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REVIEW

Insights into the cellular mechanisms of desiccation tolerance among angiosperm resurrection plant species

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ABSTRACT

Water is a major limiting factor in growth and reproduction in plants. The ability of tissues to survive desiccation is commonly found in seeds or pollen but rarely present in vegetative tissues. Resurrection plants are remarkable as they can tolerate almost complete water loss from their vegetative tissues such as leaves and roots. Metabolism is shut down as they dehydrate and the plants become apparently lifeless. Upon rehydration these plants recover full metabolic competence and 'resurrect'. In order to cope with desiccation, resurrection plants have to overcome a number of stresses as water is lost from the cells, among them oxidative stress, destabilization or loss of membrane integrity and mechanical stress. This review will mainly focus on the effect of dehydration in angiosperm resurrection plants and some of the strategies developed by these plants to tolerate desiccation. Resurrection plants are important experimental models and understanding the physiological and molecular aspects of their desiccation tolerance is of great interest for developing drought-tolerant crop species adapted to semi-arid areas.

Key-words: *Craterostigma wilmsii*; cell wall; dessication tolerance; oxidative stress; plant growth regulators; resurrection plants; water stress.

INTRODUCTION

Desiccation tolerance is defined as the ability to dry to an equilibrium with ambient air and to revive following the loss of all the protoplasmic water when moisture is available (Bewley 1979; Proctor & Pence 2002). Most higher plants are able to produce structures such as seeds or pollen, which are tolerant to desiccation, but only a small number of plants, termed resurrection plants, possess desiccation-tolerant vegetative tissues. These plants are widespread and found in most taxonomic groups ranging from pteridophytes to dicotyledons with the exception of gymnosperms (Gaff 1971; Oliver 1996).

Although some mechanisms are common to all desiccation-tolerant cells, there are also major differences in the strategies developed by these plants to cope with desiccation. In general the more tolerant bryophytes are termed 'fully desiccation tolerant' (Oliver & Bewley 1997) as tolerance is constitutive and is not affected by the rate of drying. In contrast, some of the less tolerant bryophytes and many desiccation-tolerant vascular plants are termed 'modified desiccation-tolerant plants' as tolerance is induced in the course of slow drying (Oliver & Bewley 1997). In order to tolerate desiccation, resurrection plants must be able to limit the damage associated with dehydration, to maintain physiological integrity in the dried state and to mobilize mechanisms upon rehydration to repair damage caused during desiccation and subsequent rehydration (Bewley & Krochko 1982).

The majority of resurrection plants were originally described in the 1970s (Gaff 1971; Gaff & Ellis 1974; Gaff & Churchill 1976; Gaff & Latz 1978). They are mainly found in seasonally arid subtropical and tropical regions. Most of the desiccation-tolerant angiosperms such as *Myrothamnus flabellifolius* (Child 1960), *Xerophyta* spp. or *Craterostigma* spp. (Gaff 1977) are native to Southern Africa (especially South Africa, Namibia and Zimbabwe) or to Australia, as is the case for *Borya nitida* (Gaff & Churchill 1976). Most of the earlier studies of desiccation tolerance were undertaken on mosses and ferns and more especially on the moss *Tortula ruralis* (reviewed by Bewley 1979 and Bewley & Krochko 1982). Physiological and anatomical characterizations of resurrection plants have been well investigated in species such *B. nitida* (Gaff, Zee & O'Brien 1976; Hetherington, Smillie & Hallam 1982), *Craterostigma wilmsii* (Sherwin & Farrant 1996; Vicré *et al*. 1999; Vicré 2001; Cooper & Farrant 2002), *Eragrostis nindensis* (Vander Willigen *et al*. 2001, 2003, 2004) or *M. flabellifolia* (Sherwin & Farrant 1996; Farrant & Kruger 2001) and most of the molecular aspects of desiccation tolerance have been studied in *Craterostigma plantagineum* (Bartels *et al*. 1990; Bartels & Salamini 2001; Hilbricht, Salamini &

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Bartels 2002; Phillips *et al*. 2002), *Xerophyta viscosa* (Mundree *et al*. 2000; Mowla *et al*. 2002; Garwe, Thomson & Mundree 2003) and *Xerophyta humilis* (Collet *et al*. 2003).

The stress that resurrection plants have to overcome in order to survive desiccation can be classified into three main categories: (1) mechanical stress associated with loss of turgor (Iljin 1957); (2) destabilization or loss of membrane integrity (Vertucci & Farrant 1995); and (3) oxidative stress related to disruption of metabolism (Seel, Hendry & Lee 1992a,b; Smirnoff 1993; Farrant 2000). Mechanisms that confer desiccation tolerance include the production of non-reducing sugars and the synthesis of dehydrin proteins as well as the use of free radical scavenging systems. Desiccation tolerance is a complex phenomenon and the understanding of the protection mechanisms involved in the drying and the resurrection process is still patchy. Excellent reviews have been recently published on the molecular, signalling and regulatory aspects of desiccation tolerance (Hoekstra, Golovina & Buitink 2001; Phillips, Oliver & Bartels 2002; Ramanjulu & Bartels 2002; Mundree *et al*. 2002). The aim of the review is to present an overview of recent advances on the study of desiccation tolerance mechanisms with a particular interest in the cellular aspects of desiccation.

RESPONSES TO DEHYDRATION

Morphological changes upon dehydration

Water stress produces major changes in the morphology of the desiccation-tolerant plants (Gaff 1989; Scott 2000; Farrant *et al*. 2003; Vander Willigen *et al*. 2003). One of the most obvious responses to desiccation in these plants is the curling or folding of their leaves upon dehydration. The leaves of *X. humilis* are flat and grasslike (Fig. 1). Upon dehydration the leaf blades fold in half along the midrib and only the abaxial surfaces are exposed to the light which is thought to serve to reduce light absorbed by the leaf in the desiccated state (Hallam & Luff 1980; Sherwin & Farrant 1998). In fully hydrated *C. wilmsii* plants, the leaves are green and expanded (Fig. 1). As the plant dries, leaves progressively curl inward and become tightly folded with only the abaxial surfaces of the outer whorl of older leaves exposed to sunlight (Sherwin & Farrant 1998; Farrant 2000; Vicré 2001). It is thought that a number of morphological modifications associated with dehydration are adaptations of resurrection plants to minimize damage from light (and consequent free radical stress) in the dry tissues. Leaf movements occurring during dehydration in resurrection plants have been suggested to reduce the

Figure 1. Morphological changes in the resurrection plant *X. humilis* (a and b) and *C. wilmsii* (c and d) during dehydration. *Xerophyta humilis* in the hydrated state possess green flat leaves (a). As the plant dries (b), leaves fold half along the midrib. Fully hydrated *C. wilmsii* plants (c) have green expanded leaves; (d) dried plants have curled leaves, only older abaxial surfaces of outermost ring of leaves are exposed to the sunlight. (from Vicré *et al*. 2004. With permission).

effective transpiring surface during early stage dehydration and/or to prevent excessive irradiation of air-dry younger tissue (Gaff 1989; Farrant 2000). In support of this, Farrant *et al*. (2003) have demonstrated that *C. wilmsii* does not survive drying in light if the leaves were manually prevented from folding.

Effects of dehydration at cellular levels

Changes in shape at the whole plant level are often accompanied by a large reduction in size and an important decrease in cell volume. Dehydration causes a considerable reduction in cell volume in *C. wilmsii* (Farrant 2000). Preparation of tissues for scanning electron microscopy (SEM) using cryomethods allows comparisons between tissues of hydrated and dry leaves. Such anhydrous fixation techniques were successfully applied for the first time to dry *C. wilmsii* tissues (Vicré *et al*. 1998). At the cellular level, hydrated leaf cells are characterized by a rounded shape in both spongy and palisade parenchyma cells (Fig. 2a). In contrast, cells from dried leaves are folded and highly shrunken (Fig. 2b). It is interesting to note that cell wall undulations are apparent in three dimensions without any regular pattern or orientation (Fig. 2b). Only xylem, being more rigid, maintains its original shape. Figures 2c and d show surface views of the epidermis from hydrated and dry tissues, respectively. In Fig. 2c stomata and glands are visible. Epidermis of dry leaves appears convoluted and highly folded (Fig. 2d). Glands are apparent in the upper and lower epidermis (Fig. 3). They are composed of several units as seen at higher magnification (Fig. 3b). Their presence has not been previously reported and their function is unknown but we assume that they may play a role in desiccation tolerance. It is very interesting to note that in the dried leaves, these glands are trapped in the epidermal folds (Fig. 2d). These glands are likely to be localized where the initial folding occurs (Vicré 2001; Vicré *et al*. 2004).

STRATEGIES OF RESURRECTION PLANTS TO COPE WITH VARIOUS STRESSES

Involvement of plant growth regulators

Plant growth regulators are known to play major roles in drought stress. In the leaves of the resurrection plant *C. wilmsii* dehydration induces a rapid but slight, decrease of the content of the cytokinins, zeatin and zeatin riboside (Fig. 4a & b). Levels of both cytokinins are maintained low during initial dehydration but increased markedly when plants were dried below 20% relative water content (RWC). Upon rehydration both zeatin and zeatin riboside contents decline progressively to initial levels after 70%

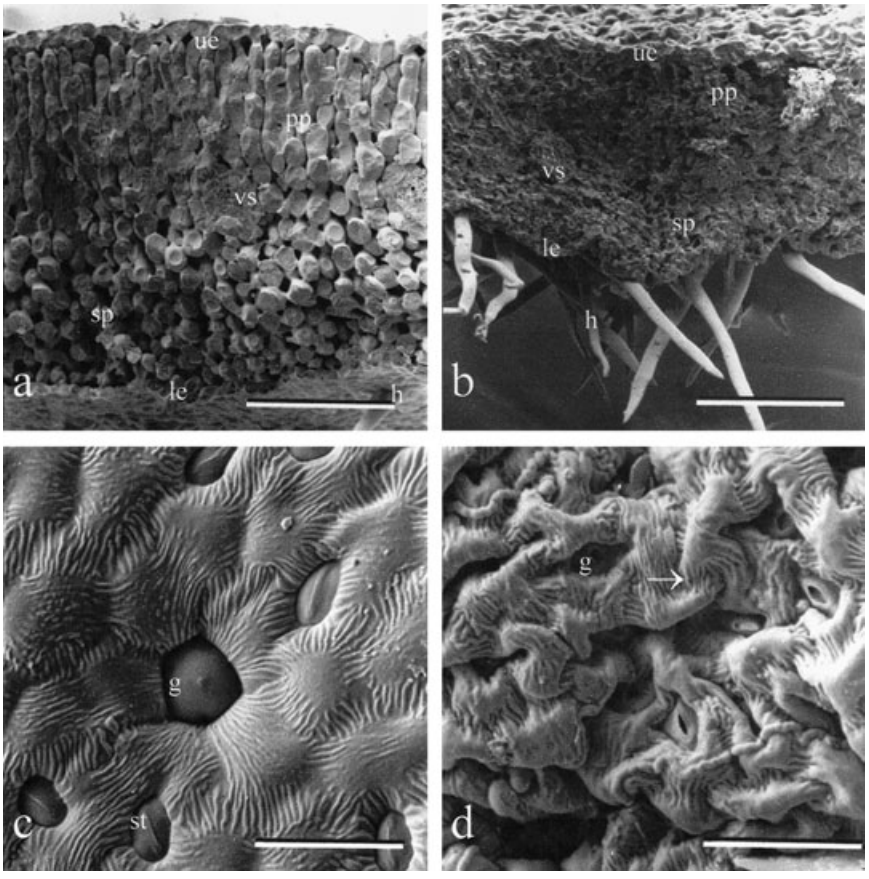

Figure 2. Cryoscanning electron micrographs of hydrated and dried *C. wilmsii* leaves. (a, b) Transverse sections of hydrated (a) and dried (b) *C. wilmsii* leaves. (c, d) Surface view of the epidermis of hydrated (c) and dried (d) leaves. Glands are present in the epidermis. Note that in the dry leaves, the stomata are largely open. g, gland; h, hair; le, lower epidermis; st, stomata; sp, spongy parenchyma; pp, palissadic parenchyma; ue, upper epidermis; vs, vessels; →, cell wall folding. Bars: a, b = 200 μm; c, d = 40 μm (From Vicré *et al*. 2004. With permission).

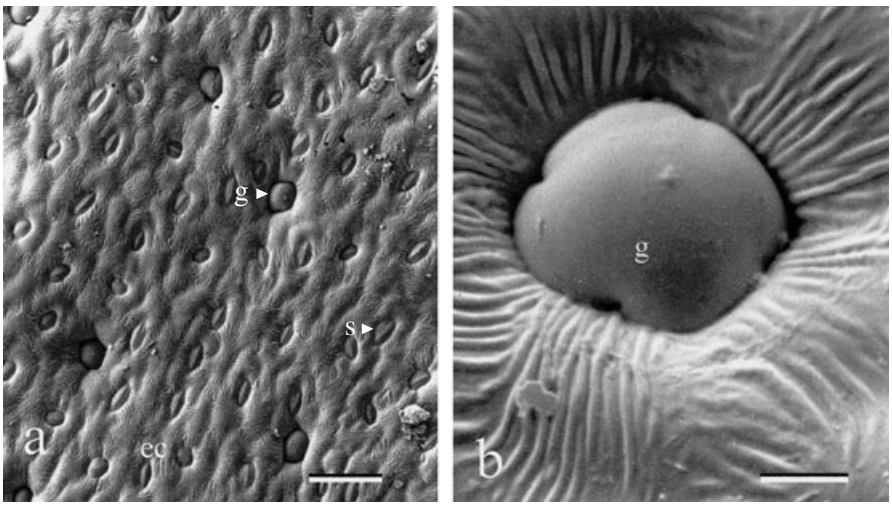

Figure 3. Scanning electron micrographs showing the upper epidermal surface of hydrated leaves of *C. wilmsii.* (a) Epidermis presents numerous glands and stomatas. (b) Gland at higher magnification. g, gland; s, stomata; ec, epidermal cell. Bars: a = 60 μm; b = 8 μm (from Vicré 2001).

RWC. These results suggest that zeatin and zeatin riboside are involved in desiccation tolerance of *C. wilmsii,* and most probably in recovery of metabolism during rehydration (Vicré 2001). Indole-3-acetic acid (IAA) content increases markedly during dehydration of *C. wilmsii* (Fig. 4c). This increase is initiated very early during dehydration and the maximum is detected at a RWC of 20%; a second increase occurs during rehydration (Vicré 2001). The role of IAA in

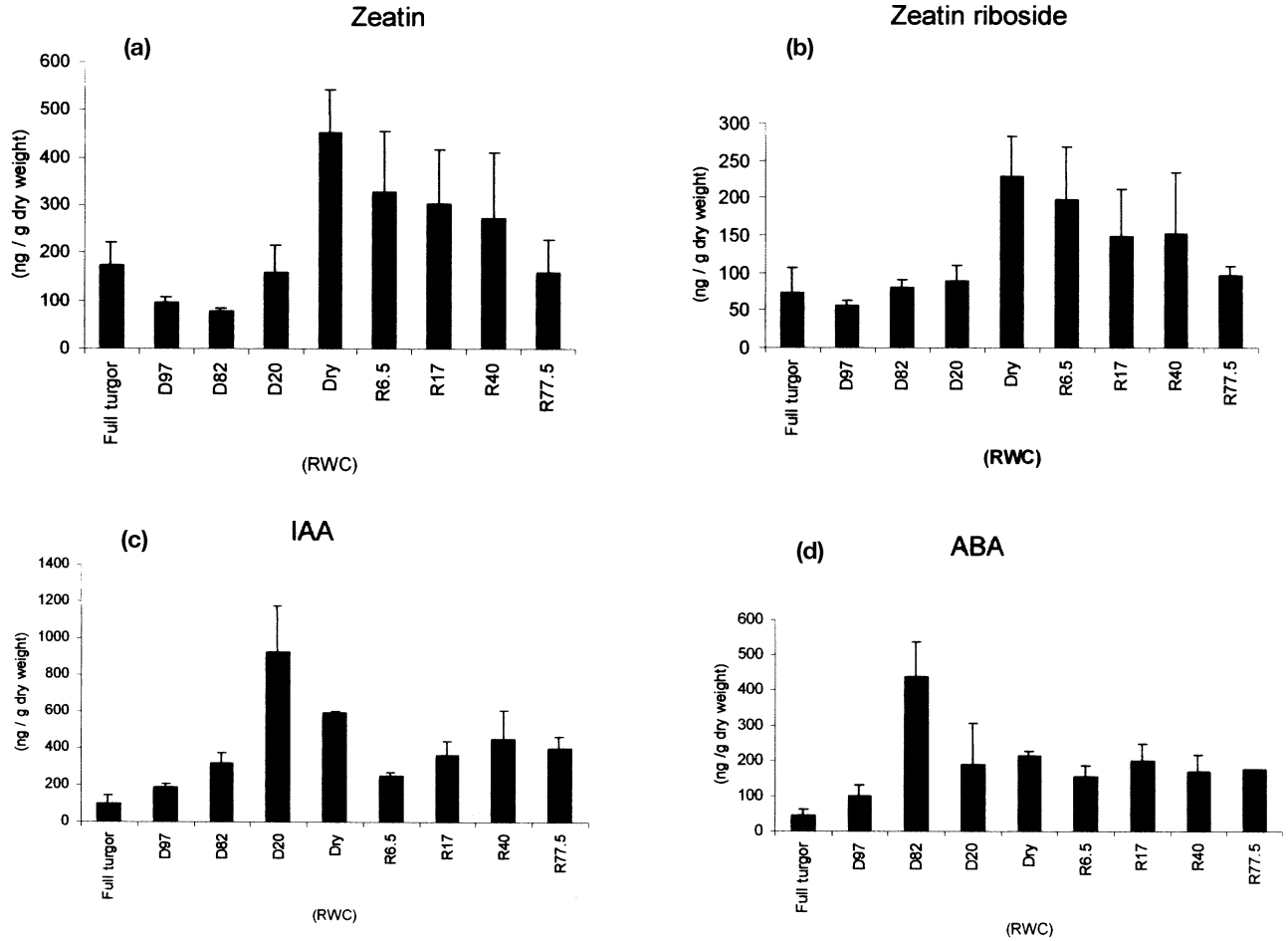

Figure 4. Hormone content in hydrated and dry leaves of *C. wilmsii.* Leaves were analysed using radioimmunoassay (RIA) technique. The free forms of the following plant hormones cytokinins zeatin (a) and zeatin riboside (b), auxin (c) and abscisic acid (d) were determined at different water content during dehydration and rehydration. The results are the average on triplicates performed on two different lots of plants (from Vicré 2001).

desiccation tolerance is still not clear. Many studies have shown the effect of IAA on cell wall enzymes (Inouhe & Nevins 1991; Xu *et al*. 1995; Wu *et al*. 1996) and IAA might play a role by acting to regulate some cell wall enzymes in *C. wilmsii* during desiccation. However, the effects of IAA on cell wall enzymes during the process of dehydration remain to be investigated.

It is now well established that one of the earliest responses to stress in plants is the accumulation of abscisic acid (ABA). The response of ABA to water stress is extremely rapid in the case of *C. wilmsii* (Fig. 4d), in which a slight decrease in relative water content (RWC 97%) resulted in increased levels of this hormone (Vicré 2001). ABA plays a key role in induction of desiccation tolerance (Neale *et al*. 2000). Bartels *et al*. (1990) demonstrated that application of exogenous ABA was able to induce desiccation tolerance in callus from *C. plantagineum*. ABA has been shown to be associated with expression of several dehydration- regulated genes in resurrection plants (Bartels *et al*. 1990, 1992; Michel *et al*. 1993; Nelson, Salamini & Bartels 1994; Ingram & Bartels 1996; Phillips *et al*. 2002). The mechanisms induced by ABA and the components of the signalling pathway have been mainly studied in *C. plantagineum* by D. Bartels' group (Bartels *et al*. 1990; Kirch, Nair & Bartels 2001; Hilbricht *et al*. 2002; Phillips *et al*. 2002) and in *Borya constricta* and *Sporobolus stapfianus* by D. Gaff's group (Gaff & Loveys 1984; Neale *et al*. 2000). It has been shown that application of exogenous ABA induces desiccation tolerance in callus from *C. plantagineum* as well as the expression of many dehydration-regulated genes in several resurrection plants (Bartels *et al*. 1990, 1992; Michel *et al*. 1993; Nelson *et al*. 1994; Ingram & Bartels 1996; Phillips *et al*. 2002). ABA is also thought to be involved at least partially in dehydration-activated signal transduction (Shinozaki & Yamaguchi-Shinozaki 1997; Frank *et al*. 1998). Recent results on gene expression suggest the coexistence of ABA-dependent and ABA-independent signalling pathways interacting to induce gene expression (Shinozaki & Yamaguchi-Shinozaki 1997). The ABA-inducible genes present specific ABA-responsive elements termed ABRE's in their promoter regions (Mundree *et al*. 2002). *CDeT27-45*, a *lea*-like gene from *C. plantagineum* whose transcripts accumulate during drying or in response to exogenous ABA, provided a useful model to study ABA-dependent signalling pathways (Bartels *et al*. 1990; Piatkowski *et al*. 1990). Michel *et al*. (1993) identified the specific ABA responsive region of the *CDeT27-45* promoter and Nelson *et al*. (1994) discovered that the ABA-induced expression of *CDeT27-45* is dependant on DNA-binding activity by nuclear proteins. More recently Hilbricht *et al*. (2002) identified a novel SAP-domain transcription factor (CpR18) binding to the promoter region of *CDeT27-45*. Furini *et al*. (1997) also isolated a gene (*CDT-1*) which is regulated by ABA and is able to activate a pathway inducing desiccation tolerance in *C. plantagineum.* Other transcription factors such as homeodomain-leucine zipper (HD-ZIP) proteins were shown to be inducible by ABA (Frank *et al*. 1998). Interestingly Frank *et al*. (1998) have isolated two HD-ZIPs family genes (*CPHB-1* and *CPHB-2*) showing different responses to ABA. Whereas both transcripts accumulated during drying in *C. plantagineum*, only the transcript level of *CPHB-2* increased in response to ABA. The phytohormone ABA plays a major role in desiccation tolerance not only by inducing regulatory proteins but also by regulating the expression of functional proteins such as LEA proteins (Piatkowski *et al*. 1990; Ingram & Bartels 1996). In a recent study, Phillips *et al*. (2002) reported on a novel gene family involved in maintenance of chloroplast integrity and whose expression is rapidly induced in response to dehydration and exogenous ABA treatment. Furthermore, several aquaporins which are up regulated by dehydration are also inducible by ABA in *C. plantagineum* (reviewed in Ramanjulu & Bartels 2002).

How do resurrection plants cope with oxidative stress?

Interruption of the metabolism due to water stress causes a number of perturbations, the most critical being oxidative stress. Oxidative stress is the result of drying-induced disruption of the electron transport which causes oxygen free radical production. The major sites of such production are the mitochondria and chloroplasts. When desiccation occurs in the light, there is an active production of singlet oxygen by transfer of excitation energy from chlorophyll to oxygen. Since this energy cannot be dissipated via photosynthetic pathways it is used to photo-reduce oxygen, initiating the free radical generating process and formation of reactive oxygen species (ROS) (Seel *et al*. 1992a,b; Smirnoff 1993). The formation of ROS, and more especially superoxide (O_2^-) and hydroxyl (OH^-) radicals, results in damage to essential cellular components such as nucleic acids, polysaccharides, proteins and membrane lipids (reviewed in Mundree *et al*. 2002). Hence, to prevent damage associated with oxidative stress, resurrection plants appear to have evolved various protective mechanisms which vary among plant species (discussed below). It has been hypothesized that, for angiosperm resurrection plants, these mechanisms are more preventive during drying than repairing upon rehydration (Oliver & Bewley 1997; Oliver, Wood & O'Mahony 1998).

Some resurrection plants such as *Xerophyta* species lose their chlorophyll and thylakoid membranes during desiccation. These plants are termed poikilochlorophyllous plants (PDT) and many of them are monocotyledons (Gaff 1977; Hetherington *et al*. 1982; Tuba *et al*. 1994; Sherwin & Farrant 1996; Farrant *et al*. 2003). This strategy avoids free radical formation caused by energy transfer from excited chlorophyll to oxygen but has the disadvantage that the photosynthetic system has to be resynthesized *de novo* upon rehydration which retards the recovery rate. The homoiochlorophyllous desiccation-tolerant plants (HDT) retain chlorophyll and maintain their photosynthetic apparatus during dehydration and tend to undergo morphological changes during drying to protect their tissues from oxidative stress (Sherwin & Farrant 1998; Farrant *et al*.

1999; Farrant 2000; Koonjul *et al*. 2000). A significant part of the photosynthetic apparatus is preserved during desiccation which allows a quicker recovery during rehydration (Sherwin & Farrant 1996, 1998; Kranner *et al*. 2002). As chlorophyll is retained in the desiccated state, homoiochlorophyllous plants also need better antioxidant protection against free radical attack compared with the poikilochlorophyllous plants (Kranner *et al*. 2002).

Resurrection plants minimize the formation of ROS by not only preventing the opportunity for light/chlorophyll interaction but also by quenching these processes via antioxidants (Farrant *et al*. 2003). Resurrection plants upregulate various antioxidant protectants during drying (Kranner *et al*. 2002). When exposed to direct sunlight, cells of both HDT and PDT plants accumulate pigment such as carotenoids or anthocyanins during drying (Sherwin & Farrant 1998; Farrant 2000). It has been hypothesized that for HDT plants such as *Craterostigma* species, these pigments could act as a 'sun-screen' masking the chlorophyll from excessive radiation (Gaff 1989; Sherwin & Farrant 1998). Furthermore, by acting as antioxidants, they can also minimize damage caused by free radicals (Smirnoff 1993; Farrant 2000). Enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (AP) and glutathione reductase (GR) are known to regenerate antioxidants (ascorbate, glutathione) and also accumulate in leaves of these plants during drying (Ingram & Bartels 1996; Sherwin & Farrant 1998). In *X. viscosa*, activities of AP, GR and SOD were found to increase during dehydration (Sherwin & Farrant 1998). More recently Mowla *et al*. (2002) identified in *X. viscosa* a novel stress-inducible antioxidant enzyme, XvPer1, belonging the peroxiredoxin group of enzymes. XvPer1 is of the subtype 1Cys-Prx (reviewed in Mundree *et al*. 2002). The enzyme 1Cys-Prx has been reported to be expressed in the nuclei of immature embryo and aleurone cells of angiosperm seeds but it has never been found in vegetative tissues even under stress conditions. Mowla *et al*. (2002) observed that it was transcribed soon after *X. viscosa* had been exposed to environmental or abiotic stresses (dehydration, heat or cold) but was absent in healthy unstressed plants. Immunofluorescence studies showed that XvPer1 is localized within the nucleus of dehydrated leaf cells suggesting its implication in the protection of nucleic acids against oxidative injury (Mowla *et al*. 2002; Mundree *et al*. 2002). Furthermore Kirch *et al*. (2001) have recently isolated an aldehyde dehydrogenase gene *Cp-ALDH* from *C. plantagineum*. Aldehydes are highly reactive and toxic components which are formed during abiotic stress. The authors suggested that the aldehyde dehydrogenase Cp-ALDH could be involved in the detoxification processes of damaging aldehydes and could play a role in oxidative stress protection.

Maintenance of integrity of the subcellular milieu

Sugars as protectants of membranes and cytoplasm

During dehydration stress, resurrection plants accumulate carbohydrates such as trehalose or more particularly sucrose (Bianchi *et al*. 1991; Drennan *et al*. 1993; Whittaker *et al*. 2001). Trehalose occurs predominantly in desiccation-tolerant less complex organisms including some vascular plants whereas sucrose is found in all angiosperms and mosses studied to date (Kaiser & Gaff 1985; Drennan *et al*. 1993; Ghasempour *et al*. 1998; Scott 2000; Whittaker *et al*. 2001). The leaves of the resurrection plant *M. flabellifolius* contain high amounts of trehalose, which is not common in higher plants, and sucrose. The amount of these two sugars increases considerably during dehydration, suggesting they are probably involved in desiccation tolerance (Bianchi *et al*. 1993; Drennan *et al*. 1993). Whittaker *et al*. (2001) found that upon dehydration the hexokinase activity increased significantly during sucrose accumulation in *X. viscosa* and this was correlated with a removal of glucose and fructose. These authors have also shown the importance of hexokinases in the mobilization of sucrose during rehydration for purposes of recovery metabolism (Whittaker *et al*. 2002). In *C. plantagineum*, an extremely high concentration of the unusual C8 sugar 2-octulose occurs in hydrated leaves (Bianchi *et al*. 1992). During drying, the level of this sugar declines and this is inversely proportional to the accumulation of sucrose (Ingram & Bartels 1996). These authors have proposed that 2-octulose is converted into sucrose upon drying. This conversion is correlated with an increase in the expression of sucrose synthase and sucrose phosphate synthase (Ingram *et al*. 1997; reviewed in Ramanjulu & Bartels 2002).

The presence of water is a key element to maintain the assembly of phospholipids into biological membranes and for the correct conformation of proteins. Recent work on desiccation tolerance in seeds (reviewed in Leprince & Hoekstra 1998) provides evidences that accumulation of sugars is essential to avoid protein denaturation and to preserve membrane integrity. As the water is lost from the cells, the cytoplasmic content becomes highly viscous and molecular interactions which can cause protein denaturation and membrane fusion are likely to happen. Compatible solutes such as trehalose and sucrose have been shown to prevent these adverse molecular interactions from occurring. Upon early drying, the sugars are preferentially excluded from proteins and membranes and this process allows these macromolecules to be coated by a water layer which provides hydration and maintains their native conformation. When disappearance of this water shell occurs upon further dehydration, the sugar molecules act as a substitute for water as they can bind the proteins and the lipids via hydrogen interactions thus stabilizing the protein structure and membranes in a glassy cytoplasm (reviewed in Hoekstra *et al*. 2001). Under severe dehydration, the cytoplasm vitrifies and becomes brittle but with the physical properties of a liquid (i.e. the glassy state). At this stage of dehydration, a mixture of carbohydrates including sucrose together with other molecules is mainly involved in the stabilization of the cytoplasm in a glassy matrix (Buitink, Hemminga & Hoekstra 2000).

Late embryogenesis abundant proteins and desiccation tolerance

Late embryogenesis abundant (LEA) proteins represent an important group that is well known to accumulate during the late stage of embryogenesis or in response to dehydration. They are divided into different groups according to sequence similarity and biochemical properties. Dehydrins, also termed 'LEA D11 family' consist of the most studied group of LEA proteins (Ingram & Bartels 1996; Ramanjulu & Bartels 2002) and they have been especially well described during embryogenesis and cold acclimatation (van Zee *et al*. 1995; Close 1996; Bravo *et al*. 1999; Ismail, Hall & Close 1999).

Dehydrins and LEA proteins have been shown to accumulate in resurrection plants during drying (Bartels *et al*. 1993; Michel *et al*. 1994; Velasco, Salamini & Bartels 1994; Alamillo & Bartels 1996; Ndima *et al*. 2001). The exact role of these proteins in desiccation tolerance is still unclear. Dehydrins are thought to be mainly structural stabilizers and possess chaperonin-like properties (for review Hoekstra *et al*. 2001). Dure *et al*. (1989) proposed that the hydroxyl groups on the surface of these polypeptides might be a substitute for water, so maintaining the integrity of macromolecules and membranes. Charged amino acids in such proteins may also serve to neutralize the increasing concentration of ions during desiccation (Dure 1993). In the resurrection plant *C. plantagineum*, Schneider *et al*. (1993) reported the presence of three desiccation-related proteins localized in the cytosol and two others in the chloroplast, one in the stroma, the other one associated with thylakoid membranes. Recently, Koag *et al*. (2003) have shown that the dehydrin DHN1 from maize was able to bind to lipid vesicles containing acidic phospholipids and that this association leads to an increase of α-helicity of the dehydrin. The authors suggested that DHN1 could stabilize the membranes under stress. LEA proteins possess high numbers of polar residues and it is thought that they protect other proteins and macromolecules by conferring a preferential hydration during the first stage of dehydration. After further dehydration, their own hydroxylated residues could replace the loss of water according to the 'replacement water' theory (for review Hoekstra *et al*. 2001).

Mechanical stress

One of the major stresses plants have to overcome in order to survive desiccation is the mechanical stress that occurs as the water is lost from the cells (Iljin 1957). A very similar stress happens upon rehydration when the water rushes into the cells. In the hydrated state, plant cells are characterized by having a single or several large water-filled vacuoles. When plants dry, the water is lost from vacuoles and cytoplasm shrinks creating tensions between the plasmalemma and the more rigid cell wall which cannot undergo a corresponding shrinkage in surface area. This can result in a tearing of the plasmalemma and thus irreversible damage to the cells.

Osmolyte accumulation preserves a normal osmotic pressure

Microscopical studies have shown that during drying a fragmentation of the main vacuole into several small vacuoles occurs in many of the resurrection plants (Gaff, Zee & O'Brien 1976; Hallam & Luff 1980; Farrant & Sherwin 1997; Farrant 2000; Vander Willigen *et al*. 2003, 2004). In the resurrection plants *C. wilmsii*, *E. nindensi*s, *M. flabellifolius* and *X. humilis*, the main water-filled vacuoles present in hydrated cells are replaced by small vacuoles filled with non-aqueous substances (Farrant 2000; Vander Willigen *et al*. 2004). It is proposed that accumulation of osmolytes such as sugars and proline in the vacuoles during drying might offers a mechanism to preserve a normal osmotic pressure in the cells and prevent mechanical stress (Tymms & Gaff 1979; Bohnert, Nelson & Jensen 1995; Farrant 2000; Vander Willigen *et al*. 2004).

Importance of the cell wall in preserving cell integrity

Folding and structural alterations of the cell wall

For some of the resurrection plants such as the modified desiccation-tolerant spike moss *Selaginella lepidophylla* and the *Craterostigma* species (Fig. 5), upon drying, cell walls from leaf tissues fold in along with the cell contents and become highly convoluted (Sherwin 1995; Platt, Oliver & Thomson 1997; Thomson & Platt 1997). When the plant is rehydrated, cells return to their original volume without apparent injury. It has been proposed by Farrant & Sherwin (1997) that the folding of the cell wall could be a strategy developed by the plant to avoid the tearing of the plasmalemma from the cell wall and to maintain its integrity during drying. If this is the case, wall folding could be a mechanism to minimize mechanical stress.

Such folded cell walls are also a common feature in dry seeds and the manner of the cell wall collapse is characteristic for a given species. Webb & Arnott (1982) suggested that this wall folding in seeds is essential for preserving the structural integrity of the tissue and to retain its viability upon rehydration. It was suggested that this folding depends on cell wall composition and structure.

Both biochemical and immunocytochemical studies show that the overall cell wall composition of *C. wilmsii* leaves was similar to that of other dicotyledonous plants with respect to the pectin content. Biochemical data indicated that leaves were characterized by a large proportion of homogalacturonan and the occurrence of rhamnogalacturonans. Immunogold labelling revealed a specific distribution within cell walls depending on the nature of pectins. β(1–4) galactans recognized by the antibody LM5 were mostly associated with the cell wall domain close to the plasma membrane (Vicré *et al*. 1999). A similar distribution was found for the polysaccharide bupleuran IIC and rhamnogalacturonans II (RGII). Relatively low methylesterified pectins, detected by JIM5, were abundant and equally distributed in parenchyma cell walls (Vicré 2001). This result might be surprising as JIM5 is often associated with middle

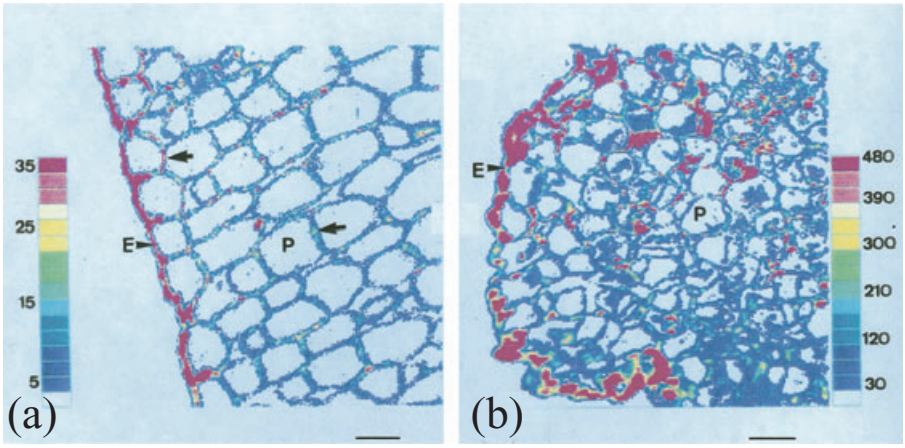

Figure 5. Visualization of calcium distribution in transverse sections of hydrated (a) and dry (b) *C. wilmsii* leaves using SIMS technique. Calcium is mostly associated with the cell walls (←). However the dry tissue has a much more higher signal compared with hydrated tissues (note the different values in the two colour scales). The false colour on the scales indicates the ions intensities (counts for an acquisition time of 5 min) within an image. (E and arrowhead) epidermis; (P) parenchyma cells. Bars: a, b = 30 μm (from Vicré 2001).

lamella and cell junctions (Knox *et al*. 1990). The epitope recognized by anti-PGA/RG1 antibodies was essentially located in the middle lamella area and cell junctions as was described for other tissues (Moore & Staehelin 1988).

The structure of the hemicellulosic polysaccharide xyloglucan (XG) was characterized to be XXGG-type lacking the fucose substitution (Vicré *et al*. 2004) like XG from Solanaceous plants (Vincken *et al*. 1997) and the storage XG of tamarind seeds (York *et al*. 1993). The hemicellulose XG was found in the whole cell wall although cell junctions were sometimes less abundantly labelled (Vicré *et al*. 1999).

Immunocytochemical analysis and quantification showed an increase in labelling of XG and unesterified pectins PGA/RG1 upon drying which was not apparent with the other wall components such as β(1–4) galactans or RGII (Sherwin *et al*. 1997; Vicré *et al*. 1999; Vicré 2001). Biochemical data suggest that modifications of PGA/RG1 pectin labelling could be attributed to modifications of pectin solubilization and/or changes in the organization and bonding with other wall components rather than to specific changes in amount of these polysaccharides.

Marked changes were observed in the hemicellulosic wall fractions from dried plants compared with hydrated ones (Vicré *et al*. 2004). The most conspicuous change was a decrease in glucose content in the hemicellulosic fraction of dry plants. In addition, XG from the cell wall of dry leaves was relatively more substituted with galactose than in hydrated walls. As a consequence, XG of hydrated plants, with a higher proportion of less substituted domains would appear to be richer in Glc content in comparison with its Xyl and Gal contents. If this were the case, cleavage and/or partial degradation of XG could possibly allow modification in the mechanical properties of *C. wilmsii* cell walls, increasing its elasticity in response to dehydration. In this context, it is worth noting that Sherwin (1995) demonstrated that the leaves of *Craterostigma nanum* become more elastic during dehydration. Considering the large degree of shrinkage in cell volumes and the greater capacity of the wall to fold, it seems reasonable to expect that the cell walls of leaves of *C. wilmsii* would be remarkably elastic during drying in order to prevent any irreversible damage.

Calcium content of the cell wall

Calcium is known to play a major role in cell growth and development in higher plants. One of the most important functions of this ion is its involvement in cross-linking of pectic polysaccharides to form 'egg-box' structures that confer rigidity to the cell wall (Carpita & Gibeaut 1993).

We have quantified Ca^{2+}, using secondary ion mass spectrometry (SIMS) technology and found an important increase in calcium in the cell walls in dry leaves of *C. wilmsii* in comparison with hydrated leaves (Vicré *et al*. 1998; Vicré 2001) (Fig. 5). Such a cell wall-specific increase was not seen in dehydrated leaves of *Saintpaulia ionantha*, a desiccation-sensitive plant we studied recently (Gibouin *et al.* unpublished). Energy dispersive spectrometry (EDS) analysis on both frozen-hydrated and freeze-dried samples also confirms this increase, at least for epidermal cells. Micro-PIXE (proton-induced-X-ray emission) analyses of freeze-dried leaves reveal an increase in Ca^{2+} concentration in parenchyma of dry leaves in comparison with hydrated leaves. This result is an average of a small region of tissue and therefore the increase could be associated either with the cell wall, with the cytoplasm or with both compartments. To determine if this ionic increase in dry cell walls was specific for calcium, the content of others ions was also assessed. Together the results for K^+ and P did not reveal any particular increase of these ions in the cell wall of dry plants compared to hydrated ones. Thus, the Ca^{2+} increase in cell walls of dry plants is probably not due to a general increase in the ionic concentration during dehydration. Such an increase of Ca^{2+} in walls of dry plants may have an impact on the mechanical properties of the cell walls during dehydration. More specifically Ca^{2+} might be necessary to cross-link cell wall polymers such as acidic pectins and further stabilize cell wall architecture in the dry state (Vicré 2001).

These findings indicate that dehydration induces significant alterations in the polysaccharide content and structure of the cell wall of *C. wilmsii*, which in turn may be involved in the modulation of the mechanical properties of the wall during dehydration (Vicré *et al*. 2004). Upon rehydration, *C. wilmsii* mesophyll cell walls were not able to unfold

properly if mRNA or protein synthesis was inhibited by distamycin A or cycloheximidine, respectively (Cooper & Farrant 2002). Furthermore, if leaves were rapidly dried and then rehydrated to 100% RWC in distamycin A, the cell walls were still highly folded. Although it is conceivable that many of the proteins needed for the reversal of this process are synthesized before or during desiccation, it appears that newly synthesized components are also required upon rehydration. Together these data strongly suggest that cell wall folding and unfolding are a well-controlled phenomenon in *C. wilmsii* rather than a simple collapse of the cells.

CONCLUSION

Analysis of desiccation tolerance in resurrection plants such as *C. plantagineum* and *X. viscosa* suggests that some of the molecules involved in tolerance mechanisms are similar to those involved in desiccation-tolerance of orthodox seeds (Farrant & Sherwin 1997; Bartels & Salamini 2001; Mundree *et al*. 2002). Indeed, the shutting down of metabolism on drying and the recovery after a period of desiccation suggests parallels with maturation of seeds and their subsequent germination (Proctor & Pence 2002). Although most of the plants have a stage in their life cycle that can tolerate desiccation, resurrection plants are remarkable as they can use the same mechanisms to protect their vegetative tissues such as leaves and roots when extreme drought stress conditions occur.

By using a molecular approach, genes specifically expressed during desiccation and rehydration stages have been identified (Bernacchia, Salamini & Bartels 1996; Mundree *et al*. 2000; Bartels & Salamini 2001; Mowla *et al*. 2002; Collett *et al*. 2003). More work is necessary to define gene functions and understand the complex regulation of their expression but results to date have already provided attractive insights into the protective mechanisms involved in desiccation tolerance. Although resurrection plants are of no immediate economic value to agriculture, they provide unique model systems to investigate possible mechanisms for improving drought tolerance of crop plants (Mundree *et al*. 2002). Successful genetic engineering of metabolic pathways for a number of compatible solutes such as sorbitol, mannitol and trehalose, as well as the synthesis of several individual antioxidant enzymes, for example SOD, catalase and AP have been reported in transgenic plants (for review see Kranner *et al*. 2002; Ramanjulu & Bartels 2002). Very interestingly, Xu *et al*. (1996) discovered that the ABA-inducible gene *HVA1* from barley seeds which encodes a group 3 LEA protein was able to confer drought tolerance to transgenic rice plants.

Drought is one of the major problems facing agriculture in Africa and it has been reported than only 11.6% of the land of South Africa is suitable for growing crops (reviewed in Mundree *et al*. 2002). It is clear that understanding the molecular basis of desiccation tolerance in the resurrection plants is of great importance and represents promising prospects to allow subsequent transformation of agronomically important African crop species.

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