Acquisition and Loss of Desiccation Tolerance

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I. INTRODUCTION

The ability to withstand complete loss of cellular water is an unusual feature in life; yet it is an adaptive feature of seeds of many species. These seeds are termed “orthodox,” because they can be stored for years under cold dry conditions. Desiccation tolerance is not a universal aspect of seeds. Several plant species from tropical rainforests, temperate forests, and riparian environments produce seeds that are sensitive to desiccation. Because these seeds are difficult to store for more than a single growing season, they are often called “recalcitrant” (Roberts, 1973).

The ability to tolerate desiccation is not limited to seeds, although in spermatophytes, it appears to be more prevalent in the propagating structures such as pollen, seeds, dormant buds, and somatic embryos (Bewley, 1979; Levitt, 1980b; Bewley and Oliver, 1992; Crowe et al., 1992). Desiccation tolerance of vegetative tissues is more common in the lower orders (mosses and algae), and it has been suggested that tolerance in these tissues is based on repair of structures rather than protection, as is hypothesized for seeds (Bewley and Oliver 1992). Despite the widespread occurrence of desiccation tolerance, very little is known about the mechanisms whereby tissues are able to survive dehydration. We could reason that tolerance to desiccation is difficult to achieve and perhaps very energetically costly; otherwise all tissues should be able to adapt to this extreme stress. Vegetative tissues are subject to drying at unpredictable intervals, and thus the mechanisms of tolerance must be readily activated in response to the stress. In contrast, orthodox seeds undergo desiccation in a programmed manner at the termination of their development. Desiccation tolerance is acquired during development of orthodox seeds and it is lost after germination. The purpose of this chapter is to review our understanding of the possible mechanisms of desiccation tolerance in seeds. We will discuss some of the anatomical, biochemical, and biophysical events associated with the onset and loss of desiccation tolerance in orthodox seeds and compare these with developmental aspects of recalcitrant seeds.
II. CHALLENGES OF THE DESICCATED ENVIRONMENT

Water has physical properties that make it an ideal biological solvent; thus it plays many roles in the processes of life. Because it is an incompressible fluid, it can fill cells and organelles, giving them structure. The fluid environment provided by water allows for the diffusion of substrates to the active sites of enzymes. Hydrophilic and hydrophobic interactions stabilize macromolecular conformations and allow for the sequestering of cellular constituents. Water is a reactant or product in many important reactions. Evidence suggests that water may inhibit deleterious reactions by preventing molecules from interacting; in this sense, water also serves as a protectant of macromolecular structure. Because of its myriad roles, water controls the level of metabolic activity in plants (Clegg, 1978; Hegarty, 1978; Adams and Rinne, 1980; McIntyre, 1987; Leopold and Vertucci, 1989). Thus the loss of water will profoundly affect the nature of physical and biochemical reactions (e.g., Karel, 1975; Adams and Rinne, 1980; Leopold and Vertucci, 1989; Bryant and Wolfe, 1992; Crowe et al., 1992). An organism that can tolerate desiccation must be able to prevent, slow down, and/or repair the deleterious reactions induced by the removal of water.

In order to understand the mechanisms of tolerance, it is necessary to understand the nature of damage when water is removed from organisms. We have approached this by reviewing the effects of dehydration at cellular and subcellular levels.

A. Metabolic Stresses

As water is removed from the cell, the concentration of solutes is increased, and eventually the fluidity of the aqueous medium declines. These changes affect the metabolic status of the cell. The changes in metabolic activity are believed to occur at specific moisture levels (Leopold and Vertucci, 1989; Clegg, 1978) (Fig. 1). Critical moisture levels have been postulated for germination metabolism (Palit, 1987), germination (McIntyre, 1987; Hegarty, 1978; Palit, 1987), continued embryogenesis (Adams and Rinne, 1980; Galau et al., 1991; Rosenberg and Rinne, 1986; Finkelstein and Crouch, 1986; Morris et al., 1991; Fisher et al., 1988; Xu et al., 1990), and the cessation of growth (Adams and Rinne, 1980; Saab and Obendorf, 1989) and cell division (Adams and Rinne, 1980; Myers et al., 1992). Below a moisture level of about −1.5 MPa, tissues no longer grow or expand (e.g., McIntyre, 1987; Hegarty, 1978; Levitt, 1980b), and protein and nucleic acid synthesis patterns change (e.g., Dell’Aquila and Spada, 1992; Dhindsa and Cleland, 1975; Skriver and Mundy, 1990). This slight desiccation may induce production of protectants (Close and Chandler, 1990; Dure et al., 1989; Skriver and Mundy, 1990; Dhindsa, 1991; Chen and Burris, 1990; Blackman et al., 1991, 1992; Bewley, 1979; Kermode, 1990; Bewley and Oliver, 1992). Greater levels of desiccation can result in metabolic imbalances. At about 0.45 g H₂O/g dm or about −3 MPa* (according to data from Dell’Aquila, 1992), protein synthesis ceases and repair processes become inoperative (Dhindsa and Cleland, 1975; Osborne, 1983; Dell’Aquila, 1992; Clegg, 1978; Adams and Rinne, 1980). Respiratory activity continues until tissues are dried below about 0.25

*In the interest of using similar units throughout the paper, water potential is used to describe moisture level. To achieve different water contents at water potentials = −11 MPa, biological materials are usually equilibrated to different relative humidities. Water potentials are then calculated from the equation \( \Psi = R/T \ln(RH/100) \). This treatment assumes that the molar volume of water does not change at extremely low water contents. In this paper, water potentials can be converted back to relative humidity with the equation \( RH = \exp(-0.0074 \cdot \Psi) \)).
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<th>HYDRATION LEVEL</th>
<th>6% water&lt;sup&gt;1&lt;/sup&gt;</th>
<th>-150 MPa</th>
<th>25% water&lt;sup&gt;2&lt;/sup&gt;</th>
<th>-17 MPa</th>
<th>45% water&lt;sup&gt;3&lt;/sup&gt;</th>
<th>-3 MPa</th>
<th>&gt;70% water&lt;sup&gt;5&lt;/sup&gt;</th>
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<td>osmotic excursions</td>
<td>protein synthesis I nucleic acid repair</td>
<td>protein synthesis II</td>
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<td>DESCENTION DAMAGE</td>
<td>free radical production II</td>
<td>enzymatic degradation</td>
<td>unregulated catabolism</td>
<td>free radical production III</td>
<td>interbilayer interactions</td>
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<td>nonbilayer phase transitions</td>
<td>bilayer phase transitions</td>
<td>immature embryos and seedlings die</td>
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<td>concentrated or pore solution</td>
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**Figure 1** Hydration levels in seeds. There appear to be critical moisture levels at which discrete changes in metabolic activity, damage induced by desiccation, and the properties of water are observed. Here, we present critical moisture levels based on literature cited in this paper. The water potentials given are approximate and require more detailed research, especially for levels 4 and 5. Protein synthesis I and II represent different levels of gene expression. Similarly, free radical production I and II represent different mechanisms of production. The moisture levels for these are also conjectural at this point.

<sup>1</sup>g/g or -11 MPa (Leopold and Vertucci, 1989; Vertucci, 1989). At moisture levels between -3 and -11 MPa (about 0.45 to 0.25 g H<sub>2</sub>O/g dm), catabolic activities continue unabated and processes utilizing the high-energy intermediates are impeded (Leopold and Vertucci, 1989; Vertucci, 1992). These cells may utilize food reserves and accumulate toxins, such as free radicals (LePrince et al., 1990b; 1992; Hendry et al., 1992; McKersie et al., 1988). Desiccation tolerant organisms must be able to withstand such changes in metabolism (Levitt, 1980b; LePrince et al., 1990b, 1992; Rogerson and Matthews, 1977).

Cells may be more susceptible to the rate of desiccation rather than to loss of water per se (Pritchard, 1991; Pammenter et al., 1991; Berjak et al., 1993; compare Probert and Brierley, 1989 with Kovach and Bradford, 1992; Finch-Savage, 1992a). When held at moisture levels where only catabolic activities occur, cells that are innately desiccation tolerant may suffer damage because they have been subjected to the stress of an unregulated metabolism for a longer time period than if dried rapidly. It is therefore
important to realize that the ability to tolerate desiccation must be considered in the context of how the tissue was dried.

B. Mechanical Stresses

One of the first obvious indications of water stress in cells is a loss of turgidity. Under extreme drought, the cells collapse (reviewed by Levitt, 1980a,b). A nonrigid cell wall is required to minimize the tension between the plasmalemma and the cell wall induced by the negative turgor pressure (Ilijin's theory of mechanical stress, reviewed by Levitt, 1980a,b). Cell walls of seed tissues appear to be adapted to accommodate the changes in cell volume (Webb and Arnott, 1982).

The contraction of cells during dehydration can result in the loss of membrane material (reviewed by Steponkus and Webb, 1992, and Hincha and Schmitt, 1992). Meryman (1974) proposed that cells could not contract beyond a "minimum critical volume" without loss of membrane function upon rehydration. Ilijin in the 1920s and 1930s (reviewed by Levitt, 1980a,b) observed that cells that were more tolerant of desiccation had only minor reductions in volume when desiccated, and this could be achieved in small cells (about 100 to 1000 \( \mu \text{m}^3 \)), which give relatively large surface-area-to-volume ratios (ca. 1:1) or in cells where accumulated dry matter fills the space previously occupied by water. According to Ilijin's hypothesis of mechanical damage, reduced vacuolar space would also enhance tolerance to desiccation. Furthermore, organelle geometry appears to be an important feature in vegetative tissues possessing desiccation tolerance (Bewley, 1979; Levitt, 1980b; Kaiser, 1982; Oertli, 1986; Bergstrom et al., 1982; Oliver and Bewley, 1984).

During contraction, the plasma membrane vesiculates, and the loss of surface area can result in lysis or liposomes that fail to swell upon subsequent rehydration (Steponkus et al., 1990; Steponkus and Webb, 1992; Hincha and Schmitt, 1992; Johnson-Flanagan and Singh, 1986). The degree of contraction that is tolerated and the amount of membrane material that is irreversibly lost when small vesicles leave the plasmalemma is probably affected by the plasma membrane lipid composition. For example, during acclimation to cold, the phospholipid composition of the plasmalemma changes (Liljenberg and Kates, 1985; Uemura and Steponkus, 1989; Steponkus et al., 1990). Increased cold-hardiness is associated with greater desiccation tolerance (Levitt, 1980a,b), and so one may reason that membrane compositional changes during acclimation to either stress may be comparable. Indeed, increases in the level of unsaturated fatty acids in the phospholipid component is detected during cold acclimation, and addition of these lipid moieties to nonacclimated protoplasts enhances their tolerance to fluctuating water potentials, or "osmotic excursions" (Uemura and Steponkus, 1989). Organisms that are tolerant to desiccation must have a mechanism that allows the reduction of the protoplasmic volume upon dehydration and the subsequent retrieval of the material that may have been lost during vesiculation.

A consequence of protoplasm contraction during dehydration is that the surface-area-to-volume ratio of the protoplast increases. While Ilijin's hypothesis suggests that a relatively high ratio (about 1:1) is associated with desiccation tolerance, theoretical considerations suggest that a very high ratio (such that membranes are within a few nm of each other) contributes to deforming stresses that can alter membrane properties (Wolfe, 1987; Bryant and Wolfe, 1989, 1992). When water is removed from cells, membrane systems become packed together and the interbilayer interactions can cause membrane
fusion and/or phase transitions (Lis et al., 1982; Wolfe, 1987; Bryant and Wolfe, 1992; Steponkus and Webb, 1992; Webb and Steponkus, 1993). Organelles with high membrane contents, such as chloroplasts and mitochondria, are particularly susceptible to this type of stress (Wolfe, 1987; Bryant and Wolfe, 1992). Organisms that tolerate dehydration must be able to cope with the mechanical stress resulting from membrane apposition by avoiding the stress (spacers between membranes) or diminishing the strain by optimizing surface-area-to-volume ratios of membranes.

C. Phase Transitions of Lipids

1. Induction by Decreasing Water Content

When moisture levels are considerably reduced, the structure of nucleic acids, proteins, and polar lipids can be altered because the hydrophilic and hydrophobic interactions that stabilize conformations are weakened (Leopold, 1986; Crowe et al., 1987; Carpenter and Crowe, 1988). Membrane systems are considered particularly susceptible to dehydration damage (Crowe et al., 1986, 1987, 1988, 1992; Leopold, 1986; Kerhoas et al., 1987; Wolfe, 1987; Caffrey et al., 1988; McKersie et al., 1988; Bryant and Wolfe, 1989, 1992; Steponkus and Webb, 1992; Quinn, 1985). The losses of membrane function have been attributed to "demixing" (segregation of the various components) of membrane constituents with different hydration characteristics and/or to phase transitions of the polar lipid component (Quinn, 1985; Crowe et al., 1987; 1992; Wolfe, 1987; Steponkus and Webb, 1992; Bryant and Wolfe, 1989, 1992; Webb et al., 1993). Two types of phase transitions are commonly reported: lamellar liquid crystalline to gel in which the bilayer configuration is maintained, and lamellar liquid crystalline to hexagonal in which a nonbilayer structure is formed. A water potential of about -12 MPa or a water content of about 0.20 g/g dm appears to be a critical moisture level for membranes changes in unprotected systems (Lis et al., 1982; Crowe et al., 1987, 1992; Wolfe, 1987; Steponkus and Webb, 1992; Bryant and Wolfe, 1989, 1992; Webb et al., 1993; Webb and Steponkus, 1993). Greater levels of dehydration are required for membrane aberrations in very cold-hardy cells and in desiccation tolerant pollen (Webb and Steponkus, 1993; Hoekstra et al., 1992).

Since lipid phase transitions are reversible, they may not be lethal in themselves. In fact, liquid crystalline to gel transitions have been reported in desiccation tolerant organisms (Hoekstra et al., 1991, 1992; Crowe et al., 1992). However, "demixing" of membrane constituents can lead to the loss of protein in some portions of the membrane or even the irreversible efflux of membrane-bound proteins (Hincha and Schmitt, 1992; Quinn, 1985; Senaratna et al., 1987; Kerhoas et al., 1987; Webb and Steponkus, 1993). In recalcitrant Camellia sinensis embryos, lipids undergo reversible transitions with lethal drying, but the changes in protein structure are not reversible (Sowa et al., 1991). Demixing may also lead to the formation of nonbilayer structures (Quinn, 1985; Crowe et al., 1986, 1989, 1992; McKersie et al., 1989; Hoekstra et al., 1992; Steponkus and Webb, 1992; Bryant and Wolfe, 1989, 1992). Irreversible damage is associated with the formation of hexagonal phases (Gordon-Kamm and Steponkus, 1984; Crowe et al., 1986, 1987, 1989, 1992; McKersie et al., 1989; Hoekstra et al., 1992; Webb et al., 1993), probably because it leads to membrane fusion, loss of cell compartmentation, and irreversible membrane disorganization.

The possibility of membrane phase transitions and the nature of the transitions are determined by the molecular geometry of the liposome and its polar lipid components, the presence of other membrane constituents (proteins and/or sterols), the temperature,
and the degree of dehydration (e.g., Quinn, 1985; Small, 1986; Bryant and Wolfe, 1989, 1992; Steponkus and Webb, 1992; Webb et al., 1993). Formation of nonbilayer configurations also requires that several membrane systems be in close proximity so that lipids from different lamellar systems interact (Li et al., 1982; Steponkus and Webb, 1992; Webb and Steponkus, 1993). Upon dehydration, the lamellar gel phase is favored by polar lipids with rectangular-shaped head groups such as phosphatidylcholine, and the hexagonal phase is favored by polar lipids with more triangular-shaped head groups such as phosphatidylethanolamine (Quinn, 1985; Small, 1986). Saturated long chain fatty acids and saturated free fatty acids tend to increase the transition temperature (given the same water content), thus promoting the formation of gel phase domains (Small, 1986; McKersie et al., 1988, 1989; Crowe et al., 1989, 1992). On the other hand, the formation of hexagonal phases is more common if the hydrocarbon chains are unsaturated, if there are free fatty acids present, or if the polar lipid is extremely dehydrated (water potentials less than –150 MPa, or water contents less than about 0.03 g/g) (Quinn, 1985; Small, 1986; Bryant and Wolfe, 1989, 1992; Crowe et al., 1989, 1992; McKersie et al., 1989; Hockstra et al., 1992; Webb et al., 1993). Bilayers containing sterols or proteins generally have low-energy phase transitions over a broader range of temperatures (Quinn, 1985; Small, 1986). This indicates that these molecules profoundly affect the phase behavior of the polar lipids. The phase behavior of a membrane is also affected by the degree of curvature of the vesicle (Mason et al., 1983; Gaber and Sheridan, 1982; Small, 1986), suggesting that the geometry of the membrane vesicles formed during cell contraction may influence their stability.

2. Induction by Peroxidation

The phase behavior of membranes can change as a consequence of the unregulated chemical reactions that occur when tissues are dried. The reactions are believed to be peroxidative and result in lower levels of fatty acid unsaturation, lipid hydroperoxides and their byproducts, and free fatty acids (Chan, 1987; Priestley, 1986; McKersie et al., 1988). Lipid peroxidation products can be formed at low moisture levels enzymatically (Karel, 1975; Vertucci and Leopold, 1987b; Priestley, 1986; Leopold and Vertucci, 1989) or nonenzymatically by free radicals (McKersie et al., 1988; Hendry et al., 1992; Rockland, 1969) produced from the partial reduction of oxygen in active mitochondria (Leprince et al., 1990a, 1992; Cakmak et al., 1993; Puntarulo et al., 1988), from metal ions exposed within heme proteins (Chan, 1987; Eriksson, 1970; Labrude et al., 1987), or from incident radiation (Conger et al., 1966). The free radicals accumulate because the scavenging systems are not very effective in the dehydrated state (Hendry et al., 1992; Dhindsa, 1991; LePrince et al., 1990b; McKersie et al., 1988; Arrigoni et al., 1992; Cakmak et al., 1993). Because free fatty acids cause membrane fusions at all moisture levels regardless of the presence of protectants (Crowe et al., 1989; McKersie et al., 1989), they are considered quite destabilizing to the membrane structure (McKersie et al., 1988). While lipids are particularly susceptible to peroxidative attack, the by-products of these reactions also affect protein and nucleic acid function (Dizdaroglu, 1991; Gardner, 1979; Kanner and Karel, 1976; Kanazawa et al., 1975; Witz, 1989; Wolff et al., 1986).

D. Ultra Dry Conditions

If cells can survive the immediate stress imposed on the structure of organelles and macromolecules when water is removed, they are often termed desiccation tolerant.
However, several studies have shown that complete removal of water destabilizes protein structure (Labrude et al., 1987; Sanches et al., 1986), promotes lipid peroxidation (Rockland, 1969; Karel, 1975; Kanner and Karel, 1976), and hastens the rate of seed deterioration (Conger et al., 1966; Vertucci and Leopold, 1987; Vertucci and Roos, 1990). While these processes are often regarded as "aging," we view them as desiccation injury, because the rate of deterioration is a function of the water content (Vertucci and Roos, 1990; Hoekstra et al., 1992; Hong and Ellis, 1992a). The mechanism of damage upon complete removal of water is unknown; however, it has been suggested to make cellular constituents more susceptible to the deleterious effects of an oxidizing atmosphere (Conger et al., 1966; Rockland, 1969; Karel, 1975; Bewley, 1979; Sanches et al., 1986; Labrude et al., 1987; Vertucci and Roos, 1990). An alternative hypothesis is that the removal of water allows molecular species to interact so that phase changes are induced (Quinn, 1985; Crowe et al., 1992; Hoekstra et al., 1992; Bruni and Leopold, 1992b). By coating the surfaces of macromolecules, water may prevent interactions of highly reactive species.

Clearly, there are a series of challenges associated with surviving in an anhydrous environment (Fig. 1). Truly desiccation tolerant tissues will have mechanisms to tolerate all the anatomical, biochemical, and biophysical changes that result when hydrated cells are dried. Consequently, desiccation tolerance must be viewed as a complex phenomenon with perhaps several interactive components. One could postulate that for a tissue to be truly tolerant, all of the components must be present; lack of one or more could lead to intermediate levels of tolerance.

III. SEED DEVELOPMENT AND LEVELS OF DESICCATION TOLERANCE

Because water affects the condition of cells in so many ways, tissues that survive its removal are likely to have a combination of strategies to limit the damage resulting from dehydration. It is logical, then, to view desiccation tolerance as a quantitative feature (Pritchard, 1991; Berjak et al., 1989, 1992, 1993; Finch-Savage, 1992b; Sun and Leopold, 1993), and the level of tolerance as a function of the effectiveness of the series of strategies that the tissue possesses. For example, a cell may be able to tolerate the mechanical stresses of dehydration but may lack sufficient protective solutes to survive severe desiccation or prolonged periods in the desiccated state. Alternatively, an extremely desiccation sensitive cell may have ample protectants but may be so highly vacuolated that it cannot survive the mechanical stress of dehydration to even moderate water potentials. This argument implies that when desiccation tolerance is not achieved (in a recalcitrant seed, for example), we must look for several possible reasons. Studies of the degree to which tissues can be dried, the effect of different drying protocols, and the longevity of tissues in the dry state may give indications of the limiting factor to desiccation tolerance for that particular tissue.

A. Developmental Stages

For both recalcitrant and orthodox seeds, the relative level of desiccation tolerance changes throughout development so that embryos become more tolerant as they mature and less tolerant as they germinate (Adams et al., 1983; Berjak et al., 1989, 1992, 1993; Berry and Bewley, 1991; Bewley, 1979; Dasgupta et al., 1982; Farrant et al., 1986, 1988, 1989; Finch-Savage, 1992b; Fischer et al., 1988; Hong and Ellis, 1990; Kermode and
Bewley, 1985; Long et al., 1981; Pritchard, 1991; Rogerson and Matthews, 1977; Rosenberg and Rinne, 1986; Sargent et al., 1981; Sun and Leopold, 1993; Tompsett and Pritchard, 1993; Welbaum and Bradford, 1989). However, only orthodox seeds achieve considerable tolerance of desiccation. The acquisition of tolerance is presumably developmentally controlled (Kermode, 1990; Bewley and Oliver, 1992; Galau et al., 1991). In their review, Galau and coworkers (1991) divided postdifferentiation embryogenesis into five stages based on the appearance of molecular markers: (1) maturation, (2) postvascular separation (PVS),* (3) predesiccation, (4) desiccation, and (5) quiescence. These (and other) authors suggest that desiccation tolerance is acquired during the PVS stage.

Studies of the effect of premature harvest on seed vigor and viability suggest that maximum desiccation tolerance (defined as survival after complete removal of water and maintenance of vigor in the desiccated state) is achieved only upon the successful completion of the first three stages of embryogenesis and the rapid completion of the fourth stage. A recent report suggests that complete maturation is required for *Acer platanoides* to survive complete desiccation (Hong and Ellis, 1992a). While tolerance to desiccation is acquired continuously during seed maturation (see references above), several orthodox seeds acquire maximum vigor and longevity in dry storage a time after maximum dry matter accumulation (Demir and Ellis, 1992; Ellis et al., 1987; Fisher et al., 1988; Welbaum and Bradford, 1989). Recalcitrance appears to be a product of either an abbreviated PVS stage (progression toward germination processes following abscission) (Berjak et al., 1990; Farrant et al., 1985, 1986, 1988, 1992a, 1993c) or an early termination of development (Finch-Savage, 1992b). Thus we suggest that embryos that do not achieve maximum desiccation tolerance either lack events occurring in stages 1 through 3 that confer tolerance or are shed (and initiate germination) prior to completing these stages. The developmental changes that occur during embryogenesis allow us to study the process by which embryos acquire and lose their tolerance to desiccation (Fig. 2).

At the early stages of development, embryos are extremely sensitive to dehydration stress (Rogerson and Matthews, 1977; Long et al., 1981; Dasgupta et al., 1982; Berjak et al., 1993). There is little information regarding how much water is actually required, but sufficient quantities to allow cell division are certainly necessary. Thus we propose that water potentials greater than −1.6 MPa (Myers et al., 1992) are required.

During the maturation phase, the embryo accumulates dry matter and becomes germinable. Coincident with these changes, tolerance of low water potentials increases (see references above). In orthodox seeds, there is a transition, at a particular stage in the developmental pathway, from a relatively desiccation intolerant to a tolerant state (Kermode, 1990). This transition can be prematurely induced or prolonged by environmental and chemical manipulations (e.g., Blackman et al., 1991, 1992; Johnson-Flanagan et al., 1991, 1992). Once induced, the immature embryo can become fairly tolerant of desiccation within a few days (Galau et al., 1991; Kermode, 1990; Blackman et al., 1992; Johnson-Flanagan, 1992). Because this high level of tolerance is achieved so rapidly, it is difficult to evaluate the true level at the time when the embryo is harvested and thus the exact timing of the programmed developmental switch. Although often not documented in experimental procedures, the time interval between harvest (or shedding) and

*In their paper, Galau et al. (1991) use the term "post-abscission," which may be confused with shedding of seeds. The stage these authors describe is believed to be initiated by the loss of the vascular connection between the parent and the embryo.
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Figure 2  A schematic diagram of the desiccation tolerance of developing seeds in relationship to the changing requirements for different types of water. The solid line represents the moisture level below which drying is lethal for orthodox seeds. Dashed lines represent recalcitrant embryos' responses to drying. Text outside the solid lines represents different types of desiccation damage, and text within the solid lines represents mechanisms to resist the damage. Developmental processes are described along the abscissa; the timing of these is not represented in the figure and will depend on the species as well as on environmental factors. Processes that are starred (*) do not occur in recalcitrant seeds, and so it is expected that the corresponding protective mechanisms also do not occur.

Experimental manipulation and the drying time course are necessary to evaluate whether embryos are in a desiccation tolerant stage or are inducible to a desiccation tolerant stage.

We hypothesize that embryos from recalcitrant seeds have an abbreviated postvascular separation phase, or even lack one. While such seeds may become increasingly tolerant of drying as maturation proceeds (Berjak et al., 1992, 1993; Finch-Savage, 1992b; Pritchard, 1991; Tompsett and Pritchard, 1993), they remain hydrated and metabolically active throughout development (Berjak et al., 1992, 1993; Farrant et al., 1992b, 1993c). Recalcitrant seeds appear to initiate germination-related metabolism shortly after shedding (Farrant et al., 1988, 1992b; Berjak et al., 1989) and, in Avicennia marina, 10 to 15 days before shedding (Farrant et al., 1993a). As germination events progress, the seeds become increasingly sensitive to drying (Farrant et al., 1986; Berjak et al., 1989, 1992, 1993).
Thus the timing of the onset of germination and the rate at which it proceeds can determine the level of desiccation sensitivity in these seeds (Farrant et al., 1986; 1988; 1989; 1992b).

B. Maximum Desiccation Tolerance of Different Seed Types

For many seeds, the timing of maximum dry matter accumulation and the acquisition of maximum desiccation tolerance of the embryonic axis are within days (Berry and Bewley, 1991; Demire and Ellis, 1992; Ellis et al., 1987; Fischer et al., 1988; Kermode and Bewley, 1985; Sun and Leopold, 1993; Long et al., 1981; Dasgupta et al., 1982; Berjak et al., 1992; Finch-Savage, 1992b; Tompsett and Pritchard, 1993; Welbaum and Bradford, 1989). Notable exceptions may be barley and wheat grains, which appear to be quite tolerant at fairly early stages of dry matter accumulation (Bartels et al., 1988; Rasyad et al., 1990). The critical moisture level to which mature embryos can be dried without inducing desiccation damage is generally species dependent and serves as a tool to define whether a seed has orthodox, recalcitrant, or intermediate (Ellis et al., 1990a, b) storage behavior.

The minimum moisture level for survival of some recalcitrant seeds (Camellia, Cacao, Hevea, Quercus, Aesculus, Litchi, Euphoria, Landolphia, and some Acer) corresponds to about −11 MPa (or about 0.2 to 0.3 gH₂O/gdm) (Berjak et al., 1992, 1993; Finch-Savage, 1992a, b; Hong and Ellis, 1990; Pammenter et al., 1991, 1992; Pritchard, 1991; Tompsett and Pritchard, 1993; Xia et al., 1992; Chin et al., 1981; Ray and Sharma, 1987; Poulsen, 1992; Pence, 1992). To achieve this low level of hydration without killing the tissue, embryos at the correct developmental stage must be dried rapidly (Berjak et al., 1993; Pammenter et al., 1991; Pritchard, 1991), perhaps because the rapid drying limits deleterious effects of an "uncontrolled metabolism" (Pritchard, 1991; Pammenter et al., 1991; Berjak et al., 1993; Vertucci, Pammenter and Berjak, unpublished) or because it prevents further development of the embryo into a germinating and less desiccation tolerant state (Farrant et al., 1985, 1986, 1989; Berjak et al., 1989).

In a number of cases, the critical moisture level for survival is about 0.6 to 0.8 gH₂O/gdm. Mature embryos of some recalcitrant seeds endemic to tropical areas (i.e., Avicennia marina, Podocarpus henkelii, and Castanospermum australe), seeds that have been stored under humid conditions (and so have developed past the stage of maximum desiccation tolerance), or embryos that have been dried too slowly are in this category (Farrant et al., 1985, 1988, 1989; Berjak et al., 1992; 1993; Pammenter et al., 1991, 1992). The water potential for this moisture level has not been determined; using isotherms derived for other embryonic tissues (Grange and Finch-Savage, 1992; Dell’Aquila, 1992), a water potential between −1.5 and −2.5 MPa can be estimated. It seems plausible that seeds in this category either lack or have lost some fundamental property required for desiccation tolerance.

The phenomenon of intermediate storage behavior has only recently been defined (Ellis et al., 1990a, b). Embryos that cannot withstand the synergistic effects of low moisture levels and low temperatures fall into this category [e.g., papaya (Ellis et al., 1991), Elaeis (Ellis et al., 1990b), Zizania (Kovach and Bradford, 1992; Vertucci, unpublished) and also partially germinated seeds (Hong and Ellis, 1992b)]. We have suggested that seeds with intermediate storage characteristics lose viability rapidly when stored at moisture levels less than about −150 MPa, while seeds with orthodox characteristics lose viability slowly (i.e., they age) at moisture levels below this value (Vertucci and Roos, 1990, 1993). The considerable desiccation tolerance of seeds with
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intermediate storage behavior may be a result of a completed PVS stage (as has been documented for *Zizania* using protein markers) (Bradford and Chandler, 1992). Since tolerance of virtually complete removal of water is enhanced during the very final stages of seed development (Hong and Ellis, 1992a), one may speculate that seeds with intermediate storage characteristics lack the predesiccation stage described by Galau and coworkers (1991). It is intriguing to think that seeds can survive absolute dehydration only if the entire embryogenic program is completed. It is plausible that seeds adapted for very harsh environments (e.g., desert species) have stringent regulation of the latter stages of embryogenesis to ensure their completion.

IV. CRITICAL MOISTURE LEVELS AND THE STATES OF WATER

A. Water Properties and Hydration Levels of Desiccation Tolerance

In the previous section, we showed that while the level of desiccation tolerance changed with development, there were several critical moisture levels. During histodifferentiation, embryos would not be expected to survive water potentials less than about $-1.6 \text{ MPa}$, the water level at which cell division is inhibited (Myers et al., 1992). This corresponds to the permanent wilting point for many species (Levitt, 1980b). At their maximum level of tolerance, most recalcitrant seeds do not survive below a water potential of about $-11 \text{ MPa}$, even when dried very rapidly. Orthodox seeds and seeds with intermediate storage behavior survive to moisture levels as low as about $-150 \text{ MPa}$.

These critical moisture levels correspond to the critical moisture levels for metabolic activity (Fig. 1). Discrete changes in metabolic activity with moisture content are hypothesized to be associated with discrete changes in the physical properties of water (Clegg, 1978; Rupley et al., 1983; Leopold and Vertucci, 1989; Vertucci, 1989, 1990, 1992; Bruni et al., 1989). This hypothesis is based on the observation that the characteristics of water change with the degree of hydration and suggests that with increased levels of hydration, water becomes progressively capable of fulfilling the specific functions required for life's processes. Thus upon the loss of water with certain properties, an essential function provided by water is no longer possible. A tissue that is not damaged by the removal of a certain type of water has developed mechanisms to tolerate or avoid that particular stress (Fig. 2).

At least five types of water can be distinguished from calorimetric and motional properties (Clegg, 1978; Vertucci, 1990; Rupley et al., 1983), although recent evidence suggests that this may be an oversimplification (Bruni and Leopold, 1992a). Type 5 is dilute solution water and is detected at water potentials greater than about $-2 \text{ MPa}$ (the corresponding water content varies for mature seed tissues between 0.6 to 0.9 g/gdm). Type 4 is believed to be concentrated solution or capillary water and is detected at water potentials between $-2$ and $-4 \text{ MPa}$ or water contents between 0.7 and 0.45 g/gdm. Type 3 is suggested to form bridges over hydrophobic moieties on macromolecules. It is detected at water potentials between $-4$ and $-11 \text{ MPa}$ or water contents between 0.45 and 0.25 g/gdm. Type 2 water has glassy characteristics and is believed to have strong interactions with polar surfaces of macromolecules or hydroxyl groups of solutes. It is detected at water potentials between $-12$ and $-150 \text{ MPa}$ or water contents between 0.25 and 0.08 g/gdm. Type 1 water corresponds to the theoretical level at which water binds to macromolecules as a structural component. It occurs at water potentials less than $-150$
MPa or water contents less than about 0.08 g/gdm (actual water content depends on the chemical composition (Vertucci and Roos, 1990) (Fig. 1).

The correspondence of different types of water with critical moisture levels for survival can lead to an understanding of the essential function of water that is the limiting factor for desiccation tolerance. Type 5 is probably required for turgor in seeds (Vertucci, 1990). The lethal removal of type 5 water from very immature and germinated embryos and perhaps throughout development of highly recalcitrant embryos such as *Avicennia marina* (Farrant et al., 1993b) suggests that these tissues cannot survive the mechanical stresses associated with drying. Mature embryos of orthodox seeds and many recalcitrant seeds can survive drying to within the third level of hydration but do not survive prolonged periods at this hydration level. Presumably this is because unregulated catabolic activities occur, which lead to the degradation of macromolecules and the accumulation of toxins (Vertucci, 1992). The complete removal of type 3 water is associated with membrane structural changes (Crowe et al., 1987 1992; Wolfe, 1987; Steponkus and Webb, 1992; Bryant and Wolfe, 1989, 1992). Mature orthodox seeds can either resist or endure these perturbations. However, even at their maximum level of tolerance, recalcitrant seeds or pollens generally do not survive below this moisture level (Pammenter et al., 1991; Berjak et al., 1992, 1993; Pritchard, 1991; Finch-Savage, 1992a,b; Tompsett and Pritchard, 1993; Kerhoas et al., 1987). The removal of type 1 water appears to be lethal to seeds with intermediate storage behavior (Ellis et al., 1990a,b; 1991; Kovach and Bradford, 1992) and can also affect the long-term viability of some orthodox seeds and pollen (Vertucci and Roos, 1990; 1993; Hoekstra et al., 1992).

B. Comparison of States of Water in Desiccation Tolerant and Sensitive Tissues

Comparisons of the properties of water in mature recalcitrant and orthodox seeds showed no major differences between the two types of embryos (Vertucci, 1990; Pammenter et al., 1991; Berjak et al., 1992; 1993). This led us to the conclusion that desiccation tolerance was not a result of the amount of structured water, as has been proposed previously (Adams and Rinne, 1980; Berjak et al., 1984; Vertucci and Leopold, 1987; Welbaum and Bradford, 1989; Grange and Finch-Savage, 1992); rather desiccation tolerance involves the ability to lose a considerable proportion of hydration water.

The implication that desiccation intolerance reflects the inability to survive the loss of structural water leads to the suggestion that the nature of the structural water or the interaction of the water with macromolecular surfaces differs in desiccation tolerant and intolerant cells. There are only a few studies that have dealt with this hypothesis. Comparisons of the properties of water in desiccation tolerant and sensitive tissues suggested that water in desiccation sensitive tissues (immature embryos, seedlings, or recalcitrant seeds) had a greater tendency to be released from subcellular surfaces upon lethal drying or freezing (Leopold and Vertucci, 1986; Vertucci and Leopold, 1987; Vertucci et al., 1991; Welbaum and Bradford, 1989; Bruni and Leopold, 1991, 1992a; Berjak et al., 1993). The loss of structured water may be either a cause or a consequence of crystallization or denaturation of other cellular components (Leopold and Vertucci, 1986; Vertucci et al., 1991; Berjak et al., 1993; Koster, 1991). Since the experiments leading to this suggestion killed the tissues, it is impossible to attribute these findings to differences in desiccation tolerance rather than an effect of death. The suggestion that desiccation damage leads to changes in molecular organization may also describe the loss
of viability when orthodox seeds are dried to water contents corresponding to type 1 water (Vertucci and Roos, 1990, 1993; Bruni and Leopold, 1992b). Currently, our working hypothesis is that water and macromolecules requiring water for structural stability can demix in desiccation sensitive cells. For some reason, this separation is less likely to occur in desiccation tolerant tissues. This hypothesis is analogous to the hypothesis that phase separation in membrane lipids is a cause of desiccation damage (discussed previously). The analogy may not be coincidental, as the same physical processes may be driving the two reactions.

C. Summary

The schematic diagram in Fig. 2 describes how critical moisture levels for survival are developmentally achieved and how drying protocols affect the apparent level of desiccation tolerance. With maturation, embryos become progressively more tolerant of turgor loss and can withstand the associated change in their metabolism. If an immature embryo is held within the fourth water level, metabolism required for further development is possible (Fig. 1) and so that embryo can mature and acquire a more tolerant state (Fig. 2). Thus slow drying of immature embryos so that they are maintained within this hydration level for several days can enhance desiccation tolerance (Adams et al., 1983; Bewley and Oliver, 1992; Blackman et al., 1992; Chen and Burris, 1990; Hoekstra et al., 1989; Kermode and Bewley, 1985; Oishi and Bewley, 1992; Senaratna et al., 1989; LePage-Degivry and Garello, 1991; Compton et al., 1992; Saranga et al., 1992). On the other hand, holding fully mature embryos at a similar moisture level will allow those embryos to proceed with germination processes (e.g., Berjak et al., 1989; Palit, 1987). In this case, slow drying results in a lower level of desiccation tolerance (Berjak et al., 1989; Farrant et al., 1986, 1989, 1993b). If the embryo is held at the third level of hydration, it can respire but it cannot continue an integrated metabolism (Vertucci, 1989; Leopold and Vertucci, 1989; Vertucci, 1992). Although the embryo can survive the immediate stress of partial desiccation, the uncontrolled metabolism can lead to rapid death. The position of the dashed lines in Fig. 2 is expected to be species dependent and affected by the rate of drying as well as the time at a given moisture level. Only orthodox seeds, at a precise developmental stage, are able to tolerate the removal of water in levels 2 and 3.

We are faced with the questions of what developmentally associated events enable seeds to tolerate the removal of water that is essential for growth and metabolism, and what the specific events are that enable orthodox seeds to tolerate removal of water that is essential for the maintenance of macromolecular structure (i.e., enter the lower square in Fig. 2). Insight into these mechanisms of desiccation tolerance can possibly be gained by observations of events surrounding the onset and loss of tolerance in both orthodox and recalcitrant seeds.

V. ANATOMICAL AND BIOCHEMICAL ASPECTS OF DEVELOPING SEEDS: THEIR POSSIBLE CONTRIBUTION TOWARD DESICCATION TOLERANCE

In contrast to orthodox seeds, there are few studies on the development of recalcitrant seeds. Thus linkages between the two types of seeds in terms of their relative desiccation tolerance are difficult. Much of the evidence cited for recalcitrant seeds comes from a
study on the development of the highly recalcitrant seeds of the mangrove *Avicennia marina* (Farrant et al., 1992a,b; 1993a,b,c), and it is expected that other seeds described as recalcitrant will have characteristics intermediate between *Avicennia* and orthodox seeds. Mutants of corn and *Arabidopsis* that produce desiccation sensitive seeds (Neill, 1986; Koornneef et al., 1989) are also useful for comparative purposes. In general, the events that occur during histodifferentiation are essentially similar in both desiccation sensitive and tolerant seeds; differences between the two types arise during the later stages of embryogenesis (Farrant et al., 1992b; 1993c).

A. Ultrastructural Changes

1. Axis Differentiation

During histodifferentiation, the embryonic tissues are highly metabolically active; there is extensive cell division, respiratory levels are high, and sub cellular organelles such as mitochondria and chloroplasts have well differentiated internal membranes (Bain and Mercer, 1966; Klein and Pollock, 1968; Rogerson and Matthews, 1977; Bewley and Black, 1985; Farrant et al., 1992b; Berjak et al., 1992, 1993). Following this stage, there is a period of expansion by increased vacuolation of the embryo tissues. During these stages, the tissues of all seed types are highly sensitive to desiccation (Rogerson and Matthews, 1977; Long et al., 1981; Dasgupta et al., 1982; Berjak et al., 1993) (Fig. 2).

2. Vacuolation

Histodifferentiation is followed by a period of reserve accumulation, during which high levels of ergastic material accumulate in the cytoplasm and organelles of the embryo (storage tissues in particular). Vacuoles become filled with storage proteins, and the extent of vacuolation is generally reduced. As mentioned previously, most seeds become increasingly tolerant of water loss during this process (Fig. 2). The relative increase in tolerance may be related to changes in cellular anatomy resulting from a switch from structurally oriented metabolism (formation of new membrane and organelles) to anabolic accumulation of nutrient reserves (reviewed by Kermode, 1990). The packaging of reserves in the cytoplasm and consequent reduction in total vacuolar volume may alleviate some of the mechanical stresses imposed by dehydration (Bewley, 1979; Levitt, 1980b; Berjak et al., 1984; Kermode, 1990). Consistent with this idea is the correlation between the amount of nutrients available and the degree of desiccation tolerance in somatic embryos (Anandarajah and McKersie, 1990; Lai et al., 1992; Compton et al., 1992). Furthermore, the low quality of shrunken-2 maize kernels has been related to inefficient starch deposition (Cobb and Hannah, 1986). For orthodox seeds, near maximum tolerance of desiccation occurs about when reserve accumulation is complete and the cells are so filled with reserves as to preclude visualization of other cytoplasmic detail (Bain and Mercer, 1966; Klein and Pollock, 1968; Briarty et al., 1969). Different recalcitrant seeds show varying degrees of vacuolation (Berjak et al., 1989; Farrant et al., 1989; Dodd et al., 1989). Some recalcitrant seeds, such as *Araucaria angustifolia*, appear to accumulate large quantities of reserves and are minimally vacuolated. The extremely desiccation sensitive embryos of *Avicennia marina* remain highly vacuolated throughout development; storage proteins and lipid are not accumulated, and starch levels remain minimal (Farrant et al., 1992a,b; 1993b). Desiccation tolerance is lost as seed germinate and become vacuolated due to the utilization of storage reserves (Bewley, 1979; Kermode, 1990; Sargent et al., 1981; Berjak et al., 1992, 1993). Kermode (1990) suggested that
there was a critical level of dry matter deposition required to withstand the mechanical stresses of desiccation. There appears to be a correlation between extent of vacuolation (and the amount of complex reserves accumulated) and the degree of sensitivity to desiccation, but this correlation does not always hold. Some recalcitrant seeds remain sensitive to the loss of types 2 and 3 water in spite of minimal vacuolar space (Farrant et al., 1989). Furthermore, desiccation tolerance can be induced in immature embryos with unfilled vacuoles (Kermode, 1990; Galau et al., 1991; Blackman et al., 1992). It is also possible that changes in chromatin structure are associated with changes in desiccation sensitivity (Crevecoeur et al., 1988; Deltour and Jacqmad, 1974). Since the onset of cell division and increased vacuolation are often coincident in germinating seeds, distinguishing between these two possibilities is difficult.

3. Dedifferentiation of Organelles

During the latter stages of dry matter accumulation in orthodox seeds, there is a general dedifferentiation of subcellular organelles such as mitochondria, chloroplasts, and polysomes (Priestley, 1986; Fincher Chabot and Leopold, 1982; Dasgupta et al., 1982; Klein and Pollock, 1968; Schneider et al., 1993). "Degraining" (loss of thylakoid membranes) of orthodox embryos is coincident with the onset of extreme desiccation tolerance (Long et al., 1981; Galau et al., 1991) but is not a universal phenomenon, as several seeds (e.g., Pisum sativum) remain green throughout the maturation process. In general, there appears to be a correlation between dismantling of internal membrane structure and polysomes and the onset of desiccation and freezing tolerance (Bewley, 1979; Bergstrom et al., 1982; Hetherington et al., 1982; Oquist and Martin, 1986; Schneider et al., 1993). The reduction of membrane surface area in organelles does not imply that these organelles become dysfunctional. Electron transport occurs at very low moisture levels (Vertucci et al., 1985; Leopold and Vertucci, 1989; Vertucci, 1989; Hetherington et al., 1982); but biochemical and fluorescence studies suggest different structures in dry and hydrated photosynthesizing (Papegeorgiou, 1975) and respiratory (Pantarullo et al., 1987; Atucci et al., 1991; Dizengremel and Tuquet, 1984) complexes. The reduction in internal membrane surfaces may be a mechanism to slow metabolism and avoid the physical consequences of drying highly membranous systems (Wolfe, 1987; Bryant and Wolfe, 1992). Alternatively and/or additionally, the enforced cessation of respiratory metabolism may prevent the accumulation of damaging levels of high-energy intermediates (LePrince et al., 1992). A slight and transient dedifferentiation of mitochondria was noted in some recalcitrant embryos at their most desiccation tolerant state (Berjak et al., 1992, 1993); however, in general, the organelles in maturing recalcitrant seeds remain structurally complex and metabolism is not switched off. These observations might lead to speculation that the ability to dedifferentiate subcellular organelles and switch off metabolism in a programmed manner is necessary to attain desiccation tolerance. However, as dedifferentiation occurs coincident with the onset of the desiccation stage, it is difficult to assess whether it is a prerequisite for, and a mechanism of, desiccation tolerance or merely a consequence of the drying treatment (the embryo already being tolerant as a result of other processes).

4. Membrane Reserves

During the latter stages of dry matter accumulation, lipid bodies are deposited along the periphery of the plasmalemma in a number of species (Priestley, 1986; Dasgupta et al., 1982; Fincher Chabot and Leopold, 1982; LePrince et al., 1990a). It has been suggested
that these droplets serve as reservoirs for lipids that will be required when the plasmalemma expands during imbibition and germination. A ready supply of membrane material would help alleviate some of the mechanical stresses observed during exposure to low water potentials. Similar droplets have been observed in mature embryos of some recalcitrant seeds (Berjak et al., 1993) but not in others (Berjak et al., 1992; Farrant et al., 1992b).

B. Biochemical Changes

In addition to ultrastructural changes, there are numerous biochemical changes that occur during the late stages of development in seeds. Several molecules are observed to accumulate in orthodox seeds when tolerance is acquired, and these are degraded when tolerance is lost. Perhaps these molecules protect subcellular surfaces and so confer maximum desiccation tolerance.

1. Proteins

Patterns of protein expression during seed development have been studied intensively in a number of species. In desiccation tolerant seeds, a set of hydrophilic and robust proteins (they remain soluble during boiling) accumulate during the latter stages of development, and their expression ceases during germination (Kermode, 1990; Galau et al., 1991; Blackman et al., 1991, 1992; Bradford and Chandler, 1992; Ried and Walker-Simmons, 1993). The function of these late-embryogenesis-accumulated or LEA proteins is unknown: they have no apparent catalytic activity, but their highly conserved nature, physical properties, and abundance argue for a role in desiccation tolerance (Kermode, 1990; Bewley and Oliver, 1992; Blackman et al., 1991, 1992; Bradford and Chandler, 1992; Gomez et al., 1988; Mundy and Chua, 1988; Close and Chandler, 1990; Ried and Walker-Simmons, 1993), possibly by binding to macromolecular structures (Dure et al., 1989). During embryogenesis of recalcitrant seeds of *Avicennia marina*, the patterns of protein synthesis do not change discretely (Farrant et al., 1992a) as is observed in orthodox embryos between the maturation and postvascular separation phase (Galau et al., 1991). Although hypothesized to be generally lacking in desiccation sensitive seeds (Bradford and Chandler, 1992, Farrant et al., 1992a), the proteins are detected in *Zizania palustris*, a seed with intermediate storage behavior (Bradford and Chandler, 1992), and preliminary evidence suggests that they are present in recalcitrant embryos of *Quercus robur* (Finch-Savage and Bewley, personal communication). The expression of LEA proteins or their transcripts alone is not sufficient for tolerance (Blackman et al., 1991; Johnson-Flanagan et al., 1992; Ried and Walker-Simmons, 1993). Since desiccation tolerance is a quantitative trait, the amount of LEA proteins may determine the level of tolerance, or the combination of LEA proteins and other protectants may be required (Blackman et al., 1992).

It has recently been shown that another class of “stress” proteins, heat shock proteins, are expressed during the natural course of seed development in the absence of applied stress (Almoguera and Jordano, 1992; Helm and Abernethy, 1990; Vierling, 1991). Heat shock proteins are believed to have a role in stabilizing protein conformation (Lindquist and Craig, 1988) and thus have been suggested to play a role also in the mechanism of desiccation tolerance in seeds. Their role is seen as either preserving or repairing macromolecular structure during dehydration or rehydration, respectively (Helm and Abernethy, 1990). In this regard, heat shock was shown to enhance the desiccation
tolerance of alfalfa somatic embryos (Anandarajah and McKersie, 1990; Senaratna et al., 1989).

Another abundant and highly conserved protein, integral to the vacuolar membrane, has been shown to accumulate in developing seeds and disappear during seed germination (Johnson et al., 1989). This protein, believed to be a transport channel (Johnson et al., 1990; Hofte et al., 1992), has also been implicated in the mechanism of desiccation tolerance (Johnson et al., 1989). Recent work suggests that a homolog found in vegetative tissues serves as a water channel and may be important in osmotic adjustment (Maurel et al., 1993).

Thus there are a number of proteins linked with desiccation tolerance. Some of these appear to be relatively general, being expressed under a variety of stresses (e.g., desiccation, freezing, or heat) in vegetative as well as embryonic tissues. A deeper understanding of the nature and extent of these proteins might give greater understanding to stress tolerance in general.

2. Carbohydrates

Changes in carbohydrates also occur during seed and pollen development and germination. Generally, as seeds or pollen mature, the monosaccharide content decreases and the oligosaccharide content increases (Amutis and Pollard, 1977; Blackman et al., 1992; Castillo et al., 1990; Chen and Burtis, 1990; Adams et al., 1982; Hoekestra and van Roekel, 1988; Hoekestra et al., 1989; LePrince et al., 1990a, 1992; Lowell and Kuo, 1989; Handley et al., 1983; Saranga et al., 1992). The reverse trend is observed during germination or pollen tube elongation, cotton being an exception (Koster and Leopold, 1988; Kuo et al., 1988). In a variety of orthodox seeds, sucrose, raffinose-family oligosaccharides, and monosaccharides contribute between 1 and 12% of the dry mass (Amutis and Pollard, 1977; Kuo et al., 1988; Handley et al., 1983).* In mature orthodox seeds, sucrose contents range between 15 to 90% of the soluble carbohydrates, and monosaccharides are usually there in trace amounts. Correlations between high concentrations of nonreducing sugars and desiccation tolerance of orthodox seeds (Adams et al., 1982; Koster and Leopold, 1988; Chen and Burtis, 1990; Blackman et al., 1982; LePrince et al., 1990a, 1992), pollen (Hoekestra and van Roekel, 1988; Hoekestra et al., 1989), desiccation tolerant vegetative tissues (Schwab and Gaff, 1990; Bianchi et al., 1993, and references therein), and animal systems (Madin and Crowe, 1975) has led to the suggestion that they may play a role in the mechanism of desiccation tolerance. There are several ways in which sugars have been proposed to act in conferring tolerance.

a. The “Water Replacement Hypothesis.” This hypothesis (e.g., Clegg, 1986; Crowe et al., 1992) suggests that the hydroxyl groups of sugars substitute for water and provide the required hydrophilic interactions for membrane and protein stabilization. In artificial bilayer systems, the sugars bind to the polar head groups, separating the individual lipid molecules and weakening the van der Waals attractions among the fatty acyl chains. This results in the maintenance of the bilayer liquid-crystalline structure of the membrane even at very low hydration levels (Crowe et al., 1987, 1988, 1992; Hoekestra et al., 1989, 1991, 1992; Quinn, 1989; Bryant and Wolfe, 1992). The disaccharide trehalose appears to be most effective at maintaining conformation after desiccation (Crowe et al., 1987, 1992), perhaps because it is sterically compatible with the arrangement of phos-

*In the cited papers, sugar contents were expressed on a defatted dry mass basis. To convert to a dry mass basis, lipid contents from Sinclair and DeWit (1975) were used.
phatidylocholine head groups in lipid bilayers (Gaber, 1986). Trehalose does not occur in most angiosperm seeds, but the high sucrose content in many of these seeds has led to the suggestion that sucrose also can supply the hydrogen bonding required to prevent lipid phase transitions (Leopold and Vertucci, 1986; Crowe et al., 1987, 1992; Hoekstra and van Roekl., 1988; Hoekstra et al., 1989, 1991; Caffery et al., 1988). It is unknown whether sucrose actually binds to membranes, as is proposed for trehalose. The protective effects of sucrose during drying may be obtained through nonspecific interactions between sugars, water, and/or polar lipids so that the interface between the two phases is sufficiently stable (Quinn, 1989; Bryant and Wolfe., 1992; Crowe et al., 1992).

b. The “Glass Formation Hypothesis.” Aqueous glasses can be formed by nonspecific interactions between hydroxyl groups of solutes and water when a solution is dried or cooled sufficiently to change from a supersaturated liquid to a superviscous fluid (Franks, 1982). Usually used in the context of cryopreservation, aqueous glass formers have been defined by how they alter the properties of water (i.e., prevent its crystallization). Because of their extreme viscosity, it was proposed that aqueous glasses can prevent molecular movement and hence maintain cellular constituents in a “stasis” (Burke, 1986). Aqueous glasses have been detected in dried seed materials (Bruni and Leopold, 1991, 1992a,b; Vertucci, 1990; Williams and Leopold, 1989) and in sugar mixtures of composition similar to that in maize embryos (Koster, 1991); however, the evidence suggesting that sugars cause the glasses observed in vivo, especially at low water potentials, is lacking. Since glasses only slow the inevitable changes in chemistry and physical state, they are said to be kinetically, but not thermodynamically, stable.

In the context of desiccation tolerance, the effectiveness of a glass will be a function of its stability. Evidence that the stability of aqueous glasses contributes to the level of desiccation tolerance comes from differences in the physical properties of water (Bruni and Leopold, 1991) and shapes of moisture sorption isotherms between dried desiccation tolerant (alive) and dried desiccation sensitive (dead) tissues (Leopold and Vertucci, 1986; Vertucci and Leopold, 1987; Welbaum and Bradford, 1989) (also discussed in Sec. IV.B). The hyperbolic shape of isotherms from the desiccation intolerant tissue suggests that a crystallization event occurred (Labuza, 1984) (this may be interpreted broadly to mean a change in molecular organization of sugars or a structural change in macromolecules, both brought about by changes in water-solute/surface interactions); the limited mobility of the water fraction in damaged tissues (Bruni and Leopold, 1991) confirms this suggestion.

Sugars are good glass formers at subzero temperatures (Franks, 1982), and so it has been suggested that they are important in the stabilization of the aqueous phase during desiccation at suprafreezing temperatures (Burke, 1986; Koster and Leopold, 1988; Koster, 1991; Williams and Leopold, 1989; Bruni and Leopold, 1991, 1992a,b). Pure sucrose solutions, when concentrated to levels correlated with desiccation tolerance [about 10–15% of the dry mass or a ratio of 5 g/g polar lipid (Blackman et al., 1992; Crowe et al., 1987, 1988, 1992; Hoekstra and vanRoekl., 1988; Hoekstra et al., 1989, 1991; Koster and Leopold, 1988; LePrince et al., 1990a; Bianchi et al., 1993)], are not stable and tend to crystallize (as is observed when candy gets stale), thus eliminating most of the protective benefits. The addition of raffinose, which has limited protective capacities beyond osmotic adjustment (Hincha and Schmitt, 1992; Crowe et al., 1987), prevents the crystallization (Smythe, 1967; Caffrey et al., 1988; Koster, 1991) and provides better protection than sucrose alone (Caffrey et al., 1988). Thus a combination of sugars may be important to
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stabilize the protecting effect of sucrose, and raffinose has been implicated in this role (Leopold and Vertucci, 1986; Chen and Burris, 1990; Koster and Leopold, 1988; Koster, 1991). However, a combination of sucrose and raffinose or any other sugar does not appear to be mandatory. Sucrose can occur in combination with trehalose or it may comprise as much as 97% of the soluble carbohydrate in some seeds and desiccation tolerant organisms (Bianchi et al., 1993 and references therein; Schwab and Gaff, 1990; Hoekstra and van Roekl, 1988; Hoekstra et al., 1989, 1991; Kuo et al., 1988). It has also been suggested that a combination of protective sugars and proteins are required for desiccation tolerance (Blackman et al., 1992).

c. Problems with Current Hypotheses of “Sugars as Protectants.” While both the water replacement and the glass formation hypotheses present provocative models for how sugars may protect cellular constituents, a number of arguments can be made that suggest that they are not the only mechanism of protection. High levels of soluble sugars accumulate, in the appropriate concentrations and ratios, in developing and mature recalcitrant seeds (Berjak et al., 1989; Dodd et al., 1989; Farrant et al., 1992b, 1993c; Finch-Savage, 1992a), in somatic embryos (Saranga et al., 1992), and in desiccation sensitive mutants of Arabidopsis (Ooms et al., 1992); yet these tissues remain intolerant of drying. Desiccation tolerance in mutants of Arabidopsis can be manipulated by treatment with an ABA analog without a commensurate change in sugar composition (Ooms et al., 1992). Also maize seeds with very high soluble sugar contents often have low vigor after drying (e.g., Cobb and Hannah, 1986), a trait that may be indicative of incomplete desiccation tolerance (Demir and Ellis, 1992; Hong and Ellis, 1992a). Finally, experiments correlating the concentration of sucrose and oligosaccharides with desiccation tolerance were conducted using seeds that accumulate high concentrations of soluble carbohydrates. It should be noted that these high sugar contents are not a general trend in orthodox seeds (Kuo et al., 1988): total soluble sugars can make up as little as 1% of the dry mass of some orthodox seeds (significantly lower than the hypothesized requirement of about 10%), and raffinose is observed in trace quantities in several cereal grains and dicotyledonous seeds.

For both the water replacement and glass formation hypotheses, there is also little correlation between the moisture level at which damage occurs and the moisture level where protection is expected. While there is an intriguing relationship between the critical moisture level for several recalcitrant seed species that are rapidly dried (Pammenter et al., 1991, 1992) and the critical moisture content for membrane phase transitions (Sec. II.C.2), and that these membrane perturbations are prevented by sugars (Sec. V.B.2.a), the relationship does not always hold. Water replacement by sugars would be ineffective in tissues that are extremely sensitive to desiccation (damaged to 0.8 to 0.6 g H₂O/g dry weight), since damage occurs at much higher water contents than membrane aberrations are expected (compare critical moisture levels in Farrant et al., 1985, 1986 and Berjak et al., 1992, 1993 with critical moisture levels in Wolfe, 1987; Lis et al., 1982; Bryant and Wolfe, 1992; Crowe et al., 1987, 1992). Similarly, dehydration-related damage often occurs at hydration levels far higher than those at which aqueous glasses are detected (compare moisture levels/temperature combinations for damage in Berjak et al. (1989, 1992, 1993) and Pammenter et al. (1991) with glass transition phase diagrams (Williams and Leopold, 1989; Bruni and Leopold, 1992a,b; Koster, 1991)).

Glass formation in desiccation tolerant tissues supposedly protects them from desiccation damage by desiccation avoidance (Burke, 1986) (i.e., the solution is so viscous
that water molecules cannot diffuse out). However, the moisture level of orthodox seeds is easily and reversibly manipulated by relative humidity (e.g., Vertucci and Roos, 1990), suggesting that the seeds equilibrate to ambient conditions and that a metastable state is not maintained. Furthermore, the tendency toward glass formation, as measured by the amount of unfrozen water, does not differ substantially in recalcitrant and orthodox seeds (Pammenter et al., 1991; Berjak et al., 1992, 1993) or in the sugar extracts of germinated and ungerminated seeds at the required temperature/moisture combinations (Koster, 1991). From these arguments, we would suggest that considerable tolerance of drying is already present in mature orthodox embryos before glass formation occurs, and that it is unlikely that the mere presence of sugars alone will confer desiccation tolerance. However, it is possible that glass formation (which would occur naturally when orthodox seeds dry) will confer stability to the subcellular environment in the dry state, and that removal of the glass, either by hydration to level 3 or by dehydration to level 1, would jeopardize this stability (Vertucci and Roos, 1990, 1993; Bruni and Leopold, 1992b).

d. Alternative Hypotheses. The concerns about the role of soluble carbohydrates in desiccation tolerance do not rule out their probable effect. It may be argued that sugars in orthodox seeds are properly located to give efficient protection, and sugars in recalcitrant seeds sequestered in compartments (such as vacuoles) where they are unable to protect cytoplasmic membranes. Recent studies have suggested that, at a given water content, there are “pools” of water with different properties; one of these water types may be essential to macromolecular structure and may be lacking in recalcitrant seeds (Bruni and Leopold, 1992a). It is also possible that the high surface area of membranes in recalcitrant seeds promotes lethal bilayer-bilayer interactions at moisture contents greater than where sugars are effective. Alternatively, the production of free fatty acids by free radicals formed during drying of desiccation sensitive tissues (McKersie et al., 1988; LePrince et al, 1992) may make sugars ineffective protectants against membrane disorganization (McKersie et al., 1989).

It has also been proposed that oligosaccharides are important for desiccation tolerance, not because they serve as protectants of cellular structures, per se but because they reduce the pool of monosaccharides (Loomis et al., 1979; Koster and Leopold, 1988; LePrince et al., 1992; Rogerson and Matthews, 1977). It is a general observation that, during the latter stages of maturation in orthodox seeds, monosaccharide levels are reduced, possibly by the preferential formation of oligosaccharides. Lowering monosaccharide content results in a reduction in respiratory substrates and may impose metabolic quiescence (thus limiting a source of free radicals) prior to drying (LePrince et al., 1992; Rogerson and Matthews, 1977). Alternatively, high levels of monosaccharides such as glucose, fructose, and galactose may contribute to Maillard-type reactions that disrupt protein structure (Karel, 1975; Loomis et al., 1979; Koster and Leopold, 1988; Wetlauffer and Leopold, 1991). These reactions are particularly important at water potentials between −15 and −150 MPa (Karel, 1975; Wetlauffer and Leopold, 1991), the moisture level where there are few metabolic controls (Leopold and Vertucci, 1989; Vertucci, 1989). Maillard reactions have been implicated in seed deterioration at high moisture levels (Wetlauffer and Leopold, 1991), a condition that is analogous to very slow drying of tissues. Finally, sucrose and other sugars may protect cellular constituents by scavenging free radicals (Smirnoff and Cumbes, 1989), which are destructive when desiccation sensitive tissues are dried (McKersie et al., 1988; Hendry et al., 1992; LePrince et al., 1990b; 1992). In sum, high levels of oligosaccharides may be an important mechanism of establishing
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metabolic control at moisture levels where many reactions occur, but there is no integrated metabolism. At present, these hypotheses have not been tested by comparing the level of mono- and oligosaccharides in recalcitrant seeds.

3. Lipids

Numerous studies have demonstrated that membranes are particularly susceptible to structural changes during desiccation and that alteration of membrane structure can be particularly damaging. Based on these findings, one would predict that there should be changes in the lipid component of orthodox seeds to accommodate the mechanical and biochemical stresses. Although changes have been inferred (Le Page-Degivry and Garello, 1991), there have been few studies that actually document changes in membrane components during seed development, or especially whether recalcitrant and orthodox seeds differ. Phosphatidylycercolamines (PE) can undergo lethal hexagonal phase changes with drying. The high level of PE in Gramineae pollen grains (about 35% of polar lipids) may be responsible for their recalcitrant behavior (Hoekstra et al., 1989). High ratios of phosphatidylcholine (PC) to PE may be expected in the desiccation tolerant condition, as it may help preserve the membrane bilayer structure at low water contents. A doubling of PC-to-PE ratios is observed during preconditioning of maize grains (Chen and Burris, 1991) and acclimation of oat roots to water stress (Liljenberg and Kates, 1985). Similar changes in head group composition were not observed in leafy tissues (Steponkus et al., 1990; Huner, 1988), but these tissues have considerably lower levels of PE (about 11%) and are rarely subjected to the degree of dehydration experienced in pollen or seed tissues. In mature soybean and corn embryos and in Typha pollen, there is a relatively high PE content (about 20%) (Senaratna et al., 1987; Chen and Burris, 1991; Hoekstra et al., 1991). This large amount of PE is surprising in a recalcitrant tolerant tissue and suggests that protection against formation of nonbilayer phases is required. Without protection, membranes with this amount of PE undergo a bilayer to hexagonal phase change at about 0.04 g/g or~125 MPa (Webb et al., 1993), which corresponds to the moisture content at which intermediate and orthodox seeds experience damage.

The effects of changes in the level of fatty acid saturation on desiccation tolerance is unclear. Increased levels of unsaturated fatty acids can have beneficial effects by increasing tolerance to moderately low water potentials (osmotic excursions) (Uemura and Steponkus, 1989) and decreasing the likelihood of liquid crystalline to gel phase transitions (which could lead to demixing) (Quinn, 1985; Small, 1986; Crowe et al., 1992). Alternatively, unsaturated fatty acids could have a detrimental effect by increasing the possibility of lethal hexagonal changes (Quinn, 1985; Small, 1986; Bryant and Wolfe, 1989, 1992). A consistent trend toward loss of linolenic acid (18:3) occurs during the latter stages of seed maturation (Cherry et al., 1984; Chen and Burris, 1991; Dutta and Appelqvist, 1991; Hoekstra and vanRoeckl, 1988; Horbowicz and Obendorf, 1992; Johnson-Flanagan et al., 1991; Dahmer et al., 1991; Khor and Chan, 1988). These changes might also be important to the longevity of seeds in the dry state, since unsaturated lipids are believed to be most sensitive to peroxidative reactions (reviewed by Priestley, 1986; Hoekstra and McKersie, 1990; Chan, 1987). However, there is conflicting evidence that this is truly the case (e.g., McKersie et al., 1988, 1990).

4. Antioxidative Systems

Since susceptibility to peroxidation may increase with drying (Bewley, 1979; LePrince et al., 1990b, 1992; Hendry et al., 1992; Rockland, 1969; Dhandia, 1991; McKersie et al.,
1988), one may reason that free radical scavenging systems are an important part of
desiccation tolerance. Vegetative tissues with greater drought or desiccation tolerance
appear to have more efficient antioxidative enzyme systems (Hendry et al., 1992; Dhindsa,
1991; Pastori and Trippi, 1993). Antioxidant systems in developing embryos depend on the
species and tissue (embryonic axis versus storage tissues) as well as the developmental status
of the embryo (Cakmak et al., 1993; Arrigoni et al., 1992; Hendry et al., 1992; LePrince
et al., 1990b). Ascorbate and ascorbate oxidizing enzymes are plentiful during the early
stages of embryogenesis, are less significant during the maturation stages, and then become
increasingly important again with germination (Arrigoni et al., 1992; Cakmak et al., 1993;
LePrince et al., 1990b). Changes in activity and importance of catalase, superoxide
dismutase, and glutathione reductase with embryogenesis and germination are variable
(Arrigoni et al., 1992; Cakmak et al., 1993; LePrince et al., 1990b; Puntarulo et al., 1988),
but there appears to be a general trend toward increasing activity of enzyme scavenging
systems with increasing mitochondrial activity. This is consistent with the primary function
of these enzyme systems: to metabolize peroxides produced from about 1% of the oxygen
consumed from unstressed mitochondria (Puntarulo et al., 1988). Thus as mitochondrial
activity declines with drying, the requirement for these enzymes systems may be alleviated.

Enzyme systems that process peroxides leaked from mitochondria may be inefficient
at processing other sources of free radicals. Perhaps this is the reason for their decline
during the latter stages of embryo maturation when enhanced desiccation tolerance is
acquired but respiratory activity declines. In this case, antioxidants such as tocopherol
(LePrince et al., 1990b; Hendry et al., 1992; McKersie et al., 1988; Senaratna et al.,
1985), sucrose (Smirnoff and Cumbes, 1989), or phytate (Graf et al., 1987) may be more
effective. In orthodox seeds, free radical scavengers accumulate during maturation and
are lost during germination (Senaratna et al., 1985; LePrince et al., 1990a,b; Koster and
Leopold, 1988; Lowell and Kuo, 1989; Murray, 1984).

Among the few studies conducted in this area, there appears to be a trend toward
presence and effectiveness of antioxidants and desiccation tolerance. For example, ascorbic
citric might be considered an antioxidant, but it also serves as a source of free
radicals in the presence of metal ions (McKersie et al., 1990). While not found in mature
orthodox embryos of maize or Vicia faba (LePrince et al., 1990b; Arrigoni et al., 1992),
ascorbate is found in high concentrations in recalcitrant axes of Quercus robur (Hendry
et al., 1992). In contrast, the level of tocopherol [a lipid soluble chemical that slows the
initiation of autoxidation of lipids (Chan, 1987; McKersie et al., 1990)] is about ten times
less in Q. robur than in orthodox embryos of corn and soybean (compare Hendry et al.,
1992, with Priestley et al., 1980, and LePrince et al., 1990b). In spite of the low level of
tocopherol in Q. robur, it was the primary free radical scavenger in axes, enzymes
being more important in the cotyledons (Hendry et al., 1992). These authors therefore
concluded that the axis was more sensitive to desiccation than the cotyledons. However,
in the preceding paragraph we suggested that at low water potentials, antioxidants were
more effective than enzyme-mediated free radical scavenging systems. This suggestion is
supported by the observation that when axes of Q. robur were severed from the
cotyledons, they survived to lower water contents (Finch-Savage, 1992a); apparently,
toxic elements were produced in, and transported from, the cotyledons.

5. Abscisic Acid
The plant growth regulator abscisic acid (ABA) appears to play an important role in the
development of desiccation tolerance. ABA has also been implicated in the response of
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vegetative tissues to water stress (Bewley and Oliver, 1992; Hetherington and Quatrano, 1992). In general, ABA levels increase during the late stages of development of orthodox seeds and decline only during maturation drying (King, 1982; Quatrano, 1987; Kermode, 1990). Exogenous application of ABA induces desiccation tolerance to developing embryos (Bartels et al., 1988; Anandarajah and McKersie, 1990; Johnson-Flanagan et al., 1992; Meurs et al., 1992; Hetherington and Quatrano, 1992; LePage-Degivry and Garello, 1991; Saranga et al., 1992; Senaratna et al., 1989) and prolongs the desiccation tolerant phase in mature embryos (Blackman et al., 1991). Application of fluridone, an inhibitor of ABA synthesis, results in embryos with viviparous characteristics (Oishi and Bewley, 1992; Xu et al., 1990). Mature embryos of recalcitrant seeds of Avicennia marina, Theobroma cacao, or Quercus robur have low levels of ABA (Farrant et al., 1993a,c; Pence, 1991; Finch-Savage et al., 1992b). In addition, double mutants of Arabidopsis and corn that are both lacking in and insensitive to ABA produce desiccation sensitive seeds (Koornneef et al., 1989; Neill et al., 1986).

The mechanism whereby ABA induces tolerance is not clear. Evidence suggests that it may be important in inducing or maintaining the postvascular separation or predesiccation stages (stages according to Galau et al., 1991) that may be abbreviated in recalcitrant seeds. In keeping with its role as a plant growth regulator, ABA may act as a signal-transducer for the transcription of protectants (Dure et al., 1989; Skriver and Mundy, 1990; Kermode, 1990; Ried and Walker-Simmons, 1993). Exogenous application of ABA to seedlings or immature embryos induces mRNAs for proteins associated with water stress (Kermode, 1990; Skriver and Mundy, 1990; Blackman et al., 1991; Johnson-Flanagan et al., 1992; Thomann et al., 1992). Studies on ABA double mutants and recalcitrant seeds might give insights into the genetic control of desiccation tolerance. Presumably, mutants have the genetic ability for desiccation tolerance; their inability to survive desiccation lies in the lack of, or insensitivity to, the signal that induces protection. This could also be the situation in some recalcitrant seeds; or the information required for tolerance could be absent from the genome entirely.

VI. EMBRYO RESPONSE(S) TO DEHYDRATION: SUMMARY OF HYPOTHESES FOR THE MECHANISMS OF DESICCATION TOLERANCE

The removal of water from cells can result in many levels of damage. During development both recalcitrant and orthodox seeds acquire the ability to withstand some of the deleterious effects of dehydration. Orthodox seeds are able to survive nearly complete desiccation, and we have reviewed the anatomical and biochemical changes that occur in these seeds that might serve as the foundation for hypotheses as to how this is possible. Hypotheses used to explain desiccation tolerance in vegetative tissues often include extensive repair processes, while those for orthodox seeds are viewed mostly as protection against desiccation damage (Bewley and Oliver, 1992). To withstand such extensive drying, it is likely that orthodox seeds must be able to protect and repair cellular constituents. We believe that the ability to repair requires some level of structural integrity, and thus we present the major hypotheses explaining desiccation tolerance in the context of mechanisms that stabilize the subcellular organization of the tissues.

If the mechanism of desiccation tolerance is under genetic control, then it is
presumably missing or nonfunctional in recalcitrant seeds or mutants that produce desiccation sensitive seeds. The major differences between these seed types is that, at a specific developmental stage, orthodox seeds are able reversibly to switch off metabolism (and the genome) and stabilize the subcellular organization so that they can tolerate the loss of types 3 and 2 water (enter the lower square in Fig. 2). Recalcitrant seeds can tolerate varying degrees of water loss, but they do not switch off metabolism (Berjak et al., 1992, 1993; Farrant et al., 1992b) and they cannot enter the lower square in Fig. 2. They remain metabolically active throughout development; on shedding, metabolism progresses immediately toward germination (Berjak et al., 1989; Farrant et al., 1989, 1992b).

Embryos are most sensitive to drying during histodifferentiation and after germination (Fig. 2), when they have not yet attained the competence for extreme drying, or have lost the competence, respectively. During these stages the embryos are highly metabolically active and the tissues are somewhat vacuolated. Drying results in the interruption of metabolism, which becomes lethal, possibly because of the buildup of high-energy intermediates of metabolism that have toxic effects (McKersie et al., 1988; LePrince et al., 1992). Because embryos from recalcitrant seeds are always metabolically active and never achieve the competence for complete desiccation, this type of damage probably occurs during dehydration unless drying is extremely rapid (e.g., Parmeter et al., 1991).

Most embryos (Avicennia marina being an exception) become more tolerant of dehydration during dry matter accumulation. This may be due to the increased stabilization of the subcellular environment by packaging of reserves. The accumulation of these reserves may minimize mechanical stresses of dehydration (an analogy may be made here with how fragile items are packaged for shipping). Furthermore, the increased deposition of dry matter gradually decreases the cytoplasmic volume, perhaps imposing metabolic quiescence as well as a spatial separation of membranes that might otherwise interact. Thus we hypothesize that desiccation tolerance may be a quantitative feature partially related to the extent of reserve accumulation.

While the ability to minimize mechanical damage must contribute considerably to the ability to tolerate drying, it appears to be insufficient to account for absolute tolerance of drying. In order to tolerate near dryness (i.e., to lose structure-associated water and enter the lower square in Fig. 2), there must be the ability to protect and stabilize subcellular surfaces. It is believed that these protective mechanisms are put into place during the postvascular separation and predesiccation stages (stages according to Galau et al., 1991), which are apparently lacking or abbreviated in recalcitrant seeds. The nature of biochemical constituents (proteins, sugars, and lipids) that may prevent changes in macromolecular structure upon desiccation have been reviewed here. The extent to which each candidate might contribute is yet unknown.

Since current research has shown that there are limits to desiccation tolerance, it is likely that protection against desiccation damage involves slowing down, rather than stopping, deleterious reactions that are inevitable. From a thermodynamic standpoint, this means that the maintenance of metastable states (in the aqueous and lipid phases and in the aqueous/lipid dispersion) is the key to survival, and that the attainment of equilibrium (i.e., crystallization and phase separations) results in death. Geometric considerations (e.g., shape, size, and separation of reactive species) and the presence of protectants (e.g., glass formers and emulsifying agents) establish metastable states. Desiccation tolerance may be a function of how well these are maintained.
VII. CONCLUSION

The acquisition of desiccation tolerance is clearly a complex phenomenon, involving the interaction of metabolic and/or structural adjustments that allow cells to undergo extensive water loss with minimum damage. Lack of one or more of these features might result in differing degrees of tolerance. However, complete protection against damage may be impossible. The capacity to reverse such inevitable damage must also be an integral part of desiccation tolerance. The essence of true desiccation tolerance is possibly the ability to maintain sufficient structural integrity to repair damage when water is available once again.

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