Seed Science Research

cambridge.org/ssr

Dry architecture: towards the understanding of the variation of longevity in desiccation-tolerant germplasm

Daniel Ballesteros[1], Hugh W. Pritchard[1] and Christina Walters[2]

[1]Department of Comparative Plant and Fungal Biology, Royal Botanic Gardens, Kew, Wellcome Trust Millennium Building, Wakehurst Place, Ardingly RH17 6TN, UK and [2]USDA-ARS National Laboratory for Genetic Resources Preservation, 1111 South Mason Street, Fort Collins, CO 80521, USA

Review Paper

Cite this article: Ballesteros D, Pritchard HW, Walters C (2020). Dry architecture: towards the understanding of the variation of longevity in desiccation-tolerant germplasm. *Seed Science Research* **30**, 142–155. https://doi.org/10.1017/S0960258520000239

Received: 22 November 2019
Revised: 16 May 2020
Accepted: 18 May 2020
First published online: 9 September 2020

Key words:
lipid crystallization; oxidative stress; photosynthetic stress; seeds; solidified cytoplasm; spores

Author for correspondence:
Daniel Ballesteros,
E-mail: d.ballesteros@kew.org

Abstract

Desiccation-tolerant (DT) plant germplasm (i.e. seeds, pollen and spores) survive drying to low moisture contents, when cytoplasm solidifies, forming a glass, and chemical reactions are slowed. DT germplasm may survive for long periods in this state, though inter-specific and intra-specific variation occurs and is not currently explained. Such variability has consequences for agriculture, forestry and biodiversity conservation. Longevity was previously considered in the context of morphological features, cellular constituents or habitat characteristics. We suggest, however, that a biophysical perspective, which considers the molecular organization – or structure – within dried cytoplasm, can provide a more integrated understanding of the fundamental mechanisms that control ageing rates, hence the variation of longevity among species and cell types. Based on biochemical composition and physical–chemical properties of dried materials, we explore three types of the interplay between structural conformations of dried cytoplasm and ageing: (1) cells that lack chlorophyll and contain few storage lipids may exhibit long shelf life, with ageing probably occurring through slow autoxidative processes within the glassy matrix as it relaxes; (2) cells with active chlorophyll may die quickly, possibly because they are prone to oxidative stress promoted by the photosynthetic pigments in the absence of metabolic water and (3) cells that lack chloroplasts but contain high storage lipids may die quickly during storage at −20°C, possibly because lipids crystallize and destabilize the glassy matrix. Understanding the complex variation in structural conformation in space and time may help to design strategies that increase longevity in germplasm with generally poor shelf life.

Introduction

Diverse plant germplasm, including seeds, pollen and fern spores, have different functional traits that influence whether they survive drying (DT, desiccation tolerant) and how long they survive in the dry state (longevity). These traits are critical for successful *ex situ* conservation of germplasm (Wyse et al., 2018; Ballesteros et al., 2019; Colville and Pritchard, 2019; Pence et al., 2020). The ability to survive desiccation is a common trait among seeds, pollen and fern spores. An estimated 90% of angiosperm species produce DT seeds (Wyse and Dickie, 2017), DT appears to be widespread among pollen of most plant families studied (Hoekstra, 2002; Franchi et al., 2011; Nebot et al., 2018) and most fern spores are DT (López-Pozo et al., 2018).

DT is often characterized as survival to moisture levels <0.1 g H_2O gDW^{-1} or drying to relative humidity (RH) <50% (Leprince and Buitink, 2010). Germplasm lifespan when stored dry (i.e. longevity) varies with storage conditions, specifically the surrounding temperature and RH (Ellis and Roberts, 1980; Buitink et al., 1998; Ballesteros et al., 2017, 2019) and possibly gaseous environment (Gonzalez-Benito et al., 2011; Groot et al., 2015). Lethal ice does not form in germplasm dried to these levels and so freezer storage is widely used with the aim of preserving living germplasm for the long-term (e.g. FAO, 2014; Ballesteros and Pence, 2018).

However, life cannot be preserved indefinitely during dry/cold storage, and seeds, pollen and fern spores eventually die. Seeds from numerous crops might be expected to survive for nearly 100 years (Walters et al., 2004, 2005c). Long surviving germplasm provides an opportunity to understand how drying and low-temperature interact with and affect the functional stability of cells and tissues. Rapid ageing was detected in about 26% of seed accessions from wild species stored for over 20 years under conventional freezer conditions at Kew's Millennium Seed Bank (i.e. dried at 15°C and 15% RH then placed at −20°C) (Probert et al., 2009), and many crop seed accessions have a relatively short lifespan in dry and cold storage (Walters et al., 2005c; Li and Pritchard, 2009; Colville and Pritchard, 2019). In addition, significant ageing was detected within 25 years for dry seeds and fern spores stored at

CAMBRIDGE UNIVERSITY PRESS

liquid nitrogen (LN) temperatures (Walters et al., 2004; Ballesteros and Pence, 2017; Ballesteros et al., 2019), despite early supposition that the low temperature of LN could extend viability 'forever'.

Short lifespans are worrisome because aged germplasm must be regenerated or recollected, substantially reducing the efficacy and efficiency of gene banking operations for agriculture and conservation (Walters et al., 2005c, 2010; Li and Pritchard, 2009; Probert et al., 2009; Nagel and Börner, 2010; Hay and Probert, 2013; Colville and Pritchard, 2019). Some crop seeds, such as lettuce, celery and peanut, are known for relatively short shelf life compared to longer surviving species such as wheat or peas (Walters et al., 2005c).

Considerable variation in seed longevity occurs within and among species, and even seed lots harvested in different years. DT seeds of some species, such as orchids, poplars, willows and elm deteriorate especially quickly (Pritchard and Seaton, 1993; Ballesteros and Pence, 2017; Davies et al., 2018); pollen (Hoekstra, 2005) and some fern spores (Ballesteros et al., 2019) are notorious for fast ageing. This is important as it has recently been suggested that relatively short seed lifespan seems to be a common trait in a majority of species in diverse collections and storage conditions (Colville and Pritchard, 2019).

The characteristics within dry cytoplasm that result in differences in ageing rate among diverse species and cell types are poorly understood. In this paper, we take a molecular and biophysical perspective to investigate the status of life in dry cytoplasm of DT germplasm in order to postulate about the processes responsible for ageing and why there is a variation of lifespans among cells. We aim to explore whether there are specific physicochemical characteristics in some dry seeds, fern spores and pollen that make them predisposed to fast ageing. Elucidating these traits can help to predict short lifespans and ultimately devise post-harvest procedures and banking options that extend germplasm longevity.

Acquisition of DT and longevity

DT and longevity are determined in seeds during the mid and late stages, respectively, of the embryogenic development programme (Walters et al., 2010; Dekkers et al., 2015; Sano et al., 2016; Leprince et al., 2017, and references within). Embryo cells accumulate specific compounds that are associated with cells' ability to tolerate extreme water stress; for example, low molecular weight antioxidants, complex carbohydrates such as oligosaccharides of the raffinose family for species within Fabaceae, late embryogenesis abundant proteins (LEAs), and heat-shock proteins (HSPs). Moreover, structures within cells either dismantle or fortify during development and maturation. Changes to cell structures include folded cell walls, condensed chromatin and dismantled thylakoids of chloroplasts (Webb and Arnott, 1982; Vertucci and Farrant, 1995; Hoekstra, 2005; Carranco et al., 2010; Nagel et al., 2015; Sano et al., 2016; Arif et al., 2017; Leprince et al., 2017; Pereira Lima et al., 2017; López-Pozo et al., 2018). DT cells tend to be undifferentiated, having relatively small if any vacuoles and considerable deposits of dry matter reserves (Farrant et al., 1997; Pérez et al., 2012; Walters, 2015). These structural changes presumably reduce metabolic activity while also mitigating mechanical stress of cell shrinkage incumbent when cells lose water (Walters, 2015).

The molecular and structural changes to embryonic cells during maturation appear guided by suites of genes that are activated or inhibited in a highly regulated co-expression network (Leprince et al., 2017, and references therein). Gene regulation and longevity are also influenced by maternal effects, such as environmental cues and growing conditions of the parent plant (Carranco et al., 2010; Nagel et al., 2015; Sano et al., 2016; Arif et al., 2017; Leprince et al., 2017; Pereira Lima et al., 2017).

Germplasm that acquires DT survives water removal and the consequent changes in structure and function within cells (Walters et al., 2010; Walters, 2015; Sano et al., 2016; Leprince et al., 2017). As cellular constituents compress when cells dehydrate, reacting substrates concentrate and molecules are no longer dispersed within the cell. Without protection, there is greater opportunity to interact with neighbouring molecules to destabilize important cellular machinery through oxidations, cross-linking and structural relaxations (Walters et al., 2010; Ballesteros and Walters, 2011, 2019; Fleming et al., 2017). The ability of dry cytoplasm to avoid or ameliorate ageing reactions, maintain function and survive becomes crucial and is a critical feature of longevity – not dying while dry. The properties of the cytoplasm that forms, *vis-a-vis* the amount of molecular movement that is allowed and the reactivity of adjacent molecules, defines the persistence of dried germplasm (Walters et al., 2010; Ballesteros and Walters, 2011, 2019; Walters, 2015).

Biochemistry of seed ageing

Ageing-associated degradation is mostly characterized by an accumulation of oxidized molecules (e.g. Kranner et al., 2006; Rajjou et al., 2008). Reactions involving free radicals (FR) or reactive oxygen species (ROS) are often implicated in ageing (Wang et al., 2015; Nagel et al., 2019). These ubiquitous molecules are particularly hungry for electrons, abstracting them from other organic molecules, and becoming reduced while the molecule that lost an electron is oxidized. Antioxidants are particularly generous and readily donate electrons, which prevents ROS from seeking electrons and damaging molecules critical to cell machinery. The diversity of products, such as volatile organic carbons (VOC), DNA and RNA fragments, loss in antioxidant redox potential and carbonylated proteins, supports growing evidence that all cellular constituents are potential substrates or targets for ageing reactions and that an initial oxidation event can cascade into molecular fragmentation or cross-linking (Fig. 1; Walters et al., 2010; Colville et al., 2012; Nagel et al., 2015; Mira et al., 2016; Fleming et al., 2017, 2018).

The reduction–oxidation reactions associated with ageing are usually not catalysed by enzymes and so require the presence of highly energetic (i.e. mobile) molecules that can diffuse through the cytosol and reach relatively distant targets (Roudaut et al., 2004). Alternatively, neighbouring molecules may be so confined that the close proximity allows enhanced interaction, as is the case of oxidation of proteins by auto-oxidized lipids in microencapsulated oils (Velasco et al., 2003), deamidation of proteins (Manning et al., 2010) and reduction of disulphide bonds of amino acids by nearby photo-excited tryptophan (Miller et al., 2003). Given a constant pressure of oxidation, the placement of antioxidants adjacent to core machinery or to block the pathway of diffusing oxidizers is critical (Halliwell and Gutteridge, 1999; Walters et al., 2010).

Diverse reactions are increasingly reported in dry, viable germplasm before a change in physiology (i.e. mortality). For example, VOC, mostly indicative of lipid peroxidation (Mira et al., 2010, 2016), fragmented nucleic acids (Kranner et al., 2011; Fleming

https://doi.org/10.1017/S0960258520000239 Published online by Cambridge University Press

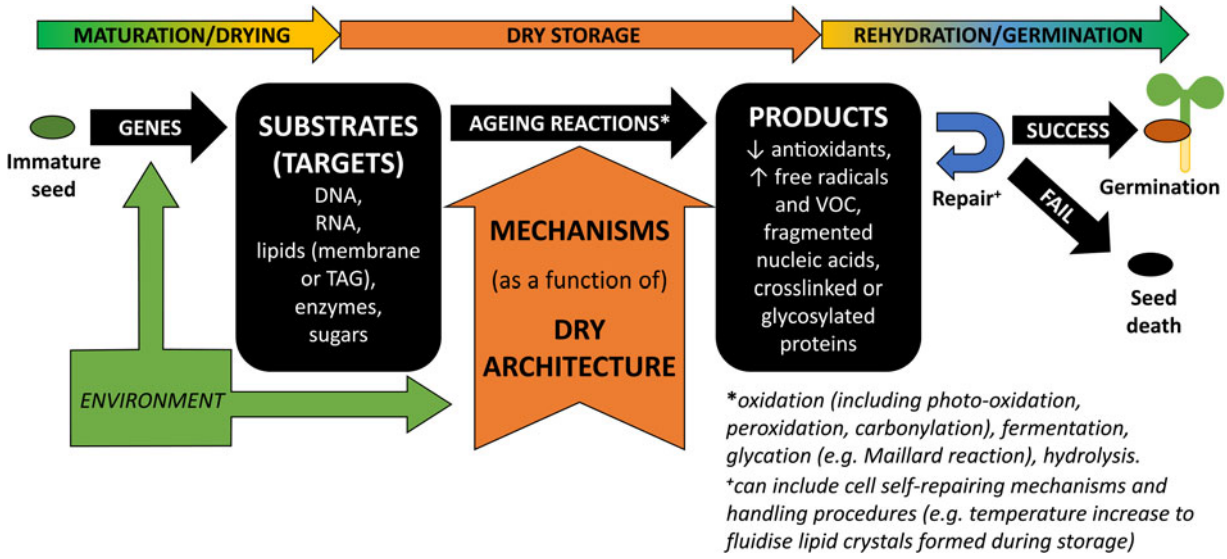

Fig. 1. Schematic representation of the substrate-dependent ageing mechanisms that generate products impacting on whether a seed can or cannot germinate after storage. During seed development and maturation/drying, diverse specific biochemical compounds are accumulated in the cells. These are determined by activation/inhibition of a variety of genes but are also influenced by environmental cues (Nagel et al., 2015; Sano et al., 2016; Arif et al., 2017; Leprince et al., 2017; Pereira Lima et al., 2017). Some of these biochemical compounds are correlated to the acquisition of seed longevity and will be the cell's substrates or targets for the ageing reactions, which include oxidation (including photo-oxidation, peroxidation and carbonylation), fermentation, glycation (e.g. Maillard reaction) and hydrolysis (Rajjou et al., 2008; Kranner et al., 2011; Colville et al., 2012; Nagel et al., 2015; Mira et al., 2016; Sano et al., 2016; Leprince et al., 2017). Ageing reactions lead to the formation of diverse 'products' that have been used to probe and predict seed longevity (e.g. Kranner et al., 2006; Mira et al., 2016; Fleming et al., 2018). During dry storage, environmental factors such as moisture and temperature are going to be the main drivers for ageing in DT seeds and the maintenance of a solid cytoplasm (a.k.a. glassy state) is the basis for seed bank strategies (FAO, 2014; Walters, 2015).

et al., 2017, 2018), decrease in pH, likely due to membrane deterioration (Nagel et al., 2019) and loss of glutathione reducing capacity (Kranner et al., 2006), occur in seeds that maintain germination potential. Loss of antioxidant capacity of glutathione appears to trigger irreversible, deleterious changes that have been correlated with programmed cell death (PCD) (Schafer and Buettner, 2001; Kranner et al., 2006). Oxidized molecules of DNA, lipids, carbohydrates and proteins are readily found in dry cells that have lost viability and it is unclear whether these changes are a cause or consequence of mortality (Walters, 1998; McDonald, 1999; Rajjou et al., 2008; Kalemba and Pukacka, 2014; Mira et al., 2016; Nagel et al., 2019). Dead plant cells can be distinguished from live ones by glutathione half-cell reduction potential increasing to up to −180 to −160 mV (Kranner et al., 2006; Nagel et al., 2019), which likely signals the cell's inability to buffer an oxidizing environment.

Eventually, dry germplasm loses germination potential and the question arises about the cause of the discontinuity between viable and inviable. In other words, is lost function caused by a culmination of minor damage that eventually has a major effect, or by a sudden weakening of protective mechanisms that lead to failure of critical cellular machinery? Are all molecules in the cell equally susceptible to degradation or are some molecules more prone to damage, and what damage has greatest impact on viability? Indeed, the period that viability is maintained (i.e. longevity) may be highly dependent on the variation in susceptibility of different molecules to ageing reactions and to the impact of different damaged molecules on function. Therefore, the organization and chemical composition of the cytoplasm is likely to have profound effects on the longevity trait (Priestley, 1986; Horbowicz and Obendorf, 1994; Hoekstra, 2005; Walters et al., 2005c, 2010; Probert et al., 2009; Nagel et al., 2015; Walters, 2015). The mechanism of the protection is likely to vary depending on whether ageing reflects specific or random events, and this distinction might provide insight into the unexplained variation of longevity within and among cell types, species and growth environments.

The role of glassy matrices in survival and deterioration of dried germplasm

The quiescence of dry organisms makes it difficult to pinpoint the moment of death from desiccation or ageing as well as the particular reaction that caused mortality. This quiescence is a consequence of being dry, which leads to a metabolic shutdown as the cytoplasm transitions from fluid to solid. Solidification limits diffusion of molecules that comprise the matrix thereby inhibiting most reactions, especially those that are catalysed by enzymes (Roudaut et al., 2004; Fernández-Marin et al., 2013; Nagel et al., 2019).

Diffusion is limited in solids because molecules are pressed together when water is removed, entrapping each other and slowing down movement (Fig. 2). At some point, the flow of molecules stops. Molecules within the cytosol or cytoplasm of cells are diverse (Fig. 2a). About 50% of the dry matter is protein, of which half could be storage proteins and up to 20% of the protein in DT cells may contain intrinsically disordered proteins (IDPs), such as LEA and HSPs. Soluble carbohydrates (such as sugars including trehalose, raffinose and stachyose) comprise up to 5–10% of the dry mass, and ions comprise about 2%. The cytosol also contains other diverse macromolecules, such as antioxidants and RNAs. Upon drying, the cytosol and internal compartments of organelles thicken and cellular constituents compress and interact with each other and with membrane surfaces (Fig. 2b), essentially locking them into position. Eventually, the matrix stabilizes into a structure that holds its shape and, in DT cells, preserves function of lipid bilayers, organelles and cytosolic

https://doi.org/10.1017/S0960258520000239 Published online by Cambridge University Press

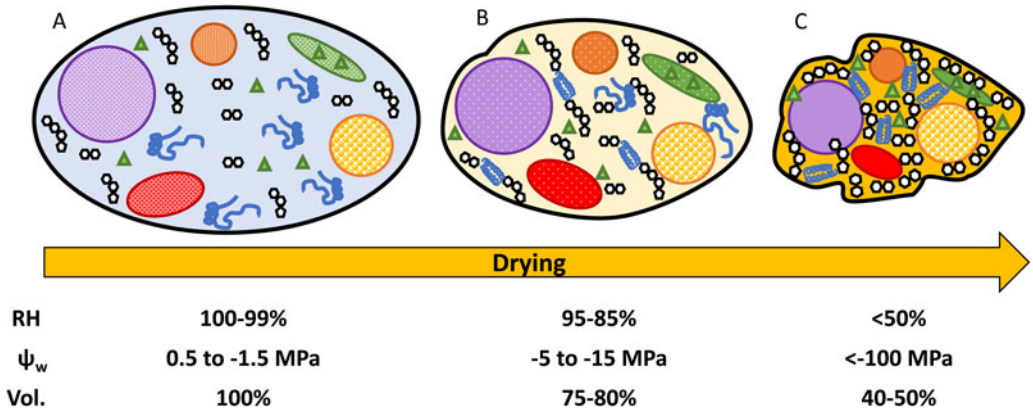

Fig. 2. Schematic representation of the glass formation in a plant cell during drying at diverse relative humidity (RH). Cell's water potential and volume change from initial are also indicated. (a) Cell cytoplasm before drying: the cytosol of the cells contains a mixture of sugars (trehalose, raffinose and stachyose: white hexagonal figures), proteins (e.g. LEAs and other IDP, blue lines) and other biochemical and metabolites (e.g. antioxidants, green triangles); organelles with fluid contents are immersed in this cytosol. (b) As cell dries <95% RH, cell volume decreases up to 75%, cell wall folds, LEAs modify their 3D structure, and with sugars and ions, by forming a gel, encapsulate other cell constituents and structures, such as cell organelles; organelle content also jellifies as water is removed. In this stage, voids form in the aqueous cellular matrix. (c) Cell cytoplasm after drying: the jelly cytosol vitrifies forming the glass and locking the cytoplasmic contents, containing among diverse biomolecules, vitrified (solid figures) or fluid (bubbly figure) organelles (Walters, 2004, 2015; Walters and Koster, 2007; Thalhammer et al., 2010, 2014; Walters et al., 2010; Ballesteros and Walters, 2011; Cornette and Kikawada, 2011; Hincha and Thalhammer, 2012; Boothby et al., 2018).

macromolecules (Fig. 2c; Koster et al., 2000; Wolkers et al., 2001; Cicerone et al., 2003; Walters and Koster, 2007; Buitink and Leprince, 2008; Thalhammer et al., 2010, 2014; Walters et al., 2010; Ballesteros and Walters, 2011; Cornette and Kikawada, 2011; Hincha and Thalhammer, 2012; Walters, 2015; Bremer et al., 2017; Boothby et al., 2018). In contrast, in cells of DS seeds, the translocation PLDα1 to the membrane facilitates the production of phosphatidic acid (PA) and destabilization of the plasma membrane before the matrix stabilizes (Chen et al., 2017).

When the diverse solutes of the cytosol compress during drying, the structure that forms is disorganized and comprises of pores having different shapes and sizes. Hence, dried cytoplasm is called a glass or glassy matrix, to distinguish it from crystalline solids in which molecules pack together in a regular pattern forming uniform-shaped pores. Solidification to glass is called a glass transition or vitrification, and the temperature at which this occurs is abbreviated T_g. The reverse solid-to-fluid transition is called plasticization and water is a potent plasticizer of cytoplasmic glasses, meaning that it reduces T_g by increasing pore size so that immobilized molecules can relax. At room temperature and moisture contents corresponding to about 50% RH, the cytoplasm is, on average, near the fluid-to-solid transition (Buitink and Leprince, 2008; Ballesteros and Walters, 2011, 2019). However, cell components are not uniformly dispersed within the cytoplasm. Localized differences in the nature and concentration of molecules can lead to localized differences in fluid-to-solid transitions. This was described for natural deep eutectic solvents (NADES), which maintain high fluidity in a concentrated solution (Choi et al., 2011). Furthermore, heterogeneity of pores can create mobility islands where the localized diffusional motion of small molecules and the relaxation of large molecules is allowed (Roudaut et al., 2004). Gaseous molecules, which are not part of the solid matrix, are still able to diffuse in a solid if pores are large enough. Pore size also affects short-range motion that persists in solids such as stretching, bending, rotation and vibration of atoms and ligands (Cicerone et al., 2003; Roudaut et al., 2004; Ballesteros and Walters, 2011). These short-range fast motions can lead to a reorientation of side chains of large molecules and ultimately intermolecular interactions or further relaxation of the structure (Walters, 2004; Ballesteros and Walters, 2019). Therefore, both the composition and packing efficiency of molecules have profound effects on the ultimate physical and chemical stability of the matrix.

Moreover, the physical structure of non-water soluble constituents, like lipids, is hardly influenced by water. Oil droplets remain fluid within cells until exposed to low temperatures (Crane et al., 2003; Ballesteros et al., 2018, 2019). Cells with high triacylglycerol (TAG) components may be analogous to composite materials that are engineered from polymers and plastics. Combining components with different physical–chemical properties can improve strength, or reduce mass or cost. However, composite materials may be prone to failure when environmental conditions change, causing the components to separate. Co-existence of fluid and solid cellular regions could also have serious implications for the nature and kinetics of ageing reactions by facilitating long-range diffusion of ROS (Fryars et al., 2018), allowing catabolic reactions more typical of deterioration under fluid conditions (Velasco et al., 2003) and encouraging molecular realignment and pore reconfiguration (Ballesteros et al., 2019).

Water tends to remain mobile in glassy systems (Roudaut et al., 2004) and can be removed by exposure to extremely low RH. Under very dry conditions (<10% RH), water can act as an anti-plasticizer to reduce molecular mobility and increase structural stability (Pittia and Sacchetti, 2008). This duality of water as plasticizer or anti-plasticizer, depending on the proximity of conditions to T_g, might explain increased ageing rates in seeds that have been excessively dried (Vertucci et al., 1994). The mechanical properties of seeds appeared more stable at low temperatures in the presence of some water and complete drying tended to increase molecular mobility (Ballesteros and Walters, 2011). Other low weight molecules solutes, such as glycerol, dimethyl sulphoxide and ethylene glycol, can also act as anti-plasticizers to stabilize molecular relaxations and local motions (Cicerone et al., 2003).

The susceptibility and rate of degradation of diverse biological molecules differ in solid matrices and fluid systems owing to the

https://doi.org/10.1017/S0960258520000239 Published online by Cambridge University Press

nature and spatial scale of molecular motion and interactions (Cicerone et al., 2003; Velasco et al., 2003; Roudaut et al., 2004). Analogously, we may expect that the mechanisms that cause ageing and eventual mortality in dry seeds may differ from cells that are fluid and allow reactions that are dominated by long-range diffusive motion (Walters, 1998; Ballesteros and Walters, 2011; Fernández-Marin et al., 2013; Nagel et al., 2019).

The predominant motions within glasses are short range, which increases the likelihood of cross-linking or fragmentation (Roudaut et al., 2004; Ballesteros and Walters, 2011, 2019; López-Pozo et al., 2019). For example, solidified peptides and proteins are susceptible to deamidation, which hydrolyses the amide side chains of asparagine and glutamine (Manning et al., 2010). Cross-linking can occur between reducing sugars and peptides in close proximity (i.e. glycation) (Povey et al., 2009). Light-induced oxidation of solidified protein mixtures is common and can result in a number of breakdown products (Miller et al., 2003; Manning et al., 2010). These reactions arise from slight molecular rearrangements within the molecule (Cicerone et al., 2003; Roudaut et al., 2004; Ballesteros and Walters, 2011).

The small movements of molecules within the glassy matrix can be a source of 'physical ageing' of the structure, which has been implicated in the deterioration of foods, plastics and pharmaceutical compounds (e.g. Cicerone et al., 2003; Roudaut et al., 2004; Kucera et al., 2013; Cowie and Arrighi, 2014). Over time, molecules that were entrapped by neighbouring molecules budge (or relax) and begin to fill in the pores. The rate of compression reflects molecular mobility within the matrix, which is determined by temperature, molecular packing, presence of plasticizers and anti-plasticizers and the innate flexibility and space-filling properties of constituents of the matrix (Cicerone et al., 2003; Roudaut et al., 2004; Walters, 2004; Walters et al., 2010; Ballesteros and Walters, 2011, 2019).

Slow diffusion of oxygen or other gaseous ROS molecules through the large pores of glassy matrices is an important factor in the deterioration of polymers and biomolecules (Roudaut et al., 2004). Photodegradation of proteins depends on the presence of molecular oxygen (Miller et al., 2003) and auto-oxidation is facilitated in the presence of mobile ROS (Minemoto et al., 2001; Velasco et al., 2003). Volatilized by-products of oxidized fatty acids can react with surrounding molecules, including proteins, which may lead to eventual impairment of catalytic function (Velasco et al., 2003).

To summarize, dehydrating cytoplasm eventually leads to a solid with heterogeneous properties and domains of high and low fluidity due to the diverse mixture of cellular constituents as well as the imperfect packing. DT organisms survive the immediate effects of cell shrinkage and molecular compression that caused solidification, but eventually, succumb with time to deteriorative reactions that are highly dependent on molecular mobility and proximity. We expect the nature of deteriorative reactions to differ in fluid and solid cytoplasm because restricted diffusive motion in structures affects the probabilities of different molecular interactions. Structure and mobility within a solid are inextricably related. Here, we make the case that the structure of the solid matrix is a critical factor in the long-term survival of DT organisms.

Dry architecture of dry germplasm

Solid matrices stabilize cytoplasm by slowing reactions, but the protection provided is not indefinite. Deterioration occurs and ultimately causes mortality. Duration of survival (i.e. longevity) varies considerably among organisms (Priestley, 1986; Hoekstra, 2005; Walters et al., 2005a,c; Probert et al., 2009; Nagel and Börner, 2010; Ballesteros et al., 2019). The intrinsic differences in longevity among cell types and species suggest that chemical reactivity varies in glassy matrices or that cells have different tolerances to chemical reactivity.

Biochemical composition, in terms of total protein, lipid and carbohydrates, does not provide strong insights into the susceptibility of seeds to ageing, though some trends emerge that suggest that cell structures are an important factor in the longevity of dried germplasm. For example, long-lived Fabaceae seeds lack storage lipids and have highly degraded photosystems, for example, peas and beans (Farrant et al., 1997; Walters et al., 2005c). On the other hand, short-lived cells maintain well-developed photosystems, for example, chlorophyllous seeds and fern spores (Roqueiro et al., 2010; Ballesteros et al., 2019). In addition, some seeds and fern spores with high lipid content age faster in the freezer at −20°C than in the refrigerator at 5°C (Dussert et al., 2001; Crane et al., 2003; Hor et al., 2005; Ballesteros et al., 2019; Fleming et al., 2019).

In this paper, we hypothesize that different structural conformations promote distinctive ageing mechanisms in dry cells (e.g. Ballesteros et al., 2018), particularly under preservation conditions used by most seed banks (−20°C; FAO, 2014), and that this can lead to faster than expected ageing. Three main conformations of dry cells are presented to illustrate how chlorophyll content and storage lipids can affect physical–chemical properties: (1) non-chlorophyllous cells lacking TAG (Fig. 3a), (2) chlorophyllous cells lacking TAG (Fig. 3b) and (3) non-chlorophyllous cells with TAG (Fig. 3c).

*Non-chlorophyllous cells with low TAG (**Fig. 3a**)*

Photosynthetic pigments, including chlorophyll and carotenoids, become degraded during maturation and drying of DT cells (Vertucci and Farrant, 1995; Leprince et al., 2017). During embryogenesis, DT cells also accumulate high amounts of protein, carbohydrates or TAG, replacing more than 50% of the cellular water with these dry matter reserves (Farrant et al., 1997; Walters et al., 2010; Pérez et al., 2012; Walters, 2015). About one-third of angiosperm taxa, including representatives in Poaceae and Fabaceae, favour protein and carbohydrate dry matter, synthesizing less than a 10% of accumulated dry matter as TAG (Fig. 4a); half of these accumulate less than 5% TAG (Royal Botanic Gardens Kew SID, 2018). The accumulated dry matter could be a plentiful substrate for oxidation within the glassy matrix, with proteins and starch possibly serving as scavengers of ROS (Fig. 5). TAG in these cells may be susceptible to peroxidation (Fig. 5), but the densely packed protein bodies and starch granules probably slow transport of oxidized lipid by-products from the small and dispersed lipid droplets.

The determined or predicted lifespan of seeds with non-chlorophyllous cells and <10% TAG ranges broadly from <10 to >400 years for specific dry and cold storage conditions (Walters et al., 2005c). We postulate that variation in longevity arises from different properties of the solid matrices among dried seeds. For example, a comparison of mechanical properties of cells within dried embryonic axes pea and soybean, both non-chlorophyllous containing <10% TAG, suggest that soybean, which ages faster, has a more fragile matrix. This means that molecules are less densely spaced in soybean and allow more molecular relaxation, especially in the presence of water

https://doi.org/10.1017/S0960258520000239 Published online by Cambridge University Press

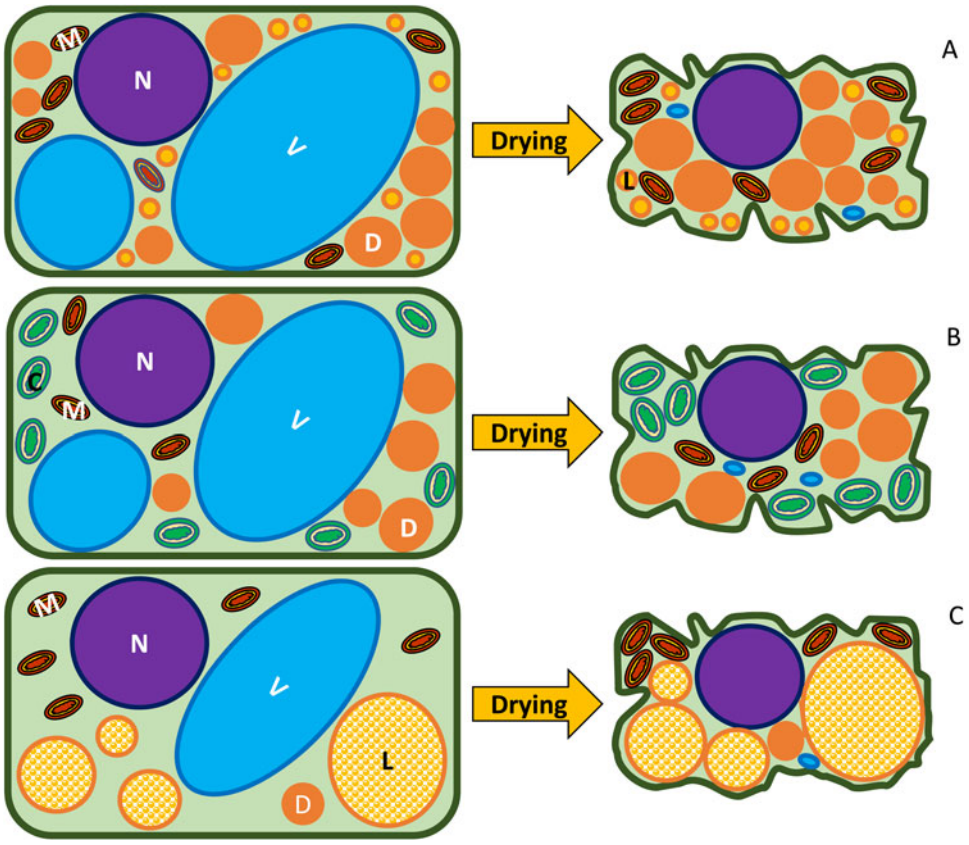

Fig. 3. Schematic representation of three basic structural cell conformations before and after drying. During drying, aqueous vacuoles (V) are reduced and mostly disappear, organelles come in close proximity, some may shrink [e.g. the nucleus (N) and mitochondria (M)], and cell walls are slightly folded. (a) Non-chlorophyllous cells with very low TAG: cytoplasm is occupied by dry matter (D), including protein storage bodies, starch and sometimes small amount of lipid droplets (L). Internal membranes, such as the endoplasmic reticulum and the Golgi apparatus, are not represented for simplicity of the model but are considered as part of N and D, respectively. (b) Chorophyllous cells with low TAG: chloroplasts (C) are not degraded in the maturation phase and are present. (c) Non-chlorophyllous cells with high storage lipids: cytoplasm is occupied by dry matter (D), including some protein storage bodies and starch but mostly by lipid droplets (L).

(Ballesteros and Walters, 2019). Larger pore size and greater flexibility of molecules within the soybean matrix could allow greater diffusion of volatile ROS and greater local interactions between molecules to enhance cross-linking with neighbouring molecules, for example, glycation and carbonylation of LEAs and HSPs (Rajjou et al., 2008; Kalemba and Pukacka, 2014). Differences in the stability of solid structures can, therefore, affect biochemical reactivity and hence ageing rates. The composition of molecules within the glassy matrix surely contribute to fragility, and it is compelling to think that soluble carbohydrates and/or IDPs, which provided early foundations of glass formation in the biological literature, may protect cells by scaffolding a fragile matrix (e.g. Buitink et al., 2000; Koster et al., 2000; Wolkers et al., 2001; Buitink and Leprince, 2008; Hincha and Thalhammer, 2012; Boothby et al., 2018).

*Chlorophyllous cells with low TAG (**Fig. 3b**)*

Degradation of photosynthetic pigments sometimes does not occur in DT plant germplasm and dried cells retain active chlorophyll (Fig. 3b). About 16% of Australian dicotyledon species (from a sample of approximately 300 species) produced mature seeds with chlorophyllous cells (Wright et al., 2000). Fern spores from Equisetaceae, Hymenophyllaceae, Onocleaceae and Osmundaceae are chlorophyllous, as well as all grammatid ferns within Polypodiaceae, representing significant portions of the fern flora in Mesoamerica, the Neotropics and Africa-Madagascar (Sundue et al., 2011). Dried seeds and spores with chlorophyllous cells also tend to have low TAG content (Fig. 3b; Lloyd and Klekowski, 1970; Ballesteros et al., 2019). Chlorophyllous cells tend to have intact chloroplasts and are distinguished from cells that are simply green. For example, dried pea cotyledons have one-tenth of the chlorophyll of Salix cotyledons (Cheng et al., 2004) and thylakoids are disassembled giving them lower capacity for electron transfer beyond light-harvesting pigments as a result of the reorganization of the chloroplast structure (Vertucci et al., 1985).

Seeds and spores with chlorophyllous cells often tend to age quickly compared to non-chlorophyllous germplasm. Some examples include seeds of *Salix* and *Populus* ssp., seeds from mutant lines of Arabidopsis and *Medicago truncatula* that retain chlorophyll, and chlorophyllous fern spores (Lloyd and Klekowski, 1970; Maroder et al., 2000; Popova et al., 2013; Ballesteros and Pence, 2017; Ballesteros et al., 2017, 2019; Leprince et al., 2017). Ageing of dry cells and proteins is faster in the light compared to darkness (Vertucci et al., 1994; Khan et al., 1996; Manning et al., 2010; Roqueiro et al., 2010; Ballesteros et al., 2018). Intact photosynthetic apparatus can enhance the presence of ROS and FR, especially in the presence of light (Fig. 6). Even under dry conditions, pigments can absorb

https://doi.org/10.1017/S0960258520000239 Published online by Cambridge University Press

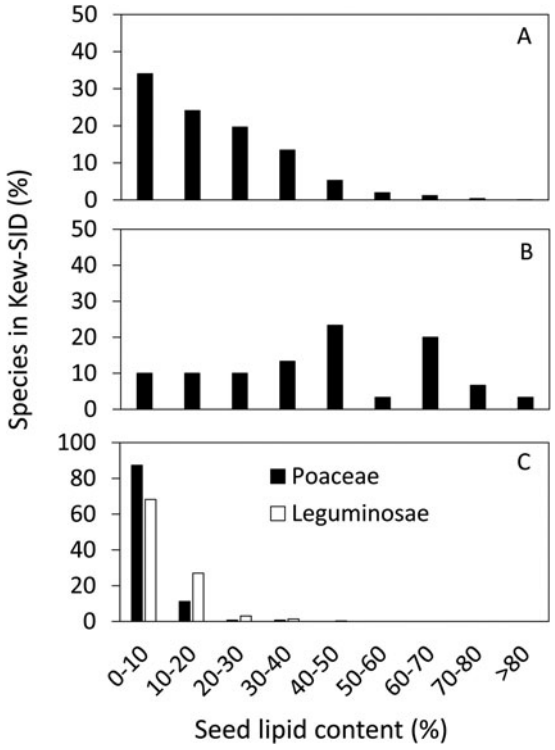

Fig. 4. Lipid (TAG) composition in seeds from 2,865 species that are listed in the Kew Seed Information Database that are (a) orthodox (DT) and (b) considered 'intermediate' between orthodox and recalcitrant (not DT). In (c), a comparison of lipid composition is provided for seeds within Poaceae (black bars) and Fabaceae (white bars), both families producing predominantly DT seeds.

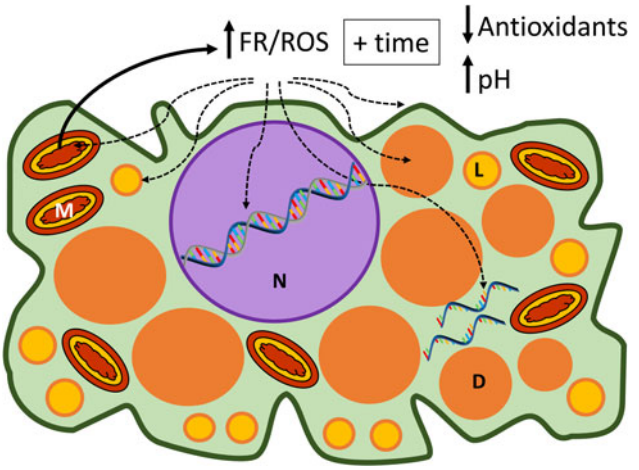

Fig. 5. A schematic model of ageing in dry cells that begins with accumulated ROS and FR from background levels or impaired metabolism during drying. With time, the efficiency of the antioxidant machinery declines and leads to autocatalytic reactions and damaged molecules. ROS and FR attack of abundant starch or storage proteins are not as deleterious as attack on genetic (DNA and RNA), structural (e.g. membranes, carbohydrates and soluble proteins) or metabolic (e.g. mitochondria, enzymes and membrane proteins) components (Davies, 2005; Kranner et al., 2006; Bailly et al., 2008; Rajjou et al., 2008; Manning et al., 2010; Kalemba and Pukacka, 2014; Mira et al., 2010, 2016; Fleming et al., 2017, 2018; Nagel et al., 2019). Increased pH in the dry cytoplasm (Nagel et al., 2019) may compromise rehydration/germination or alter the rate of protein glycation within the glassy matrix (Povey et al., 2009). M, Mitochondria; N, Nucleus; L, lipid bodies; D, Dry matter (e.g. protein bodies and starch).

light and transduce the excited state through Photosystem II and I (Vertucci et al., 1985). Photo-excited pigments release electrons that become strong oxidizers in the absence of water or antioxidant protection (Fig. 6a; Heber et al., 2006; Kranner et al., 2008; Roqueiro et al., 2010; Ballesteros et al., 2018; Verhoeven et al., 2018). The light harvested by intact photosynthetic systems increases the production of FR which then begins attacking thylakoid membranes (Roqueiro et al., 2010). Even in darkness, germplasm with chlorophyllous cells tend to age quickly, and we speculate that this can be attributed to highly efficient electron transfer among pigments and within thylakoids of Photosystem II (PSII) (Fig. 3b). Multiple double bounds of photosynthetic pigments are regions of high electron density that allow electron jumps, even under dry conditions (Vertucci et al., 1985; Lebkuecher, 1997), but these electron-dense molecules are extremely susceptible to ROS and FR and can serve as 'ground-zero' for autocatalytic cascades. Chlorophyllous cells may have insufficient levels of molecular antioxidant defenses (Kranner et al., 2002, 2010; Roqueiro et al., 2010; Ballesteros et al., 2018). Failure to down-regulate light-harvesting machinery can exacerbate oxidation stress by increasing FR levels before cells desiccate (Vertucci and Farrant, 1995; Bailly, 2004).

Formation of glassy structures that are fragile or allow a lot of local movement may also explain fast ageing of chlorophyllous seeds and fern (Walters, 2004; Ballesteros and Walters, 2011; Ballesteros et al., 2017, 2019; López-Pozo et al., 2019). However, parallel Arrhenius plots of ageing rate for chlorophyllous and non-chlorophyllous spores argue against differences in temperature-dependency of long-range motions (Ballesteros et al., 2019).

Non-chlorophyllous cells with high storage lipids (Fig. 3c)

Many plant species accumulate TAG within cells of seeds, pollen or spores, likely as a main source of energy for germination (Lloyd and Klekowski, 1970; Bewley and Black, 1994; Graham, 2008). Two-thirds of taxa listed in the Kew SID (Royal Botanic Gardens Kew Seed Information Database - SID, 2018) produce seeds with over 10% TAG, and 9% of these accumulate more than 40% dry matter as TAG. TAG is encapsulated in lipid droplets of diverse composition, size and number, depending on species (Tzen et al., 1993; Fig. 7a). TAG content in non-chlorophyllous fern spores ranges between 20 and 50% depending on species (Ballesteros and Walters, 2007; Ballesteros et al., 2017).

There is no correlation between seed longevity and TAG content (Priestley, 1986; Walters et al., 2005c; Probert et al., 2009; Nagel et al., 2015; Mira et al., 2019). Nevertheless, TAG continues to be linked to poor storage quality. Historically, this association arises from rancidity problems of unsaturated oils extracted from seeds or known differences in keeping quality of pea and beans (low TAG) compared to soybean and peanuts (high TAG). Higher free fatty acid content and lost polyunsaturation of fatty acids are found in long-dead seeds (Walters, 1998; McDonald, 1999) and VOC from lipid oxidation are detected before mortality (Mira et al., 2010, 2016). Moreover, the physical properties of TAG, related to crystallization behaviour, change during storage (Vertucci, 1992; Walters et al., 2005b; Ballesteros et al., 2019). As is true for the suite of chemical changes detected in preserved germplasm, there is insufficient evidence to link changes of TAG specifically to the event(s) causing mortality (Walters, 1998; Fleming et al., 2018).

Despite unclear relationships between chemical changes of TAG and seed ageing, the different physical–chemical properties of TAG and the aqueous-based cytoplasm that surrounds lipid bodies

https://doi.org/10.1017/S0960258520000239 Published online by Cambridge University Press

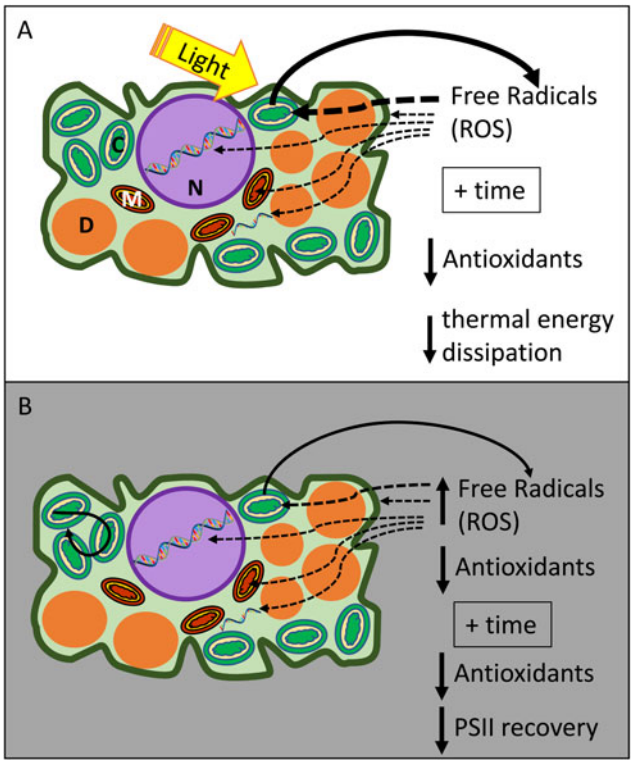

Fig. 6. A schematic model of the ageing mechanisms in the dry state for seeds and spores with chlorophyllous cells in the presence of light (a) or in the dark (b). In (a), light is absorbed by the pigments of the photosynthetic apparatus, and when water is absent, free radicals are produced and threaten to oxidize components of the photosynthetic apparatus and other cellular components such as membranes or the genetic material. This oxidative stress is evident over time as thermal energy dissipation mechanisms and antioxidant protection are reduced (Heber et al., 2006; Kranner et al., 2008, 2010; Roqueiro et al., 2010; Ballesteros et al., 2018; Verhoeven et al., 2018). In (b), high accumulation of free radicals during maturation/drying threaten to oxidize diverse cellular components (including the PSII), particularly when antioxidant protection is initially reduced or is reduced over time (Roqueiro et al., 2010; Ballesteros et al., 2018). These mechanisms may be potentiated by a non-stable and highly mobile glassy matrix in the cytoplasm (Ballesteros and Walters, 2011; Ballesteros et al., 2019) that facilitates cross-linking reactions within the photosynthetic apparatus and diffusion of ROS and other small molecules across the cell (Roudaut et al., 2004; Ballesteros and Walters, 2011; López-Pozo et al., 2019). M, Mitochondria; N, Nucleus; C, chloroplast; D, Dry matter (e.g. protein bodies and starch).

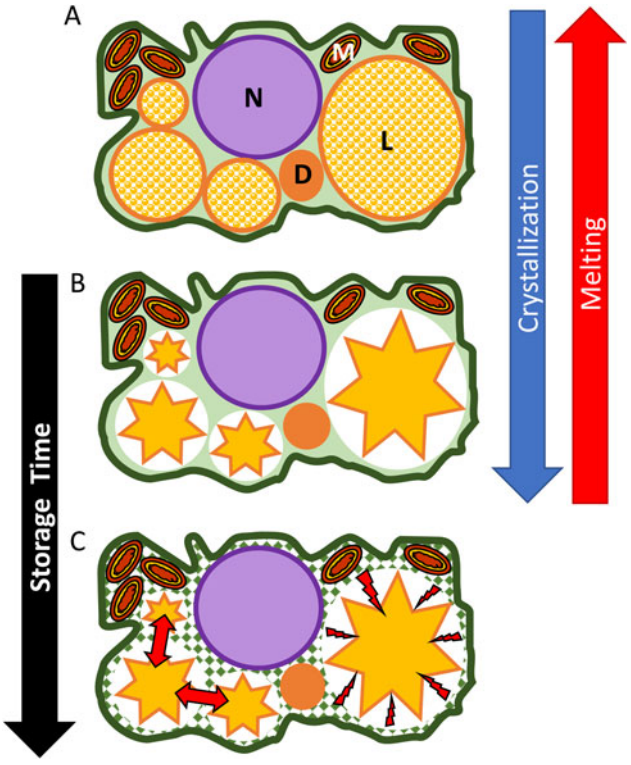

Fig. 7. A schematic model of the mechanisms of deterioration in the dry state for seeds and spores with non-chlorophyllous cells with high storage lipids. When dry seeds and spores (**a**) are exposed to low temperatures (i.e. −20°C) storage lipids (L) crystallize (**b**). Lipid crystallization (**b**) can be reverted (**a**) by warming to the melting temperature given by the specific lipid (triacylglycerol) composition (e.g. Crane et al., 2003, 2006; Ballesteros et al., 2018; PCP). When seeds or spores are stored at −20°C (**b**) crystallization of storage lipids continue progressing into the most stable lipid crystal forms (Ballesteros et al., 2018; PCP). Lipid crystallization involves volume reduction, which could create pores and spaces between the solid cytoplasm and the compressed lipid droplet (**c**). These pores could facilitate lipid droplet restructuration and merging upon melting (represented by double arrows) but also could lead to structural collapse (red rays) and/or changes in the glassy properties of the dry cytoplasm (squared pattern) (Leprince et al., 1998; Crane et al., 2006; Shimada et al., 2008; Walters, 2015). All these structural changes over time could also facilitate ROS diffusion and make cellular constituents more prone to oxidative stress. M, Mitochondria; N, Nucleus; D, Dry matter (e.g. protein bodies and starch).

presents a compelling chapter in the story of dry architecture. While water-soluble regions of the cell shrink and solidify during drying at room temperature, TAG does not, but rather maintain volume and stay fluid. TAG solidify when cooled, usually to sub-zero temperatures (e.g. Vertucci, 1992; Crane et al., 2003; Walters et al., 2005b; Ballesteros and Walters, 2007; Ballesteros et al., 2019; Mira et al., 2019). Especially with rapid cooling, TAG initially forms disordered crystals; but as annealing time increases, crystals recrystallize into more ordered, lower energy forms, creating ever denser, lower volume oil droplets (Metin and Hartel, 2005; Fig. 7b,c). As TAG crystals shrink, gaps between the oil droplets and solidified cytoplasm grow, potentially destabilizing the matrix or allowing larger ROS molecules to penetrate. Cytoplasm with regions of TAG might be considered as a 'composite' in materials sciences contexts. Composite materials are comprised of components with distinct properties, usually engineered to improve strength or reduce mass or cost of a structure. However, when the separate components have incompatible physical–chemical properties and temperature changes, components can separate and the composite structure fails.

An analogous structural failure has been used to explain the detrimental effects of −20°C in germplasm from diverse species (Crane et al., 2003, 2006; Walters, 2015). The syndrome has been used to explain 'intermediate' storage physiology, which was classically defined as seeds that do not survive the combined stress of desiccation and low temperature and so are inappropriate for conventional freezer storage (Ellis et al., 1991, FAO, 2014). Seeds from some *Coffea* ssp. (Ellis et al., 1990; Dussert et al., 2001; Eira et al., 2006), *Cuphea* ssp. (Crane et al., 2003, 2006), *Citrus* ssp. (Hor et al., 2005), papaya (Ellis et al., 1991) and orchids (Pritchard and Seaton, 1993), as well as spores from some fern species (Ballesteros, 2011), can be damaged by exposing them to freezer temperatures. TAG is a usual feature in the cells of germplasm characterized as having 'intermediate' storage physiology. Seeds in over 80% of species categorized as 'intermediate' contain >20% TAG, and 57% of 'intermediate' species produce seeds with >40% TAG (Royal Botanic Gardens Kew SID, 2018). Most are from the tropical origin. Damage can be expressed with either

https://doi.org/10.1017/S0960258520000239 Published online by Cambridge University Press

Table 1. Genebanking strategies to maintain germplasm viability, their influence in the different dry architectures and suggestions to improve longevity

Genebanking strategies to maintain germplasm viability (as per FAO, 2014)	Influence in the different dry architectures: CCLL, NCLL and NCHL[a]	References
Decrease moisture	Plant germplasm longevity increases as the germplasm moisture content decreases in all three dry architectures. However, drying germplasm beyond certain critical moisture content provides little or no additional benefit to longevity or can accelerate ageing rates.	Harrington (1972), Ellis and Roberts (1980), Walters (1998), FAO (2014) and Ballesteros et al. (2017)
Decrease temperature	Plant germplasm longevity increases as the storage temperature decreases in all three dry architectures. However, NCHL may die quickly during storage at standard genebank conditions (around −20°C), possibly because lipids crystallize and destabilize the glassy matrix.	Harrington (1972), Ellis and Roberts (1980), Walters (1998), FAO (2014) and Ballesteros et al. (2019)
Suggestions to improve longevity		
Adjusting moisture in the function of storage temperature	Optimum moisture levels vary among organisms and storage temperature, but they do not seem to be related to the cell's dry architecture. Regarding temperature, it has been shown that lowering the storage temperature increases the optimum germplasm moisture content level, which suggests there might be danger of over-drying germplasm when dried at room temperatures and stored at very low (cryogenic) temperatures. This can be applied to all three dry architectures.	Vertucci et al. (1994), Walters (1998), Walters et al. (2005a) and Ballesteros et al. (2017)
Lowering storage temperature	Liquid nitrogen temperatures (−176 to −196°C) have been demonstrated to increase seed and fern spore longevity in all three types of cells compared to −20°C storage.	Walters et al. (2004), Ballesteros and Pence (2017) and Ballesteros et al. (2019)
	Storing NCHL germplasm at temperatures that are outside the range for which lipid crystallization reactions are observed (e.g. liquid nitrogen) helps to the stabilization of the storage lipids, for example: - Long-term storage in liquid nitrogen not only provided larger longevity to oily samples but also maintained the typical lipid crystallization pattern that samples had before storage. - For some species (e.g. Brassicaceae), storage temperatures above the lipid melting temperature (so when lipids are fluid) may be beneficial compared to the standard −20°C storage.	Pritchard and Seaton (1993), Crane et al. (2006), Ballesteros et al. (2019) and Mira et al. (2019)
Controlling cooling rate	Rapid cooling rates seem to be negative for liquid nitrogen storage of dry NCHL seeds from soybean and sunflower.	Vertucci (1989)
Applying heat treatment after cold storage	*Cuphea* ssp. seeds (NCHL) were successfully germinated after −18°C storage and thawing up to 45°C to allow full melting of storage lipids and avoid sensitivity to imbibitional damage after storage.	Crane et al. (2003, 2006)
Shortening of post-harvest procedures to reduce the concentration of free radicals produced during drying or in the lag-time from collection to storage	Fast and gentle seed handling during collection and during post-harvest procedures (e.g. cleaning and drying) has been suggested to maximize the longevity of dry CCLL seeds from *Salix* and *Populus* ssp. The same principle could also be applied to NCHL and NCLL but more research is needed to determine the efficiency, as the longevity of some seeds may benefit from a post-harvest maturation step.	Roqueiro et al. (2010) and Ballesteros and Pence (2017)
Decreasing oxygen levels and reducing exposure to light	The storage atmosphere may play an important role. For example, the absence of light and a reduction of oxygen in the storage atmosphere maximized longevity for CCLL, and maybe these conditions should be extended to diverse steps during the post-harvest management of the germplasm (e.g. cleaning and drying). Interestingly, liquid nitrogen storage creates an	Halliwell and Chirico (1993), Roqueiro et al. (2010), Gonzalez-Benito et al. (2011), Groot et al. (2015), Mira et al. (2016) and Ballesteros et al. (2018)

(*Continued*)

https://doi.org/10.1017/S0960258520000239 Published online by Cambridge University Press

Table 1. (*Continued.*)

Genebanking strategies to maintain germplasm viability (as per FAO, 2014)	Influence in the different dry architectures: CCLL, NCLL and NCHL[a]	References
	anoxic storage atmosphere where oxidative reactions are likely to be reduced. The same principle could also be applied to NCHL and NCLL to reduce oxidative stress (including photo-oxidation), but more research is needed to determine the efficiency.	
Modifying cellular properties to create more stable structures, for example:		
Application of cytoplasm stabilizers (anti-plasticizers) to increase the strength of the seed glasses before drying and storage	Glycerol, an important constituent in cryoprotectant cocktails, appears to reduce local motion and may provide structural protection to cryopreserved materials beyond its recognized role in suppressing ice formation. Dimethyl sulphoxide and ethylene glycol can also quench molecular relaxations and local mobility. More research is needed.	Cicerone et al. (2003) and Ballesteros et al. (2014, 2019)
Manipulation of the maternal environment during seed development using abiotic factors (e.g. light)[b]	Lettuce seed (NCHL) longevity was modified by the management of light conditions during seed maturation in the mother plants. Application to CCLL and NCLL might also be useful, but more research is needed to determine the abiotic factors on the maternal environment that can increase seed longevity.	Contreras et al. (2008, 2009)
Manipulation of the seed development using biotic factor (e.g. auxins or the introduction of modified genes related to heat-shock transcription factors)[b]	Heat-Shock transcription Factor A9 (HSFA9) was helpful in protecting the photosynthetic apparatus from oxidative stress during drying in sunflower seedlings. This principle could be applied to CCLL, but more research is needed. Application to NCHL and NCLL might also extend longevity in seeds, but more research is needed to determine the efficiency.	Carranco et al. (2010) and Almoguera et al. (2012)
Developmental modifications in order to generate cells with a particular concentration of oleosins and/or a specific lipid droplet distribution[b]	Low oleosin content negatively affects germination after drying and cooling. More research is needed.	Leprince et al. (1998) and Shimada et al. (2008)

[a]CCLL, Chlorophyllous Cells with very Low or no storage Lipids; NCHL, Non-Chlorophyllous cells with moderate or High storage Lipids; NCLL, Non-Chlorophyllous cells with very Low or no storage Lipid.
[b]These modifications of the cellular properties to create more stable structures for preservation may diverge from genebanking principles to not alter genetic identity or may be particularly difficult for wild germplasm when there is a small window of opportunity to collect.

short duration in the freezer (Crane et al., 2003, 2006) or as storage time increases, and so takes on characteristics of ageing (Walters, 2015; Ballesteros et al., 2019; Fleming et al., 2019). A different interaction between water and crystallized or fluid TAG might exacerbate damage to imbibing cells (Crane et al., 2003, 2006). Oleosins, amphiphilic proteins that surround and stabilize the lipid droplet (Leprince et al., 1998; Shimada et al., 2008), likely reorganize or eject during contraction of the crystallized TAG (Leprince et al., 1998; Walters, 2015), which can lead to coalescence of the oil bodies upon hydration, deformation of nuclei, and ultimately defective germination and seed mortality (Shimada et al., 2008; Fig. 7c). The interrelationships between TAG properties, lipid body geometry and oleosin interactions with solidified cytoplasm will provide greater insight into ageing mechanisms of non-chlorophyllous cells with TAG (Huang, 1996; Leprince et al., 1998; Shimada et al., 2008, 2018; Walters et al., 2010; Ballesteros and Walters, 2011; Walters, 2015).

Genebanking strategies to maintain germplasm viability

Strategies to preserve DT germplasm have historically involved controlling the moisture and temperature environment (Table 1) and have been modelled to illustrate, what we now understand, as the exponential relationships between environmental factors and viscosity of the cytoplasm prior to solidification, that is, above T_g (Harrington, 1972; Ellis and Roberts, 1980). Poignantly, Harrington's Hundred Rule (safe storage occurs when the sum of RH and temperature in Fahrenheit is less than 100) advocates storage conditions that nearly parallel water plasticization effects on cytoplasm (Walters, 1998). However, once the cytoplasm has solidified, the relationships between viscosity and environmental factors of temperature and moisture are expected to change from those observed in the more fluid cytoplasm (Walters, 2004). Moreover, within solids, or glasses, the mobility of molecules differs considerably with the environment (Ballesteros and Walters, 2011), and this is a factor of both the composition and organization of molecules within the dried cytoplasm (Walters, 2015; Ballesteros and Walters, 2019). The diversity of solid-state structures that form in diverse cells and species during preservation is surely key to the variation in longevity that is observed in nature (e.g. Shen-Miller et al., 1995; Sallon et al., 2020) and under controlled genebanking conditions (e.g. Walters et al., 2005c; Nagel and Börner, 2010). New strategies to predict the longevity of preserved germplasm, and

https://doi.org/10.1017/S0960258520000239 Published online by Cambridge University Press

possibly to extend it, must account for stability and reactivity within solids.

Developing advanced strategies to preserve germplasm requires a focus on both environmental controls as well as properties intrinsic to the cell (Table 1). For example, lowering the storage temperature to cryogenic temperatures is a good way to expand germplasm longevity beyond the time accrued by the standard seed storage temperatures (Walters et al., 2004; FAO, 2014; Ballesteros and Pence, 2017; Ballesteros et al., 2019). However, neither low moisture nor temperature will confer infinite stability within the solid (Dickie et al., 1990; Walters, 2004; Walters et al., 2005a; Ballesteros and Walters, 2011, 2019). Water is a plasticizer near T_g, but an anti-plasticizer well below T_g, which explains the dual effects manifested by faster ageing under extremely dry conditions (Vertucci et al., 1994; Walters, 1998; Walters et al., 2005a; Ballesteros et al., 2017). With the current understanding of the diversity of structure and motion in cellular glasses, as well as the interaction of temperature, it would be reasonable to postulate that optimum moisture levels vary among organisms and storage temperature.

Temperature also has conflicting effects on structure and mobility within solids. Diffusive motion is certainly limited by cooling to cryogenic temperatures; however, there is a greater disparity between the structure formed with open pores at T_g and the equilibrium structure that is far denser at lower temperatures, and this disparity serves as a driving force for molecular movement (Walters, 2004). Moreover, the temperature has less effect on short-range motions, which are likely involved in continued deterioration of cryogenically stored germplasm (Walters, 2004; Walters et al., 2004, Ballesteros et al., 2019). In other words, low temperature alone will not stop ageing reactions. Indeed, specific cold temperatures might facilitate ageing reactions through a change in the dry architecture brought about by lipid crystallization. That said, storage at cryogenic temperature provides a powerful tool to induce compatibility among aqueous and TAG components, that have very different physical–chemical properties at higher sub-zero temperatures.

In addition to temperature and moisture, modifications in the physicochemical environment where seeds are processed and stored to reduce the source of FR and ROS may also be beneficial to increase longevity (Table 1). Some examples are the reduction of the oxygen levels within the storage containers (Gonzalez-Benito et al., 2011; Groot et al., 2015), or the reduction of the exposure to light across the post-harvest procedures (Roqueiro et al., 2010; Ballesteros and Pence, 2017; Ballesteros et al., 2018).

Modifying cellular properties to create more stable structures for preservation (Table 1) may diverge from genebanking principles to not alter genetic identity or may be particularly difficult for wild germplasm when there is a small window of opportunity to collect. Our greatest insights might come from studies of germplasm that are particularly long-lived. One solution may involve an early onset of quiescence to reduce high-energy metabolites that are a source of FR and ROS. Cellular constituents that increase the density of cytoplasm above T_g may induce quiescence while also making glasses stronger and less likely to relax below T_g. Additionally, anti-plasticizers stiffen molecules so they are less likely to move (Table 1). These can be engineered as ligands on large molecules that fill space or additions of small molecules like glycerol, dimethyl sulphoxide and ethylene glycol that quench molecular relaxations and local mobility (Cicerone et al., 2003).

Conclusions

The cells of preserved germplasm successfully transition from fluid to solid. The structure of the solid impedes molecular mobility, thereby, limiting chemical reactivity that leads to ageing. Nevertheless, ageing of preserved germplasm occurs, albeit slowly, and eventually leads to mortality. Here, we have reviewed the solidification process and have argued that structures formed as DT organisms dry can vary considerably among diverse organisms and that these differences can contribute to differences in how long preserved germplasm persists (i.e. longevity). We have identified some structural features that tend to reduce longevity. Chlorophyllous cells appear to retain intact photosynthesizing machinery, which possibly increases their risk to oxidative damage either by residual high-energy intermediates or by the high content of molecules particularly vulnerable to electron abstraction. Non-chlorophyllous cells with high levels of storage lipids are comprised of components with very different physical–chemical properties that make them prone to structural failure with changes in temperature. A temperature anomaly near −20°C, manifested by faster than expected mortality, may arise when the lipids crystallize, causing aqueous and lipid domains to rip apart. The diversity of solid cellular structures in preserved germplasm may require the implementation of additional treatments to stabilize the dry architecture of cells that are intrinsically unstable. A greater understanding of the variation in structural conformations in space and time will lead to improved strategies that increase germplasm longevity in cells with short lifespans.

Acknowledgements. The authors acknowledge Udi Liu (RBG Kew) for her help sourcing data from the SID for Fig. 4. Royal Botanic Gardens, Kew receives grant-in-aid from Defra, UK. Garfield Weston Foundation under the Global Tree Seed Bank Project funded the trip to the ISSS 2nd seed longevity workshop where the basis of this paper was presented for the first time.

References

Almoguera C, Prieto-Dapena P, Personat J-M, Tejedor-Cano J, Lindahl M, Diaz-Espejo A and Jordano J (2012) Protection of the photosynthetic apparatus from extreme dehydration and oxidative stress in seedlings of transgenic tobacco. *PLoS ONE* **7**, e51443. doi:10.1371/journal.pone.0051443.

Arif MAR, Nagel M, Lohwasser U and Börner A (2017) Genetic architecture of seed longevity in bread wheat (*Triticum aestivum* L. *Journal of Biosciences* **42**, 81–89.

Bailly C (2004) Active oxygen species and antioxidants in seed biology. *Seed Science Research* **14**, 93–107.

Bailly C, El-Maarouf-Bouteau H and Corbineau F (2008) From intracellular signaling networks to cell death: the dual role of reactive oxygen species in seed physiology. *Comptes Rendus Biologies* **331**, 806–814.

Ballesteros D (2011) Conservation of fern spores, pp. 165–172 *in* Kumar A; Fernández H and Revilla-Bahillo A (Eds) *Working with ferns. Issues and applications*, New York, USA, Springer.

Ballesteros D and Pence VC (2017) Survival and death of seeds during liquid nitrogen storage: a case study on seeds with short lifespans. *CryoLetters* **38**, 278–289.

Ballesteros D and Pence VC (2018) Fern conservation: spore, gametophyte, and sporophyte ex situ storage, in vitro culture, and cryopreservation, pp. 227–249 *in* Fernández H (Ed.) *Current advances in fern research*, Cham, Switzerland, Springer.

Ballesteros D and Walters C (2007) Calorimetric properties of water and triacylglycerols in fern spores relating to storage at cryogenic temperatures. *Cryobiology* **55**, 1–9.

Ballesteros D and Walters C (2011) Detailed characterization of mechanical properties and molecular mobility within dry seed glasses: relevance to the physiology of dry biological systems. *The Plant Journal* **68**, 607–619.

https://doi.org/10.1017/S0960258520000239 Published online by Cambridge University Press

Ballesteros D and Walters C (2019) Solid-state biology and seed longevity: a mechanical analysis of glasses in pea and soybean embryonic axes. *Frontiers in Plant Science* **10**, 920.

Ballesteros D, Naidoo S, Varghese B, Berjak P and Pammenter N (2014) Towards understanding the protection afforded by cryoprotectants commonly used in the cryopreservation of zygotic explants of recalcitrant seeds. *Cryobiology* **69**, 518.

Ballesteros D, Hill LM and Walters C (2017) Variation of desiccation tolerance and longevity in fern spores. *Journal of Plant Physiology* **211**, 53–62.

Ballesteros D, Narayan S, Varghese B and Sershen (2018) Photo-oxidation modulates green fern spore longevity during dry storage. *Plant Cell Tissue and Organ Culture* **133**, 165–175.

Ballesteros D, Hill LM, Lynch RT, Pritchard HW and Walters C (2019) Longevity of preserved germplasm: the temperature dependency of aging reactions in glassy matrices of dried fern spores. *Plant and Cell Physiology* **60**, 376–392.

Bewley JD and Black M (1994) *Seeds: physiology of development and germination* (2nd edn). New York, Plenum.

Boothby TC, Piszkiewicz S, Mehta A, Brozena A, Tapia H, Koshland D, Holehouse A, Pappu R, Goldstein B and Pielak G (2018) Gelation and vitrification of tardigrade IDPs. *Biophysical Journal* **114**, 560A–561A.

Bremer A, Wolff M, Thalhammer A and Hincha DK (2017) Folding of intrinsically disordered plant LEA proteins is driven by glycerol-induced crowding and the presence of membranes. *FEBS Journal* **284**, 919–936.

Buitink J and Leprince O (2008) Intracellular glasses and seed survival in the dry state. *Comptes Rendus Biologies* **331**, 788–795.

Buitink J, Walters C, Hoekstra FA and Crane J (1998) Storage behavior of *Typha latifolia* pollen at low water contents: interpretation on the basis of water activity and glass concepts. *Physiologia Plantarum* **103**, 145–153.

Buitink J, Hemminga MA and Hoekstra FA (2000) Is there a role for oligosaccharides in seed longevity? An assessment of intracellular glass stability. *Plant Physiology* **122**, 1217–1224.

Carranco R, Espinosa JM, Prieto-Dapena P, Almoguera C and Jordano J (2010) Repression by an auxin/indole acetic acid protein connects auxin signaling with heat shock factor mediated seed longevity. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 21908–21913.

Chen H-Y, Yu X-M, Pritchard HW and Li W-Q (2017) Phospholipase Dα1-mediated phosphatidic acid production is a key determinant of desiccation-induced viability loss in seeds. *Plant, Cell & Environment* **41**, 50–63.

Cheng M, Mcphee KE and Baik B (2004) Bleaching of green peas and changes in enzyme activities of seeds under simulated climatic conditions. *Journal of Food Science* **69**, 511–518.

Choi YH, van Spronsen J, Dai Y, Verberne M, Hollmann F, Arends IW, Witkamp G-J and Verpoorte R (2011) Are natural deep eutectic solvents the missing link in understanding cellular metabolism and physiology? *Plant Physiology* **156**, 1701–1705.

Cicerone MT, Tellington A, Trost L and Sokolov A (2003) Substantially improved stability of biological agents in dried form: the role of glassy dynamics in preservation of biopharmaceuticals. *BioProcess International* **1**, 36–47.

Colville L and Pritchard HW (2019) Seed life span and food security. *New Phytologist* **224**, 557–562.

Colville L, Bradley EL, Lloyd AS, Pritchard HW, Castle L and Kranner I (2012) Volatile fingerprints of seeds of four species indicate the involvement of alcoholic fermentation, lipid peroxidation, and Maillard reactions in seed deterioration during ageing and desiccation stress. *Journal of Experimental Botany* **63**, 6519–6530.

Contreras S, Bennett MA, Tay D and Metzger JD (2008) Maternal light environment during seed development affects lettuce seed weight, germinability, and storability. *Horticultural Science* **43**, 845–852.

Contreras S, Bennet MA, Metzger JD, Tay D and Nerson H (2009) Red to far-red ratio during seed development affects lettuce seed germinability and longevity. *Horticultural Science* **44**, 130–134.

Cornette R and Kikawada T (2011) The induction of anhydrobiosis in the sleeping chironomid: current status of our knowledge. *IUBMB Life* **63**, 419–429.

Cowie JMG and Arrighi V (2014) Physical aging of polymer blends, pp. 1357–1394 *in* Utracki L and Wilkie C (Eds) *Polymer blends handbook*. Dordrecht, Springer.

Crane J, Miller AL, Van Roekel JW and Walters C (2003) Triacylglycerols determine the unusual storage physiology of *Cuphea* seed. *Planta* **217**, 699–708.

Crane J, Kovach D, Gardner C and Walters C (2006) Triacylglycerol phase and 'intermediate' seed storage physiology: a study of *Cuphea carthagenensis*. *Planta* **223**, 1081–1089.

Davies MJ (2005) The oxidative environment and protein damage. *Biochimica et Biophysica Acta* **1703**, 93–109.

Davies RM, Dickie JB and Ballesteros D (2018) Evaluation of short-lived seeds' cryopreservation as alternative to conventional seed banking. *Cryobiology* **85**, 140–141.

Dekkers BJ, Costa MCD, Maia J, Bentsink L, Ligterink W and Hilhorst HW (2015) Acquisition and loss of desiccation tolerance in seeds: from experimental model to biological relevance. *Planta* **241**, 563–577.

Dickie JB, Ellis RH, Kraak HL, Ryder K and Tompsett PB (1990) Temperature and seed storage longevity. *Annals of botany* **65**, 197–204.

Dussert S, Chabrillange N, Rocquelin G, Engelmann F, Lopez M and Hammon S (2001) Tolerance of coffee (*Coffea* spp.) seeds to ultra-low temperature exposure in relation to calorimetric properties of tissue water, lipid composition, and cooling procedure. *Physiologia Plantarum* **112**, 495–504.

Eira MTS, Amaral da Silva AE, De Castro RD, Dussert S, Walters C, Bewley JD and Hilhorst HWM (2006) Coffee seed physiology. *Brazilian Journal of Plant Physiology* **18**, 149–63.

Ellis RH and Roberts EH (1980) Improved equations for the prediction of seed longevity. *Annals of Botany* **45**, 13–30.

Ellis RH, Hong TD and Roberts EH (1990) An intermediate category of seed storage behaviour? I. Coffee. *Journal of Experimental Botany* **41**, 1167–1174.

Ellis RH, Hong TD and Roberts EH (1991) Effect of storage temperature and moisture on the germination of papaya seeds. *Seed Science Research* **1**, 69–72.

FAO (2014) *Genebank standards for plant genetic resources for food and agriculture*. Rome, Food and Agriculture Organization of the United Nations.

Farrant JM, Pammenter NW, Berjak P and Walters C (1997) Subcellular organization and metabolic activity during the development of seeds that attain different levels of desiccation tolerance. *Seed Science Research* **7**, 135–144.

Fernández-Marín B, Kranner I, Sebastián MS, Artetxe U, Laza JM, Vilas JL, Pritchard HW, Nadajaran J, Minguez F, Becerril JM and García-Plazaola JI (2013) Evidence for the absence of enzymatic reactions in the glassy state. A case study of xanthophyll cycle pigments in the desiccation-tolerant moss *Syntrichia ruralis*. *Journal of Experimental Botany* **64**, 3033–3043.

Fleming MB, Richards CM and Walters C (2017) Decline in RNA integrity of dry-stored soybean seeds correlates with loss of germination potential. *Journal of Experimental Botany* **44**, 34–49.

Fleming MB, Patterson EL, Reeves PA, Richards CM, Gaines TA and Walters C (2018) Exploring the fate of mRNA in aging seeds: protection, destruction, or slow decay? *Journal of Experimental Botany* **69**, 4309–4321.

Fleming MB, Hill LM and Walters C (2019) The kinetics of ageing in dry-stored seeds: a comparison of viability loss and RNA degradation in unique legacy seed collections. *Annals of Botany* **123**, 1133–1146.

Franchi GG, Piotto B, Nepi M, Baskin CC, Baskin JM and Pacini E (2011) Pollen and seed desiccation tolerance in relation to degree of developmental arrest, dispersal, and survival. *Journal of Experimental Botany* **62**, 5267–5281.

Fryars S, Limanton E, Gauffre F, Paquin L, Lagrost C and Hapiot P (2018) Diffusion of redox active molecules in deep eutectic solvents. *Journal of Electroanalytical Chemistry* **819**, 214–219.

Gonzalez-Benito ME, Perez-Garcia F, Tejeda G and Gomez-Campo C (2011) Effect of the gaseous environment and water content on seed viability of four Brassicaceae species after 36 years storage. *Seed Science and Technology* **39**, 443–451.

Graham IA (2008) Seed storage oil mobilization. *Annual Review of Plant Biology* **59**, 115–42.

Groot SPC, de Groot L, Kodde J and van Treuren R (2015) Prolonging the longevity of ex situ conserved seeds by storage under anoxia. *Plant Genetic Resources* **13**, 18–26.

Halliwell B and Chirico S (1993) Lipid peroxidation: its mechanism, measurement, and significance. *American Journal of Clinical Nutrition* **57**, 715–724.

Halliwell B and Gutteridge JMC (1999) *Free radicals in biology and medicine* (3rd edn). Oxford, UK, Oxford University Press.

Harrington JF (1972) Seed storage longevity, pp. 145–245 *in* Kozlowski TT (Ed.) *Seed biology*, vol. **III**. New York, USA, Academic Press.

Hay FR and Probert RJ (2013) Advances in seed conservation of wild plant species: a review of recent research. *Conservation Physiology* **1**, cot030. https://doi.org/10.1093/conphys/cot030.

Heber U, Lange OL and Shuvalov VA (2006) Conservation and dissipation of light energy as complementary processes: homoiohydric and poikilohydric autotrophs. *Journal of Experimental Botany* **57**, 1211–1223.

Hincha DK and Thalhammer A (2012) LEA proteins: IDPs with versatile functions in cellular dehydration tolerance. *Biochemical Society Transactions* **40**, 1000–1003.

Hoekstra FA (2002) Pollen and spores: desiccation tolerance in pollen and spores of lower plants and fungi, pp. 185–205 *in* Black M and Pritchard HW (Eds) *Desiccation and survival in plants: drying without dying*. Wallingford, UK, CABI Publishing.

Hoekstra FA (2005) Differential longevities in desiccated anhydrobiotic plant systems. *Integrative and Comparative Biology* **45**, 725–733.

Hor YL, Kim YJ, Ugap A, Chabrillange N, Sinniah UR, Engelmann F and Dussert S (2005) Optimal hydration status for cryopreservation of intermediate oily seeds: Citrus as a case study. *Annals of Botany* **95**, 1153–1161.

Horbowicz M and Obendorf RL (1994) Seed desiccation tolerance and storability: dependence on flatulence-producing oligosaccharides and cyclitols—review and survey. *Seed Science Research* **4**, 385–405.

Huang AHC (1996) Oleosins and oil bodies in seeds and other organs. *Plant Physiology* **110**, 1055–1061.

Kalemba EM and Pukacka S (2014) Carbonylated proteins accumulated as vitality decreases during long-term storage of beech (*Fagus sylvatica* L.) seeds. *Trees* **28**, 503–515.

Khan MM, Hendry GA, Atherton NM and Vertucci-Walters CW (1996) Free radical accumulation and lipid peroxidation in testas of rapidly aged soybean seeds: a light-promoted process. *Seed Science Research* **6**, 101–107.

Koster K, Lei YP, Anderson M, Martin S and Bryant G (2000) Effects of vitrified and nonvitrified sugars on phosphatidylcholine fluid-to-gel phase transitions. *Biophysical Journal* **78**, 1932–1946.

Kranner I, Beckett RP, Wornik S, Zorn M and Pfeifhofer HW (2002) Revival of a resurrection plant correlates with its antioxidant status. *The Plant Journal* **31**, 13–24.

Kranner I, Birtić S, Anderson KM and Pritchard HW (2006) Glutathione half-cell reduction potential: a universal stress marker and modulator of programmed cell death? *Free Radical Biology and Medicine* **40**, 2155–2165.

Kranner I, Beckett R, Hochman A and Nash TH III (2008) Desiccation tolerance in lichens: a review. *The Bryologist* **111**, 576–593.

Kranner I, Minibayeva FV, Beckett RP and Seal CE (2010) What is stress? Concepts, definitions and applications in seed science. *New Phytologist* **188**, 655–673.

Kranner I, Chen H, Pritchard H, Pearce S and Birtić S (2011) Internucleosomal DNA fragmentation and loss of RNA integrity during seed ageing. *Plant Growth Regulation* **63**, 63–72.

Kucera SA, Felton LA and McGinity JW (2013) Physical aging in pharmaceutical polymers and the effect on solid oral dosage form stability. *International Journal of Pharmaceutics* **457**, 428–36.

Lebkuecher JG (1997) Desiccation-time limits of photosynthetic recovery in *Equisetum hyemale* (Equisetaceae) Spores. *American Journal of Botany* **84**, 792–797.

Leprince O and Buitink J (2010) Desiccation tolerance: from genomics to the field. *Plant Science* **179**, 554–564.

Leprince O, van Aelst AC, Pritchard HW and Murphy DJ (1998) Oleosins prevent oil-body coalescence during seed imbibition as suggested by a low-temperature scanning electron microscope study of desiccation-tolerant and-sensitive oilseeds. *Planta* **204**, 109–119.

Leprince O, Pellizzaro A, Berriri S and Buitink J (2017) Late seed maturation: drying without dying. *Journal of Experimental Botany* **68**, 827–841.

Li DZ and Pritchard HW (2009) The science and economics of ex situ plant conservation. *Trends in Plant Science* **14**, 614–621.

Lloyd RM and Klekowski EJ Jr (1970) Spore germination and viability in Pteridophyta: evolutionary significance of chlorophyllous spores. *Biotropica* **2**, 129–137.

López-Pozo M, Fernández-Marín B, García-Plazaola JI and Ballesteros D (2018) Desiccation tolerance in ferns: from the unicellular spore to the multi-tissular sporophyte, pp. 401–426 *in* Fernández H (Ed.) *Current advances in fern research*. New York, Springer.

López-Pozo M, Ballesteros D, Laza JM, García-Plazaola JI and Fernández-Marín B (2019) Desiccation tolerance in chlorophyllous fern spores: are ecophysiological features related to environmental conditions? *Frontiers in Plant Science* **10**, 1130.

Manning MC, Chou DK, Murphy BM, Payne RW and Katayama DS (2010) Stability of protein pharmaceuticals: an update. *Pharmaceutical Research* **27**, 544–575.

Maroder HL, Prego IA, Facciuto GR and Maldonado SB (2000) Storage behaviour of *Salix alba* and *Salix matsudana* seeds. *Annals of Botany* **86**, 1017–1021.

McDonald MB (1999) Seed deterioration: physiology, repair and assessment. *Seed Science and Technology* **27**, 177–237.

Metin S and Hartel RW (2005) Crystallization of fats and oils, pp. 45–76 *in* Shahidi F (Ed.) *Bailey's industrial oil and fat products* (6th edn). Hoboken, Nueva Jersey, John Wiley & Sons, Inc.

Miller BL, Hageman MJ, Thamann TJ, Barron LB and Schöneich C (2003) Solid state photodegradation of bovine somatotropin (bovine growth hormone): evidence for tryptophan-mediated photooxidation of disulfide bonds. *Journal of Pharmaceutical Sciences* **92**, 1698–709.

Minemoto Y, Adachi S and Matsuno R (2001) Effect of relative humidity during storage on the autoxidation of linoleic acid encapsulated with a polysaccharide by hot-air-drying and freeze-drying. *Food Science and Technology Research* **7**, 91–93.

Mira S, González-Benito ME, Hill LM and Walters C (2010) Characterization of volatile production during storage of lettuce (*Lactuca sativa*) seed. *Journal of Experimental Botany* **61**, 3915–3924.

Mira S, Hill LM, González-Benito ME, Ibáñez MA and Walters C (2016) Volatile emission in dry seeds as a way to probe chemical reactions during initial asymptomatic deterioration. *Journal of Experimental Botany* **67**, 1783–1793.

Mira S, Nadarajan J, Liu U, González-Benito ME and Pritchard HW (2019) Lipid thermal fingerprints of long-term stored seeds of Brassicaceae. *Plants* **8**, 414.

Nagel M and Börner A (2010) The longevity of crop seeds stored under ambient conditions. *Seed Science Research* **20**, 1–12.

Nagel M, Kranner I, Neumann K, Rolletschek H, Seal CE, Colville L, Fernández-Marín B and Börner A (2015) Genome-wide association mapping and biochemical markers reveal that seed ageing and longevity are intricately affected by genetic background and developmental and environmental conditions in barley. *Plant, Cell & Environment* **38**, 1011–1022.

Nagel M, Seal E, Colville L, Rodenstein A, Un S, Richter J, Pritchard HW, Börner A and Kranner I (2019) Wheat seed ageing viewed through the cellular redox environment and changes in pH. *Free Radical Research* **53**, 641–654.

Nebot A, Pritchard HW and Ballesteros D (2018) Desiccation tolerance and the hydration window for the cryopreservation of woody species' pollen. *Cryobiology* **85**, 139.

Pence VC, Ballesteros D, Walters C, Reed B, Philpott M, Dixon K, Pritchard H, Culley T and Vanhove A-C (2020) Cryobiotechnologies: tools for expanding long-term ex situ conservation to all plant species. *Biological Conservation*. In press.

Pereira Lima JJ, Buitink J, Lalanne D, Rossi RF, Pelletier S, da Silva EAA and Leprince O (2017) Molecular characterization of the acquisition of longevity during seed maturation in soybean. *PLoS ONE* **12**, e0180282.

Pérez HE, Hill LM and Walters C (2012) An analysis of embryo development in palm: interactions between dry matter accumulation and water relations in *Pritchardia remota* (Arecaceae). *Seed Science Research* **22**, 97–111.

Pittia P and Sacchetti G (2008) Antiplasticization effect of water in amorphous foods. A review. *Food Chemistry* **106**, 1417–1427.

Popova EV, Han SH, Moltchanova E, Pritchard HW and Hong YP (2013) Systematic overestimation of Salicaceae seed survival using radicle emergence in response to drying and storage: implications for ex situ seed banking. *Acta Physiologiae Plantarum* **35**, 3015–3025.

Povey JF, Perez-Moral N, Noel TR, Parker R, Howard MJ and Smales CM (2009) Investigating variables and mechanisms that influence protein integrity in low water content amorphous carbohydrate matrices. *Biotechnology Progress* **25**, 1217–1227.

Priestley DA (1986) *Seed aging: implications of seed storage and persistence in the soil*. Ithaca and London, Comstock Publishing.

Pritchard HW and Seaton PT (1993) Orchid seed storage: historical perspective, current status, and future prospects for long-term conservation. *Selbyana* **14**, 89–104.

Probert RJ, Daws MI and Hay FR (2009) Ecological correlates of ex situ seed longevity: a comparative study on 195 species. *Annals of Botany* **104**, 57–69.

Rajjou L, Lovigny Y, Groot SPC, Belghazi M, Job C and Job D (2008) Proteome-wide characterization of seed aging in Arabidopsis: a comparison between artificial and natural aging protocols. *Plant Physiology* **148**, 620–641.

Roqueiro G, Facorro GB, Huarte MG, de Celis ER, Garcia F, Maldonado S and Maroder H (2010) Effects of photooxidation on membrane integrity in *Salix nigra* seeds. *Annals of Botany* **105**, 1027–1034.

Roudaut G, Simatos D, Champion D, Contreras-Lopez E and Le Meste M (2004) Molecular mobility around the glass transition temperature: a mini review. *Innovative Food Science & Emerging Technologies* **5**, 127–134.

Royal Botanic Gardens, Kew. (2018) *Seed Information Database (SID). Version 7.1*. Available from: http://data.kew.org/sid/ (accessed November 2018).

Sallon S, Cherif E, Chabrillange N, Solowey E, Gros-Balthazard M, Ivorra S, Terral J-F, Egli M and Aberlenc F (2020) Origins and insights into the historic Judean date palm based on genetic analysis of germinated ancient seeds and morphometric studies. *Science Advances* **6**, eaax0384.

Sano N, Rajjou L, North MH, Debeaujon I, Marion-Poll A and Seo M (2016) Staying alive: molecular aspects of seed longevity. *Plant and Cell Physiology* **57**, 660–674.

Schafer FQ and Buettner GR (2001) Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radical Biology and Medicine* **30**, 1191–1212.

Shen-Miller J, Mudgett MB, Schopf JW, Clarke S and Berger R (1995) Exceptional seed longevity and robust growth: ancient sacred lotus from China. *American Journal of Botany* **82**, 1367–1380.

Shimada TL, Shimada T, Takahashi H, Fukao Y and Hara-Nishimura I (2008) A novel role for oleosins in freezing tolerance of oilseeds in *Arabidopsis thaliana*. *The Plant Journal* **55**, 798–809.

Shimada TL, Hayashi M and Hara-Nishimura I (2018) Membrane dynamics and multiple functions of oil bodies in seeds and leaves. *Plant Physiology* **176**, 199–207.

Sundue MA, Vasco A and Moran RC (2011) Cryptochlorophyllous spores in ferns: nongreen spores that contain chlorophyll. *International Journal of Plant Sciences* **172**, 1110–1119.

Thalhammer A, Hundertmark M, Popova AV, Seckler R and Hincha DK (2010) Interaction of two intrinsically disordered plant stress proteins (COR15A and COR15B) with lipid membranes in the dry state. *Biochimica et Biophysica Acta* **1798**, 1812–1820.

Thalhammer A, Bryant G, Sulpice R and Hincha DK (2014) Disordered cold regulated proteins protect chloroplast membranes during freezing through binding and folding, but do not stabilize chloroplast enzymes in vivo. *Plant Physiology* **166**, 190–201.

Tzen JTC, Cao YZ, Laurent P, Ratnayake C and Huang AHC (1993) Lipids, proteins, and structure of seed oil bodies from diverse species. *Plant Physiology* **101**, 267–276.

Velasco J, Dobarganes C and Márquez-Rui G (2003) Variables affecting lipid oxidation in dried microencapsulated oils. *Grasas y Aceites* **54**, 304–314.

Verhoeven A, García-Plazaola JI and Fernández-Marín B (2018) Shared mechanisms of photoprotection in photosynthetic organisms tolerant to desiccation or to low temperature. *Environmental and Experimental Botany* **154**, 66–79.

Vertucci CW (1989) Effects of cooling rate on seeds exposed to liquid nitrogen temperatures. *Plant Physiology* **90**, 1478–1485.

Vertucci CW (1992) A calorimetric study of the changes in lipids during seed storage under dry conditions. *Plant Physiology* **99**, 310–316.

Vertucci CW and Farrant JM (1995) Acquisition and loss of desiccation tolerance, pp. 237–271 *in* Kigel J and Galili G (Eds) *Seed development and germination*. New York, NY, Marcel Dekker.

Vertucci CW, Ellenson JL and Leopold AC (1985) Chlorophyll fluorescence characteristics associated with hydration level in pea cotyledons. *Plant Physiology* **79**, 248–252.

Vertucci CW, Roos EE and Crane J (1994) Theoretical basis of protocols for seed storage III. Optimum moisture contents for pea seeds stored at different temperatures. *Annals of Botany* **74**, 531–540.

Walters C (1998) Understanding the mechanisms and kinetics of seed aging. *Seed Science Research* **8**, 223–244.

Walters C (2004) Temperature-dependency of molecular mobility in preserved seeds. *Biophysical Journal* **86**, 1253–1258.

Walters C (2015) Orthodoxy, recalcitrance and in-between: describing variation in seed storage characteristics using threshold responses to water loss. *Planta* **242**, 397–406.

Walters C and Koster KL (2007) Structural dynamics and desiccation damage in plant reproductive organs, pp. 251–280 *in* Jenks MA and Wood AJ (Eds) *Plant desiccation tolerance*. Iowa, USA, Blackwell Publishing.

Walters C, Wheeler L and Stanwood PC (2004) Longevity of cryogenically stored seeds. *Cryobiology* **48**, 229–244.

Walters C, Hill LM and Wheeler LJ (2005a) Dying while dry: kinetics and mechanisms of deterioration in desiccated organisms. *Integrative and Comparative Biology* **45**, 751–758.

Walters C, Landre P, Hill L, Corbineau F and Bailly C (2005b) Organization of lipid reserves in cotyledons of primed and aged sunflower seeds. *Planta* **222**, 397–407.

Walters C, Wheeler LM and Grotenhuis JM (2005c) Longevity of seeds stored in a genebank: species characteristics. *Seed Science Research* **15**, 1–20.

Walters C, Ballesteros D and Vertucci VA (2010) Structural mechanics of seed deterioration: standing the test of time. *Plant Science* **179**, 565–573.

Wang Y, Li Y, Xue H, Pritchard HW and Wang XF (2015) Reactive oxygen species (ROS)-provoked mitochondria-dependent cell death during ageing of elm (*Ulmus pumila* L.) seeds. *The Plant Journal* **81**, 438–452.

Webb MA and Arnott HJ (1982) Cell wall conformation in dry seeds in relation to the preservation of structural integrity during desiccation. *American Journal of Botany* **69**, 1657–1668.

Wolkers WF, McCready S, Brandt WF, Lindsey GG and Hoekstra FA (2001) Isolation and characterization of a D-7 LEA protein from pollen that stabilizes glasses in vitro. *Biochimica et Biophysica Acta* **1544**, 196–206.

Wright IJ, Clifford HT, Kidson R, Reed ML, Rice BL and Westoby M (2000) A survey of seed and seedling characters in 1744 Australian dicotyledon species: cross-species trait correlations and correlated trait-shifts within evolutionary lineages. *Biological Journal of the Linnean Society* **69**, 521–547.

Wyse SV and Dickie JB (2017) Predicting the global incidence of seed desiccation sensitivity. *Journal of Ecology* **105**, 1082–1093. doi:10.1111/1365-2745.12725.

Wyse SV, Dickie JB and Willis KJ (2018) Seed banking not an option for many threatened plants. *Nature Plants* **4**, 848–850.