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Minireview

Physiological and molecular insights into drought tolerance

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Water is a major limiting factor in world agriculture. In general, most crop plants are highly sensitive to even a mild dehydration stress. There are however, a few genera of plants unique to Southern Africa, called "resurrection plants" which can tolerate extreme water loss or desiccation. We have used *Xerophyta viscosa*, a representative of the monocotyledonous resurrection plants to isolate genes that are associated with osmotic stress tolerance. Several genes that are differentially expressed, and that confer functional sufficiency to osmotically-stressed *Escherichia coli* are being studied at the molecular and biochemical levels. In this review, we use this as a basis to discuss the physiological and molecular insights into drought tolerance.

Key words: Drought stress, reactive oxygen species, osmoprotectants, abscisic acid, transcription factors.

INTRODUCTION

Water is a fundamentally important component of the metabolism of all living organisms, facilitating many vital biological reactions by being a solvent, a transport medium and evaporative coolant (Bohnert et al., 1995). In plants and other photoautotrophs, water plays the additional role of providing the energy necessary to drive photosynthesis. Water molecules are split, in a process termed autolysis, to yield the electrons that are used to drive the energy yielding photosystem II reaction centre (Salisbury and Ross, 1992a). One of the major consequences of drought stress is the loss of protoplasmic water leading to the concentration of ions such as Cl^- and NO_3^-. At high concentrations these ions effectively inhibit metabolic functions (Hartung et al., 1998). Additionally, the concentration of protoplasmic constituents and the loss of water from the cell lead to the formation of what is termed a glassy state. In this state whatever liquid is left in the cell has a very high viscosity, increasing the chances of molecular interactions that can cause protein denaturation and membrane fusion (Hartung et al., 1998; Hoekstra et al., 2001). Plants thus require the genetically encoded ability to ensure the maintenance of cellular turgor and metabolic functions.

Our research group aims to develop transgenic plants, both crops and grasses, able to withstand drought. The source of genes is the desiccation-tolerant plant *Xerophyta viscosa* Baker (Family Velloziaceae) which belongs to a small group of angiosperms, referred to as "resurrection plants" because they are capable of tolerating extremes of desiccation (Farrant, 2000; Gaff, 1977). *X. viscosa* can be dehydrated to 5% relative water content (RWC) and upon rewatering the desiccated plant rehydrates completely within 80 hours, resuming full physiological activities (Sherwin and Farrant, 1998). The genes that will be discussed in this review include ones that code for an antioxidant, *XVPer1*; a subunit c-like protein of the vacuolar H^+-adenosine triphosphatase, *VATP1XV*; galactinol synthase, *XVGols*; aldose reductase, *ALDRXV4*; a cell membrane binding protein, *XVSAP1*; and a transcription factor, *DREB*1A. To determine the effects of the expression of these genes in monocots we first introduce them into the grass *Digitaria sanguinalis* for which we have developed a transformation system (Chen et al.,

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1998). Thereafter, if results are positive, the genes are transformed into crops such as maize (*Zea mays*). To do the same for dicots we first introduce them into *Arabidopsis thaliana* and *Nicotiana tobacum*.

Desiccation tolerance is a complex phenomenon, involving the co-ordinated expression of a large number of genes (Walters et al*.,* 2002). It is thus likely that in order to obtain drought tolerant plants more than one gene will need to be co-expressed. For instance, expression of *XVPer1,* which is thought to protect DNA from reactive oxygen species, together with *XVSAP1*, which probably prevents membrane leakage, *XVGols* and *ALDRXV4,* both osmoprotectants, might result in a suite of proteins that together confer drought tolerance.

SOME PHYSIOLOGICAL AND STRUCTURAL CHANGES AT THE WHOLE PLANT AND CELLULAR LEVEL

Since the response of plants to drought is complex and diverse (for reviews see Alpert and Oliver, 2002; Levitt, 1980; Walters et al., 2002), it is unrealistic to consider that there is a single "gene for drought tolerance" and hence the need for an understanding of the physiological response of plants under water-limited conditions is of critical importance.

Unlike "normal" plants, which maintain water potentials above that of the surrounding environment and attempt to continue to function during water limited periods, desiccation-tolerant plants use a very different strategy: the vegetative tissues lose all free water and then rehydrate once water becomes available again. This unusual ability to survive extreme water loss in the vegetative tissues has been observed in only approximately 100 angiosperms (Gaff, 1977). Although these resurrection plants are of no immediate economic value to the agricultural industry, a greater understanding of this phenomenon may provide further insights into possible mechanisms for improving drought tolerance of crop plants. In fact, there are numerous means by which different plants respond to drought (summarised in Figure 1).

The response of resurrection angiosperms to water deficits contrasts with that of most other plants (which are unable to survive extreme water loss). The former have a number of protection mechanisms, instated during drying, to minimize the stresses associated with

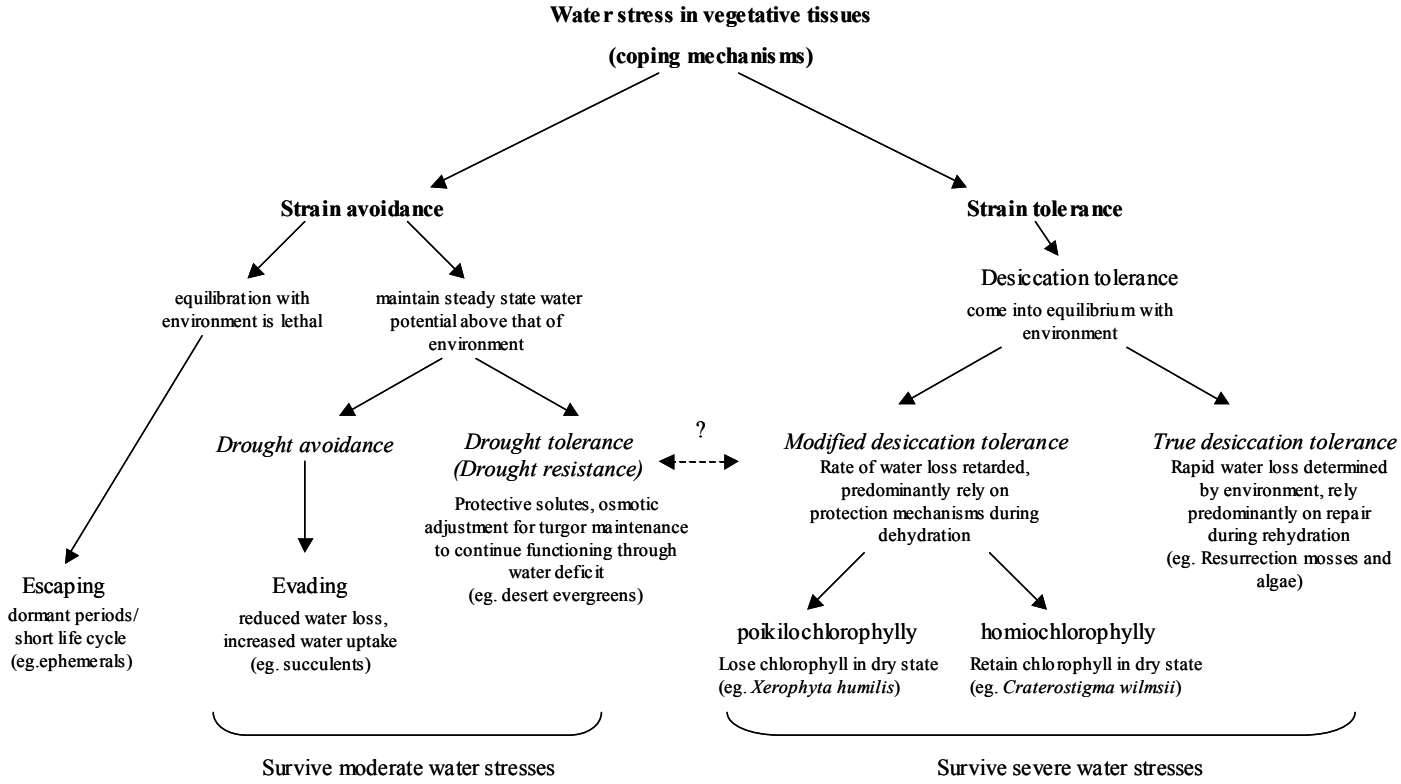

Figure 1. Classification of mechanisms utilized by plants in coping with water stress.

severe cellular water deficit (reviewed by Farrant, 2000; Gaff, 1989; Oliver et al., 1998; Vertucci and Farrant, 1995). One of the associated stresses in photosynthetically active tissues is light. Under water limiting conditions, excitation energy harnessed by chlorophyll cannot be dissipated via photosynthesis and can lead to the formation of oxygen free radicals, which if left unquenched, can cause considerable subcellular damage (Halliwell, 1987; Kaiser, 1987; Larson, 1988; Smirnoff, 1993). Within the resurrection angiosperms, two distinct patterns in the manner in which they protect themselves from photo-oxidative damage are known. Homiochlorophyllous angiosperms (such as *Craterostigma wilmsii* and *Myrothamnus flabellifolius*) protect their chlorophyll from light by leaf folding and anthocyanin accumulation whereas poikilochlorophyllous angiosperms (such as *Xerophyta viscosa* and *Eragrostis nindensis*) disassemble chloroplasts and chlorophyll on drying (Farrant, 2000; Gaff, 1989; Oliver et al*.,* 1998). Since these protection mechanisms (shading or dismantling of the photosynthetic machinery) are unique to resurrection plants, the corresponding controlled reduction in the net photosynthetic rate during water deficits is not seen in other higher plants (Figure 2). Although the response of other higher plants can vary (for review see Lawlor and Cornic, 2002), the shutdown in carbon assimilation is usually as a consequence of the water limitation itself. Interestingly in plants which are able to continue growing under moderate water deficits (such as *E. curvula*), photosynthesis only plummets at the lethal relative water content of that species. As water becomes limiting in the environment, cellular volume in plant tissues is reduced. In desiccation-sensitive plants, once turgor is lost, the mechanical strain on the cellular membranes and cell walls usually results in cytorhysis (cell wall collapse) and membrane damage, which is irreparable (Vander Willigen et al., 2001a). However, in the resurrection plants, the mechanical stress associated with cell volume reduction is counteracted by a number of different protection mechanisms. In some species such as *M. flabellifolius, C. wilmsii* (Farrant, 2000; Vicre et al., 1999) and *E. nindensis* (Vander Willigen et al., 2001b)*,* mesophyll cells show significant cell volume reduction associated with a regulated phenomenon of cell wall folding. In other resurrection angiosperms like *X. humilis, X. viscosa* (Farrant, 2000; Mundree and Farrant, 2000) and *E. nindensis* (Vander Willigen et al., 2001b), bundle sheath cells maintain cell volume by multiple (small) vacuole formation. Water is replaced within these vacuoles by compatible solutes such as proline (Vander Willigen et al., 2002).

Apart from these structural changes which occur in response to drying, a number of biochemical modifications are necessary to protect against metabolic imbalances caused by cellular water loss. Some of these are reviewed below.

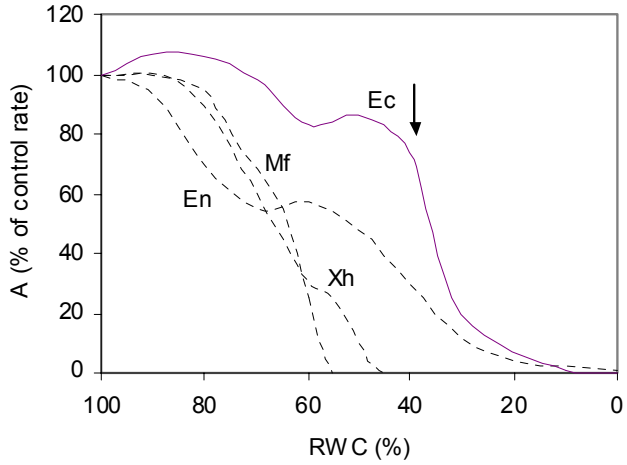

Figure 2. Net photosynthetic rates during drying of the resurrection angiosperms *M. flabellifolius* (Mf), *X. humilis* (Xh) and *E. nindensis* (En) and a drought tolerant grass, *E. curvula* (Ec) expressed as a percentage of the hydrated leaf tissue. Arrow indicates the relative water content (RWC) beyond which Ec cannot recover.

THE ROLE OF REACTIVE OXYGEN SPECIES IN DROUGHT STRESS

A common aspect of most adverse environmental conditions is the increased production of reactive oxygen species (ROS) within several subcellular compartments of the plant cell (Van Breusegem et al., 2001). ROS can occur as by-products of regular cellular metabolism such as in photosynthesis. However, under stress, their formation is usually exacerbated. Drought stress leads to the disruption of electron transport systems and thus under water deficit conditions the main sites of ROS production in the plant cell are organelles with highly oxidizing metabolic activities or with sustained electron flows: chloroplasts, mitochondria and microbodies. Within the photosynthetic apparatus, photosystem II (PS II) is affected most by drought stress, particularly within the oxygen-evolving complex and the reaction centres (Toivonen and Vidaver, 1988; He et al., 1995).

In general, ROS (particularly superoxide and hydroxyl radicals) are damaging to essential cellular components such as DNA, proteins and lipids. Lipid peroxidation disrupts the membrane integrity of the plant cell. As a result, essential solutes leak out of organelles and from the cell, causing disruption in membrane function and metabolic imbalances. DNA is the blueprint for both

future form and function. Any damage to its integrity could mean that proteins that would have been essential for optimal function of the plant will not be synthesised. Similarly, denaturation of important proteins essential for biochemical reactions leads to the whole plant being negatively affected and unable to cope.

Plants have evolved complex protective mechanisms to prevent the damage initiated by free radicals. The primary constituents include antioxidant enzymes such as superoxide dismutase, catalases and peroxidases, and free radical scavengers such as carotenoids, ascorbate, tocopherols and oxidized and reduced glutathione (GSSG and GSH, respectively) (Price et al., 1994). Superoxide dismutase regulates the cellular concentration of O_2^- and H_2O_2. The latter is broken down by catalases and peroxidases. Under moderate stress conditions, the radicals are efficiently scavenged by this antioxidant defence system. However, in periods of more severe stress in desiccation-sensitive plants, the scavenging system becomes saturated by the increased rate of radical production, and damage is inevitable.

Resurrection plants, in addition to structural changes which minimise ROS formation (outlined above), upregulate various antioxidant protectants during drying and rehydration (Farrant, 2000; Kranner et al., 2002; Sherwin and Farrant, 1998). In *X. viscosa*, activities of ascorbate peroxidase, glutathione reductase and superoxide dismutase were found to increase during dehydration (Sherwin and Farrant, 1998).

A Novel Stress-inducible Antioxidant in *X. viscosa*

By differential screening of a cDNA library of *X. viscosa*, a novel stress-inducible antioxidant enzyme, XvPer1, has been identified (Mowla et al., 2002). It is classified as a peroxiredoxin (Prx). Although it belongs to a phylogenetically old group of enzymes, its catalytic function has only recently been investigated (Dietz et al., 2002). Four different clusters of related proteins are distinguished within Prx: 1Cys-Prx, 2Cys-Prx, type II-Prx, and Prx Q. All Prx are characterized by one or two conserved cysteine residue(s) essential for the catalytic function. XvPer1 is of the sub-type 1Cys-Prx. Although the cellular function of the 1Cys-Prx is not completely understood, its protein structure has been elucidated (Stacy et al., 1999). It is a fairly small protein (~24 kDa) and is highly conserved in organisms as widely divergent as the *Archea* bacteria, plants and humans (Kang et al., 1998). The first plant 1Cys-Prx was discovered while investigating dormancy in seeds (Goldmark et al., 1992). Since then it has been found to be expressed in the nuclei of immature embryo and aleurone cells of angiosperm seeds, but absent in vegetative tissues under both normal and stress conditions (Stacy et al., 1996). A unique feature of the *X. viscosa* 1Cys-Prx is that it is expressed in the vegetative tissues under stress conditions and is absent in healthy unstressed plants. It is transcribed soon after the plant is exposed to various environmental and abiotic stresses such as dehydration, heat, cold, highlight and abscisic acid (ABA) (Mowla et al., 2002).

We postulate that *X. viscosa*, being a resurrection plant, has certain “seed-specific” behaviours, such that it can remain dormant in a dry state for long periods of time without sustaining major tissue damage. Hence it is not surprising that certain “traditionally” seed-specific genes are expressed in leaf tissues of the plant when under stress. Furthermore, immunolocalization studies localized XvPer1 to the nucleus of dehydrated *X. viscosa* cells. Previous functional assays on 1Cys-Prx have revealed that it is a DNA-protecting enzyme (Stacy et al., 1996). A rice 1Cys-Prx, over-expressed in transgenic tobacco, enhanced resistance against oxidative stress (Lee et al., 2000). This gene is thus of great relevance in our aim to develop drought-tolerant plants.

THE EFFECTS OF DROUGHT STRESS ON ION HOMEOSTASIS

Homeostasis can be broadly defined as an organismal response to an environmental change in which some aspect of the plant’s internal condition is maintained relatively constant, or is allowed to vary only within limits in spite of more drastic changes in that same factor in the environment (Salisbury and Ross, 1992b). When applying this definition to ion homeostasis in plants it implies that plants employ a means of coping with environmental fluctuations in ion concentrations at the cellular, tissue and whole plant level. According to the United Nations Environmental Programme approximately 50% of cropland in the world is affected by salinity (Flowers and Yeo, 1995). In arid conditions farmers need to irrigate their crops more and the water acts as a source of Na^+ (Serrano et al., 1999). When the water evaporates, ions such as calcium and magnesium often precipitate into carbonates and in most cases Na^+ remains as the dominant cation. Salinity stress is very often represented by and attributed to an increase in intracellular Na^+. Drought and salinity impose an osmotic stress on plants by decreasing the chemical activity of water and causing loss of turgor within the cell.

The Role of Vacuoles in Ion Homeostasis

The plant vacuole primarily maintains cellular turgor pressure along with other functions such as giving the cell shape and rigidity, increasing the cellular surface

area to facilitate efficient photosynthesis and absorption of nutrients and storage of various compounds such as sugars, polysaccharides, organic acids, amino acids, pigment compounds and compounds that could be toxic to the cell if released into the cytoplasm (Taiz, 1992). The vacuole also plays a vital role in maintaining ion homeostasis between itself and the cytoplasm. This is achieved by facilitating the functioning of ion pumps, ion channels, H^+-pyrophosphatases (PPase) and vacuolar H^+-adenosine triphosphatases (V-ATPase). Ion transporters and their regulatory systems fulfil several crucial physiological roles such as establishing and maintaining intracellular ion concentrations within the optimal range for normal cellular functions. In plant cells this translates into high concentrations of K^+ and magnesium; and low concentrations of Na^+, protons and calcium (Serrano et al., 1999). Why these relative ionic ratios are preferred within the cell is not yet fully understood, but it can be stated that a neutral pH is preferred for the optimal functioning of proteins and a high magnesium/calcium ratio favours the solubility of phosphate compounds. High intracellular concentrations of chloride and Na^+ ions are toxic to cell systems because they interfere with the hydrophobic-electrostatic forces which assist molecules to maintain their native state. Ion transporters create cell turgor, which is facilitated by high K^+. The generation of electrochemical gradients across membranes energises the active transport of nutrients and ions into the cytoplasm, using membrane-bound H^+-cotransporters.

Cellular H^+ pumping to Cope with Ionic Stress; P-ATPase and V-ATPase

Compartmentalization of cytotoxic ions from the cytoplasm is necessary to facilitate the function of specific ion ratios as signal determinants. This is largely achieved by H^+ adenosine triphosphatases (H^+ ATPases) located on the plasma membrane (P-ATPases), or localised on the vacuole tonoplast (V-ATPases) and H^+ pyrophosphatases (PPases). Essentially, these pumps establish an H^+ electrochemical potential across their respective membranes, which can be used as the driving force for secondary active transport. They also establish membrane potential gradients that facilitate electrophoretic ion flux. As our research concentrates on the V-ATPase from *X. viscosa,* this complex will be discussed in greater detail.

The V-ATPase is a multiheteromeric subunit protein with an accumulative molecular mass of over 700kDa, a cytoplasmic catalytic domain (V1) and a tonoplast-anchoring domain (V0). V-ATPases are commonly composed of at least 11 subunits arranged in a head/stalk/base fashion (Ratajczak, 2000). V1 domain subunits bind and conduct the catalytic cleavage of ATP, the energy source for the translocation of H^+ into the vacuolar lumen. V0 is primarily composed of 6-12 copies of a 16 kDa proteolipid protein, also referred to as subunit c (Ward and Sze, 1992). Subunit c homologues share common characteristics such as four transmembrane domains and a conserved glutamic acid residue in the fourth transmembrane domain which functions in binding the H^+ to be translocated. When the glutamic acid residue 137 in the *Saccharomyces cerevisiae* subunit c homologue, VMA3, was substituted with any non-negatively charged amino acid, V-ATPase activity was completely lost (Ratajczak, 2000). Copies of subunit c arrange themselves into a ring-like H^+-translocating pore. The H^+-electrochemical potential across the tonoplast generated by V-ATPases is used to drive the secondary transport of ions such as Na^+ into the vacuole by an NHX1-like Na^+/ H^+ antiporter (Serrano and Rodriguez-Navarro, 2001).

The expression and regulation of V-ATPase subunits has been investigated in a range of plants, but of particular interest is the regulation of subunit c homologues in response to salinity stress. In the halophyte *Mesembryanthemum crystallinum* (common ice plant) it has been shown that the activity of V-ATPase increased when the plant was treated with NaCl (Ratajczak et al., 1994; Tsiantis et al., 1996). Subunit c transcripts were found in leaves and roots of 6-week-old *M. crystallinum* treated with 350mM NaCl (Tsiantis et al., 1996). In whole plants and in a cell suspension culture of *Beta vulgaris* subunits A and c showed differential expression in response to NaCl (Kirsch et al., 1996; Lehr et al., 1999). Subunit c homologue *Vac1* transcripts in the moss *Tortula ruralis,* increased in the polysomal RNA fraction, but the VAC1 protein levels remained at a steady state (Chen et al., 2002).

In our research, cDNA levels of a subunit c homologue isolated from the resurrection plant *X. viscosa* were significantly upregulated when the plant was subjected to desiccation stress (Mundree and Farrant, 2000). This protein, VATP1XV (formerly referred to as XV5, Mundree and Farrant, 2000) shares high homology at the amino acid level to other subunit c homologues and has all the properties of a subunit c-like protein (unpublished data). By upregulating the expression of *vatp1xv* in salinity stressed plants the auto-assembly of native V-ATPase subunits should be stimulated. Functional V-ATPases complexes would act to translocate protons into the lumen, establishing the electrochemical gradient for active transport of solutes into the vacuole. This would then stabilize the osmotic imbalance across the tonoplast.

THE ROLE OF OSMOPROTECTANTS

One of the mechanisms that plants use to combat the detrimental effects of water loss is to synthesise compatible solutes, typically certain polyols, sugars, amino acids, betaines and related compounds (Bohnert et al., 1996; Ramanjulu and Bartels, 2002). By definition compatible solutes are synthesised in response to osmotic stress and can occur at high intracellular concentrations without hindering normal cellular metabolism (Ramanjulu and Bartels, 2002). The properties of compatible solutes facilitate the maintenance of favourable turgor pressure during water stress and in addition may serve as protective agents by stabilising proteins (Figure 3) (Carpenter et al., 1990). Compatible solutes have also been shown to function as free radical scavengers, protecting DNA from the degradative effects of reactive oxygen species (Akashi et al., 2001).

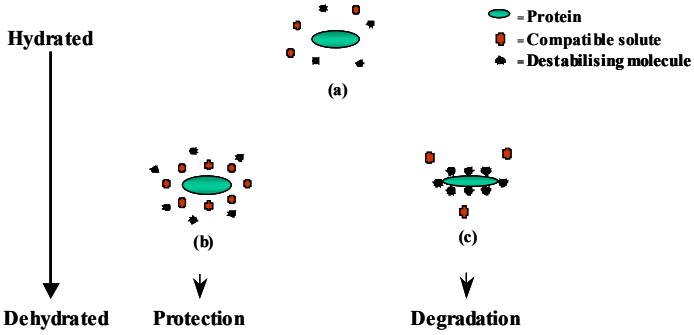

Figure 3. diagram illustrating the function of compatible solutes. (a) In the hydrated state, the presence of water reduces the interaction of destabilising molecules. (b) In tolerant cells the synthesis of compatible solutes preferentially excludes the binding of destabilising molecules and stabilises native protein conformation. (c) In sensitive cells the lack of compatible solutes results in the preferential binding of destabilising molecules to the protein surface, leading to degradation. (Adapted from Hoekstra et al., 2001).

Of the genes identified thus far in *X. viscosa* two drought responsive cDNAs, encoding a galactinol synthase (*XVGols*) and an aldose reductase (*ALDRXV4*) may be important components in compatible solute biosynthesis. *XVGols* was found to be up-regulated in the leaves of *X. viscosa* during drought stress. GolS enzymes represent the first step in the synthesis of Raffinose Family Oligosaccharides (RFOs), major soluble carbohydrates occurring in the seeds and other vegetative tissues of plants (Peterbauer et al., 2002). Galactinol, an α-galactoside of myo-inositol, is an unusual molecule found exclusively in plants (Peterbauer et al., 2002). Biosynthesis of galactinol is catalysed by the galactosyltransferase galactinol synthase, which utilises myo-inositol and UDP-galactose as substrates (Sprenger and Keller, 2000).

RFOs are derivatives of sucrose to which galactosyl units are added to the glucose moiety of sucrose, via α-(1,6) linkages. The most common are the trisaccharide raffinose, the tetrasaccharide stachyose and the pentasaccahride verbascose (Bentsink et al., 2000). The first step in the biosynthesis of RFOs is the reversible transfer of the galactosyl residue from the donor molecule, galactinol, to sucrose. This reaction results in the formation of raffinose, the first member in the family, which serves as the acceptor molecule for the transfer of another galactosyl residue from galactinol, to form stachyose. The reactions are catalysed by the enzymes raffinose synthase and stachyose synthase respectively (Peterbauer et al., 2001). Although higher derivatives of RFOs are known to exist, very little is known about their biochemistry.

RFOs have been extensively characterised as principle agents in carbon translocation in plants (Bentsink et al., 2000; Haritatos et al., 2000; Peterbauer et al., 2001; Sprenger and Keller, 2000). These studies have, in addition, observed a correlation between RFO accumulation and cold, drought or salinity stress, implying a role for RFOs in stress adaptation. Three stress responsive *GolS* genes out of seven *GolS* genes in *A. thaliana* have been identified (Taji et al., 2002). Two of these were found to be up-regulated by drought and high salinity stresses whilst the third was found to be induced by cold stress. Transgenic *A. thaliana* plants that overexpressed one of the drought responsive genes were found to contain elevated levels of both galactinol and raffinose and displayed improved drought tolerance relative to wild type plants. This result implicates potential roles for galactinol and raffinose as compatible solutes. The presence of multiple *GolS* genes with modulated expression in response to various stresses is highly likely. In *Ajuga reptans,* two functional GolS isoforms involved in carbon translocation have been identified (Sprenger and Keller, 2000). Our work has identified *XVGolS*, as well as a second *GolS* isolated from a cDNA library generated from cold stressed *X. viscosa* leaves (unpublished data). Future work examining the levels of galactinol and raffinose during drought stress in *X. viscosa* and *D. sanguinalis* plants that over-express XVGolS will help in understanding the role that RFOs play in desiccation tolerance.

Aldose reductases (ARs) are NADPH-dependent monomers belonging to the aldo-keto reductase superfamily (Bohren et al., 1989). These enzymes are responsible for the reduction of sugars to their corresponding alcohols and have broad catalytic

activities on aldose sugars and aldehydes. ARs serve as the rate limiting enzymes in polyol biosynthetic pathways and are involved in the synthesis of sorbitol from glucose-6-phosphate via the intermediate sorbitol-6-phosphate (Rakowitz et al., 2002; Ko et al., 1997).

In our research northern and western blot analyses showed that the AR isolated from *X. viscosa* (*ALDRXV4*) was expressed in the leaves during drought stress. Levels of the polyol sorbitol were also found to be elevated in the leaves during drought stress (Mundree et al., 2000). The accumulation of sorbitol has been demonstrated in some higher plants subjected to chronic hyperosmosis (Ahmad et al., 1979). We additionally showed that ARs from *X. viscosa* leaves utilise glucose as a substrate. It is likely that ARs in *X. viscosa,* such as *ALDRXV4,* function in polyol biosynthesis during drought stress, converting glucose-6-phosphate to sorbitol.

In many higher plants under dehydration stress, carbohydrate metabolism is shifted to favour the conversion of other sugars to sucrose (Norwood et al., 1999; Whittaker et al., 2001). We have studied the effect of dehydration on sucrose accumulation in the resurrection plants *X. viscosa*, *Sporobolus staphianus* and C*raterostigma wilmsii* (Cooper and Farrant, 2002, Whittaker et al., 2001; Mundree and Farrant 2000). In all cases increases in sucrose accumulation in response to drought stress was observed, albeit at varying levels for the different species - implicating a role for sucrose in the acquisition of desiccation tolerance in these plants. Sucrose is thought to function as a typical osmoprotectant, stabilising cellular membranes and maintaining turgor. As an easily metabolisable reducing sugar, sucrose may serve as an immediate energy source upon rehydration.

The successful engineering of metabolic pathways for a number of compatible solutes such as glycine betaine, sorbitol, mannitol, trehalose and proline have led to reported results of transgenic plants which display increased resistance to drought stress, high salinity and cold stress (for review see Chen and Murata, 2002). It is thus likely that compatible solute biosynthesis is a key mechanism which enables drought tolerant plants to survive the debilitating effects of water loss to the cell. Engineering *ALDRXV4* and *XVGolS* into drought sensitive plant species such as the monocot grass *D. sanguinalis,* would help to investigate the functions of these enzymes in osmolyte biosynthesis and provide the basis for subsequent transformation of agronomically important African crop species such as maize.

CELL MEMBRANE STABILITY

The cell membrane plays an important role in maintaining cell integrity, being involved in signal transduction and ion homeostasis during drought stress conditions. Osmotic shock to osmosensitive cells leads to irreversible damage of the cell membrane. On the other hand osmotolerant cells can survive water stress only if they have inherent mechanisms involved in membrane stability. Osmotic shock involves an automatic amphiphile-induced membrane perturbation at the onset of drying and has been implicated to have a signalling function. The signal induced by the membrane perturbation leads to expression of late embryogenesis abundant proteins and heat shock proteins in seeds (Hoekstra et al., 2001). Upon dehydration, cytoplasmic amphiphilic compounds increase in concentration and partition into membranes and cause disturbance in both tolerant and sensitive cells. In the intermediate phase, in tolerant cells, the presence of solutes (sugars) keeps the membrane surface preferentially hydrated and prevents membrane fusion. Below 0.3 gH_2O/g dry weight, sugar molecules in tolerant cells replace water in the hydration shell of the membrane, thereby maintaining the spacing between phospholipid molecules.

Garwe et al. (2002) have suggested that a cDNA, *XVSAP1*, from a cDNA library from dehydrated leaves of *X. viscosa*, plays a role in membrane stability. XVSAP1 shares high identity with the K^+ transporter family. The predicted XVSAP1 consisted of a highly hydrophobic protein with two membrane lipoprotein lipid attachment sites. The presence of these sites supports the concept that XVSAP1 associates closely with the cell membrane and could be one of the components involved in the repair of membrane damage resulting from water deficit.

TRANSCRIPTIONAL FACTORS TARGETING STRESS RESPONSIVE GENE EXPRESSION

Genes induced under water-deficit stress can be divided into two groups. The first code for proteins directly involved in protection, the second for proteins involved in regulation of signal tranduction and gene expression. Following cellular perception of water loss, a signalling mechanism must be activated to induce specific genes (Bray, 1997). Different conditions induce different stress-induced genes and thus there must be several different signalling mechanisms. One of the major signals operating during water stress is the plant hormone abscisic acid, ABA. Studies on ABA have shown that this hormone mediates various developmental and physiological processes that affect the agronomic performance of crop plants such as embryo maturation and germination as well as the response of vegetative tissues to osmotic stress (Ramagopal, 1987; Zeevaart and Creelman, 1988). ABA increases as a result of water stress and has important

roles in the tolerance of plants to drought, high salinity and cold.

Expression patterns of drought-induced genes are complex. Some genes respond to the stress very rapidly, whereas others are induced slowly after the accumulation of ABA. Most of the genes that respond to drought are also induced by exogenous application of ABA (Shinozaki and Yamaguchi-Shinozaki, 1997). There are some genes that are induced by drought stress but are not responsive to exogenous ABA treatment. These findings suggest the existence of both ABA-independent and ABA-dependent signal transduction cascades between the initial signal of drought or cold stress and the expression of specific genes (Bray, 1997). These dehydration inducible genes contain potential abscisic acid responsive elements (ABRE's) with the sequence (PyACGTGGC) in their promoter regions. An ABRE functions as a *cis*–acting DNA element involved in ABA regulated gene expression and was first identified in wheat *Em* and rice *rab* genes. An ABRE-DNA-binding protein EmBP-1 was shown to encode a bZIP protein. The G-box resembles the ABRE motif and functions in the regulation of plant genes in a variety of environmental conditions. A coupling element is required to specify the function of the ABRE constituting an ABA responsive complex in the regulation of the *HVA22* gene (Shen and Ho, 1995). Other *cis*-acting elements that function in ABA responsive gene expression not only under drought stress conditions but also in seed desiccation are the Sph box and the MYB and MYC binding sites of the *Arabidopsis rd22* gene. More recently a transcriptional factor (CpR18) from the resurrection plant *Craterostigma plantagineum* which binds to the promoter region necessary for ABA mediated expression of the *CDeT27-45* gene was isolated. This gene has a 29 bp (AAGCCCAAATTTCACAGCCCGATAACCG) *cis*-regulatory region present in the drought inducible *CDe27-45* gene promoter of *C. plantagineum* (Hilbricht et al*.,* 2002).

Genes induced by drought, but which do not require ABA, include *rd29A*, *kin1*, *cor6.6* and *cor47* (Shinozaki and Yamaguchi-Shinozaki, 1997). The *rd29A* gene from *A. thaliana* was extensively analysed and a 9 base pair conserved sequence (TACCGACAT), termed dehydration responsive element (DRE) was found to be essential for the regulation of the induction of *rd29A* under stress conditions. DRE does not function as an ABRE and therefore is involved in ABA independent gene expression. DRE related motifs including C repeat (CRT), which contains a CCGAC core motifs are thought to be expressed in the absence of ABA. Protein factors that bind to DRE have been cloned using the yeast one hybrid screening method. All these proteins contain a conserved DNA binding motif, for example, the ethylene response element binding protein (EREBP) and AP2 proteins, which are involved in ethylene responsive gene expression and floral morphogenesis, respectively. Other DRE-binding proteins called *DREB*1A and *DREB*2A, also specifically bind and activate transcription of genes containing the DRE sequence of *A. thaliana* (Kasuga et al*.,* 1999).

Results have shown that co-operative action of *cis*-elements and the promoter configuration is crucial for regulation by abscisic acid (Busk and Pagès, 1998). Furthermore, several elements are organ and species-specific. Recent studies of the chromatin structure of abscisic acid-responsive genes point to the importance of induction of transcription by coactivators or by phosphorylation or dephosphorylation of transcription factors. Three main levels of regulation modulate transcription factor activity. (i) Transcription factors can be sequestered in the cytoplasm and rendered inactive through lack of access to their target sequences. Phosphorylation of the factor itself or a cytoplasmic anchor protein allows translocation of the transcription factor into the nucleus where it acts. (ii) The DNA binding activity of nuclear transcription factors can be modulated by phosphorylation either positively or negatively. (iii) Phosphorylation can affect the interaction of transcription factor transactivation domains with the transcriptional machinery (Hunter and Karin, 1992). These possibilities are by no means mutually exclusive, and in principle phosphorylation at multiple sites by different protein kinases can result in regulation at several distinct levels.

DRE is essential for the regulation of dehydration responsive gene expression and was found to function as a *cis*-acting element involved in the induction of *rd29A* expression by low temperature in *A. thaliana* (Liu et al., 1998). It is important to understand how two different stress signals, drought and cold, are transmitted separately in plant cells to activate DRE-dependent transcription of the *rd29A*/*cor78* gene. Expression of the *DREB*1A cDNA under the control of the 35S cauliflower mosaic virus (CaMV) promoter in transgenic *Arabidopsis* plants gave rise to strong constitutive expression of the stress-inducible genes, and increased tolerance to freezing, salt, and drought stresses (Liu et al., 1998). The overexpression of the *DREB*1A cDNA not only induced strong expression of the target genes under unstressed conditions but also caused 'dwarfed' phenotypes. However, expression of the *DREB*1A cDNA under the control of the *rd29A* promoter produced transgenic *Arabidopsis* plants that exhibited improved stress tolerance as well as improved growth under unstressed conditions (Kasuga et al*.,* 1999). In contrast, overexpression of the *DREB*2A cDNA induced weak

expression of the target genes under unstressed conditions and caused growth retardation of the transgenic plants (Liu et al., 1998). These results indicate that the two independent families of *DREB* proteins function as *trans*-acting factors in two separate signal transduction pathways under low temperature and dehydration conditions.

DRE was also found to function in stress response in tobacco plants (Yamaguchi-Shinozaki and Shinozaki, 1993; Yamaguchi-Shinozaki and Shinozaki, 1994), which suggests the existence of similar regulatory systems in tobacco and other crop plants. DRE related motifs have been reported in the promoter regions of cold inducible *Brassica napus* and wheat genes (Jiang et al., 1996; Ouellet et al., 1998). These observations suggest that both the *DREB*1A cDNA and the *rd29A* promoter can be used to improve the dehydration, salt, and freezing tolerance of agriculturally important crops by gene transfer (Kasuga et al., 1999).

CONCLUDING REMARKS

It is clear that insights into the physiology and molecular biology of drought tolerance in resurrection plants such as *X. viscosa* will enable us to determine which genes could, either singly or in combination, confer some degree of similar tolerance to transgenic plants. Drought is one of the major problems facing agriculture in sub-Saharan Africa. For example, according to the Food and Agricultural Organisation, only 11.6% (14.2 million hectares) of the land in South Africa is suitable for growing crops and 93% (13.17 million hectares) of this is already used for agriculture. The economic losses from insufficient water supply are major and considerable increase in agricultural productivity can be brought about by the production of genetically modified drought tolerant crops.

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