2 assimilation fall has been observed in *X. scabrida* due to stomatal closure and loss of chlorophyll as observed by fluorescence imaging; this ultimately leads to a halt in photosynthesis (Tuba 2008).

When the leaves of *X. viscosa* had lost about 60% of their water content through the first 4 days of drying, no photosynthetic activity were found by that time (Tuba et al. 1996; Tuba 2008). The recovery of CO_2 assimilation during rehydration process in *Haberlea rhodopensis* and *Ramonda serbica* can be correlated well with an increased stomatal conductance and Rubisco activity. The photosynthetic activity was significantly reduced with the desiccation in *C. plantagineum*, while its rate is further resumed after rehydration (Dinakar et al. 2012).

Conductance rate of water through the xylem vessels directly influences the stomatal responses in plants under water stress conditions (Salleo et al. 2000). Alterations in leaf turgidity in terms of water would translate into a signal that might consequently lead to stimulate the changes in osmotic potential of guard cells during scarcity in water supply (Buckley and Mott 2002). Stomata regulate the xylem pressure and prevent them going beyond its cavitation threshold (Jackson et al. 2000; Buckley and Mott 2002).

Stomatal aperture is also regulated by other hormones besides ABA, either in isolation or acting in association with ABA. Stomatal sensitivity is decreased towards ABA with the increase in cytokinin concentration in the xylem that promotes stomatal opening directly (reviewed by Wilkinson and Davies 2002). On the contrary, root cytokinin (zeatin and zeatin riboside) was found to be decreased due to partial root zone drying, thus an increase in xylem ABA brings about a reduction in stomatal conductance (Stoll et al. 2000). Stomatal regulation through ABA involves both its long-distance transport and modulations at the guard cells to a given dose of hormones (Wilkinson and Davies 2002). Xylem sap and leaf tissue pH exhibit an increase due to high evaporative demands caused by a high deficit in water vapour pressure of the air, high light intensity and high leaf temperature.

2.6.2 Photosynthesis

In resurrection plants, photosynthesis is shut down during desiccation. The downregulation of photosynthesis-related gene expression (Bockel et al. 1998) along with the degradation of photosynthetic structures contributes a significant reduction in this process. As already explained earlier, despite changes observed in photosynthetic pigment distribution, homoiochlorophyllous species retain its chlorophyll and thylakoid membranes structures (Alamillo and Bartels 2001). However, dismantling of chlorophyll and thylakoid structures favours the poikilochlorophyllous species to remain safe by preventing the accumulation of reactive oxygen species (Tuba et al. 1998).

Reactive oxygen species (ROS) production and accumulation during water stress condition are contributed majorly by chloroplasts (Moran et al. 1994; Kranner and Lutzoni 1999). Closed stomata limit the intake of CO_2 to chloroplasts, thus inhibiting carbon fixation and causing overexcitation of chlorophylls which ultimately transfers excess energy to oxygen, giving rise to ROS (Inźe and Van Montagu 1995; Kranner and Lutzoni 1999; Navari-Izzo and Rascio 1999).

It is well known that photosynthesis is affected by a number of genes during desiccation. The decreased rubisco activity is largely dependent on the activity of

Rubisco activase and stromal ATP/ADP ratio than to the changes at protein level. Under normal conditions, rubisco activase transcripts were shown to be abundantly expressed in leaves suggesting the plant's preparation before dehydration in *C. plantagineum* (Dinakar et al. 2012). Different species vary in their responses to shutting down the photosynthesis during dehydration in resurrection plants. For this it is important to maintain the membrane intactness or their repairing for carrying on the electron transport reaction during dehydration. In desiccation-tolerant plants, thylakoid membranes and their functions were found to be recovered completely upon rehydration (Dinakar et al. 2012).The thylakoid membrane intactness and the ratio of pigment–protein complexes were maintained in dehydrated *Boea hygrometrica* and *H. rhodopensis* despite a decrease in photosynthetic carbon fixation and the PS II functions (Georgieva et al. 2007; Deng et al. 2003). Another protective mechanism was also observed in *R. serbica* leaves upon desiccation where the PS II activity was significantly decreased to maintain membrane integrity (Deng et al. 2008). The low levels of psbA transcripts, required for the initial assembly of PS II and also important component of reaction centre protein D1, are stably maintained during desiccation in *X. humilis*. The necessary transcript for the recovery of PS II is already stored in order to immediately translate within first few hours of rehydration (Collett et al. 2003). Light-harvesting complex (LHC) protein, which is a primary chlorophyll-binding protein and responsible for the maintenance of chloroplast stability, is considered to be induced under desiccation (Schneider et al. 1993; Neale et al. 2000). Other proteins which were found to be involved in chloplast stability in leaves of *Craterostigma plantagineum* (Bartels et al. 1992; Alamillo and Bartels 2001) and *Sporobolus stapfianus* (Neale et al. 2000) exhibited higher similarities with ELIPs (early light-inducible proteins). It has been suggested that the enzymes involved in Calvin cycle are more affected by dehydration with that of the membrane-bound electron transport reactions (Georgieva et al. 2007).

Homoiochlorophyllous plants rapidly resume photosynthesis upon rehydration (Drazic et al. 1999) because their photosynthetic structure is almost maintained in recoverable form during dehydration. At the same time, the event of photoexcitation of chlorophyll persists which is further responsible for ROS production. In order to limit the rise in ROS and to safeguard the cells from their consequences, various structural and biochemical modifications occur in dehydrating cells. Antioxidant system of chloroplast provides first level of defence in dehydrating cells. Various enzymes and antioxidants scavenge the free radicals produced in cells during the process of desiccation (Pandey et al. 2010).

Nonradiative dissipation of excessive excitation energy is another approach to limit the levels of ROS. The carotenoids of the xanthophyll cycle contribute in this process (Ruban and Horton 1999) through the de-epoxidation of violaxanthin to zeaxanthin that either directly or indirectly dissipates solar radiation as heat (Alamillo and Bartels 2001; Kranner et al. 2002).

The strategy which allows poikilochlorophyllous plants to degrade their photosynthetic structures during desiccation also offers an advantage of reducing the cellular sources of ROS in the dry state (Proctor and Tuba 2002). However, it takes time to resume their photosynthesis after rehydration because the entire

photosynthetic apparatus needs to be rebuilt after rehydration (Sherwin and Farrant 1996; Tuba et al. 1994, 1998). A programmed event that transforms the chloroplast into a "desiccoplast" which is devoid of any pigment and thylakoid membrane follows well-defined metabolic pathways (Sherwin and Farrant 1998; Tuba et al. 1998). While this process happens, "desiccation respiration" of mitochondria provides energy for this organelle transformation. Unlike chloroplast, mitochondria do not undergo structural or functional alterations until a severe degree of cell dehydration is reached (Tuba et al. 1996). In order to meet the energy demands for rebuilding the chloroplast structure, respiration follows a very quick resumption in poikilochlorophyllous plants on rehydration (Vander Willigen et al. 2001). Despite all these preventive measures, poikilochlorophyllous plants also face the risk of ROS production which arises from respiration maintained in conditions of stress. Thus, to combat these deleterious effects, a preventive and protective system activates against free oxygen radicals during dehydration in poikilochlorophyllous resurrection plants. Large quantities of anthocyanins were produced in drying leaves of *Eragrostis nindensis* (Vander Willigen et al. 2001). Similar behaviour of leaf folding and increased anthocyanin production was also observed in *Xerophyta humilis* (Farrant 2000). There was an increased level of the antioxidative enzymes, e.g. ascorbate peroxidase (APX), glutathione reductase (GR) and superoxide dismutase (SOD), observed in *Xerophyta viscosa* during desiccation (Sherwin and Farrant 1998). A new desiccation-inducible antioxidant enzyme which corresponds to a form of 1-cys peroxiredoxin was found in leaves of *Xerophyta viscosa* (Ndima et al. 2001; Mowla et al. 2002). A group of highly conserved class of thiol-specific antioxidant enzymes is represented by peroxiredoxins (Prxs), which is present in all organisms ranging from archaebacteria to plants and mammals (Chae et al. 1994; Kozak 1999). Two conserved residues of cysteines are present at the N-terminus of this protein, which are responsible for peroxide reduction. In plants, the 2-cys Prxs which acts in conjugation with photosynthetic machinery in chloroplast is also responsible for scavenging free radicals (Baier and Dietz 1997). Because of the fact that 1-cys Prxs are exclusively expressed in seeds during storage and their complete disappearance after germination and their absence in vegetative tissues even after dehydration stress, this suggests that the main function of these enzymes is to protect the seed tissues that survive desiccation from oxidative stress (Haslekas et al. 1998). Now the interesting fact is the identification of the Prx in leaves of *Xerophyta viscosa* (XvPer1) undergoing dehydration which exhibits approx 70% sequence identity with the seed-specific 1-cys peroxiredoxins (Aalen et al. 1994; Haslekas et al. 1998; Lewis et al. 2000), and this was the only instance which shows 1-cys Prx noticeable in vegetative tissues. Its transcripts were observed only under dehydration and upon other stresses including heat and high light responsible for producing increased levels of ROS. This protein (XvPer1) also ensured its nuclear location in *X. viscosa*, thus indicating its protecting role (Mowla et al. 2002).

2.7 Biochemical Adaptations

2.7.1 Antioxidative Defences

Reactive oxygen species (ROS) possess readily available unpaired electron and thus considered as highly reactive molecule. Oxygen, being highly oxidising, gets readily converted into highly reactive oxygen and nitrogen species, namely, singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet -}$), the hydroxyl radical ($^{\bullet}OH$) and nitric oxide ($NO^{\bullet}$). The process of electron transfer to oxygen in ground state ($^3O_2$) during electron transport chains of respiration and photosynthesis is responsible for production of ROS (Ananyev et al. 1994; Navari-Izzo and Rascio 1999). At cellular level, ROS are also produced under non-stress conditions, but its level does not exceed beyond the safe values through the defence system of the cell including superoxide dismutase (SOD), catalase (CAT) and peroxidise (POX) and free radical scavengers, such as carotenoids, tocopherols, ascorbate and glutathione (Navari-Izzo et al. 1997a, b; Loggini et al. 1997; Alscher et al. 1997). If level of ROS exceeds its normal values, it can cause lipid peroxidation and protein and nucleic acid denaturation that finally lead to destruction of subcellular structures (Mundree et al. 2002; Walters et al. 2002; Vicre et al. 2004; Berjak 2006). The occurence of ROS, being an unavoidable consequence to desiccation stress, is frequent in both seeds (Hendry 1993; Kranner et al. 2006) and resurrection plants (Kranner et al. 2006). The antioxidative activity was found to be increased in response to dehydration or rehydration in both desiccation-sensitive and desiccation-tolerant plants, but generally it is important to mention that a well-defined control of oxidative damage is seen in the desiccation-tolerant species as compared to the sensitive ones which exhibit clear signs of oxidative damage (Dhindsa and Matowe 1981).

The activity of defence enzymes, namely, superoxide dismutase (SOD) and catalase, exhibited fourfold increase in the desiccation-tolerant moss *Tortula ruralis* than in the sensitive *Cratoneuron filicinum* (Dhindsa and Matowe 1981). Drought-tolerant *Tortula ruraliformis* exhibited induced response of superoxide dismutase (SOD) and catalase activities as compared to relatively sensitive *Dicranella palustris*, but peroxidise and ascorbate peroxidase (AP) activities were somewhat greater in the sensitive ones than in the DT species suggesting importance of antioxidants (tocopherols and glutathione) than that of active oxygen-processing enzymes in determining desiccation tolerance. During the process of rehydration after 24 h, the leaves of the DT grass *Sporobolus stapfianus* showed twofold induced activities of glutathione reductase (GR) and dehydroxy ascorbate reductase (DHAR), while AP decreased by half (Sgherri et al. 1994). However, the content of total ascorbate doubled, and glutathione increased 50-folds on drying in the dicotyledon *Boea hygroscopica*. In *S. bryopteris*, various defence enzymes like CAT, SOD and APX, along with invertase and proline, were found to be significantly increased during dehydration (Pandey et al. 2010).

Although the activities of antioxidative enzymes, namely, APX, GR and SOD, were found to be increased in *Craterostigma wilmsii*, *Myrothamnus flabellifolia* and *Xerophyta viscosa*, during drying or rehydration phase at some point, their detailed

pattern of response varied greatly among these species (Sherwin and Farrant 1998; Farrant 2000). Despite having several evidences, it is slightly difficult to explain any stable universal pattern in responses of antioxidants and antioxidant enzyme systems to desiccation and rehydration in resurrection plants. Although dry *S. lepidophylla* and *P. polypodioides* employ the strategy of leaf curling to limit photodamage during rehydration, this was not found to be different from non-DT plants (Lebkuecher and Eickmeier 1993; Muslin and Homann 1992). Based on all evidences, it has been suggested that DT plants generally encounter the problem at source rather than by initiating high activity of defence enzymes after the event.

The chloroplast is also taken care by the glutathione system for protection from photo-oxidative damage. By forming glutathione–protein complex (GSSP), glutathione (GSH) not only provides protection to thiol groups from irreversible formation of intramolecular disulphide bonds, it also scavenges free radicals in response to desiccation (Kranner and Grill 1996; Navari-Izzo et al. 1997a; Kranner et al. 2002).

2.7.2 Proteomic Alterations

The "omics" technologies provide a platform for measuring quantitative abundance of biological molecules in such a way, thus making it possible to easily visualise and compare the differential responses among desiccation-tolerant and desiccation-sensitive species with that of their respective controls. Various approaches including transcriptomics, proteomics and metabolomics have enabled us to visualise and compare the complete digital information of the modulations taking place in transcripts, proteins and metabolites, respectively, during the process of desiccation followed by rehydration, thus providing a flow chart of metabolic events. Various "omics" approaches have been applied to study the molecular changes occuring during desiccation tolerance in resurrection plants (Table 2.1).

Some proteins which are specifically induced in response to desiccation provide cellular protection and help in recovery after desiccation in vascular plants. The late embryogenesis abundant proteins (LEAs), which are responsive to ABA and constitute a group of dehydrins, have been isolated as cDNA clones from the desiccation-tolerant *Craterostigma* tissues (Bartels et al. 1993; Ingram and Bartels 1996). These proteins provide cellular protection to seeds during desiccation and water deficits (Bray 1993). Similarly, *Sporobolus stapfianus* also exhibited several drying-induced cDNA coding for proteins related to LEAs (Blomstedt et al. 1998).

LEA proteins, including dehydrins (Close 1996), are hydrophilic in nature and help in preserving the membrane structure and also provide protein protection in conjugation with disaccharides. These proteins help sugars in maintaining cell vitrification and thus forming a glasslike situation with a very slow/negligible movement inside cell suggesting their primarily important role in determining the physical properties of intracellular glasses rather than their sugar component (Buitink and Leprince 2004).

Two groups of dehydrins, namely, LEA II or LEA D-11, and six different groups of LEA proteins were identified on the basis of expression pattern and sequence

Table 2.1 Various omics studies carried out in resurrection plants and sister group comparisons between desiccation-tolerant and desiccation-sensitive plants

Approach	Desiccation-tolerant species	Desiccation-sensitive species	References
Resurrection plants			
Transcriptome analysis	*Craterostigma plantagineum*		Rodriguez et al. (2010)
Transcriptome and metabolomic analysis	*Haberlea rhodopensis*		Gechev et al. (2013)
Proteomic analysis	*Selaginella tamariscina*		Wang et al. (2010)
Proteomic analysis	*Selaginella bryopteris*		Deeba et al. (2016)
Proteomic analysis	*Xerophyta viscosa*		Ingle et al. (2007)
Proteomic analysis	*Boea hygrometrica*		Jiang et al. (2007)
Proteomic analysis	*Sporobolus stapfianus*		Oliver et al. (2011a)
Metabolomic analysis	*Selaginella lepidophylla*		Yobi et al. (2013)
Sister group comparison			
Metabolomic comparison	*Sporobolus stapfianus*	*Sporobolus pyramidalis*	Oliver et al. (2011a)
EST sequencing and comparison	*Selaginella lepidophylla*	*Selaginella moellendorffii*	Iturriaga et al. (2006)
Metabolic comparison	*Selaginella lepidophylla*	*Selaginella moellendorffii*	Yobi et al. (2012)

similarity (Campbell and Close 1997; Cuming 1999). These proteins were found to be associated with different cellular compartments and form a complex including nucleoprotein complexes to endomembranes either in cytosol, nucleoplasm and chloroplasts at the subcellular level (Puhakainen et al. 2004). Dehydrins were found to act as surfactant on the macromolecules, thus providing a hydration buffer on them and protecting them from coagulation by preserving their structural integrity, and this could be possible because of their unique molecular features which include the abundance of glycine, the absence of cysteine and tryptophan and the presence of amphipathic α-helices (Close 1997). These proteins were also found to protect the macromolecules by sequestering ions and renaturing unfolded proteins and act as free radical scavengers (Bray 1993; Cuming 1999; Hara et al. 2004).

The LEA proteins have also found to be constitutively expressed or induced by several other stresses including cold, osmotic stress or exogenous abscisic acid or can even be expressed constitutively (Wise and Tunnacliffe 2004). Some LEA genes are constitutively recruited for ameliorating the deleterious effects of stress condition and thus also found to be induced abundantly in response to desiccation in *Haberlea rhodopensis* (Gechev et al. 2013). But unlike *Haberlea*, LEA genes were exclusively induced only during desiccation in leaves of *C. plantagineum* and not in

fully hydrated tissues (Rodriguez et al. 2010). ELIPs (early light-inducible proteins) play an important role in chloroplast stability in homoiochlorophyllous drought-tolerant plants like *Craterostigma plantagineum* (Bartels et al. 1992) and *Sporobolus stapfianus* (Neale et al. 2000).

The ELIPs which are thylakoidal chlorophyll-binding proteins (discussed under photosynthesis section) exhibit similarity with the HLIPs (high light-induced proteins) and the LHC (light-harvesting complex) proteins of photosystems (Montaṅe and Kloppstech 2000). These proteins have a protective function against photo-oxidative damage of the photosynthetic apparatus (Hutin et al. 2003). These proteins are thought to bind chlorophylls, thus keeping free pigments under strict control during the high light stress (Hutin et al. 2003). These proteins might also be participating in the non-photochemical quenching of light energy by binding with zeaxanthin (Krol and co-authors 1999). A plastid-targeted protein (CpPTP) was also found to express in leaves of *Craterostigma plantagineum* during dehydration (Phillips et al. 2002). These proteins thought to have the ability to modulate the chloroplast genes expression by interacting with plastid DNA (Phillips et al. 2002).

For plant's ability to control the exchange of water between cell and environment, it is important to adjust the permeability of cell membrane during the drying periods. The channel proteins of cell membrane, called aquaporins, facilitate the movement of water across the membrane (Maurel and Chrispeels 2001). These proteins constitute a family of hydrophobic membrane proteins, which are also called as major intrinsic proteins (MIPs). These occupy different subcellular locations, such as the tonoplast intrinsic proteins (TIPs) and the plasma membrane intrinsic proteins (PIPs). (Tyerman et al. 2002). The zones with high water flow in rapid cell division and enlargement areas are enriched with these aquaporins (Suga et al. 2001). The expression and activity of aquaporins under water stress is under strict control (Yamada et al. 1997; Martinez-Ballestra et al. 2000). This directly indicates that aquaporin expression and activity plays an important role in desiccation tolerance mechanisms. One TIP and several PIPs have been found to be differentially expressed during dehydration in the leaves of *Craterostigma plantagineum* (Mariaux et al. 1998). The *Cp-PIPa* gene transcript exhibits the varied response during different stages of dehydration, one being expressed more in the early phase, while another predominates in the late stage of desiccation. This suggests its role for the preparation for rehydration. A TIP has also been reported to be expressed during desiccation in the leaves of *Sporobolus stapfianus* (Neale et al. 2000). A tonoplast aquaporin, namely, TIP 3;1, has also been reported to be specifically expressed in dried leaves of *Eragrostis nindensis* (Vander Willigen et al. 2004). In fact, TIP 3;1 proteins (formerly called α-TIPs), being unique to orthodox seeds (Maurel et al. 1995), had never been found in vegetative tissues. The TIP 3;1 is considered to be permeable to glycerol and small proteins also in addition to water and participate in the mobilisation of solutes during the process of germination (Maurel and Chrispeels 2001). The TIP3;1 helps in mobilising sugars, proteins and other solutes accumulated inside the small vacuoles of bundle sheath during rehydration in dried leaves of *Eragrostis nindensis* (Vander Willigen et al. 2004).

2.7.3 Osmolyte

Osmolytes constitute a group of organic solutes, including a variety of low molecular weight compounds, e.g. amino acids, sugars, polyhydric alcohols, tertiary sulphonium and quaternary ammonium compounds. Osmolytes help in maintaining functional physiological state of cell, and their biosynthesis is affected by environmental factors at any developmental stage of the plant. Despite maintaining osmoregulation, osmolytes play a major role in scavenging reactive oxygen species, thus protecting subcellular structures. Osmolytes in the cytoplasm provide protection to cell membranes, proteins and metabolic machinery, thus preserving subcellular structure from damage(s) of dehydration (Rhodes and Samaras 1994; Rathinasabapathi 2000; Slama et al. 2015).

A coordinated regulation of biosynthesis and catabolic pathways leads to osmolyte accumulation that is considered as a determining factor for their mode of action. Osmolytes are thought to function for rapid and short-term fluctuations (accumulation/degradation, e.g. proline), as well as for longer periods also (e.g. betaines) (Gagneul et al. 2007). Osmolyte production and consumption follow a metabolic dynamics in such a way that its terminal/end products are also as much important as of osmolyte. For example, proline biosynthesis and catabolism lead to rapid and efficient consumption or release of reducing power (Szabados and Savouré 2010).

Despite showing varied biochemical/metabolic nature, osmolyte accumulation also exhibits species-specific varied responses to seasonal patterns. Various studies have been undertaken pertaining to osmolyte production in different resurrection plants (Table 2.2). In addition, level of osmolytes is also influenced by changes in growth period, developmental stage and organ and environmental factors (Hare et al. 1998; Murakeözy et al. 2003). Growing conditions and nutritional and environmental challenges also influence the distribution of osmolytes among various cell compartments (Gagneul et al. 2007).

Osmolytes include a number of osmoprotective compounds, ranging from mono-, di-, oligo- and polysaccharides (glucose, fructose, sucrose, trehalose, raffinose); sugar alcohols (sorbitol, mannitol, glycerol, inositol and methylated inositols); amino acids (proline, pipecolic acid); other betaines (glycine betaine, β-alanine betaine, choline *O*-sulphate) and tertiary sulphonium compounds (dimethylsulphoniopropionate, DMSP) (Rhodes et al. 2002; Ashraf and Foolad 2007). With the influence of various abiotic stresses, these different osmoprotective compounds were found to accumulate in higher plants (Ahmad et al. 1981).

These compounds are metabolically inactive (safe) molecules produced from the primary metabolic pathways (Rhodes et al. 2002). Proline, synthesised mainly from glutamate and also ornithine, was found to hyperaccumulate in plants experiencing with salt and drought stress (Kishor et al. 2005).

Proline is responsible for reducing cytoplasmic acidosis and maintaining NADP+/NADPH ratios at the required level for the respiration and photosynthetic process (Hare and Cress 1997). For the adequate regeneration of NADPH, the pentose phosphate pathway requires higher concentrations of NADP+, thus facilitating the supply of ribose-5-phosphate for photosynthesis and for purines synthesis as well (Kishor et al. 2005).

Table 2.2 Various osmolytes in vegetative tissues of resurrection plants under hydrated and desiccated conditions

Species	Hydrated condition	Dehydrated/desiccated condition	References
Craterostigma plantagineum, *Craterostigma wilmsii*	2-Octulose	Sucrose	Bianchi et al. (1991), and Cooper and Farrant (2002)
Haberlea rhodopensis	Sucrose raffinose	Sucrose, raffinose, maltose, verbascose, stachyose	Djilianov et al. (2011), and Gechev et al. (2013)
Myrothamnus flabellifolia	Fructose, glucose	Sucrose, arbutin, glucosylglycerol	Bianchi et al. (1993)
Boea hygroscopica	Glucose, fructose, sucrose, inositol	Sucrose	Bianchi et al. (1991)
Sporobolus stapfianus	Glucose, fructose, sucrose	Sucrose, raffinose, stachyose	Oliver et al. (2011a)
Xerophyta viscosa	Galactinol, Myoinositol	Sucrose, Raffinose	Peter et al. (2007)
Selaginella lepidophylla	Trehalose, sucrose and glucose	Trehalose, sucrose and glucose	Yobi et al. (2012)

Glycine betaine, one of the quaternary ammonium compounds, is considered as the highly abundant osmoprotective compound known to accumulate in plants under salinity, drought, heat and cold stress (Ashraf and Harris 2004; Hanson et al. 1991; Lokhande and Suprasanna 2012). Choline, the precursor molecule for glycine betaine synthesis, gets converted into glycine betaine through the sequential enzymatic action of choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH), respectively. There are other pathways known for glycine betaine synthesis, such as the direct N-methylation of glycine, but the choline pathway is considered to be found in all glycine betaine-accumulating plant species (Ashraf and Foolad 2007; Fitzgerald et al. 2009).

The production of organic osmolytes such as sucrose, polyols, glycine betaine or proline follows one common strategy which is found in both desiccation-tolerant and desiccation-sensitive species (Bray 1997; Geigenberger et al. 1997; Pelah et al. 1997; Clifford et al. 1998; Hare et al. 1998; Mundree et al. 2000; Gao et al. 2004). The preferential exclusion of these organic solutes on the surface of membrane and protein provides protection to them against moderate water loss (Hare et al. 1998; Hoekstra et al. 2001).

2.7.4 Sugars

Sugars are considered to be a major component of desiccation tolerance (Scott 2000; Oliver 1996: Alpert and Oliver 2002). Carbohydrate metabolism undergoes major changes upon drying that can be directly correlated with the desiccation tolerance.

Cellular protection in fully desiccation-tolerant mosses (*Tortula ruraliformis* and *T. ruralis*) is majorly employed by the free sugar, sucrose (Toldi et al. 2009), which accounts for approximately 10% of the dry weight for *T. ruralis* gametophytic cells that is sufficient to provide membrane protection during drying, at least in vitro.

Moreover, the content of sucrose does not change on a dry weight basis regardless of the fact that plant is subjected to drying or rehydration in the dark or light, suggesting that it is important for cells to maintain sufficient amounts of sucrose (Bewley et al. 1978). The fully desiccation-tolerant mosses that maintain high sucrose content constitutively do not require increasing its content on dehydration (Smirnoff 1992). However, dehydration-specific induction in the level of sucrose was found in modified desiccation-tolerant plants (Vicre et al. 2004; Ingram et al. 1997; Buitink and Leprince 2004). A comparative metabolic analysis of desiccation-tolerant and desiccation-sensitive species *Eragrostis nindensis* demonstrated that sucrose, being the major carbohydrate found upon desiccation in angiospermic resurrection plants (Cooper and Farrant 2002), exhibited enhanced accumulation in dried leaves of tolerant species, while the sensitive species did not accumulate sucrose (Illing et al. 2005).

Sucrose helps in maintaining the drying cells stabilised either by direct interactions with macromolecules and membranes or by reversibly immobilising the cytoplasm to the extent of slow-flowing glasslike vitrified liquid (Buitink and Leprince 2004).

A significant rise in sucrose metabolising enzyme, namely, class-I sucrose synthase genes, was found in *C. plantagineum* during the dehydration or abscisic acid (ABA) treatment, suggesting an increased glycolytic demand (Kleines et al. 1999). This increase was found to be paralleled by the increase in the abundance of cytosolic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) during desiccation or ABA treatment (Velasco et al. 1994). This increase in mRNA levels of class-I sucrose synthase and GAPDH ultimately leads to the higher protein and enzyme contents. The eight-carbon carbohydrate, 2-octulose, is converted into the sucrose with the functional enzyme complex of transketolase–transaldolase in *C. plantagineum* in response to dehydration (Bernacchia et al. 1995), and this enzyme complex might alternatively enhance pentose-to-hexose flux, thus providing more hexose for glycolysis. This process might be a preparation for the cell to meet the demand of ATP and NADH during recovery (Kleines et al. 1999). Extremely high concentrations of the unusual C8 sugar, 2-octulose, are found under normal conditions in *C. plantagineum* and *C. Wilmsii*, acting as a temporary storage carbohydrate. This sugar is converted to sucrose during dehydration, providing much needed energy to plants. The conversion of octulose to sucrose shares up to 80% of the carbohydrates in desiccated leaves of *Craterostigma*. Although *C. plantagineum* plants synthesise starch during photosynthesis, the percent share of starch as storage material is very less as compared with that of 2-octulose which is small (Norwood et al. 2000). Similarly in *Sporobolus stapfianus*, with the concomitant decrease of glucose and fructose levels, sucrose was found to significantly increase upon desiccation (Ghasempour et al. 1998). Despite the induced accumulation of sucrose and raffinose, other carbohydrates including stachyose, maltotetraose and myoinositol have also been found to accumulate upon desiccation. Glucose-6-phosphate that

stores phosphate during dehydration is responsible for the synthesis of maltotetraose and myoinositol (Oliver et al. 2011a). Maltose, verbascose and stachyose are among the other unique sugars apart from sucrose which were found to increase upon desiccation in *H. rhodopensis* (Gechev et al. 2013). The dehydration-specific accumulation of the arbutin, glucosylglycerol and trehalose was reported from *M. flabellifolia.* However, trehalose, sucrose and glucose are among the metabolites found to increase in *Selaginella lepidophylla* during desiccation which account for 50% of the total metabolites.

The oligosaccharides from raffinose family, being widely distributed nonstructural carbohydrates in the plant kingdom, have been considered to act as antistress agent throughout the whole life cycle of plants either in vegetative or reproductive tissues (Peterbauer et al. 2002; Peters et al. 2007; Taji et al. 2002; Pennycooke et al. 2003). These compounds have the ability to accumulate in large quantities without affecting primary metabolic processes (Peters et al. 2007).

The non-reducing sugar trehalose significantly contributes to vegetative desiccation tolerance in plants by providing protection against a variety of stresses in different organisms (Goddijn et al. 1997; Gaff 1989; Drennan et al. 1993; Bianchi et al. 1993). Being an unreactive sugar, trehalose is known for its strong stability which results from very low energy (1 kcal mol^{1}) of the glycoside oxygen bond joining the two hexose rings in it (Schiraldi et al. 2002). Thus, the primary metabolism and the energy homeostasis of the cell are not considered to be affected by trehalose.

Reports from the transgenic plants grown for the enhanced trehalose expression exhibited varied responses. In some cases plants had shown to increase tolerance against abiotic stresses in terms of sustained growth (Garg et al. 2002), but it cannot be generalised, because the expression of the *S. cerevisiae* trehalose-6-phosphate synthase gene in potato resulted in drought tolerance, but with slow growing, retarded plants (Stiller et al. 2008). Several studies on plants with modified trehalose expression had revealed that trehalose-inducible genes are also associated with jasmonic acid and ethylene-dependent stress signalling pathways (Bae et al. 2005a, b). The expression modulation of *Arabidopsis* trehalose- 6-phosphate synthase gene simultaneously influences the sugar sensing and photosynthate partitioning (Gomez et al. 2005; Kolbe et al. 2005).

2.8 Molecular Mechanism of Desiccation Tolerance

2.8.1 Transcription Factors

Generally proteins, functional byproduct of dehydration-induced genes, are responsible for preventing cellular damages and thus participate in antioxidant defence of cell machinery upon desiccation. There are various techniques and algorithms assembled in order to sequencing the entire transcriptome by deep RNA sequencing (RNA-Seq) even without a reference genome; therefore this is also applicable to resurrection plants. Various gene expression and EST sequence studies have been performed in variety of resurrection plants such as the moss Tortula ruralis (Zeng et al. 2002; Oliver et al. 2004); the club moss Selaginella lepidophylla and

Selaginella tamariscina (Iturriaga et al. 2006); the monocot species Sporobolus stapfianus (Neale et al. 2000), X. viscosa (Mundree et al. 2000; Mowla et al. 2002), X. humilis (Collett et al. 2003, 2004; Illing et al. 2005) and X. villosa (Collett et al. 2004); and the dicot species C. plantagineum (Bockel et al. 1998).

The identified transcripts of *C. plantagineum* and *H. rhodopensis* at diffrent physiological stages revealed highest similarities to the genes of *Vitis vinifera*, *Ricinus communis* and *Populus trichocarpa* (Dinakar and Bartels 2013).

The enzymes related to carbohydrate metabolism, proteins with protective properties, regulatory proteins such as transcription factors and kinases and signalling molecules are encoded by transcription factor induced upon desiccation stress (Dinakar and Bartels 2013).

The abundance of the transcripts generally varies between desiccation-tolerant and desiccation-sensitive plants. As an example, the LEA-like CDeT11-24 transcript exhibits varied response between desiccation-tolerant *C. plantagineum* (higher abundance) and desiccation-sensitive species of *Lindernia subracemosa* plants (lower abundance) suggesting unstable or lower rate of accumulation of this transcript in *L. subracemosa* during dehydration. The transcript expression level is influenced by promoter cis elements which was found to be absent in *L. brevidens*; that's why the accumulation of transcript CDeT11-24 exhibited lower abundance upon dehydration (van den Dries et al. 2011). This strategy strengthens these results which follow the hypothesis that high level of dehydration-specific gene expression is governed by selection of promoter cis elements. In *Sporobolus stapfianus* as well as in *X. humilis*, a large number of genes related to the antioxidant pathway were found to be significantly enhanced during desiccation (Neale et al. 2000; Collett et al. 2004). In addition to induced expression of antioxidant genes, expression of genes from other pathways also provided protection to some resurrection plants. The desiccation-induced antioxidant gene encoding 1-Cys peroxiredoxin (XvPer1) in *X. viscosa* exhibited sequence identity (70%) with the seed-specific dormancy-related 1-Cys peroxiredoxin in *Arabidopsis* (AtPer1; Mowla et al. 2002). The transcripts of thiamine biosynthesis and aldehyde dehydrogenase (CpALDH) were found to be upregulated upon desiccation and thus contributed to antioxidant defence in *C. plantagineum* (Rodriguez et al. 2010).

Several genes, for galactinol and stachyose synthase, are found constitutively present in *C. plantagineum* and *H. rhodopensis* leaves under normal conditions, but on dehydration these get additionally induced (Rodriguez et al. 2010; Gechev et al. 2013) suggesting the important role of oligosaccharides in providing protection to the cells upon dehydration. Various sugar metabolism-related transcripts encoding sucrose synthases (SuS) and sucrose–phosphate synthases (SPS) have also been found to be increased in *C. plantagineum* during dehydration (Ingram and Bartels 1996; Kleines et al. 1999). Various isoforms of transketolases, considered as key enzymes of the reductive and oxidative pentose phosphate pathways, are responsible for the synthesis of intermediates for sugar phosphate which ultimately help in different metabolic pathways of cell on desiccation. There were three isoforms of transketolase in *C. plantagineum* including *tkt3*, *tkt7* and *tkt10*; out of which one is constitutively expressed and also involved in octulose synthesis (Rodriguez et al. 2010; Willige et al.

2009), while other one, e.g. *tkt7*, was abundantly found in rehydrating tissues, and lastly, *tkt10* is exclusively expressed in fully hydrated tissues. Two isoforms of tkt were also observed in *H. rhodopensis*, but they exhibited differential expression in its carbohydrate metabolism with that of *C. plantagineum* (Gechev et al. 2013).

Expansins are the proteins responsible for the enhanced extensibility of cell wall, and the transcripts of this protein were found to be induced upon dehydration in *C. plantagineum* (Jones and McQueen-Mason 2004). Although some genes encoding for xyloglucan endotransglucosylases, pectin esterases and pectate lyases are constitutively expressed in *H. rhodopensis* in hydrated conditions, on approaching towards the desiccation phase, the expression of these genes were switched off, but simultaneously transcripts for laccase genes, which is involved in lignin biosynthesis, were only expressed in desiccated tissues suggesting a cell wall remodelling approach during desiccation (Gechev et al. 2013).

The above studies suggested that a catalogue of the regulatory genes can be prepared by transcriptome analysis which provides a profile of upregulated genes during desiccation, but there is another problem to understand at present how these genes interact with other pathways and which target genes they regulate.

2.9 Signalling Cascade

The environmental signals are perceived and followed by the secondary messenger production that in turn modulates the levels of intracellular Ca^{2+}, thus making a cascade of protein phosphorylation which ultimately assigns the proteins involved in cellular protection or transcription factors controlling the expression of stress-related genes. These signalling events ultimately lead to the production of the genes which may contribute in the generation of regulatory molecules like the phytohormone, abscisic acid (ABA). The phytohormone ABA accumulation under various kind of abiotic stress is one of the earliest responses observed in plants (Dinakar et al. 2012).

2.9.1 ABA Dependent

Various abiotic stresses including drought, low temperature and hypoxia induce the biosynthesis of abcisic acid (ABA) in the shoot and root of the plant. The epoxidation of zeaxanthin is the precursor event for ABA biosynthesis in roots which leads to the formation of epoxy xanthophyll precursors with the help of zeaxanthin epoxidase (ZEP) enzyme (Audran et al. 1998). Dehydration-induced expression of ZEP mRNA has been observed to increase by sevenfold in roots after 8 h of desiccation, while it does not report to increase in leaves (Audran et al. 1998; Bray 2002). There is sufficient amount of violaxanthin present in the leaves, so the gene induction for ABA biosynthesis is not required in leaves as compared to roots (Bray 2002).

The ABA hormone, which is considered to coordinate various metabolic processes during stress condition, also modulates the expression of most of the genes involved in acquisition of desiccation tolerance (Wilkinson and Davies 2002).

Dehydration-specific induction of ABA molecules serves as signalling molecule in most of the resurrection plants (Hartung et al. 1998). The genes which are thought to be induced by ABA contain a specific region of ABA-responsive elements (ABRE) in their promoter region (Mundree et al. 2002). Dehydration or the treatment of exogenous ABA stimulates the accumulation of *CDeT27-45*, a *LEA*-like gene in *C. plantagineum*, thus suggesting a model to study ABA-dependent signalling pathways (Bartels et al. 1990; Piatkowski et al. 1990). Dehydration-specific induction in the level of ABA varies with the different species of resurrection plants; as an example, generally it leaps by three- to sevenfold in the leaves upon desiccation (Bernaccia and Furini 2004), but it may increase by 20 times as compared with the control values in drying leaves of *Chamaegigas intrepidus* (Schiller et al. 1997). Moreover, exogenously applied ABA can also stimulate the adaptive responses in plants which was supposed to be induced by dehydration (Kleines et al. 1999; Frank et al. 2000; Kirch et al. 2001; Mowla et al. 2002), and dedifferentiated callus tissue of *Craterostigma plantagineum* also exhibited desiccation tolerance by exogenously applied ABA (Bartels and Salamini 2001; Bernacchia and Furini 2004). Some workers have identified the ABA-responsive region of the *CDeT27-45* promoter and also proposed that *CDeT27-45* expression depends on DNA-binding activity by nuclear proteins (Michel et al. 1994; Nelson et al. 1994). It has also been found that a novel SAP-domain transcription factor (CpR18) binds with the *CDeT27-45* promoter region (Hilbricht et al. 2002). Abcisic acid is found to regulate the expression of a gene called *CDT-1* which is supposed to activate a pathway inducing desiccation tolerance in *C. plantagineum* (Furini et al. 1997). The transcription factors, namely, homeodomain–leucine zipper (HD-ZIP) proteins, were shown to be induced by ABA; however, some genes of HD-ZIP family (CPHB-1 and CPHB-2) exhibited differential responses to ABA, one being nonresponsive to ABA (CPHB-1) and other one increased their accumulation in response to ABA in *C. plantagineum* (Frank et al. 1998). ABA not only modulates the regulatory proteins but also influences the expression of functional proteins such as LEA proteins that collectively lead to desiccation tolerance (Piatkowski et al. 1990; Ingram and Bartels 1996). The application of exogenous ABA or dehydration induces a novel gene family which was found to be involved in maintenance of chloroplast integrity, and its expression is very rapid in response to these treatments. Moreover, the expressions of several aquaporins are found to be upregulated by dehydration and ABA as well in *C. plantagineum* (reviewed in Ramanjulu and Bartels 2002).

Late embryogenesis abundant (LEA) proteins are also known to be ABA responsive and accumulate in vegetative organs during desiccation stress conditions. The vegetative tissues of resurrection plants were found to accumulate high levels of dehydrins under both drought and ABA induction. As dehydrins provide protection during the early phase of dehydration, that's why they accumulate in the cytoplasm and plastids during the first phases of cell drying (Rascio and Rocca 2005).

The process of leaf development is influenced by expression of expansin which can also manipulate the leaf shapes (Pien et al. 2001). However, the process of expansin accumulation in response to desiccation is not evident. The tomato and

barley mutants with ABA deficiency exhibited reduced leaf expansion with an increase in xylem sap pH which might act as drought signal governed by ABA-mediated mechanism (Bacon et al. 1998).

2.9.2 ABA Independent

ABA plays various roles in induction of desiccation tolerance, but there are several other signalling pathways and metabolic processes which are not under the control of ABA; they are considered as ABA independent. There are some dehydration-induced genes which are not regulated by ABA. As an example, the *CPHB-1* gene of a transcription factor and phospholipase D genes both are induced in response to drying but are shown to be insensitive to ABA in *Craterostigma plantagineum* (Frank et al. 1998; Frank et al. 2000). Similarly, the expression of the *XVSAP1* gene is only influenced by dehydration but did not show any effects of ABA in *Xerophyta viscosa* (Garwe et al. 2003). Furthermore, it has also been shown that the mechanism of desiccation tolerance follows an ABA-independent pathway in the leaves of *Sporobolus stapfianus* which were dried down to 60% RWC (Neale et al. 2000).

These observations have indicated that, in addition to ABA-mediated processes, other signalling pathways must also exist that serve to protect the deleterious effects of dehydration in order to provide the drought tolerance in resurrection plants but that involve yet unidentified molecular signals (Bray 2002).

2.10 Genetic Transformation of Desiccation-Tolerant Plants

There are several studies available on genetic engineering of drought tolerance pertaining to various traits including osmolytes metabolism, protective proteins, ROS scavenging proteins etc. (Umezawa et al. 2006). However, gene bank only provides information based on putative functions of gene on the basis of sequence similarity. It is mandatory to obtain in vivo proof after scruitinising transgenic plants with either higher or lower abundances of genes associated with desiccation tolerance (Toldi et al. 2006). Although genetic transformation procedures are having some limitation with vegetative desiccation-tolerant plants because of the extreme sensitivity to tissue culture level manipulations during the establishment of their plant regeneration and genetic transformation systems (Table 2.3), there are some resurrection plants for which plant regeneration protocol has been established, e.g. *C. plantagineum* (Toldi et al. 2002: Furini et al. 1994), *R. myconi* (Toth et al. 2004), *H. rhodopensis* (Djilianov et al. 2005) and *Lindernia brevidence* (Smith-Espinoza et al. 2007). The problems frequent necrosis, hyperhydrated leaves and polyphenol secretion into the culture media under suboptimal conditions are some of the limitations during the plant regeneration and genetic transformation process. Therefore, in order to develop non-lethal selection

Table 2.3 Advantages and disadvantages of the most abundant protective agents associated with vegetative desiccation tolerance in terms of practical application

Protection agent	Metabolism	Advantage	Disadvantage
Sucrose	Carbohydrate	Carbon and energy source, osmoprotectant	Possible interference with growth and osmoregulation
Trehalose	Carbohydrate	Osmoprotectant, signal metabolite	Possible interference with sugar sensing and photosynthetic performance
RFOs	Carbohydrate	Osmoprotectant, antistress agent, metabolically inactive, synergistic effects	None
LEAs	Protein	Hydration buffer, protein and membrane protectant	The most specific and most determinative LEAs are still missing
ABA	Mevalonate	Growth regulator, signal metabolite	Possible interference with plant development and growth, contrasting effects

Source: Toldi et al. (2009)

strategies and modified tissue culture media with relatively lesser amount of macro- and micro-elements and different antioxidant agents, it is mandatory to establish low stress transformation technologies for nearly all resurrection plants (Toldi et al. 2006). The biochemical preinduction of *vir* genes enhances the colonisation of wounded surface by the transforming *Agrobacterium* cells which is shown to be an additional strategy for increasing the transforming frequency. This could be possible because of the application of low-pH liquid medium with acetosyringone, aldose-type sugar source and organic nitrogen during the infection phase. Therefore, for a reliable genetic transformation method for resurrection plants, it is important to establish these modifications along with the appropriate tissue culture system. These knowledges can be further used to induce resistance in recalcitrant species (Toldi et al. 2009).

2.11 Evaluation of Plant Responses to Drought: Methodological Tools

There are several areas covered for evaluating plant's responses to drought including leaf gas exchange process, growth analysis and in vitro determination of enzymes, metabolites and gene expression patterns. However, evaluation of plant's responses to stress is revolutionised over the years by the methodological advances in various aspects.

Advances in genomics and proteomics provided a platform for understanding the molecular mechanisms underlying plant response to stress, but there are other tools, for example, stable isotope and thermal or fluorescence imaging, which can be used to study the plant responses within their natural habitat under any stress condition.

2.11.1 Genome-Wide Tools

The study of global transcriptome response of plants to various stresses including abiotic and biotic stress could be possible because of the advancements in high-throughput DNA sequencing methods which possibly made the sequencing complete for the genome projects including *Arabidopsis* (the first genome) and draft sequence of rice genome for both indica (Yu et al. 2002), japonica (Goff et al. 2002) and *Selaginella moellendorffii* (one of smallest plant genomes known to date: ~110 Mb; Wang et al. 2005; Banks et al. 2011). The DNA chips (DNA microarray technology) is considered as very promising tool in the current scenario of stress genomics studies in which cDNA gene probes (generally produced by expressed sequence tags (ESTs)) are being spotted onto a glass slide with the help of printing robots. Complementary DNAs (cDNAs) taken from control samples as well as from stressed samples are fluorescently tagged and mixed in equal amounts and agonistically hybridised to the probes in the microarray slide. The amount of hybridised probes in control is quantified relatively with the stressed one with a high resolution scanner (Brown and Botstein 1999). By using this approach, it was studied that how many of key regulatory genes have changed expression wise for the induction of CAM metabolism in *Mesembryanthemum crystallinum* and there were 60 functionally unknown ESTs found in *M. crystallinum* leaves (Maroco et al. 2000).

2.11.2 Proteomic Tools

Genome-wide tools provide a high-throughput data for the differential changes in gene expression for evaluating the plant response to drought. There is a drawback of this technique by which gene expression is prevented to translate it into functional proteins through posttranscriptional and posttranslational events. Therefore, this is a prerequisite of researchers to obtain a platform based on differential abundance of proteins. Traditionally, a technique of two-dimensional polyacrylamide gel electrophoresis (2D PAGE) is used to separate proteins according to their isoelectric points in their first dimension and at molecular weight basis in the second dimension. Since this technique includes several extraction and separation steps during its processing and proteins have large degree of diversity, thus this is considered as having a drawback of low reproducibility. This is also a labour-intensive process and involves the issues with loading capacity and limitation in protein detection on 2D gel. These flaws in 2D GE lead the development of new alternative due to the requirement of high-throughput proteome analysis in the present post-genomic era. There is another technique named difference gel electrophoresis (2D-DIGE) which is known to overcome many of these problems and provide relatively more precise and sensitive quantitative proteomics studies. CyDyes are normally cyanine dyes with a reactive group of *N*-hydroxysuccinimidyl ester which binds covalently with the ε-amino groups of lysine residues in proteins. Dye concentrations are optimised in such a way that only one dye molecule attaches per protein. Due to the limitations of the current in-gel digestion as well as the detection limits for various mass

spectrometers, it is almost difficult to identify the lesser abundant proteins on 2D-DIGE. There is yet another advanced combination of multidimensional chromatography which involves the protein separation coupled with mass spectrometry techniques for the functional characterisation of proteins (Washburn and Yates 2000. A variety of protein ligands are also used for microarrays (protein chips) which allow for the investigation of a high-throughput functional determination of proteins (MacBeath and Schreiber 2000). Fluorescent tags are used to label the proteins in a mixture and identified then by their positional coding or by further mass spectrometry, and this technique is useful where ligands are unknown for the proteins (Zhou et al. 2001). There are other studies pertaining to the quantitative proteomic changes during drought (Koh et al. 2015) in *Brassica napus* and combined effects of drought with heat stress (Zhao et al. 2016) in maize leaf. Such type of studies can be conducted in resurrection plants as well.

2.11.3 Stable Isotope

Stable isotope has become a powerful tool to study plant responses to the environment changes, from cellular to ecosystem level. Isotopic effects originate by analysing the differences in physical and chemical properties between heavy (e.g. ^{13}C or ^{18}O) and lighter isotope (e.g. the more common ^{12}C or ^{16}C). After the confirmation that plant material depletion occurs in heavier isotope of carbon (^{13}C) in terms of CO_2 in the air, it's the first time when the natural abundances of stable isotopes get into the notice in plant science in the middle 1900s. Lately, it was found that discrimination against the heavier carbon isotope was more in C_3 plants as compared to C_4 and CAM plants. The modelling of ^{13}C fractionation during photosynthesis in C_3 plants has been studied in details during the early 1980s (Farquhar et al. 1982). There were other heavy element isotope studies including oxygen ($^{18}O/^{16}O$) and hydrogen (D/H), which also contributed to the studies of plant water relations. Theoretical and practical knowledge of the use of stable isotope has exponentially increased with the advancements in mass spectrometry techniques (Griffiths 1998). The discrimination of the carbon isotopic composition occurs during photosynthesis as well as in respiration, so this is considered a complex process to study.

Utilisation of glycine and serine were studied as nitrogen sources in the roots of *Zea mays* and a resurrection plant *Chamaegigas intrepidus* by using NMR spectroscopy and stable isotope labelling. The various pathways of these amino acid utilisation were studied by using *C. intrepidus* and maize (*Zea mays*) root tips which were incubated with [^{15}N]glycine, [^{15}N]serine and [$^{2\text{-}13}C$]glycine (Hartung and Ratcliffe 2002).

2.11.4 Thermal and Fluorescence Imaging

There are various setbacks of destructive sampling or in situ measurements faced during the past for stress monitoring in plants. Therefore, it is a need of present time to develop almost accurate and non-obtrusive methods. Improved capacity for data

processing and analysis of several imaging techniques in the present time has allowed us to monitor the changes in physiological state of plants in a nonintrusive way (Buschmann and Lichtenthaler 1998). With these techniques it is possible to study the spatio-temporal localisation stress responses of plants, thus providing a better understanding of the dynamics of plant performance under field conditions. There are various advanced imaging technologies, including thermal imaging, fluorescence imaging and multi- or hyperspectral reflectance imaging. In order to study plant–water relations, thermal imaging is used to measure leaf surface temperature specifically for stomatal conductance, because the rate of transpiration is a determining factor of leaf temperature. Fluorescence signals which are good indicator of blue or actinic light absorption from plants are measured by fluorescence imaging. Moreover, the physiological parameters of plants including photoassimilation and non-photochemical quenching are estimated by combining an actinic light source with brief saturating blue pulses.

Advances in these recent technologies extended their usage to field conditions (Jones et al. 2009). Screening of a large number of phenotypes at a time is made possible because of the high working efficiency of these techniques, suggesting their importance in crop breeding programme, in crop management techniques (e.g. field irrigation scheduling) or in supporting models that simulate the impact of global climate change on natural and agroecosystems.

The process of thermography enables the measurements of the leaf temperature and their canopies which is directly dependent on rate of transpiration and thus can be used as an indicator of stomatal conductance and stress intensity (Chaerle and Van Der Straeten 2001). This technique is ahead over direct methods of stomatal conductance measurements, in its ability to focus the spatial heterogeneity of stress response over a leaf or canopy. The investigation of any kind of "hypersensitive response" to any infection before it became apparent is quantified with the help of this technique (Chaerle and Van Der Straeten 2001). It has also been used to screen for differential distribution and responses in stomatal apertures to identify mutants with altered stomatal function (Merlot et al. 2002).

The drawback of the thermographic approach is in the acquisition of temperature data that are truly representative of the crop (Jones 1999). Using image analysis software along with thermal imaging can solve this problem under controlled conditions. However, stomatal behaviour study or any kind of stress responses in the field is very difficult to interpret because of the variation in environmental conditions (Jones 1999). The photosynthetic activity and pigment contents have been studied by chlorophyll fluorescence imaging in the resurrection plants *Ramonda serbica* and *Ramonda nathaliae* following dehydration and rehydration (Gashi et al. 2013). In the resurrection plant *Haberlea rhodopensis*, it has been shown that lumenal substance protects the thylakoid against the damaging combined effects of light and drought Georgieva et al. (2012).

In order to powerfully probe the photosynthetic functioning in plants, the modulated fluorometers have been used for the measurements of chlorophyll fluorescence which provide greater potential when combined with reflectance or thermal imagery (Schreiber et al. 1986). For getting a detailed spatial analysis of stress-induced

inhibition of photosynthesis, fluroscence imaging is used for preparing quantitative maps of photosynthetic electron transport activity over the leaf (Meyer and Genty 1999). This technique has been proven very helpful to screen and identify mutants in altered PSII photochemistry and xanthophyll cycle (Niyogi et al. 1998).

2.12 Conclusion and Future Perspective

In the present chapter, it has been shown that the resurrection plants possess an extraordinary mechanism of desiccation tolerance for surviving in a very extreme water conditions in such a way that after almost complete water loss in vegetative organs, they resume their biological activities after rehydration. This outstanding capability of resurrection plants could be possible because of simultaneous interaction of various mechanisms and pathways required to cope with the numerous problems as a result of cell drying. There are various studies on morphological, physiological and molecular standpoints in resurrection plants which contribute greatly for improving the knowledge of their adaptive strategies. However, there are still a number of desiccation-related genes and proteins providing cell protection against water loss damage which need to be elucidated. It has been shown that environmentally induced adaptive strategies of desiccation-tolerant resurrection plants share its traits with the developmentally induced desiccation tolerance of seeds, suggesting that almost similar line of tolerance mechanisms is followed by both. This is also in agreement with the fact that inductive desiccation tolerance in seeds as well as in resurrection plants evolved directly or indirectly, from a primitive kind of constitutive desiccation tolerance found in bryophytes.

Transcriptome and proteome analysis revealed various genes and proteins with potential role in triggering desiccation tolerance; similarly metabolic profiling provided detailed descriptions of metabolic processes involved in the functioning of stress responses. Performing systematic analyses of resurrection plants is still an intricate task technically as well as intellectually. However, with the help of transcriptome and proteome, partial profiling of candidate genes could be possible in elucidating their functions for molecular breeding programmes. Although sugar is very important for most of the metabolic process, manipulating its expression (upregulation or downregulation) could lead to serious threat for balancing the osmotic adjustment, energy metabolism and growth and development. On the contrary, transgenic plants with enhanced trehalose synthesis do not seem to obstruct with the primary metabolism unlike sucrose, but with the other pathways including jasmonic acid/ethylene signalling. This can mislead the signals which ultimately disturb the plant responses in general.

As far as the specific LEA genes are concerned, although, known to provide tolerance against various abiotic stressors, they have already been quite extensively utilised in molecular breeding programmes, no field trials have still been conducted under real field situations. This aspect needs further studies for identifying the most effective types of LEA genes.

Several signalling and metabolic genes need to be modified in order to achieve desiccation tolerance, but the constraints for the entire high-throughput technology are 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 (Toldi et al. 2009) and is currently available for both monocotyledonous and dicotyledonous plants. Moreover epigenetic mechanisms should be studied in resurrection plants to explain their role in modifying response of these plants to desiccation stress.

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