Review

Unraveling the Mechanistic Basis for Control of Seed Longevity

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Unraveling the Mechanistic Basis for Control of Seed Longevity

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Abstract: Seed longevity, which holds paramount importance for agriculture and biodiversity conservation, continues to represent a formidable frontier in plant biology research. While advances have been made in identifying regulatory elements, the precise mechanisms behind seed lifespan determination remain intricate and context-specific. This comprehensive review compiles extensive findings on seed longevity across plant species, focusing on the genetic and environmental underpinnings. Inter-species differences in seed lifespan are tied to genetic traits, with numerous *SEED LONGEVITY-ASSOCIATED GENE*s (*SLAG*s) uncovered. These *SLAG*s encompass transcription factors and enzymes involved in stress responses, repair pathways, and hormone signaling. Environmental factors, particularly seed developmental conditions, significantly modulate seed longevity. Moreover, this review deliberates on the prospects of genetically engineering seed varieties with augmented longevity by precise manipulation of crucial genetic components, exemplifying the promising trajectory of seed science and its practical applications within agriculture and biodiversity preservation contexts. Collectively, our manuscript offers insights for improving seed performance and resilience in agriculture's evolving landscape.

Keywords: seed longevity; transcription factor; molecular breeding

1. Introduction

Seed longevity, the inherent ability of seeds to remain viable during storage, plays a pivotal role in the perpetuation of successful plant reproduction [1]. Gradual loss of viability over time is an inherent aspect of seed aging, driven by degradation processes that ultimately reduce seedling emergence and vigor [2]. Since seeds serve as the primary vehicle for plant propagation, maintaining seed longevity is essential not only for sustaining agricultural productivity but also for conserving plant genetic diversity [3]. For cultivated crops specifically, enhanced seed longevity ensures higher probabilities of achieving optimal germination rates and sturdy seedling establishment, thereby contributing to improved crop output [2,3]. Against the backdrop of climate change, which imposes shifting selection pressures and drives changes in plant population genetics, seed conservation becomes a vital strategy in preventing the extinction of species or loss of plant communities that may not adapt or migrate at equal speeds [4]. Both *in situ* and *ex situ* seed conservation practices are thus recognized as indispensable for protecting global plant biodiversity [5]. Therefore, a thorough understanding of the complex factors influencing seed longevity carries profound ecological, agronomic, and economic implications.

Many plant species demonstrate impressive resilience to harsh environmental conditions when stored in a desiccated state [6]. Under such conditions, seeds enter a dormant phase where metabolic activity is drastically curtailed, yet their potential to germinate remains intact over extended periods [7,8]. Notable examples from botanical history abound, showcasing extraordinary seed longevity: date palm seeds (*Phoenix dactylifera*) have been carbon-dated to around 2000 years old [9], sacred lotus

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(*Nelumbo nucifera*) seeds have retained viability after 1300 years [10], and canna (*Canna compacta*) seeds have germinated after 600 years [11]. Inspired by seminal experiments like William Beal's seed burial test initiated over a century ago, researchers continue to explore the mysteries of seed longevity [12], seeking answers to why certain seeds can survive for centuries longer than others [7].

Accumulating research delves into the molecular underpinnings of seed longevity, investigating how specific genes might confer this exceptional durability. Studies in *Arabidopsis*, rice, barley, maize, wheat, lettuce, oilseed rape, and tobacco, among other species, have uncovered genetic determinants of seed longevity [13–22]. An extensive body of work, particularly in *Arabidopsis*, a widely used model organism, has pinpointed *SEED LONGEVITY ASSOCIATED GENE*s (*SLAG*s) (Table 1). Manipulating these genes using molecular techniques has shown promise in altering seed longevity under experimental settings.

Table 1. Genes Involved in Regulating Seed Longevity in *Arabidopsis*.

Locus	Gene	Effect	Pathway	References (PubMed ID)
AT4G13250	NYC1	enhance	Chlorophyll degradation	22751379
AT3G48190	ATM	decrease	DNA repair	27503884
AT5G40820	ATR	decrease	DNA repair	27503884
AT3G05210	ERCC1	enhance	DNA repair	35858436
AT1G16970	KU70	enhance	DNA repair	35858436
AT5G57160	LIG4	enhance	DNA repair	20584150
AT1G66730	LIG6	enhance	DNA repair	20584150
AT1G25580	SOG1	decrease	DNA repair	35858436
AT1G21710	OGG1	enhance	DNA repair	22473985
AT2G31320	PARP1	enhance	DNA repair	35858436
AT5G22470	PARP3	enhance	DNA repair	24533577
AT1G14410	WHY1	enhance	DNA repair	37351567
AT2G02740	WHY3	enhance	DNA repair	37351567
AT5G64520	XRCC2	enhance	DNA repair	35858436
AT1G34790	TT1	enhance	Flavonoid biosynthesis	10677433
AT5G48100	TT10	enhance	Flavonoid biosynthesis	10677433
AT5G42800	TT3	enhance	Flavonoid biosynthesis	10677433
AT3G55120	TT5	enhance	Flavonoid biosynthesis	10677433
AT5G07990	TT7	enhance	Flavonoid biosynthesis	10677433
AT4G09820	TT8	enhance	Flavonoid biosynthesis	10677433
AT3G28430	TT9	enhance	Flavonoid biosynthesis	10677433
AT3G24650	ABI3	enhance	Hormone, ABA	12231895
AT3G18490	ASPG1	enhance	Hormone, ABA	29648652
AT5G45830	DOG1	enhance	Hormone, ABA	17065317
AT2G36610	ATHB22	enhance	Hormone, GA	24335333
AT5G65410	ATHB25	enhance	Hormone, GA	24335333
AT1G14440	ATHB31	enhance	Hormone, GA	24335333
AT1G80340	GA3OX2	enhance	Hormone, GA	24335333
AT2G01570	RGA1	decrease	Hormone, GA	24335333
AT1G14920	RGA2	decrease	Hormone, GA	24335333
AT1G66350	RGL1	decrease	Hormone, GA	24335333
AT3G03450	RGL2	decrease	Hormone, GA	24335333
AT5G17490	RGL3	decrease	Hormone, GA	24335333
AT1G09570	PHYA	decrease	Light	27227784
AT2G18790	PHYB	decrease	Light	27227784
AT2G45970	CYP86A8	enhance	Lipid biosynthesis	32519347
AT3G47860	AtCHL	enhance	Lipid peroxidation	23837879
AT5G58070	AtTIL	enhance	Lipid peroxidation	23837879

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AT1G55020	LOX1	decrease	Lipid peroxidation	28371855
AT1G28440	AtHSL1	enhance	LRR-RLK	35763091
AT2G27500	BG14	enhance	Metabolism Carbohydrate	36625794
AT2G47180	GOLS1	enhance	Metabolism Galactose	26993241
AT1G56600	GOLS2	enhance	Metabolism Galactose	26993241
AT1G30370	AtDLAH	enhance	Metabolism Lipid	21856645
AT2G19900	NADP-ME	enhance	Metabolism Malate	29744896
AT4G15940	AtFAHD1a	decrease	Metabolism Oxoacid	33804275
AT4G02770	PSAD1	enhance	PHOTOSYSTEM	32519347
AT1G62710	β-VPE	enhance	Protein catabolism	30782971
AT2G26130	RSL1	enhance	Protein degradation	24388521
AT5G45360	SKIP31	enhance	Protein degradation	37462265
AT5G53000	TAP46	enhance	Protein dephosphorylation	25399018
AT3G25230	ROF1	enhance	Protein isomerization	22268595
AT5G48570	ROF2	enhance	Protein isomerization	22268595
AT3G48330	PIMT1	enhance	Protein repair	19011119
AT3G57520	AtSIP2	decrease	Raffinose catabolism	34553917
AT4G02750	SSTPR	enhance	RNA modification	32519347
AT1G19570	DHAR1	enhance	ROS detoxification	32519347
AT1G05250	PRX2	enhance	ROS detoxification	31600827
AT2G41480	PRX25	enhance	ROS detoxification	31600827
AT5G64120	PRX71	enhance	ROS detoxification	31600827
AT5G47910	RBOHD	decrease	ROS production	32519347
AT1G19230	RBOHE	decrease	ROS production	32519347
AT1G64060	RBOHF	decrease	ROS production	32519347
AT3G17520	LEA	enhance	Seed development	32519347
AT5G44120	CRUA	enhance	Seed storage protein	26184996
AT1G03880	CRUB	enhance	Seed storage protein	26184996
AT4G28520	CRUC	enhance	Seed storage protein	26184996
AT4G36920	AtAP2	enhance	TF AP2/EREBP	10677433
AT5G53210	SPCH1	enhance	TF bHLH	32519347
AT2G34140	CDF4	enhance	TF DOF	27227784
AT1G29160	COG1	enhance	TF DOF	31600827
AT4G00940	DOF4.1	decrease	TF DOF	35845633
AT5G42630	ATS	enhance	TF G2-LIKE	10677433
AT1G79840	GL2	enhance	TF HB	10677433
AT1G62990	KNAT7	decrease	TF HB	32519347
AT5G54070	AtHSFA9	enhance	TF HSF	32683703
AT5G15800	AGL2	decrease	TF MADS	32519347
AT1G18710	MYB47	enhance	TF MYB	32519347
AT1G21970	LEC1	enhance	TF NF-YB	19754639
AT2G38470	WRKY33	enhance	TF WRKY	26410298
AT4G32770	VTE1	enhance	Tocopherol biosynthesis	15155886
AT2G18950	VTE2	enhance	Tocopherol biosynthesis	15155886
AT1G73190	TIP3.1	enhance	Transmembrane transport	26019256
AT1G17810	TIP3.2	enhance	Transmembrane transport	26019256

To systematize research efforts and facilitate further exploration, a comprehensive literature review has led to the assembly of information on *SLAG*s and their corresponding mutants across multiple species. This collective endeavor has resulted in the development of a dedicated database (https://ngdc.cncb.ac.cn/lsd/slag_mutant.php) [23], providing a rich resource and a firm groundwork for advancing knowledge into the molecular intricacies of seed longevity. This valuable tool enables researchers to delve deeper into the mechanisms that allow certain seeds to defy time, enduring for

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centuries, and paves the way for targeted interventions to enhance seed survival and preserve biodiversity.

2. Molecular Genetics Governing Seed Longevity

2.1. Transcription Factors in Regulating Seed Longevity

Transcriptional regulation serves as a pivotal coordinator governing diverse developmental processes and adaptive responses to a wide array of environmental challenges in plants [24,25]. At the epicenter of this regulatory mechanism are transcription factors (TFs), which exert profound control over fundamental facets of plant physiology [25,26]. Within the context of seed longevity, TFs constitute key regulators that dictate the expression of genes involved in maintaining seed vigor and viability over extended periods of storage and dormancy (Figure 1). Through their ability to bind to specific DNA sequences and modulate gene expression patterns, these TFs orchestrate a complex network that contributes to seed longevity and resilience.

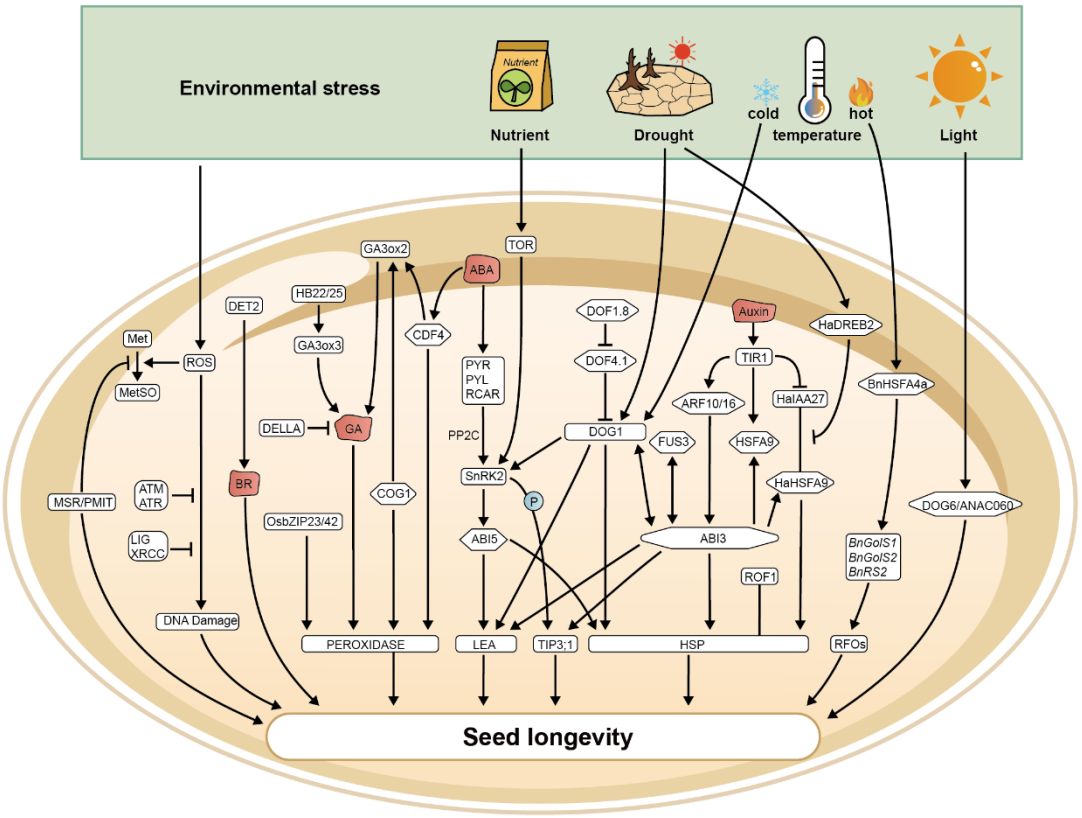

Figure 1. Influence of Endogenous and Exogenous Signals on Seed Longevity and Underlying Regulatory Mechanisms. Seed longevity is influenced by a variety of environmental cues such as nutrient status, temperature, moisture, and light, as well as internal signals like plant hormones. Endogenous and exogenous stress factors have the ability to trigger diverse types of damage, such as DNA damage and protein damage, which in turn compromise seed longevity or vigor. Multiple plant hormones, such as ABA and GA, regulate seed longevity through modulation of transcription factors. ABA, Abscisic Acid; GA, Gibberellic Acid; BR, Brassinosteroids; ROS, Reactive Oxygen Species; PMIT, Protein-L-isoaspartate (D-aspartate) O-methyltransferase; ATM, ATAXIA TELANGIECTASIA MUTATED; ATR, ATAXIA TELANGIECTASIA AND RAD3-RELATED; CDF, CYCLING DOF FACTOR; COG1, COGWHEEL1; TOR, TARGET OF RAPAMYCIN; DOF, DNA BINDING WITH ONE FINGER; DOG1, Delay of Germination 1; HSFA9, heat shock factor A9; ROF1, ROTAMASE FKBP 1; HSP, heat shock protein; TIP3;1, ALPHA-TONOPLAST INTRINSIC PROTEIN; TIR1,

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TRANSPORT INHIBITOR RESPONSE 1. The arrow represents promotion, while the vertical line stands for inhibition. P, Phosphorylation.

The plant-specific TF ABSCISIC ACID-INSENSITIVE3 (ABI3) has emerged as a critical player in orchestrating seed dormancy and longevity via ABA-dependent pathways, as evidenced by extensive studies in *Arabidopsis* [27]. Mutations in the *ABI3* gene lead to aberrant seed maturation, compromising dormancy, desiccation tolerance, and longevity, often accompanied by impaired chlorophyll breakdown [28]. ABI3 exerts its regulatory influence by binding to the evolutionarily conserved RY motif [CATGCA(TG)] prevalent within the promoter regions of numerous seed-specific genes [29]. Notably, *AtHSFA9* and *TONOPLAST INTRINSIC PROTEIN 3;1* (*TIP3;1*), both bearing RY motifs in their promoter sequences, serve as downstream targets of ABI3 and contribute significantly to seed longevity enhancement. While AtHSFA9 is a seed-specific heat shock factor that bolsters longevity upon activation [30], TIP3;1, a seed-specific aquaporin, also contributes positively to longevity under ABI3 regulation [31]. Loss of function of AtHSFA2 or AtHSFA9 significantly reduces seed longevity in Arabidopsis, whereas overexpresion of *AtHSFA2* or *AtHSFA9* leads to the increased accumulation of heat shock proteins (HSPs) and superior seed longevity [32]. As a chaperone of HSFA2, HSP90 interacts with ROTAMASE FKBP 1 (ROF1) and ROF2 to bolster seed longevity [33] (Figure 1). Accordingly, disruption of *ROF1*/*ROF2* results in increased sensitivity to accelerated aging and poor germination under adverse conditions [33]. Moreover, the orthologs of *AtHSFA9* across various plant species consistently demonstrate their ability to augment seed longevity. For instance, the overexpression of *Helianthus annuus HSFA9* (*HaHSFA9*) or *Medicago truncatula HSFA9* (*MtHSFA9*) results in enhanced seed thermo-tolerance and longevity, thus presenting promising candidates for molecular breeding interventions [34,35]. The interplay between TFs further underscores the complexity of seed longevity regulation. *Helianthus annuus DROUGHT RESPONSIVE ELEMENT BINDING FACTOR 2* (*HaDREB2*), an AP2/ERBP family member, amplifies the seed longevity effects of *HaHSFA9* when co-expressed, potentially by disrupting the suppressive interaction between HaHSFA9 and AUXIN-RESPONSIVE PROTEIN 27 (HaIAA27), a protein encoded by the *AUXIN/INDOLE-3-ACETIC ACID* (*Aux/IAA*) gene, thereby liberating HaHSFA9's function to promote longevity [36,37]. However, HaDREB2 alone does not increase seed longevity without HaHSFA9.

Expanding the scope, research continues to reveal the multifaceted roles of additional TF families in regulating seed longevity. Members of the DNA BINDING WITH ONE FINGER (DOF) family, which are plant-specific TFs with a broad spectrum of biological functions [38], have been implicated in modulating seed longevity. Genetic evidence from the *Arabidopsis dof4.1* loss-of-function mutant shows enhanced seed viability following artificial aging treatments, suggesting that DOF4.1 operates as a negative regulator of seed longevity [39]. Transcriptomic analysis unveiled that the expression of *DELAY OF GERMINATION* (*DOG1*), a positive regulator of seed maturation and longevity [40,41], is upregulated in the *dof4.1* mutant compared to wild type plants, suggesting that DOF4.1 may negatively regulate seed longevity by repressing *DOG1* [39]. Conversely, COGWHEEL1 (COG1/DOF1.5) and CYCLING DOF FACTOR 4 (CDF4/DOF2.3) serve as positive regulators, with their overexpression conferring resistance to seed deterioration in Arabidopsis [42,43]. COG1 enhances seed longevity possibly by increasing expressions of peroxidase genes based on the transcriptomic analysis of *cog1-2D*, a gain-of-function mutant with increased seed longevity [42]. Co-expression network analysis identified the TFs, *WRKY3* and *NFXL1*, as components involved in seed longevity, as loss-of-function mutants of *wrky3* and *nflx1* exhibit reduced seed longevity [44]. Genome-wide association study (GWAS) revealed several TFs, including MYB TF (MYB47), MADS box TF (SEPALLATE 3, SPE3), and homeodomain (HB) TF (KNOTTED-LIKE HOMEOBOX OF ARABIDOPSIS THALIANA 7, KNAT7), as positive regulators of seed longevity [45]. The *athb25-1D* dominant *Arabidopsis* mutant, with higher expression of *HOMEOBOX PROTEIN 25*, displays improved seed longevity [46], further supporting the involvement of HB TFs in seed longevity regulation.

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High-throughput RNA sequencing has identified SEUSS, a transcriptional corepressor linked to embryonic development [47], as being significantly upregulated in aged seeds of *Astronium fraxinifolium* [48]. While this suggests a potential role in seed longevity, the exact regulatory mechanisms remain unclear. In the floral meristem, SEUSS is known to interact with APETALA1 (AP1) and SHORT VEGETATIVE PHASE (SVP) to repress homeotic gene expression, thereby preventing premature differentiation of the floral meristem [47]. Given this, it is plausible that in the context of seed longevity, SEUSS may be recruited by specific transcription factors to form a complex that fine-tunes the expression of genes related to seed longevity.

Seed vigor and longevity are pivotal for enhancing grain quality and germplasm conservation in crops. RNA-seq co-expression regulatory network analyses identified the bZIP transcription factors *bZIP23* and *bZIP42* as candidate genes for seed longevity in rice [49]. The overexpression of *bZIP23* robustly elevates seed vigor, a process linked to the activation of *PEROXIDASE 1A* (*PER1A*), unveiling a bZIP23-PER1A-mediated detoxification pathway that fortifies seed vigor [49]. This finding underscores the potential of targeted manipulation of key TFs through genome editing as a viable strategy to boost seed vigor, overall seed quality, and ultimately crop yield. In conclusion, transcriptional regulation, particularly through TFs like ABI3, DOF, MYB, MADS box, HB, and bZIP family proteins, constitutes a sophisticated network that deeply impacts seed longevity and vigor. These discoveries provide fertile ground for innovative agricultural advancements and germplasm conservation practices in crop species.

2.2. Impact of DNA Damage Repair on Seed Longevity

Seeds in their desiccated state possess extraordinary survival capabilities, yet they face a significant challenge as substantial DNA damage accumulates during storage, accelerating seed aging and impairing vigor [50]. To unravel the intricate defense mechanisms against this damage, researchers have turned to mutants with altered DNA repair-related genes [1,51]. This line of inquiry has shed light on the essential contribution of specific elements within the DNA repair pathway to seed longevity (Figure 1). Key players in this context include the proteins ATAXIA TELANGIECTASIA MUTATED (ATM) and ATM AND RAD3-RELATED (ATR) [52], SUPPRESSOR OF GAMMA 1 (SOG1) [53], DNA LIGASE 4 and 6 (LIG4/6) [14,54], KU70, X-RAY REPAIR CROSS COMPLEMENTING 2 (XRCC2), POLY(ADP-RIBOSE) POLYMERASE 1 (PARP1) and PARP3, EXCISION REPAIR CROSS COMPLEMENTING-GROUP 1 (ERCC1) [53], 8-OXOGUANINE (8-OXOG) and DNA GLYCOSYLASE 1 (OGG1)[55], as well as WHIRLY 1 (WHY1) and WHY3 [56].

Among the various forms of DNA damage, double-strand breaks (DSBs) are particularly detrimental [57]. The recognition of DSBs sparks intricate intracellular signaling cascades governed by the protein kinases ATM and ATR [58,59]. Mutant plants lacking functional ATM are more sensitive to DSBs and exhibit early-onset leaf senescence [60]. An intriguing observation is that seeds from ATM mutants germinate more rapidly than wild-type seeds following accelerated aging under harsh conditions of high temperature and humidity [52]. Despite this faster germination, aged ATM mutant seeds show a high prevalence of chromosomal abnormalities. Moreover, seedlings arising from these aged seeds experience reduced survival rates and slower development of true leaves compared to wild-type seedlings, emphasizing ATM's vital role in maintaining the genomic integrity of the germinating embryo [52].

Organisms combat DSBs using two primary repair pathways: homologous recombination (HR) and non-homologous end-joining (NHEJ) [61]. When subjected to accelerated aging, mutant lines deficient in either HR (*xrcc2-1*, *why1 why3*) or NHEJ (*ku70-1*, *ku80-3*, *lig4 lig6*) pathways exhibit a marginally delayed germination [53,54,56]. Additionally, base excision repair (BER) and nucleotide excision repair (NER) pathways, exemplified by mutants *arp1* and *ogg1* (BER) and *ercc1* (NER), also play a role in maintaining seed viability under stress, showing slightly delayed germination under similar conditions [53]. In summary, seed longevity is critically dependent on the proper functioning of DNA repair pathways, especially those involving ATM, ATR, and other associated components. Mutations in these genes can impact seed vigor and chromosomal stability. The ATM mutants,

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although demonstrating accelerated germination, suffer from compromised chromosomal integrity, revealing a delicate balance between rapid germination and genomic fidelity [52]. By deepening our understanding of the role DNA repair plays in seed aging, we can develop informed strategies to enhance seed viability and bolster crop resilience.

2.3. Role of Protein Repair or Homeostasis in Maintaining Seed Longevity

Reactive oxygen species (ROS) cause oxidative damage to seed proteins during storage, affecting their structural integrity and functionality, a typical hallmark of aging [62]. Among the amino acids, Methionine (Met) is notably susceptible to oxidation, transforming into S- and R-diastereomers of methionine sulfoxide (MetSO) [63,64]. Methionine sulfoxide reductase (MSR), consisting of MSRA and MSRB subtypes that specifically reduce Met-S-SO and Met-R-SO back to Met, respectively [65], counteracts this oxidation. MSR activity positively correlates with seed longevity across various plant species and its expression levels [66–68]. In aged rice seeds, reduced MSR activity and elevated MetSO content correlate with decreased seed vigor [66]. Overexpression of the seed-specific enzyme, *OsMSRB5*, effectively diminishes MetSO formation, thereby enhancing seed vigor and longevity by optimizing ROS balance [66], underscoring the critical role of MSR in sustaining seed longevity.

Simultaneously, cellular proteins inherently incur covalent damage that contributes to aging [69]. During seed aging, damaged proteins accrue abnormal isoaspartate (isoAsp) residues, degrading seed vigor and longevity. This issue is addressed by Protein-L-isoaspartyl methyltransferase (PIMT), which repairs these residues and positively affects seed longevity across several plant taxa [70–73]. PIMT activity, predominantly observed in seeds, is instrumental for seed vigor and longevity. Elevating *PIMT* gene expression boosts seed longevity and germination vigor in *Arabidopsis* and chickpea [70,73]. Analogously, overexpression of *OsPIMT1* in rice reduces isoAsp accumulation, thus improving embryo viability and extending seed longevity [72]; conversely, loss of PIMT function leads to decreased seed vigor under stress conditions. The pivotal part played by PIMT in extending seed longevity underscores its critical importance in combating the detrimental effects of isoAsp accumulation during seed senescence.

Seeds can survive extreme desiccation for millennia by entering a state of quiescence. This involves accumulating protective storage proteins and lipids through intricate adjustments in protein homeostasis. Recently, reseachers found that disruption of proteostasis tiggerred by mutations of type-II metacaspase (MCA-II) proteases compromises seed longevity in *Arabidopsis* [74]. MCA-II mutant seeds fail to confine the AAA ATPase CDC48 (CELL DIVISION CYCLE 48) to the endoplasmic reticulum, leading to the accumulation of misfolded proteins and compromised seed viability. The localization of CDC48 to the endoplasmic reticulum is contingent upon MCA-II-mediated cleavage of PUX10 (ubiquitination regulatory X domain–containing 10), an adaptor protein that regulates the association of CDC48 with lipid droplets. PUX10 cleavage facilitates the dynamic shuttling of CDC48 between lipid droplets and the endoplasmic reticulum, a critical regulatory mechanism for maintaining spatiotemporal proteolysis, lipid droplet dynamics, and overall protein homeostasis. Interestingly, removing the PUX10 adaptor in MCA-II mutant seeds partially restores proteostasis, CDC48 localization, and lipid droplet dynamics, thereby extending seed lifespan [74]. This work reveals a novel proteolytic module essential for seed longevity.

2.4. Role of RFOs in Regulating Seed Longeivity

Raffinose family oligosaccharides (RFOs), including raffinose, stachyose and verbascose, play a critical role in seed longevity and vigor [75–79]. During seed maturation, RFOs accumulate alongside other compounds such as sucrose and LEA proteins, contributing to desiccation tolerance and the preservation of cellular integrity. This, in turn, enhances seed longevity and vigor [75–79].

Galactinol synthase (GolS), a key enzyme in RFO biosynthesis, is upregulated during seed development, leading to increased RFO levels [75]. Overexpression of *Cicer arietinum CaGolS1/2* or *Arachis duranensis AdGolS3* in *Arabidopsis* has been shown to improve seed vigor and longevity [75,80]. Additionally, mutations in *GolS* genes can lead to reduced galactinol levels and decreased seed

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lifespan [79]. The critical role of RFOs in longevity is further underscored by the *zmdreb2a* mutant in maize, which exhibits decreased seed longevity due to reduced expression of *ZmRS* (*raffinose synthase*), a gene responsible for raffinose synthesis, and consequently lower RFO accumulation [77]. Interestingly, the relationship between RFOs and seed vigor is complex and may vary between plant species. In *Arabidopsis*, the total RFO content and RFO/sucrose ratio, rather than individual RFO amounts, are positively correlated with seed vigor [76]. In contrast, in maize, raffinose appears to be the primary RFO associated with seed vigor [76]. The expression of *GolS* is regulated by various factors. Heat shock cis-elements (HSEs) have been identified in the promoter of *BnGolS1* in *Brassica napus* [21], suggesting regulation by heat shock factors (HSFs). BnHSFA4a, a heat shock transcription factor, binds to these HSEs and activates *BnGolS1* expression. Additionally, BnHSFA4a can directly regulate the expression of other genes involved in RFO biosynthesis, such as *BnGolS2* and *BnRS2*, further enhancing RFO production and improving seed longevity and stress tolerance [21].

RFOs are hydrolyzed during seed germination, but the specific genes involved in this process are not fully understood. Maize alkaline α-galactosidase 1 (ZmAGA1) is a key enzyme responsible for RFO hydrolysis [81]. Overexpression of *ZmAGA1* enhances seed germination under stress conditions but may also negatively impact seed aging tolerance [81], suggesting a potential trade-off between seed germination and longevity. In support of this observation, integrated quantitative trait locus (QTL) analyses for seed longevity in *Arabidopsis* reveal a negative correlation between seed longevity and seed dormancy [14]. In summary, RFOs play a crucial role in seed longevity and vigor. Their biosynthesis is regulated by factors such as GolS and HSFs, while the hydrolysis of RFOs during germination is mediated by enzymes like ZmAGA1. Understanding the complex interplay between RFO biosynthesis, hydrolysis, and seed quality is essential for developing strategies to improve seed longevity and vigor.

2.5. Hormonal Regulation of Seed Longevity

Phytohormones play a central role in orchestrating the complex series of events during seed maturation, profoundly impacting essential quality attributes such as germination potential, dormancy, and longevity [82]. Advances in molecular-genetic, biochemical, and pharmacological research have progressively uncovered the detailed contributions of phytohormones to seed longevity and the underlying regulatory mechanisms (Figure 1).

2.5.1. ABA: A Central Regulator of Seed Longevity

Among the phytohormones, abscisic acid (ABA) is a pivotal regulator intricately involved in controlling seed longevity, dormancy, and desiccation tolerance [27]. Deficiencies in ABA synthesis and signaling components significantly impinge on seed longevity, as demonstrated in several studies [1,13,83,84]. For example, the aba1 mutant, unable to produce epoxy-carotenoid precursors necessary for ABA biosynthesis, exhibits drastically reduced ABA levels compared to wild-type plants in Arabidopsis. Dominant mutations like abi1-1 and abi2-1, which affect genes coding for type 2C protein phosphatases (PP2C), interfere with ABA signaling by inhibiting SUCROSE NON-FERMENTING 1-RELATED PROTEIN KINASE 2 (SnRK2), leading to attenuated ABA responsiveness. Notably, ABA-deficient mutants (*aba1*) and ABA-insensitive mutants (*abi1-1* and *abi2-1*) display reduced desiccation tolerance and longevity in Arabidopsis [85].

The perception of ABA begins with the engagement of intracellular receptors, specifically pyrabactin resistance 1 (PYR1) and PYR1-like (PYL) proteins, which form complexes with clade A PP2Cs, ultimately activating SnRK2 protein kinases [86]. Activated SnRK2s then modulate the expression of ABA-responsive genes by phosphorylating transcription factors like ABA-responsive element-binding factors (ABFs) [86]. Accordingly, mutants devoid of functional PYR/PYL, SnRK2, or ABF2/3/4 display compromised longevity relative to wild-type plants. Interestingly, SnRK2.6 regulates seed longevity by phosphorylating aquaporin PIP2;1 [86], suggesting that ABA controls seed longevity through regulating aquaporin function at both transcriptional and post-translational levels.

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2.5.2. Impact of Auxin on Seed Longevity

Auxin, a pivotal plant hormone, exerts a sophisticated influence on the attainment of seed longevity [37,87,88]. During the maturation of *Arabidopsis* seeds, there is a concurrent escalation and spatial distribution of auxin signaling inputs and outputs within the embryo, tightly aligned with the seed's journey towards achieving longevity [88]. Experimental supplementation of auxin during the maturation phase has been demonstrated to enhance seed longevity. Mutants with dysfunctional auxin biosynthesis pathways consistently exhibit altered longevity, reflecting a clear dose-response relationship that is tied to the intensity of auxin signaling activity [88]. The identification of a conserved gene network related to seed longevity, enriched with the cis-regulatory element ARFAT, an auxin response factor binding site, underscores the direct link between auxin signaling and the acquisition of longevity [44]. Moreover, biochemical evidence reveals that auxins enhance seed longevity by destabilizing the HaIAA27 protein and thus stimulating *HSFA9* expression [37], providing additional insights into the molecular mechanisms underlying auxin regulation of seed longevity.

Auxin's downstream actions intersect with the ABA signaling cascade within the embryo. It has been found that auxin promotes the expression of *ABI3* and its LEA protein target, *EARLY METHIONINE1* (*EM1*), with ABI3 activity shown to be dysregulated in the auxin biosynthesis mutant *cyp79b2* [88]. More importantly, the beneficial influence of external auxin application on seed longevity during development is negated in a*bi3-1* mutants, underscoring the synergy between auxin and ABA pathways [88]. Beyond its interaction with the ABA signaling pathway, auxin may also directly modulate genes pertinent to seed longevity, implicating its involvement through both ABA-dependent and independent routes. This dual regulatory mechanism suggests that auxin plays a multifaceted role in the complex regulatory web governing seed longevity.

2.5.3. Influence of Gibberellins (GA) on Seed Longevity

GAs are known for their prominent role in triggering seed germination and subsequent growth. Comparative analyses of higher longevity (HL) and lower longevity (LL) varieties after natural aging have led to the identification of specific long-lived mRNAs in rice, including the gibberellin receptor gene GID1. Seeds store various long-lived mRNAs, some of which are crucial for the early stages of germination and, consequently, for seed longevity. These findings suggest that gibberellin signaling plays a role in seed longevity [89]. Genetic analysis has shown that overexpression of *ARABIDOPSIS THALIANA HOMEOBOX 25* (*AtHB25*) in an *Arabidopsis* 'activation tagging' line collection resulted in elevated levels of active gibberellins and increased transcripts of the gibberellin biosynthesis gene *GIBBERELLIN 3-OXIDASE 2* [46]. This augmented gibberellin activity was correlated with improved resistance to controlled deterioration tests (CDT). Of note, GA3-treated plants and the quintuple DELLA mutant, characterized by persistent gibberellin responses, displayed stronger CDT resistance, pointing to a potentially positive role of gibberellins in enhancing seed longevity [46]. However, conflicting evidence arises from mutants like *ga1-3*, which is defective in gibberellin synthesis, and the gibberellin-insensitive *gai* mutant, neither of which showed decreased germination following prolonged dry storage when compared to wild-type plants [90]. This inconsistency highlights that while there is suggestive evidence for gibberellins' participation in seed longevity, more research is needed to definitively establish their precise role.

Despite the wealth of research affirming ABA's role in seed longevity, the contribution of other hormones remains less clear-cut [91]. Studies have shown that mutants resistant to ethylene and jasmonic acid do not significantly lose viability after long-term storage, implying a limited role for these hormones in regulating longevity [83]. On a separate note, recent findings indicate that brassinosteroids (BR) might negatively affect seed longevity during the priming process, a controlled treatment designed to improve germination performance [92]. Seeds from BR-deficient mutants such as *cyp85a1/a2* and *det2* demonstrate prolonged longevity post-priming in *Arabidopsis* [92], suggesting a possible connection between BR signaling and seed longevity, an area that merits further investigation.

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2.6. Seed Dormancy and Longevity: Positive and Negative Correlations

Seed dormancy and longevity are both critical traits for plant survival and agricultural productivity. Ideally, a favorable correlation between these traits would benefit both natural ecosystems and crop cultivation. However, studies have reported both positive and negative correlations between seed dormancy and longevity [93].

The testa of higher plant seeds protects the embryo against adverse environmental conditions. Mutations in the *TRANSPARENT TESTA* genes have been shown to decrease seed dormancy and longevity in *Arabidopsis* due to increased seed coat permeability and altered flavonoid deposition [93]. Disruptions in cutin biosynthesis and deposition, caused by mutations in genes such as *GPAT4/8*, *DCR*, *LACS2*, or *BDG1*, also compromise seed dormancy and longevity in *Arabidopsis* [94]. Mutants in the *VTE1* gene, which is involved in tocopherol-mediated antioxidant activity, exhibit decreased levels of both dormancy and longevity in *Arabidopsis* [95]. Interestingly, a loss-of-function mutation in *RBOHD*, which encodes an NADPH oxidase, results in increased dormancy and longevity in *Arabidopsis* compared to the wild type [45,96]. Additionally, using a tetragenic system, researchers discovered that natural genes controlling seed dormancy are also involved in the regulation of soil seed bank longevity in rice [97]. These findings suggest a positive correlation between seed dormancy and longevity.

Higher-order *DELLA* mutants in a gibberellin-deficient background (*ga1-3*) exhibit reduced dormancy due to the constitutive activation of gibberellin signaling in *Arabidopsis* [98]. Conversely, the *DELLA* quintuple mutant demonstrates increased resistance to accelerated aging, likely attributable to enhanced seed coat mucilage production [46]. Disruption of *CYP707A1/A2*, which are involved in ABA catabolism, results in enhanced dormancy but reduced longevity in *Arabidopsis* [99]. Similarly, the aspartic protease ASPG1, which is responsible for seed reserve mobilization, shows increased dormancy but decreased longevity in mutants with reduced proteolytic activity [100]. Auxin biosynthesis mutants, such as *taa1*, *tar1*, and *yuc1*, display reduced dormancy but increased longevity in *Arabidopsis* [88]. Additionally, quantitative trait locus (QTL) analyses in recombinant inbred line populations have revealed a negative correlation between seed dormancy and seed longevity in *Arabidopsis* [14]. These findings suggest that ABA catabolism and auxin biosynthesis play pivotal roles in the observed negative correlation between seed dormancy and longevity.

In summary, existing genetic analyses have demonstrated both positive and negative correlations between seed dormancy and longevity. However, it is challenging to interpret these contradictory findings, given that few studies have explicitly examined the relationship between these two traits. Nonetheless, based on the limited research available, it is possibly reasonable to hypothesize that the regulation of seed dormancy and longevity involves both shared and independent signaling pathways.

3. Environmental Regulation of Seed Longevity

Seed longevity is intricately influenced by a multitude of environmental factors in conjunction with genetic determinants [101,102] (Figure 1). The environment experienced by the maternal plant during seed maturation, along with the conditions following harvest and throughout storage, plays a critical role in dictating seed viability [17,103]. Key environmental parameters that significantly affect seed longevity include temperature, humidity, light exposure, and oxygen concentration [104]. Soil attributes, such as pH levels and mineral content, also have profound effects on seed survival within the soil seed bank [105,106]. Additionally, the seed microbiome, composed of endophytes and pathogens, subtly adjusts the seed microenvironment and defense mechanisms, thus influencing seed longevity [107]. Our understanding of seed longevity in cultivated crops primarily stems from studies employing wet aging conditions to assess seed vigor. By contrast, a broader range of dry storage conditions has been applied to wild species. Despite this, our understanding of how environmental factors interact with molecular regulators of longevity remains incomplete.

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3.1. Influence of Temperature on Seed Longevity

Storage temperature primarily impacts seed longevity through its modulation of enzymatic activities within the seed [108]. Elevated temperatures and moisture levels intensify seed metabolism, whereas lower temperatures modify the phenylpropanoid composition and permeability of the seed coat, as observed in *Arabidopsis* mutants *transparent testa* and reduce longevity [93]. Consequently, precise temperature management is essential to optimize seed longevity during storage. The temperature-sensitive DOG1, a key regulator of seed dormancy in *Arabidopsis*, also influences seed longevity [40]. DOG1 promotes seed longevity by upregulating a variety of genes, including *HSP*s and *LEA*s, partly through the activation of *ABI5* expression and in concert with ABI3 signaling [109]. Moreover, DOG1 protein levels fluctuate with seed maturation temperature, and the loss of DOG1 function hampers seed dormancy induction at low maturation temperatures [110]. It is plausible that DOG1 orchestrates seed longevity responses to temperature via similar mechanisms.

Furthermore, the effect of temperature on seed longevity varies across species and genotypes [13,105,111]. Warm temperatures during seed development tend to boost longevity in alpine species and A*rabidopsis*, yet they might be detrimental to rice and *Medicago truncatula* [108,112]. On the other hand, low temperatures during seed development might diminish longevity in *Arabidopsis*, but do not seem to have a noticeable effect on *Medicago truncatula*, potentially due to species-specific adaptations [108].

3.2. Water Availability

Water availability has a nuanced and context-dependent impact on seed longevity, which varies according to species and the intensity of water stress [113]. Soybean seeds experiencing water stress during maturation produce mature green seeds that correlate with decreased longevity [114]. Drought similarly reduces seed longevity in Medicago truncatula, even without an obvious chlorophyll retention phenotype [108]. However, in Brassica rapa, withholding irrigation during early seed filling stages actually accelerates the accrual of seed longevity, enabling longer viability under dry storage [104]. Peanuts exemplify the species-specific response where drought stress during seed development can lead to increased longevity [101,113].

The effect of water availability on seed longevity is complex and stage-dependent. Developing seeds possess a remarkable ability to adjust their maturation processes in response to water availability, thereby mitigating potential losses in longevity [113]. Field experiments on wheat, simulating varying rainfall patterns across growth stages, demonstrate the adaptability of seed development in response to water availability [115]. Field experiments on wheat, simulating rainfall fluctuations at different developmental stages, demonstrate the plasticity of seed development programs in response to water availability [116]. An increase in seed water content due to wetting during development can decrease post-harvest longevity, yet allowing seeds to re-dry naturally restores much of this lost longevity [116], underscoring the intricate interaction among water availability, seed development, and longevity.

Although the exact molecular mechanisms behind water-regulated seed longevity are not fully understood, ABA signaling pathways appear to play a pivotal role in mediating seed maturation and dormancy under water stress [117]. Further exploration into these pathways is expected to shed light on the intricate regulatory networks that govern seed longevity in variable environmental contexts.

3.3. Light Exposure

During seed filling, embryos actively absorb 20-30% of light, particularly enriched in green and far-red wavelengths [118]. Photosynthesis is essential for energy production required for seed reserve accumulation, with chloroplasts adapting their pigment composition and photosystem activity to cope with shade conditions [104]. As seeds mature and gain longevity, chloroplasts disintegrate, and chlorophyll molecules undergo specialized degradation mechanisms differing from those seen in senescent leaves [101,119].

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To ensure longevity, it's crucial to manage photochemical reactions during chloroplast breakdown to avoid the build-up of toxic compounds [120]. Photoperiod and light intensity during seed development both influence seed longevity, with evidence pointing to their regulatory roles in pathways related to longevity [14]. In Arabidopsis, light perception involves genes underlying the DOG3 and DOG6 loci, hinting at a genetic basis for light-mediated longevity control [117]. Future research focused on cloning and characterizing these genes will likely uncover more about the intricate interplay between light perception and seed longevity regulation.

3.4. Nutrient Supply

Nutrient availability in the soil and the mother plant's nutritional state profoundly influences seed yield and germination traits, reflecting a sophisticated interplay between genetics and environmental elements like temperature and light [13,101]. Various plant species, including tomato, Arabidopsis, and oilseed rape, provide evidence for the impact of nitrate, phosphate, and sulfate availability on seed traits [104]. Although the direct connection between nutrient availability and seed longevity has been less studied, new evidence suggests a potential link.

In barley, seeds harvested from plants grown under optimal nutrient conditions demonstrated enhanced longevity compared to those from nutrient-limited environments [101]. Nitrogen availability has been found to affect seed longevity in Arabidopsis, with higher nitrate levels corresponding to longer seed lifespans [121]. Changes in amino acid and glucoronate contents, along with alterations in gene transcripts linked to cell wall metabolism, emphasize the impact of nutrient availability on seed composition and longevity [122]. Metabolic sensors like the Target of Rapamycin (TOR) complex and SnRK1 complex are crucial for integrating nutrient and energy signals to regulate seed development and longevity [123]. Mutant plants lacking these sensors exhibit decreased resistance to aging, highlighting the importance of metabolic sensing pathways in regulating seed longevity [101]. Thus, a deeper understanding of the intricate balance between nutrient availability, metabolic sensing, and seed longevity necessitates further investigation into the underlying molecular mechanisms.

3.5. Oxygen Level

Oxygen levels during storage significantly affect seed longevity by modulating the formation of reactive oxygen species (ROS) and consequent oxidative damage to macromolecules [124]. High oxygen levels are associated with increased chromosomal abnormalities and reductions in seed viability and vigor [125,126]. Research on Vicia faba and soybean seeds stored under elevated oxygen pressures demonstrates the harmful effects of high oxygen levels, leading to rapid loss of germination capacity [127].

On the other hand, reduced oxygen levels can extend seed longevity during storage. Ultra-dried Brassica seeds maintained viability for over three decades when kept in a modified atmosphere with lowered oxygen levels [128]. These results highlight the significance of oxygen regulation for maintaining seed viability during storage and point to the promise of modified atmosphere storage techniques for seed preservation.

4. Strategies to Enhance Seed Longevity

Current research highlights that seed longevity is determined not only by external environmental influences but also by intricate genetic factors [84]. The revelation of numerous genes deeply involved in seed longevity pathways raises the possibility of engineering seed varieties with enhanced longevity by targeting specific genetic elements. Additionally, considering that seeds often encounter suboptimal storage conditions that undermine their viability, exploring feasible methods to restore vigor to aged seeds is crucial for agriculture, laboratory research, and plant biodiversity conservation.

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4.1. Extension of Seed Longevity Through Molecular Genetics

Genetic regulation plays a significant role in controlling seed longevity, thus offering a route to manipulate this trait by altering gene expression using molecular genetics or genome editing technologies. Researchers have probed the molecular foundations of seed longevity, pinpointing essential genes (*SEED LONGEVITY-ASSOCIATED GENE*, *SLAG*) and pathways. For example, genes involved in antioxidant defense systems, such as *SUPEROXIDE DISMUTASE* (*SOD*), *CATALASE* (*CAT*), and *PEROXIDASES* (*POD*), help mitigate oxidative stress and preserve seed viability during storage [129]. Genes responsible for synthesizing and managing storage compounds, like LEAs and HSPs, have also been shown to significantly affect seed longevity [130].

With recent breakthroughs in genome editing, such as CRISPR-Cas9, scientists can now make precise adjustments to the genetic makeup of seeds. For example, editing the *FUSCA3* (*FUS3*) gene, which acts as a key regulator of seed maturation and longevity [44,131–133], has proven effective in enhancing seed storability and germination vigor in *Arabidopsis*. Similarly, CRISPR/Cas9-mediated knockout of the *LIPOXYGENASE 10* (*OsLOX10*) gene in rice led to increased seed longevity compared to wild-type under artificial aging conditions [134]. Knockout of type-II metacaspase (MCA-II) proteases via CRISPR in *Arabidopsis* disrupts proteostasis in seeds, thereby compromising seed longevity [74]. Leveraging molecular genetics and genome editing opens up the possibility of designing seeds with improved longevity characteristics tailored to specific environmental conditions or storage regimens. However, more research is needed to fully understand the complex genetic networks that govern seed longevity and to optimize the application of these techniques in crop improvement projects. Additionally, it's crucial to remember that gene editing can have unexpected consequences due to the complex nature of gene function. For example, although a knockout of the *RBOHD* gene can positively impact seed dormancy and longevity [45,96], it may also lead to undesirable phenotypes like reduced growth rate and weakened immunity in *rbohd* mutants [135].

4.2. Extending Seed Longevity Through Seed Priming

Seed priming, a technique that partially activates germination processes without fully initiating germination, is known to improve seed performance and enhance stress tolerance in crop plants [136–142]. This process aids in facilitating cellular repair mechanisms, which contributes to improved seedling vigor and crop yield [51]. Studies on leek (*Allium porrum*) and *Brassica oleracea* seeds have shown that priming is linked to heightened rates of DNA synthesis and accelerated cell division, leading to more rapid germination and quicker establishment of seedlings [143,144]. Moreover, priming induces changes in gene expression, such as the upregulation of DNA repair pathways and the increased activity of the protein repair enzyme L-ISOASPARTYL METHYLTRANSFERASE [145]. These molecular adjustments help reduce chromosomal abnormalities and enhance the overall quality of germination [51].

However, priming can occasionally diminish the storability or longevity of seeds. For example, the beneficial effects of seed priming were evident only for the first 15 days of storage at 25°C in rice [146]. Beyond this period, the performance of the primed seeds declined, becoming even poorer than that of the non-primed seeds. The detrimental effects of storing the primed seeds at 25°C were associated with impaired starch metabolism within the rice seeds [146,147]. To tackle this challenge, researchers have developed an innovative priming method aimed at enhancing seed survival rates and maintaining seed longevity through the use of biologically active compounds, tested using *Arabidopsis* seeds [148]. Their findings indicated that priming with cell cycle inhibitors, such as mimosine, aphidicolin, hydroxyurea, and oryzalin, significantly improved both the survival rate and storability of seeds [148]. This suggests that the progression of the cell cycle during priming serves as a critical checkpoint affecting seed storability. By modulating this checkpoint through the inhibition of cell cycle progression, it may be possible to develop priming methods that preserve seed longevity while simultaneously boosting other aspects of seed performance.

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4.3. Revitalizing Old Seeds

Despite the importance of optimal storage, real-world constraints such as infrastructure limitations, natural disasters, and logistical issues can hinder the provision of perfect seed storage environments. This is especially true for rare or experimentally conserved germplasm that may have suffered from subpar storage conditions, reducing their viability. Therefore, developing methods to revive even a portion of these seeds carries immense value.

Aged or poorly stored seeds often suffer from energy depletion, reduced enzyme activities, and hypoxic conditions during germination [101,149], necessitating targeted interventions to restore vital components. Hydrogen peroxide, for instance, has been successfully used to resuscitate four-year-old squash (*Cucurbita pepo*) seeds by acting as an oxygen supplement [150]. Beyond direct chemical treatments, *in vitro* tissue culture techniques present a promising avenue to rescue the germination potential of immature and aged cucurbit seeds. This method extracts embryos from deteriorating seeds and cultivates them in a nutrient-rich, sterile environment conducive to germination, with success partially hinging on the selection, concentration, and synergistic combination of specific plant growth regulators. Ethylene supplementation, for example, has been shown to expedite germination in aged *Brassica napus* seeds [151], while cucumber seed regeneration has been effectively achieved with the combined use of 1-naphthaleneacetic acid and 6-benzylaminopurine [152,153]. Low doses of epibrassinolide have also been found to improve germination rates in pepper (*Capsicum annuum*) seeds [154]. GA3 holds potential for enhancing seed germination when applied externally, though its effectiveness is highly dependent on dosage - lower concentrations can stimulate germination, while higher amounts can inhibit it [155,156] Interestingly, alternative gibberellin compounds like GA4/7 may outperform GA3 in promoting cucurbit seed germination, suggesting potential benefits over conventional GA3 usage [155]. Notably, the Zn-specific chelator TPEN (N, N, N′, N′-Tetrakis (2-pyridylmethyl) ethylenediamine) can significantly delay the aging process of the seeds by regulating the levels of glutathione [157], suggesting that free metal ions released due to the loss of membrane integrity may be both a consequence and a contributing factor to seed aging.

While rescue strategies play a vital role in rejuvenating aged seeds, the fundamental importance of proper seed storage cannot be underestimated. To optimally preserve laboratory seeds, meticulous attention must be paid to keeping seed density low in centrifuge tubes, using hermetically sealed containers for cold storage, maintaining ideal relative humidity levels to prevent moisture damage (Weigel and Glazebrook, 2002). When it comes to preserving laboratory seeds, optimal storage necessitates meticulous attention to several key aspects: first, ensuring minimal seed density within centrifuge tubes, thereby minimizing the risk of accelerated deterioration; second, employing hermetically sealed containers for long-term preservation in cold environments, and maintaining a relative humidity level to prevent moisture-induced damage, and incorporating silica gel pellets to act as a desiccant, absorbing excess moisture and prolonging seed lifespan and viability (Weigel and Glazebrook, 2002). Moreover, the strategic integration of silica gel pellets into the storage system serves as an effective desiccant measure, which actively absorbs excess moisture and thereby extends the lifespan and viability of the seeds (Weigel and Glazebrook, 2002). By combining stringent storage practices with these revitalization techniques, researchers can maximize the likelihood of aged seeds germinating successfully while safeguarding against the multiple factors contributing to seed deterioration and loss of vigor over time.

5. Challenges, Questions and Approaches

While advancements in biotechnology and molecular biology have led to significant insights into the mechanisms governing seed vigor and longevity, numerous challenges persist in translating these discoveries into practical applications. One of the primary obstacles in enhancing seed vigor and longevity is the complexity of the underlying biological processes. These processes involve intricate networks of genetic, metabolic, and environmental interactions that are not yet fully understood [13]. For instance, the role of ROS in seed aging is well-documented, but the precise

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mechanisms by which ROS damage cellular components and the ways to mitigate this damage remain areas of ongoing research [62]. Another challenge is the variability among different plant species and genotypes. Seeds of various species exhibit different sensitivities to environmental stressors and storage conditions, making it difficult to develop universal strategies for improving seed quality [158]. Additionally, there is a need for more comprehensive understanding of how abiotic stresses, such as temperature and humidity, interact with seed physiology to affect longevity [101]. Moreover, economic constraints and the lack of standardized protocols for seed testing and evaluation pose significant barriers to progress[159]. There is a continuous need for cost-effective and reliable methods to assess seed quality, which can be applied globally, from small-scale farmers to large agribusinesses [160,161].

To address these challenges, an interdisciplinary approach is required, combining genetics, biochemistry, and agronomy with biotechnology and precision agriculture [13,162]. Strategies could include the use of genetic engineering to introduce or enhance protective mechanisms against ROS, the development of species-specific storage guidelines based on detailed physiological studies, and the creation of robust seed quality assessment tools [160]. Furthermore, global collaboration and sharing of resources and knowledge could accelerate the discovery of effective solutions.

While enhancing seed longevity offers clear benefits, such as improved storage stability and extended viability, it is essential to consider the potential downsides. One significant concern is the possible trade-off between seed longevity and germination vigor. Seeds engineered for extended shelf life might exhibit reduced field performance due to alterations in metabolic pathways that affect germination efficiency and early seedling establishment [2]. Additionally, increasing seed longevity could inadvertently select for traits that delay germination, potentially leading to uneven crop stands and reduced uniformity in planting populations, which are detrimental to crop management and yield consistency [14,84].

Furthermore, the genetic modifications required to achieve enhanced longevity might have unintended consequences on plant health and susceptibility to diseases. For instance, changes in seed composition could impact the plant's natural defense mechanisms against pathogens and pests [163]. Lastly, there is an ecological consideration; seeds with extended viability might persist in the soil longer, potentially outcompeting native species and disrupting local ecosystems if they are not properly managed [164].

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Abbreviations

The following abbreviations are used in this manuscript:

SLAG	SEED LONGEVITY-ASSOCIATED GENE
ABI3	ABSCISIC ACID-INSENSITIVE3
TIP3;1	TONOPLAST INTRINSIC PROTEIN 3;1
ROF1	ROTAMASE FKBP 1

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DREB2	DROUGHT RESPONSIVE ELEMENT BINDING FACTOR 2
IAA27	AUXIN-RESPONSIVE PROTEIN 27
DOG1	DELAY OF GERMINATION
COG1	COGWHEEL1
CDF4	CYCLING DOF FACTOR 4
PER1A	PEROXIDASE 1A
ATM	ATAXIA TELANGIECTASIA MUTATED
ATR	ATM AND RAD3-RELATED
SOG1	SUPPRESSOR OF GAMMA 1
LIG4	DNA LIGASE 4
XRCC2	X-RAY REPAIR CROSS COMPLEMENTING 2
PARP1	POLY(ADP-RIBOSE) POLYMERASE 1
ERCC1	EXCISION REPAIR CROSS COMPLEMENT-ING-GROUP 1
8-OXOG	8-OXOGUANINE
DSBS	double-strand breaks
HR	homologous recombination
ROS	Reactive oxygen species
SnRK2	SUCROSE NON-FERMENTING 1-RELATED PROTEIN KINASE 2
ABA	abscisic acid
EM1	EARLY METHIONINE1
AtHB25	ARABIDOPSIS THALIANA HOMEOBOX 25
TOR	Target of Rapamycin
SOD	SUPEROXIDE DISMUTASE
CAT	CATALASE
POD	PEROXIDASES

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