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REVIEW

Orthodoxy, recalcitrance and in-between: describing variation in seed storage characteristics using threshold responses to water loss

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Abstract

Main conclusion **Discrete categories of seed physiology can be explained through a unified concept of the structural and molecular mobility responses within cells to drying.**

Tolerance of desiccation is typically described by a threshold or low water content limit to survival. This convention provides fairly good distinction between orthodox and recalcitrant seeds, which show thresholds of less than about 0.07 and greater than about 0.2 g H_2O g DW^{-1}, respectively. Threshold water contents, however, are not direct measures of the intensity of water stress tolerated by seeds, nor are they measures of cell response to water stress. More direct criteria, that accommodate both spatial and temporal effects of water loss, are required to explain variation of desiccation tolerance and longevity in seeds from diverse genetic backgrounds and growth conditions. This essay presents the argument that changes in cellular volume directly quantify primary responses to desiccating stress in a context that also links damage, as cellular constituents compress, and protection, as compressed molecules form stabilizing structure. During desiccation, fluid cytoplasm solidifies, and the newly formed spatial relationships among molecules determine whether and how long viability is maintained. The diversity of seed behaviors suggests complexity and opportunity to discover molecules and mechanisms that regulate survival and perception of time in cells that lack metabolic function.

Keywords Cell shrinkage · Configurational entropy · Desiccation · Desiccation tolerance · Glass formation · Plasmolysis · Temperature · Threshold

Abbreviations

DW Dry weight
LN Liquid nitrogen

Introduction

The distinction between orthodox and recalcitrant seeds provides an important management criterion for storing seeds in genebanks (FAO 2013). Orthodox seeds are generally dried to low water contents and placed in a freezer at −20 °C, known as "conventional" storage. In contrast, recalcitrant seeds must be protected and stored cryogenically, usually in liquid nitrogen (LN) (Walters et al. 2013). This functional definition is not generally adopted in the seed biology literature. Rather, orthodoxy and recalcitrance are usually considered as categorical designations of desiccation tolerance (e.g., Royal Botanic Gardens Kew 2015): orthodox seeds survive drying to low water contents and recalcitrant seeds do not survive appreciable drying; 'low' and "appreciable" having somewhat nebulous definitions. Despite increasing recognition of variation in

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seed responses to desiccation within and among seed categories (e.g., Berjak and Pammenter 2008), we still tend to treat both desiccation stress and response as qualitative features. Our experiments often apply an arbitrary level of stress and measure response categorically, as surviving or not surviving.

The overall goal of most research on desiccation tolerance is to characterize the 'tolerance phenotype' so that we can infer the underlying developmental patterns and genetic or environmental factors that regulate the trait. Defining tolerance—the ability to prevent, decrease or repair injury (Levitt 1980)—has actually been difficult because the nature of injury(ies) is still largely conjectural. The spatial (nanometer–millimeter) and temporal (picoseconds–seconds) scales at which water influences cell activities suggest that perturbations resulting from water removal will also be broad in scale. In other words, wherever and whenever we look we will find effects of desiccation and hence, opportunities for protection. A conceptual model of the primary and subsequent effects of water loss may lead to more comprehensive assessments of cell and organism response to desiccation and better elucidation of the factors that limit survival as well the role of various gene products in conferring protection.

To that end, this essay considers cell responses to water loss in a general manner that encompasses structure and molecular mobility over broad spatial and temporal scales. This perspective enables us to incorporate evidence of injury, such as membrane phase changes and metabolic dysfunction, with hypothesized protection mechanisms, such as accumulation of compatible solutes and glass formation. The overall objective is to provide a means to characterize water stress and cell response to water stress in a way that allows desiccation tolerance to be evaluated quantitatively and consistently among species and diverse tissue types. If accomplished, the categories of seed orthodoxy and recalcitrance can be replaced with a classification scheme that provides insights about the diverse spectrum of seed response to dehydration, including the unique ability of some seeds to dry out and survive for centuries.

The orthodox-recalcitrant seed paradigm

The original concept of orthodox seed behavior uses a mathematical model to describe expected responses of drying and cooling (Justice and Bass 1978; Ellis and Roberts 1980) (Fig. 1). Importantly, the model incorporated the factor of time by expressing response as duration of survival (i.e., longevity). These models quantify the general pattern of increased longevity of orthodox seeds with decreased water content and/or temperature—that is,

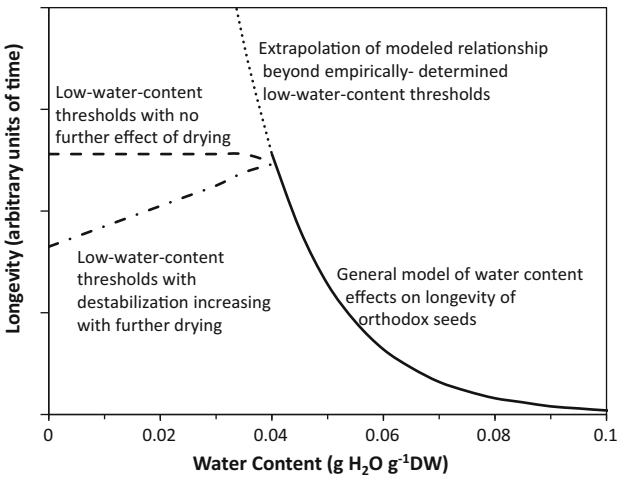

Fig. 1 Modeled behavior of the effects of water on seed longevity for a hypothetical orthodox seed. *Solid curve* an empirically determined relationship, in this case a doubling of longevity for a 0.01 g g^{-1} decrease in water content (Justice and Bass 1978). The modeled relationship exhibits a threshold or low water content limit, which occurs in this hypothetical seed at 0.04 g H_2O g DW^{-1}. *Dashed curves* the low water content limit to the modeled relationship, showing no (Ellis et al. 1989, 1990a; Ellis and Hong 2006) or decreasing (Vertucci and Leopold 1987; Vertucci and Roos 1990; Walters et al. 2005a) longevity with further reductions of water content. *Dotted curve* the modeled relationship extrapolated above empirically determined limits. Modeled behavior follows expected decreases in cytoplasmic viscosity near the glass transition (Sun and Leopold 1994)

chemical or physical reactions that lead to lost viability tend to slow down when orthodox seeds are dried or cooled.

Though characterized by the pattern of increased longevity with increased drying, orthodox seeds deviate from this pattern at threshold water contents that range between 0.03 and 0.07 g H_2O g^{-1} DW (Vertucci and Leopold 1987; Ellis et al. 1989, 1990a; Vertucci and Roos 1990; Walters 1998; Walters et al. 2005a; Ellis and Hong 2006). At water contents less than the threshold water content, longevity is either unaffected or decreases as water content approaches zero (i.e., absolute dryness) (Fig. 1). Threshold water contents mark the moisture level at which seed aging rates are minimized. Even still, seeds continue to deteriorate regardless of the environmental conditions. Seeds that have better protection against damage that occurs under dry conditions survive longer; hence, seed longevity is a manifestation of desiccation tolerance and is conventionally treated as a quantitative trait. Factors that affect differences in maximum seed longevity among seed lots or species are under intense investigation (Rajjou and Debeaujon 2008; Rajjou et al. 2008; Schwember and Bradford 2010; Nguyen et al. 2012; Nagel et al. 2015), and the lack of reliable methods to assess response to drying among different time scales hampers this effort.

A commonly used method to assess potential seed longevity under dry conditions makes use of the faster aging observed when seeds are moist. This approach, referred to as 'accelerated aging' or 'controlled deterioration', assumes that the factors that regulate response to water content on both spatial and temporal scales are identical across diverse genetic backgrounds and seed development conditions (Ellis and Roberts 1980), an assumption that has been disputed with evidence of interacting effects of moisture content among genetic lines (Niedzielski et al. 2009; Schwember and Bradford 2010; but see Rajjou et al. 2008). With current understanding, we have limited ability to predict seed biology under anhydrous conditions using assessments made under moist conditions (Walters et al. 2010; Ballesteros and Walters 2011).

Sometimes, the water content of seeds used in accelerated aging experiments exceeds the established limits of inference for longevity models (aptly referred to as the high water content limit), and seed longevity increases with increasing water content (Ibrahim and Roberts 1983). At the high water content limit, low oxygen tensions appear to play a role in viability loss (Ibrahim and Roberts 1983; Leprince et al. 2000; Walters et al. 2001). The value of the high water content limit is often assumed to be common among diverse species and is marked by the water content required for respiration (Vertucci and Leopold 1984; Roberts and Ellis 1989).

Recalcitrant seeds were originally distinguished from orthodox seeds by humans' inability to usefully extend storage life by drying (Roberts 1973). Below the high water content limit of longevity models, both orthodox and recalcitrant seeds deteriorate; recalcitrant seeds dying nearly instantly. Interestingly, the values of the high water content limit in orthodox seeds and the onset of near-instant mortality in recalcitrant seeds overlap for diverse species, ranging between 0.20 and 0.3 g H_2O g^{-1} DW. The low water content limit that marks the onset of mortality in recalcitrant seeds is often used to quantify differences in desiccation tolerance among tissue types, maturity stages, ecotypes, species and assay conditions (Tweddle et al. 2003; Daws et al. 2006a; Berjak and Pammenter 2008; Xia et al. 2014) (Fig. 2). Does variation in these threshold moisture levels indicate differences in the amount (or kind) of damage that cells tolerate or differences in the amount of drying that induces a similar and lethal level of damage?

It may be tempting to use drying time as a measure of desiccation tolerance in whole recalcitrant seeds. This practice confounds assessments of responses to level and duration of stress. Moreover, many recalcitrant seeds possess large, fleshy organs and thick covering layers surrounding the embryo as mechanisms to resist water loss (Daws et al. 2006b; Xia et al. 2012). In the classical sense,

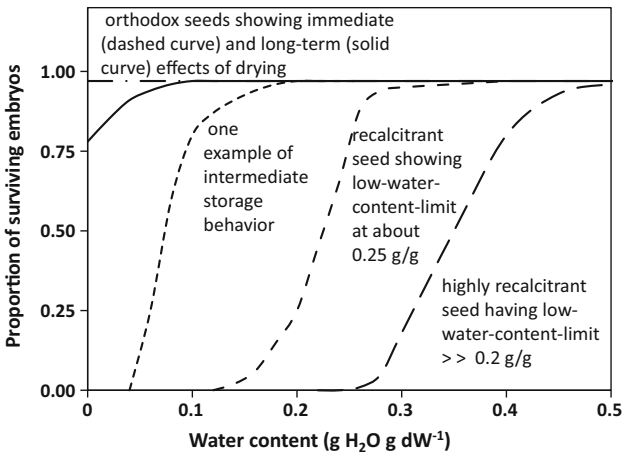

Fig. 2 The relationship between survival and water content in hypothetical seeds from different seed storage categories. Seeds express damage when dried below threshold water contents. The lower limit of the threshold water content for recalcitrant seeds is about 0.2 g H_2O g DW^{-1} (*dashed curve*), but thresholds for recalcitrant seeds range broadly above this value, depending on the tissue, maturity, ecotype and species. Orthodox seeds do not immediately exhibit low water content thresholds (*dot-dash curve*), but over time show a break in longevity relationships (*solid curve*) as described in Fig. 1. Seeds classified in the intermediate category sometimes show intermediate threshold water contents (Sacandé et al. 2000; Hor et al. 2005; Eira et al. 2006; Pérez et al. 2012). Threshold water contents are an indirect measure of water stress (which is more accurately expressed as water potential). Variation of threshold water contents among seed categories implies variation in the amount of stress tolerated and illustrates the quantitative nature of desiccation tolerance

these features contribute to desiccation avoidance rather than tolerance per se (Levitt 1980). Converting drying time to water content usually reveals higher threshold water contents (i.e., less tolerance) with slower drying (Walters et al. 2001; Berjak and Pammenter 2008). This general feature can be explained by either progress towards germination, which increases sensitivity to water loss, or accumulation of toxic compounds from the degenerative metabolism incumbent with accelerated aging. The confounding effects demonstrate that cell responses to desiccation are expressed both structurally and temporally. It is nearly impossible to disassociate these two parameters when considering desiccation damage and tolerance mechanisms.

Orthodox and recalcitrant seeds both exhibit threshold water contents, and further drying impacts survival (Fig. 2). The two seed types are conventionally distinguished by the water content ranges of the thresholds, which are distinct. Many studies of desiccation tolerance or seed aging identify specific pairs of molecules that protect and need protecting. And, many of the specific effects identified for both desiccation damage and seed aging are the same, as are the respective protection mechanisms

(Vertucci and Farrant 1995; Walters 1998; Black et al. 2006; Berjak and Pammenter 2008; Kranner et al. 2010). These commonalities suggest that the nature and intensity of damage by desiccation and aging may be similar among diverse seeds, and not specific to a range of water contents or a particular set of molecules or organelles. From this perspective, seed storage categories are distinguished by the amount of water each can lose without experiencing lethal damage and by the timeframe for lethal damage to accumulate. If true, we need to re-examine our conventions for quantifying desiccation response, especially using water content limits. Threshold water contents describe the amount of water remaining in cells before damage is expressed; they do not describe the amount of stress imposed (i.e., water potential) nor the primary response of cells (the amount of water removed).

In-between orthodox and recalcitrant seed storage categories

The last two decades brought increasing exploration of seed behavior in wild species and increasing recognition that the confines of the orthodox and recalcitrant seed paradigm are too restrictive. So-called "intermediate" seeds were discovered in the early 1990s (Ellis et al. 1990b, 1991a, b). As the name implies, intermediate seeds exhibit characteristics of both orthodox and recalcitrant seeds, illustrating that our convention of using two exclusive categories—desiccation tolerant and intolerant—oversimplifies matters (Royal Botanic Gardens Kew 2015). Characterizing temporal responses to drying, implicit in the original definitions of orthodox and recalcitrant seeds, provides a framework to explore the conceptual advance made possible by the discovery of intermediate seeds.

The intermediate condition can be manifested in a number of ways including (1) seeds that are able to tolerate drying to lower water contents than recalcitrant seeds, but not as low as orthodox seeds (Fig. 2). (Ellis et al. 1991a, b; Sacandé et al. 2000; Hor et al. 2005; Eira et al. 2006; Pérez et al. 2012); (2) seeds that exhibit anomalous longevity responses to temperatures between +10 and −30 °C (Ellis et al. 1991a, b; Hor et al. 2005; Eira et al. 2006; Crane et al. 2006; Pérez et al. 2012); or (3) seeds that have short lifespans no matter how they are dried or cooled (Mondoni et al. 2010; Michalak et al. 2015). Water content thresholds and time for lethal damage to accumulate are cross-cutting parameters used to characterize all seed types. In addition, low temperature effects are necessarily invoked in the study of intermediate seeds (Royal Botanic Gardens Kew 2015). Temperature effects are also an essential component of orthodox and recalcitrant seed behavior, and this essay will return to that point.

The existence of an intermediate category of post-harvest physiology demonstrates natural variation in seed responses to water loss, despite discrete seed storage categories. This natural variation leads to questions about the nature of metabolism and developmental processes that confer different threshold water contents or longevity responses at these thresholds among seed types. Assignment of seeds to particular categories is based on seed responses at full maturity, before germination begins. This is a difficult task because many recalcitrant seeds lack clear punctuation between maturation and germination (Berjak and Pammenter 2008). Increasing evidence also suggests variation in embryo development among ecotypes (Dussert et al. 2000; Tweddle et al. 2003; Daws et al. 2006a; Berjak and Pammenter 2008; Xia et al. 2014), which can confound seed classifications made at the taxonomic level (Royal Botanic Gardens Kew 2015).

Response to desiccation stress from a structural perspective

Variation of seed response to desiccation is rooted within the embryogenic program (Vertucci and Farrant 1995; Walters and Koster 2007; Berjak and Pammenter 2008). During embryogenesis, seed cells experience large changes in structure and composition that are coincident with changes in cellular responses to desiccation. Numerous gene products are hypothesized to confer protection during desiccation and storage, for example, structural proteins and house-keeping or regulatory genes (Mouillon et al. 2008; Mène-Saffrané et al. 2010; Chatelain et al. 2012; Verdier et al. 2013; Personat et al. 2014; Dekkers et al. 2015).

Accumulation of dry matter reserves during embryo development is a major cause of structural and compositional changes in cells and have important, but nonspecific, protective benefits during desiccation and storage (Farrant et al. 1997). Food reserves entering embryonic cells replace fluid volume with solid volume. As a consequence, metabolic capacity of the cell declines because there is less fluid volume. Water content (i.e., the proportion of water relative to dry matter) within cells declines precipitously during dry matter accumulation because of the complementary processes of water removal and dry matter loading. However, water potential (i.e., the availability of water molecules to participate in reactions) remains relatively constant (Farrant and Walters 1998; Pérez et al. 2012). Hence, by accumulating food reserves, embryos are able to effect water loss without imposing water stress. In the process, cells tend towards a more solid matrix.

Structure is stabilized by solidification. In general chemistry class we learned that solids maintain their shape,

in contrast to fluids that take the shape of their container. Hence, in a completely fluid environment, water loss causes massive changes in cell shape; the cell shrinks. Shrinkage is controlled during dehydration by loading dry matter into cells. For example, cells filled with 20 and 60 % dry matter lose up to 80 % and only 40 % volume, respectively, during dehydration. As unprotected cells shrink, cellular constituents redistribute and compress, concomitantly increasing chemical potential of biomolecules (Fig. 3). Plasmalemma may be ripped from the cell wall, interrupting inter-cell communications. Membrane systems may come into close proximity and alter partitioning capacity within the cell (Walters et al. 2002). Localized organization of biomolecules may be perturbed, potentially signaling defense responses or interrupting linked reactions required for integrated metabolism (Hyman and Simons 2012). Cells that are packed with accumulated dry matter experience less of these changes and are more tolerant to desiccation than highly vacuolated cells (Farrant et al. 1997; Walters et al. 2002; Walters and Koster 2007). Loading cells with dry matter is metabolically costly, and may explain why desiccation tolerant organisms tend to be small or rare (Alpert 2006) or why this vestigial trait is retained for the most critical stage of the plant life cycle, when it must reproduce, disperse and establish (Oliver et al. 2005).

Most of our current understanding about damage by desiccation is, in some way, associated with compression of cell constituents due to cell shrinkage (Walters et al. 2002; Walters and Koster 2007). Changed volume, therefore, becomes an appealing general measure of cellular response to water stress, which also has relevance in thermodynamic terms because it allows us to link structure with energy (Shamblin et al. 1999). A threshold loss of volume or surface area of more than 50 % was once proposed to be lethal to cells (Meryman 1974), but has not been tested more thoroughly (except see Steponkus et al. 1995). Hence, proportion of cell volume occupied by dry matter at the onset of dehydration can provide a quantitative tool to predict the amount of water loss that can be tolerated.

Why glasses are important

As cells compress during dehydration, cellular constituents begin to interact and form loose associations. Further compression leads to molecular rearrangement within the

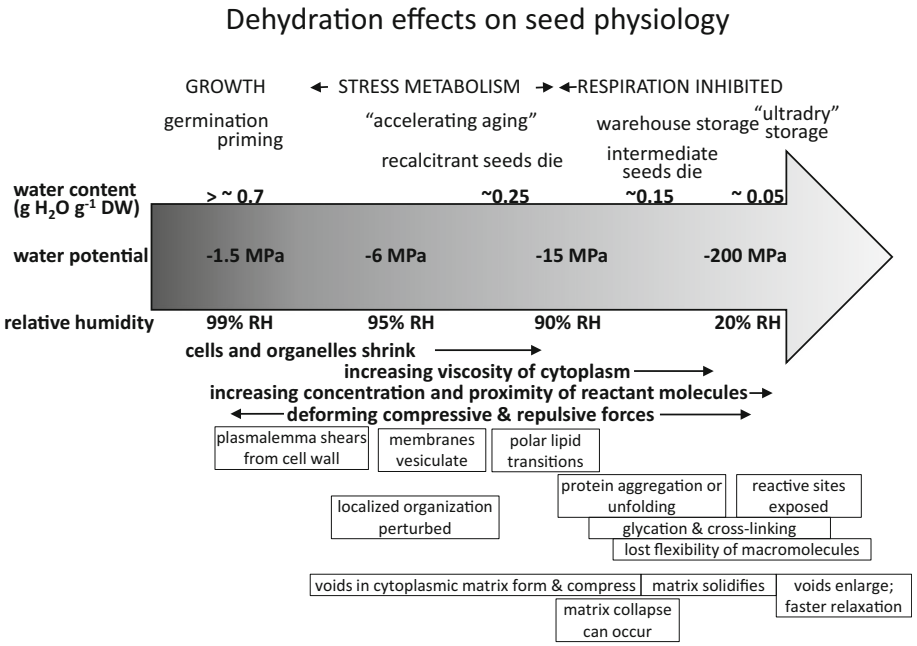

Fig. 3 A schematic diagram of seed cell response to water stress (adapted from Walters and Koster 2007). Water stress ranges from 0 MPa (pure fluid water) to $-\infty$ (no water). At $\psi_w > -10$ MPa, large changes in water content occur with small changes in water potential (see example PV curves, Walters et al. 2001; Xia et al. 2014). At $\psi_w < -15$ MPa, water stress is more easily described by relative humidity and can be roughly inferred by seed water content (see example sorption isotherms, Walters 1998). The primary response of cells during water stress is cell shrinkage which leads to secondary effects of changed solute concentrations, increased cytoplasmic viscosity and deforming forces, all of which can affect physiological function as indicated in the top part of the diagram. It is tempting to link threshold water contents (Fig. 2) with the onset of a particularly damaging event, such as a phase change within membranes, and to propose that lower threshold water contents indicate greater capacity to resist that deformation. This essay presents an alternative model in which the primary response to desiccation stress, cell shrinkage, is mitigated in cells that survive the initial stress of desiccation

cellular matrix to increase packing efficiency; loose associations strengthen, concomitantly trapping molecules and impeding their movement. Eventually, molecules within the matrix become immobilized, and further dehydration does not allow for easy compression because of steric hindrance among the molecules. The structure within the cellular matrix becomes fixed; the material is able to support its own weight and hold shape; it becomes a solid. Packing of molecules is not perfect in this type of solid, also known as a glass. Rather, molecules align irregularly and form pores.

Formation of a glass is inevitable in drying cells whether they are tolerant or intolerant of desiccation (Fig. 3). Cytoplasm becomes glassy when dried to about 0.06–0.12 g H_2O g DW^{-1} assuming ambient temperature of about 20–25 °C (Ballesteros and Walters 2011), which is the glass transition temperature (T_g) typically observed for biomolecules with that amount of water (Angell 2002). Obtaining this low water content has debilitating effects on cell structures in intolerant cells. In contrast, tolerant cells shrink less and are able to maintain spatial relationships among key biostructures until the glass finally stabilizes the structure (Wolkers et al. 1998). Initially, we sought to link desiccation tolerance with 'good' glass formers that vitrified at relatively high water contents (Buitink and Leprince 2008). Sucrose and other sugars were excellent candidates because they showed potent protective properties in three-component systems (Angell 2002). However, these solutes form so-called 'fragile' glasses (sensu Angell 2002), meaning that the highly fluid ↔ glassy transition is abrupt and might not accommodate organized positioning of constituents or prevent rapid demixing during environmental fluctuations in moisture or temperature. Glasses within orthodox seeds do not resemble sucrose glasses; they are comprised of complex materials (Buitink and Leprince 2008) and transition from fluid ↔ glass over broad temperature and moisture ranges indicative of "strong" glasses (Walters 2004; Ballesteros D and Walters C, data not shown). The ability to buffer viscosity during dehydration and hydration likely provides additional stability to cell structures, and molecules such as lea-like proteins + sugars or NADES may have special capacity to slowly fix in structure and maintain it under conditions when glasses form and melt (Walters et al. 1997; Wolkers et al. 2001; Mouillon et al. 2008; Choi et al. 2011; Rivera-Najera et al. 2014).

Very, very slowly, molecules within glassy matrices shift to fill pore spaces and improve packing efficiency. This continual "relaxation" of the fixed structure is a defining property of the glass, and why we consider molecular structure and mobility as two sides of the same coin. The rate at which molecules within a glass shift position is related to strength of molecular interactions and void volume, which are, in turn, determined by composition, drying process, water content and temperature.

The size of the void volume in glasses defines both the amount of molecular appression that occurred during cell shrinkage and the potential for relaxation within the glassy matrix. A low density glass might provide transient protection from desiccation damage, but this protection may disappear relatively quickly if the glass tends to relax rapidly. Large void volumes also allow ligands to move independently of the molecular backbone that is constrained within the glass. Shifting positions of ligands through vibration or rotational motion can create the opportunity for reaction among molecules in close proximity. Hence, the structure and mobility of molecules within glasses explains discrete changes in reaction kinetics as glasses plasticize (Vertucci and Leopold 1987; Vertucci and Roos 1990; Mira et al. 2010; Colville et al. 2012). One can imagine sophisticated regulation of some types of reactions by diversifying glassy structure within different regions of the cell or seed.

Conceptual model that incorporates spatial and temporal effects of water loss

The underpinning thesis of this essay is that differences in post-harvest physiology among diverse seeds can be explained quantitatively through general spatial and temporal consequences of dehydrating cells. Changes in molecular proximity are roughly approximated by volume changes within the cells as they shrink. This concept is represented by a horizontal axis describing volume lost upon water removal and ranges from 100 % (i.e., the material is a droplet of pure fluid water) to 0 % (i.e., the material has no space occupied by water) (Fig. 4). A working hypothesis is that desiccation tolerance depends on glasses forming in the cellular matrix before a critical volume loss of 50 % (Meryman 1974). Hence, recalcitrant and immature embryos are separated from mature orthodox seeds by a vertical line bisecting the horizontal axis near 50 % volume loss (though there may be natural variation in this value). With prolonged dry matter accumulation, seeds generally regarded as recalcitrant can traverse the vertical line; likewise, premature harvests might push orthodox seeds to the right.

The vertical axis describes temporal effects of water loss through changes in viscosity, and it is bisected by the horizontal axis at the viscosity typical of a glass at its transition (10^{14} poise) (Angell 2002). The quadrats above the horizontal axis reflect the time for the glassy matrix within seed cells to relax. Seeds exhibiting intermediate syndromes of storage might be expected to lie near the intersection of the two axes, suggesting that drying these

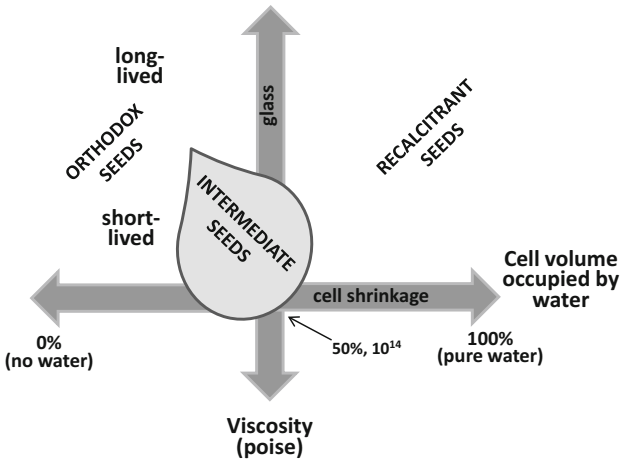

Fig. 4 A model describing general spatial and temporal effects of water loss in seeds from different seed storage categories. *Horizontal axis* the cell volume occupied by water before the desiccation stress is applied. Highly vacuolated cells will lie to the far *right* and cells packed with dry matter will lie to the *left*. The *horizontal axis* is bisected by a *vertical axis* that describes the viscosity of the cytoplasmic matrix or the tendency of molecules to maintain structure. The axes intersect at a threshold volume change, hypothesized to be near 50 % volume occupied by water (the hypothesized threshold for volume loss, Meryman 1974) and 10^{14} poise, the average viscosity at T_g (Angell 2002). Fluid behavior exhibited below the horizontal axis is mostly relevant to this essay in the context of "fragile" and "strong" glasses (sensu Angell 2002). The properties of the solid glassy matrix formed during drying or cooling determine position along the vertical axis. Long-lived and short-lived orthodox seeds are hypothesized to form more and less stable glasses, respectively. Intermediate seeds fall close to the 50 % volume axis, suggesting that they can exceed the volume threshold by excessive drying, rapidly relaxing glasses, or contraction of TAG during cold treatments

cells places them at a precarious nexus that is near the volume threshold in a glass that relaxes relatively rapidly compared to orthodox seeds. The model presents the hypothetical argument that short- and long-lived orthodox seeds are quantitatively distinguished by the glass relaxation rates, with slowest relaxing glasses resulting in the longest surviving seeds.

The role of triacylglycerols

This essay argues that the accumulation of dry matter is an essential component of desiccation tolerance because it regulates the extent that cell shrink before molecules are sufficiently appressed to form stabilizing glasses. Triacylglycerols (TAG) accumulate into lipid bodies during seed development, and will have little effect on properties of aqueous glasses that form at about 20–25 °C. Unlike molecules within the aqueous glass, molecules within the TAG phase retain fluidity. The presented model of seed longevity (Fig. 4) presupposes an inverse correlation between molecular mobility and longevity. TAG content is not correlated with seed longevity per se (Walters et al. 2005b; Probert et al. 2009); however, a role for TAG in deteriorative reactions should not be ruled out. The TAG phase provides a near endless reservoir of small, polar and reactive molecules that can migrate to the edges of the aqueous glassy domain and effect chemical change.

Intermediate storage physiology was discovered when TAG crystallized in seeds during low temperature storage (Crane et al. 2006). We speculate that the contraction of the lipid body, consequent from crystallization, has a destabilizing effect on the glassy matrix at the interface of lipid and aqueous domains. The voids created along the periphery of the lipid body would free molecules once fixed into place by steric hindrance, and their movement would cause shifts within the glassy matrix or allow reaction with neighboring molecules. Therefore, seeds that appeared to be orthodox at higher storage temperatures show anomalous temperature responses when stored at temperatures that induce lipid crystallization (Fig. 4).

Temperature effects

As described in the previous section for TAG, a relevant and highly familiar effect of lowering temperature is crystallization. Unlike glass formation, during crystallization, a sufficient number of like-molecules self-assemble and pack into a regular framework that causes a discrete change in volume. The intermolecular bonds that form cause an abrupt release of enthalpy and reduction in molecular mobility.

Crystallization of water (i.e., freezing or ice formation) is lethal, and so has a major impact on seed storage practices. There is sufficient concentration of water molecules in seeds containing more than 0.2–0.3 g H_2O g DW^{-1} to allow ice to form within relevant timeframes, and this usually occurs at temperatures between −10 and −40 °C, depending on the water content (Walters et al. 2007). Because recalcitrant seeds do not survive drying below water contents that limit water freezing, they are at high risk of lethal ice formation when stored in conventional freezers as orthodox seeds are stored. Alternatively, excised portions of recalcitrant seeds are dried slightly and cooled rapidly to glass-forming temperatures (Wesley-Smith et al. 2014).

Treatment of recalcitrant seeds implies that crystallization can be avoided during low temperature treatment, in favor of glass formation. The process of supercooling and glass formation is a matter of intense research in the materials sciences literature, and is mostly beyond the scope of this essay. In brief, as temperature decreases/increases,

molecules tend to compress/expand, analogous to above descriptions for drying/hydration. Much of the literature on temperature effects is concerned with viscosity changes at T_g, which occur abruptly and less abruptly in fragile and strong glasses (sensu Angell 2002), respectively.

For both orthodox and recalcitrant seeds, effective seed storage occurs at temperatures below T_g. This is generally below 20–25 °C for orthodox seeds dried below 0.06–0.12 g H_2O g DW^{-1} (Figs. 3, 4) and below about −100 °C in recalcitrant seeds containing more than 0.3 g H_2O g DW^{-1} (Ballesteros and Walters 2011). At temperatures below T_g, change in viscosity follows Arrhenius kinetics (Walters 2004). The Arrhenius equation assumes the pre-exponential factor (which roughly describes the amount of movement in the glass) and the temperature coefficient (which roughly describes the minimum energy required for movement) are constant over narrow temperature ranges (Black et al. 2006); understanding the factors that affect variation of Arrhenius parameters over broader temperature ranges and among different seed glasses will be informative.

At temperatures approaching T_g, water, which is a plasticizer of biological glasses, has interacting effects with temperature. Plasticizing solutes loosen intermolecular constraints, enlarge pores and allow molecules to move a bit more—effects also observed during an incremental increase in temperature. The interaction between water content and temperature is often portrayed in phase diagrams, more accurately termed plasticization curves, which show decreasing T_g with increasing water content (Sun and Leopold 1994; Angell 2002; Walters 2004; Buitink and Leprince 2008; Ballesteros and Walters 2011). The interdependence of glass properties with moisture and temperature is, arguably, the predominant discussion point of glasses in biological contexts (Buitink and Leprince 2008). This interdependence makes it possible to obtain similar glass properties by slightly adjusting water content for temperature conditions, and vice versa. Though plasticization of seed glasses was not understood at the time that orthodox longevity models were developed (Justice and Bass 1978; Ellis and Roberts 1980), these models are consistent with plasticization effects (Sun and Leopold 1994). Storage conditions recommended in Harrington's 'Hundreds Rule' [the sum of RH and temperature (in degrees Fahrenheit) should be less than 100] (Justice and Bass 1978) and ultradry technology (use extreme drying to achieve comparable longevity as freezer storage) (Ellis et al. 1990a; Ellis and Hong 2006; FAO 2013) appear valid within the vicinity of T_g. Moisture ranges that flank relevant plasticization effects have been determined empirically as the high and low water content limits of longevity equations (Fig. 1).

Conclusions

Current categories depicting orthodox and recalcitrant seed storage behavior provide a useful dichotomy to base management decisions about seed storage conditions. In a physiological context, these categories may present a false dichotomy that limits a unified consideration of the broad spectrum of organism responses to desiccation stress and survival under anhydrous conditions. This essay seeks a new convention that quantifies cell response to desiccating stresses using both spatial and temporal scales. Primary response to water stress is considered spatially by expressing the amount of water lost from cells in terms of volume change. This practice represents a departure from current convention that expresses desiccation tolerance in terms of threshold amounts of water remaining in cells. Acknowledging volume changes allows us to simultaneously consider damage from compressing cellular constituents and stabilization from increasing intermolecular associations found in glasses. A different dichotomy of seed responses to desiccation is introduced that is based on whether or not a stabilizing glass forms before cell volume shrinks to a threshold level, currently hypothesized as 50 %. Formation of the glass merely changes the question of survival from "if" to "when." Cell constituents continue to compress in the glassy matrix, albeit slowly, and the movement is either a direct cause or an indicator of damage that continues in dried biological structures. In effect, the glasses that form during desiccation serve as biological clocks that keep time based on the spatial properties bestowed during desiccation.

This perspective of desiccation stress and survival invites the discovery of cell properties and constituents that regulate the spatial and temporal effects of water removal. Effects will be manifested in a solid rather than fluid matrix, which is a new frontier for biological investigation. Gene products that fill space or act as plasticizers or antiplasticizers are good candidates for exploration. These molecules may be distributed throughout the organism or locally concentrated to give nearly limitless variation in seed responses to the environment.

Author contribution Christina walters is responsible for the synthesis of information and new ideas presented in this paper.

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