Minireview

Biochemical and molecular responses to water stress in resurrection plants

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A small group of angiosperms, known as resurrection plants, can tolerate extreme dehydration. They survive in arid environments because they are able to dehydrate, remain quiescent during long periods of drought, and then resurrect upon rehydration. Dehydration induces the expression of a large number of transcripts in resurrection plants. Gene products with a putative protective function such as LEA proteins have been identified; they are expressed at high levels in the cytoplasm or in chloroplasts upon dehydration and/or ABA treatment of vegetative tissue. An increase in sugar concentration is usually observed at the onset of desiccation in vegetative tissue of resurrection plants. These sugars may be effective in osmotic adjustment or they may stabilize membrane structures and proteins. Regulatory genes such as a protein translation initiation factor, homeodomain-leucine zipper genes and a gene probably working as a regulatory RNA have been isolated and characterized. The knowledge of the biochemical and molecular responses that occur during the onset of drought may help to improve water stress tolerance in plants of agronomic importance.

Introduction

Since agriculture began, drought, or more generally inadequate water availability, has been one of the major crop production-limiting factor, causing famine and death. It continues to be a permanent constraint to agricultural production in many developing countries and causes yield reduction in developed ones (Ceccarelli and Grando 1996). Thus, in environments where water deficit occurs, understanding the mechanisms that allow plants to tolerate drought is an urgent need for agriculture.

Some angiosperms are able to cope with arid environments by mechanisms that mitigate drought stress, such as stomatal closure, partial senescence of tissues, reduction of leaf growth, development of water storage organs, and increased root length and density, in order to use water more efficiently. Water flux through the plant can be reduced or water uptake can be increased by several physiological adaptations. These mechanisms allow plants to survive in arid environments by lessening the severity of drought stress, but they do not make these plants tolerant to desiccation. In fact, with long periods of drought these plants will dehydrate and die (Scott 2000).

The modern scientific study of drought tolerance began in 1702 when Anthony von Leeuwenhoek discovered that rotifers could survive without water for months. Now it is known that a few groups of animals and a wide variety of plants can tolerate desiccation in the adult stages of their life cycle. Many lichens, bryophytes, and a few ferns can survive in a dried state. Among vascular plants a small group of angiosperms known as poikilohydric or resurrection plants (Gaff 1987) can tolerate extreme dehydration. Desiccation tolerance allows a plant to survive equilibrium with 0% air humidity and it is preserved until water becomes available. Upon watering, dried resurrection plants rapidly revive and become fully photosynthetically active within 24 h (Bernacchia et al. 1996). In addition, the processes of drying and rehydration cause only limited damage to plant tissues. Thus, resurrection plants have the advantage over other species in arid environments in that they can remain quiescent during long periods of drought. They can resurrect upon rehydration, grow and reproduce long before non-resurrecting plants have the opportunity to do so (Scott 2000).
Desiccation tolerance is common in non-vascular plants, but is rare in vascular plants, even though dried structures (i.e. seeds and pollen grains) are frequently present in most plant species. This would suggest that the genetic properties required for tolerance are present in flowering plants and that only some tightly regulated programmes of gene expression in plant development are required to induce the revival of a dried plant body (Hartung et al. 1998). The study of desiccation-tolerant plants started at the beginning of the last century (Irmscher 1912) with the botanical description of several species, and has continued until the present day, focusing on the physiological, biochemical and molecular aspects of this phenomenon.

The importance of drought resistance in determining crop productivity is well known. In arid lands or environments where drought occurs, the mechanisms of desiccation tolerance can play a role in plant survival until water becomes available. It is justifiable that a large number of scientists are interested in plant responses to water deficit at physiological, biochemical and molecular levels because this knowledge may be applied in the development of drought-tolerant agricultural species. The purpose of this minireview is to show what is currently known about resurrection plants with a special focus on the biochemical and molecular events that lead to desiccation tolerance.

Resurrection plants: definition and distribution
Angiosperms that possess the unique ability to withstand desiccation of their vegetative tissues and to revive from an air-dried state are called resurrection plants (Gaff 1987, Fig. 1). These poikilohydrous plants can experience different rates of desiccation, depending upon the water status of the environment, and they can recover uninjured from complete dryness within 24 h of contact with water. Some resurrection plants lose chlorophyll and dismantle their photosynthetic apparatus during the onset of drought and they are then defined as poikilo-chlorophyllous. Others retain chlorophyll and the photosynthetic machinery during dehydration and are therefore called homoiochlorophyllous (Tuba et al. 1998). In earlier studies, Bewley (1979) suggested that to survive in a dehydrated state, plants must be able to limit the damage, they must maintain the physiological integrity in the desiccated state, and they must possess repair mechanisms to recover from the damage upon rehydration. More recent studies (Bewley and Oliver 1992) confirmed that interpretation and suggested that desiccation-tolerant plants can also be classified into two groups based on their resistance mechanism: protection of cellular integrity or cellular repair of desiccation-induced damage.

Resurrection plants are widely distributed, they are found in all continents except Antarctica. According to Gaff (1987), they are mainly concentrated in arid climates such as southern Africa, eastern South America and western Australia, while only a few species have been found in Europe in the Balkan mountains (Stefanov et al. 1992). About 330 species of angiosperms have been found to survive desiccation (Gaff 1987; Porembski and Barthlott 2000), but no resurrection gymnosperms are known (Hartung et al. 1998). There are both monocotyledonous plants such as *Xerophyta viscosa* and *Sporobolus stapfianus* and dicotyledonous species such as *Myrothamnus flabellifolia*, *Craterostigma plantagineum* and *Chamaegigas intrepidus*. The latter is the unique example known of aquatic resurrection plants (Hartung et al. 1998). Despite their broad geographical distribution, the ecological range for resurrection plants is narrow. Usually they are found in habitats subjected to lengthy periods of drought, where rainfall is extremely sporadic, particularly on rock outcrops below 2000 m in tropical and subtropical areas, and to a lesser extent in temperate zones (Porembski and Barthlott 2000).

Resurrection plants have evolved structures and mechanisms to allow survival under extreme conditions. For instance, a recently described and very interesting aspect of the physiology of resurrection plants is the response of the xylem to desiccation. In fact, when the plant is in the air-dried state the xylem vessels are completely embolized. During rehydration, the xylem must be able to refill, avoiding the formation of air bubbles, in order to provide a supply of water to the leaves. Vulnerability of xylem to the formation of air bubbles depends on wood anatomy, particularly the size of pores in inter-vessel pits. Larger pores are more susceptible to air entry than small pores. In *M. flabellifolia* narrow reticulate xylem vessels have been observed, which cavitate upon desiccation but refill from root pressure and capillarity when water becomes available (Sherwin et al. 1998).

![Fig. 1. An example of resurrection plant: Craterostigma plantagineum.](image-url)
Although the mechanisms that allow these plants to survive a severe water stress have potential applications in agriculture, molecular and biochemical studies are to date restricted to a few species: *C. plantagineum* (reviewed in Ingram and Bartels 1996; Bartels and Salamini 2001), *S. stapfianus* (Neale et al. 2000), *X. viscosa* (Mundree et al. 2000) and the moss *Tortula ruralis* (Bewley and Oliver 1992; Oliver and Bewley 1997; Oliver et al. 2000).

**Molecular and biochemical studies**

**LEA proteins**

Many transcripts and proteins accumulate during drying in resurrection plants and some of them have been cloned and/or sequenced. They include, for instance, gene products that may play a role in protecting cytoplasmic structures during dehydration such as Late Embryogenesis Abundant (LEA) proteins. LEA proteins include a large group of proteins that accumulate in mature embryos during the onset of desiccation (Galau et al. 1986), and they can be induced in immature embryos by ABA treatment. Generally their expression is found in osmotically stressed or ABA-treated tissue (Mundy and Chua 1988) in desiccation-tolerant as well as in desiccation-sensitive plants (Skriver and Mundy 1990; Campbell and Close 1997; Svensson et al. 2002).

LEA proteins, even if quite heterogeneous, are usually rich in hydrophilic amino acids, are water soluble and can be divided into different groups based on sequence similarities and properties. For example group 1 LEA proteins are characterized by a 20-amino-acid-motif that was first found in the wheat Em protein (Litts et al. 1990; Campbell and Close 1997; Svensson et al. 2002).

Group 1 LEA proteins are hydrophilic; they share a conserved serine and lysine-rich motif and remain soluble after boiling. Group 3 LEA proteins are characterized by a motif of 11 amino acids, which is predicted to form an amphipathic α-helix probably involved in structural interactions (Dure et al. 1989). Group 4 and 5 show relatively less conserved structures and are probably involved in protecting membranes and binding water, respectively (Ingram and Bartels 1996). Sequence homologies to LEA genes from all five groups have been isolated and characterized in the resurrection plant *C. plantagineum* (Bartels and Salamini 2001). They are expressed at high levels in the cytoplasm or in the chloroplast upon dehydration and/or ABA treatment of vegetative or callus tissue. The accumulation of LEA proteins in desiccated vegetative tissues of *C. plantagineum* pointed out the similarity between seeds and resurrection plants: both systems survive in an air-dried state and revive during rehydration. In addition, the high cellular concentration of different LEA proteins in desiccated *C. plantagineum* tissues and their high hydrophilic structure suggest that they may play a role in protecting cellular structures during drought stress.

A cellular protection mechanism is also present in the moss *T. ruralis*, whose cellular integrity is maintained during drying, allowing a rapid recovery of the photosynthetic activity upon rehydration (Proctor and Smirnoff 2000). Interestingly, it has been reported that dehydrin-type LEA proteins accumulate constitutively also in the hydrated state of vegetative tissue of *T. ruralis* (Bewley et al. 1993). By using purified antibodies raised against corn seedling dehydrin, it was shown that *T. ruralis* produces two dehydrins of 90 and 35 kDa. They are present constitutively and do not appear to increase during rapid or slow drying (Bewley et al. 1993). This fact may indicate that different mechanisms of drought tolerance are present in Bryophytes and in vascular plants. Nevertheless, *T. ruralis* gametophytes recover at a much slower rate if the water loss occurs rapidly and this suggests that this moss can activate system(s) during the desiccation phase that can decrease the time necessary to recover upon rehydration.

**Carbohydrate metabolism**

In addition to the synthesis of proteins, an increased concentration of sugars is usually observed in seeds of many species at the onset of desiccation (Leprince et al. 1993) and also in vegetative tissues of resurrection plants such as *C. plantagineum* (Bianchi et al. 1991), *Ramonda myconi*, *Haberlea rhodopensis* (Müller et al. 1997), *S. stapfianus* (Albini et al. 1994) and others. Studying the mechanisms by which diverse organisms such as bacteria, fungi and yeast can withstand desiccation, Crowe and Crowe (1986) reported that in *Saccharomyces cerevisiae* trehalose was effective in preserving the integrity of membranes during desiccation and rehydration. This sugar is extremely rare in plants where sucrose and other sugars may play a similar role. Sugars may be effective in osmotic adjustment during water loss, but they may prevent the cells by causing, during severe desiccation, glass formation with the mechanical properties of a solid. This phenomenon has been observed, for instance, in maize seeds and has been correlated with their viability (Williams and Leopold 1989). In addition, sucrose may preserve the integrity of the lipid bilayer during dehydration (Crowe and Crowe 1986).

It has been observed that in many desiccation-tolerant plants, independently of the species of soluble sugar accumulated in fully hydrated tissues, sucrose is invariably present at high levels in the dried state (Leprince et al. 1993). A particular case is represented by the leaves of *C. plantagineum*. During desiccation, the unusual eight-carbon sugar 2-octulose, representing approximately 90% of the total sugar in fully hydrated leaves, is converted in sucrose, which then represents about 40% of the DW (Bianchi et al. 1991).

2-Octulose is the main photosynthetic storage sugar that *C. plantagineum* leaves build up during the day.
During dehydration, 2-octulose is rapidly converted into sucrose without any significant alteration in the overall sugar content. Little is known about the mechanism of this conversion. Nevertheless, it has been shown to correlate with an increased synthesis of sucrose-metabolizing enzymes. Specific isoenzymes of sucrose synthase and sucrose phosphate synthase are in fact differentially expressed during this phase, along with an upregulation of glyceraldehyde dehydrogenase (reviewed in Ingram and Bartels 1996; Bartels and Salamini 2001). Interestingly, fully hydrated roots of *C. plantagineum* accumulate the oligosaccharide stachyose and its level does not appear to change significantly during dehydration. Because there is no additional information on the sugar composition of *C. plantagineum* dehydrated roots, it is not known if these also accumulate sucrose during dehydration or rely on the presence of this oligosaccharide for their desiccation tolerance.

Sucrose is also the only available sugar for cellular protection in *T. ruralis*. The amount of this sugar in gametophytic cells is usually 10% of the DW. Wet-dry episodes do not induce changes in sucrose concentration, thus indicating that this moss needs to maintain sufficient amount of this sugar in the event of desiccation (Oliver and Bewley 1997). Other resurrection plants rely on different carbohydrate sources for their survival: in *X. viscosa* for instance, glucose and fructose accumulate as leaves dry, while in *Ramonda* it was reported that starch content declines as sucrose accumulates during drying (Müller et al. 1997).

In *C. plantagineum* plants, the rehydration process has been studied in terms of metabolic activity and gene expression profiles (Bernacchia et al. 1995, 1996). A fast recovery of full photosynthetic activity and of respiration was observed upon rewaturing, along with a restart of the synthesis of related proteins (Bartels and Salamini 2001). Differential screenings also revealed a particular behaviour of transketolase-encoding genes in rehydrating *C. plantagineum* leaves. In fact, two genes coding for putatively cytosolic transketolases are upregulated during recovery, while the plastid-localized one appears to be expressed independently of the water content of the plant (Bernacchia et al. 1995). These results suggest a possible role of transketolases in the sugar conversions taking place during the dehydration-rehydration cycle, even if questions about the nature of the metabolic pathway are still open.

**Role of ABA**

The involvement of ABA in the desiccation tolerance of resurrection plants is well known. The ABA content has been measured in fully hydrated and dehydrated leaves of several resurrection plants (Gaff and Loveys 1984; Schiller et al. 1997; Bartels and Salamini 2001). In general a 3- to 7-fold increase of leaf ABA content was observed upon desiccation; moreover, when leaves of *M. flabellifolia* and *Borya nitida* were too rapidly dehydrated, the increase in ABA was not observed and leaves did not survive after rehydration (Gaff and Loveys 1984). As reported, leaves of *C. plantagineum* do not require ABA treatment to establish or to increase drought tolerance (Bartels and Salamini 2001); however, a feature of this species is that a pretreatment with ABA prior to dehydration is essential to induce desiccation tolerance in dedifferentiated callus tissue.

Most of the proteins studied so far showing an increased accumulation during drought in resurrection plants are also induced by an exogenous ABA treatment. Genetic studies with *Arabidopsis thaliana* mutants (Parcy et al. 1994) defective in their sensitivity to ABA or with an altered ABA biosynthetic pathway also confirmed the role of ABA in inducing gene expression in response to water stress. Furthermore, different types of cis-acting elements relevant to ABA and/or drought have been extensively analysed (Skriver et al. 1991; Yamaguichi-Shinozaki and Shinozaki 1994). In the resurrection plant *C. plantagineum*, the promoters studied are derived from LEA-like genes induced by dehydration and ABA (Furini et al. 1996; Ingram and Bartels 1996). These promoters were analysed in transgenic *Arabidopsis* and tobacco plants and resulted expressed in seeds and pollen in the dry state. This observation suggests that in vegetative tissues of *C. plantagineum* the signal transduction pathway from water stress to gene expression requires the activation of specific genes, which in angiosperms are expressed only in dried organs.

*Arabidopsis* abscisic acid-insensitive *abi3* mutant seeds do not acquire desiccation tolerance and remain nondormant (Ooms et al. 1993). Further studies (Parcy et al. 1994) indicated that the *ABI3* encodes a transcriptional factor that regulates the expression of several genes during seed development. The *ABI3* gene is specifically expressed in seeds but its ectopic expression leads to the accumulation of seed-specific transcripts in transgenic *Arabidopsis* plantlets in response to ABA. In addition, it has been observed (Furini et al. 1996) that the ectopic expression of *ABI3* also conferred the ability to induce *C. plantagineum* promoter activity of LEA-like genes in vegetative tissues of transgenic *Arabidopsis* after ABA treatment. These results led to the hypothesis that a gene product necessary for the induction of ABA-and/or desiccation-inducible genes accumulates in vegetative tissue of *C. plantagineum* while its analogous counterpart accumulates in seeds of angiosperms. An *ABI3* homologue was isolated from *C. plantagineum* to verify if this gene may be responsible for the activation of dehydration- and/or ABA-inducible genes. The encoded product was, indeed, able to induce LEA-like genes in transient expression assays, but its expression was not detected in vegetative tissue of *C. plantagineum* (Bartels and Salamini 2001), indicating that another factor may be involved in the induction of drought-tolerance genes.

Genes induced at very low levels during the early stages of desiccation have been isolated from the resurrection grass *Sporobolus stapfianus*. The *SDG134c* gene, encoding an eIF1 protein translation initiation factor, was detected at very low levels in hydrated leaves and...
The different responses observed in resurrection plants upon water stress.

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the transcript level increased and persisted during drought (Neale et al. 2000). In desiccation-tolerant plants, many components essential for re-initiation of metabolic activity are present in the dried tissue. It was suggested (Neale et al. 2000) that SDG134c is required during the rehydration process that leads to full metabolic activity within several hours.

Homeodomain-leucine zipper (HD-ZIP) genes are specific for plants and are involved in the regulation of developmental processes and induced by biotic and abiotic stresses. From C. plantagineum two HD-ZIP family II genes were isolated and their products were shown to interact in a yeast (S. cerevisiae) two-hybrid system (Frank et al. 1998). These genes, CPHB-1 and CPHB-2, are of particular interest because both are induced by dehydration but show different responses to ABA: CPHB-1 was not induced by the phytohormone, while the transcript level of CPHB-2 increased upon ABA treatment. This suggests that ABA-dependent and ABA-independent pathways are responsible for adaptation to dehydration in C. plantagineum (Table 1). In fact, previous studies on Arabidopsis ABA mutants have revealed the existence of different water-stress signal-transduction pathways. ABA regulates one mechanism, while the other works independently of the phytohormone (Shinozaki and Yamaguchi-Shinozaki 2000). These observations strengthen the hypothesis that similar mechanisms underlying water-stress responses exist in resurrection and in non-resurrection plants.

Other genes that act as putative regulators during the dehydration stress have been isolated from C. plantagineum. For example, Myb-related genes and a heat-shock transcriptional factor (for a review see Phillips et al. 2002) are induced by dehydration. A further gene isolated via T-DNA activation tagging is implicated in the process of desiccation tolerance: CDT-1 is a member of a repeated gene family that, when transcribed at high level in callus tissue, leads to the constitutive activation of desiccation-related genes. This gene upregulation enables callus cells to survive desiccation without an ABA pre-treatment (Furini et al. 1997). CDT-1 mRNA does not carry a long ORF starting with ATG and no protein product was detected by in vitro translation. Therefore, further experiments were designed to verify whether CDT-1 might be involved in the activation of the signal pathway that leads to desiccation tolerance via regulatory RNA or via a small polypeptide. Preliminary results suggest (Furini et al., unpublished data) that a putative peptide encoded by CDT-1 is not required for conferring desiccation tolerance in C. plantagineum callus tissue, therefore strengthening the hypothesis that the CDT-1 transcript may be responsible for the observed phenotype.

Conclusions

The importance of drought resistance in determining crop productivity is well known. In arid lands or environments where drought occurs, the mechanisms of desiccation tolerance may promote plant survival until soil moisture levels improve. Thus, studies of these mechanisms have potential applications in the development of desiccation-tolerant crops. Although more work is still necessary, many genes that play a possible role in desiccation tolerance have been isolated from resurrection plants and the function of the encoded proteins have been established for several of them. Molecular responses to dehydration such as the expression of LEA genes and changes in sugar metabolism and regulatory pathways have been extensively studied.

It is known from molecular marker analysis (Quarrie 1996) that tolerance to water stress is a quantitative trait; nevertheless all the knowledge so far achieved with the available gene technology opens up new opportunities for improving stress tolerance in crop plants. For instance, in transgenic Arabidopsis plants carrying the transcriptional activator DREB1a driven by an abiotic stress-inducible promoter, induction of LEA genes was observed and these plants were more tolerant to water stress (Kasuga et al. 1999).

The use of cDNA microarrays to identify drought- and cold-inducible genes in Arabidopsis allowed the isolation of stress-induced genes that were not previously identified (Seki et al. 2001). This technology may be applied for the isolation of new genes involved in the signal transduction pathway that leads resurrection plants to survive extreme water stress.

References


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