



Age-Dependent Oxidative Stress And Seed Viability In *Arabidopsis thaliana*

Shashi Bhushan*

ABSTRACT

Seed longevity is a vital determinant of plant survival, agricultural productivity, and genetic resource conservation. However, seed viability progressively declines during storage, largely due to oxidative stress. In this study, we investigated the relationships between hydrogen peroxide (H_2O_2) accumulation, peroxidase activity, and germination performance in *Arabidopsis thaliana* seeds subjected to long-term storage. Seeds were stored at 4 °C in darkness for durations ranging from 23 to 536 days. Biochemical assays were performed to quantify H_2O_2 concentration using the Amplex Red Hydrogen Peroxide/Peroxidase Assay, while peroxidase activity was assessed in the same extracts. Germination frequency was determined on Murashige and Skoog (MS) medium under controlled conditions. Results revealed a pronounced increase in H_2O_2 levels with extended storage, peaking at 354 days, followed by a decline at later stages. This pattern suggests that ROS accumulation initially exceeds antioxidant buffering capacity, triggering oxidative stress and cellular damage, while the subsequent decline reflects metabolic inactivity and extensive viability loss. Peroxidase activity displayed a distinct temporal pattern, with maximum activity at 256 days, preceding the H_2O_2 peak. This indicates an induced but transient antioxidant defense that was not sustained during prolonged storage. Germination rates correlated with these biochemical changes: seeds maintained >90% germination up to 54 days, but declined to ~40–50% at 354 days, and <30% by 536 days. Collectively, our findings highlight the central role of oxidative stress in seed deterioration. The temporal misalignment between ROS accumulation and peroxidase activity underscores the imbalance between oxidative challenge and antioxidant response as a key driver of seed aging and loss of viability.

Keywords: *Arabidopsis thaliana*, seed aging, hydrogen peroxide, peroxidase, germination, oxidative stress.

*Corresponding Author: shashilbhushan@gmail.com

Dept. of Botany, Anjabit Singh College, Bikramganj, Veer Kunwar Singh University Ara, Bihar

DOI: <https://doi.org/10.66690/ijoasar.2026.v1i101>

How to Cite: Bhushan, S. (2026). Age-dependent oxidative stress and seed viability in *Arabidopsis thaliana*. Int. J. Allied Sci. Res, 1(1), 1–6.

INTRODUCTION

Seed longevity is a fundamental component of plant survival and has direct implications for crop establishment, agricultural productivity, and the long-term conservation of plant genetic resources. Seeds stored in gene banks are expected to retain viability for extended periods; nevertheless, even under carefully controlled conditions, they gradually lose vigor and germination potential. This deterioration is a progressive

and complex process influenced by genotype, seed developmental history, moisture status, temperature, storage environment, and duration of storage (McDonald, 1999; Walters et al., 2005; Pirredda et al., 2023). Understanding the biological events that accompany this decline is therefore important not only for seed physiology but also for the efficient management of germplasm collections.

Oxidative imbalance has emerged as one of the major processes associated with seed ageing. During storage and subsequent rehydration, reactive oxygen species (ROS), including superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH), can arise from residual metabolic activity, mitochondrial dysfunction, and other redox reactions. At controlled concentrations, ROS are not exclusively harmful; they participate in signalling pathways involved in dormancy release, germination, stress responses, and cellular adaptation. However, when ROS production exceeds the capacity of antioxidant and repair systems, redox homeostasis is disrupted and oxidative damage accumulates in membranes, proteins, and nucleic acids (Bailly, 2004; El-Maarouf-Bouteau & Bailly, 2008; Kurek et al., 2019). Recent reviews continue to place ROS-mediated damage and impaired redox regulation at the centre of the molecular processes associated with seed deterioration (Zhang et al., 2021; Pirredda et al., 2023; Wang et al., 2024).

Hydrogen peroxide deserves particular attention in this context. Compared with highly reactive free radicals, H_2O_2 is relatively stable and can diffuse across cellular compartments, allowing it to function both as a signalling molecule and as a mediator of oxidative injury. Its biological effect consequently depends on concentration, cellular location, and the capacity of the seed to remove it. Accumulation of H_2O_2 during ageing has been associated with lipid peroxidation, protein oxidation, DNA damage, and progressive loss of seed vigor (El-Maarouf-Bouteau & Bailly, 2008; Kurek et al., 2019). Thus, monitoring H_2O_2 provides a useful biochemical approach for examining changes in redox status during prolonged storage.

Seeds possess an extensive antioxidant network that limits the potentially damaging effects of ROS. This system includes enzymatic components such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and other peroxidases, together with low-molecular-weight antioxidants including ascorbate and glutathione. Rather than functioning as isolated components, these molecules operate as an interconnected redox-buffering system that maintains

ROS within physiologically tolerable limits (Scandalios, 2005; Gill & Tuteja, 2010). The glutathione–ascorbate system is particularly important in maintaining cellular redox balance, while peroxidases contribute to the removal of H_2O_2 and other peroxide substrates. During ageing, however, antioxidant capacity may become insufficient relative to the increasing oxidative burden, allowing damage to accumulate (Kibinza et al., 2006; Zhang et al., 2021).

The relationship between ROS and germination is therefore not simply a matter of ROS being beneficial in young seeds and harmful in old seeds. A controlled increase in ROS can form part of the signalling network that promotes dormancy release and radicle emergence. Oracz et al. (2007) demonstrated that ROS production and protein oxidation participate in mechanisms associated with dormancy alleviation. More broadly, ROS have been described as having dual functions throughout the seed life cycle: moderate levels can contribute to signalling and germination, whereas excessive accumulation can compromise cellular integrity and viability (Bailly, 2004; El-Maarouf-Bouteau & Bailly, 2008). This concentration-dependent behaviour makes the balance between ROS generation, scavenging, and repair particularly important when interpreting seed-ageing responses.

As storage progresses, oxidative damage can become increasingly interconnected with other forms of cellular deterioration. Membrane integrity may decline, mitochondrial function can become impaired, energy metabolism may be disrupted, and damage to proteins and DNA can accumulate. Recent work has emphasized that seed ageing is better understood as a network of interacting processes rather than as the consequence of a single biochemical lesion (Zhang et al., 2021; Pirredda et al., 2023). ROS are closely linked with mitochondrial dysfunction, macromolecular oxidation, antioxidant depletion, and programmed cell-death-associated processes, thereby contributing to the gradual transition from reduced vigor to complete loss of viability (Kurek et al., 2019; Wang et al., 2024).

Arabidopsis thaliana provides a particularly useful experimental system for investigating these mechanisms

because its seed biology, genetics, and molecular responses have been extensively characterized. Studies in *Arabidopsis* have demonstrated that longevity is influenced by redox regulation, DNA repair, hormonal signalling, protective proteins, and other mechanisms operating during seed maturation and storage (Waterworth et al., 2015; Pirredda et al., 2023). Recent research has further reinforced the importance of coordinated antioxidant, repair, and protective pathways in determining how effectively seeds withstand prolonged storage (Wang et al., 2024).

Against this background, the present study examined *A. thaliana* Col-0 seeds maintained under controlled cold and dark conditions for different storage periods. Particular emphasis was placed on the relationship between H₂O₂ accumulation, peroxidase-associated activity, and germination capacity. By examining biochemical and physiological responses across a broad storage timeline, the study sought to identify changes associated with progressive seed ageing and to clarify the relationship between redox imbalance and the loss of germination potential. Such information may contribute to a better understanding of oxidative processes involved in seed deterioration and provide useful biochemical indicators for assessing seed physiological status during storage.

2. MATERIALS AND METHODS

2.1. Seed Material and Storage Conditions

Seeds of wild-type *Arabidopsis thaliana* ecotype Columbia-0 (Col-0) were used throughout the study. Mature seeds originating from the same seed lot were used for all experimental treatments to minimize variation arising from differences in genotype, seed development, or initial physiological status.

Following maturation, seeds were transferred to airtight vials and maintained at 4 °C in complete darkness. These conditions were selected to minimize metabolic activity and to maintain the seeds in a stable storage state. Seed longevity is strongly influenced by temperature, moisture, and storage environment; therefore, maintaining uniform conditions was essential for comparing responses across storage periods (Walters et al., 2005; Zhang et al., 2021).

Eleven storage durations were examined: 23, 54, 195, 227, 256, 275, 311, 354, 438, 467, and 536 days. These time points provided a broad temporal series encompassing relatively recent storage through extended storage. For each sampling point, seeds were collected

from the same experimental storage conditions and subjected to biochemical and germination analyses.

2.2. Quantification of Hydrogen Peroxide

Hydrogen peroxide content was determined using the Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions with minor modifications. The Amplex Red system is based on the oxidation of the non-fluorescent Amplex Red reagent to the fluorescent product resorufin in a reaction catalysed by horseradish peroxidase in the presence of H₂O₂. The resulting fluorescence is proportional to the amount of H₂O₂ present within the assay range.

For each sampling point, seeds were rapidly frozen and finely powdered in liquid nitrogen. One hundred milligrams of frozen seed material was homogenized in 1 mL sodium phosphate buffer (pH 7.4). The homogenate was centrifuged at 13,000 rpm for 5 min at 4 °C, and the resulting supernatant was carefully collected for analysis. An aliquot of 50 µL of the supernatant was transferred to a 96-well microplate. The reaction mixture was supplemented with 100 µM Amplex Red reagent (10-acetyl-3,7-dihydroxyphenoxazine) and 0.2 U/mL horseradish peroxidase (HRP). Reaction mixtures were incubated for 30 min at room temperature in darkness to minimize light-associated changes in the fluorescent reaction product.

Fluorescence was subsequently recorded using a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at an excitation wavelength of 560 nm and an emission wavelength of 590 nm. H₂O₂ concentrations were calculated from a standard curve generated using known concentrations of hydrogen peroxide processed under identical assay conditions. Appropriate blanks and standards were included during each analysis.

The measurement of H₂O₂ is particularly relevant to seed-ageing studies because oxidative imbalance and ROS accumulation are closely associated with loss of seed quality during storage (Bailly, 2004; Kurek et al., 2019; Zhang et al., 2021).

2.3. Peroxidase-Associated Activity Assay

Peroxidase-associated activity was determined using the corresponding fluorescence-based reaction system under the experimental conditions established for the seed

extracts. Enzyme activity was expressed as relative units (RU) based on the change in fluorescence over time.

Because peroxidases contribute to the removal of H₂O₂ and form an important component of the antioxidant network, changes in their activity can provide information about the capacity of stored seeds to respond to oxidative pressure. The activity of antioxidant enzymes, including peroxidases and catalase, has previously been associated with changes in seed quality during ageing (Goel et al., 2003; Kibinza et al., 2006; Zhang et al., 2021).

For comparisons among storage treatments, enzyme activity was normalized to the quantity of seed extract used in the reaction. All measurements were performed under identical assay conditions, and technical blanks were included to account for background fluorescence.

Note: If the laboratory protocol used here was specifically designed to measure endogenous peroxidase activity rather than H₂O₂ concentration, the exact substrate concentration, reaction kinetics, and unit definition should be reported. The Amplex Red/HRP reaction itself is primarily a highly sensitive H₂O₂ detection system and should not automatically be interpreted as a direct measurement of endogenous peroxidase activity.

2.4. Seed Germination Assay

Germination capacity was assessed using Murashige and Skoog (MS) basal medium, a standard culture system for *Arabidopsis* seed germination (Murashige & Skoog, 1962). Before plating, seeds were surface sterilized by immersion in 70% ethanol for 1 min, followed by treatment with 5% sodium hypochlorite containing 0.1% Tween-20 for 10 min. The seeds were subsequently rinsed five times with sterile distilled water to remove residual sterilizing agents.

Sterilized seeds were distributed onto MS agar medium in sterile Petri dishes. The plates were maintained at 4 °C in darkness for 48 h to provide a uniform stratification treatment. Cold stratification is commonly used in *Arabidopsis* to synchronize germination by reducing differences in dormancy status among seeds.

Following stratification, the plates were transferred to a controlled growth chamber maintained at 22 ± 1 °C, with a light intensity of approximately 6000 lux, a 16 h light/8 h dark photoperiod, and 78% relative humidity. Germination was monitored under identical environmental conditions for all storage treatments.

A seed was scored as germinated when the radicle had visibly emerged through the seed coat. Germination frequency was calculated after 7 days according to the following equation:

$$\text{Germination Frequency} = \left(\frac{\text{Number of Germinated Seeds}}{\text{Total Number of Seeds Plated}} \right) \times 100$$

Each storage treatment included at least three independent biological replicates, with 50–100 seeds per replicate. The use of a common seed lot and identical germination conditions enabled differences in germination frequency to be interpreted primarily in relation to storage duration and associated physiological deterioration.

2.5. Statistical Analysis

All measurements were obtained from at least three independent biological replicates and are presented as mean ± standard error (SE). Statistical analysis was performed to determine whether storage duration significantly affected H₂O₂ concentration, peroxidase-associated activity, and germination frequency.

Differences among storage treatments were evaluated using one-way analysis of variance (ANOVA). When the ANOVA indicated a significant treatment effect, Tukey's honestly significant difference (HSD) test was applied for post hoc pairwise comparisons. Statistical significance was considered at $p < 0.05$.

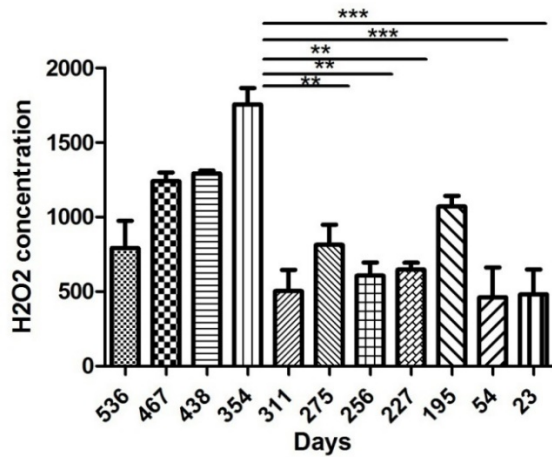
The statistical approach was selected because the principal experimental factor was storage duration, represented by multiple independent treatment levels. Prior to interpretation, the data were examined for consistency with the assumptions underlying the selected analysis. Where appropriate, residual distribution and variance homogeneity should be assessed before applying ANOVA.

Graphical presentation and statistical analyses were performed using GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). The resulting biochemical and physiological datasets were subsequently compared to determine whether changes in H₂O₂ accumulation and peroxidase-associated activity were accompanied by corresponding alterations in seed germination capacity.

3. RESULTS

3.1. Hydrogen Peroxide (H₂O₂) Accumulation during Seed Storage

Hydrogen peroxide content varied considerably across the storage period and showed an overall tendency to increase during the intermediate stages of ageing (Figure 1). Seeds stored for 23 and 54 days contained comparatively low amounts of H₂O₂, with values remaining below approximately 600 units. As storage progressed, H₂O₂ gradually accumulated and reached its



highest level at 354 days, where values were approximately 1800–1900 units. This increase was Figure 1. H₂O₂ concentration in *Arabidopsis thaliana* seeds following different storage durations.

3.2. Peroxidase Activity during Seed Storage

Peroxidase-associated activity exhibited a dynamic and distinctly non-linear pattern over the storage period (Figure 2). Seeds stored for 23 days showed relatively high activity, approximately 1800 units. Activity subsequently varied between approximately 700 and 1100 units during much of the intermediate storage period.

A pronounced increase was observed at 256 days, when peroxidase-associated activity exceeded 2000 units, representing the highest value recorded in the experiment. Activity remained relatively high at 275 days, reaching approximately 1800 units. In contrast, seeds stored for 227 days displayed a marked reduction, with activity falling to approximately 600 units, the lowest value among the storage treatments.

During the later storage period, from approximately 354 to 536 days, peroxidase-associated activity remained within a comparatively narrow range of approximately 900–1100 units. Thus, unlike H₂O₂, which reached its maximum at 354 days, the strongest peroxidase response occurred earlier, at 256 days.

statistically significant compared with the earlier storage treatments ($p < 0.01$ – 0.001).

Relatively elevated H₂O₂ concentrations were also detected in seeds stored for 438 and 467 days, with values of approximately 1200 units. Interestingly, the maximum observed at 354 days was not maintained during the longest storage intervals. Instead, H₂O₂ levels decreased progressively after the 354-day time point and were below 1000 units in seeds stored for approximately 438–536 days.

capacity are progressively altered (Kurek et al., 2019; Zhang et al., 2021).

The temporal pattern therefore indicates that H₂O₂ accumulation was not strictly linear throughout storage. Rather, the data suggest a pronounced oxidative phase during intermediate ageing, followed by a reduction in detectable H₂O₂ at advanced storage stages. Such non-linear behaviour is biologically plausible because ROS concentrations reflect the balance between their generation, detoxification, sequestration, and the physiological state of the seed. ROS are recognized as important regulators of seed ageing, but their abundance can change as cellular metabolism and antioxidant

This temporal separation is noteworthy. It suggests that the antioxidant response was dynamic and may have been activated before the maximum accumulation of H₂O₂. However, because the present experiment measured peroxidase-associated activity rather than transcript abundance or individual peroxidase isoforms, the observed increase cannot by itself be attributed to transcriptional induction of a particular gene. Recent evidence indicates that antioxidant responses during seed ageing are heterogeneous; peroxidase activity, in particular, does not necessarily follow the same trajectory as other antioxidant enzymes (Nickas et al., 2025).

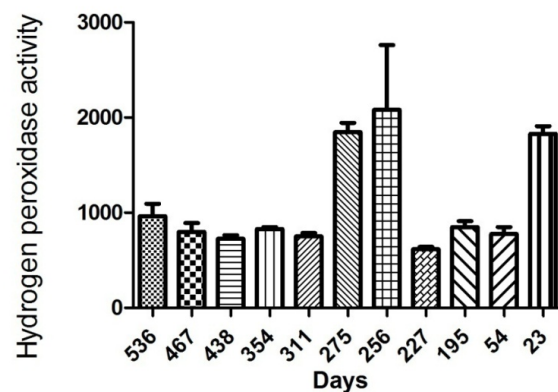


Figure 2. Peroxidase-associated activity in *Arabidopsis thaliana* seeds following different storage durations.

3.3. Seed Germination during Storage

Germination capacity declined progressively with increasing storage duration (Figure 3). Seeds stored for 23–54 days retained high germination, exceeding 90%. A moderate reduction became apparent during intermediate storage, with germination values decreasing to approximately 70–80% between 195 and 275 days.

A more pronounced loss of viability occurred thereafter. By 354 days, germination had declined to approximately 40–50%, indicating substantial deterioration in seed performance. The decline continued during prolonged storage, and germination fell below 30% in seeds stored for 438–536 days.

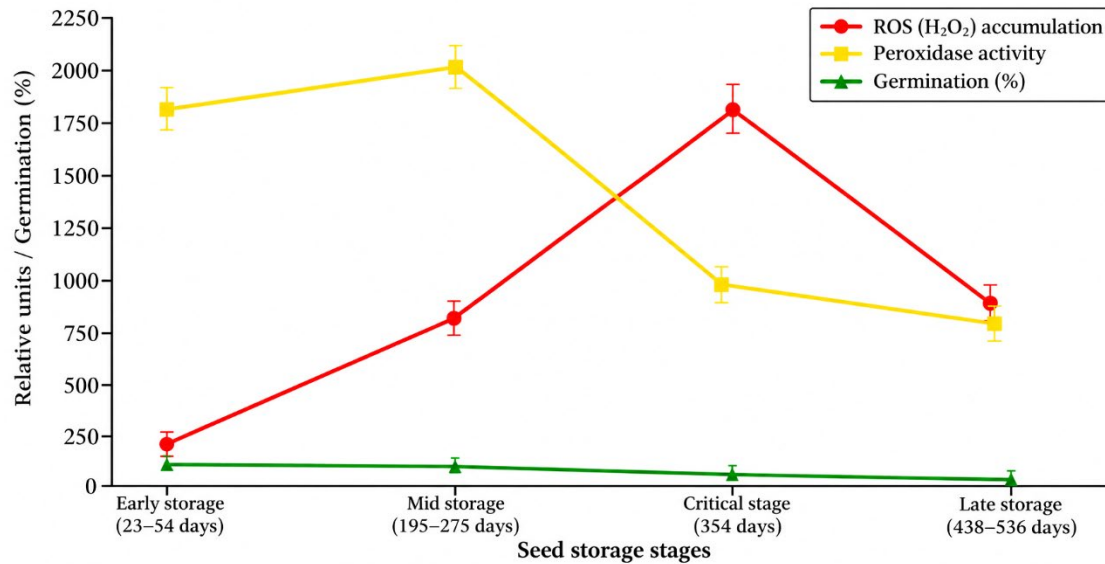


Figure 3. Relationship among storage duration, H_2O_2 accumulation, peroxidase-associated activity, and germination capacity in *Arabidopsis thaliana* seeds.

3.4. Relationship among H_2O_2 Accumulation, Peroxidase Activity, and Germination

Taken together, the three datasets reveal distinct temporal patterns during seed storage. H_2O_2 accumulation reached its maximum at 354 days, whereas peroxidase-associated activity showed its highest value earlier, at 256 days. Germination, meanwhile, declined progressively across the storage series.

The earlier increase in peroxidase-associated activity may represent a compensatory response to increasing oxidative pressure. In other words, the antioxidant system appears to have responded before the highest H_2O_2 concentration was reached. Nevertheless, this

The progressive reduction in germination occurred alongside substantial changes in the redox-related parameters. Seeds with the highest germination capacity were associated with comparatively low H_2O_2 concentrations, whereas the period of greatest H_2O_2 accumulation coincided with a substantial reduction in germination. These observations are consistent with the established relationship between oxidative imbalance and declining seed quality, although the present data alone do not demonstrate that H_2O_2 is the sole cause of reduced germination (Zhang et al., 2021; Waterworth et al., 2024).

response was not sufficient to prevent the subsequent loss of germination capacity.

The apparent temporal mismatch between antioxidant activity and H_2O_2 accumulation is particularly interesting because seed ageing is increasingly regarded as a dynamic interaction between ROS production, antioxidant buffering, macromolecular damage, and repair capacity rather than as a simple accumulation of oxidants.

Accordingly, the present findings suggest that 256–354 days may represent an important transition period in the physiological ageing of the seeds, during which antioxidant responses remain detectable but are increasingly unable to maintain the redox balance

required for preservation of germination potential. This interpretation should be regarded as a working physiological model rather than proof of a causal threshold, since additional measurements of lipid peroxidation, protein oxidation, DNA damage, antioxidant gene expression, and cellular viability would be required to establish the underlying mechanism.

4. DISCUSSION

Seed ageing is a progressive deterioration process in which physiological performance gradually becomes compromised during storage. The present study demonstrates that this process in *Arabidopsis thaliana* is accompanied by coordinated but temporally distinct changes in H₂O₂ concentration, peroxidase-associated activity, and germination capacity. The results are consistent with the current view that seed longevity depends on the maintenance of redox homeostasis together with effective protection and repair of cellular macromolecules.

Oxidative changes during seed ageing

A prominent feature of the present dataset was the accumulation of H₂O₂ during intermediate storage. Concentrations increased from less than approximately 600 units in the youngest storage treatments to approximately 1800–1900 units at 354 days. This pattern supports the involvement of oxidative processes during seed deterioration. ROS have long been associated with seed ageing, where they can influence membrane lipids, proteins, nucleic acids, and cellular structures. Importantly, ROS are not intrinsically detrimental. At controlled concentrations they also act as signalling molecules involved in dormancy release and germination; it is the loss of redox control and excessive accumulation that becomes damaging (Bailly, 2004; El-Maarouf-Bouteau & Bailly, 2008; Li et al., 2022).

The maximum H₂O₂ concentration at 354 days coincided with a substantial decline in germination, which had fallen from greater than 90% during early storage to approximately 40–50%. This temporal association is consistent with reports linking oxidative imbalance with membrane deterioration, impaired metabolic function, macromolecular oxidation, and declining seed viability (Kurek et al., 2019; Zhang et al., 2021).

Nevertheless, the subsequent decline in H₂O₂ concentration after 354 days requires careful interpretation. A reduction in the measured concentration does not necessarily indicate recovery of redox

homeostasis. At advanced stages of deterioration, changes in metabolic activity, ROS production, scavenging capacity, compartmentalization, and chemical consumption of H₂O₂ could all contribute to the observed pattern. Since the present study did not directly measure cellular respiration, membrane integrity, programmed cell death, or total ROS production, the lower H₂O₂ levels at 438–536 days should not be interpreted as evidence that oxidative stress had been alleviated. This distinction is important because severely aged seeds may simultaneously exhibit low detectable H₂O₂ and very poor germination capacity.

Antioxidant response and peroxidase activity

Peroxidase-associated activity followed a different trajectory from H₂O₂. Its maximum occurred at 256 days, approximately 98 days before the highest H₂O₂ concentration was detected. This temporal pattern may indicate an adaptive antioxidant response during the earlier stages of deterioration. Peroxidases participate in peroxide detoxification and form part of the broader antioxidant network that includes catalase, ascorbate peroxidase, glutathione-dependent systems, and other redox-regulating components.

The finding that peroxidase activity increased before the H₂O₂ maximum is compatible with a model in which antioxidant defences are initially mobilized to buffer increasing oxidative pressure. Similar concepts have been proposed in seed ageing studies, although the precise response differs according to species, seed condition, storage environment, and ageing protocol (Kibinza et al., 2006; Zhang et al., 2021).

An important point emerging from recent literature is that antioxidant enzymes do not necessarily show a uniform decline as seeds age. A 2025 systematic review of physiological and biochemical responses during seed ageing found that antioxidant activities generally changed with deterioration, but peroxidase activity could behave differently from several other antioxidant components (Nickas et al., 2025). This observation provides useful context for the present data, in which peroxidase-associated activity remained measurable even when germination had fallen substantially.

The sharp reduction observed at 227 days followed by a strong increase at 256 days further emphasizes that the antioxidant response was not simply proportional to chronological age. Such fluctuations may arise from changes in enzyme stability, substrate availability, post-translational regulation, or differences in the

physiological state of individual seed populations. Consequently, the observed pattern is better described as a dynamic antioxidant response than as a straightforward age-dependent decline.

Oxidative imbalance and loss of germination capacity

The most biologically relevant outcome of the present experiment was the progressive reduction in germination. Seeds stored for only 23–54 days retained more than 90% germination, whereas prolonged storage reduced germination below 30% by 438–536 days. This decline is consistent with the established understanding that seed ageing ultimately compromises the cellular processes required for successful germination.

The relationship between oxidative stress and germination is complex. During the early stages of germination, ROS can act as signalling molecules and contribute to the transition from dormancy to active growth. Excessive ROS, however, can cause lipid peroxidation, protein oxidation, DNA lesions, and disruption of cellular membranes. Thus, the physiological outcome depends on whether antioxidant and repair systems can keep oxidative damage within tolerable limits (Li et al., 2022; Zhang et al., 2021).

In the present experiment, the pronounced decline in germination during the period of elevated H₂O₂ suggests that redox imbalance may be one component of the deterioration process. The results are therefore consistent with a model in which oxidative stress contributes to, rather than independently determines, the loss of seed viability.

Genome integrity and seed longevity

Oxidative damage is particularly important because nucleic acids are among the cellular targets affected during seed ageing. Accumulation of DNA lesions can interfere with genome stability and compromise the successful transition from the dry state to germination. Recent reviews emphasize that DNA repair and DNA damage-response pathways are integral components of seed longevity (Waterworth et al., 2024).

Evidence from *Arabidopsis* provides direct support for this concept. AtOGG1, a DNA glycosylase involved in the repair of oxidized guanine residues, contributes to protection against oxidative DNA damage, and enhanced AtOGG1 expression has been associated with increased resistance to seed deterioration (Waterworth et al., 2012). In addition, studies of ageing *Arabidopsis* seeds indicate that DNA damage-response pathways influence germination and post-germination development, with

chromosomal-break repair being particularly important for seed longevity.

These findings provide a plausible mechanistic link between oxidative imbalance and declining germination in the present experiment. However, because DNA damage was not directly measured here, the contribution of genome instability remains a mechanistic interpretation rather than a demonstrated experimental outcome. Direct assessment of 8-oxoG, DNA strand breaks, comet assay parameters, or expression of DNA repair genes would be necessary to establish this relationship experimentally.

A proposed model of progressive seed deterioration

When the present findings are considered together, they support a four-stage conceptual model of ageing. During early storage, seeds retain high germination potential and relatively low H₂O₂ accumulation. During the intermediate phase, oxidative pressure increases and the antioxidant system appears to respond, as reflected by the pronounced peroxidase-associated activity at 256 days. The subsequent period, centred around 354 days, is characterized by maximum H₂O₂ accumulation and a substantial loss of germination capacity. During advanced storage, germination continues to decline despite the absence of a corresponding further increase in measured H₂O₂.

This final stage is particularly informative. It indicates that seed deterioration cannot be inferred from a single oxidative marker. Once damage has accumulated, processes such as membrane dysfunction, protein modification, mitochondrial impairment, DNA lesions, and inadequate repair may continue to influence viability even when a particular ROS measurement begins to decrease (Waterworth et al., 2024; Wang et al., 2024).

The overall pattern therefore supports the concept of progressive loss of redox and cellular homeostasis, rather than a simple linear increase in oxidative stress. Recent work on seed longevity similarly emphasizes interactions among redox regulation, macromolecular protection, repair mechanisms, hormonal control, and genetic background (Pirredda et al., 2023; Waterworth et al., 2024).

Implications for seed conservation

The present findings have potential implications for monitoring seed quality during storage. Germination testing remains the most direct measure of viability, but biochemical indicators such as H₂O₂ concentration and

antioxidant activity may provide additional information about physiological deterioration before complete viability loss becomes evident. Reviews of seed ageing increasingly support the use of multiple biochemical and molecular indicators rather than relying on germination percentage alone (Zhang et al., 2021; Nickas et al., 2025). The observed divergence between H₂O₂ and peroxidase-associated activity also suggests that a combination of markers may be more informative than either parameter individually. Future studies could strengthen this model by simultaneously monitoring catalase, ascorbate peroxidase, glutathione status, lipid peroxidation, protein carbonylation, mitochondrial activity, DNA damage, and expression of selected antioxidant and DNA-repair genes.

Overall, the data indicate that seed ageing in *Arabidopsis thaliana* is accompanied by a dynamic relationship between oxidative status, antioxidant response, and germination capacity. The early increase in peroxidase-associated activity may represent an adaptive response, whereas the later accumulation of H₂O₂ and progressive loss of germination suggest that protective mechanisms become increasingly insufficient. The findings therefore support a model in which disruption of redox homeostasis contributes to the broader network of molecular and physiological events underlying seed deterioration.

ACKNOWLEDGMENTS

I sincerely thank Dr. R. Baskar, Department of Biotechnology, Indian Institute of Technology Madras (IIT Madras), for providing the opportunity to undertake this work in his laboratory. I am also grateful to IIT Madras for providing access to the research facilities, instrumentation, and laboratory resources that supported the experimental work. Their support and the availability of these facilities were valuable to the successful completion of the study.

REFERENCES

1. Bailly, C. (2004). Active oxygen species and antioxidants in seed biology. *Seed Science Research*, 14(2), 93–107.
2. Chen, H., Chu, P., Zhou, Y., Li, Y., Liu, J., Ding, Y., Tsang, E. W. T., Jiang, L., Wu, K., & Huang, S. (2012). Overexpression of *AtOGGI*, a DNA glycosylase/AP lyase, enhances seed longevity and abiotic stress tolerance in *Arabidopsis*. *Journal of Experimental Botany*, 63(11), 4107–4121.
3. El-Maarouf-Bouteau, H., & Bailly, C. (2008). Oxidative signaling in seed germination and dormancy. *Plant Signaling & Behavior*, 3(3), 175–182.
4. Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, 48(12), 909–930.
5. Goel, A., Goel, A. K., & Sheoran, I. S. (2003). Changes in oxidative stress enzymes during artificial ageing in cotton (*Gossypium hirsutum* L.) seeds. *Journal of Plant Physiology*, 160(9), 1093–1100.
6. Kibinza, S., Bazin, J., Bailly, C., Farrant, J. M., Corbineau, F., & El-Maarouf-Bouteau, H. (2011). Catalase is a key enzyme in seed recovery from ageing during priming. *Plant Science*, 181(3), 309–315.
7. Kurek, K., Plitta-Michalak, B., & Ratajczak, E. (2019). Reactive oxygen species as potential drivers of the seed aging process. *Plants*, 8(6), 174.
8. Li, W., Niu, Y., Zheng, Y., & Wang, Z. (2022). Advances in the understanding of reactive oxygen species-dependent regulation on seed dormancy, germination, and deterioration in crops. *Frontiers in Plant Science*, 13, 826809.
9. McDonald, M. B. (1999). Seed deterioration: Physiology, repair and assessment. *Seed Science and Technology*, 27(1), 177–237.
10. Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, 15(3), 473–497.
11. Nickas, J., N'Danikou, S., Shango, A. J., & Kilasi, N. (2025). Physio-biochemical response of vegetable seeds to ageing: A systematic review. *Plant Physiology and Biochemistry*, 228, 110223.
12. Oracz, K., El-Maarouf-Bouteau, H., Farrant, J. M., Cooper, K., Belghazi, M., Job, C., Job, D., Corbineau, F., & Bailly, C. (2007). ROS production and protein oxidation as a novel mechanism for seed dormancy alleviation. *The Plant Journal*, 50(3), 452–465.
13. Pirredda, M., Fañanás-Pueyo, I., Oñate-Sánchez, L., & Mira, S. (2024). Seed longevity

- and ageing: A review on physiological and genetic factors with an emphasis on hormonal regulation. *Plants*, 13(1), 41.
14. Rajjou, L., Duval, M., Gallardo, K., Catusse, J., Bally, J., Job, C., & Job, D. (2012). Seed germination and vigor. *Annual Review of Plant Biology*, 63, 507–533.
 15. Scandalios, J. G. (2005). Oxidative stress: Molecular perception and transduction of signals triggering antioxidant gene defenses. *Brazilian Journal of Medical and Biological Research*, 38(7), 995–1014.
 16. Walters, C., Wheeler, L. M., & Grotenhuis, J. M. (2005). Longevity of seeds stored in a genebank: Species characteristics. *Seed Science Research*, 15(1), 1–20.
 17. Waterworth, W. M., Balobaid, A., & West, C. E. (2024). Seed longevity and genome damage. *Bioscience Reports*, 44(2), BSR20230809.
 18. Waterworth, W. M., Bray, C. M., & West, C. E. (2015). The importance of safeguarding genome integrity in germination and seed longevity. *Journal of Experimental Botany*, 66(12), 3549–3558.
 19. Ye, T., Ma, T., Chen, Y., Liu, C., Jiao, Z., Wang, X., & Xue, H. (2024). The role of redox-active small molecules and oxidative protein post-translational modifications in seed aging. *Plant Physiology and Biochemistry*, 213, 108810.
 20. Zhang, K., Zhang, Y., Sun, J., Meng, J., & Tao, J. (2021). Deterioration of orthodox seeds during ageing: Influencing factors, physiological alterations and the role of reactive oxygen species. *Plant Physiology and Biochemistry*, 158, 475–4