Seed Longevity and Deterioration

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Abstract

The fundamental deteriorative processes that lead to loss of seed viability contrastingly vary between desiccation insensitive (orthodox) and desiccation sensitive seeds (recalcitrant). Orthodox seeds which undergo maturation drying are bestowed with protective mechanisms which guard the seeds against deterioration. They include the accumulation of antioxidants, non-reducing sugars, protective proteins such as late embryogenesis abundant proteins, heat-shock proteins, lipocalins, hormones and chemical protectants (raffinose family oligosaccharides, flavonoids, lignins, vitamin E). The nuclear DNA is packed denser and chlorophyll is degraded. Besides, the cytoplasm is capable of transitioning between liquid and glassy state depending on the moisture content of the seeds aiding in the maintenance of seed viability potential. In the dry seeds, the glassy state of the cytoplasm ensures the stabilization of cellular components by arresting cell metabolism. However, even with low moisture content and a glassy state of cytoplasm, reactive oxygen species generated due to the presence of oxygen in the storage atmosphere may cause the ageing of seed. As the seed moisture content increases, mitochondrial respiration gets activated, also leading to increased production of reactive oxygen species, owing to inefficient mitochondrial activity. The reactive oxygen species lead to the oxidation of essential molecules such as DNA, RNA, proteins and lipids. Further, mitochondrial membranes also get oxidized, leading to reduced aerobic respiration potential. When the damage is not substantial, orthodox seeds are capable of repairing the molecular damages that accumulate during storage, enabling the seeds to partially

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overcome the damages and extend their longevity. This includes activation of repair of cell membranes, DNA, RNA, proteins and mitochondria as the seeds imbibe water.

Unlike the orthodox seeds, the recalcitrant seeds are largely devoid of protective mechanisms which guard the seeds against rapid deterioration. The recalcitrant seeds are shed from the mother tree at high moisture content while they are metabolically active. After dispersal, the seeds undergo deteriorative changes during drying due to the damage to the cytoskeleton (physical damage), besides reactive oxygen species-induced damage due to lack of antioxidant activity (metabolism-induced damage). Even when maintained under high moisture content, seeds exhibit dysfunction of the cell organelles and extensive vacuolization predisposing the seeds to deterioration. Thus, recalcitrant seeds are prone to deterioration either under low or high moisture content.

Keywords

Orthodox longevity behaviour · Reactive oxygen species · Seed deterioration · Seed ageing · LEA proteins · Glassy state · Recalcitrant seed

1 Introduction

Seed, the basic unit of propagation, serves a crucial role in the evolution and survival of higher plants. Acquisition of desiccation tolerance during the late maturation stage bestows upon orthodox seeds a unique property of remaining viable for a prolonged period in a dry quiescent state. However, as all living organisms, seeds also undergo ageing, gradually losing vigour and viability, until they eventually die. Seed longevity refers to the period from maturation to the loss of seed viability in dry storage. Seed storage potential is highly related to the environmental conditions of storage, the physiological status of seeds as well as genetic factors. Certain plant species have shown a remarkably long longevity period for their seeds, e.g. lotus (*Nelumbo nucifera*) seeds have remained viable for nearly 1300 years (Shen-Miller et al. 2002) and a *Phoenix dactylifera* seed could germinate even after 2000 years (Sallon et al. 2008). The variation in the shelf life of seeds may be due to differences in the biochemical components of these seeds, which may be attributed to their genetic constitution. Seeds of certain species have a very short storage life. For instance, seeds of trees belonging to *Dipterocarpaceae* as those from *Shorea robusta* remain viable only for 7–10 days (Saha et al. 1992).

A characteristic and contrasting seed storage behaviour among plant species has led to the classification of seeds into two prominent types viz., recalcitrant and orthodox seeds (Bewley et al. 2013). The recalcitrant seeds are 'desiccation-sensitive'. On maturation these seeds do not undergo drying or undergo drying only to a limited extent, therefore they are shed from the trees with relatively high moisture content of 0.4–4.0 g water/g and often high metabolic activity. For that reason, the post-harvest life of the recalcitrant seed is very short, extending from just few days to

few months, depending on the species and storage conditions. Some recalcitrant seeds from temperate regions, as those of *Quercus suber* (cork oak) can be stored for about a year at temperatures around 0 °C, due to slowed down metabolic activity, but eventually die due to depletion of reserve carbohydrates (SPC Groot, personal observations). Recalcitrant seeds from tropical species do not withstand such cold storage and consequently have a very short shelf life. On the other hand, the orthodox seeds, which are desiccation-insensitive undergo maturation drying to reach a moisture content of <15%, before being shed from the mother plant. They can generally be stored at ambient conditions for a very long period ranging from months to many years. A third category with intermediate seed storage behaviour has been identified for seeds that can withstand desiccation but not storge at sub-zero temperatures (Ellis et al. 1990).

The loss of seed viability during storage occurs due to the onset of deterioration processes. Seed ageing or seed deterioration is commonly described as an irreversible, cumulative and inexorable process (McDonald 1999) that can cause the build up of cellular damages, and result in delayed seedling emergence, reduced ability to withstand stresses, and ultimately loss of viability (Zhang et al. 2021). Limited damage can often be repaired, but as this will take energy and time, it will result in a slowing down of average germination rate, accounting for protrusion of the radicle. Substantial deterioration may still allow this embryonic root protrusion but may result in seedling abnormalities and in case of extreme deterioration of seeds, no radicle protrusion and seedling formation occurs.

The process of seed deterioration distinctly varies between the orthodox and recalcitrant seeds. Wide variations of longevity are observed among species, genotypes and populations (Clerkx et al. 2004; Walters et al. 2005; Probert et al. 2009), which to a large extent are attributed to the differences in their biochemical or biophysical characteristics (Walters et al. 2010). Irrespective of the inherent storage potential of the seeds, all the orthodox seeds show extended viability under cool and dry storage, while at elevated temperatures and relative humidity, the seed storability is greatly reduced (Ellis and Hong 2006). Seed longevity duration is governed mainly by two factors, these are the genetic constitution—which is manifested in the chemical constitution and structural features of the seed parts, and the environment in which the seed is stored. Among the environmental factors, relative humidity (RH) of the storage air which directly influences the seed moisture content and the temperature are the two primary factors influencing seed longevity besides the gaseous composition of the storage environment (Roberts 1972). Hence, seed longevity depends on environmental and seed factors.

2 Seed Factors

The storage potential of the orthodox seeds has been attributed to cellular protective mechanism. At the end of seed development, orthodox seeds undergo the maturation processes, with corresponding decline in moisture level and suspension of their metabolic activities. The dry state is called as quiescent state which aids in the

seed storage potential. The protective mechanisms imposed during late seed maturation include the accumulation of antioxidants, non-reducing sugars, and protective proteins such as late embryogenesis abundant (LEA) proteins, heat shock proteins (HSPs) and lipocalins. The nuclear DNA is packed denser and chlorophyll is degraded. Seed storage proteins can act as primary targets of oxidation, thereby helping in buffering of reactive oxygen species (ROS) generated during dry storage. Since chlorophyll levels can be measured very sensitively using its fluorescent properties, chlorophyll fluorescence levels of individual seeds can be used as a marker for seed maturity for those seeds that contain chlorophyll during development, as most do. The lower the level of fluorescence, higher the level of seed maturity.

2.1 Role of Chemical Protectants in Various Tissues

The main damaging factor during seed storage is oxidative stress induced by ROS. Molecular and enzymatic antioxidants are therefore essential in seed longevity. Under dry conditions, enzymes cannot access the ROS due to restricted molecular mobility within the cytoplasm (Gerna et al. 2022). Under these dry conditions, seeds rely on low molecular weight antioxidants as tocochromanols (tocopherols and tocotrienols), ascorbate (vitamin C) and glutathione. Seeds are often rich in tocopherols, including vitamin E (alfa-tocopherol), which are lipophilic antioxidants, that play an important role in preventing oxidation of storage and membrane lipids. Vitamin E-deficient *Arabidopsis* mutants have a considerably reduced seed longevity (Sattler et al. 2004). Ascorbate and glutathione are the main water soluble antioxidants present in seeds. At the low water content of dry seeds, enzymatic regeneration of the antioxidants is not possible and eventually the antioxidant pool will get exhausted (Groot et al. 2012; Gerna et al. 2022). Exposing seeds to higher RH environment, or by imbibition, will allow enzymatic ROS scavenging activity by glutathione-reductase, superoxide dismutase, peroxidases and catalases. These enzymes also play a role in regeneration of molecular antioxidants. A study with barley seeds showed that tocopherol and glutathione levels decrease during seed ageing, both under dry genebank storage and during controlled deterioration at 45 °C and 75% RH (Roach et al. 2018).

During seed maturation, the cells accumulate sucrose and raffinose family oligosaccharides (RFOs), which are raffinose, stachyose and verbascose. These sugars have been suggested to play a role in the formation of the glassy state and thereby improving seed longevity (Bernal-Lugo and Leopold 1992; Vandecasteele et al. 2011). Galactinol is the direct precursor of raffinose and a positive genetic correlation between seed galactinol content and longevity has been shown for Arabidopsis, tomato and cabbage, while its role was confirmed by shorter longevity shown by seeds from Arabidopsis galactinol synthase mutants (de Souza Vidigal et al. 2016). In that study Arabidopsis and tomato seed longevity was tested at 85% RH and 40 °C, while the cabbage seeds had been stored in paper bags at 20 °C

without RH control. Galactinol has also been shown to enable protection against oxidative stress in Arabidopsis leaves (Nishizawa et al. 2008).

Late embryogenesis abundant proteins (LEAs) and heat shock proteins (HSPs) are synthesized at the end of seed maturation. They play a role in seed longevity, by stabilization of the glassy cytoplasm, protecting structural proteins, condensation of chromatin and dismantling of thylakoids in chloroplasts (Sano et al. 2015; Ballesteros et al. 2020).

The seed coat is a maternal tissue which surrounds the embryo and nutritive tissues, forming both a physical and biochemical layer of protection. The seed coat cells become dead at the end of the seed development. Metabolites accumulated during seed development determine the composition and structure of the seed coat and influence the chemical and mechanical protection to the seed and the longevity potential. The polyphenols found in the seed coat are flavonoids, lignins and lignans. During initial seed development polymeric colourless compounds accumulate in the vacuoles of the innermost layer or endothelium cells. Later during desiccation, they are oxidized into a brown pigment by polyphenol oxidase to form flavonoids called flavonols. The flavonoids act as antioxidants and scavenge the ROS eventually reducing the oxidative stress. In rapeseed (*Brassica napus*) the dark-pigmented seeds survive longer under accelerated ageing conditions (Zhang et al. 2006). Peroxidation of flavonoids accumulated in seed coats may cause browning and reduced water permeability of the seed coat. The proanthocyanidins (PAs) (also known as condensed tannins) present in the seed coat can also have antimicrobial properties, thereby providing a chemical barrier against infections by fungi. In cowpea (*Vigna unguiculata*), PAs were also found to be poisonous to bruchid larvae and prevented their infestation (Lattanzio et al. 2005).

Lignin is a polymer of monolignol units which are rich in flax seeds. It is known to protect the seeds against mechanical stress (Capeleti et al. 2005) besides having antioxidant properties. Defence-related proteins which accumulate in testa of Arabidopsis and soybean (*Glycine max*) are polyphenol oxidases, peroxidases and chitinases (Moïse et al. 2005; Pourcel et al. 2005).

2.2 Role of Hormones

Hormones such as abscisic acid, auxin and gibberellic acid are reported to be involved in acquisition of seed longevity. In Arabidopsis, the loss of functional mutants of ABI3 (ABSCISIC ACID INSENSITIVE 3) and LEC1 (LEAFY COTYLEDON 1) produced seeds that lost their viability within few weeks of harvest (Sugliani et al. 2009), implying that these LAFL transcription factors influenced the longevity of seeds. ABI3 is also shown to regulate seed de-greening by induction of *STAYGREEN* (Delmas et al. 2013) and induce acquisition of seed longevity. ABI3 and LEC1 were reported to directly or indirectly regulate proteins such as HSP and LEA besides storage proteins which were linked with seed longevity. The alternative splicing of *ABI3* mRNA is expected to be associated with acquisition of seed

longevity in Arabidopsis and many other species because the relative abundance of transcripts of full-length *ABI3i* decreased as the seeds entered maturation drying.

ABI5 is yet another important regulator of longevity in some plants such as *Medicago truncatula* and pea (Zinsmeister et al. 2016). The seeds of *abi5* mutants of these species showed 40–60% reduction in longevity compared to wild-type, in dry storage. ABI5 regulates LEA proteins and raffinose family oligosaccharide (RFO) synthesis which plays a role in conferring stability to the seeds in dry state. It is also known to regulate antioxidant levels in seeds during storage, especially tocopherol profile (Zinsmeister et al. 2016).

ABI5 acts downstream of ABI3 and regulates AtEM gene expression (Lopez-Molina et al. 2002) suggesting that interactions existed between ABI3 and PYR/PP2C/SnRK2 signalling pathways. ABA regulates aquaporins which are involved in transport of water and other small molecules including H_2O_2. ABA may control water relations and H_2O_2 accumulation via ABI3 modulation of aquaporins, thereby contributing to seed longevity (Sano et al. 2015).

Auxin is also involved in the regulation of seed longevity (Righetti et al. 2015; Carranco et al. 2010). Exogenous application of auxin during maturation had increased the seed longevity period. In the embryo, auxin signalling was found to be associated with ABA signalling pathway. Auxin is proven to induce the expression of *ABI3* and its LEA protein target EARLY METHIONE1 (EM1) since their expression was deregulated in the auxin biosynthesis mutants. Auxin could also regulate the genes involved in longevity directly (Pellizzaro et al. 2019).

Gibberellins offer resistance against seed deterioration by reinforcing the seed coat (Bueso et al. 2014). Increased tolerance of seeds to accelerated ageing was observed when there was an overexpression of ARABIDOPSIS THALIANA HOMEOBOX 25 (ATBH25) leading to increased accumulation of gibberellins and transcripts of the gibberellin biosynthesis gene GIBBERELLIN 3—OXIDASE 2 (Bueso et al. 2014). However, since mutants with defective gibberellin synthesis had also recorded high seed longevity as that of wild-type, the role of gibberellin seemed to be inconclusive (Clerkx et al. 2004).

Brassinosteroids (BR) may also positively influence seed longevity since it has been reported that the BR-deficient mutants *cyp85at/a2* and *det2* recorded significantly longer longevity period than the wild type (Sano et al. 2017).

3 Storage Factors

3.1 Moisture Content, Water Activity or Equilibrium Relative Humidity

The main driver for seed deterioration during storage of orthodox seeds is humidity. Water is important for most chemical and enzymatic reactions. Oxidation of lipids, proteins and nucleic acids, important building blocks of living organisms, is stimulated by moisture, oxygen and temperature. In the oily or lipophilic part, deterioration is due to oxidation of the unsaturated fatty acids in the oil bodies and

membranes. Whereas in the non-oily, or hydrophilic part, deterioration is mainly due to oxidation of proteins, DNA and RNA and cross-linking of macromolecules. An initial rule of the thumb for the quantitative effects of humidity on seed ageing was formulated by Harrington (1972), stating that when seed moisture content is between 5% and 14%, each 1% decrease in seed moisture doubles the shelf life of the seeds.

While studying the effect of moisture on seed ageing, a clear distinction should be made between seed moisture content and water activity or storage relative humidity (RH). Traditionally seeds were characterized by data on their moisture content concerning the seed trade. Also, seed technologists were used to defining the humidity level of the seeds in moisture content, either on a fresh or dry-weight basis. But the seed moisture content does not define the availability of water in the non-oily part of the seeds, and hence the deterioration processes and rates at which these reactions are taking place. Imagine castor bean (*Ricinus communis*) seeds containing 50% oil and 10% moisture content on a wet basis, which will mean a moisture content of 20% in the non-oily part. In comparison, seeds from a common bean (*Phaseolus vulgaris* L.) that contain only 2% oil will experience at the same total seed moisture content only about 10% moisture in their non-oily part. As a consequence, the physiological conditions of both seeds will differ, despite their similar seed moisture content. Seed oil content does not only vary between crops but can also vary between varieties (Yao et al. 2020) and production conditions. In the food industry, it is common to use water activity (a_w) as a measure of the moisture status of commodities, including seeds. When in equilibrium with the humidity of the surrounding air, the a_w is more or less linearly related to the relative humidity RH, be it that a_w is expressed between 0 and 1.0 and the RH in percentages. At the first Seed Longevity Workshop of the International Society of Seed Science (Wernigerode, Germany, 5–8 July 2015) it was concluded that for studies on seed ageing it is better to compare seeds based on their a_w or equilibrium RH (eRH) instead of their moisture content.

According to the Seed Viability Equation, seed survival prolongs with decreasing seed moisture contents, but there is a limit to this. In fact at very low moisture levels, under so-called ultra-dry storage conditions, which equals eRH levels below around 15–20%, seed deterioration could be faster (Chai et al. 1998). This is likely because of a faster rate of lipid oxidation, as has been described in food science (Labuza and Dugan 1971).

3.1.1 Glassy or Liquid Cytoplasm

Non-reducing sugars such as sucrose and raffinose family oligosaccharides (RFOs) are stored in seeds as energy sources during the seed development process. Raffinose and stachyose are efficient inhibitors of sucrose crystallization. During late seed maturation, drying leads to transformation of cell cytoplasm from a fluid state to the viscosity of glass, thereby disrupting the normal crystal matrices (Koster and Leopold 1988). The glassy state has an extremely low molecular mobility that enables arresting of cell metabolism and stabilization of cellular components. This in turn reduces the deteriorative processes and results in the extension of seed longevity (Sun 1997). At 25 °C for at least four species with varying seed oil

content, the transition from a cytoplasmic glass phase to a more viscous cytoplasm is reported to occur at an RH between 44% and 49%, which is lower than the RH levels that leads to loss of viability in recalcitrant seeds (Buitink and Leprince 2008). Glass formation is therefore not the mechanism for desiccation tolerance, but it enables the orthodox seeds to withstand long term survival under dry conditions.

Upon incubation at higher RH levels or imbibition, the seeds absorb moisture and the cytoplasm can first become viscous, also called rubbery and subsequently liquid, allowing enzyme activity and resumption of metabolic activities. With increasing temperatures, this transition occurs even at a lower RH. The rubbery phase is a damaging state as the hydration level increases the rate of deleterious chemical processes, while it is still too low for the active repair of the cellular damage (Bewley et al. 2013). After transition towards a liquid cytoplasm, enzymes can become active. At higher levels of eRH, more enzymes may become active. With storage of peanuts at different RH levels in hermetically closed containers, oxygen levels significantly start to decline from an RH of 70% onwards, but the oxygen consumption considerably speeds up with a further increase of the RH, indicating onset of aerobic respiration activities from eRH of around 70%, which accelerates with the increase in RH (Groot et al. 2022).

The glass phase transition is crucial for the physiological status of the seeds and the response to environmental conditions. A common error made in the studies on seed ageing is that the analyses of enzyme activity are performed on liquid extracts obtained from dry stored seeds. Extracts made from dry stored seeds can indeed have some enzyme activity, but that only shows that these enzymes present in these seeds can be activated in a watery extract. These enzymes may include those involved in the scavenging of reactive oxygen species (ROS), repair of damage or respiration activity providing cellular energy. However, this does not mean that they are active in the dry seed. Only in the liquid cytoplasmic state these enzymes can become active in situ, while their actual activity still depends on the available water. Some enzymes will require full hydration during imbibition to express maximum activity.

3.2 Temperature

Seeds deteriorate faster at higher temperature conditions, as these accelerate the rate of chemical oxidation. For this reason, gene banks are recommended to dry and store their valuable germplasm at sub-zero temperatures (Rao et al. 2006). More expensive seeds of horticultural species are generally stored in temperature and humidity-controlled warehouses, set at 15 °C and 30% RH. As mentioned above, increasing the temperature decreases the a_w at which glass phase transition occurs. A second rule of thumb formulated by Harrington stated that the storage life approximately doubles for every 5 °C decrease in temperature (Harrington 1972). Ellis et al. (1989) suggested that chemical reactions that take place in the stored seeds are dependent mainly on moisture content of the seed as well as the storage temperature. Indeed it has been shown for many desiccation-tolerant seeds that by lowering the storage temperature, seed longevity can be improved. Genes and Nyomora (2018), for

example, reported that the seeds of *Escoecaria bussei* could retain the seed viability for a period of 9 months when stored at 15 °C, while they lost the germination potential within 3 months, if stored at 30 °C. Strelec et al. (2010) also observed that wheat seeds stored at 40 °C had a greater decrease in germination and vigour compared to seeds stored at 25 °C. Seeds with an intermediate seed storage behaviour can withstand desiccation but are sensitive to storage at sub-zero temperatures (Ellis et al. 1990). An example is seeds from oil palm (*Elaeis guineensis* Jacq.) (Ellis et al. 1991).

3.3 Oxygen

Most organisms need oxygen to survive and allow metabolic activity, but seeds in a state of glassy cytoplasm under dry conditions are not metabolically active and they do survive without the need of oxygen (O_2) (González-Benito et al. 2011). Only above a water potential of about −14 MPa providing oxygen becomes essential to sustain respiration (Roberts and Ellis 1989). Under dry storage conditions, molecular oxygen is a main source of ROS. The internal environment of seeds is rich in metal ions such as Fe^{2+}, Cu^{2+} and Zn^{2+}, needed for metabolism in the emerging seedling as cofactors for enzymes, including those involved in respiration and photosynthesis. Interaction of molecular oxygen with these metal ions results in the formation of ROS (Fig. 1) (Hayyan et al. 2016). These ROS can react with organic molecules, resulting in oxidation of lipids, proteins and nucleic acids. Therefore, the reason for seed deterioration under dry conditions is oxidation. The higher the oxygen concentration, the faster the ageing of seeds (Groot et al. 2012). In the food industry it is common practice to limit food oxidation, mainly lipid oxidation that would otherwise result in a rancid taste, by packaging under controlled atmosphere with low oxygen levels or even anoxia. Indeed, low oxygen or anoxia storage under dry conditions can extend seed longevity considerably (González-Benito et al. 2011; Schwember and Bradford 2011; Gerna et al. 2022).

To a lesser degree, ROS can also be generated in dry seeds by autocatalytic lipid oxidation. Under humid storage conditions, when seeds possess liquid cytoplasm,

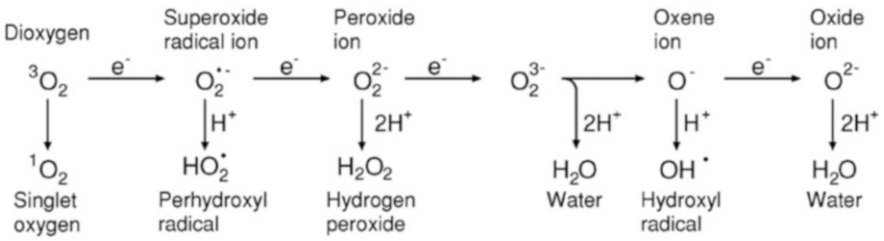

Fig. 1 Generation of ROS by energy transfer or sequential reduction of triplet oxygen. (Original picture: Apel and Hirt 2004)

mitochondrial respiration activity can start, and ROS may also be generated by the inefficient mitochondrial activity.

3.4 Pests and Pathogens

In addition to the direct influence of RH, temperature and oxygen on seed deterioration, these also play an important role in the survival and multiplication of pests and pathogens, which impacts the loss of seed viability. Fungi can severely deteriorate seeds during storage. They can grow in and on the seeds at about 70% RH with an increased rate at higher humidity levels, but they are considerably reduced in their survival at temperatures below zero (Roberts 1972). Although storage under reduced oxygen levels can strongly inhibit fungal growth, fungi like *Aspergillus* spp. can still proliferate at 0.5% oxygen, be it at a lower rate (Hall and Denning 1994).

Insects as the rice weevil and cowpea bruchid can induce considerable damage to storage grains and pulses. Some insects can survive and propagate under rather dry conditions, till about 30% RH (Roberts 1972), but insects are sensitive to sub-zero temperatures and hypoxia. Both conditions are recommended as alternative for chemical fumigation (Kalpana et al. 2022; Kandel et al. 2021). However, it should be considered that the eggs or pupae can have, related to their low metabolic activity, a greater tolerance compared to the adult insects and modified atmospheric treatments can take some weeks, also depending on the temperature.

4 Modelling Seed Ageing

Ageing of seed lots should be considered on a population level, in which the frequency of seeds capable of radicle protrusion decreases over storage time, with a sigmoidal curve. When this curve is plotted on a probit scale, the decline in frequency of surviving seeds will show a straight line (Ellis and Roberts 1980). The angle of this line may vary between species. Variation in storage environments will also change the angle of this line, decreasing in case of less deteriorating conditions. Plotting survival curves of seed lots using probit transformation can aid in estimating the p50 value, the storage time resulting in a 50% decline in viability, to characterize the effect of genetics or seed treatments on the tolerance to ageing under specific conditions (Hay et al. 2018). According to Ellis and Roberts (1980), the viability of the seed population after a certain storage period can mathematically be defined by the equation

$$v = K_i - p/\sigma,$$

The percentage viable seeds (v) are equal to the initial viability (K_i, or intercept with the Y-axis) decreased by the seed death expected during the storage period (p) according to the slope ($1/\sigma$) of the line. To estimate the survival of seeds from

specific species under different storage conditions, Ellis and Roberts (1980) have developed the Seed Viability Equation:

$$\log \sigma = K_E - C_W \log m - C_H T - C_Q T^2,$$

Here K_E is a species constant that accounts for the differences in storability among species, m is the seed moisture content (in percentage on fresh weight basis), and T is the storage temperature (°C). C_W, C_H and C_Q are species-specific constants, empirically determined by storing seeds at different moisture levels in hermetically sealed pouches at different temperatures. Related to the large efforts and time needed for these experiments, it should be considered that these constants have been determined for only limited number of species and most often for only a single seed lot of that species. A comparison of eight species indicated that the temperature constants C_H and C_Q are rather similar and the constant C_W for moisture content is common when expressed in relation to the equilibrium RH instead of absolute moisture content (Bewley et al. 2013).

The Seed Information Database maintained by the Royal Botanic Gardens, Kew, UK (http://data.kew.org/sid/viability/) has compiled available data on these species-specific constants, along with tools to calculate estimated seed longevities, under different seed moisture contents and temperature conditions, and the seed moisture content at a certain RH, based on the seed oil content.

Limited data have been published on the quantitative effect of oxygen on seed ageing, but very strong shelf life-extending effects have been reported for seeds of *Brassica* species where seed viability had not or hardly declined after almost 40 years storage under a combination of ultra-dry storage and anoxia (González-Benito et al. 2011). Preliminary data of experiments with dry storage of primed celery seeds under different oxygen levels and temperatures shows that halving the oxygen level during storage increases shelf life with a factor of 1.6, and this effect was independent of the storage temperature (Groot et al. unpublished data). It was observed that reducing the oxygen level from 21% (air) to 1% extends shelf life almost eight times.

5 Estimating Seed Longevity

Estimating the potential longevity or storability of commercial seed lots is of great significance in the seed trade. The viability equations and nomographs based on these were attempts to provide a reliable estimate of the shelf life of seed lots based on their initial germination (%), and specified regimes of seed moisture content or storage RH (%) and storage temperatures. Though useful to some extent, these tools could not differentiate between the lots of similar germination but variable vigour status. For managing the commercial seed inventory, it is important to estimate the shelf life of all seed lots of a given species or determine the efficacy of seed treatments on subsequent storability. To provide answers in short periods of time, experiments are performed to estimate storability at high humidity and temperature

conditions. Such 'accelerated ageing' (AA) and 'controlled deterioration' (CD) tests are often performed under humidity levels between 75% and 100% RH and at temperatures around 40 or 50 °C. When seed lots showing large differences in tolerance to ageing are compared, they generally show similar rankings in these tests. However, for more subtle differences in tolerance, these tests seem to fail in showing a reasonable relationship with the shelf life of seeds under commercial storage conditions. Such deviations are more prominent in the case of primed seeds, which exhibit a fast decline in longevity under dry storage. The main reason for this lack of correlation is that commercial seed storage is performed under dry conditions, with a glassy cytoplasm, while in the accelerated ageing and controlled deterioration tests the cytoplasm is in a liquid state. Seed storage experiments performed at high RH levels can possibly provide more reliable information on the storage behaviour of high moisture seeds. When the same seeds are stored under dry conditions with a glassy cytoplasm, their relative ageing pattern can be quite different.

Since oxygen is a third factor associated with accelerating seed ageing under dry conditions, an alternative seed ageing treatment has been developed by storing seeds under an elevated partial pressure of oxygen (EPPO) in pressure tanks under 200 times the normal air pressure, without increasing the seed water content or storage temperature (Groot et al. 2012). This test has successfully been employed to analyse genetic variation in seed longevity with seeds from Arabidopsis (Buijs et al. 2020) and barley (Nagel et al. 2016). However, more extensive testing is needed with seeds of contrasting morphological, chemical and physiological characteristics to validate the robustness of this test.

6 Types and Causes of Seed Deterioration

Damage induced during storage of seed with a glassy cytoplasm is due to reactive oxygen species (ROS) that can induce oxidation of essential molecules, such as DNA, RNA, proteins and lipids. DNA oxidation results in strand breaks, that can only be repaired upon imbibition. Protein oxidation, or carbonylation, occurs mainly with the storage proteins, that are less likely to be protected against oxidation compared to structural proteins and enzymes. As such storage proteins may act as ROS scavengers. Oxidation of the unsaturated membrane phospholipids results in increasing their melting point and cross-linking, both make the cell and organellar membranes less flexible. This creates more risks on leakage when water passes the membranes upon imbibition, especially at lower temperatures or at large differences in water potential. Because of the latter, it is recommended to humidify aged seeds by storing them overnight at 100% RH before sowing. Oxidation of the mitochondrial membranes results in reduced aerobic respiration potential and the need for anaerobic respiration to sustain the supply of energy. Ethanol production during seed imbibition is inversely related to seed vigour (Woodstock and Taylorson 1981). Indeed, seeds stored dry for prolonged periods in the presence of oxygen show more

anaerobic respiration during early germination, compared to seeds stored for a shorter period or under less deteriorating conditions (Kodde et al. 2012).

7 Repair Mechanisms

Protective mechanisms involved in repair of damage to the cell membranes, DNA, RNA, proteins and mitochondria are rapidly activated in the seeds once they imbibe water during the early hours of seed germination.

With respect to RNA, pre-existing or stored mRNAs are involved in resumption of metabolic activity in seeds immediately after imbibition (Bewley et al. 2013). Reduction of total RNA content and RNA integrity are associated with loss of germination ability of seeds. RNA is more vulnerable to oxidation damage compared to DNA since it possesses only a single strand. Damaged mRNA blocks translation, and this loss of translational activity in imbibed seeds is correlated with loss of seed longevity (Sugliani et al. 2009). The molecular mechanisms which are associated with repair of RNA have not yet been elucidated (Sano et al. 2015).

Similar to DNA and RNA, spontaneous damage to proteins, such as oxidations or covalent modifications, take place during seed ageing, which culminates in loss of protein function (Sugliani et al. 2009). The major damage of protein is due to oxidation of methionine to methionine sulfoxide by the ROS resulting in damage of protein (Stadtman 2006). The repair of oxidized proteins happens due to reduction of methionine sulfoxide by methionine sulfoxide reductase (MSR) (Weissbach et al. 2005). Therefore, MSR repair system plays a major role in preservation of seed longevity. Spontaneous covalent modification of proteins results in conversion of L-aspartyl or asparaginyl residues to abnormal Zisoaspartyl residues. L-Isoaspartyl O-methyl transferase (PIMT) is capable of repairing these residues. High PIMT activity has been reported with germinating sacred lotus seeds which show remarkable seed longevity duration (Shen-Miller et al. 2002). Thus, repair of protein appears to play a significant role in extending the longevity of seeds under dry storage.

8 Storability of Recalcitrant Seeds

Recalcitrant seeds possess high moisture content and are metabolically active when shed from the mother plant on maturation. They are largely devoid of seed protective mechanism which can enable the seeds to withstand desiccation and induce greater longevity potential. Delahaie et al. (2013) reported that critical LEA proteins, which contribute to the desiccation tolerance and extended storability of seeds, were absent in the recalcitrant seeds of *Castanospermum australe*. However, most recalcitrant seeds reportedly do accumulate LEA proteins and respond to ABA, drought or temperature (Farrant et al. 1996), although they may not be effective at very low seed water contents wherein seed would have already lost viability. Further, the organelles of a mature recalcitrant seeds were found to be in a highly differentiated

state and they also undergo specific ultra-structural changes resembling the modifications that occur during the early stages of seed germination (Berjak and Pammenter 2000). The changes include development of mitochondria and deposition of starch, dense material in the plastids, mitochondrial development and appearance of golgi bodies in root primordia and strong development of polysomes. This reveals that 'dessication sensitive' recalcitrant seeds lack the ability to "switch off" the metabolic activity by undergoing maturation drying after attaining maturity, unlike the 'desiccation insensitive' orthodox seeds, eventually leading to poor seed storage potential.

The physiological basis of viability loss in recalcitrant seeds has been reviewed by Umarani et al. (2015). During dehydration the cytoskeleton of these seeds suffer physical or mechanical damage due to lack of intracellular support, which does not reassemble upon rehydration, resulting in viability loss. These seeds are also more prone to '*metabolism-induced damage*' because the embryos of seeds, when shed from the mother tree, are metabolically active with high respiration rate. An uncontrolled activity of ROS coupled with concurrent failure of antioxidant system also leads to generation of free radicals and destruction of metabolic system of the cells. Further, even if maintained with high seed moisture content, dysfunction of the cell organelles, extensive vacuolization, consumption of reserves, dysfunction of mitochondria (Pammenter and Berjak 2000), presence of unstacked golgi bodies, dilated endoplasmic reticulum cisternae and abnormal vacuoles become rampant (Motete et al. 1997). The confluence of all these changes results in acute damage to cell metabolism, culminating in the loss of viability of recalcitrant seeds.

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