Desiccation Tolerance in the Resurrection Plant *Craterostigma plantagineum*. A Contribution to the Study of Drought Tolerance at the Molecular Level[1]

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Adverse environmental conditions restrict the productivity and the range of habitats available to plants. This represents a severe economic constraint on agricultural production. Plants as sessile organisms have evolved a wide spectrum of adaptations to cope with the challenges of environmental stress. Quite often, however, adaptation mechanisms themselves adversely affect yield parameters, and a compromise between biomass production and environmental fitness has to be accepted. One major factor that limits the productive potential of higher plants is the availability of water. The International Water Management Institute predicts that by the year 2025, one-third of the world's population will live in regions that will experience severe water scarcity (www.iwmi.org). Therefore, it has become imperative for plant biologists to understand the mechanisms by which plants can adapt to water deficit while retaining their capacity to serve as sources of food and other raw materials.

Water deficit can affect plants in different ways. A mild water deficit leads to small changes in the water status of plants, and plants cope with such a situation by reducing water loss and/or by increasing water uptake (Bray, 1997). The most severe form of water deficit is desiccation—when most of the protoplasmic water is lost and only a very small amount of tightly bound water remains in the cell.

Both forms of water deficit have been studied at the molecular level using a variety of experimental systems. Arabidopsis has been extensively studied as a model plant that tolerates moderate water deficit. Genes involved in many different pathways are expressed in response to water stress in Arabidopsis, and the molecular complexity of the process is best illustrated by recent microarray experiments (Seki et al., 2001). Mutant analysis has greatly contributed to our knowledge of the mode of gene regulation under stress, and it has become obvious that a network of signal transduction pathways allows the plant to adjust its metabolism to the demands imposed by water deficit (Shinozaki and Yamaguchi-Shinozaki, 2000; for review, see Kirch et al., 2001b). These studies raise two major questions: (a) Do diverse plants use different pathways to respond to the stress?, and (b) Do variable degrees of water stress activate different metabolites?

Most flowering plants cannot survive exposure to a water deficit equivalent to less than 85% to 98% (v/v) relative humidity during their vegetative growth period, although desiccation is an integral part of the normal developmental program of most higher plants in the context of seed formation. Only a few plants possess desiccation-tolerant vegetative tissues; these include a small group of angiosperms, termed resurrection plants (Gaff 1971), some ferns, algae, lichens, and bryophytes.

Some of these species can equilibrate the leaves with air of 0% (v/v) relative humidity. Resurrection plants can be revived from an air-dried state and are often poikilohydrous, i.e. their water content varies with the relative humidity in the environment. Resurrection plants are found in ecological niches with limited seasonal water availability, preferentially on rocky outcrops at low to moderate elevations in tropical and subtropical zones (Porembski and Barthlott, 2001). It has been estimated that around 200 species of resurrection plants may exist, mainly in Southern Africa, Australia, India, and South America (W. Barthlott, personal communication). The physiological basis of desiccation tolerance in resurrection plants is complex. Some mechanisms may vary between different species; for example, some species retain chlorophyll during dehydration, whereas others lose their chlorophyll.

Studies aimed at understanding the molecular basis of desiccation tolerance have focused on a few species representing different groups: the dicotyledonous South African *Craterostigma plantagineum* (Bartels et al., 1990), the monocotyldonous species *Sporobulus stapfianus* (Neale et al., 2000), and the moss *Tortula ruralis* (Oliver and Bewley, 1997). Most information is available on *C. plantagineum*, which will be the main focus of this review. For this plant, the

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phenomenon of desiccation tolerance is shown in Figure 1.

C. PLANTAGINEUM AS AN EXPERIMENTAL SYSTEM

The attraction of *C. plantagineum* as an experimental system is due to the fact that desiccation tolerance is expressed in vegetative tissues and in undifferentiated callus cultures. This allows one to compare gene expression in two systems with the same genetic background—in the absence of developmental complications, such as those that may arise when acquisition of desiccation tolerance during seed development is investigated. Callus from *C. plantagineum* is not intrinsically desiccation tolerant, but it acquires tolerance after it has been cultured on medium containing the plant hormone ABA (Bartels et al., 1990). Treatment of callus with ABA induces the expression of a set of mRNAs comparable with that activated upon drying in the whole plant. *C. plantagineum* is suited for molecular studies because it can be genetically transformed by *Agrobacterium tumefaciens* (Furini et al., 1994) and it is suited for transient expression analysis using protoplasts or a ballistic approach.

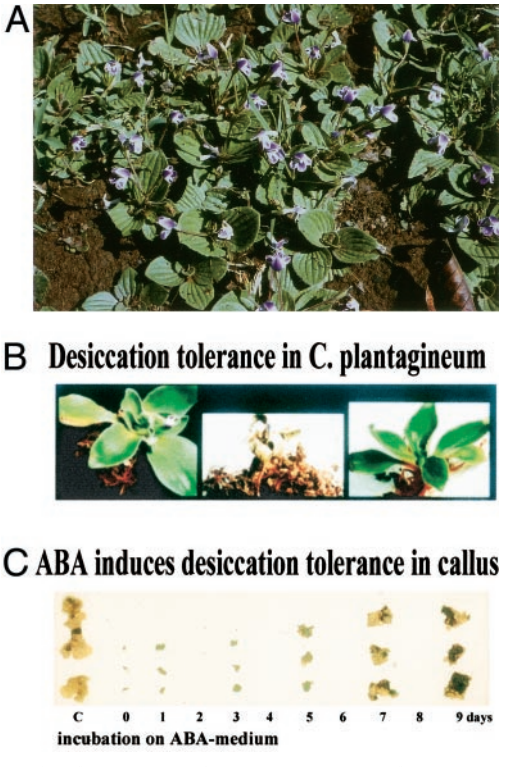

Figure 1. The desiccation-tolerant resurrection plant *Crateostigma*. A, *C. pumilum* plants in their native habitat in Kenya (photograph courtesy of Dr. W. Barthlott, University of Bonn). B, Effect of desiccation on a *C. plantagineum* fully turgid plant, a desiccated plant, and a plant rehydrated for 12 h. C, Callus from left to right: untreated, 0 to 7 d. Abscisic acid (ABA)-treated callus, dried and regrown.

Studies with *C. plantagineum* have shown that the physiological state of the plant before the onset of drying appears to be critical for survival. The plants only develop the ability to survive desiccation, if water loss occurs slowly enough to allow their metabolism to adapt by activating a specific program of gene expression. If dehydration occurs too rapidly, plants do not acquire tolerance to desiccation. Most changes in gene expression occur during dehydration and relatively few are observed during the rehydration phase. Thus, many dehydration-specific gene products have been isolated but very few rehydration-specific proteins are known (Bernacchia et al., 1996). This is in contrast to observations made on *T. ruralis*, a representative of the desiccation-tolerant bryophytes, which survives rapid desiccation. Here, the major changes in gene expression occur during the first hours of rehydration (Wood and Oliver, 1999). In this case, changes in gene expression in response to dehydration and rehydration are regulated at the translational level, resulting in a change in the complement of mRNAs selected for protein synthesis from a relatively constant mRNA pool (Wood and Oliver, 1999). These findings support the suggestion that desiccation tolerance in bryophytes differs from that in *C. plantagineum* in being mainly a rehydration-induced cellular repair response (Oliver and Bewley, 1997).

MOLECULAR AND METABOLIC CHANGES IN *C. PLANTAGINEUM* DURING THE DEHYDRATION/REHYDRATION CYCLE

In *C. plantagineum*, dehydration leads to drastic changes in gene expression and carbohydrate metabolism, increases in ABA levels and photosynthesis-related processes, and changes in cell ultrastructure. This plant reacts within the first 2 h to dehydration, and the metabolic changes are initiated before any signs of wilting are apparent. All of the dehydration-induced processes are reversed during rehydration. In this review, we will mainly focus on the processes that occur during dehydration because these appear to be important for cellular protection and probably also for recovery during rehydration.

PHOTOSYNTHESIS AND ENERGY METABOLISM

Water relations in plants are regulated to a large extent via the opening and closure of stomata. Water deficit leads to closure of stomata and at the same time to a decrease in intercellular CO_2 concentration. As a consequence of the lower CO_2 availability, carbon assimilation is inhibited and ultimately photosynthetic capacity is lost. This observation is common to dehydration-sensitive and drought-tolerant species (Schwab et al., 1989). These physiological

changes are reflected at the molecular level: The steady-state levels of transcripts related to photosynthesis are down-regulated in response to water stress, e.g. the transcript for the small subunit of the Rubisco enzyme in *C. plantagineum* (Bernacchia et al., 1996). However, a particular feature of resurrection plants is that they recover full photosynthetic activity following rewatering. Respiration is quickly restored in *C. plantagineum* on rewatering when the water content reaches about 20% of its initial value. This allows us to conclude that protective mechanisms must be in place that maintain the integrity of the photosynthetic machinery in the dried plant tissue. This inference correlates well with observations that several putative protective proteins accumulate in plastids of *C. plantagineum*, which are targeted to the stroma and to thylakoid membranes (Schneider et al., 1993).

CARBOHYDRATE METABOLISM

In many organisms, bacteria and yeasts in particular, high concentrations of carbohydrates are observed in dry tissues, and a contribution of carbohydrates to desiccation tolerance has been proposed. This is supported by in vitro studies showing that a wide range of biomolecules is less susceptible to denaturation when dehydrated in the presence of sugars (Crowe et al., 1992). In seeds of higher plants, a correlation has been observed between the accumulation of soluble sugars and the acquisition of desiccation tolerance (Leprince et al., 1993). In *C. plantagineum*, a remarkable change in carbohydrate metabolism occurs on dehydration (Fig. 2). The unusual C8 sugar octulose is present in large quantities (up to 90% of the soluble sugars, corresponding to up to 400 mg g^{-1} of lyophilized leaf material) in photosynthetically active leaves. Upon dehydration, the octulose level declines and conversely Suc accumulates; the reverse is observed during rehydration (Bianchi et al., 1991). Despite the qualitative change in sugar composition, the overall sugar content is similar in hydrated and dried leaves. The accumulation of Suc in dehydrated tissues seems to be a common theme in different resurrection plants, although different metabolic routes may be used for the synthesis of Suc. It is interesting that the sugar composition in roots of *C. plantagineum* does not change drastically with water availability. The tetrasaccharide stachyose is the dominant sugar, comprising more than 50% of the total sugar content, and octulose is only found in small amounts in untreated and in dehydrated

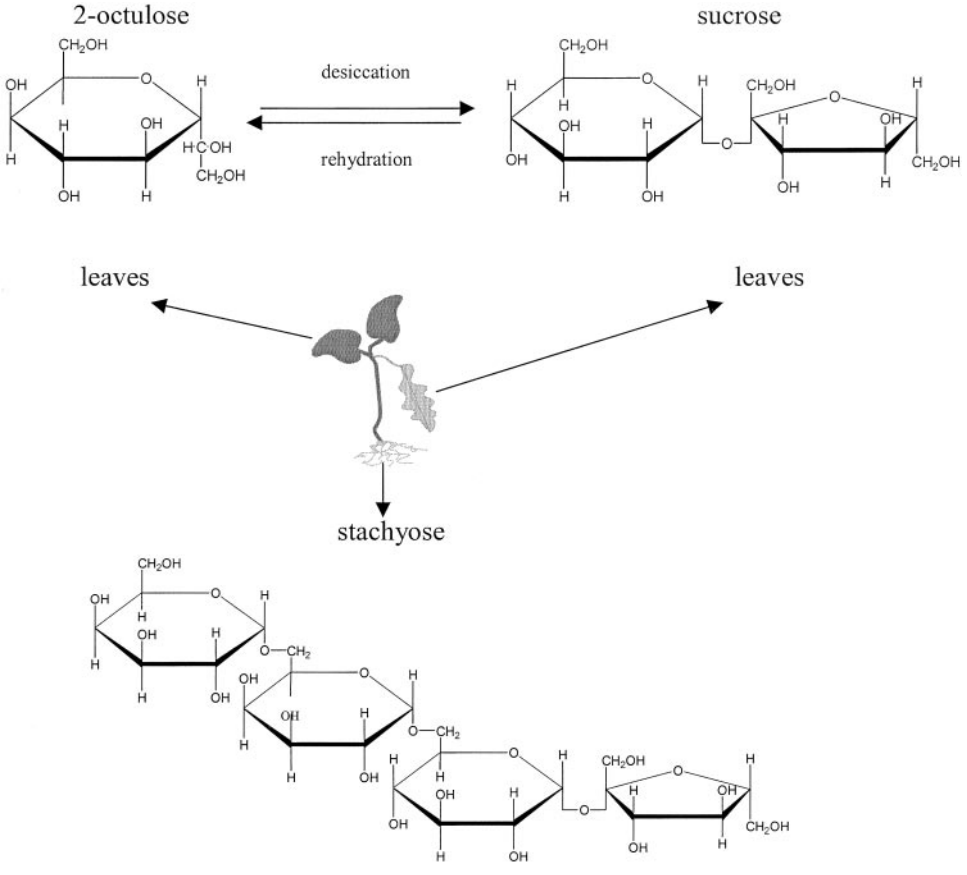

Figure 2. The most prominent sugars found in *C. plantagineum*. 2-Octulose is predominant in untreated leaves and is converted to Suc upon dehydration. Stachyose is the major sugar in both untreated and dried roots.

roots. Octulose is probably a product of photosynthesis that accumulates in leaves during the light period, but is partially metabolized at night. Because octulose has been found in the phloem sap, it is likely that it is transported from leaves to roots (Norwood et al., 2000).

To understand the biochemical basis for this switch in sugar metabolism, the expression of genes encoding sugar-metabolizing enzymes was investigated. *C. plantagineum* possesses a set of genes encoding isoenzymes of Suc synthase and Suc phosphate synthase (Ingram et al., 1997; Kleines et al., 1999), which are differentially expressed. Besides these enzymes and the dehydration-induced expression of cytosolic glyceraldehyde dehydrogenase, it has been proposed that transketolase contributes to the conversion of octulose to Suc. In addition to a constitutively expressed, plastid-localized transketolase, induction of two genes for transketolases during rehydration has been reported (Bernacchia et al., 1995). In *C. plantagineum*, two main questions concerning the carbohydrate metabolism are still open: (a) What enzymes are involved in the biosynthetic pathway leading to octulose?, and (b) Does Suc act as a protectant in the dehydrated leaves?

THE TRANSCRIPTIONAL RESPONSE TO DEHYDRATION

The basic pattern of changes in gene expression that occur in response to dehydration can be summarized for *C. plantagineum* as follows: (a) Transcripts accumulate to high levels during dehydration and disappear early during rehydration, (b) transcripts accumulate transiently during the initial dehydration phase, (c) transcripts decline during dehydration, and (d) transcripts remain unchanged in response to dehydration.

It is estimated from transcript profiling that several hundred genes probably are differentially expressed in response to dehydration (Bockel et al., 1998). The identified genes encode compounds that can be assigned to diverse metabolic pathways, although their precise function has not yet been demonstrated. The following main groups can be distinguished: (a) genes encoding proteins with protective properties, (b) genes encoding membrane proteins involved in transport processes, (c) genes encoding enzymes related to carbohydrate metabolism, (d) genes encoding regulatory molecules, such as transcription factors, kinases, or other putative signaling molecules, and (e) genes that show no homologies to known sequences. Studies on tissue-specific expression patterns and subcellular localizations have revealed specific cellular distributions of RNAs and proteins that appear to correlate with their predicted functions (Phillips et al., 2001).

PROTEINS WITH PROTECTIVE PROPERTIES

This group includes the proteins that accumulate most abundantly in different tissues of *C. plantagineum* during dehydration. Its major representatives include late embryogenesis-abundant (LEA) proteins (Schneider et al., 1993), some proteins with enzymatic function, e.g. an aldehyde dehydrogenase (Kirch et al., 2001a), and also some small heat shock proteins (Alamillo et al., 1995). LEA proteins are a heterogenous group of proteins found universally in plants, which were first discovered because they accumulate to high levels during late stages of embryo development. Synthesis of LEA proteins is associated with dehydration in seeds and with water deficit in vegetative tissues (Cuming, 1999). Dehydration-elicited expression of LEA genes is not restricted to resurrection plants but has been extensively reported also for non-tolerant plants.

LEA proteins are divided into different classes based on conserved sequence motifs. In general, LEA proteins are hydrophilic and have a biased amino acid composition, mostly lacking Cys and Trp. They are typically highly water soluble, and they often remain soluble after boiling. Despite extensive studies, our knowledge of the biochemical function of LEA proteins is still rudimentary, but molecular and biochemical features strongly suggest a protective role for them. A protective function correlates with their cellular distribution: LEA polypeptides are found in all cell types, accumulating abundantly in cytoplasm or plastids (Schneider et al., 1993). This protective role is further corroborated by Wolkers et al. (1998), who showed that LEA proteins may be anchors in a structural network stabilizing cytoplasmic components during pollen drying. Two approaches have been used to demonstrate that LEA proteins function as cellular protectants: in vitro protection assays with purified proteins and in planta studies in which LEA proteins were overexpressed. The results generally support a protective role (Xu et al., 1996), although contradictory observations have also been reported (Iturriaga et al., 1992). One conclusion that can be drawn from functional analysis indicates that individual LEA proteins make only a small contribution to dehydration tolerance, whereas the coordinated synthesis of the complete set of proteins probably plays a central role. Biochemical experiments, including structural analysis of native proteins, are needed to understand the role of each specific LEA protein. It is remarkable that *LEA* genes contain sequence motifs that are conserved in all higher plants. This strict conservation during evolution indicates that these motifs define functional units within these proteins.

Thus, our studies suggest that desiccation tolerance in vegetative tissues in *C. plantagineum* is probably not due to structural genes that are unique to resurrection plants, but that relevant genes are also present in the genome of non-tolerant plants. The

difference between tolerant and non-tolerant plants is likely to reside in the expression patterns and is likely to be at least in part a quantitative characteristic with respect to LEA genes. A comparison of dehydration-induced gene expression in the tolerant *C. plantagineum* with a non-tolerant close relative may provide further evidence for this hypothesis.

REGULATION OF GENE EXPRESSION DURING DEHYDRATION

An understanding of gene regulation is particularly important in the case of a multigenic trait like desiccation tolerance because different regulatory pathways determine the expression of a whole set of genes. Knowledge of regulatory circuits is scarce; individual factors have been characterized, but their interaction with other molecules within the network is for the most part unknown. Several experimental approaches have been followed to identify molecules involved in the activation of gene expression in response to stress. Most information is derived from promoter analyses and from differential screening procedures. Mutants have contributed to understanding gene regulation in Arabidopsis (Shinozaki and Yamaguchi-Shinozaki, 2000). A mutational approach to desiccation tolerance in *C. plantagineum* is quite difficult because of the polyploid nature of its genome.

One molecule that is central to dehydration-regulated gene expression is the plant hormone ABA. Exposure of *C. plantagineum* plants and callus to exogenous ABA induces genes that are otherwise activated by dehydration. Specifically in callus tissue, ABA is required to induce the genes needed for the expression of tolerance. The regulation of gene expression by dehydration and ABA involves several signaling pathways and different cis-acting elements in the stress-responsive genes.

PROMOTER ANALYSIS

Promoters of LEA-type genes and of genes encoding dehydration-inducible enzymes have been analyzed and compared. Comparisons of the promoter sequences did not reveal obvious common motifs. Therefore, several promoters were analyzed in transgenic tobacco (*Nicotiana tabacum*) and Arabidopsis plants to define functional cis-elements. The promoters tested were found to be highly active in seeds and pollen, but two out of three promoters were not active in vegetative tissues of Arabidopsis or tobacco (Furini et al., 1996; Velasco et al., 1998). Only the promoter of the gene *CDeT6-19* was inducible by dehydration or ABA in vegetative tissues of a heterologous plant. However, ectopic expression of the Arabidopsis ABI-3 gene product leads to activation of the other two promoters in Arabidopsis leaves upon ABA treatment (Furini et al., 1996; Velasco et al., 1998). *ABI-3* encodes a transcription factor that is active during seed development in Arabidopsis. In view of the fact that *C. plantagineum* synthesizes in vegetative tissues many LEA-type transcripts that are closely related to those of *LEA* genes expressed during seed maturation, an *ABI-3* homolog was isolated from *C. plantagineum* to test the possibility that such a gene might be responsible for transcriptional activation of LEA-type genes in vegetative tissues of *C. plantagineum*. A gene closely related to the Arabidopsis *ABI-3* gene was isolated, and its product was indeed able to transactivate *LEA* genes in transient expression assays (Chandler and Bartels, 1997). However, expression of the ABI-3 homolog was not detected in fully developed leaves of *C. plantagineum*. Therefore, other transcription factors must be involved in the activation of *LEA* genes.

In many genes regulated by ABA and osmotic stress, one or more ABA response elements (ABREs) play a key role in promoter activity. The ABREs have a core ACGT-containing G-box motif. It is assumed that transcription factors of the basic Leu zipper type bind as dimers to the ABREs. Modified core ABRE elements are present in LEA gene promoters in *C. plantagineum*, but they do not seem to be the major determinant of ABA responsiveness. Instead, e.g. in the CDeT27-45 promoter, other elements have been identified that bind nuclear proteins in an ABA-dependent fashion and are essential for activation of a reporter gene in response to ABA (Kirch et al., 2001b).

Molecules with putative transcriptional activities or with a putative signaling role have been defined in our laboratory in the course of various differential screening experiments. Their identification is based on sequence homology to known molecules. Transcripts encoding these molecules are induced during the early phase of dehydration and/or by ABA treatment, thus suggesting an involvement in the dehydration response. In this way, genes for several potential transcription factors were isolated, and it was shown that members of diverse gene families participate in the response of *C. plantagineum* to dehydration. These include genes for Myb and a heat shock transcription factor, members of the homeodomain Leu zipper (HDZIP) family, a phospholipase D (PLD), a kinase, and the gene *CDT-1*. These genes represent single building blocks in a complex regulatory network, but in most cases their target genes are still elusive and it is not known whether and, if so, how individual regulatory pathways interact with each other. In the following section, three examples of studies on such *C. plantagineum* factors will be described.

HDZIP GENES

HDZIP genes are specific for plants and are thought to regulate developmental processes, and

responses to environmental cues ranging from light perception to pathogen-induced and abiotic stress. HDZIP proteins are characterized by the presence of a DNA-binding homeodomain adjacent to a Leu zipper motif that mediates protein-protein interactions (Ruberti et al., 1991). The activity of HDZIP proteins resides primarily in binding of the homeodomain to specific HDE recognition sequences present in promoters of target genes. Several HDZIP genes that are regulated by dehydration have been isolated from *C. plantagineum*, indicating that this family of transcription factors is likely to play a major role in modulating gene expression during dehydration. Research in Arabidopsis supports this idea. Two HDZIP genes from *C. plantagineum*, *CPHB-1* and *CPHB-2*, are of particular interest because their products were shown to interact in a yeast (*Saccharomyces cerevisiae*) two-hybrid system (Frank et al., 1998). Both transcripts were inducible by dehydration, but only *CPHB-2* was responsive to ABA. This suggests that the two genes act in different pathways of the regulatory network, an ABA-mediated pathway and an ABA-independent pathway. Cross talk between these two pathways should be possible through the interaction of the two HDZIP proteins. Although no target genes for the HDZIP proteins have been definitively identified, active binding sites for HDZIP proteins were found in the promoters of at least two desiccation-regulated genes.

THE NOVEL *CDT-1* GENE

Because of its ploidy level, the best way to obtain mutants in *C. plantagineum* is to induce dominant mutations. The ABA-dependent induction of desiccation tolerance in the callus system offers the opportunity to screen for such dominant mutants. The *C. plantagineum* leaf discs are transformed by infection with *A. tumefaciens* cells bearing a T-DNA vector containing enhancer fragments of the 35S-cauliflower mosaic virus promoter. The enhancers are inserted randomly into the genome and, if present in appropriate positions, should lead to gene activation. Among thousands of regenerated calli, a transgenic callus was selected that was capable of surviving desiccation without prior addition of ABA to the medium. Analysis of RNA transcripts showed that genes were constitutively expressed in the mutant callus, which in non-transformed calli can only be induced by ABA treatment. The gene targeted by the T-DNA, which led to the constitutive activation of desiccation-related genes, was isolated and named *CDT-1* (Furini et al., 1997). *CDT-1* is present in multiple copies in the genome of *C. plantagineum* and its expression can be induced by ABA in non-transformed callus and plants. The most surprising feature of the gene is that it has only a very short open reading frame encoding a putative polypeptide of 22 amino acids. To date, it has not been possible to demonstrate the presence of a polypeptide encoded by *cDT-1*, and further experiments support the hypothesis that CDT-1 may act as a regulatory RNA. Besides *CDT-1*, another example for a gene with such properties is the *ENOD40* gene from *Medicago sativa*, which controls the organogenesis of *Rhizobium meliloti*-induced N_2-fixing nodules (Crespi et al., 1994). No sequence homolog to *cDT-1* was identified in the Arabidopsis genome, but this does not exclude the possibility that Arabidopsis encodes transcripts that may function at the RNA level. On the other hand, the function of *cDT-1* may be specifically related to the highly sensitive and effective set of reactions that protect *C. plantagineum* from the effects of severe desiccation.

ACTIVATION OF PLD: AN EARLY EVENT IN THE DEHYDRATION RESPONSE

Recently, phospholipid metabolism has been suggested to be involved in plant responses to various forms of osmotic stress (Munnik and Meijer, 2001). The formation of phospholipid-based signaling molecules may well be among the primary events in the signaling cascade that leads from the perception of water stress to stress-adapted metabolism. In the context of the desiccation response in *C. plantagineum*, PLD is of particular interest. PLD catalyzes the hydrolysis of a structural phospholipid, phosphatidylcholine, and other phospholipids, to form phosphatidic acid (PA). PA in turn regulates protein kinases or small GTP-binding proteins. In *C. plantagineum*, PLD activity is induced within minutes by dehydration (Frank et al., 2000). The PLD activity is specific for dehydration and is not induced by ABA. Two cDNA clones (*CpPLD-1* and *CpPLD-2*) encoding PLDs have been isolated. The *CpPLD-1* transcript is constitutively expressed, whereas *CpPLD-2* is responsive to dehydration (Frank et al., 2000). Analysis of PLD proteins indicates a complex regulation at the level of expression and cellular distribution depending on the physiological status of the plant. The constitutively expressed *CpPLD-1* is likely to be involved in early responses to dehydration, producing PA as a second messenger that transmits the stress signal. *CpPLD-2* may be involved in phospholipid metabolism and the membrane rearrangements that are observed as a consequence of cellular desiccation.

Multiple forms of PLD exist in Arabidopsis, and a dehydration-responsive PLD was also recently reported from this plant (Katagiri et al., 2001). This PLD shows low sequence similarity with the PLDs from *C. plantagineum*. The Arabidopsis PLD that is structurally most closely related to the products of the *C. plantagineum* genes is not responsive to water deficit in vegetative tissues. Instead, it is associated with senescence, and is reported to be present in guard cells, regulating the closure of stomata, and is thus involved in controlling water relations (Sang et

al., 2001). The comparison of the PLDs in two plant species, *C. plantagineum* and Arabidosis, demonstrates that despite close sequence homologies, expression patterns and gene functions have diverged during evolution. The adaptation of *C. plantagineum* to cope with extreme desiccation may have resulted from the recruitment of more genes to dehydration-responsive regulons than are devoted to this task in a plant that only tolerates a moderate degree of water stress.

CONCLUSIONS

Although more work is necessary to define gene functions and dissect the complex regulation of gene expression, the genes isolated and characterized from *C. plantagineum* to date give us many intriguing insights into the protective mechanisms that determine desiccation tolerance. The studies on *C. plantagineum* extend our knowledge of the responses of plants to dehydration, providing a wider perspective on significance of the information gained from the extensive studies of Arabidopsis. A comparison of the information obtained from *C. plantagineum* and Arabidopsis can help to answer the following question: Is the mechanism of desiccation tolerance in resurrection plants comparable with the mechanism used in the seeds of most higher plants or has *C. plantagineum* recruited unique genes to protect its vegetative tissues?

In view of technological advantages, it is a safe bet that most advances in general understanding of basic patterns of growth and performance of plants will be obtained from studies on Arabidopsis. However, these studies will not be sufficient to explain the adaptation of *C. plantagineum* to extreme drought. The information available from Arabidopsis research is often useful in identifying genes isolated from *C. plantagineum*. Nevertheless, careful comparative studies have to be performed because several cases (e.g. see the section on PLD above) have taught us that sequence homology does not always imply functional identity.

Research on *C. plantagineum* has uncovered novel features. This is the case for the unusual and biotechnologically very interesting accumulation in leaves of the C8 sugar octulose, which seems to be restricted to a small group of plants. An understanding of the underlying mechanism may teach us new lessons in carbohydrate technology.

A discovery that would not have been possible without the research on *C. plantagineum* was the isolation of the *CDT-1* gene. Although the function of the *CDT-1* gene is not understood, it is likely that it acts as a regulatory RNA. This points to novel molecular mechanisms that may have played a particularly important role in the evolution of adaptation to extreme environmental stress.

The analysis of desiccation tolerance in *C. plantagineum* suggests that, at least to a large extent, the same molecules are involved in the tolerance mechanism in seeds and in vegetative tissues of *C. plantagineum*. This is important because it means that the genetic information for desiccation tolerance is present in the genome of most, if not all, higher plants. Differences in the control of gene expression probably account for the restriction of desiccation tolerance to specific stages of seed development. Identification of the molecular switches that determine the spatial and temporal patterns of gene expression induced during the acquisition of desiccation tolerance in seeds may, in the future, lead to the programmed control of the desiccation response also in vegetative organs.

Research on *C. plantagineum* has allowed us to answer several questions that are important in understanding biodiversity and plant adaptation to ecological niches. The combination of data from studies on the genetic model plant Arabidopsis and on diverse plant species should help us to understand one of the most amazing inventions in plant biology—the ability to survive without water.

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