seeds

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Review

The Effects of Storage Conditions on Seed Deterioration and Ageing: How to Improve Seed Longevity

Françoise Corbineau

Biologie des Semences, UMR7622 Biologie du Développement, IBPS, Sorbonne-Université, 4 Place Jussieu, F-75005 Paris, France; francoise.corbineau@sorbonne-universite.fr

Abstract: Seeds are classified as either: orthodox, seeds that tolerate dehydration; recalcitrant, seeds that are high in moisture content and cannot withstand intensive desiccation; or intermediate, seeds that survive dehydration but die during dry storage at low temperatures. Seed lifespan depends on the seed category and also varies from one species to another. The rate of loss of vigor and viability of orthodox seeds depends mainly on temperature and seed moisture content (MC); the lower the MC and storage temperature, the longer the longevity. Ultimately, storage in liquid nitrogen or seed ultra-drying by well-adapted processes should allow for long-term storage. The ageing of orthodox seeds is associated with numerous forms of cellular and metabolic damage (membrane integrity, energy metabolism, and the impairment of DNA, RNA, and proteins) in which reactive oxygen species play a prominent role. Interestingly, priming treatment can reinvigorate aged seeds by restoring the antioxidant systems. The storage of recalcitrant seeds is very difficult since they must be placed in a wet medium to avoid dehydration and at temperatures low enough to prevent germination but warm enough to avoid chilling injury. A better understanding of the mechanisms involved in ageing is necessary to identify markers in order to estimate seed longevity.

Keywords: seed longevity; orthodox; recalcitrant and intermediate seeds; ageing; regulation of ageing; markers of ageing; seed banks

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1. Introduction

Seeds are remarkable organs that are essential in the life of humans and animals since they ensure plant reproduction. They are the natural forms of preservation of higher plants, designed to perpetuate the species through the germination process. They are also used as direct sources of food or in various industries such as oil, starch, and flour manufacturing and malting. Seed storage is the best technology for preserving and conserving plant biodiversity. For agronomical use, seeds must be stored under conditions that maintain high seed quality (i.e., viability and vigor) [1–7].

Decline in seed viability and seed quality (i.e., germinability and vigor) depends on their tolerance to dehydration and is regulated by three important factors: seed moisture content in equilibrium with the relative humidity of the atmosphere, the storage temperature, and the gaseous environment [1,4–12]. For the majority of the species classified as orthodox (i.e., seeds that tolerate dehydration [13]), a study found that seed longevity increased when reducing the seed moisture content and decreasing the temperature [1,2,7,10,11,14,15]. However, for species classified as recalcitrant, i.e., seeds that do not tolerate dehydration [13,14,16], seed longevity is generally short—from a few weeks to a few months. Such seeds must be stored at high moisture content (between a 20 and 70% fresh weight basis) and at a temperature between 7 and 17 °C and −3 and 5 °C for species of tropical origin and those of temperate climate origin, respectively [17].

Oxidative damage due to ROS' (reactive oxygen species) reactivity towards cell macromolecules, including proteins, sugars, lipids, and nucleic acids, has been demonstrated

to be associated with ageing [1,3,7,12,18–25]. However, a better understanding of the cellular, biochemical, and molecular mechanisms associated with ageing could lead to the identification of new markers of the loss of viability during storage.

The objectives of this review are (1) to indicate the lifespan of seeds described in the literature, (2) to characterize the effects of environmental factors (temperature, moisture content, and oxygen) on seed longevity in order to extend seed viability and to propose storage conditions to improve seed longevity, and (3) to better understand the cellular, biochemical, and molecular mechanisms associated with ageing, which could suggest markers of loss of viability during storage.

2. Biological Categories of Seeds

There exist two main biological types of seeds that have been termed "orthodox" and "recalcitrant" seeds [13]. The majority of seeds referred to as orthodox require desiccation tolerance during their development, allowing them to be stored for long periods under air-dry storage [3,26]. On the other hand, recalcitrant seeds have a high moisture content at shedding and do not tolerate desiccation; therefore, they are able to be stored in a dry state [18,27–29]. More recently, a third category of seeds, named "intermediate", has been defined [30,31]; they survive the loss of water, but they become damaged and die during dry storage at low temperatures. *The Compendium of Information on Seed Storage Behaviour* [17,32] recognizes these three biological types.

"Orthodox" seeds generally undergo dehydration prior to shedding; they are desiccation tolerant and can be stored successfully in a dehydrated state at a temperature below freezing for very long periods (decades or longer). At maturity (or harvest), they contain no more than 10–15% water and survive drying to very low moisture content (3–5%). They therefore tolerate subsequent storage at sub-zero temperatures [33]. Orthodox seeds acquire desiccation tolerance relatively early during their development and usually before the maturation drying phase. Several metabolic changes occur with respect to the protection of seed cells against dehydration damage [18,26,33–35]. In particular, carbohydrate metabolism [36,37] and specific proteins (dehydrins, late embryogenesis abundant proteins: LEA, and heat shock proteins: HSPs) [33] seem to be involved in this process. Some soluble sugars, such as sucrose and oligosaccharides (raffinose, stachyose, and verbascose), might also play an important part in this process by facilitating the stabilization of lipids and proteins in cell membranes or by promoting the vitrification of water and then the protection of cytosolic structures [35,37,38].

"Recalcitrant" seeds are desiccation intolerant. They are highly hydrated at shedding and do not survive drying below a moisture content between 30–65% depending on the species [39]. They include seeds mostly produced by tropical or subtropical species from fruit crops: *Litchi chinensis* (litchi), *Euphorbia longan* (longan), *Garcinia mangostana* (mangosteen), *Mangifera indica* (mango), and *Nephelium lappaceum* (rambutan), species used as beverages: *Cacao theobroma* (cocoa) and *Coffea robusta* (coffee), plantation crops: *Hevea brasiliensis* (rubber), *Elaeis guineensis* (oil palm), and *Cocos nucifera* (coconut), and many timber species belonging to the Dipterocarpaceae family (Table 1) [27,39–41]. They also concern temperate species such as *Quercus* spp. (oak), *Juglans* spp. (walnut), *Castanea* spp. (chestnut), *Corylus avellana* (filbert), and *Salix* spp. (willow) (Table 1).

These species are highly hydrated at shedding and cannot survive drying below a certain critical moisture content [28,42] (Table 2, [43–60]). Sensitivity to dehydration is often different for the whole seed and isolated embryos. For example, seeds of *Araucaria angustifolia* die when the water content decreases to 25% [46], while isolated embryos die when the water content decreases to about 40% [47]. In natural conditions, these seeds rapidly die if they cannot germinate as soon as they fall to the ground. Such seeds cannot be stored by means of conventional methods and place constraints on our ability to store them, both in the long term for germplasm conservation and in the short term for seed trade or storage from one season to the next [61,62].

Table 1. Examples of recalcitrant seeds from temperate and tropical species. Modified from [27,39–41].

Origine	Species	Family
Temperate	*Acer saccharinum*	Sapindaceae
	Acer pseudoplatanus	Sapindaceae
	Aesculus hippocastanum	Hippocastanaceae
	Castanea spp.	Fagaceae
	Corylus avellana	Corylaceae
	Juglans spp.	Juglandaceae
	Quercus sp.	Fagaceae
	Populus spp.	Salicaceae
	Salix spp.	Salicaceae
Tropical	*Araucaria* spp.	Araucariaceae
	Avicenia marina	Avicenniaceae
	Camellia sinensis	Theaceae
	Cocos nucifera	Arecaceae/Palmaceae
	Euphorbia longan	Euphorbiaceae/Sapindaceae
	Garcinia mangostana	Clusiaceae/Guttiferae
	Hevea brasiliensis	Euphorbiaceae
	Hopea odorata	Dipterocarpaceae
	Litchi chinensis	Sapindaceae
	Mangifera indica	Anacardiaceae
	Nephelium lappaceum	Sapindaceae
	Persea americana	Lauraceae
	Shorea roxburghii	Dipterocarpaceae
	Shorea talura	Dipterocarpaceae
	Symphonia globulefera	Guttifereae
	Theobroma cacao	Steruliaceae

Table 2. Examples of recalcitrant seeds and the moisture content below which they die. Modified from [43–60].

Species	Minimum Water Content (% of Dry Matter)	References
Acer pseudoplatanus (European sycamore)	30–45	[43,44]
Acer saccharinum (silver maple)	30–35	[45]
Araucaria angustifolia (Parana pine)	25–35	[46,47]
Clausena lansium	33–35	[48,49]
Euphorbia longan (longan)	25–30	[50]
Hevea brasiliensis (hevea)	20–25	[51]
Hopea odorata	20–25	[52]
Litchi chinensis (litchi)	20–30	[48,50,53]
Mangifera indica (mango tree)	30–35	[50,52,54]
Quercus petraea (sessile oak)	30–60	[55]
Quercus robur (pedunculate oak)	30–48	[56,57]
Quercus rubra (Red oak)	60–75	[55]
Shorea roxburghii	17–30	[52]
Symphonia globulifera	37–40	[52,58]
Theobroma cacao (cocoa tree)	45–50	[59,60]

Another characteristic of recalcitrant seeds, in particular seeds of tropical origin, is that many of them are chilling sensitive [63]. Table 3 shows that tropical recalcitrant seeds of *Hevea brasiliensis*, *Hopea odorata*, *Mangifera indica*, *Shorea roxburghii*, and *Symphonia globulifera* do not tolerate temperatures below 12–15 °C, while seeds of *Dryobalanops aromatica* and *Shorea talura* are less sensitive to low temperatures and tolerate 5 °C. In the case of temperate species, the high moisture content of the seeds does not allow storage at negative temperatures. Sycamore seeds are completely killed at a temperature below −26 °C, while acorns of pedunculate oak with a moisture level of 40–45% fresh weight basis are killed at −7–−9 °C. Suszka et al. [55] recommend −3 °C to maintain viability and avoid germination. The storage temperature must not be below −3 °C for silver maple and sycamore.

Table 3. Examples of cold-sensitive recalcitrant seeds and the lowest temperature limit that they can tolerate. From [62,63].

Species	Temperature Limit (°C)
Cedrela odorata (Spanish cedar)	10
Dryobalanops aromatica (Bornean camphol tree)	5
Hevea brasiiensis (rubber)	15–16
Hopea odorata (Chengal pasir)	12
Mangifera indica (mango tree)	12
Shorea roxburghii (Lac tree)	12–15
Shorea talura (Jalari tree)	4
Symphonia globulifera (Buckwax tree)	15

"Intermediate" seeds: The term intermediate is used for seeds that do not behave exactly like orthodox or recalcitrant seeds. They tolerate a greater degree of dehydration than typical recalcitrant seeds but are less tolerant to dehydration than usual orthodox seeds. When dry, such seeds lose viability more rapidly at 0 °C or −20 °C than at warmer temperatures such as those around 15 °C [30,31]. *Coffea* spp. [30,31], oil palm (*Elaieis guineensis*) [64], *Carica papaya* [65], and *Azadirachtaidica* (neem) [66] seeds belong to this category. It is important to underline that there is wide variation in the desiccation sensitivity of intermediate seeds that has been evaluated or quantified by the water content at which half of the initial viability is lost, i.e., within the genera Coffea [67,68].

The repartition of the three categories of seeds (orthodox, intermediate, and recalcitrant) depends on the family. At the family level, the percentage of recalcitrant seeds varies from 77.1–80.0% in the Lauraceae and the Fagaceae to less than 2% in the Melastomataceae, with it being around 65.4% in the Sapotaceae, 42.1–48.8% in the Clusiaceae and the Moraceae, and 25.8–31.0% in the Arecaceae and Sapindaceae [1,69]. All of these families also present orthodox seeds: 3.8% (Sapotaceae), 17.4% (Fagaceae), 27.8% (Aracaceae), 50–51.2% (Maraceae and Rutaceae), 57.9% (Clusiaceae), 63.1% (Sapindaceae), and 83.9% (Myrtaceae). The majority of angiosperms (Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Ericaceae, Gentianaceae, Poaceae, Ranunuculaceae, Scrophylariaceae, and Solanaceae) have 95–100% orthodox seeds [69]. The intermediate seeds characterize some species such as the Arecaceae, the Rutaceae, the Sapotaceae, and the Zingiberaceae [69].

3. The Lifespan of Seeds

The lifespan or longevity of seeds varies greatly depending on the species; some survive for very long periods of time in the soil or under ambient conditions whereas others die rapidly—surviving less than 3 years. Ewart [70] in a treatise entitled "On the longevity of seeds" arbitrarily divided seeds into three classes according to their period of survival under natural conditions: macrobiotic, mesobiotic, and microbiotic seeds.

Macrobiotic seeds are defined as seeds capable of surviving for more than 15 years in the soil or under ambient conditions, but seeds with longevity exceeding 50 or 100 years are far from rare. They are often hard seeds (i.e., seeds with seed coats impermeable to water). This has been shown by Becquerel [71] who succeeded in germinating very old seeds from the collections of the Natural History Museum of Paris and estimated their longevity as 55 years (*Melilotus lutea*), 63–68 years (*Cytisus austriacus, Lavatera pseudo-olbia, Ervum lens,* and *Trifolium arvense*), 81–86 years (*Mimosa glomerata, Cytisus biflorus,* and *Astragalus massiliensis*), and a period of 100–158 years (*Cassia multijuga* and *Dioclea pauciflora*) [1]. Evidence from hundreds to thousands of years of seeds has been described [1]. Lotus (*Nelumbo nucifera*, the Indian lotus) seeds are also well known for their longevity of several hundred years [1]. For example, Dum [72] also achieved the germination of *Chenopodium album* and *Spergula arvensis* seeds that were about 1700 years old. The literature reveals that the record is held by *Lupinus arcticus* seeds which successfully germinated despite being more than 10,000 years old [73]. However, such long longevity must be regarded with skepticism without direct dating of the seeds [1,34]. Radiocarbon dating allows us to determine the age of seeds at about 2000 years for the date (*Phoenix dactylifera*) [74], at 1300 years for lotus (*Nelumbo nucifera*) [75], and at 600 years for canna (*Canna compacta*) [76]. The myth concerning the longevity of mummy grains discovered in Egyptian tombs and supposed to remain able to germinate is in fact a mistake or a hoax [1,34].

Mesobiotic seeds have a lifespan of 3 to 15 years. The great majority of the species fall under this category [1,77]. They include species with a longevity of 3–5 years, such as rape, bean, pea, carrot, cyclamen, and nasturtium, or longer (5–15 years) such as celery, sugar-beet, cabbage, chicory, and cereals (wheat, oat, and barley).

Microbiotic seeds survive at most 3 years under natural conditions. All recalcitrant seeds (see Table 1) and orthodox oleaginous seeds exhibit such short longevity. In this group, we can cite seeds of vegetables (onion, leek, fennel, and parsley) or ornamental species (dahlia, delphinium, petunia, and viola) [1,77,78].

Although this classification gives information concerning the putative survival behavior of numerous species, it is debatable because it does not take into account the main factors of storage (temperature and seed moisture content). Indeed, seed survival is both genetically and environmentally controlled. Depending on the conditions of storage, microbiotic seeds could become mesobiotic or macrobiotic ones.

4. Loss of Seed Viability

4.1. Change in Viability during Storage: Viability Equations

At harvest, the initial seed viability is a product of the seed history through development on the mother plant [1,34]. Subsequent seed longevity depends on post-harvest treatments (drying, cleaning, sorting, coating, etc.) and the conditions of storage (temperature, moisture content, and oxygen availability) [34]. The viability equations are based on fitting a negative cumulative normal distribution to viability percentages. The conversion of the negatively sigmoidal curve obtained to probits linearizes the curve [1,2,34,78] (Figure 1).

Roberts and Coll [13,79–81] developed and generalized a descriptive equation, known as the "viability equation", taking into account the conditions of seed storage on the loss of viability:

$$v = Ki - p/10^{(KE - (Cw \times \log m) - (CH \times t) - (CQ \times t2))}$$

where v represents the probit % germination after p days of storage; Ki is the probit of initial germinability for the seed lot; K_E, C_w, C_H, and C_Q are constants depending on the species; m is the seed moisture content (expressed on a fresh weight basis); and t is the storage temperature in °C.

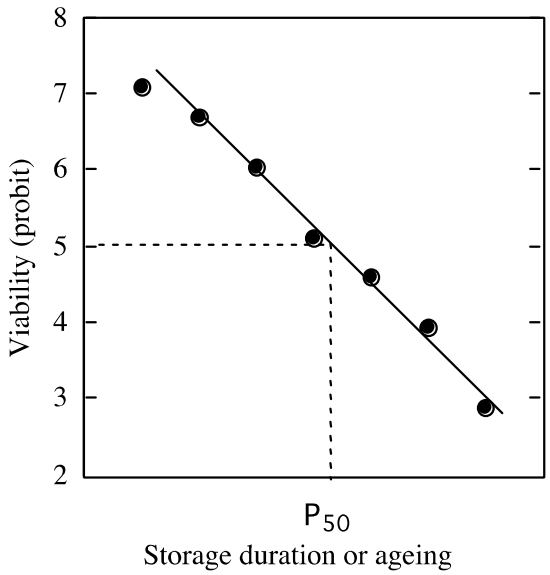

Figure 1. Theoretical curve of loss of viability in probit during dry storage. A probit value of 5 determines the half-viability period. From [77].

This equation indicates that longevity increases as the storage temperature decreases. Dickie et al. [82] working on eight species (barley, chickpea, cowpea, soybean, elm, mahogany, terb, and lettuce) placed over a wide range of storage conditions (temperature from −13 to 90 °C; moisture content from 1.8 to 25% fresh weight) demonstrated that the temperature coefficients (C_H and C_Q) of the equation do not differ significantly between these species, with them being equal to 0.0329 and 0.000478, respectively, i.e., the effect of temperature on longevity appears to be similar. In contrast, the effect of moisture content on longevity evaluated by the coefficient C_W differs between species [83–85]. This variation in C_W results in part from differences in seed composition; for example, the higher the seed oil content, the lower the value of C_W [1,2]. Data obtained by Ellis et al. [84,85] indicate that longevity is doubled for each 8.4–8.7% reduction in seed equilibrium in relative humidity between 90 and 10% [2].

Roberts [13,79] derived a simple mathematical equation, allowing us to calculate the P_{50} value or half-viability period.

$$\text{Log } P_{50} = K_v - C_1 m - C_2 t$$

where P_{50} is the time taken for 50% of the seed population to lose viability, m is the moisture content expressed on a fresh weight basis, t is the temperature in °C and K_v, and C_1 and C_2 are constants. Figure 1 shows a theoretical curve of loss of viability in probit during ageing, and Table 4 gives some examples of an estimated half-viability period of seeds from various cultivated species stored under open storage conditions in a temperate climate with the mean temperature at about 10 °C and the average RH at about 60–75% [1,86]. Under these conditions, the P_{50} value varies from 3.4–4.1 years (parsley, parsnip, and celery) to 10.5–15.9 years (lucerne, French bean, garden pea, and broad bean) and 24.5 years for tomato. Generally, seeds containing a high amount of starch (e.g., cereals) can be stored well (7.2–12.9 years), but this is not the case for rye (4.5 years). Seeds containing high levels of oil are often considered to be relatively short-lived, but this link between the amount of seed oil and P_{50} is debatable: for example, tomato seeds that contain a high level of oil (more than 30%) have high longevity (P_{50} = 24.5 years) whilst onion seeds that are poor in oil (often less than 10%) are difficult to store (a P_{50} of about 5.4 years). On the other hand, oily seeds such as soybean and sunflower are characterized by a short P_{50} (3.4 and 5.4 years, respectively), while the P_{50} of rape seeds is about 13.9 years.

It is often difficult to compare the data concerning seed longevity across species and seed lots because the conditions of storage are different depending on the laboratory. For example, when seeds were stored at 5 °C with 5 ± 2% water in the USDA National Seed Storage Laboratory, the P_{50} calculated [86] was higher than that obtained in open storage (as indicated in Table 4). It reaches 53 years for *Helianthus annuus* (sunflower), 36 years for *Secale cereale* (rye), 30 years for *Glycine max* (soybean), 22 years for *Lactuca sativa* (lettuce), and 18 years for *Allium cepa* (onion) and is longer than 80 years for *Medicago sativa* (lucerne), *Pisum sativum* (garden pea), and *Lycopersicon esculentum* (tomato).

Table 4. Estimated half-viability period (P_{50}) of some cultivated species. The seeds are stored under open storage conditions in a temperate climate where the mean temperature is 10 °C and the average RH is about 60–75%. Modified from [1,77,86].

Type of Species	Species	P_{50} (Years)
Cereals	*Avena sativa* (oat)	12.9
	Hordeum vulgare (barley)	7.2
	Triticum aestivum (wheat)	7.6
	Secale cereale (rye)	4.5
	Zea mays (corn)	9.6
Legumes	*Glycine max* (soybean)	3.4
	Medicago sativa (lucerne)	10.5
	Phaseolus vulgaris (French bean)	15.9
	Pisum sativum (garden pea)	15.8
	Vicia faba (broad bean)	15.6
Other crops	*Beta vulgaris* (beet)	16.5
	Brassica napus (rape)	13.9
	Helianthus annuus (sunflower)	5.4
	Nicotiana tabacum (tobacco)	10.3
	Allium cepa (onion)	5.4
Vegetables	*Apium graveolens* (celery)	4.1
	Cichorium intybus (endive)	5.4
	Cucumis sativus (cucumber)	4.9
	Daucus carota (carrot)	6.6
	Lactuca sativa (lettuce)	6.4
	Lycopersicon esculentum (tomato)	24.5
	Pastinaca sativa (parsnip)	4.1
	Petroselinum crispum (parsley)	3.4

Hay et al. [87,88] discussed and described the most widely adopted protocols that are used to measure seed longevity, in particular, a "comparative longevity protocol" established at the Millennium Seed Bank (MSB) of the Royal Botanic Gardens, Kew [89]. This protocol suggests choosing 60% RH and 45 °C; these conditions allow one to obtain data within an acceptable length of time.

4.2. Modulation of Viability by Storage Conditions

As indicated by the "improved viability equation", loss of seed viability is mainly regulated by seed moisture content and temperature during storage. The storage of orthodox seeds follows two rules [1,2,4,7,34,79,80]:

- For each 1–2% decrease in seed moisture content (when the MC ranges between 5 and 14%), the seed storage life is doubled;
- For each 10 °F (5.6 °C) decrease in seed storage temperature (between 0 °C and 50 °C), the seed storage life is doubled.

However, maximum seed longevity is achieved at a critical low moisture content limit for the application of the viability equation, and drying the seeds below this critical value does not improve seed longevity [8,30,84,85,90,91]. It varies among species and depends on the seed composition, in particular the lipid content and temperature. It is for example about 2% (fresh weight basis) in *Arachis hypogea* [85] and 6.2% in *Pisum sativa* [84], and it varies in an inverse relationship with the lipid content [8,84,85]. Maximum survival after 4–5 years at ambient temperature was determined to be in the range of 1.8–2.5% for sesame, 4.3–5% for soybean, and 7.6–9.7% for wheat [92]. In addition, this critical moisture content also decreases with increasing temperature, with it ranging between 3 and 4% at high temperatures and 4 and6% at ambient temperature [93].

The availability of oxygen (i.e., hermetic vs. open storage) can also influence seed viability during storage [94–96]. Oxygen is generally detrimental to seed viability maintenance, but the beneficial effect of low oxygen or anaerobic conditions depends on the conditions of ageing. Ellis and Hong [95] indicate that this negative effect increases as the seed moisture content decreases. Storage in N_2 (i.e., in the absence of oxygen) is advantageous for pea, broad beans, and barley [97], but it has no significant effect on cabbage, onion, and red clover [98]. In the case of non-primed and primed lettuce seeds stored in low RH (33%), longevity was extended in an anaerobic environment, but this effect was less under storage in controlled deterioration (75% RH, 50 °C) [96]. In onion, this beneficial effect of anaerobic conditions is only observed in primed seeds [96].

4.3. Procedures for Long-Term Storage in Genebanks

Improving seed storage techniques is a major research focus of the International Plant Genetic Resources Institute (IPGRI). Guidelines for long-term conservation recommend storage at −20 °C ± 4 °C and 15 ± 3% RH, considered a conventional method [99]. Cryopreservation is also a possible technique to prolong the longevity of orthodox seeds with short lifespans [100]. In contrast, recalcitrant and intermediate seeds require cryopreservation [7,101,102].

To achieve the appropriate moisture content, Kew recommends equilibrating the seeds to 15% RH at 15 °C (www.rbgkew.org.uk/, accessed on 1 November 2023), Ellis et al. [30,84,85] suggest equilibrating the seeds to 10% RH at 20 °C, and Vertucci and Roos [90,103] propose to equilibrate the seeds at 20–25% RH at storage temperature. Storing seeds in hermetically sealed containers to maintain their water content is also recommended.

Most orthodox seeds also show long longevity when ultra-dried or freeze-dried. One advantage of ultra-drying or freeze-drying is that seeds can be stored at room temperature, but they must be maintained under vacuum in tightly closed containers or bags impermeable to water vapor. Table 5 shows that freeze-drying gives excellent results with the seeds of some vegetable species compared to storage under ambient conditions [62,77]. However, large seeds, such as pea, soybean, and bean, can be damaged by freeze-drying, with cracks in the cotyledons occurring at thawing or during seed re-imbibition. The results obtained with vacuum-dried seeds (seeds dried under vacuum for 3 days at 20 °C) and freeze-dried seeds (seeds immersed in liquid nitrogen and placed under vacuum for 3 days at 20°) and then stored under vacuum in the presence of silica gel for 12–19 months at 5, 20, and 30 °C clearly indicated that the responsiveness of ultra-dried seeds to storage depends on the species and the temperature of storage. Ultra-dried seeds of lettuce and leek remained viable after 12 months of storage although the germination rate of leek seeds and freeze–dried lettuce seeds was slightly reduced. In onion and lamb's lettuce seeds, ultra-drying resulted in a marked decrease in seed viability after 12 months of storage, and this deleterious effect was reinforced by increasing the temperature of germination. In addition, ultra-dried seeds stored for 12 months were more sensitive to accelerated ageing treatment (40–45 °C, 100% RH) suggesting that the seeds became progressively less vigorous during storage.

Table 5. Comparison of the longevity of seeds from some vegetable species stored in the open air or freeze-dried and stored under vacuum at ambient temperature. C, control non-freeze-dried seeds; FD, freeze-dried seeds. Modified from [62,77].

Species	Germination (%) after Storage for									
	0 (Harvest)		4 Years		8 Years		12 Years		20 Years	
	C	FD	C	FD	C	FD	C	FD	C	FD
Allium cepa (onion)	92	92	8	72	2	74	2	76	0	68
Asparagus officinale (asparagus)	93	96	0	62	0	60	2	35	0	25
Cichorium intybus (endive)	96	90	4	100	0	68	0	65	0	85
Foeniculum officinale (fennel)	100	83	4	72	0	50	0	56	0	50
Lonicera caprifolium (honeysuckle)	90	92	60	100	50	78	38	71	32	96
Papaver somnifera (opium poppy)	92	90	80	100	16	72	0	60	0	80
Portulaca oleracea (purslane)	84	100	100	100	69	96	7	95	0	80
Trifolium repens (white clover)	100	100	100	100	90	88	82	81	100	100
Valerianella olitoria (lamb's lettuce)	88	96	4	100	0	80	0	84	0	72

5. Damage Occurring during the Dehydration of Recalcitrant Seeds

A sequence of irreversible cellular and metabolic damage is associated with the dehydration of desiccation-intolerant tissues. Table 6 [47,104] shows as an example the deleterious events occurring during the desiccation of recalcitrant *Araucaria angustifolia* embryos. Dehydration results in a decrease in various metabolic activities, among which loss of the ability to incorporate methionine in proteins is one of the earliest indicators of cell deterioration. Dehydration for 0.5 h and 2 h is sufficient to reduce the protein synthesis by about 25% and 75% in embryonic axes, respectively [47,104]. Injury to the plasmalemma and intracellular membranes also seems to be the primary lesion induced by dehydration and results in an increase in electrolyte leakage and a decrease in the activity of ACC oxidase, allowing the conversion of ACC into ethylene. A decrease in the ability to oxidase ACC into ethylene should be considered a good marker of membrane injury since the in vivo activity of ACC oxidase is known to depend on membrane integrity [105]. Reduced moisture content of axes below 55–60% results in a 50% decrease in ACC-dependent ethylene production, which is almost nil below 50% moisture content [47,104]. Electrolyte leakage also increases progressively during dehydration in *Quercus robur* [106], silver maple and areca palm [107], and *Landolphia kirkii* [108]. In *Araucaria angustifolia* axes, it reached about 50% after 6 h of dehydration, i.e., when all embryos have become unable to germinate (Table 6). Energy metabolism decreases after the first 1–2 h of desiccation (Table 6, [104]). However, respiration is markedly affected only after 6 h of desiccation, when embryos have lost their ability to germinate (Table 6); therefore, this parameter cannot then be considered as a marker of damage induced by dehydration.

Strong evidence exists regarding the involvement of active oxygen species (AOS) in injury following the desiccation of recalcitrant seeds, but it is often difficult to know whether the accumulation of these highly reactive compounds is a cause or a consequence of the loss of viability [1,21,23,109]. In studies on temperate recalcitrant species, *Quercus robur* showed that a rapid accumulation of free radicals (measured by electron paramagnetic resonance, EPR) and a rise in lipid peroxidation occurred in the embryonic axis when their moisture content fell below 40–45% [110,111]. The loss of viability during the dehydration of other recalcitrant seeds such as *Castanea sativa* [112], *Aesculus hippocastanum* [112], *Shorea*

robusta [113] and *Theobroma cacao* [59], and germinated maize [114] and wheat seeds [115] is associated with an accumulation of free radicals and/or a decrease in the efficiency of the cellular antioxidant systems.

Table 6. Sequence of some cellular and metabolic events occurring in embryonic axes of *Araucaria angustifolia* during the desiccation of embryos at 25 °C and 55% RH. Modified from [47,104]. The mean moisture content of freshly isolated embryos was about 120%. Around 50% of the embryos were dead when their moisture content (MC) had fallen by about 60–65%. The critical MC (*) at which viability (evaluated by germination) was completely lost was around 25–30%.

Duration of Desiccation (h)	Moisture Content (% Dry Weight)	Cellular and Metabolic Events
0–0.5	90–95	- 25% decrease in protein synthesis
0.5–1	70–75	- 50% decrease in protein synthesis
1–1.5	55–60	- 50% decrease in ACC conversion to ethylene
1–2	50–55	- 20–40% decrease in energy charge (ATP + 0.5 ADP)/(ATP + ADP + AMP)
1.5–2	40–45	- 75% decrease in protein synthesis
2–114	30–45 *	- Leakage of 25% of total leachable electrolytes
4–6	20–27 *	- Leakage of 50% of total leachable electrolytes
6–7	20–22	- 50% decrease in respiratory activity (O_2 uptake) and 80% decrease in energy charge

It seems therefore that critical features of desiccation intolerance are the inability to maintain the physiological integrity of membranes in the dry state and to limit free radical damage during dehydration due to a decrease in efficiency of the scavenging enzyme activity and/or the antioxidant compounds [1,21,28,109]. Global approaches (proteomic, metabolomic, transcriptomic, and genomic analyses) could also provide information on and aid in the identification of the mechanisms involved in the loss of viability as a response to dehydration (review [116] and references therein).

6. Physiological and Biochemical Events Associated with the Ageing of Orthodox Seeds

6.1. Loss of Vigor during Ageing

During ageing, seed viability first slowly decreases, then follows a sharp decline, and finally, is lost (Figure 2). Seed vigor is a trait required to obtain rapid and uniform seed germination and seedling emergence in wide environmental conditions [1,3,20,62,117,118]. As seeds accumulate damage, they start to show a delay in germination. For example, in wheat grains, accelerated ageing carried out at 45 °C and 100% relative humidity results in a slight decrease in viability after 7 days, a 50% loss of viability after about 10 days, and a complete loss of viability of the seed population after 14 days (Figure 2A, curve 2). Seed vigor evaluated by the germination rate obtained after 3 days starts to decrease after 3 days of accelerated ageing, i.e., when the viability of the seed batch is still 100% (Figure 2A, curve 1). Figure 2B shows, in the case of lettuce seeds, that ageing also results in poor seedling development even when seeds remain able to germinate.

Figure 3 shows the effects of temperature (Figure 3A) and different levels of oxygen (Figure 3B) on the germination ability of rape seeds after various durations of accelerated ageing at 45 °C and 100% RH; the longer the ageing treatment, the narrower the temperature range, allowing for germination, and the stronger the seed sensitivity to low oxygen tension seeds. In summary, ageing is characterized by two physiological phases: (1) a loss of seed vigor which can be reversible, in particular by priming treatment (see Section 6.3), and (2) a loss of viability resulting from the accumulation of damage that becomes progressively irreversible.

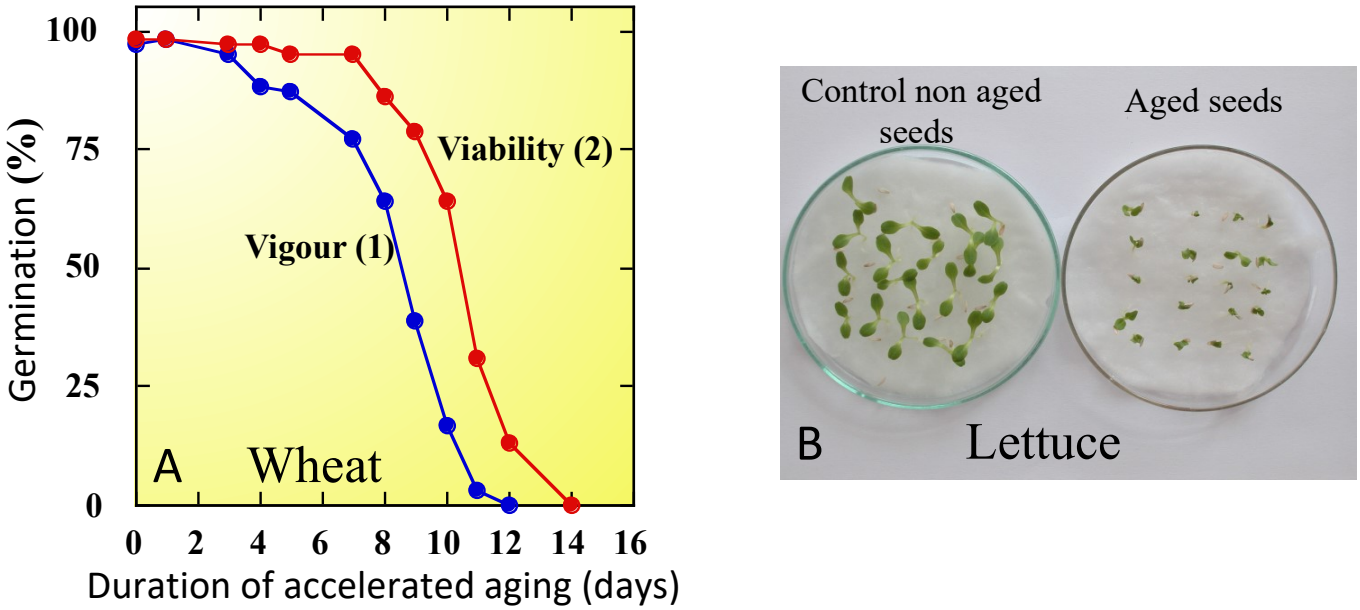

Figure 2. Loss of vigor and viability of orthodox seeds during ageing. (**A**) Loss of vigor (1) and viability (2) of wheat grains during accelerated ageing at 45 °C and 100% relative humidity; (**B**) seedlings obtained within 7 days at 20 °C from control non-aged lettuce seeds and aged lettuce seeds. Both batches are 100% viable.

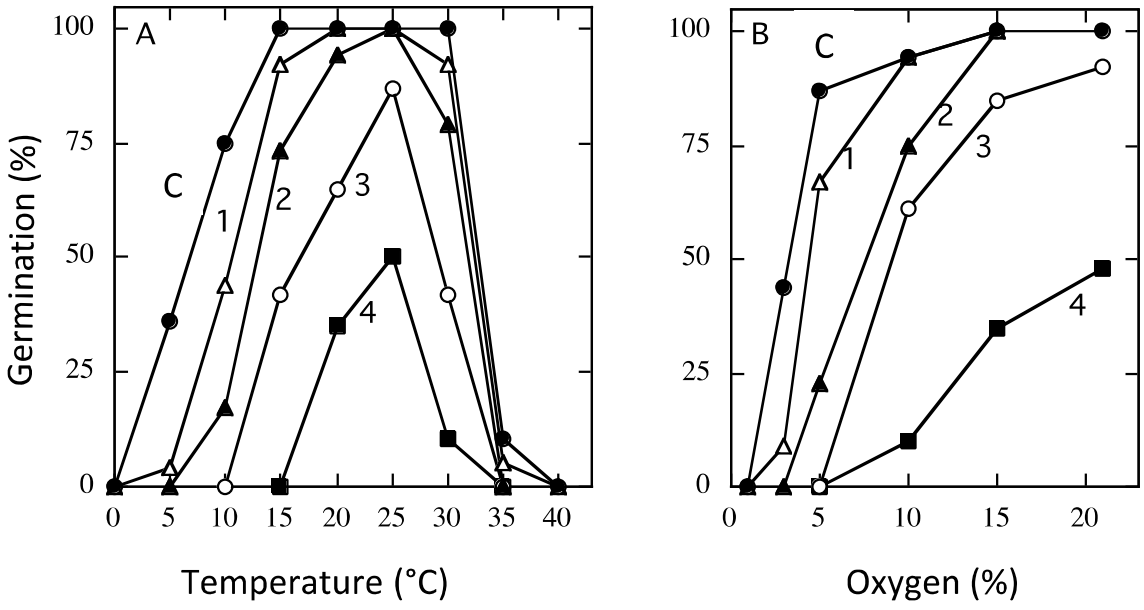

Figure 3. Effects of ageing at 45 °C and 100% relative humidity on rape seeds' sensitivity to temperature and oxygen availability. (**A**) The effects of temperature on the germination percentages obtained after 7 days in air with non-aged seeds (C) or seeds aged for 7 h (1), 17 h (2), 24 h (3), and 48 h (4); (**B**) the effects of oxygen on the germination percentages obtained after 7 days at 25 °C with non-aged seeds (C) or seeds aged for 7 h (1), 17 h (2), 24 h (3), and 48 h (4). From [77].

6.2. Cellular and Metabolic Deterioration during the Ageing of Orthodox Seeds

Numerous reviews on free radical processes provide evidence that the loss of viability during the ageing of orthodox seeds is associated with damage to various macromolecules, including membrane phospholipids, proteins, and nucleic acids, and damage to the nucleus and cytoplasmic organelles [1,3,7,12,19–21,62,79,117–119]. Figure 4 shows the major biochemical changes that occur in orthodox seeds during ageing. These alterations cause

mitochondrial dysfunction and then a decrease in ATP synthesis, damage to proteins through thiol oxidation and carbonylation resulting in modification of ion transport, alteration of ion gradients, and changes in enzyme activity. Lipid peroxidation at the origin of end products such as malondialdehyde (MDA), hexane, pentane, and conjugated dienes results in the loss of membrane integrity [3,7,119]. In addition, damage to RNA and DNA caused by ROS accumulation leads to altered gene expression and, finally, to an increase in chromosomal aberrations [12,21,25,120–123].

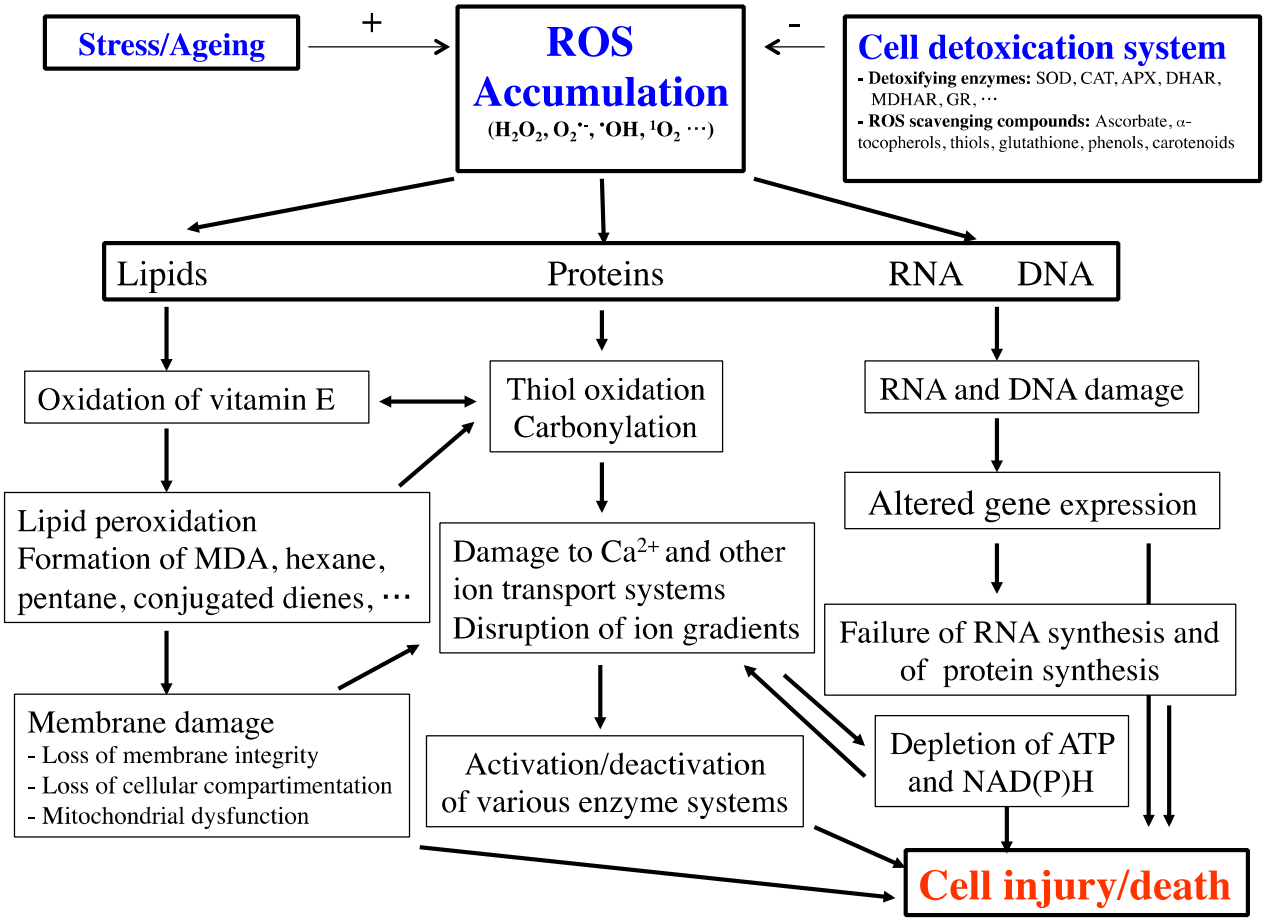

Figure 4. Major biochemical changes that occur in orthodox seeds during ageing. Adapted from [1,3,7,20,62,77,117–119]. Ageing results from the accumulation of ROS (H_2O_2: hydrogen peroxide; $O_2^{\cdot-}$: superoxide anion; $^{\cdot}OH$: hydroxyl radical; $^1O_2$: singlet oxygen) due to a reduction in the efficiency of the cell detoxication systems through the activity of the detoxifying enzymes (SOD: superoxide dismutase; CAT: catalase; APX: ascorbate peroxidase; DHAR: dehydroascorbate reductase; MDHAR: monodehydroascorbate reductase; GR: glutathione reductase) and the presence of ROS-scavenging compounds such as ascorbate (vitamin C), monodehydroascorbate, dehydroascorbate, α-tocopherol (vitamin E), thiols, reduced glutathione, phenolic compounds, and carotenoids. The accumulation of ROS causes seed deterioration (1) by affecting lipid peroxidation and leading to the formation of MDA (malondialdehyde), hexane, pentane, and conjugated dienes and the loss of membrane integrity, cellular compartmentation, and mitochondrial dysfunction, (2) through the damage of proteins through thiol oxidation and carbonylation resulting in modification of ion transport and ion gradients and changes in the activity of various enzymes, and (3) through damage at the level of RNA and DNA resulting in alterations in gene expression and the failure of RNA synthesis and protein synthesis. All of these alterations result in a loss of seed vigor and, progressively, the loss of seed viability.

Ageing is a result of an imbalance between ROS synthesis and the efficiency of the antioxidant defense systems [1,3,7,19,21,62,109]. The reduction of oxygen results in the synthesis of the superoxide radical ($O_2^{-\cdot}$) that can further form hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\cdot}$) [3,20,21]. H_2O_2 may also result from the non-enzymatic reduction of $O_2^{-\cdot}$ in the presence of H^+ ions or from the action of catalase on $O_2^{-\cdot}$ [109]. There exist numerous cellular protective mechanisms against reactive oxygen species. They involve free radical- and peroxide-scavenging enzymes, such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), dehydroascorbate (DHA), glutathione reductase (GR), and antioxidants such as ascorbate (vitamin C), α-tocopherol (vitamin E), thiols, phenolic compounds, and carotenoids [3,7,21,109,119]. SOD is generally considered a key enzyme involved in the regulation of intracellular concentrations of free radicals such as $O_2^{\cdot -}$ and peroxides that can lead to lipid peroxidation [124]. Peroxides can also be removed through catalase activity or the ascorbate–glutathione cycle involving ascorbate peroxidase, dehydroascorbate reductase, and glutathione reductase [3,21]. These systems require NADPH, usually produced by the pentose phosphate pathway.

Lipid peroxidation has been demonstrated to be the primary factor leading to an alteration in membrane permeability [125,126]. The progressive loss of membrane integrity is demonstrated in aged seeds by an increase in the leakage of cell components in the external medium by an increase in the conductivity of the external solution [127,128]. MDA accumulation has been associated with the loss of viability in soybean [129], Norway maple [130], and sunflower [127,131,132]. On the contrary, lipid peroxidation evaluated by MDA content is not associated with seed deterioration in wheat [133,134], maize [135], and pigeon pea [136]. It is important to underline that the sources of ROS depend on the physiological state of the seeds; it is necessary to identify the process involved in seeds stored at low moisture content and in seeds after imbibition [21]. During seed storage, lipid autoxidation can generate primary free radicals, resulting in the degradation of lipids, mRNA, DNA, and proteins, and the activities of antioxidant enzymatic systems are not sufficient to remove the ROS. Prolonged storage or storage in unfavorable conditions (high temperature and relative humidity, for example during accelerated ageing or controlled deterioration) results in an increase in free radical production and a decrease in antioxidant enzyme activities and the antioxidant pool [21,137,138]. The imbibition of aged seeds results in the resumption of metabolism, in particular respiration in mitochondria (the main source of ROS production), but the antioxidant systems altered during storage are then inefficient in removing secondary free radicals and new damage against cell macromolecules. The loss of viability resulting from a concomitant accumulation of ROS and a decrease in the antioxidant protective mechanisms is observed in numerous species [1,20,127,137,139] but depends on the seed moisture content. In sunflower, for example, below 0.21 g H_2O g DW^{-1}, H_2O_2 accumulates rapidly, but without lipid peroxidation; on the other hand, at higher water content, the loss of viability is a result of lipid peroxidation [132]. In wheat aged at 45 °C and 100% RH, i.e., at an embryo moisture content close to 50–60% DW, the loss of viability is attributed to H_2O_2 production without MDA accumulation [134]. At low MC, i.e., in seeds in equilibrium in region 1 of the sorption isotherm, auto-oxidation reactions are responsible for ageing [6]; at higher MC (i.e., in region 2 of the sorption isotherm), enzymatic oxidations may be improved and contribute to cellular damage. In sunflower seeds, at MC higher than 0.25 g H_2O g^{-1} DW, mitochondrial respiration occurs and can be considered to be the major source of ROS production [132].

Damage to proteins by ROS can correspond to a reversible oxidative modification of Lys, Arg, Pro, or Thr residues (i.e., carbonylation) or can be reversed through the actions of thioredoxins, peroxiredoxins, glutaredoxins, or methionine sulfoxide reductase [3,140]. In 1978, Cheah and Osborne [141] demonstrated that nuclear DNA undergoes progressive cleavage to lower molecular weight fragments. More recently, in sunflower seeds, RAPD (random amplification of polymorphic DNA) analysis showed that DNA alterations are regulated by moisture content; DNA damage and PCD-like DNA fragmentation are observed in aged seeds when the moisture content is high [22]. RNA is much more vulnerable

to oxidative damage than DNA because of its single-stranded structure, its cytoplasmic location, and the absence of repair systems [142]. The oxidation of bases has been identified, with guanine being the most commonly oxidized base in RNA. The oxidation of guanine results in the production of 8-hydroxyguanosine, the amount of which was determined to regulate the protein-selective translation involved in the alleviation of seed dormancy in sunflower [143].

The review by Fu et al. [12] on recent papers on seed biology summarizes the biomarkers available for assessing seed ageing, including the expression of genes involved in anti-oxidative mechanisms and DNA and protein repair.

6.3. Repair

Priming (osmo- and hydro-priming) and seed treatment with various compounds, such as H_2O_2, ascorbic acid, or salicylic acid, can promote the germination of aged seeds through different physiological and molecular effects [137–139,144]. Osmo-priming improves the germination of low-vigor or aged seeds in various species such as wheat [145], cauliflower [146], tomato [147], sunflower [139], pepper [148], and rape [77]. In rape, for example, osmo-priming at 25 °C in PEG solution at −2 MPa increases the germination of aged seeds; this improvement effect increases with the duration of treatment, and after 6 days of priming, the aged seeds germinate as well as the non-aged ones [77]. In the case of sunflower seeds, the reinvigoration of aged seeds during priming is associated with a decrease in lipid peroxidation and the recovery of the detoxifying enzyme (superoxide dismutase, catalase, or glutathione reductase) activities [139]. In addition, CAT has been found to be a key determinant in sunflower seed recovery since aminotriazol, an inhibitor of CAT, during priming suppresses the beneficial effect of the treatment [149].

Different DNA ligase genes [150] and protein L-isoaspartyl methyltransferase (PIMT) [151,152] are required for DNA and protein repair, respectively. In Arabidopsis, the reduced viability of the simple mutant *atlig6* associated with the altered expression of PIMT1 highlights the potential role of ligase and PIMT in ageing.

7. Conclusions

As with most vegetative organs, the dehydration of recalcitrant seeds induces irreversible cellular damage. The long-term storage of these seeds will only be possible when methods are devised for blocking germination or growth, without leading to excessive dehydration or the risk of chilling injury. Wet storage of these seeds is difficult since the temperature must be low enough to prevent germination or reduce the seedling growth rate but high enough to avoid the risk of chilling injury, which can lead to the death of seeds or seedlings. At temperatures high enough to avoid chilling injury, growth is usually still too fast for prolonged storage. Cryopreservation might be a technique that will allow for the long-term storage and preservation of biodiversity [50,100].

On the contrary, orthodox seeds have developed very efficient biological mechanisms that allow them to maintain cellular integrity in a dehydrated state. Their extraordinary ability to tolerate desiccation enables them to survive for a very long time and greatly facilitates their ability to be stored under air-dry conditions typical of genebanks. A better understanding of the biochemical, cellular, and molecular mechanisms involved in the loss of seed vigor and then in seed deterioration allows us to suggest various markers of seed quality [153]. The wide characterization of seed ageing using biochemistry, transcriptomics, proteomics, and metabolomics allows us to suggest new markers and propose a model in order to predict/estimate seed longevity.

Seed ageing is a complex biological trait integrating numerous physiological, metabolic, and mitochondrial signals which are interconnected [12]. The biochemical events associated with this process largely depend on the conditions of ageing and underline the key role of water content in the mechanisms involved in seed deterioration or protection against ageing damage. Consequently, accelerated ageing, which consists of placing seeds at 35–45 °C and a high relative humidity (75–100%), and controlled deterioration, which is

carried out by treating seeds at warm temperatures and a precise water content (between 19–24% fresh weight), used in research as methods to estimate longevity or to evaluate seed tolerance to storage, cannot be used as a model to understand the mechanisms involved in seed ageing in the dry state. Numerous research works indicate that free radical effects can be reduced by a free radical scavenger and antioxidant compounds and that the balance between free radical production and detoxification mechanisms control seed ageing. A better understanding of DNA, mRNA, and protein oxidative modifications, protection, and repair mechanisms are promising in order to manipulate seed longevity. Multidisciplinary approaches including genomics, transcriptomics, proteomics, and metabolomics are required to be able to optimize the conditions of storage to prolong seed viability and propose a model to improve seed longevity and evaluate seed storability.

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