Molecular and environmental factors regulating seed longevity

Julia Zinsmeister[1], Olivier Leprince[2], Julia Buitink[2]

1 Centre for Research in Agricultural Genomics (CRAG), Bellaterra (Barcelona), Spain,
2 Institut de Recherche en Horticulture et Semences, Agrocampus Ouest, INRA, Université d'Angers, SFR 4207 QUASAV, 49070 Beaucouzé, France

Correspondence: Julia Buitink (julia.buitink@inra.fr)

Abstract

Seed longevity is a central pivot of the preservation of biodiversity, being of main importance to face the challenges linked to global climate change and population growth. This complex, quantitative seed quality trait is acquired on the mother plant during the second part of seed development. Understanding what factors contribute to lifespan is one of the oldest and most challenging questions in plant biology. One of these challenges is to recognize that longevity depends on the storage conditions that are experimentally used because they determine the type and rate of deleterious conditions that lead to cell death and loss of viability. In this review, we will briefly review the different storage methods that accelerate the deteriorative reactions during storage and argue that a minimum amount of information is necessary to interpret the longevity data. Next, we will give an update on recent discoveries on the hormonal factors regulating longevity, both from the ABA signaling pathway but also other hormonal pathways. In addition, we will review the effect of both maternal and abiotic factors that influence longevity. In the last section of this review, we discuss the problems in unraveling cause-effect relationship between the time of death during storage and deteriorative reactions leading to seed ageing. We focus on the three major types of cellular damage, namely membrane permeability, lipid peroxidation and RNA integrity for which germination data on seed stored in dedicated seed banks for long period times are now available.

Introduction

Seeds of the vast majority of plant species terminate their development by losing most of their cellular water content and reach a state of anhydrobiosis, where cellular activities and metabolism are suspended and the cellular content is solidified. This dry state confers to the seeds the extraordinary property to escape from time and space, necessary for the dissemination of the species and underpinning global agriculture and food security as it secures the planting value for the next sowing season. The capacity to remain alive during storage, or longevity, and the benefits of keeping seeds in a dry state to ensure establishment of a new crop was recognized as early as -300 BC [1]. It has been exploited as a vehicle for the long-term *ex situ* conservation of plant germplasm in over 1750 dedicated gene banks holding altogether ca. 7.4 million accessions [2-4]. Maintaining this genetic diversity alive is necessary for breeders to produce new varieties for tomorrow's needs and constitutes an insurance policy to preserve threatened species [5]. For this purpose, seeds are stored at a constant low temperature (-20°C) and low humidity (3-7% moisture). Unlike these dry cold conditions, in the soil seeds of wild species will survive for decades while being exposed to rehydration-dehydration cycles and a range of temperatures and to pathogens. This seedbank longevity is understood as an adaptive trait to seasonality and aridity [6-9].

Understanding what factors contribute to seed lifespan is one of the oldest and most challenging questions in plant biology (reviewed by [10]). One of these challenges is to recognize that longevity depends on the storage conditions that are experimentally used because

they determine the type and rate of deleterious conditions that lead to cell death and loss of viability. Here, we will briefly review the different storage methods that accelerate the deteriorative reactions during storage and argue that a minimum amount of information needs to be provided to correctly interpret the longevity data.

Longevity is progressively acquired during seed maturation [9, 11, 12], after the induction of desiccation tolerance. Depending on the species, the acquisition of longevity can be can be spread out over several weeks after seed filling and can even still occur after harvest [9, 13]. Longevity acquisition is regulated by hormonal and maternal factors, and is under strong influence of the environment. In this review, an update of the current knowledge of these regulatory mechanisms is presented that is complementary to recent excellent reviews describing the protective factors involved in longevity [7, 9, 14, 15]. In the last section of this review, we discuss problems in unravelling cause-effect relationship between the time of death during storage and deteriorative reactions leading to seed ageing. We focus on the three major types of cellular damage, namely membrane permeability, lipid peroxidation and RNA integrity, for which germination data on seeds stored in dedicated seed banks for long period times are now available. Next to longevity, developing seeds acquire dormancy, a physiological state that blocks germination despite favorable conditions. Although genetic links have been established between both longevity and dormancy [16], the latter trait will not be further developed in this review.

Seed longevity in a nutshell

Over the past decades, it has become clear that there are numerous factors contributing to seed longevity. To provide a background for the different sections in this review, Figure 1 gives a summary of these factors. They include protective factors encompassing the seed coat, whose compounds limit the diffusion of oxygen to the embryo, a range of lipophilic and water soluble antioxidant compounds, stress proteins (Heat Shock Protein (HSP), Late Embryogenesis Abundant (LEA) proteins) with moonlight functions, RNA binding proteins that conserve mRNA in the dry seeds and non-reducing sugars (sucrose and oligosaccharides) that prevent protein denaturation, protect membranes, and participate to the formation of a glassy state (Fig. 1). Longevity depends also on mechanisms that repair genome lesions, amino acid modifications and aggregated proteins. For more detail we refer the reader to recent excellent reviews describing the protective factors involved in longevity [7, 9, 14, 15]. Longevity is a multifactorial trait, evident from the multiple genetic factors have been identified using Quantitative trait locus (QTL) and Genome-Wide Association Study (GWAS) approaches using historical data from long-term storage or conditions that accelerate seed ageing in a range of crops such as wheat [17, 18], soybean [19, 20], oilseed rape [21-23], maize [24], rice [25-27], barley [28], tobacco [29], lettuce [30] and Arabidopsis [16]. In these studies, different storage / aging protocols have been used, giving rise do different QTL, which complicates a comparative analysis of the data. In the next section, the importance of the storage protocol to assess longevity will be discussed.

‘Wet’ vs ‘dry’ ageing

Experimentally, assessing seed longevity on a tractable scale requires to increase at least one of the storage conditions that influences it, namely seed water content (or relative humidity [RH] at which seeds were equilibrated), temperature and oxygen availability. Ageing protocols used to assess the actual lifespan of the seeds and its extrapolation to colder or drier conditions are important issues. There is a wide range of temperature / RH combinations during storage conditions that are used by laboratories and a wide range of wordings to describe them, such as

ambient or natural ageing, accelerated ageing or controlled deterioration (reviewed by Hay *et al.,* 2018 [31]). This makes it difficult to compare the molecular responses to seed ageing across studies, or even within a species. Indeed, storage conditions will determine the type of ageing reactions and the underlying damage that will lead to cell death. For example, storage over water vapor at 100% relative humidity (RH) and 42-45°C will allow both the resumption of respiration as seeds are progressively imbibing and a heat stress leading to protein denaturation, whereas storage at 75% RH at 35°C does not allow respiration to occur because the cytoplasm is too viscous to allow molecular motion necessary to produce ATP. In barley, the biochemical changes occurring during loss of viability spanning over 4 years of storage in the laboratory conditions (50% RH, 20°C) were not identical to those occurring during the loss of viability at 75% RH, 45°C [32]. Similar conclusions have been reported using biophysical approaches [33] or genetic approaches [30, 34]. Therefore, great care must to be taken in extrapolating mechanisms or genes identified under conditions that accelerate ageing to conditions that will be found in the soil, seed banks or the laboratory bench.

Recently, a new ageing method was introduced to accelerate ageing by increasing the oxygen concentration during storage by elevating the pressure to 18 kPa using scuba diving tanks [35]. Whether this accelerated ageing can be construed as a means to mimic deterioration during storage in ambient conditions remains an open question. Variations in pressure during the experiment is itself detrimental. Barley seeds exposed to high nitrogen pressure for 6 weeks showed a significant number of abnormal seedlings with white shoots and no roots compared to unaged controls [36]. It is likely that a high pressure disturbs the physico-chemical and structural properties of the cytoplasmic glass as demonstrated in other glass systems. For example, in a hydrous silicate glass, an increase in pressure induces the H_2O molecules to act as a network former rather than a modifier, making the glass elastically stiffer [37]. In meat, an increased pressure decreases lipoxygenase activity [38], whereas this is known to be an enzyme that contributes to seed ageing [39, 40].

Hay and colleagues [31] recommended that seed ageing protocols should be designed based on the potential downstream use of the findings and the biological significance of longevity. In an effort to contribute towards this objective, Figure 2 presents the minimal information necessary for an unambiguous interpretation of an ageing experiment. In soil seedbanks, seeds are exposed to hydration-dehydration cycles, allowing some metabolism to occur because the cytoplasm remains fluid [7]. This could be referred to as “wet ageing”, corresponding to storage at a RH above 80% and temperatures ranging from 4 to 45°C. Also classified in this category are the “accelerated ageing” and “controlled deterioration” tests. These are two different and specific tests defined by the International Seed Testing Association as a means to evaluate seed performance during germination rather than longevity *per se* (see [31] for the detailed protocols). An intermediate storage condition that is close to ‘dry’ aging or bench storage corresponds to 75% RH and 30-35°C. These conditions will bring the cytoplasm in a highly viscous, rubbery state, with deteriorative reactions severely slowed down, but still being slightly above the glass transition temperature of the cytoplasm. Finally, we consider that storing seed at or below 70% RH corresponds to a “dry” ageing. This corresponds to conditions used in 1) gene banks where seeds are stored under constant dry conditions (<35% RH) and low temperature (4°C/-20°C) where the cytoplasm is solidified and in a glassy state with restricted molecular mobility and 2) ambient storage or storage under laboratory conditions (22-25°C, 50-70% RH). These latter conditions correspond to the limit where short scale molecular mobility is allowed but not long range slow diffusive motion (or α relaxations) [33, 41]. Typically, the upper limit of RH/temperature combinations for a dry ageing that would represent a good compromise between the above conditions and those leading to a manageable loss of viability are 60-75% RH and 35-45°C. Good correlations were obtained for longevity of seeds of the tree *Astronium fraxinifolium* that were stored at 60%, 65% and 70% RH [42]. The boundaries of what is “wet” or “dry” are difficult to set. They depend on the temperature

and are likely a continuum from short-range molecular mobility and long-range diffusive motion within the cells [33, 41, 43].

Effects of maternal tissues on seed longevity

The contribution of the maternal-derived outer tissues of the seed to its longevity can be substantial. These tissues include the seed coat that differentiated from the ovule integuments during development and, if present, the pericarp, which is derived from the ovule. The endosperm is a triploid tissue that develops into a storage organ in endospermic seeds and to a thin layer surrounding the embryo, controlling germination, in non-endospermic seeds. The endosperm has an increased dosage of the maternal genome. The importance of the endosperm in seed longevity has been shown indirectly by meta-analyses of seed traits. Within 197 species across the globe [44] and 172 Australian species [8], life span of endospermic seeds stored at 45°C and 60% RH were on average 3.3-fold lower than non-endospermic seed. The positive effect of reduced endosperm on seed longevity was recently confirmed on the panel of 39 grassland species from a single habitat [45]. Why the presence of endosperm would reduce longevity remains unknown. Floris and Anguillesi (1974) [46] concluded that in wheat, the aged endosperm had a "debilitating" effect on the aging embryo by leaking some unknown toxic substance. Intriguingly, when freshly harvested embryos were imbibed in the presence of an isolated endosperm that had first been aged for 3 years in ambient conditions, they carried more chromatin damage during imbibition than embryos imbibed in the presence of non-aged endosperm [46]. It is noteworthy that in cereals, endosperm dies during maturation drying or very fast in the dry state, probably due to insufficient protective compounds. An exhaustive -omics comparison of embryo and endosperm in rice revealed that the endosperm has no or decreased amount of polypeptides with an important role in seed longevity such as protective proteins (several LEA proteins, small HSPs, lipocalin, an antioxidant) and repair proteins (PIMT, protein-L-isoaspartate O-methyltransferase and methylsulfoxide reductase, Figure 1, [47]). Therefore, it will be an important future research goal to better understand the influence of endosperm on seed longevity.

Pioneering work by Debeaujon and colleagues [48] established that mutants of Arabidopsis seeds defective in genes regulating seed coat composition and properties exhibited decreased life span compared to wild type. The worst impact on longevity is the absence of the two integument layers in the *ats* mutant [48]. The *TT* (*TRANSAPRENT TESTA*) genes encode maternal regulators of seed coat development and enzymes involved in flavonoid synthesis. Seeds of mutants defective in these genes also have a reduced longevity when stored at ambient conditions or at high RH / temperature combinations [12, 48, 49]. Next to a decreased longevity, these mutants also exhibited an increased permeability to tetrazolium salts, suggesting that the primary role of the seed coat is to isolate the embryo from the storage environment such as oxygen and water. The origin of the increased permeability has been recently elucidated using Arabidopsis mutants that are defective in genes involved in the biosynthesis of very long chain fatty acids. It was found that seeds in these biosynthesis mutants were defective in a cutin layer between the endosperm and the seed coat [49]. In Arabidopsis, this cutin layer is only 300 nm thick and is made of abundant deposits of aliphatic polymers of glycerol esterified to long-chain fatty acids and hydroxyacids along with proanthocyanidins [49, 50]. In these mutants, wet ageing at 82% RH, 42°C induced an increase in peroxidized lipids and decreased tocopherols, suggesting that the cutin layer prevents oxygen diffusion through the embryo [49]. Whether this protection occurs during dry storage remain to be assessed. Originally thought to be of endosperm origin [49], the deposition of a cutin actually originates from the maternal inner seed coat and is deposited to the surface of the endosperm under the control of TT1 and TT16 [50, 51]. In Arabidopsis, the formation of cutin occurs very early after fertilization. It is probably triggered by a signal originating from the fertilization of the central cell under the relief of the

FERTILIZATION INDEPENDENT SEED Polycomb group repressive mechanism. Its original function is to prevent the fusion of tissues so that the seed coat is not attached to the embryo and also ensure proper embryonic growth [51]. The presence of this cutin layer may also influence the communication between the embryo and maternal tissues during development. Possibly, cutin does not act alone to provide a barrier separating the embryo from the surrounding environment. Indeed, suberin is another hydrophobic polyester located in the outer layer of the seed coat that provides impermeability to oxygen and water during seed imbibition [52, 53]. Its role in seed longevity has not been investigated yet.

Hormonal regulation and signaling pathways involved in longevity

Seed maturation and the underlying developmental programs are under the control of the so called LAFL transcriptional regulators which include the plant-specific family of B3 domain transcription factors ABI3 (ABSCISIC ACID INSENSITIVE3), FUS3 (FUSCA3), and LEC2 (LEAFY COTYLEDON2) and the LEC1 (LEAFY COTYLEDON1), a member of the NFYB protein family (see [54] for a review). In Arabidopsis, loss of function mutants of ABI3 and LEC1 produce seeds that lose their viability during the first few weeks after harvest [55], implying that these transcription factors regulate the acquisition of longevity. However, these transcription factors do not act alone in regulating seed longevity. They form complexes between them and with other proteins yet to be identified and act redundantly [54, 55], making it difficult to unravel their precise role in seed longevity.

Downstream of ABI3 are several genes that are implicated in seed longevity (Figure 1), determined mostly by wet ageing. They include the seed specific *HEAT SHOCK FACTOR A9* (*HSFA9*), controlling the developmentally regulated expression of *sHSP* [56]. This transcription factor is part of the genetic program controlling resistance again a deterioration induced by incubating wet seeds (28% moisture) for 2 days at 50°C [57, 58]. Likewise, induction of Mt-*HSFA9* expression was also correlated with the acquisition of longevity both in *M. truncatula* [59] and in soybean [60]. In Arabidopsis, ABI3 also regulates seed degreening via the induction of *STAYGREEN* [61], a process also associated with the acquisition of longevity. By introgressing loci from different Arabidopsis ecotypes into *abi3-5* and *lec1-3* lines and restoring longevity, Sugliani et al. 2009 [55] showed that ABI3 and LEC1 regulate directly or indirectly and in a redundant manner a set of protective proteins such as small HSP and LEA proteins as well as storage proteins that are associated with longevity [11, 62].

An additional layer of complexity in the regulation of longevity involves alternative splicing of *ABI3* mRNA. In developing Arabidopsis seeds, the relative abundance of transcripts encoding the full-length *ABI3* decreases between 14 and 20 days after pollination (*i.e.* dry mature seeds), concomitantly with the acquisition of seed longevity. Also, during that period, transcripts of a spliced form, *ABI3-β*, increases and becomes preponderant in dry seeds [65]. ABI3-β encodes a truncated protein that contains two of the four functional domains (A1 and B1), but whose function in seed maturation is unknown. This splicing event is controlled by SUPRESSOR OF ABI3-5 (SUA1 to SUA4), a RNA binding protein that is involved in seed longevity. Arabidopsis seeds of double mutant *sua1 abi3-5* exhibited a longevity during dry storage that was similar to wild type seeds, and restored the reduced longevity phenotype found for *abi3-5* seeds [63].

During maturation, ABI3 is not the only gene to be alternatively spliced. Twenty percent of the genes expressed between 14 and 20 Days after pollination (DAP) are alternatively spliced and enriched in Gene Ontology (GO) functions associated with mRNA catabolic processes [64]. Alternative splicing affects a higher percentage of genes at 20 DAP compared to 14 DAP, possibly representing a mechanism that allows the rapid end of seed maturation once desiccation is achieved. Considering that spliced forms of ABI3 have been detected in seeds of many species (see references in [64]), alternative splicing might represent an important and

conserved form of longevity regulation that deserves further attention. Taking advantage of the slow acquisition of longevity in the developing legume seed *M. truncatula*, a conditional-dependent network of global transcription interactions revealed distinct co-expression modules related to the successive acquisition of desiccation tolerance and longevity [59]. The desiccation tolerance module was associated with abiotic stress responsive genes (typically LEA proteins), whereas the longevity module was enriched in genes involved in RNA processing and translation [59], in concordance with an increased splicing activity at the end of maturation [64]. This approach was taken a step further by incorporating into the network the environmental effects on the acquisition of longevity in Medicago and genes co-regulated with the acquisition of longevity in Arabidopsis [12] (Figure 3). While 85% of the genes in the module associated with the acquisition of desiccation tolerance were deregulated in Mt-*abi3* mutants, less than 50% of the genes present in the longevity module were found to be downstream targets of Mt-ABI3, suggesting that these genes are regulated via alternative pathways that remain to be elucidated.

Another player of the ABA signaling pathway, ABI5, has also been shown to be an important regulator of longevity in legumes such as *M. truncatula* and pea seeds [65]. In both species, seeds of *abi5* mutants were desiccation tolerant but exhibited a 40 to 60% reduction in longevity during dry storage (75% RH) compared to wild-type. ABI5 is a b-Zip transcription factor of the ABF (ABRE-BINDING FACTOR) family that in response to ABA regulates many genes involved in conferring stability in the dry state such as genes encoding LEA proteins and Raffinose Family Oligosaccharide (RFO) synthesis [65-67]. It also regulates the seed antioxidant status because compared to wild type seeds, Mt-*abi5* seeds were more sensitive to oxygen during storage and exhibited an altered tocopherol profile [65]. Interestingly, both in legumes and in Arabidopsis, ABI5 regulates photosynthesis and chlorophyll metabolism genes [65, 68]. As a result, in *abi5* mutants of pea and Medicago, mature seeds retained significant amounts of chlorophylls [65, 68]. In oily chlorophyllous seeds, the presence of chlorophyll in mature seeds is an undesirable trait leading to reduced seed vigor and longevity [61, 69, 70]. In Arabidopsis, *abi5* seeds did not exhibit a longevity phenotype or a green seed phenotype [65], probably because of the action of redundant bZIP transcription factors homologous to ABI5, altogether forming the ABF family [67, 68].

While in Arabidopsis, ABI5 is acting downstream of ABI3 during seed germination to regulate growth arrest upon drought stress [71], it remains to be established whether this is the case during seed development or whether ABI5 acts independently of ABI3 [66]. In *M. truncatula*, Mt-*ABI5* expression was not deregulated in Mt-*abi3* mutants [59]. Furthermore, in this species, ABI3 and ABI5 have complementary roles in inducing desiccation tolerance [72]. In Arabidopsis, ABI5 appears to be regulated by DOG1 (DELAY OF GERMINATION 1) [67]. Seed transcriptomes of these mutants showed an intriguing overlap, with genes involved in longevity being deregulated such as the molecular chaperone genes encoding LEA proteins and HSP. Consistent with this, *dog1* mutants exhibited a reduced life span when stored under dry laboratory conditions [73]. Recently, major progress has been made in elucidating the regulatory role of DOG1. DOG1 is a heme binding protein that interacts with ABA HYPERSENSITIVE GERMINATION1 (AHG1, a group A type 2C protein phosphatases (PP2C)) to trigger an ABA response and dormancy [74]. This signaling pathway seems to act in parallel with the core ABA signaling pathway upstream of ABI3. This scenario was demonstrated in the modulation of seed dormancy in response to temperature. Whether a similar scenario can be envisaged for longevity remains to be assessed. A genetic link with DOG1 and ABI3 in the regulation of seed longevity is supported by the observations that in Arabidopsis, the *dog1-1* mutant enhances *abi3-1* phenotypes and phenocopies a severe reduction in longevity and chlorophylls retention in seeds of stronger *abi3* alleles [67]. Therefore, *DOG1*, *ABI3*, *ABI5* and homologous *ABF* genes form an important module that regulates the acquisition of

longevity in Arabidopsis. A DOG1 domain-containing protein exists in the genome of *M. truncatula* and it would be interesting to investigate its function in longevity.

While evidence is secured that longevity is regulated by the components of the ABA signaling cascade, a direct role of ABA metabolism in the acquisition of longevity is still unclear. Studies on hormone synthesis and degradation in Arabidopsis show that effects on seed longevity were only reported for *aba1-5* seeds, with a significant loss in viability observed after 4 years of storage [75]. However, two *Brassica oleracea* genotypes with allelic differences at two QTLs that result in differences in ABA content did not differ in their longevity when stored at 75% RH and 40°C [76]. Likewise, Arabidopsis mutants of ABA degradation (*cyp707a1*, c*yp707a2* and *cyp707a3*) were not affected in seed longevity as determined by a wet ageing at 80-85% RH, 37 -40°C [77, 78]. However, the role of ABA metabolism in longevity needs probably to be assessed by taking into account the environmental conditions during seed maturation. When stored at approximately the same conditions (85% RH, 40°C), seeds of the double mutants *nced6 nced9* affected in ABA biosynthesis died faster than wild type if seeds were grown at 15°C but no difference was found between then genotypes at 25°C [78]. Also, a recent exhaustive study on ABA metabolism in developing seeds of *aba-2* mutants defective in ABA synthesis demonstrated the accumulation of ABA glucosyl ester, suggesting an alternative route of ABA synthesis [79].

Auxin is another hormone that is involved in the regulation of the acquisition of longevity [12, 58, 80]. A pioneering work on identifying the regulator of HaHSA9, a seed specific heat shock factor involved in the resistance against deterioration during wet storage, led to the isolation of the sunflower AUXIN-RESPONSIVE PROTEIN 27 (HaIAA27), a short-lived transcriptional repressor of early auxin response genes. HaIAA27 repressed *HaHSFA9* and the subsequent accumulation of sHSP via direct interaction [58]. An auxin resistant form of the sunflower HaIAA27 was able to repress the activation of the *sHSP* promoter by HaHSFA9 in mature bombarded tobacco embryos and its ectopic overexpression resulted in poor germination after 2 days of storage at 28% moisture, 50°C, comparable to HSFA9 loss of function lines [58]. Our own recent work also demonstrated a direct link between auxin signaling and the acquisition of longevity [80]. This study was based on the discovery of a conserved gene module related to seed longevity (Figure 3) that contains genes that are significantly enriched in the *cis*-regulatory element ARFAT, an auxin response factor (ARF) binding site [12]. In addition, this module contains *CYP79B2,* a gene involved in a minor auxin biosynthesis pathway (*i.e.* indole-3-acetaldoxime pathway, IAOx) [81, 82]. During maturation of Arabidopsis seeds, auxin signaling input and output increased and spread progressively throughout the embryo, concomitant with the acquisition of longevity. When auxin was supplied exogenously during maturation, seed longevity increased. Consistent with this, longevity of seeds of single mutants defective in the different auxin biosynthesis pathways was altered in a dose-response manner depending on the level of auxin signaling activity, with lower signaling activity coinciding with decreased longevity [80]. The downstream effect of this auxin signaling was associated with the ABA signaling pathway in the embryo. The expression of *ABI3* and its LEA protein target *EARLY METHIONE1* (*EM1*) were induced by auxin and deregulated in the auxin biosynthesis mutants. Furthermore, the beneficial effect of exogenous auxin during seed development on longevity was abolished in *abi3-1* mutants. Besides the ABA signaling pathway, auxin might also directly regulate genes involved in longevity. Indeed, other longevity-associated genes that are not regulated by ABI3 were also affected in their expression downstream of the auxin signaling [80].

The role of gibberellins in seed longevity has also been suggested but needs to be further secured. Mutants of the transcription factor *HOMEOBOX25* (*ATHB25*) are more sensitive to wet ageing at high temperature [85]. ATHB25 positively regulates GA synthesis and reinforces the seed coat. The positive role of gibberellin in resistance against deterioration was further demonstrated by the increased resistance of seeds from GA_3-treated plants or the quintuple

DELLA mutant [83]. However, like for the studies on HSFA9, a wet ageing was used in this study, corresponding to an imbibition at 42°C at 100% RH. In addition, seeds were incubated for 4 days at 4°C before testing seed viability. Considering that such cold treatment releases dormancy, decreases ABA content and induces GA synthesis, it remains to be assessed whether a similar conclusion would have been reached if *athb25* seeds were aged under dry conditions without a 4°C imbibition. In contrast to the above observations, *ga1-3* mutant defective in gibberellin synthesis and the gibberellin insensitive *gai* mutant did not exhibit a decreased germination after 4 years of dry storage compared to wild type [75].

Recently, it emerged that brassinosteroids (BR) could modulate negatively seed longevity during priming, a controlled imbibition and drying treatment that improves germination performance [77]. After priming, seeds of the BR-deficient mutants *cyp85a1/a2* and *det2* showed significantly longer longevity than the wild type. It was suggested that the loss of longevity in primed seed is due to increased seed coat permeability, which is positively regulated, at least partly, via BR signaling. Whether this hormone is also related to maturation-induced acquisition of longevity needs investigation. There is no report mentioning the involvement of other hormones in longevity, apart from the observation that the *ethylene-resistant1* (*etr1*) and *jasmonic acid-resistant1* (*jar1-1*) mutant seeds did not show any significant viability loss after storage for 4 years [75].

Environmental regulation of longevity during seed maturation

Longevity, like many other seed traits such as seed filling and dormancy, is influenced by the abiotic environment that is experienced both by the mother plant and the zygotic tissues. Conditions that influence longevity include light, temperature, drought, and salinity (Table 1), with temperature and water availability seeming to be the dominant factors. For crops, most of our knowledge is inferred from works based on wet ageing conditions that were used to assess the planting value of the seed, whereas for wild species a wider of range of dry storage conditions were employed. Our current understanding of the environmental impact on longevity and how it affects the molecular factors contributing to longevity is fragmentary but known to strongly involve genotype x environment interactions. Environmental conditions can be experienced by the seed or the mother plant as a constraint that limits or slows down the developmental programs leading to the acquisition of longevity [6, 12, 84]. There are also significant variations in seed longevity in relation to the climate or environmental conditions for which the species is adapted. A longevity survey of 197 wild species stored at 60% RH, 45°C showed that seeds originating from species collected in hot, dry environments tend to have a longer lifespan from those found in cool, wet conditions, regardless of the phylogenetic relatedness, suggesting that species have adapted to dry climates by increasing seed longevity [44]. Concordant with this, variations in longevity were found in Silene species originating from differing climates, with a longer life span for Mediterranean accessions found in dry, hot habitats compared to alpine accessions [85]. A similar variation of longevity of Arabidopsis seeds stored under wet ageing conditions (85% RH, 35°C) was observed using DOG near isogenic lines that are the result of adaptations to local environmental conditions [78]. At a local geographic scale, environmental characteristics can also influence longevity. Plants of *Silene vulgaris* growing in warm environments produced longer-lived seeds from those from colder environments [6]. All these studies led to the conclusion that longevity might be construed as a long-term adaptation to the dry, arid environment, with the consequence that it would exert a cascading selective pressure on future generations. Whether a similar hypothesis can be posited for crops remains to be assessed. Limited information suggests that it might not be the case, at least for barley. In a survey of longevity in 175 accessions of barley including landraces and cultivars from Europe, Africa, Asia and Australia, genotypic variation in seed longevity was independent of the geographical origin of the accession or its breeding status [34].

Alternatively, intraspecific variations in longevity might represent phenotypic plasticity mediated by the mother plant as the response to the environment. This interpretation is supported by experiments showing that it is possible to decrease or increase longevity by manipulating the environment, such as temperature and drought in greenhouse conditions for *M. truncatula* [12] or growing Silene mother plants in garden outside their natural habitat [6]. Another strong argument in favor of plasticity is the observation made by [86] on the parental influence on longevity of *Wahlenbergia tumidifructa* seeds, a semi-arid species. When mother plants were submitted to cold and wet conditions before returning to optimal conditions after fecundation, longevity doubled compared to seeds obtained from mother plants grown under warm dry conditions as prezygotic conditions. Understanding the causal genes implicated in long term adaptation and/or plasticity of longevity and whether transgenerational effects are at play represent an exciting new field of investigation.

Temperature

Temperature can affect longevity positively or negatively in a species- and genetic-dependent manner. Warm temperatures during seed development of alpine species and Arabidopsis tend to increase longevity during dry storage [87-89]. In contrast, warm temperatures negatively affected seed longevity in rice [84] and *M. truncatula* [12]. Low temperature during seed development led to a reduction of seed longevity in Arabidopsis [78] but had no effect on *M. truncatula* [12], probably because this species is adapted to such conditions.

How a change in temperature imparts changes in seed longevity is not understood but likely implies mechanisms both from the mother plant and the zygote. MacGregor and colleagues [90] showed that cold temperature influences the phenylpropanoid composition and permeability of the seed coat in Arabidopsis, a maternal tissue for which defective mutants showed decreased longevity [48]. We mentioned earlier that DOG1 affects longevity in Arabidopsis [67]. In fact, DOG1 is mostly known for its function as a temperature sensor that modulates seed dormancy (references in [91]). DOG1 protein levels vary according to seed-maturation temperature, and mutations in this gene compromise the ability of the seeds to enter dormancy in response to low maturation temperatures. An exciting possibility would be that a similar mechanism could be acting to regulate the longevity response to temperature.

Water stress

Drought can be beneficial or detrimental to seed longevity according to the species and intensity of the water deficit. In soybean, a water stress during seed maturation led to mature green seeds, a trait leading to decreased longevity [70, 92]. A similar effect of drought was found on seeds of *M. truncatula*, although seeds did not show a retained chlorophylls phenotype [12]. In contrast, in *Brassica rapa*, arresting irrigation during early seed filling fastens the acquisition of seed longevity, resulting in mature seeds with a longer lifespan during dry storage [93, 94]. Similarly, in peanut, a drought stress during seed development enhanced seed longevity [95]. It is likely that water availability will have a different impact on the longevity depending on the stage of seed development because developing seeds can modulate their maturation to regain to some extent the longevity that was lost due to the water availability. This is suggested by field experiments on wheat for which rainfall was simulated by wetting the ears at different stages of development or decreasing rainfall by providing shelter [96]. Wetting ears for 30 min at all stages of seed development increased the seed water content by 5-10% only but led to reduced longevity after harvest (tested under dry storage). However, these seeds could regain considerable longevity when wetting was suspended and seeds were able to re-dry *in planta* for several days [96], highlighting the plasticity of the development program leading to longevity. The physiological causes for this behavior remain to be understood, but since ABA signaling is involved in chloroplast dismantling during seed maturation that in turn is affected by water

stress in soybean [70], ABA signaling might be important for longevity in response to water stress.

Light

During seed filling and despite the presence of surrounding layers of tissues, embryos are green and can perceive 20 to 30% of light, which is enriched in green and far red wavelengths [97]. Photosynthesis is necessary to provide NADPH and ATP for seed reserve deposition. For this purpose, chloroplasts are adapted to these shade conditions. They display a high Chla/Chlb ratio and photosystem I activity is enhanced at the expense of photosystem II [97]. During the later phase of maturation, and concomitant with the acquisition of longevity, chloroplasts are dismantled and chlorophyll molecules degraded *via* mechanisms different from those in senescing leaves [98, 99]. During this phase, light may actually be detrimental for these chlorophyllous embryos. Seeds of many species in which traces of chlorophylls remain at harvest or due to gene defects in chlorophylls degradation exhibit a decreased shelf life during wet [98] and dry storage [65, 67, 69]. While Li *et al.* [98] provide some evidence for photodamage, the role of light and chlorophylls during storage are not yet elucidated because these longevity phenotypes are also observed on seeds that are stored in the dark [65]. Perhaps, the detrimental effects of light on seeds with retained chlorophylls occur during seed maturation rather than during storage or imbibition. Despite the shade conditions provided by the seed layers, control of photochemical reactions during chloroplast dismantling induced by maturation drying might be necessary to avoid the buildup of toxic compounds that could jeopardize longevity. In support of this hypothesis, mutants defective in the assembly of the cytochrome b_6f complex or wild type seeds treated with DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea), a specific inhibitor of photosystem II activity, display a reduced lifespan during storage at 75% RH, 40°C [97].

Another clue that light might be important for longevity acquisition comes from a study on the effect of photoperiod on longevity of lettuce seeds, a species displaying photosensitivity during germination [100, 101]. Seeds produced under red-rich light conditions exhibited reduced longevity during storage at 30°C and 74% RH compared to seeds produced under far red light [100]. In Arabidopsis, light intensity but not photoperiod had a positive effect on longevity, and it was suggested that this might regulate RFO accumulation [102]. This suggests that developing seeds integrate the light signal to modulate longevity but how they do so remain unknown. In Arabidopsis, light perception involves the underlying genes of DOG3 and DOG6 loci, since seeds of the near-isogenic lines NILDOG3, NILDOG6 produced under different light conditions exhibited an altered longevity compared to the *Landsberg erecta* (Ler) ecotype [78]. In the future, cloning the genes underlying DOG3 and DOG6 loci should reveal the regulatory function of light intensity on longevity.

Nutrient availability

Nutrient availability in the soil and the status of macro- and microelements in the mother plant can affect seed yield components. Likewise, nitrate, phosphate and sulfate availability also impact various traits associated with germination performance such as dormancy in a complex interaction between genotype and other environmental conditions such as temperature and light, as shown for tomato [103], Arabidopsis [78] and oilseed rape [102-104]. Therefore, it is expected that longevity might be affected as well, although this aspect has not received much attention. In barley, longevity assessed by intermediate ageing conditions (75% RH) was higher in seeds produced from plants cultivated with an adequate nutrient supply (P, K, N, Mg and organic matter) compared to those from plants grown with a lower nutrient supply [34]. In the Arabidopsis Ler ecotype, longevity also depended on the N supply to the seeds, higher nitrate leading to longer life span when aged at 82% RH. The effect was also observed with Col for high N supply, but to a lesser extent [78]. As a result of a low N supply, contents in amino acids

and glucoronate were affected in mature dry seeds together with gene transcripts impacted in cell wall metabolism, which led the authors to suggest that the C/N balance was impacted. Interestingly, the transcriptome and metabolome of seeds grown without nitrate overlapped with those of seeds produced at 15°C [102], leaving the possibility open that temperature might directly impact the C/N balance.

Another evidence suggesting that the C/N balance is important for longevity is provided by data showing that altering metabolic sensors during seed development impacts longevity. So far, two metabolic sensors have been identified for which seeds from mutants showed decreased resistance against ageing. The first one is the Target of Rapamycin (TOR) complex [105]. In Arabidopsis, mutants of the REGULATORYASSOCIATED PROTEIN OF TOR 1B (RAPTOR1B), a conserved TOR interactor, showed a decrease in germination after one year in ambient, cold storage as well as decreased resistance against wet ageing [105]. Decreased longevity was associated with changes in hormone profiles including ABA and auxins, a reduction in sugar contents, changes in secondary metabolite profiles and seed coat composition [105]. A second sensor is the Sucrose non fermenting Related Kinase 1 (SnRK1) complex [106]. In developing *M. truncatula* embryos, silencing *MtSNF4b*, a seed-specific regulatory γ-subunit of the SnRK1 complex, led to reduced longevity during dry storage concomitant with an 80% decrease in RFO and a 60% increase in sucrose content [106]. Both sensors have crucial roles in nutrient and energy sensing and translate this information into metabolic and developmental adaptations.

The continuing challenge of assessing causes of seed ageing and future perspectives

During the past decade, our knowledge of the fate of lipids, proteins and nucleic acids during seed ageing has greatly improved. However, a causal relationship between accumulation of damage with time of death during storage remains difficult to assess, making it complicated to pin-point the origin of the deterioration during storage and to trace back the overall effect to a particular set of genes. First, when cell death occurs at some point during seed storage, this does not mean that deteriorative reactions stop. Secondly, the relative contribution of viable and non-viable individuals (seeds) or cells (within a seed) to the amount of damage incurred by the seed population remains also difficult to establish. Thirdly, it is still unknown whether deteriorative reactions occur randomly during storage and are governed by stochastic processes, or whether there are specific compounds that are degraded during storage, leading to a reproducible deteriorative path [107]. To illustrate these issues, we review three major types of cellular damage, namely membrane permeability, lipid peroxidation and RNA integrity, for which germination data on seed stored in dedicated seed banks for long period times are now available.

Membrane permeability

Using EPR spectroscopy, Golovina and colleagues [108] were able to assess membrane damage *in vivo* on a seed to seed basis in wheat seeds stored over an 18-years period. In this experiment, membrane damage was assessed by measuring the plasma membrane permeability after storage in individual embryonic axis using a method that is based on the differential permeability of the plasma membrane to a nitroxide spin probe and a polar broadening agent. The amphipatic spin probe freely crosses membranes and partitions between the hydrophobic environment of the phospholipid bilayer and hydrophilic cytoplasm, whereas the broadening agent ferricyanide, which cannot penetrate membranes, erases the cytosolic signal of the probes, leaving only the lipid signal. Model membrane systems demonstrated that the ratio between lipid and cytosol signals allowed for a quantitative assessment of membrane damage, with a high R value indicative of a nonpermeable and therefore intact membrane. Figure 4A shows that in a

population of embryonic axes from seeds germinating at 100%, R values are distributed around 8, probably reflecting the variability of cell types [108]. Concomitant with time of storage and decreased viability, the frequency distribution shifted towards lower values, indicative of increased membrane damage. However, in the dead seeds that were stored for another 9 years, the frequency distribution continued to shift to lower values, suggesting that membrane damage had continued to progress (Figure 4A).

A frequency distribution was also found in histone/DNA ratios of wheat at two storage intervals [109]. A decrease in the ratio was found in the seed lot that was retrieved after 6 years compared to 4 years of storage (Figure 4B). These authors attributed the change in ratio to histones, since nuclei were arrested in the pre-synthetic phase of the mitotic cycle (G1). However, the distribution of the ratio from both seed batches overlapped, despite the contrasting germination capacity of the seed lots (Figure 3B). It is worth investigating whether changes in this frequency distribution of damage could be described quantitatively using the population-based threshold model [110] that considers the diversity of individuals that have a determinate shelf-life during storage.

Lipid peroxidation

Our current view is that seed deterioration during storage is a result of a stochastic oxidation cascade that produces peroxides, hydroperoxides, carbonyl and nitrosyl groups that further react with neighboring molecules leading to further oxidation, fragmentation and formation of adducts. Propanal, butanal, and hexanal, which are downstream products of lipid peroxidation have been shown to be progressively produced in legume seeds during storage [111, 112]. In pea seeds, the amount of propanal and hexanal first increased up to 12 days of storage at 50°C prior to the loss of viability, then decreased by half after 25 days while viability decreased to 80% [112]. This observation gives rise to two possible interpretations: 1) complex oxidative reactions within lipids leading to accumulation of alkoxy radicals do not lead to death or 2) only a limited number of critical cells need to be damaged by lipid oxidation to cause loss of viability. In contrast, in *Lathyrus pratensis*, no apparent significant change in hexanal content was found whereas half of the seed population died during storage. Upon further aging, when 90% of the seeds had died, hexanal content increased 20-fold. Similar observations were made in seeds of lettuce, argulla and caraway [111, 113]. No obvious correlations were found between rate of ageing and apparent increase in byproducts of lipid peroxidation. Furthermore, seed ageing was not affected in a mixture of seeds emitting different amounts of these compounds, suggesting these compounds are a consequence of deterioration rather than the cause [113].

The role of lipid oxidation in seed deterioration has gained momentum from detailed analyses of the changes of the lipidome during long term dry storage of a range of genotypes of oilseed rape wheat, barley and Arabidopsis [3, 39, 40]. These authors documented complex changes in the lipidome and oxylipidome after storage, involving lipids both from oil reserves and membranes and originating both from enzyme activities and non-enzymatic degradation. In general, during long-term storage, hundreds of singly and multiply oxidized lipids of various classes increased with decreased germination after storage, following first- or second-order kinetics, reinforcing the role of oxidation and oxygen as a key factor governing seed longevity [40, 42, 114]. However, [42] pointed out that some of these oxidized lipids were already highly abundant before the onset of loss of viability, making it impossible to untangle a cause-effect relationship with the primary events triggering loss of viability. Such relationship might also be obscured by the impact that these degraded lipids might have during seed imbibition and germination. Mobilization of storage oil such as triacylglycerols appears to be more important for seedling establishment than germination [115]. Therefore, the level of oxidized triacylglycerols might not be that critical for seed germination after storage. In contrast, subtle changes in membrane phospholipid composition during storage might strongly influence its physico-chemical properties upon rehydration, leading for example to increased susceptibility

to imbibitional injury and cell death [116-118]. Further work is therefore needed to understand what triggers the pathways leading to lipid oxidation and hydrolysis and how it affects seed germination after ageing.

RNA integrity

Seed ageing during dry storage is also associated with RNA degradation, for which a complex picture starts to emerge. Correlation of ribosomal RNA integrity with storage time or storage temperature was significant for a range of species over a 50 years period, making it an interesting tool to quickly assess seed ageing [119]. However, like for lipid degradation and membrane damage, loss of rRNA integrity occurred both before and during the loss of germination, and there was not a single threshold RIN value for all species for either high viability or death. Likewise, RIN values could not distinguish germinable from nongerminable soybean seeds [119]. Perhaps, the translational machinery is very well protected in dry seeds and very low RIN values are symptoms of post-mortem decay.

Dry seeds store large amount of mRNAs as they will rely on them to synthesize proteins early during imbibition to ensure germination [120-122]. Their number range from ca. 12.000 in Arabidopsis to > 17.000 in rice, and include gene products from all different categories, but with an over-representation of genes involved in metabolism and protein synthesis (ribosomal proteins, translation initiation and elongation factors). To further understand the fate of RNA in dry seeds, Flemming et al. [119] assessed the degradation of stored mRNA in soybean seeds during dry storage using Minion technology that allows for sequencing both full-length and fragmented transcripts. They compared cohorts of seeds that were 2 years old and germinated to 100% with those that had been stored for 23 years and for which the viability declined from 61% to 80% one year prior to RNA sequencing. Four patterns of degradation were detected during long-term storage. Two categories consisted of transcripts of various lengths that degraded progressively over the 23 years storage, whereas a third category was represented by transcripts of different lengths that were equally degraded before and after storage, suggesting a fraction of degradation occurred during drying or soon after drying [119]. The fourth category was constituted by the shortest transcripts that remained intact even when seeds died. Interestingly, these transcripts might offer internal standards to normalize changes in the transcript population during seed ageing. Thus, this work offers a rich and unique set of markers that will be helpful to understand the link between seed ageing, particularly during the so-called asymptomatic phase when storage does not yet induce the loss of viability. In parallel, we need to further explore the translational efficiency of these degraded transcripts during seed imbibition, which will be a challenging task. In healthy Arabidopsis seeds, the degree and dynamics of translational regulation exhibited two temporal phases associated with seed rehydration and preparation for germination during imbibition [122]. This regulation was associated with unique mRNA features such as transcript length, secondary structure and specific motifs. It is noteworthy that in stored soybean seeds, the transcripts that remained intact during seed ageing were related to ribosomal functions, highlighting the importance of the translational machinery during germination [120, 121]. Yet the translational machinery remains sensitive to seed ageing [120], which might have causes unrelated to RNA. Dry seeds Arabidopsis knockout lines for the elongation factor eIF(iso)4G1 and eIF(iso)4G2 showed a severe reduced longevity when stored over one year in the laboratory [123]. This suggests that transcription elongating factors contribute to the survival in the dry state. Currently, the cause of transcript fragmentation and how it leads to loss of seed germination is unknown. Oxidation is strongly suspected but the chemistry remains to be elucidated. In sunflower, oxidized mRNA in the form of 8-hydroxyguanosine was found to increase by 50% during a storage period that did not kill the seeds but instead released dormancy [124]. This oxidation was not detected using total RNA, reinforcing the idea a differential sensitivity to ageing according to the RNA species. In contrast, in soybean there was no correlation between the amount of 8'OH mRNA

and with loss of viability [119]. It should be noted that like RNA, DNA integrity is also modified during storage, especially during oxidative and UV stress exposure. These damages must be overcome during germination to avoid the mutation to be spread to the offspring [15, 125]. To do so, many DNA repair proteins have been found to play a role in seed longevity in the past decades, and has been extensively reviewed by Waterworth and colleagues [15].

Being able to discriminate non-destructively in a cohort of stored seeds those that are alive and already dead would constitute a powerful tool to further understand how deterioration during aging leads to seed death during storage. The recent developments in imaging analysis, machine learning and statistics look promising developments. Multivariate analysis of spectral signatures of single cowpea seeds extracted from multispectral images after storage was able to classify seed cohorts with reasonable accuracy (68 to 98%) into different categories as aged vs. non aged, viable vs. nonviable and fast vs slowly germinating seeds [126]. However, the ageing conditions that were used were 98% RH and 45°C. These conditions are not only far away from those found in seed bank storage conditions but are permissive for respiration and protein denaturation. It remains to be assessed whether a similar predictability can be obtained from seeds stored in more appropriate storage conditions, close to the glassy state, where metabolism has stopped and molecular mobility is severely restricted.

Concluding remarks

Here, we have synthetized our knowledge on the environmental effects and signaling pathway regulating seed longevity. The main conclusion is that longevity is a highly plastic seed trait that has served species to be adapted to their environment. This is clearly established in wild species. Yet, there is a dearth of studies aiming at identifying the molecular factors that modulate longevity in response to the environment. Such knowledge would be pivotal to further understand its role as an adaptive trait. Both the mother plant and the zygote are able to regulate longevity during seed development in response to the environment, probably by interfering with ABA and auxin signaling pathways as well as metabolic regulation. Next to efforts in unravelling the molecular factors contributing to seed lifespan, the future challenge is now to understand the gene x environment interactions that govern the acquisition of longevity in crops. For this, there is a need to better describe in the environmental conditions used to grow mother plants and reconcile the storage protocols used to determine seed longevity and bring them to consensus.

Abbreviations

ABA, abscisic acid; ATP, adenosine triphosphate; BR, brassinosteroids; DAP, days after pollination; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DNA, GA, gibberellic acid; EMB, Embryogenesis; GWAS, Genome-Wide Association Study; HSP, Heat Shock Protein; LEA, Late Embryogenesis Abundant; NADPH, Nicotinamide Adenine Dinucleotide Phosphate; QTL, Quantitaive Trait Locus; RH, relative humidity; RFO, Raffinose Family Oligosaccharides; sHSP, Small Heat Shock Protein; TT, transparent testa; WC, Water content.

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Author contribution
JZ, OL and JB conceived and wrote the manuscript.

Competing interest
The Authors declare that there are no competing interests associated with the manuscript.

Figure Legends

Figure 1. Seed longevity in a nutshell.
Summary of the intrinsic and external factors contributing to seed lifespan during storage. ATM, ATAXIA TELANGIECTASIA MUTATED, HSP, heat shock protein, LEA late embryogenesis abundant protein, LIG6, LIGASE 6, MSR: methylsulfoxide reductase, PIMT, protein-L-isoaspartate O-methyltransferase, PM25: a LEA protein from the SMP family. RFO: Raffinose Family oligosaccharides. Pictograms corresponding to know environmental effects on longevity are respectively (light, water availability, temperature, nutrients). ABA, abscisic acid; AIA, auxins; DOG1, DELAY OF GERMINATION1; GA, gibberellins.

Figure 2: Information necessary for an unambiguous interpretation and reproduction of an ageing experiment to test longevity.
Knowledge of any drying treatment after harvest or pre-equilibration treatments before storing the seed lots is necessary to determine whether longevity is assessed during water sorption (*i.e.* mature seeds are imbibed to higher water content then stored) or desorption (*i.e.* mature seeds are dried to lower water content then stored [127]). Wet ageing corresponds to storage conditions above 80% relative humidity (RH) whereas dry storage corresponds to RH below 75% RH.

Figure 3. Desiccation tolerance and longevity specific gene coexpression modules identified by a gene regulatory network analysis during seed development of *M. truncatula*.
Nodes represent genes that are either upregulated during maturation in parallel to the acquisition of desiccation tolerance (DT, green) or with the acquisition of longevity, measured as the P50, the time for 50 percent of the seed population to die during storage (blue). Within each module, large squares represent transcription factors and circles, other hub genes. The upper network represents genes that are confirmed targets of MtABI3 whereas the bottom network corresponds to genes that are not regulated by MtABI3. Data are from Righetti et al. (2015) [12] and reproduced with permission ([128]. Copyright 2018, Taylor and Francis). EMB, embryogenesis. WC, water content.

Figure 4. Distribution of the progress of damage symptoms during wheat seed ageing in dry storage.
A) Distribution of membrane permeability values (R) calculated by electron paramagnetic resonance (EPR) spectroscopy in individual embryonic axes within seed lots retrieved at indicated time of storage (Y, years) and exhibiting different percentage of germination (G). For each individual axis, the R value represents the ratio between the EPR signals of a guest probe present respectively in the hydrophilic and the hydrophobic compartments of the cell in the presence of a broadening agent. The broadening agent which is water soluble cancels the signal emitted by the probe molecules that are localized in the hydrophilic compartment. Since this agent does not permeate through intact membranes, high R value indicates a partitioning of the

EPR probe between the cytosol and lipids whereas a low (or nil) R value is due the loss of the membrane integrity, allowing the broadening agent to exert its cancelling effect on the EPR hydrophilic signal. Data are from [109]. B) Distribution of histone/DNA ratios measured by histochemistry on the individual 2C nuclei from the root meristem of squashed individual axis from seed lots stored for 4 and 6 years (Y). DNA content was assessed by microscope photometry at 535 nm after staining with the Feulgen method. The same nuclei were thereafter stained with fast-green FCF and scanned for histones at 635 nm. Data are from [109].

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Table 1. Summary of studies reporting on the effect of environment on seed longevity.

Environment	Species	Aging method	Effect on seed longevity	Reference
Temperature	Arabidopsis	75%RH, 35°C and 85% RH, 40°C	Cold temperature during seed maturation negatively impacts seed longevity	12, 78
Temperature	*Orysa japonica, Oryza indica*	15% RH, 40°C	In three rice cultivars, potential longevity is higher in cool compared to warm temperatures	84
Temperature	Alpine snowbed species	60% RH, 45°C	Higher seed longevity for plants exposed to warmer temperatures compared to natural conditions	88, 89
Nutrient	*rdeum vulgare*	60% RH, 45°C and 100% RH, 43°C	Seeds from the field of lower nutrients supply display both lower resistance to AA and CD tests	34
Light	*Lactuca sativa*	74% RH, 30°C	A long day treatment increases seed longevity compared to short day treatment	100, 101
Wet/dry	*Brassica rapa*	15% RH, 40°C	Potential longevity is higher when optimal irrigation stops before the end of seed maturation	93, 94
Wet/dry	*Arachis hypogaea L.*	50-71% RH, 30°C	Drought stress during seed development enhances seed longevity	95
Wet/dry	*Triticum aestivum*	15% RH, 40°C	Dry environment during seed development enhances seed longevity	96
Wet/dry	*Glycine max*		Heat and drought during development decreases vigour and leads to stay green phenotype	70
Wet/dry x temperature	*Silene suecica* alpine and subartic populations	60% RH, 45°C	Seeds grown in dry and warm environment display higher seed longevity	85

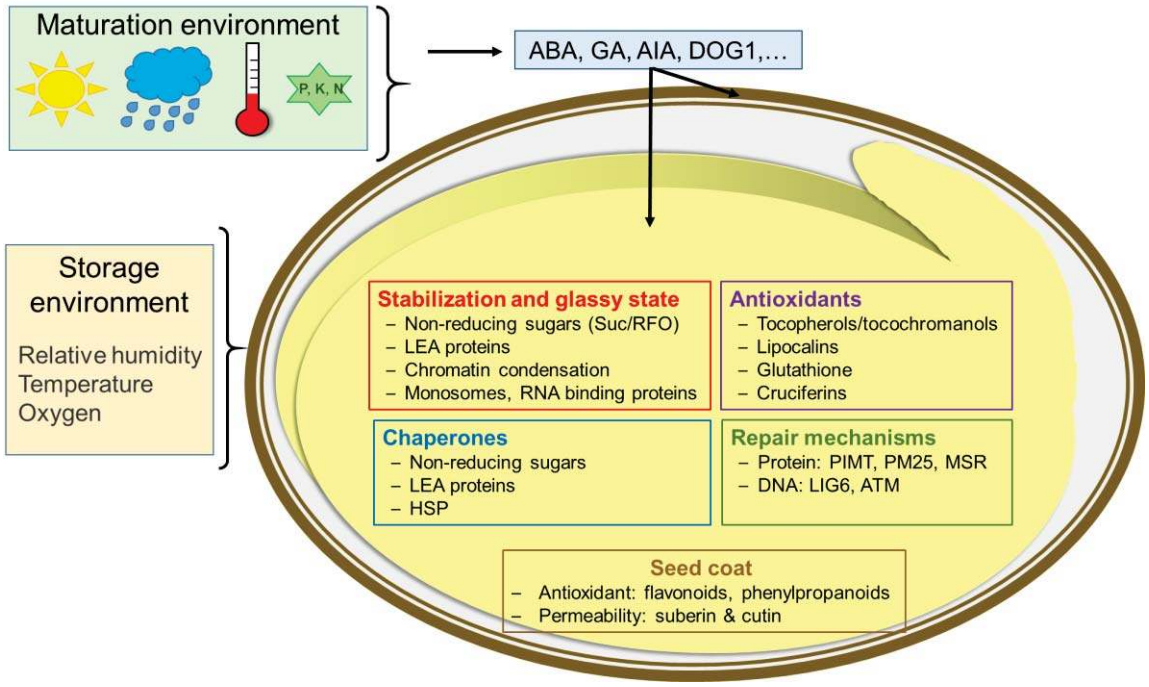

Figure 1. Seed longevity in a nutshell.
Summary of the intrinsic and external factors contributing to seed lifespan during storage. ATM, ATAXIA TELANGIECTASIA MUTATED, HSP, heat shock protein, LEA late embryogenesis abundant protein, LIG6, LIGASE 6, MSR: methylsulfoxide reductase, PIMT, protein-L-isoaspartate O-methyltransferase, PM25: a LEA protein from the SMP family. RFO: Raffinose Family oligosaccharides. Pictograms corresponding to know environmental effects on longevity are respectively light, water availability, temperature, nutrients. ABA, abscisic acid;; AIA, auxins; DOG1, DELAY OF GERMINATION1; GA, gibberellins.

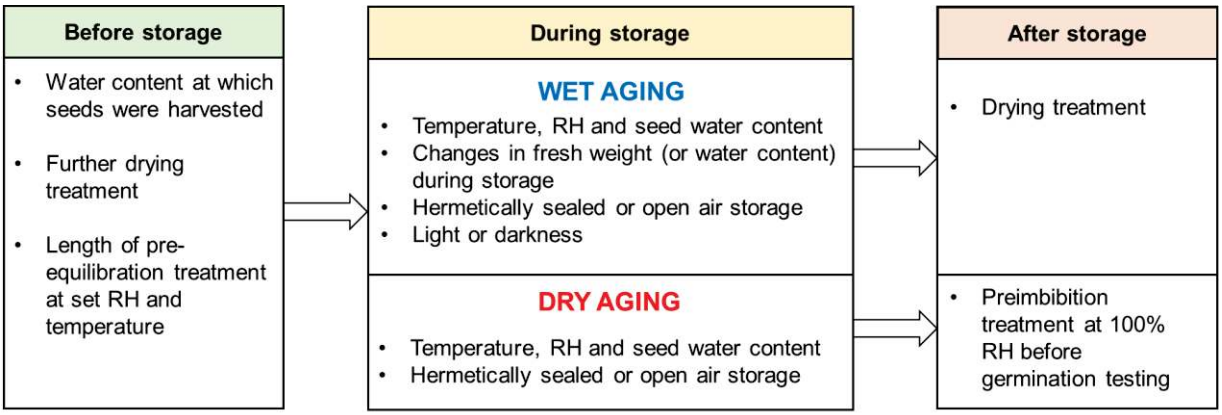

Figure 2: Information necessary for an unambiguous interpretation and reproduction of an ageing experiment to test longevity.
Knowledge of any drying treatment after harvest or pre-equilibration treatments before storing the seed lots is necessary to determine whether longevity is assessed during water sorption (i.e. mature seeds are imbibed to higher water content then stored) or desorption (i.e. mature seeds are dried to lower water content then stored) [130]). Wet ageing corresponds to storage conditions above 80% relative humidity (RH) whereas dry storage correspond to RH below 75% RH.

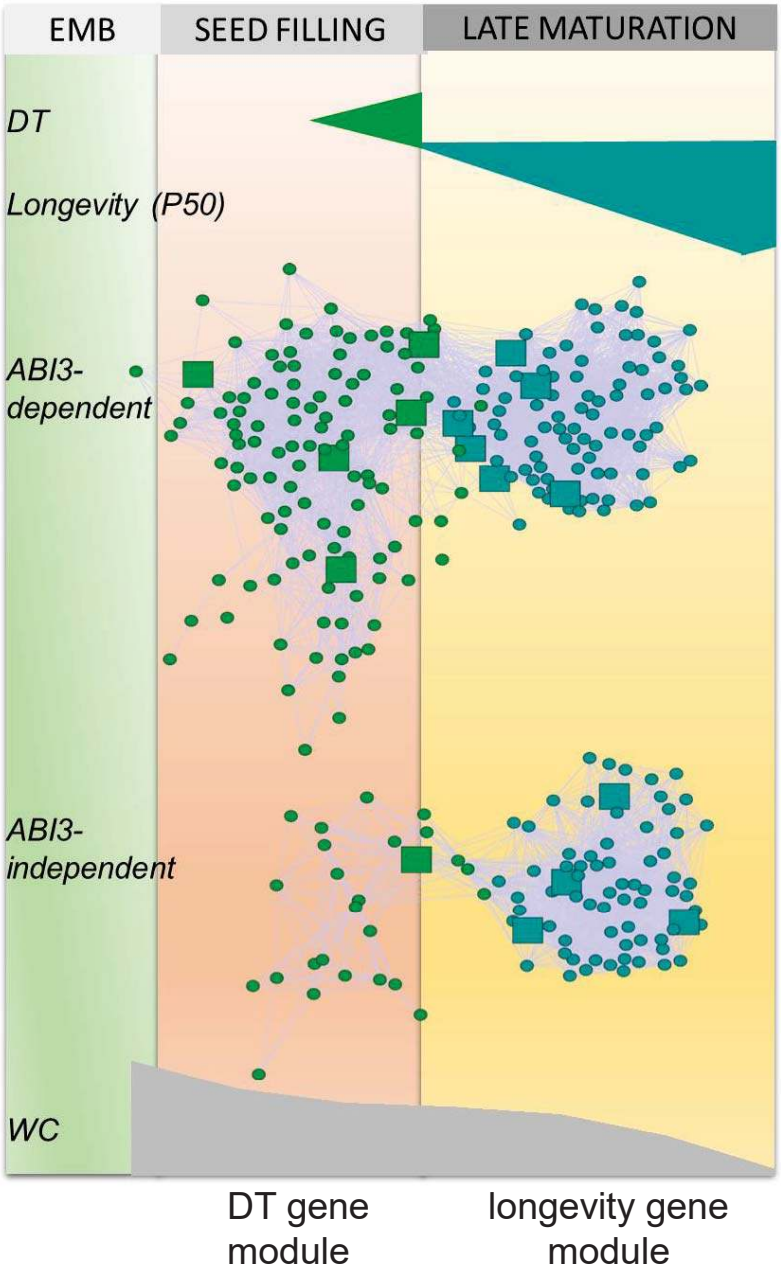

Figure 3. Desiccation tolerance and longevity specific gene coexpression modules identified by a gene regulatory network analysis during seed development of *M. truncatula*.
Nodes represent genes that are either upregulated during maturation in parallel to the acquisition of desiccation tolerance (DT, green) or with the acquisition of longevity, measured as the P50, the time for 50 percent of the seed population to die during storage (blue). Within each module, large squares represent transcription factors and circles, other hub genes. The upper network represents genes that are confirmed targets of MtABI3 whereas the bottom network corresponds to genes that are not regulated by MtABI3. Data are from Righetti et al. (2015) [12] and reproduced with permission ([128]. Copyright 2018, Taylor and Francis). EMB, embryogenesis. WC, water content.

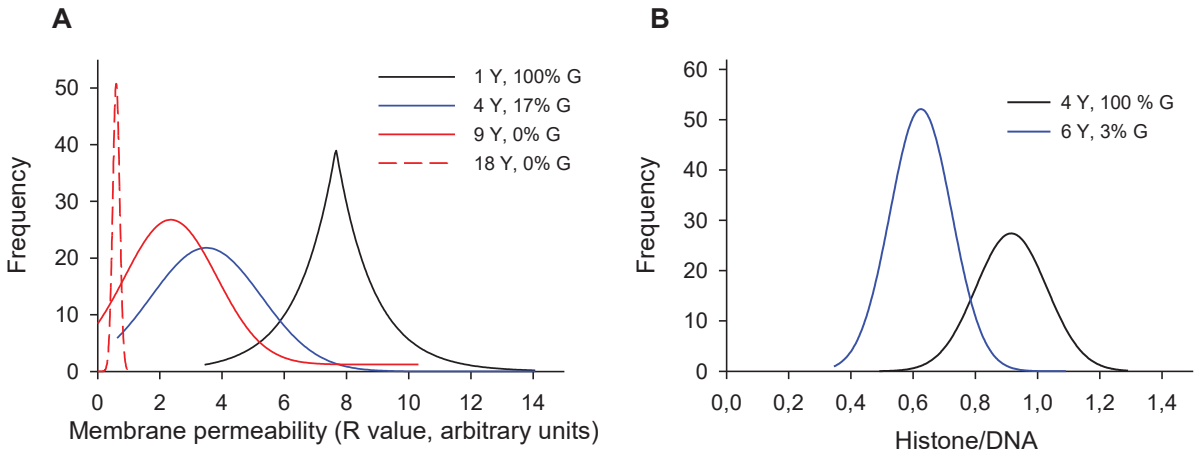

Figure 4. Distribution of the progress of damage symptoms during wheat seed ageing in dry storage.

A) Distribution of membrane permeability values (R) calculated by electron paramagnetic resonance (EPR) spectroscopy in individual embryonic axes within seed lots retrieved at indicated time of storage (Y, years) and exhibiting different percentage of germination (G). For each individual axis, the R value represents the ratio between the EPR signals of a guest probe present respectively in the hydrophilic and the hydrophobic compartments of the cell in the presence of a broadening agent. The broadening agent which is water soluble cancels the signal emitted by the probe molecules that are localized in the hydrophilic compartment. Since this agent does not permeate through intact membranes, high R value indicates a partitioning of the EPR probe between the cytosol and lipids whereas a low (or nil) R value is due the loss of the membrane integrity, allowing the broadening agent to exert its cancelling effect on the EPR hydrophilic signal. Data are from [110]. B) Distribution of histone/DNA ratios measured by histochemistry on the individual 2C nuclei from the root meristem of squashed individual axis from seed lots stored for 4 and 6 years (Y). DNA content was assessed by microscope photometry at 535 nm after staining with the Feulgen method. The same nuclei were thereafter stained with fast-green FCF and scanned for histones at 635 nm. Data are from [109].