BOTANICAL BRIEFING

Resurrection Plants and the Secrets of Eternal Leaf

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Most higher plants possess a phase in their life cycle in which tissues can survive desiccation. However, this is restricted to specialized tissues such as seeds and pollen. Resurrection plants are remarkable in that they can tolerate almost complete water loss in their vegetative tissues. The desiccated plant can remain alive in the dried state for several years. However, upon watering the plants rehydrate and are fully functional within 48 h. Underpinning this amazing ability is the capacity to accumulate large amounts of sucrose in the tissues. This sugar has the property of stabilizing enzymes and cellular structures in the absence of water. The sources of carbon that fuel sucrose synthesis are not known, but temporary carbohydrate stores and photosynthesis are the most likely candidates. On rewatering, the sucrose is metabolized rapidly as the tissues rehydrate. Increased expression of a number of genes in response to drought stress have been noted. A number of these are associated with metabolic pathways linked with primary carbohydrate metabolism. However, some genes related to LEA (Late Embryogenic Abundant) proteins have been isolated which suggests they too may play a role in maintaining tissue integrity during desiccation. How these mechanisms are integrated to enable resurrection plants to survive desiccation is discussed.

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Key words: ABA, *Craterostigma*, desiccation tolerance, poikilohydric, resurrection.

INTRODUCTION

Some higher plant species are well adapted to arid environments through mechanisms that mitigate drought stress, including both physiological and biochemical adaptations. Physiological adaptations take many forms ranging from partial senescence of tissues, to structural adaptations such as water storage organs and restrictions in surface area of aerial tissues as seen in Cactaceae and Euphorbiaceae. Biochemical adaptations range from damage limitation mechanisms to additions to photosynthetic pathways such as crassulacean acid metabolism (Smith and Bryce, 1992). All of these mechanisms are very effective and they allow plants to inhabit a wide range of arid environments, but when subjected to prolonged lack of water these plants will dehydrate and die. Although these mechanisms allow plants to lessen the severity of drought stress they do not make the plant tolerant of desiccation.

However, there is a group of higher plants known as poikilohydric or resurrection plants, which possess a uniquely effective mechanism for coping with drought stress by being desiccation tolerant. Virtually all plant species, at some point in their life cycle, are at least partially tolerant of desiccation. For example, seeds and pollen frequently lose large quantities of water during their maturation process (Bewley, 1979). But the ability of mature tissue, such as roots and leaves, to survive virtually complete desiccation is very rare. Resurrection plants survive the loss of most of their tissue water content until a quiescent stage

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is achieved. Upon watering the plants rapidly revive and are restored to their former state. This resurrection capacity is not restricted to the main meristems of these plants. Fully mature leaves can lose up to 95 % of their water content and then, upon rewatering, the leaves are rehydrated and are fully photosynthetically active within 24 h (Bernacchia *et al.*, 1996). Tissue damage through this drying and rehydration process appears to be minimal to non-existent. Unlike other plant responses to drought stress, desiccation in resurrection plants prevents growth and reproduction over the dehydrated period. Instead the plant is preserved until water becomes available; at this point it is ready to take immediate advantage of the new conditions. Thus a resurrection plant has a great competitive advantage over other species for certain ecological niches. In severely drought stressed areas, the plant can remain quiescent for considerable periods of time, but at the first substantial fall of rain it can resurrect, and grow and reproduce long before other species have the opportunity to do so.

RESURRECTION PLANTS

Small numbers of resurrection plant species are represented in most taxonomic groups, ranging from pteridophytes to dicotyledons. This topic has been reviewed thoroughly very recently by Hartung *et al.* (1998). Among these species there are both terrestrial species such as the monocotyledonous plant, *Xerophyta viscosa* (Baker) and the dicotyledonous shrub *Myrothamnus fabellifolia* (Welw.), and an aquatic species *Chamaegigas intrepidus* (Dinter). As would be expected, most resurrection plant species are native to arid
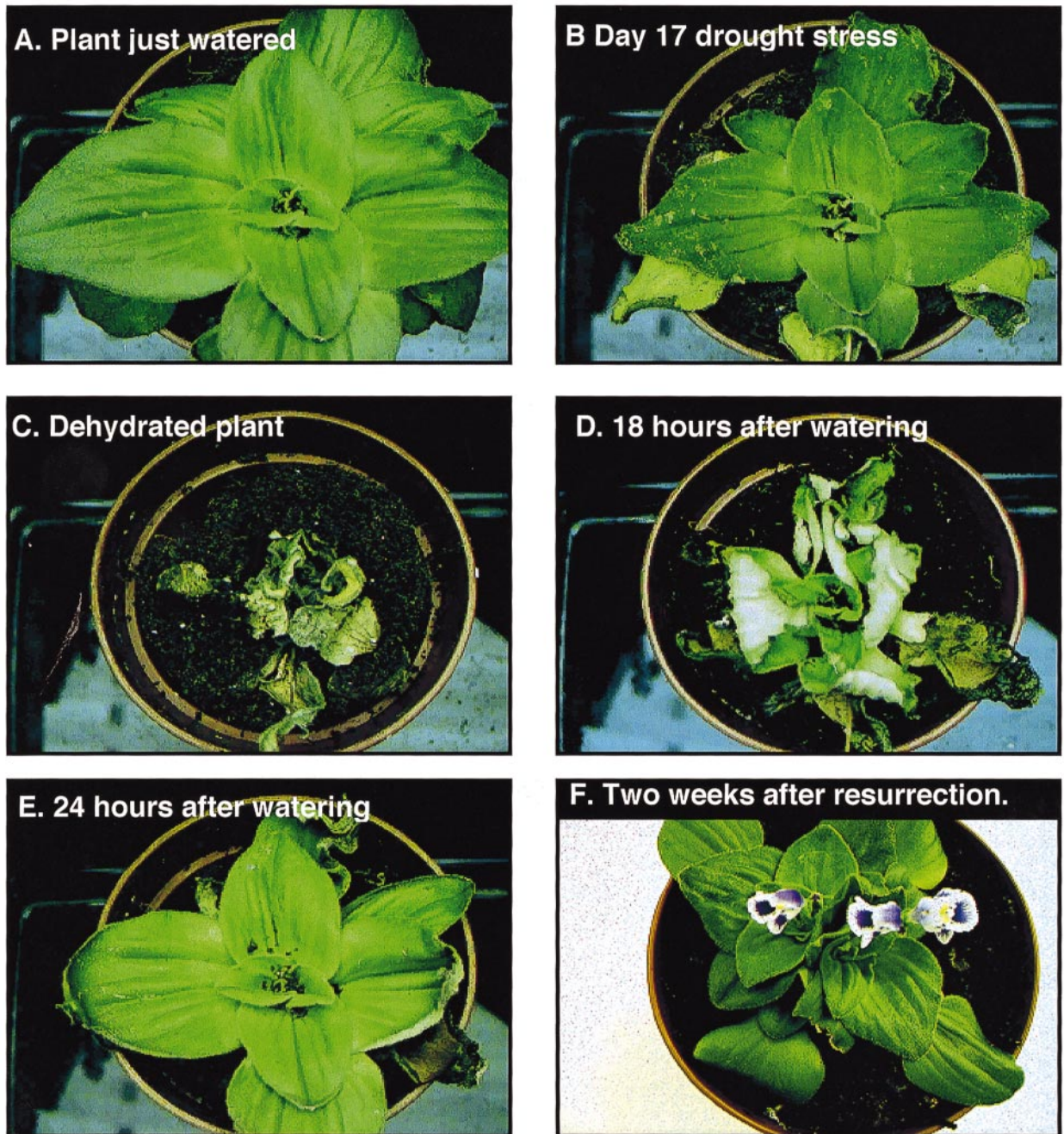
FIG. 1. A–F, Dehydration and rehydration phases of resurrection in *Craterostigma plantagineum*. On day 1 watering ceased (A). Photographs were taken of the same plant for the following 19 d. C shows the fully dehydrated plant. In D the dehydrated plant was watered and then photographs were taken of the plant regularly to follow the physiological processes up to 24 h after the initial watering. E shows a flowering *Craterostigma* plant 2 weeks after the initial watering.

climates in the world such as southern Africa, southern America, and western Australia (Gaff, 1977, 1987). Resurrection plants inhabit ecological niches that are subjected to lengthy periods of drought with periods of rain during the year. They are frequently found as denizens of rocky outcrops, possessing the ability to grow in shallow, sandy soils (Sherwin and Farrant, 1995). Their habitats often take the form of delves on rock outcrops which have the capacity to form ephemeral pools (Gaff, 1971; Gaff and Giess, 1986).

The surrounding rock surface acts to broaden the water catchment area for the plants, so only a couple of millimetres of rain can result in a large accumulation of water in pools. However, the pools formed are temporary, lasting only for a few days at best. The growth and reproduction of the plant occurs in these wet seasons, but upon drying the plants can remain dormant for considerable time periods. The aquatic plant *Ch. intrepidus* has been known to survive for up to 11 months in the dehydrated state (Hartung *et al.*,

1998). However, this period is short compared to *Craterostigma* species which have been known to last at least 2 years without water, and probably much longer.

Mechanisms that protect plants from water stress are frequently effective against other environmental stresses, and resurrection plants are no exception, for example, up to three out of five species of resurrection grasses tested have been shown to be salt tolerant (Gaff and Wood, 1988; Gaff, 1989). In addition, it has been postulated that resurrection plants should be resistant to large fluctuations in temperature (Hartung *et al.*, 1998). This is certainly a necessity for species such as *Craterostigma plantagineum* (Hochst.) which inhabits granite outcrops in southern Africa, where rock surfaces can reach over 60 °C in the dry season (Dinter, 1918). Thus the ability to survive desiccation enables resurrection plants to inhabit ecological niches uninhabitable by most higher plant species.

RESURRECTION PHYSIOLOGY

Given that resurrection plants are found in many plant families, the ability to tolerate desiccation must have arisen numerous times during evolution, and a common physiological basis of how this ability is achieved in plants is therefore unlikely. A similar situation is apparent in CAM plants. CAM species are represented in a wide range of plant families, and although the general malate shuttle
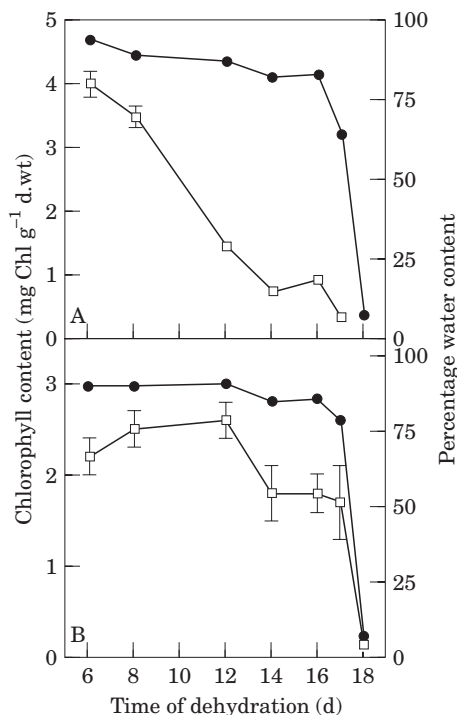

FIG. 2. Measurements of percentage water content and chlorophyll content in young and mature leaves of *Craterostigma plantagineum* during dehydration. The chlorophyll content ($\square$) and percentage water content ($\bullet$) (f.wt − d.wt)/(f.wt × 100) were calculated for *Craterostigma* plants as they were dehydrated. The leaf types are: young (less than 70 % of the final mature size) (A) and mature (B). The results are means ± s.e. from four replicate measurements from different plants.

scheme is common to all species, there are numerous metabolic differences between them (Leegood, von Caemmerer and Osmond, 1997). Thus studying a single resurrection plant is unlikely to explain what occurs in all species, but it is becoming clear that some generalizations can be made. Despite the remarkable abilities of resurrection plants only a minority of species have been studied in any detail, with most physiological and biochemical work being performed on the genus *Craterostigma*, a member of the Scrophulariaceae family. Hence *Craterostigma* has become the model subject for research.

To illustrate the resurrection process, a series of photographs and measurements of the relative water content and chlorophyll amounts in the young and mature leaves of *C. plantagineum* are shown in Figs 1 and 2. One of the most remarkable features of *C. plantagineum* is its ability to shrink during dehydration. This can be seen in the photographs (Fig. 1A–D) and this shrinkage correlates with the point in time when the leaves lose most of their water content (Fig. 2). It has been estimated that leaves of *C. plantagineum* drop to around 15 % of their original area (Hartung *et al.*, 1998). How the leaves achieve this reduction in size is not yet clear, but there is some evidence that the plasmalemma and the cell wall form a concertina that minimizes damage within and between cells. Similar shrinkage has also been observed in *Ch. intrepidus*, where leaves shrink to 25 % of their original size (Schiller *et al.*, 1999). In woody species such as *M. fabellifolia* such severe shrinkage is not seen, but the final relative water content of aerial tissues of this species in the desiccated state is remarkably similar to that reported for *Craterostigma* (RWC approx. 5–10 %).

In contrast to what is known to be occurring in the leaves, very little research has been performed on the roots of resurrection plants. In such tissues the response to drought stress is probably more rapid than in the leaves since it is the roots which will first sense a soil water deficit. In addition, the roots are very much more restricted in their ability to shrink during dehydration since they are embedded in a solid soil matrix. Thus shrinkage of roots beyond that of the soil could result in major root network damage.

Another aspect of the physiology of resurrection plants that has only just begun to receive attention is the response of the xylem to desiccation (Sherwin *et al.*, 1998). The problem is how can the tissue dry out and then reactivate on resurrection? This is no small problem since the formation of air bubbles in the xylem tissue is a well-known cause of xylem blockage (Sperry and Tyree, 1988). The solution in *M. fabellifolia* has been suggested to be narrow reticulate xylem vessels, which cavitate on desiccation of the plant, but refill from capillarity and root pressure on resurrection (Sherwin *et al.*, 1998).

Angiosperms that are resurrection plants have been subdivided into two further groups: homoiochlorophyllous plants, which retain their chlorophyll during drying (such as *Craterostigma wilmsii* (Engl.) and *M. fabellifolia*), and poikilochlorophyllous plants, which lose chlorophyll on drying (such as *X. viscosa*) (Tuba *et al.*, 1993, 1994; Sherwin and Farrant, 1995). The loss of chlorophyll in *C. plantagineum* is clearly seen in Fig. 2. The fall in chlorophyll

content in the leaves of *X. viscosa* is accompanied by the accumulation of anthocyanins (Sherwin and Farrant, 1998). The processes of dismantling the photosynthetic apparatus and the synthesis of anthocyanins are thought to be linked to the protection of plants against UV-light and from damage as a result of oxygen free radical generation during desiccation (Smirnoff, 1993; Smirnoff and Colombé, 1993; Sherwin and Farrant, 1998).

One final remarkable feature of resurrection plants is their ability to rehydrate very rapidly. The slowest documented recovery is for *M. fabellifolia*, which takes approx. 48 h to recover from the desiccated state when watered from the soil alone. Plants such as *C. plantagineum* can routinely revive within less than 24 h. This enables these plants to rapidly take advantage of changes in water availability.

METABOLIC CHANGES DURING RESURRECTION CYCLES

Numerous metabolic changes have been found to occur in resurrection plants as they dehydrate and rehydrate. However, the most thoroughly investigated is that of carbohydrate accumulation. Table 1 shows a summary of measurements of major carbohydrates present in fully hydrated and dehydrated leaves from a range of resurrection plants. In all resurrection plants sucrose is the dominant carbohydrate to accumulate. A minimum of 72 μmol g^{-1} d.wt of sucrose appears to be sufficient to protect the desiccated plant, but data are, as yet, unavailable to judge whether plants with higher final sucrose contents are more desiccation tolerant than other species. *C. plantagineum* stands out from the other species, accumulating almost three-times more sucrose than the highest of the other plant species; why there should be such an excess is not understood. Another feature that stands out from the data in Table 1 is that, for some unknown reason, measurements of sucrose and trehalose in identical plant species by different laboratories are very variable. Although it has been suggested that trehalose accumulation is also significant, the data in Table 1 provide no compelling evidence that trehalose accumulates in resurrection plants during dehydration. Only in a single case, *M. fabellifolia* (Drennan *et al.*, 1993), has trehalose been reported to accumulate to any great extent, and this finding is controversial (Bianchi *et al.*, 1993).

Accumulation of sucrose in tissues of resurrection plants has been proposed to protect the dehydrated cell (Ingram and Bartels, 1996). There are two hypotheses concerning the role of the accumulated sugars. First, the sugars could act to stabilize membranes and proteins in the dry state by maintaining hydrogen bonding within and between macromolecules (Crowe *et al.*, 1986; Allison *et al.*, 1999). Secondly, the sugars could vitrify the cell contents and stabilize internal cell structure (Crowe *et al.*, 1996). Recent evidence strongly supports a combination of these two processes enabling the maintenance of cell integrity during dehydration (Crowe *et al.*, 1998).

The accumulation of sucrose in tissues of resurrection plants may account for a large part of the acquired desiccation tolerance, but is it all that is required? The definitive experiments have yet to be performed, but indications from early experiments look promising. In experiments where isolated enzymes have been dried in the presence of sucrose there is good evidence that the enzymes remain stable in the dried state (Bustos and Romo, 1996; Suzuki *et al.*, 1997). Parallel work on isolated membrane vesicles also supports the view that sucrose preserves the integrity of the lipid bilayer during dehydration (Crowe *et al.*, 1998). Other research has investigated whether a simple living system can be desiccated. *E. coli* cells, which are desiccation intolerant, have been preserved in sucrose or trehalose (Leslie *et al.*, 1995). Although the cells exhibited poor survival rates during desiccation and over time, this may be since the *E. coli* cells do not take up the sugar sufficiently to ensure maximum protection. Data such as

TABLE 1. *Carbohydrate content in fully hydrated and desiccated leaves of a range of different resurrection plant species*

Plant species	Sucrose content		Trehalose content		Reference
	Hydrated	Dehydrated	Hydrated	Dehydrated	
	(μmol g^{-1} d.wt)				
C. plantagineum	73	2000	ND	ND	Bianchi *et al.*, 1991
M. fabellifolia	88	169	81	105	Drennan *et al.*, 1993
	383	463	148	136	Bianchi *et al.*, 1993
R. nathaliae	58	294	ND	ND	Müller *et al.*, 1997
R. myconi	96	204	ND	ND	Müller *et al.*, 1997
H. rhodopensis	60	170	ND	ND	Müller *et al.*, 1997
X. villosa	43	81	ND	ND	Ghasempour *et al.*, 1998
Tripogon jacquemontii	15	72	0·2	1·0	Ghasempour *et al.*, 1998
S. stapfianus	236	772	ND	ND	Albini *et al.*, 1994
	9	95	0·0	2·0	Ghasempour *et al.*, 1998
Desiccation intolerant species					
S. pyramidalis	45	36	NM	NM	Ghasempour *et al.*, 1998

The amounts of sucrose and trehalose in fully hydrated and dehydrated leaves of eight different species of resurrection plants and one species that is desiccation intolerant. ND, Not detectable; NM, not measured.

these have fuelled a great deal of interest in the use of sugars as a mechanism for protection of other living tissues (Pilon-Smits *et al.*, 1998; Zentella *et al.*, 1999).

Given the general nature of sucrose accumulation during tissue dehydration it appears to be a vital requirement in the desiccated state. Therefore, to understand the dehydration phase of the resurrection cycle the source for this accumulation must be identified. This area has received very little attention to date and the main evidence is circumstantial and correlative, but conclusive proof of the carbon sources is lacking. As sucrose accumulates in *C. plantagineum* similar quantities of an eight carbon carbohydrate, 2-octulose, are metabolized (Bianchi *et al.*, 1991; Ingram and Bartels, 1996). This has led to the proposal that carbon in 2-octulose is used as a source for sucrose accumulation. This conversion could account for up to 80% of the carbohydrate accumulated in leaves of *Craterostigma*. The remainder would have to be derived from alternative sources. Other resurrection plants lack the presence of 2-octulose as a possible reserve for sugar accumulation, and thus other carbohydrate sources are essential for survival. As sucrose accumulates in *Ramonda*, the starch content of the leaf declines. However, starch metabolism can only account for less than 20% of the accumulated sugar. This leaves photosynthesis and the possibility of carbohydrate reserves outside of the leaf as the other major carbon sources (Müller *et al.*, 1997). In *X. viscosa*, as leaves dry, glucose and fructose accumulate initially. There are insufficient measurements to enable comment on the source of carbon used for their accumulation but photosynthesis is the most likely candidate. As the plant desiccates, the hexoses are metabolized and sucrose accumulates as the dominant carbohydrate in the dehydrated plant. The advantage that *Craterostigma* appears to possess over the other resurrection plants is a large carbohydrate store of 2-octulose in hydrated leaves. Hence *Craterostigma* is not completely reliant upon photosynthesis for desiccation tolerance and is theoretically able to respond rapidly to sudden falls in water availability through the use of 2-octulose reserves.

The carbon source that fuels the accumulation of sugars during desiccation underpins the ability of poikilohydric plants to resurrect. Given their habitats it is clear that the environmental conditions in which the plants live can change in a short space of time (Gaff and Giess, 1986). Hence, in order to be desiccation tolerant, a plant must possess the capacity to protect itself as rapidly as possible. The synthesis of carbohydrate through photosynthesis may, under certain circumstances, be too slow to produce sufficient sugar, hence the need for other storage carbohydrates. In order to assess this we need the following data: (1) the rate of photosynthesis; (2) the amount of sugar accumulated by a species; (3) the time period over which the species is dried. This is not yet available for any species. In every instance where measurements of photosynthetic rate are provided (Schwab *et al.*, 1989; Deltoro *et al.*, 1998; Hartung *et al.*, 1998), either sugar accumulation data or the drying period are lacking.

With the huge changes in metabolic fluxes described earlier it is hardly surprising that there have been several reports documenting alterations in mRNA encoding enzymes vital for catalysis of these fluxes (Velasco, Salamini and Bartels, 1994; Bernacchia *et al.*, 1995; Ingram *et al.*, 1997; Kleines *et al.*, 1999). However, such measurements really do not tell us a great deal about the biochemistry of desiccation tolerant plants. Measurements of a whole battery of actual enzyme activities need to be performed if we are ever to understand the metabolic basis for sugar accumulation.

CHANGES IN PROTEIN EXPRESSION IN RESPONSE TO DROUGHT STRESS

Other than genes associated with metabolic pathways, there are several other genes identified as being elevated in expression during desiccation of resurrection plants. This area has been reviewed recently by Ingram and Bartels (1996), so only advances since their review will be discussed here. In *Craterostigma*, several genes have been isolated which possess homology with LEA (Late Embryogenesis Abundant) proteins; other genes have unknown functions (Piatkowski *et al.*, 1990; Ingram and Bartels, 1996). LEAs are associated with the later stages of embryo development in seeds. Since many seeds are desiccation tolerant to some degree, then the presence of LEAs in resurrection plants is not surprising. The difficulty with these proteins is the lack of an understanding of their actual role in desiccation (Baker *et al.*, 1988). From their sequences it is likely they have no functional role as enzymes, but they have been proposed to associate with internal structures of cells to stabilize them during dehydration. Thus they may act to supplement the protection afforded by sucrose accumulation. Two further genes have been isolated which encode putative homeodomain-leucine zipper proteins, CPHB-1 and -2 (Frank *et al.*, 1998). Their action is implicated in the regulation of expression of different sets of genes involved in the dehydration process in *Craterostigma plantagineum*.

Similar sets of genes have been identified in the resurrection grass *S. stapfianus*. In this species six cDNA clones have been isolated that have increased expression in leaves during drought stress (Blomstedt *et al.*, 1998). Some of these genes bear homology to genes from other plant species thought to be involved in the maintenance of cell integrity during water stress e.g. dehydrins, LEAs and thiol proteases.

One of the major difficulties in understanding the role of changes in protein expression during the resurrection cycle of poikilohydric plants is that during the dehydration phase genes also need to be expressed for the rehydration phase. Hence it is difficult to conclude at which point the protein carries out its function. Through the use of inhibitors of protein synthesis in *Xerophyta humilis* (Bak.) it has been observed that most of the components and organization for the start of the resurrection phase are already present in the plants during the dehydration phase (Dace *et al.* 1998).

SIGNAL PATHWAYS

One common feature of proteins that have been isolated and identified as being increased in terms of expression during tissue dehydration in resurrection plants has been
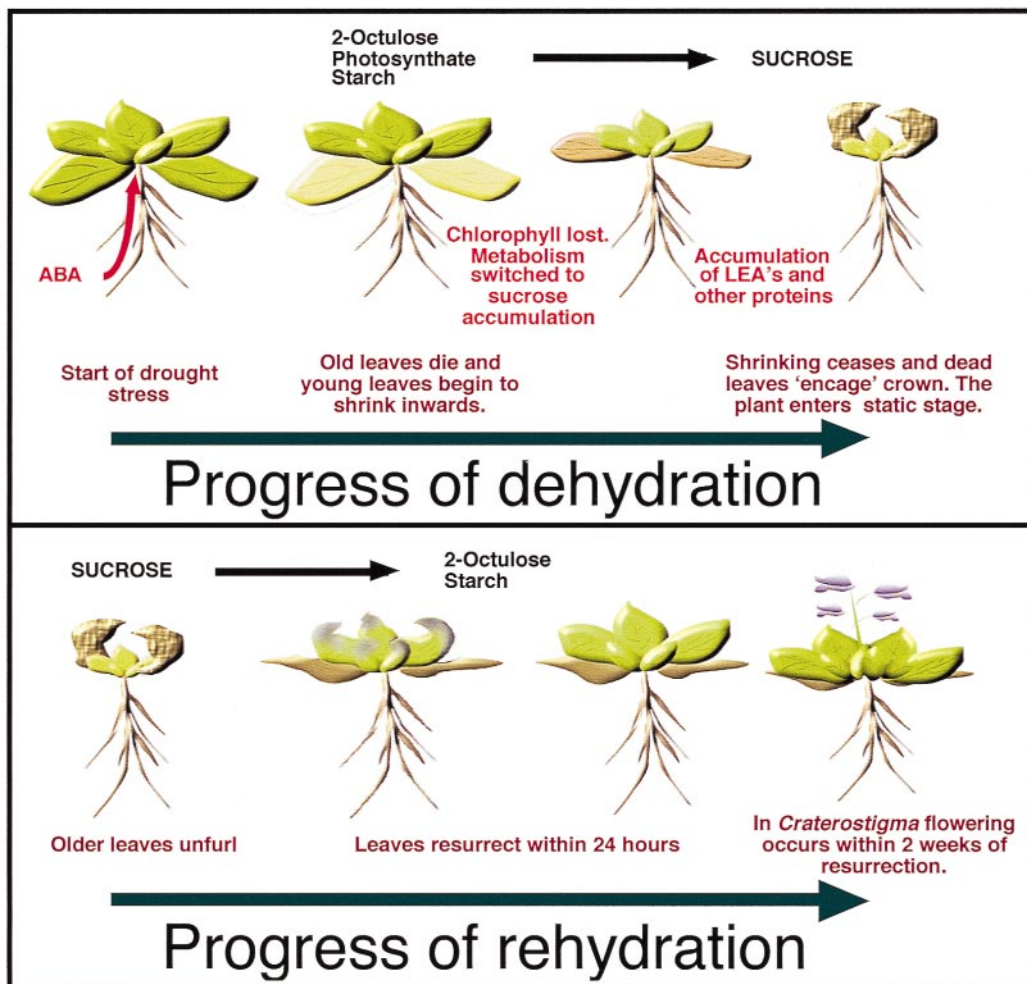
FIG. 3. Schematic representation of the dehydration and rehydration phase of the resurrection cycle in *Craterostigma plantagineum*. The scheme combines research on the accumulation of ABA (Bartels *et al*., 1990), and sucrose (Schwall *et al*., 1995) in leaves during dehydration. ABA is likely to be the first signal to leaves that water availability is diminished. The desiccation process is initiated. Upon rehydration the leaves rapidly expand and in *Craterostigma* the plant flowers within 2 weeks of resurrection.

the inducibility with abscisic acid (Bartels *et al*., 1990). The *Craterostigma* gene CdeT6-19 encodes a protein with a sequence similar to LEA D11. The promoter for this gene is induced by ABA or desiccation treatment (Michel *et al*., 1994). This has fuelled speculation that ABA forms the initial signal for metabolic events necessary for desiccation tolerance. There is evidence for this hypothesis. An increase in ABA concentration in leaves of *Craterostigma* occurs during drought stress (Bartels *et al*., 1990; Schiller *et al*., 1997). In addition, callus tissue derived in culture from leaves of *C. plantagineum* can be dehydrated and become desiccation tolerant only if treated with ABA prior to dehydration (Bartels *et al*., 1990; Chandler *et al*., 1997). Thus in the absence of roots, callus tissue can be induced to maintain viability during desiccation by addition of ABA. Furthermore, the addition of ABA to leaves of *Craterostigma* has been reported to induce sucrose accumulation, but to date there are no published data to support this statement (Schwall *et al*., 1995). Thus it is likely that ABA is one of the crucial early signals required for the survival of *Craterostigma*. A further gene has been isolated that is

implicated in the signal transduction pathway involving ABA. The gene CDT-1 can be expressed constitutively in *C. plantagineum* callus resulting in cells that no longer require ABA treatment in order to survive desiccation (Furini *et al*., 1997). CDT-1 is a novel gene sequence that may be involved in activation of the signal pathway via regulatory RNA or via a small polypeptide.

Large rises in leaf ABA content have been noted also in *Myrothamnus fabellifolia*, *Xerophyta humilis* and a large range of other resurrection plants (Gaff and Loveys, 1984; Schiller *et al*., 1997). Hence ABA may be a general signal that initiates metabolic changes for desiccation tolerance in resurrection plants. This would be very similar to the situation that occurs in other higher plants exposed to drought stress (Jensen *et al*., 1996; Hare *et al*., 1999).

SUMMARY

A summary of the ability of *Craterostigma* to survive dehydration and revive is presented in Fig. 3. The roots, being in the soil, are most likely to detect a drop in water

availability first. ABA synthesis, and release by roots is a common response in plants to drought stress. Once released, ABA could activate batteries of genes required for metabolic processes such as the accumulation of sucrose from either stored carbohydrates or through an alteration in photosynthetic carbon partitioning. In addition, the synthesis of other proteins is induced such as dehydrins and LEAs which could help to stabilize the plant cells as they lose water. Thus as the tissues dehydrate, leaves shrink, chlorophyll is degraded, sucrose accumulates and ultimately the xylem cavitates and the plants become desiccated. On addition of water, the xylem refills and cells begin to take up water and expand; enzymes present in the tissues are activated, sucrose is metabolized, and chlorophyll is resynthesized. Within 24 h the plant is restored and is reproductively active within 2 weeks.

FUTURE PERSPECTIVES

Our understanding of the resurrection process is still patchy. More research is required on a greater range of species to appreciate common elements that exist. Our understanding of the carbohydrate sources used for sucrose synthesis is incomplete and more work needs to be performed to identify these. This will allow a better understanding of the time frame required by these plants in order to become desiccation tolerant. Research into the dehydration characteristics of roots has been neglected to date. This needs to be addressed to investigate whether leaves and roots use similar mechanisms. In addition, we still do not know how a plant senses where it is in the resurrection cycle. For example, how does the plant regulate sucrose synthesis during rehydration? The use of transgenic plants to study metabolism in resurrection plants has yet to make any great headway. Reported use of transgenic *C. plantagineum* plants has been announced (Furini *et al.*, 1994, 1997), but little work has been done using this technique. In my own laboratory transgenic methods with this plant have proven generally unreliable. Thus there is still a need to develop this area more fully and exploit the potential offered by transgenic technologies. Transgenic studies of plant metabolism have proved enormously successful in C$_3$ species and there is a need to capitalize on the potential of this technology in resurrection plants (Stitt and Sonnewald, 1995; Hebers and Sonnewald, 1996).

I will leave readers with one perplexing observation on higher plants in general. Although a majority of plants have a stage in their life cycle that can tolerate dehydration, it is remarkable how few species can use these same mechanisms to protect vegetative tissues such as leaves and roots during severe drought stress conditions as resurrection plants do.

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