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Staying Alive: Molecular Aspects of Seed Longevity

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Mature seeds are an ultimate physiological status that enables plants to endure extreme conditions such as high and low temperature, freezing and desiccation. Seed longevity, the period over which seed remains viable, is an important trait not only for plant adaptation to changing environments, but also, for example, for agriculture and conservation of biodiversity. Reduction of seed longevity is often associated with oxidation of cellular macromolecules such as nucleic acids, proteins and lipids. Seeds possess two main strategies to combat these stressful conditions: protection and repair. The protective mechanism includes the formation of glassy cytoplasm to reduce cellular metabolic activities and the production of antioxidants that prevent accumulation of oxidized macromolecules during seed storage. The repair system removes damage accumulated in DNA, RNA and proteins upon seed imbibition through enzymes such as DNA glycosylase and methionine sulfoxide reductase. In addition to longevity, dormancy is also an important adaptive trait that contributes to seed lifespan. Studies in Arabidopsis have shown that the seed-specific transcription factor ABSCISIC ACID-INSENSITIVE3 (ABI3) plays a central role in ABA-mediated seed dormancy and longevity. Seed longevity largely relies on the viability of embryos. Nevertheless, characterization of mutants with altered seed coat structure and constituents has demonstrated that although the maternally derived cell layers surrounding the embryos are dead, they have a significant impact on longevity.

Keywords: Anhydrobiosis • Desiccation • Dormancy • Hormone • Longevity • Oxidation.

Abbreviations: AP, apurinic/apyrimidinic; BER, base excision repair; CDT, controlled deterioration treatment; DSB, double strand breaks; F3′H, flavonoid 3′ hydroxylase; HSP, heat-shock protein; isoAsp, isoaspartyl; LEA, late embryogenesis abundant; MSR, methionine sulfoxide reductase; PA, proanthocyanidin; PARP, poly (ADP-ribose) polymerase; PIMT, L-isoaspartyl *O*-methyltransferase; POD, peroxidase; PPO, polyphenol oxidase; QTL, quantitative trait locus; RFO, raffinose family oligosaccharide; RNAi, RNA interference; ROS, reactive oxygen species; SGR, stay-green; SSP, seed storage protein; 8-oxoG, 7,8-dihydro-8-oxoguanine.

Introduction

Most seed-producing plants can survive harsh stress conditions such as high and low temperature, freezing and desiccation in the seed stage (orthodox seeds). 'Seed longevity' is defined as the total time span during which seeds remain viable. Seed longevity is an important trait for ecology, agronomy and economy. Remarkable long-term seed longevities have been reported for sacred lotus (*Nelumbo nucifera*) (nearly 1,300 years) (Shen-Miller 2002) and for *Phoenix dactylifera* (>2,000 years) (Sallon et al. 2008), whereas other species such as onion and pepper show a relatively short longevity. It has also been reported that seed longevity varies even in a given plant species such as Arabidopsis (Bentsink et al. 2000, Clerkx et al. 2004), lettuce (Schwember and Bradford 2010), rice (Miura et al. 2002, Sasaki et al. 2005) and wheat (Landjeva et al. 2010). This suggests that seed longevity is not always a dominant selective force and there might be trade-offs depending on the plant species and environmental conditions. During long-term conservation, seed longevity depends greatly on moisture content, relative humidity, oxygen pressure and temperature of storage (Walters 1998, Groot et al. 2012). During dry storage, seed viability gradually decreases due to 'aging processes' and/or 'deterioration events'. The first symptoms are delayed seed germination and poor seedling establishment, with complete loss of viability observed as an inability to germinate, which often results in reduced crop yield (Seshu et al. 1988, Ghassemi-Golezani et al. 2010). Evaluation of seed longevity during natural aging under dry and mild temperature conditions requires a long time. Therefore, in many studies, seeds were placed under high relative humidity and high temperature to accelerate deterioration. The treatment, called 'controlled deterioration treatment (CDT)' or 'accelerated aging', mimics molecular and biochemical events that occur during natural seed aging (Rajjou et al. 2008).

In seeds, the embryo and endosperm are derived after fertilization, with the embryo going on to be the next generation after dispersal, whereas the seed coat, or testa, that surrounds the embryo and endosperm differentiates from the ovule and is of maternal origin. Embryogenesis and subsequent morphogenesis are complete by the middle of seed development. In parallel, the differentiation of the testa cell layers progresses and terminates with programmed cell death at approximately this

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stage (Haughn and Chaudhury 2005). Immature seeds could germinate when they were excised from mother plants and kept hydrated (Koornneef et al. 1989, Still et al. 1994), indicating that they had already acquired their germination potential; however, they cannot survive after desiccation. After morphogenesis, seeds enter the phase called maturation during which they acquire desiccation tolerance and water contents are gradually reduced (to <20%), before finally become quiescent. During seed desiccation, the cytoplasm of seed cells transforms from a fluid to glass viscosity. In the glassy state, cellular components are stabilized and their mobility is severely restricted, and this is indispensable for desiccation tolerance (Buitink and Leprince 2008). Desiccation tolerance of seeds contributes to seed longevity, as shown in recalcitrant seeds, which are sensitive to desiccation and cannot be stored for long periods (Ooms et al. 1993, Angelovici et al. 2010). Also, it has been reported that seed longevity gradually increases during maturation after acquisition of desiccation tolerance (Verdier et al. 2013).

As detailed above, it is well known that seed longevity decreases rapidly during storage under high relative humidity and high temperature. This is possibly due to increased fluidity of the cytoplasm that in turn promotes irreversible aggregation of denatured proteins. Oxidation of cellular molecules such as lipids, cell membranes, DNA, RNA and proteins (Osborne 1994, Bailly 2004, Rajjou and Debeaujon 2008, Rajjou et al. 2008), mediated by reactive oxygen species (ROS), also affects seed longevity (Harrison and McLeish 1954, Justice and Bass 1978, Groot et al. 2012). UV light, which can cause DNA damage, has also been reported to be harmful to seeds (Harrington 1970). As metabolic activity is extremely low in mature seeds, they must be equipped with 'protective mechanisms' against oxidative stress during seed development to prolong seed longevity. Nevertheless, even with such protective mechanisms, mature dry seeds gradually accumulate cellular damage during aging. Once seeds are imbibed, the cytoplasm of seed cells re-transforms from a glassy to a fluid state and metabolism is activated. In this condition, seeds can 'repair' the damage, thereby improving seed vigor.

Significant efforts have been made to understand the mechanisms underlying seed longevity, and recent studies have begun to elucidate the molecular aspects. In this review, we first summarize the two major mechanisms responsible for seed longevity, namely protection and repair. Although seed longevity is affected by many factors, here we focus mainly on the mechanisms involved during seed dry storage. The relationship between seed longevity and seed dormancy is then discussed. Although seed dormancy is another important adaptive trait that contributes to seed viability in a changing environment, it has been unclear whether or not these traits share common mechanisms. We also describe available evidence indicating the involvement of plant hormones in the regulation of seed longevity as they are key factors that control many other aspects of seed development, dormancy and germination. Finally, we describe the role of maternal seed tissues in longevity. The testa is an important interface between the embryo and the external environment that contributes to protection mechanisms as well as modulating seed dormancy and germination (Haughn and Chaudhury 2005). Relationships between the seed coat and longevity will be discussed based on several interesting findings reported recently.

The Protective Cellular Systems Supporting Seed Longevity

Switching off metabolic activity: the key to survival?

Survival in the dry state requires high-performance cellular protective mechanisms. An important key to keeping cells alive in limited water conditions is to reduce their metabolic activity down to a quiescent state. The most widespread strategy to abolish cell metabolism is to limit molecular mobility by accumulating soluble non-reducing sugars, thereby transforming the cytoplasm into a glassy state. Thanks to the physico-chemical properties of theses sugars, orthodox seeds can survive for decades up to millennia in extreme dehydration (Walters et al. 2005, Rajjou and Debeaujon 2008). Low temperature and low moisture content contribute to the formation of this intracellular glass. Cellular viscosity and molecular mobility within the cytoplasm have been highly correlated with seed longevity over a wide range of temperatures and water contents (Buitink et al. 2000). Glass formation in seeds is favored by the replacement of water by oligosaccharides [i.e. sucrose and raffinose family oligosaccharides (RFOs)], which disrupt normal crystal matrices (Koster and Leopold 1988). Raffinose and stachyose have been characterized as highly efficient inhibitors of sucrose crystallization, even in small amounts. In seeds, RFOs and sucrose can also serve as energy sources during germination. In effect, the rapid breakdown of RFOs was reported to occur upon seed imbibition (Blöchl et al. 2008). In the glassy state these oligosaccharides would be particularly effective at protecting against membrane damage due to dehydration by preventing fusion of adjacent vesicles during drying and by maintaining lipids in a fluid phase during the desiccation phase (Crowe et al. 1987). These sugars also protect the structure and function of labile proteins (Imamura et al. 2003), and in viable, dry seeds embryonic proteins are mostly stable and functional even after several years of storage. The glassy state prevents harmful events, such as Maillard reactions, and RFOs have been proposed to protect plant cells against oxidative damage, possibly by scavenging hydroxyl radicals (Nishizawa et al. 2008). The glassy cytoplasm is stabilized by a combination of sugars and late embryogenesis abundant (LEA) proteins that are extremely hydrophilic. Commonly associated with desiccation and abiotic stress tolerance, LEA proteins are among the intrinsically disordered proteins in aqueous solution. They undergo desiccation-induced folding during cell drying, suggesting that these proteins could carry out distinct roles under different water states. Arabidopsis comprises 51 genes encoding LEA proteins classified into nine distinct groups based on their sequence homologies (Bies-Etheve et al. 2008, Hundertmark and Hincha 2008, Hunault and Jaspard 2010). A proteomic analysis revealed that the RAB18 protein, an LEA protein of group 2 also called the

'dehydrin group', progressively decreased in accumulation in Arabidopsis seeds as artificial aging time increased and germination ability was lost, suggesting its involvement in seed longevity (Rajjou et al. 2008). Another study has confirmed this hypothesis by an RNA interference (RNAi) strategy targeting *LEA14*, a seed-specific Arabidopsis dehydrin gene (Hundertmark et al. 2011). In the RNAi lines, mRNA levels of *LEA14* as well as its homologs *XERO* and *RAB18* were reduced, and seed longevity was affected. In addition, the seed-specific biotinylated protein of 65 kDa (SBP65) in pea, a member of the group 3 LEA proteins, is mainly accumulated when embryonic cells slow down their metabolic activity from the end of maturation until the quiescent state (Duval et al. 1994, Dehaye et al. 1997). In the developing embryos of a viviparous pea mutant (*vip-1*), the levels of *SBP65* mRNA and biotinylated SBP65 protein were significantly reduced (Dehaye et al. 1997). This protein is thought to contribute to both seed desiccation tolerance and longevity by scavenging free biotin and thereby inhibiting most carboxylation reactions since biotin is a cofactor of several carboxylase enzymes. In maize, the abundance of the group 1 LEA protein named EMB564 was suggested to be related to seed viability (Wu et al. 2011). Furthermore, several other LEA proteins were previously associated with seed longevity in *Medicago truncatula* (Chatelain et al. 2012). Nevertheless, more research is needed to discover how LEA proteins contribute to modulate seed longevity.

Dealing with oxidative stress in the dry state

Seed aging is usually associated with the oxidation of macromolecules (Harman and Mattick 1976, Bailly 2004). During dry storage, seeds are subjected to progressive alteration of cell constituents (proteins, lipids, nucleic acids, sugars, etc.) by autoxidation processes such as Amadori and Maillard reactions (Murthy and Sun 2000), lipid peroxidation (Wilson and McDonald 1986) or protein carbonylation (Arc et al. 2011). In the early stages of storage, oxidative events lead to dormancy release, promote germination capacity and can be considered as a beneficial mechanism to improve seed vigor. In sunflower seed embryos, CDT under high O_2 led to rapid dormancy release and increased protein carbonylation (Morscher et al. 2015). Afterwards, if seed storage is too long, or is done in unsuitable conditions (i.e. high temperature, elevated relative humidity), the accumulation of cellular oxidative damage progressively induces a loss of seed vigor and a loss of germination capacity until irreversible death of the embryo. To promote their longevity, seeds require efficient antioxidant systems; thus, a range of protective mechanisms prevent excessive oxidation of macromolecules. It is important to distinguish between passive mechanisms including non-enzymatic ROS scavenging systems and active mechanisms including enzymatic ROS detoxification. In order to remove excess ROS accumulated during seed storage and control free radical overproduction generated by the reinitiation of metabolism upon imbibition, seeds use a set of antioxidant enzymes such as superoxide dismutases, catalases, glutathione and ascorbate peroxidases, and monodehydroascorbate, dehydroascorbate and glutathione reductases (Bailly 2004, Kumar et al. 2015). Several factors involved in redox changes have been proposed as seed viability markers (Kocsy 2015). Although, several other proteins that act as antioxidants or in related ROS signaling, such as thioredoxins, peroxiredoxins and glutaredoxins, have been identified in seeds, their roles in seed longevity remain to be described. The amount of non-enzymatic ROS scavenging systems is controlled during seed development and maturation, and can be influenced by the growth environment of the mother plant. These passive mechanisms include seed storage proteins and low molecular weight antioxidants including tocopherols, ascorbate and glutathione. Seed aging is correlated with a decline in the cellular antioxidant potential. Due to its abundance and negative redox potential, reduced glutathione (GSH) plays a major role in the regulation of the intracellular redox environment. The measurement of the reducing capacity of the oxidized glutathione (glutathione disulfide, GSSG)/GSH redox couple and the determination of its half-cell reduction potential ($E_{GSSG/2GSH}$) based on the Nernst equation were defined as useful tools for cell viability determination that can be used to monitor seed aging (Kranner et al. 2006). In barley, a strong correlation between total germination and the glutathione redox state was observed across 26 genotypes, suggesting that variations in the $E_{GSSG/2GSH}$ value could be a robust marker of seed deterioration (Nagel et al. 2015). Among other antioxidants, tocopherols (vitamin E) play an important role in maintaining seed viability as they prevent non-enzymatic lipid oxidation during seed storage. Genotypes affected in the biosynthesis of tocopherols have reduced seed longevity (Sattler et al. 2004, Giurizatto et al. 2012). In Arabidopsis, the *VTE1* (*VITAMIN E DEFICIENT 1*) gene is the limiting factor in tocopherol biosynthesis, and a close relationship between vitamin E and lipocalins has been suggested (Havaux et al. 2005). When mutation of the chloroplastic lipocalin (CHL) was combined with *vte1* mutation in an Arabidopsis double mutant, this showed hypersensitivity to high light stress and exhibited intense lipid peroxidation compared with the single mutants (Boca et al. 2014). It has been demonstrated that both AtTIL, a temperature-induced lipocalin, and AtCHL are involved in lipid protection, which is critical for stress adaptation. Interestingly, seed longevity is correlated with the accumulation of these proteins (Boca et al. 2014). Tocopherols and lipocalins would act synergistically to prevent the seed aging. Antioxidant polyphenols such as flavonoids that are present not only in the seed coat, but also in the embryo and endosperm, have been shown to increase seed longevity of Arabidopsis seeds (Debeaujon et al. 2000). It has been established that dietary polyphenols increase the lifespan of model organisms such as *Caenorhabditis elegans*, *Drosophila melanogaster* or mice through the activation of hormetic pathways (Sadowska-Bartosz et al. 2014). Nevertheless, a thorough understanding of the mode of action of polyphenols in the context of the seed requires additional studies.

Seed storage proteins (SSPs) have been described as a primary target for oxidation in seeds (Job et al. 2005, Arc et al. 2011). Generally, 12S globulin α-subunits are preferentially carbonylated over β-subunits in unaged dry seeds, while both types of subunit are fully carbonylated in aged seeds (Job et al. 2005, Rajjou et al. 2008, Kalemba and Pukacka 2014). The increased sensitivity of 12S globulin α-subunits to oxidation might

contribute to the efficient mobilization of protein bodies during seed germination via the 20S proteasome pathway (Galland et al. 2014). It was recently reported that Arabidopsis mutants affected in 12S globulin genes showed severe phenotypes for seed longevity, suggesting a role for SSPs in seed aging (Nguyen et al. 2015). Due to their abundance and their high affinity for oxidation, SSPs were proposed to be a powerful ROS scavenging system that could protect cellular components that are important for the survival of the embryo. This role for SSPs in ROS buffering during seed dry storage was linked to a chaperone function (Nguyen et al. 2015). Chaperones such as heat-shock proteins (HSPs) could be involved in the extension of seed longevity. HSPs play an important role by (i) stabilizing neosynthesized proteins to enable correct folding; (ii) protecting proteins against oxidative damage; and (iii) helping to refold proteins that have been altered during seed storage. In transgenic tobacco plants, overexpression of sunflower heat shock factor A9 (HSFA9), a heat stress transcription factor, led to enhanced accumulation of HSPs and to improved seed longevity (Prieto-Dapena et al. 2006).

A model for the protective cellular systems described in this section is summarized in **Fig. 1**.

Repair Systems During Seed Imbibition

As mentioned above, oxidative stress is one of the major factors reducing seed longevity. Dry seeds are equipped with various protection mechanisms against stress; however, after a prolonged storage period, damage accumulates gradually in DNA, RNA and proteins that are required for seed germination. Upon imbibition of quiescent dry seeds, metabolic pathways are rapidly activated and the seeds can repair the damage to initiate germination. Recent studies have shown that the ability of the seeds to repair the damage correlated with seed longevity. An overview is shown in **Fig. 2**.

Repair of DNA modifications and strand breaks

It has been reported that loss of seed longevity is often associated with the accumulation of DNA lesions such as DNA strand breaks during storage (Cheah and Osborne 1978). DNA repair mechanisms have been reported to be of paramount importance for seed germination and longevity (Balestrazzi et al. 2011, Waterworth et al. 2015).

In general, strand breaks in DNA are primarily caused by ROS, either directly through desaturation of deoxyribose units or by covalent modifications of bases (Bray and West 2005). One of the major modifications is the hydroxylation of the C-8 position of guanine to produce 7,8-dihydro-8-oxoguanine (8-oxoG) (Bray and West 2005, Biedermann et al. 2011). 8-oxoG is potentially mutagenic: it can pair with adenine (A) as well as cytosine (C), and the mispair with an A residue results in GC to TA transversion during DNA replication. It is known that a base excision repair (BER) system is involved in the removal of 8-oxoG (Bray and West 2005, Biedermann et al. 2011, Chen et al. 2012). The

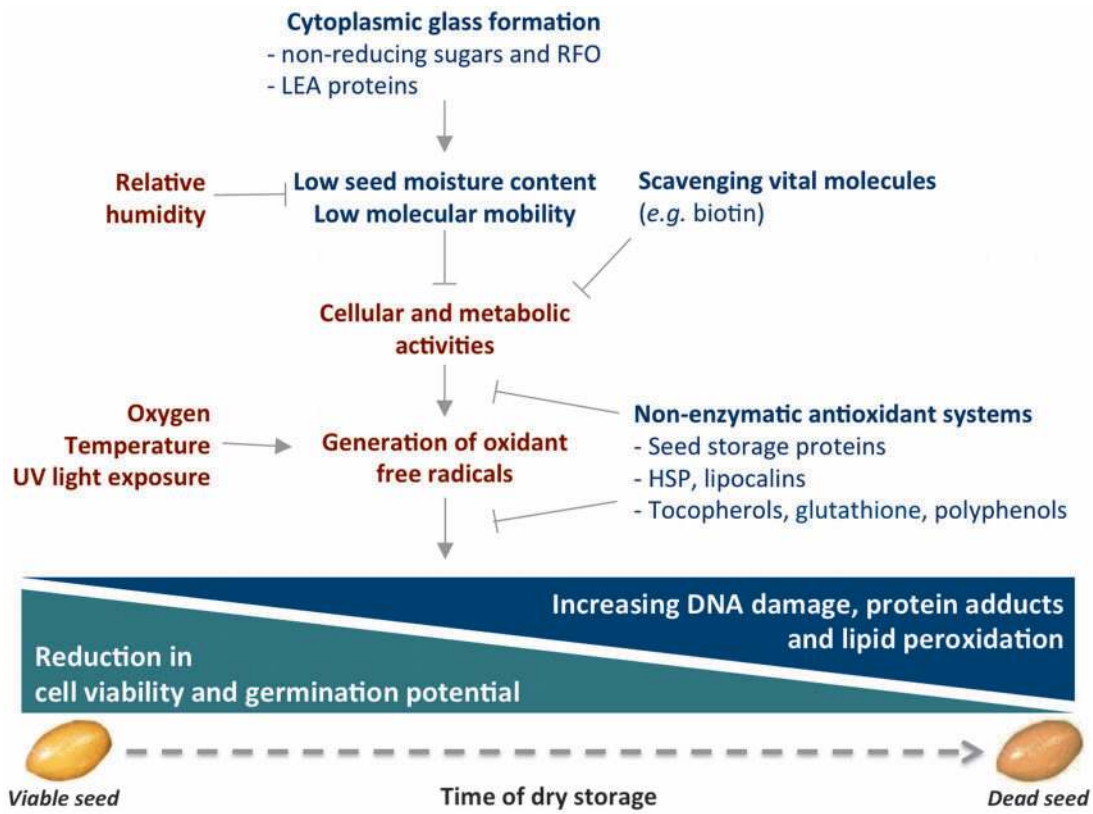

Fig. 1 Model for the cellular systems protecting seeds from injury by oxidizing free radicals during dry storage and anhydrobiosis. HSP, heat-shock protein; LEA, late embryogenesis abundant; RFO, raffinose family oligosaccharides. In dark red are factors that negatively influence seed viability during dry storage. In blue are factors that positively influence seed viability during dry storage.

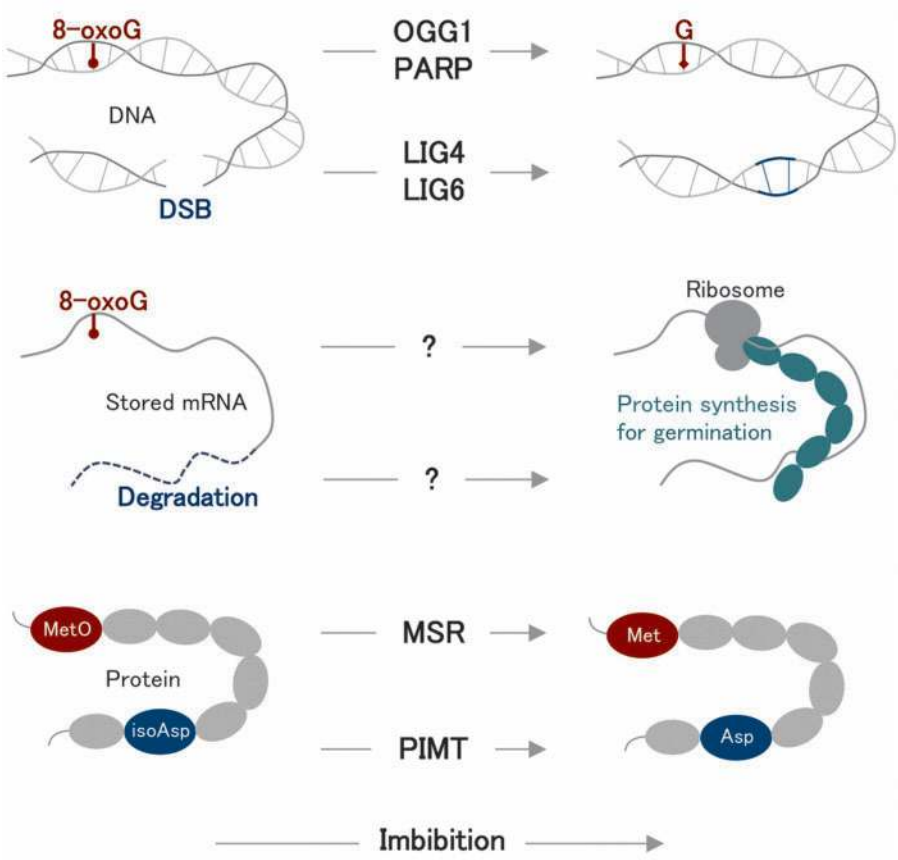

Fig. 2 Overview of seed repair systems involved in germination and longevity. Damaged DNA, RNA and protein molecules accumulate in dry seeds and are repaired upon imbibition. 8-oxG, 7,8-dihydro-8-oxoguanine; DSB, DNA double-strand break; Met, methionine; MetO, methionine sulfoxide; Asp, L-aspartyl; isoAsp, L-isoaspartyl.

first step of BER is cleavage of *N*-glycosyl-bound DNA between the base and the sugar by DNA glycosylases to excise the 8-oxoG base and generate abasic or apurinic/apyrimidinic (AP) sites. Subsequently, the AP site is hydrolyzed by an AP endonuclease at the phosphodiester bond and the DNA backbone is cleaved. Finally DNA polymerase and ligase complete the BER repair.

In Arabidopsis, *AtOGG1* encodes a bifunctional DNA glycosylase/AP lyase (Dany and Tisser 2001, Garcia-Ortiz et al. 2001). *AtOGG1* transcripts were abundant during seed desiccation and imbibition. Transgenic lines overexpressing *AtOGG1* contained significantly reduced levels of 8-oxoG in 24 h imbibed seeds and showed enhanced resistance to CDT compared with the wild type, indicating that AtOGG1-mediated BER plays an important role in seed longevity (Chen et al. 2012). In mammalian cells, poly (ADP-ribose) polymerase (PARP) is involved in the recruitment of BER-related enzymes (Biedermann et al. 2011). Of three Arabidopsis genes homologous to human *HsPARP1*, *AtPARP3* was highly expressed in seeds and the *parp3-1* mutant was susceptible to CDT compared with the wild type (Rissel et al. 2014). This again indicates that BER activity is tightly linked to seed longevity. Besides the role in BER, PARP has also been implicated in the production of nicotinamide from NAD (Hunt et al. 2004). In the Arabidopsis *nic2-1* mutant, defective in nicotinamidase NIC2 that catalyzes the conversion of nicotinamide to nicotinic acid, PARP activity is reduced possibly due to feedback inhibition by accumulated nicotinamide (Hunt et al. 2007). Germination of *nic2-1* mutant seeds was affected to a greater extent than that of wild-type seeds in the presence of the DNA-damaging agent methyl methanesulfonate. Although seed longevity of *nic2-1* was not determined, it is possible that BER mediated by PARP is regulated by NIC2 (Hunt et al. 2007).

Eukaryotes possess multiple DNA ligases and they have specific roles in cellular DNA metabolism. DNA ligase IV in yeast, mammals and plants has specific roles in the non-homologous end-joining pathway of repair of double strand breaks (DSBs) (Ellenberger and Tomkinson 2008). DSBs are serious DNA damage lesions caused directly by ROS or indirectly during the BER process. It was reported that the Arabidopsis mutant defective in DNA ligase IV (AtLIG4) and the plant-specific DNA ligase termed AtLIG6 had reduced viability after CDT compared with the wild type (Waterworth et al. 2010). Interestingly, a quantitative trait locus (QTL), *Germination Ability After Storage 6*, for seed longevity was localized in the genomic regions containing *AtLIG4* (Nguyen et al. 2012). These results suggest that DNA repair mediated by the DNA ligases might be one of the determinants of longevity.

RNA repair mechanisms remain elusive

It has been reported that mature dry seeds of Arabidopsis and rice can germinate in the presence of transcription inhibitors (Rajjou et al. 2004, Sano et al. 2012). These same studies showed that germination was blocked by translation inhibitors,

suggesting that proteins required for germination were translated from pre-existing mRNA templates in the mature dry seeds (stored mRNA or long-lived mRNA). In Arabidopsis mature dry seeds, >10,000 different mRNA species have been detected (Nakabayashi et al. 2005). In rice, mRNAs essential for germination were accumulated during the seed maturation phase (Sano et al. 2015). It has been postulated that the early translation of stored mRNAs allows seed rapidly to resume their metabolic activity upon imbibition (Brooker et al. 1978, Martin and Northcote 1981, Galland et al. 2014, Sano et al. 2015).

In deteriorating *Pisum sativum* seeds, loss of germination ability is accompanied by a reduction in total RNA content and RNA integrity (Kranner et al. 2011). RNA is more vulnerable to ROS oxidation than DNA due to its single-stranded structure. Damaged mRNA blocks translation, and loss of translational activity in imbibed seeds was correlated to loss of seed longevity (Rajjou et al. 2008). These findings indicate that the integrity of RNA, as well as DNA, impacts on seed longevity. However, molecular mechanisms such as protection and repair of damaged RNA remain to be elucidated.

Mechanism for protein repair

To translate stored mRNAs during imbibition, the proteins of the translational machinery have to be functional in mature dry seeds. As described above, the enzymatic activity of DNA repair proteins during imbibition is also important for seed longevity. Like DNA and RNA, proteins accumulate spontaneous damage such as oxidations or covalent modifications, which often leads to the loss of protein functions (Rajjou et al. 2008). Thus, protein repair systems are also a prevailing strategy for the maintenance of seed longevity.

Oxidation of methionine to methionine sulfoxide caused by ROS is one of the major forms of damage that affects proteins and is a hallmark of aging in all organisms (Stadtman 2006). Methionine sulfoxide reductase (MSR) reduces methionine sulfoxide and reversibly repairs the oxidized proteins (Weissbach et al. 2005). In two *M. truncatula* genotypes, MSR activities have been positively correlated with the longevity of seed lots having different quality levels (Châtelain et al. 2013). Also in Arabidopsis mutants or transgenic plants with altered MSR gene expression levels, a positive correlation was observed between MSR activities and seed longevity (Châtelain et al. 2013). These results suggest that the MSR repair system plays a role in the preservation of seed longevity.

Conversion of L-aspartyl or asparaginyl residues to abnormal isoaspartyl (isoAsp) residues by spontaneous covalent modification also occurs in older proteins in various organisms, resulting in loss of protein function (Lowenson and Clarke 1992). L-Isoaspartyl *O*-methyltransferase (PIMT) can repair these abnormal residues, and PIMT activity has been detected in 45 plant species from 23 families, with the activity often localized in seeds (Mudgett et al. 1997). Natural or accelerated aging of barley seeds resulted in reduced germination ability with increased levels of unrepaired isoAsp residues (Mudgett et al. 1997). Also, a high PIMT activity was observed in germinating sacred lotus that shows remarkable long-term seed longevities (nearly 1,300 years) (Shen-Miller 2002). In Arabidopsis, two genes encode PIMT (*AtPIMT1* and *AtPIMT2*) (Mudgett and Clarke 1996, Xu et al. 2004). *AtPIMT1* overexpression reduced isoAsp accumulation in seed proteins and increased seed longevity, whereas reduced AtPIMT1 levels were associated with increased isoAsp contents and loss of seed viability during CDT (Ogé et al. 2008). Moreover, Arabidopsis seeds overexpressing chickpea PIMT genes, *CaPIMT1* and *CaPIMT2*, showed enhanced seed longevity (Verma et al. 2013). This suggests that PIMT-mediated protein repair is important for seed longevity. Visualization of stably expressed green fluorescent protein-fused PIMT proteins by confocal microscopy and cell fractionation–immunoblot analysis revealed that apart from the plasma membrane, CaPIMT2 is predominantly localized in the nucleus, while CaPIMT1 is localized in the cytosol. Similar results were observed for the two *PIMT* genes from rice, designated as *OsPIMT1* and *OsPIMT2* (Wei et al, 2015). It is possible that their target proteins are also localized in different cell compartments and the two PIMTs contribute differentially to seed longevity.

Protein isomerization is likely to be a key determinant for survival in the dry state. This hypothesis is reinforced by two closely related Arabidopsis prolyl isomerases, the immunophilins ROF1 and ROF2 (ROTAMASE FKBP 1 and 2), which were shown to have an impact on seed longevity (Bissoli et al. 2012). Experiments on seed germination and longevity revealed that the *rof1 rof2* double mutant was more sensitive to accelerated aging of seeds and to germination in conditions where the regulation of intracellular pH homeostasis was disadvantageous. It will be important to ascertain the extent to which proteome integrity and its rearrangements are central mechanisms regulating seed longevity at the molecular level.

Dormancy and Longevity Regulation by Common and Distinct Signaling Pathways

Like seed longevity, dormancy is an adaptive trait that contributes to seed survival and persistence in the soil. By delaying germination after seed dispersal, dormancy allows seedling establishment under favorable environmental conditions. Most species, including Arabidopsis, exhibit a so-called physiological dormancy, which is under the control of endogenous hormonal balance (Baskin and Baskin 2004, Long et al. 2015). Physiological dormancy can be released by dry storage (after-ripening) or by hydration under specific moisture, temperature and light conditions (stratification); for instance, imbibition in cold (4°C) and dark conditions is commonly used for Arabidopsis seed stratification.

Developmental regulation of seed longevity, dormancy and desiccation tolerance

Longevity and dormancy depth are determined during seed development on the mother plant, and early genetic studies indicated that ABA was involved in the control of both traits (Ooms et al. 1993). ABA insensitivity in Arabidopsis *abainsensitive3* (*abi3*) mutants was correlated with reduced

dormancy, intolerance to desiccation and rapid viability loss during dry storage. Germination ability was impaired after only a few days of dry storage for severe alleles, whereas the leaky allele *abi3-1* could withstand a longer period of storage. Nevertheless, seed viability after 4 years of storage was extremely low for both severe and leaky alleles (Clerkx et al. 2004), and a high sensitivity to CDT was also observed in *abi3-1* seeds (Mao and Sun 2015). The combination of ABA deficiency with mild insensitivity in the *aba1-1 abi3-1* mutant led to very severe dormancy and longevity phenotypes, further suggesting that ABA controls both these processes (Ooms et al. 1993). ABI3, together with LEAFY COTYLEDON1 (LEC1), LEC2 and FUSCA3 (FUS3), is a master transcriptional regulator of seed maturation. The *abi3*, *lec* and *fus3* mutants exhibit reduced reserve storage, desiccation intolerance and precocious germination, resulting from a developmental bypass of seed maturation leading to an early transition to vegetative stage (reviewed in North et al. 2010). The simultaneous reduction of longevity and dormancy in mutant seeds was thus explained by their failure to acquire desiccation tolerance and induce dormancy during late seed development. A screen for suppressors of the *abi3-5* mutation (*sua*) led to the isolation of an *sua-1* mutant that exhibited improved longevity (Sugliani et al. 2010). Nevertheless, phenotype restoration was allele specific, since *SUA* encodes a splicing factor, whose mutation restored the production of a functional ABI3 protein in *abi3-5* only. Finally a recent study analyzing co-expression networks in *M. truncatula* seeds suggested that seed longevity was regulated by both ABI3-dependent and independent pathways (Righetti et al. 2015).

Among their pleiotropic defects, severe *abi3* mutants exhibit a stay-green (SGR) seed phenotype due to lack of Chl degradation, which is further enhanced when combined with *lec1* or *fus3* mutations (Parcy et al. 1997). In diverse crop species, including oilseed rape, Chl persistence has been correlated with reduced storability, leading to commercial losses, and is therefore used as a marker for seed quality. In Arabidopsis too, degreening alterations due to defective Chl catabolism have been correlated with reduced seed storability. Furthermore there is evidence that the expression of *NON-YELLOW COLORING1* (*NYC1*), encoding a chlorophyll *b* reductase isoform involved in Chl catabolism, is regulated by ABA, further supporting a role for ABA in these processes (Nakajima et al. 2012). Other *SGR* genes involved in degreening processes were identified in several species, including Arabidopsis, which did not exhibit longevity problems (Delmas et al. 2013). *AtSGR1* and *AtSGR2* have been shown to function downstream of *ABI3* and exclusively control seed-specific degreening, independently of other *ABI3* signaling pathways acting in seed maturation and desiccation tolerance, thus explaining their normal longevity. Another green-seeded (*grs*) mutation was selected as an enhancer of the leaky allele *abi3-1*, which affected seed longevity; however, the corresponding locus is still unknown (Clerkx et al. 2003). Taken together, current evidence does not indicate a direct link between Chl retention and an inability to withstand dry storage. In contrast, embryo photosynthetic activity is a factor that influences seed quality, and especially longevity of Arabidopsis seeds (Allorent et al. 2015). Inhibition of photosynthesis during silique development resulted in delayed germination and lower seed resistance to CDT, although reserve storage was not affected. Photosynthesis was thus suggested to improve seed fitness by preventing anoxia through oxygen production and modulating osmoprotectant accumulation.

Hormonal signaling pathways in seed longevity

A central role in seed longevity signaling has been attributed downstream of ABI3 through the seed-specific heat shock factor HSFA9 (**Fig. 3**). In Arabidopsis, the expression of this transcription factor was shown to be under ABI3-specific control and restricted to late maturation stages, concomitantly with the induction of dormancy and desiccation tolerance (Kotak et al. 2007). Furthermore, its ectopic expression in unstressed leaves induced the constitutive expression of *HSP* genes. Constitutive expression of the sunflower *HaHSFA9* had also been shown to enhance the accumulation of HSP proteins in tobacco, and to improve seed thermotolerance and resistance to CDT (Prieto-Dapena et al. 2006), confirming the potential role of this transcription factor in stress tolerance and longevity. Simultaneous heterologous expression of the DROUGHT RESPONSIVE ELEMENT BINDING factor HaDREB2, which belongs to the APETALA2/ETHYLENE RESPONSE BINDING PROTEIN (AP2/ERBP) family, further enhanced seed longevity (Almoguera et al. 2009). In a subsequent study, the HaHSFA9 transcription factor was reported to interact with the auxin/IAA (Aux/IAA) protein HaIAA27, leading to its repression. Auxin would therefore act downstream of ABI3 to enhance seed longevity by alleviating HaIAA27-mediated HaHSFA9 repression in sunflower (Carranco et al. 2010), while in Arabidopsis positively regulating seed dormancy upstream of ABI3, through AUXIN RESPONSE FACTOR10/16-mediated regulation of ABI3 expression (Liu et al. 2013) (**Fig. 3**). Finally, in accordance with reports from other species, a co-expression gene regulatory network analysis in *M. truncatula* seeds identified a *MtHSF9* homolog at the transition between desiccation tolerance and longevity modules (Verdier et al. 2013).

In contrast to *abi3*, mutants defective for ABA biosynthesis do not exhibit pleiotropic maturation phenotypes, but their reduced dormancy was frequently observed in various species (Zeevaart and Creelman 1988, McCarty 1995). Effects on seed longevity were only reported for *aba1-5* seeds, with a significant loss in viability observed after 4 years of storage, further supporting an important role for ABA in seed lifespan (Clerkx et al. 2004). ABA is perceived by a family of soluble receptors, named PYR/PYL/RCAR for pyrabactin resistance1/PYR1-like/regulatory components of ABA receptors, which bind to clade A protein phosphatases type-2C (PP2Cs) and inhibit their activity. PP2Cs act as negative regulatory components of the ABA pathway by dephosphorylation of a subset of three sucrose non-fermenting 1-related subfamily 2 (SnRK2) kinases (Cutler et al. 2010). Similarly to *abi3*, the *snrk2.2* (*srk2d*) *snrk2.3* (*srk2i*) *snrk2.6* (*srk2e*) triple mutant exhibited seed maturation phenotypes, including desiccation intolerance, absence of dormancy

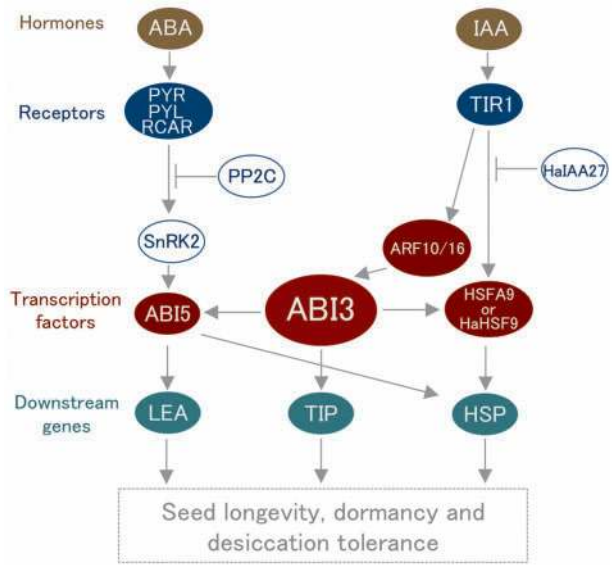

Fig. 3 A schematic model of ABA signaling pathways in seed longevity, dormancy and desiccation tolerance mediated by ABI3. The model is mainly based on analyses of Arabidopsis mutants. *HaIAA27* and *HaHSF9* are sunflower genes. Arrows and blocks indicate promotion or inhibition, respectively.

and Chl persistence, suggesting an inability to complete seed development. Moreover, seed storage under 25% humidity resulted in the loss of viability within 2 weeks (Nakashima et al. 2009). Many genes encoding LEA and HSP proteins were down-regulated in this mutant, including early methionine-labeled (EM) proteins AtEM1 and AtEM6, which are under the control of the bZIP transcription factor ABI5, a phosphorylation target of SnRK2 kinases (**Fig. 3**). Although the precise function of EM proteins is still unknown, expression of *M. truncatula EM* homologs has been correlated to the acquisition of either desiccation tolerance or longevity (Chatelain et al. 2012). Finally, ABI5 was reported to act downstream of ABI3 and regulate *AtEM* gene expression (Lopez-Molina et al. 2002), suggesting interactions between ABI3 and PYR/PP2C/SnRK2 signaling pathways (**Fig. 3**).

Aquaporins have essential functions in the transport of water and other small molecules, such as H_2O_2, and several are regulated by ABA. In vegetative tissues, SnRK2.6 phosphorylation of the plasma membrane intrinsic protein PIP2;1 contributes to ABA-regulated stomata closure (Grondin et al. 2015), whereas in seeds, recent data proved that ABI3 is involved in the regulation of genes encoding tonoplast intrinsic proteins TIP3;1 and TIP3;2 (Mao and Sun 2015). Defects in both genes reduced resistance to CDT, suggesting that ABA may control water relations and H_2O_2 accumulation via ABI3 modulation of aquaporins, thereby contributing to seed longevity (**Fig. 3**).

Although the role of ABA in seed longevity is supported by numerous studies, the implication of other hormones is less well documented. Beside the interactions of HaHSFA9 and the Aux/IAA protein HaIAA27 described above (Carranco et al. 2010), longevity genes in *M. truncatula* were found to be enriched in binding sites for auxin-binding factors (Righetti et al. 2015), implying that auxin may have a role in seed longevity. ABA is well known to control seed dormancy and germination antagonistically with gibberellin, but no viability loss after long-term storage has been observed in the gibberellin-insensitive *gai* and the gibberellin-deficient *ga1-3* mutants (Clerkx et al. 2004). Nevertheless, a screen of an Arabidopsis 'activation tagging' line collection for increased tolerance to accelerated aging identified *ARABIDOPSIS THALIANA HOMEOBOX 25* (*ATHB25*), whose overexpression increased accumulation of active gibberellins and transcripts of the gibberellin biosynthesis gene *GIBBERELLIN 3-OXIDASE 2* (Bueso et al. 2014a). The positive role of gibberellin in seed longevity was further demonstrated by the increased CDT resistance of seeds from GA_3-treated plants or the quintuple DELLA mutant that shows constitutive gibberellin responses. It has been suggested that gibberellin regulation of seed longevity was associated with changes in testa structure, this is discussed in the next section. In the same screen, a second mutant was isolated, which overexpressed a RING-type ubiquitin ligase that targets ABA receptors (Bueso et al. 2014b). Although loss of function decreased seed longevity, overexpression led to pleiotropic phenotypes, preventing the definition of its precise role. The involvement of other hormones in seed longevity remains to be established, since ethylene-resistant *etr1* and jasmonic acid-resistant *jar1-1* mutant seeds did not show any significant viability loss after storage for 4 years (Clerkx et al. 2004).

Natural variation of seed dormancy and longevity

In ABA-deficient or insensitive mutants in either Arabidopsis Landsberg *erecta* (L*er*) or Columbia (Col) genetic backgrounds, reduction of seed longevity was clearly associated with the absence of dormancy. Screens for reduced dormancy, however, identified a number of mutations that did not affect ABA levels or sensitivity. Four reduced dormancy (*rdo*) mutants were identified in the L*er* background (Leon-Kloosterziel et al. 1996, Peeters et al. 2002). *RDO2* and *RDO4* have been cloned and affect transcription efficiency. RDO2 is a transcription elongation factor, TFIIS, which interacts with the RNA polymerase II-associated factor 1 complex (Grasser et al. 2009, Liu et al. 2011), whereas *RDO4* encodes a C3HC4 RING finger protein, HISTONE MONOUBIQUITINATION 1 (HUB1), which is required for monoubiquitination of histone H2B (Liu et al. 2007). The *delay of germination1* (*dog1*) is a non-dormant mutant that was identified in the dormant near isogenic line NILDOG1-Cvi that carries a genomic region containing the *DOG1* Cape Verde Island (Cvi) allele introgressed into the L*er* background at the position of the DOG1 QTL (Bentsink et al. 2006). Despite its well-documented implication in seed dormancy, *DOG1* molecular function remains unknown; however, the higher the levels of DOG1 the longer the time required for dormancy release in freshly harvested seeds (Nakabayashi et al. 2012). Diverse tests have been performed to assess the impact of these mutations on seed longevity and have shown that *rdo4* seeds have a lower resistance to CDT, and *dog1* seeds have reduced long-term storability, while these were unaffected in *rdo1*, *rdo2* and *rdo3* (Clerkx et al. 2004, Bentsink et al. 2006, Liu et al. 2007). No clear link between dormancy and longevity can, therefore, be deduced from these genetic studies.

QTLs for seed longevity and seed dormancy have been identified in various species, but, as pointed out by Nguyen et al. (2012), the possible co-location or correlation of both traits has not been reported. A recent study specifically explored the relationship between seed dormancy and longevity (germination ability after 4–7 years of dry storage under ambient conditions), by the identification of longevity QTLs using six Arabidopsis recombinant inbred line (RIL) populations previously used to identify the *DOG* dormancy QTL (Bentsink et al. 2010, Nguyen et al. 2012). Several *Germination Ability After Dry Storage* (*GAAS*) loci co-localized with *DOG* genes; however, despite the *dog1* mutant having a shorter longevity compared with the corresponding *DOG1* background, dormancy and storability were negatively correlated for the QTLs: high dormancy was correlated with low storability and vice versa. This unexpected observation was interpreted as dormancy and longevity being alternative mechanisms to extend lifespan and whose selection would depend on the natural environment. In mild and humid climates, seeds in the soil seed bank can repair damaged macromolecules during short-term imbibition and thus dormancy would be more important for adaptation in these conditions. In contrast, in warmer and drier climates, active longevity mechanisms (e.g. protection mechanisms) would be more desirable for fitness (He et al. 2014). A similar negative relationship between dormancy and longevity was observed with regard to the impact of maternal environment on these seed traits, further supporting this hypothesis (He et al. 2014). For example, low temperature conditions during Arabidopsis seed development on the mother plant were shown to increase the dormancy of NIL*DOG1* and the *cyp707a1-1* mutant, affected in ABA catabolism, and decrease their seed longevity. It is also noteworthy that the analysis of long-term-aged seeds identified additional QTLs to those identified after CDT, indicating that CDT does not completely mimic natural aging (Nguyen et al. 2012).

Further identification of natural loci involved in regulatory networks either common to or specific to seed dormancy and seed longevity would help to understand the complex interactions between these traits, which both influence the life cycle and are of economic importance in crop species.

Seed Coat Components and Seed Longevity

The seed coat represents the interface through which the embryo interacts with the external environment; thus, its composition and structure are critical factors in seed longevity, providing chemical and mechanical protection. In certain cases, the dispersed seed is surrounded by additional maternal tissue, such as pericarp fused to the outside of the testa in achenes (**Fig. 4**), which can also contribute to seed characteristics, so their impact on longevity will also be considered here. As all of these maternally derived cells are dead at the end of seed development, structure must be established and metabolites accumulated prior to cell death. On fertilization, complex differentiation processes are initiated in cells within the integuments and metabolic processes are induced for the production of key polymers. Notably, four major types of macromolecules are found whose properties impart physical and chemical resistance to the cells: polyphenols, polysaccharides, suberin and cutin.

Seed coat polyphenols: going beyond the color

Several types of polyphenols can be found in the seed coat, and the main groups are flavonoids, lignins and lignans. In Arabidopsis, the innermost integumentary layer or endothelium accumulates proanthocyanidins (PAs) also called condensed tannins (**Fig. 4**). These polymeric flavonoids accumulate in vacuoles as colorless compounds during early seed development and become oxidized into brown pigments by the laccase-type polyphenol oxidase TRANSPARENT TESTA (TT) 10 during seed desiccation (Pourcel et al. 2005). The subepidermal cell layer undergoes secondary thickening of the inner tangential cell wall and accumulates colorless to pale yellow flavonoids called flavonols (Pourcel et al. 2005). The chalazal region also undertakes PA biosynthesis in a few specific cells (pigment strand). Several lines of evidence indicate that polyphenols influence longevity. For example, the germination of Arabidopsis mutant seeds exhibiting flavonoid defects, such as *tt* mutants with modified testa flavonoid composition, was reduced compared with the wild type after long-term ambient storage and CDT (Debeaujon et al. 2000, Clerkx et al. 2004). Furthermore, yellow-seeded mutant rapeseed (*Brassica napus*) and flax (*Linum usitatissimum*) exhibited greater reductions in germination compared with dark-pigmented seeds after accelerated aging (Diederichsen and Jones-Flory 2005, Zhang et al. 2006). Flavonoids act as antioxidants and may function by scavenging ROS and thereby contribute to limiting oxidative stress. In effect, flavonols present in the testa of *Brassica rapa*

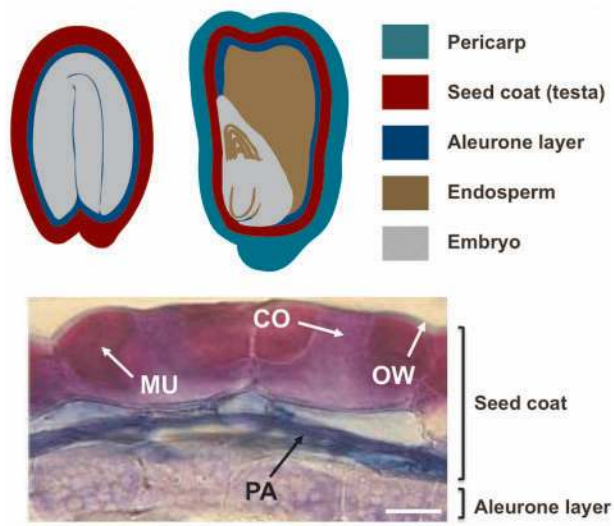

Fig. 4 Structure of maternally derived seed tissues. Schematic representation comparing the structures of an example of a true seed (upper left, Arabidopsis) and dry indehiscent fruit or achene (upper right, maize). Seed coat cell layers in a toluidine blue-stained section of a developing Arabidopsis seed when the embryo is mature (lower). Features of the epidermal cells and the endothelial cells are indicated. OW, outer primary cell wall, MU, mucilage, CO, columella formed by extensive secondary thickening around the cytoplasm, PA, proanthocyanidins. Scale bar = 10 μm.

protect the embryo from UV-B radiation (Griffen et al. 2004), probably through a combination of oxygen scavenging and a UV-B filter. In addition, PAs have been shown to induce ABA biosynthesis in developing Arabidopsis seeds and, as a consequence, *tt* mutants have less ABA in their mature seeds (Jia et al. 2012), which would also affect longevity indirectly. It has been well established that testa flavonoids are accumulated upon developmental signals (Debeaujon et al. 2000), but a recent study demonstrated that biosynthesis could also be modulated by environmental parameters. MacGregor et al. (2015) showed that seeds obtained from mother plants grown at low temperatures (16°C vs. 22°C) accumulated more PAs in the seed coat.

As well as a protective chemical arsenal, defense-related proteins, such as polyphenol oxidases or PPOs (catechol oxidases and laccases), peroxidases (PODs) and chitinases, are prevalent in the testa of Arabidopsis and soybean (*Glycine max*) (Moïse et al. 2005, Pourcel et al. 2005). The Arabidopsis TT10 laccase is present in young colorless seed coats. During seed desiccation, the oxidation of soluble PAs into quinonic compounds by TT10 might increase their capacity to bind to the cell wall, where they would have a preventive role by forming a physico-chemical protection against potential stresses. A positive correlation has been observed between PA oxidation and their cross-linking to the cell wall in pea, cotton or *Sida spinosa* seeds. During desiccation, flavonoids accumulated in seed coats are oxidized in the presence of PPOs or PODs, leading to seed coat browning and impermeability to water (Pourcel et al. 2007). The formation of antimicrobial quinones and insoluble polymers would result in the reinforcement of the seed coat as a barrier to water and oxygen permeation, mechanical damage and biotic and abiotic stresses (Pourcel et al. 2005). A systems biology approach used to identify key genes regulating the acquisition of longevity during seed maturation of *M. truncatula* and Arabidopsis has revealed many genes involved in defense (Righetti et al. 2015). Notably seeds affected in two pathogen-related transcription factors WRKY3 and NFLX1 exhibited reduced longevity, which could be related to increased testa permeability, as previously observed for *tt* mutant seeds (Debeaujon et al. 2000). Moreover, another candidate gene found by Righetti et al. (2015) was *TT7* encoding a flavonoid 3′ hydroxylase (F3′H). Transcript levels of *TT7* were not, however, significantly deregulated in the *wrky3* and *nfxl1* mutant seeds. F3′H catalyzes the transformation of the flavonol kaempferol into quercetin which has a higher antioxidant capacity, which is probably why *TT7* is also up-regulated in plants submitted to abiotic stresses such as chilling, UV-B and drought (Toda et al. 2011, Martınez-Lüscher et al. 2014). These genes confer tolerance to biotic and abiotic stress and may potentially increase seed longevity.

Lignin is a polymer of monolignol units that strengthens and waterproofs the cell wall of specialized tissue types (reviewed in Barros et al. 2015). In Arabidopsis and rapeseed, the TT10 laccase was shown to participate in seed coat lignin biosynthesis (Liang et al. 2006, Zhang et al. 2013). Previously, Debeaujon et al. (2000) had shown that the longevity of Arabidopsis *tt10* mutant seeds was significantly affected during natural aging. The lignin content in soybean testa was found to correlate with seed permeability and resistance to mechanical damage (Capeleti et al. 2005). Flax seed coats are rich in lignans, which are also thought to act as antioxidants. Although Diederichsen and Jones-Flory (2005) performed CDT on various genotypes of flaxseeds, they did not relate results to seed lignan contents, and no information is presently available with regard to the role of lignans in seed longevity.

Protecting the embryo from mechanical damage: the bodyguard

The mechanical strength of seed coat cells is determined by the composition and abundance of cell wall polymers. Certain cell layers within the maternal tissues surrounding the seed undergo structural reinforcement by secondary thickening during seed development. For example, of the five cell layers in the Arabidopsis seed coat, the two outer integument layers undergo secondary thickening (**Fig. 4**). This involves the accumulation of polysaccharides (cellulose, callose, hemicellulose and pectin), structural proteins (hydroxyproline-rich glycoproteins, proline-rich proteins and glycine-rich proteins) and polyphenols such as lignin. As mentioned above, the latter also influence cell hydrophobicity and thereby the ability of external factors to penetrate into the embryo and endosperm. The mechanical resistance of the seed coat has been associated with improved longevity, with seeds with hard coats often being long lived (reviewed in Mohamed-Yasseen et al. 1994). Furthermore, the removal of the seed coat or scarification have been documented to reduce longevity (Mohamed-Yasseen et al. 1994), and the Arabidopsis *aberrant testa shape* (*ats*) mutant that lacks two integument layers is less resistant to natural and artificial aging (Debeaujon et al. 2000, Clerkx et al. 2004). Weakening of the seed coat could affect longevity by modifying resistance to mechanical damage.

The enigma of seed mucilage in longevity

Polysaccharides are not only present as primary and secondary cell wall constituents, but in certain species are also released on seed imbibition from the outermost cell layers of the seed to form a halo of mucilage, a characteristic termed myxospermy. Although mucilage has been proposed to play diverse roles in seed physiology, none has been attributed to all types of mucilage-producing seeds and it is likely to be multifunctional. The presence of a large gelatinous mass of mucilage around the seed could provide an additional barrier to the cell layers of the integuments. The Arabidopsis *glabra 2* (*gl2*) mutant is specifically affected in the differentiation of the epidermal cells of the testa, producing insufficient mucilage polysaccharides to be released on imbibition (Rerie et al. 1994). No difference was observed in the permeability of the *gl2* testa to tetrazolium salts (Debeaujon et al. 2000), which indicates that mucilage does not affect permeability. In addition, the analysis of water uptake in mutants defective for mucilage release and/or accumulation found that the hydrogel properties of mucilage did not increase the rate of imbibition of internal seed tissues (Saez-Aguayo et al. 2014). An alternative proposition is that mucilage functions as an external water reserve that would slow down the rate of seed drying. Such prolonging of seed hydration by

mucilage would appear to play a role in maintaining seed viability in the desert plant *Artemisia*. Its physical removal from achenes resulted in lower levels of repair to γ-ray-damaged DNA when they underwent cycles of hydration and dehydration by dew deposition overnight, and this was associated with a reduced germination capacity (Huang et al. 2008, Yang et al. 2011). It will be interesting to examine mutants affected in mucilage production in other species and other habitats in order to determine if this role is widespread in myxospermous seeds.

As mentioned above, the hormonal regulation of seed coat development has recently been shown to contribute to longevity with the identification of a dominant Arabidopsis mutant, *athb25-1D*, which has improved seed longevity on aging when present in the maternal tissues (Bueso et al. 2014a). Gibberellins have previously been shown to be required for normal formation of seed coat epidermal cells, as mutants in gibberellin biosynthesis and receptors exhibit developmental defects, with reduced halos of mucilage being released on imbibition (Kim et al. 2005, Iuchi et al. 2007). As the size of mucilage halos was correlated with gibberellin contents and longevity, it was hypothesized either that gibberellin-induced mucilage production was responsible for improving longevity or, alternatively, that gibberellins improve tolerance to aging by promoting reinforcement of seed coat cell walls (Bueso et al. 2014a). Interestingly, the ABA-deficient mutant *aba1* was also reported to have reduced mucilage production (Karssen et al. 1983), indicating that in contrast to their action in other physiological processes, ABA and gibberellins might not have antagonistic effects on seed coat development.

The role of impermeabilizing waxes

Suberin and cutin are insoluble polyesters derived from fatty acids that can associate with the cell wall and reduce their permeability. In Arabidopsis seed coats, suberin is preferentially localized to the outer integuments and cutin to the inner integuments (Molina et al. 2008). In the mutants *gpat5* (*glycerol-3-phosphate 2-O-acyltransferase 5*) and *dcr-1* (*defective in cuticular ridges 1*), the synthesis of polymers typical of suberin or cutin is compromised and mutant seed coats show an increased permeability to tetrazolium salts or toluidine blue (Beisson et al. 2007, Panikashvili et al. 2009). Dormancy release by after-ripening was also longer in the *gpat5* mutant, indicating that reduced levels of these polyesters in the seed coat can modify germination characteristics. As the dormancy level can impact on viability, it could be worthwhile to examine aged *gpat5* seeds to establish how their modified permeability affects longevity. Mucilage release was also impeded in the *dcr* mutant (Panikashvili et al. 2009), and this could also have repercussions on longevity as described above. A recent study, however, found that *dcr* mucilage was released in the same manner as that of the wild type (Voiniciuc et al. 2015), suggesting variability in the manifestation of this phenotype.

Despite seed coat structure and constituents having a major impact on seed longevity, our current knowledge remains fragmentary. Nevertheless, it has already been clearly established through mutant analyses that at least some of the physicochemical characteristics of the seed coat described above increase both seed resistance to decay during storage and the level of coat-imposed dormancy. A better understanding of the genetic and molecular events taking place during testa development and differentiation will be important to improve fundamental knowledge on the important contribution of this multifunctional organ in seed longevity and open the way toward: (i) the discovery of molecular markers linked to specific parameters of testa quality, which can be used in plant breeding for the improvement of longevity; and (ii) the genetic engineering of these testa traits to fulfill requirements for seed longevity.

Concluding Remarks

Seed longevity is an important trait not only for plant life, but also for our life. In this review, we have mainly focused on four aspects affecting longevity: protection; repair; hormonal/developmental regulation in relation to dormancy; and maternal effects. Recent technological advances and the expansion of bioresources and public databases have enabled the identification of genes responsible for seed longevity. This has made a major contribution to our current understanding of the molecular mechanisms involved. Molecular-based studies will lead to improvements in the seed longevity of crop species and hence to increased agricultural productivity. Nevertheless, seed longevity is a complex trait, determined by a succession of events through seed development, storage and imbibition that are influenced by environmental conditions. The future challenge, therefore, will be to understand how these multiple factors are integrated.

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