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REVIEW ARTICLE

Biotechnological interventions for improving the seed longevity in cereal crops: progress and prospects

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ABSTRACT

Seed longevity is a measure of the viability of seeds during long-term storage and is crucial for germplasm conservation and crop improvement programs. Also, longevity is an important trait for ensuring food and nutritional security. Thus, a better understanding of various factors regulating seed longevity is requisite to improve this trait and to minimize the genetic drift during the regeneration of germplasm. In particular, seed deterioration of cereal crops during storage adversely affects agricultural productivity and food security. The irreversible process of seed deterioration involves a complex interplay between different genes and regulatory pathways leading to: loss of DNA integrity, membrane damage, inactivation of storage enzymes and mitochondrial dysfunction. Identifying the genetic determinants of seed longevity and manipulating them using biotechnological tools hold the key to ensuring prolonged seed storage. Genetics and genomics approaches had identified several genomic regions regulating the longevity trait in major cereals such as: rice, wheat, maize and barley. However, very few studies are available in other Poaceae members, including millets. Deploying omics tools, including genomics, proteomics, metabolomics, and phenomics, and integrating the datasets will pinpoint the precise molecular determinants affecting the survivability of seeds. Given this, the present review enumerates the genetic factors regulating longevity and demonstrates the importance of integrated omics strategies to dissect the molecular machinery underlying seed deterioration. Further, the review provides a roadmap for deploying biotechnological approaches to manipulate the genes and genomic regions to develop improved cultivars with prolonged storage potential.

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Introduction

Seed longevity is a quantitative trait that describes seed viability after long-term storage [1]. A seed is the unit of reproduction of a flowering plant, important for sustainable agriculture and germplasm storage. Also, seeds are critical to human nutrition and food security for future generations [2]. Since the beginning of agriculture, cereals have been majorly cultivated by the human population for food production [3]. The most cultivated cereals are *Triticum aestivum* (wheat), *Oryza sativa* (rice), *Hordeum vulgare* (barley), *Sorghum bicolor* (sorghum), *Zea mays* (corn), *Secale cereale* (rye), *Avena sativa* (oats), and several millets. Cereals are economically important crops of the graminaceous family, utilized as food and feed [4]. A significant portion of the world's population consumes cereals as a staple food to fulfill nutritional requirements [5]. Among cereals: rice, wheat, and maize provide over 60% of total food energy intake. Recently, millets are also regarded as nutri-cereals due to their better nutritional profiles than major cereal staples [6]. The estimated cereal production now stands at 2761 million tonnes, which is necessary for ensuring food security [7]. However, their production is challenged by biotic and abiotic stresses as well as poor crop standard due to low seed viability and vigor [8]. Among these factors, seed dormancy and longevity are the important post-harvest traits that affect seed quality and determine its viability [9]. Further, the potential of dormancy can be overcome by the time- and environmentally-regulated process, known as after-ripening (AR). After-ripening helps to

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Supplemental data for this article can be accessed here.

break the dormancy and affects germination potential [10]. After-ripening is a complex process that occurs in the dry seeds after harvesting and influences their dormancy and viability [10]. However, in general, increase in the age of seeds can reduce germination and vigor as the seeds' metabolic system breaks down [10].

Seed longevity is an important agronomic trait for seed germplasm storage and providing viable seeds to researchers and farmers for sustainable agriculture. The seeds of cereals are orthodox in nature, and hence remain viable for several years when appropriately stored under controlled conditions [11]. Germplasms are highly vulnerable to genetic drift during the regeneration process [12]. However, the recurrence of the regeneration procedure can be minimized by enhancing seed longevity. Further, being a complex trait, seed longevity is negatively influenced by various factors, such as the relative moisture content of seeds and storage temperature [13]. The crop yield depends on successful germination and the establishment of seed in fields. Seed longevity is the trait that determines the success of seedling establishment under diverse climatic conditions [2]. Seed storage under ambient conditions enhances the deterioration by climate-induced hot and humid weather, leading to: farmers' loss in income, crop yields, and seed security. Poor storage conditions and practices cause ~50–60% loss of cereal production worldwide [14]. Seed losses up to 50% due to storage conditions are also affected by climatic conditions. Therefore, severe losses prevail in humid tropical regions [15]. While the estimated figures represent the physical losses, the loss due to deterioration of seed quality exceeds these estimates [16]. Thus, an enormous number of seeds must be stored/sown to secure sufficient viable seeds to counter the low germination problems. Moreover, lower seed longevity significantly reduces the germination rate in fields where direct sowing is major practice during crop establishment, leading to severe yield losses [17]. Such losses, due to an imbalance between expenditure and income, increase farmers' dependency on farm-saved seeds for future crop establishment, leading to poor seedling growth and yield [18]. Consequently, understanding seed longevity is essential for farmers and seed enterprises to decide the duration for which seed lots can be stored without losing viability under certain storage conditions.

Genetic traits have been widely used in traditional breeding and genetic editing to improve the quality of grains [19]. It would be beneficial to enrich the cereal grains with crucial functional components while growing in the field to make them more stable during post-harvest storage. The genetic traits regulating various properties of seeds are very complex and diffused [20]. The advancement in omics tools contributed enormously to understanding and identifying various molecular markers related to seed longevity. Hence, advanced genomic and post-genomics techniques can be implemented to understand the molecular markers for seed longevity, followed by their application in seed longevity improvement through genetic breeding. In summary, this paper reviews various biotechnological interventions for improving the seed longevity in cereal crops and the future scope to develop new varieties with enhanced seed longevity and storage.

Key factors affecting seed longevity of cereal crops

The molecular mechanisms affecting seed longevity are conferred by various genetic determinants related to several physiological processes, including hormonal pathways, DNA repair, detoxification, reactive oxygen species (ROS) scavenging, and membrane integrity (Figure 1). Loss of seed viability in cereals is mainly affected by three factors: (i) insects and rodents; (ii) bacterial and fungal pathogens; and (iii) biochemistry of seed. Depending on the climatic conditions, cereal seeds carry a basal microbial and spore load, which is the main reason for post-harvest spoilage. The activity of these biotic factors during spoilage depends on various factors, such as moisture content of grains, temperature during storage, water availability, and storage duration (Figure 2) [21]. In addition, the seed architecture and variety play an essential role during post-harvest contamination. For example, seeds with damaged kernels are more prone to spoilage and loss of viability. Among these factors: seed moisture content, relative humidity, and storage conditions are the critical parameters affecting longevity during post-harvest storage and determine seed quality and longevity [16]. Seeds absorb moisture from the storage environment due to their hygroscopic nature, leading to enhanced insect and fungi infestation, which deteriorates seed quality and longevity [22]. Consequently, seeds are recommended to be stored at low moisture and temperature conditions [23]. Afzal et al. [24] showed a rise in moisture content of maize seed stored in polypropylene bags due to high ambient humidity, which resulted in heavy pathogen infestation leading to the deterioration of seed quality and viability. Additionally, various endogenous factors such as: hormonal balance, proteins, nucleic acids, and seed coats are crucial in regulating longevity [25]. Several studies have been

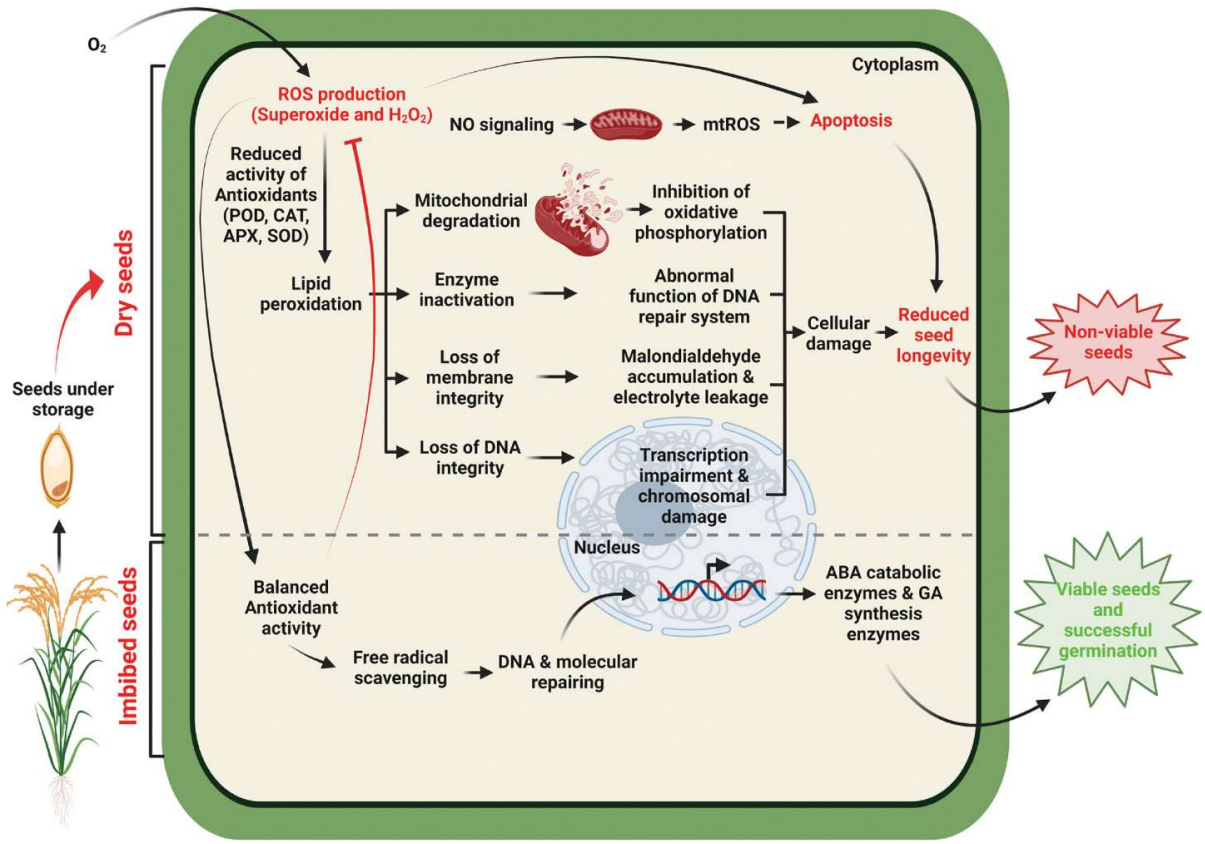

Figure 1. Pathways underlying seed deterioration during storage that affect the viability of seeds. Also, the factors playing roles in maintaining the viability are shown.

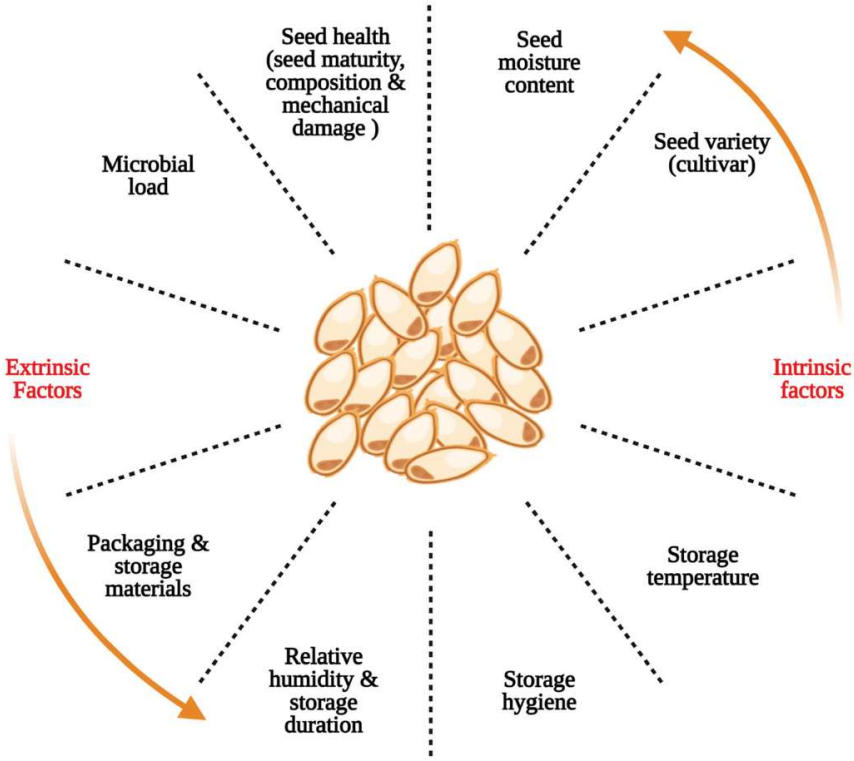

Figure 2. Intrinsic and extrinsic factors that influence the seed longevity during storage.

performed to examine the seed viability after storage at low temperature and with low moisture content. For instance, van Treuren et al. [26] reported high seed viability of barley in comparison to wheat when stored at −20 °C for 23–33 years. Likewise, Hay et al. [27] examined the viability of rice when stored at 4 °C and −20 °C. The experiment reported high germination in rice seeds stored at −20 °C, indicating improved retention of viability at this low temperature. In another study, *S. cereale* showed the least longevity among all cereal crops when stored under permafrost conditions in the coal mine since 1984 [28]. Interestingly, researchers at Michigan State University reported that some seeds placed in moist and well-aerated sand could retain viability [29,30]. This study, known as Beal's soil experiments, is the world's oldest seed longevity experiment, commenced by professor of botany and forestry at Michigan Agriculture College, Dr. William James Beal. Cereal crops vary in terms of their viability after long-term storage (Supplementary Table S1). Notably, few long-term storage experiments have been performed on cereals. For instance, *H. vulgare* and *A. sativa* retained 90% and 81% viability, respectively, after 110 years of long-term storage at 10 °C to 15 °C and 3.12% moisture content [31]. Similarly, maize also showed ∼80% germination after 12 years of long-term storage at 20.3 ± 2.3 °C and 50% relative humidity [32]. However, cereals such as *T. aestivum, S. cereale,* and *O. sativa* could not retain viability after 3 to 4 years of storage in the same storage experiments [32,33]. Earlier, 36,483 sorghum germplasm accessions conserved at ICRISAT were analyzed for seed viability [34]. The storage time for 35,221 accessions was between 5 and 21 years, and 95.6% of total analyzed accessions showed >85% seed viability. Such experiments enhance the understanding of various phenotypical features of seeds, which are essential for the management of germplasm collections. The Genebank CGIAR Research Program initiated the Seed Quality Management (SQM) project to understand and improve seed conservation research for possible long-term storage with the least cost [35].

Seed maturity also decides longevity, and immature seeds lose viability faster under similar storage conditions. In the final stages of seed maturation, various biochemical compounds necessary to maintain viability during storage are accumulated [16]. Hence, it is crucial to harvest seeds at full maturity. Compared to other cereals, millets are adapted to grow in less fertile soil with less moisture content and high environmental temperature [36]. High levels of phenolic compounds such as flavonoids are crucial in enhancing antioxidant activity and stress tolerance in *O. sativa* [37]. Millets are known to have a higher content of secondary metabolites [38], which might influence their microbial load at the time of harvesting. Even after regulating the environmental conditions during storage, filamentous fungi, mostly *Aspergillus* and *Penicillium,* threaten the stored grains. They are tolerant to low moisture and grow easily in stored grains if not controlled through chemical treatments. Insects and pests also cause significant damage to the stored seeds. The pests feed on stored seeds and destroy the germ portion and qualitative properties of seeds. The infestation of insects and pests is also affected by various factors, including moisture content, field infestation, damage in kernels, storage temperature, duration of storage, and storage systems. The damage caused by pests and insects increases the temperature in stored grains at the infestation site [39]. It produces various metabolites, enhancing the probabilities of fungal and microbial growth in stored seeds and further decreasing seed viability. The systems used during post-harvest storage of seed are also critical. The conventional storage systems for cereals are jute bags, bulk storage, or hermetic containers. For bulk storage, concrete or metal silos are often used. However, the quantity of seed stored in them is inappropriate, and hence the conditions in headspace cannot be regulated. The moisture migration inside silos results in hotspots and promotes the activity of microbes and molds, leading to the production of harmful metabolites and spoilage of seeds [36,40].

The maintenance of seed viability for future cultivation and germplasm storage is also essential. Seed longevity and vigor are related to the measure of the viability of seeds during storage. The seed health status and crop (cultivars, in particular) also play a crucial role in seed longevity and vigor. The genetic composition of seeds is a key factor in deciding health status [41]. The genetic composition also affects the synthesis of secondary metabolites, which influences many characteristics of seeds. Accumulation of a few secondary metabolites improves the quality of seeds by making them resistant to various biotic and abiotic stresses, hence improving seed longevity. Plants evolve different secondary metabolite biosynthetic pathways naturally. Such varieties are used for traditional breeding to improve the crops [42,43]. The genetic determinants of these biosynthetic pathways are potential targets to develop transgenics with the ability to synthesize important secondary metabolites [44]. For example, an increase in the growing temperatures of wheat enhances the accumulation of secondary metabolites, which further raises the antioxidant properties of wheat [45].

The increased antioxidant efficiency makes wheat more tolerant to pathogens and improves seed longevity and vigor during post-harvest storage.

Current understanding of factors affecting seed longevity

Different genetic tools and molecular markers have been widely used in modern crop breeding programs. Molecular markers such as Random Amplification of Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Simple Sequence Repeats (SSR), Single Nucleotide Polymorphisms (SNP), Cleaved Amplified Polymorphic Sequences (CAPS), etc., are very popular among agriculture scientists. Genomic approaches include Genome-Wide Association Study (GWAS), genomic selection (GS), QTL mapping, transcriptomics, transgenesis, and gene editing can support genomic-assisted crop improvement [46,47]. The availability of genomic sequence of most crops provides valuable information about various important traits that can be exploited for their improvement [48].

Several agriculturally essential traits are quantitative in nature [49]. For example, the quantitative locus (QTL) GS5 in *O. sativa* regulates: grain size, weight, and filling [50]. Higher expression of GS5 is correlated with more filling and increased weight of seed. A good understanding of crucial factors regulating the overall quality of seeds will assist in developing the varieties with excellent quality and good longevity. The GWAS study of various *O. sativa* indica varieties identified eight loci associated with seed longevity stored at 45 °C and 10.9% moisture content [51]. It is estimated that seed longevity is a complex trait, and environmental conditions highly influence it at the harvest time than any genetic trait [52]. Post-harvest drying conditions affect seed longevity, which influences the genetic analysis of this trait [52]. Several studies have identified genes related to seed longevity in *O. sativa*. For example, *Os*TPP7 (trehalose-6-phosphate phosphatase) was identified in near-isogenic lines (NIL) from the cross of Japonica (short survival time) and Aus (long survival time) parents, which has a crucial role in seed longevity [53]. However, this gene was found to be nonfunctional in the indica varieties due to large deletion [54]. Further, Miura et al. [55] identified three putative QTLs (*qLG-2, qLG-4,* and *qLG-9*) on chromosomes 2, 4, and 9, controlling seed longevity in *O. sativa*, obtained from a cross between japonica and indica varieties.

The mapping of quantitative trait loci identified 12 QTLs for germination and seedling growth as genetic determinants of seed vigor on chromosomes 7 and 9 in rice inbred lines derived from Indica and Japonica [56]. Similarly, Li et al. [57] identified six QTLs, namely: *qSS-2, qSS-3, qSS-4, qSS-6, qSS-9,* and *qSS-11,* for seed longevity in recombinant inbred lines (RILs) of *O. sativa*. Of these, four QTLs were further verified with the chromosome segment substitution lines (CSSL). Three QTLs for seed longevity were mapped on chromosomes 1, 3, and 9 using RILs from the cross of IR64 and Asominori [58]. Wang et al. [59] also identified five QTLs associated with *O. sativa* seed longevity using the RIL population. In aus variety of *O. sativa*, 26 candidate genes with significant SNP markers associated with seed longevity traits have been identified [60]. Recently, nine QTLs, namely: *qSS1-1, qSS1-2, qSS2-1, qSS3-1, qSS5-1, qSS5-2, qSS7-1, qSS8-1,* and *qSS11-1,* were identified by using a GAPIT (Genomic Association and Prediction Integrated Tool) with MLM (Mixed Linear Model) in GWAS of 456 *O. sativa* collections [61]. Studies have revealed that these genes have a potential role in various biological processes and stress responses [62,63]. Another recent work identified a gene, *OsGH3-2*, having a role in seed longevity by employing genome-wide association studies (GWAS) and linkage mapping in *O. sativa* germplasms after providing artificial and natural aging treatments [64]. In addition, the transgenic studies illustrated the negative function of *OsGH3-2* in seed longevity by influencing the abscisic acid (ABA) accumulation in *O. sativa* seeds [64]. It is known that the pericarp of the *O. sativa* grain is rich in flavonoids [37]. Lee et al. [11] identified markers on chromosome 10 located near the flavonol synthase gene in *O. sativa* aus varieties, which supports the fact that flavonoid content of pericarp and vitamin E in *O. sativa* are important factors in enhancing seed longevity. Recently, a study identified a bHLH transcription factor (TF) regulating proanthocyanidin synthesis in *O. sativa* and demonstrated their role in regulating seed longevity by minimizing oxidative stress. These findings indicated the importance of secondary metabolites like flavonoids in regulating seed longevity. The identified 45 annotated genes in a QTL from the lines generated from a cross between Azucena and Icta Motagua varieties can potentially regulate seed longevity [65]. Different *O. sativa* cultivars from various ecogeographic areas have shown variations in seed longevity [66]. Also, evidence suggests that molecular markers involved in biotic stress response have an essential role in seed longevity [67]. For instance, genes involved in maintaining the integrity of the seed coat play a significant role in regulating seed longevity [68]. The seed coat is a major structural barrier against biotic stress, and mutations in the seed

coat led to decreased seed longevity. Altogether, the studies demonstrated that genes involved in: RNA modification, hormonal pathways, detoxification, and protein-protein interactions play crucial roles in regulating seed longevity.

In cereals, the first genetic markers linked to seed longevity were identified in *O. sativa*. Further, various molecular markers have been detected in other cereals, including *T. aestivum*, *H. vulgare*, and *Z. mays*. In *T. aestivum*, microsatellite loci linked to seed longevity were identified using bi-parental (RFLP and SSR markers) and association mapping [using Diversity Arrays Technology (DArT) markers] [69,70]. Many QTLs associated with seedling vigor were detected on the chromosomes of both *T. aestivum* and *H. vulgare* [71]. Recently, Arif et al. [72] detected 24 QTLs associated with *T. aestivum* longevity. Pyramiding of these alleles causes a 12.8% enhancement in seed longevity. Similarly, Zuo et al. [73] identified 96 loci for seed vigor-related traits in *T. aestivum* using 246 RILs, which were assembled in 17 QTL-rich regions on chromosomes: 1AL, 3DL, 4AS, 2DS, 3AS (3), 3BS, 5AS, 5DS, 3BL (2), 4AL (3), 6BL, and 7AL. Subsequently, they detected 23 seed longevity loci in 166 RILs using SNP markers [74]. Both physical and chemical changes are responsible for deciding the seed longevity during storage. It was investigated that rain during various stages of seed development in *T. aestivum* significantly affects the seed longevity [75]. Additionally, in a RIL line developed from a cross between the German winter *T. aestivum* cultivars, QTL for seed longevity was identified on chromosome 1 A [76]. Several identified candidate genes associated with seed longevity were found to be involved in stress tolerance and signal transduction, providing new information regarding the genetic basis of seed longevity [74]. Recently, 49 additive QTLs were identified in a doubled haploid population of *T. aestivum*, derived from a cross between Hanxuan 10 x Lumai 14, aged under controlled environmental conditions [77]. The genes located in the identified marker were involved in: transcription, cell division during germination, lipid and carbohydrate metabolism, thereby suggesting their role in seed longevity in *T. aestivum*.

The physiological maturity of grains is crucial in deciding the seed longevity during post-harvest storage. At physiological maturity, the grains attain maximum potential longevity [78,79]. Further, some enzymatic reactions are also responsible for deteriorating the seed quality. Almost all grains have a high content of lipoxygenases (LOXs) activity, which leads to the accumulation of fatty acids in seeds [80]. The higher activity of LOX is associated with the reduced longevity of cereal seeds. Therefore, a successful reduction in the LOX activity in *T. aestivum* by generating its LOX^{RNAi} lines resulted in the improved seed quality and longevity [80]. Recently, the genetic integrity of *Pennisetum glaucum* seeds was also studied by using genomic-SSR markers [835]. In sorghum, forty-six local landraces were evaluated for seed longevity, among which seven showed stable seed longevity. These landraces were further recommended for breeding to enhance the seed longevity in this crop [81]. Similarly, stored seeds of *Z. mays* were investigated for germinations and viability, which led to the identification of polymorphic SSRs in six known genes, including *superoxide dismutase 4, pathogenesis-related protein 2, catalase 3, metallothionein1, golden plant 2,* and *opaque endosperm2* [85]. In addition, five novel candidate genes with a role in seed viability were also identified, which are already known to have a function in stress tolerance [82]. Other scientific groups have also identified heritable genetic variability for seed longevity in *Z. mays* and *Secale cereale* [83,84]. Han et al. [85] identified sixty-one QTLs linked to seed vigor and longevity in *Z. mays*. Twenty-three candidate genes with a role in seed longevity were identified in the mapped QTLs. Chromosomal regions for: QTL5-2, QTL3-2, QTL3-4, and QTL2 were found to be the hot spots linked to seed longevity traits in *Z. mays* [85]. Wu et al. [86] identified that the abundance of LEA protein named EMB564 was found to be associated with seed vigor and viability in *Z. mays*. Moreover, 13 QTLs were identified in a RIL line treated with artificial aging conditions on chromosomes: 1, 3, 4, 5, and 7, suggesting the role of these regions in regulating seed longevity in *Z. mays* [87]. In *H. vulgare*, QTL mapping identified major QTLs associated with seed vigor on chromosomes 5H, 2H, and 7H [71].

Post-genomics approaches to dissect the genomic regions underlying seed longevity

Advancement in various post-genomics approaches accelerated the study of genetic factors crucial for longevity (Figure 3). The new omics tools are capable of detecting molecular markers related to seed longevity traits in cereals. Earlier various seed deterioration/invigoration experimental methods, such as accelerated aging (AA) and controlled deterioration (CD) tests, have been used to understand seed longevity [88]. In the current scenario, post-genomics tools are available to identify various important genes and their functions. Genomics enabled the dissection of genomic regions to understand markers important for seed longevity [89]. Till now, various QTLs and genes have been identified

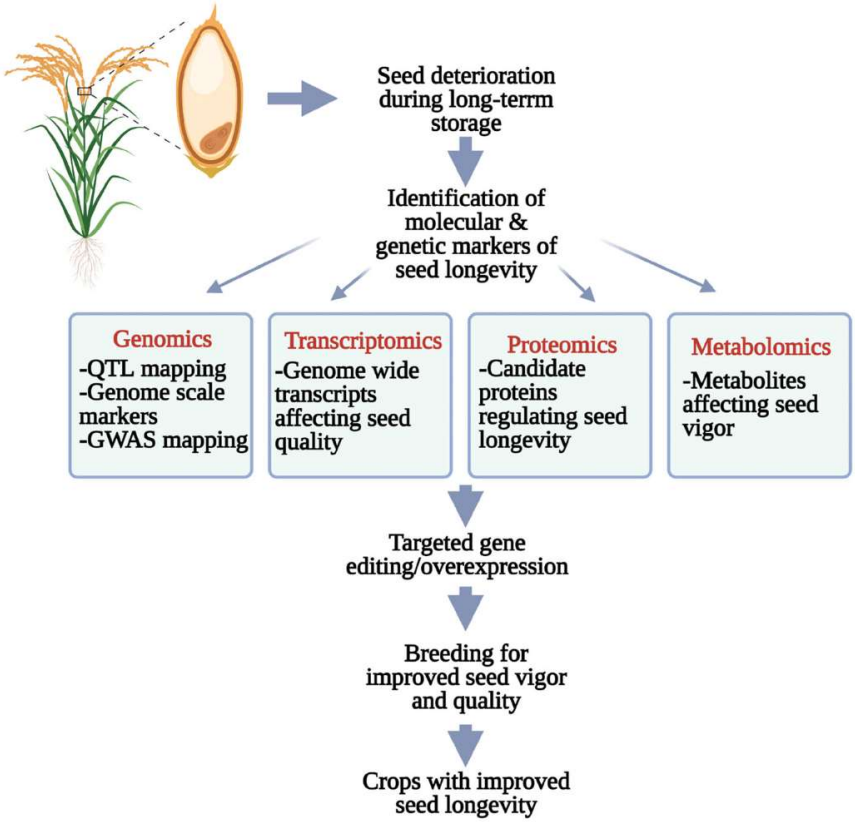

Figure 3. Next-generation breeding involving various omics approaches to understand the molecular mechanisms of seed longevity.

in various graminaceous crops, which helped to understand the molecular mechanisms regulating seed longevity (Supplementary Table S2) [53,55,58,70,72,83,84,90]. The advances in post-genomics tools contributed to the identification of candidate genes with function in seed longevity. The studies have reported numerous key factors having roles in seed longevity, which can be utilized for trait improvement through genetic breeding (Supplementary Table S2).

Transcriptomics

Transcriptome profiling has been found to be beneficial in the identification of seed vigor-related genes in the past. Chen et al. [91] detected various transcriptomic biomarkers for seed longevity using quantitative real-time PCR in *Z. mays*. The biomarkers such as: *ZmActdf, ZmUBQ, Zmβtub, Zm18S, ZmAct,* and *ZmGAPDH,* which have a crucial role in seed longevity were identified in the study. Seed aging is enhanced by the accumulation of damaged proteins, like abnormal isoaspartyl (isoAsp) in *O. sativa*. Proteins regulating the repair of these damaged proteins might have a crucial role in seed longevity. For example, Protein-L-isoaspartyl methyltransferase (PIMT) genes were investigated in *O. sativa* through transcriptome analysis [92]. Two PIMT genes, *OsPIMT1* and *OsPIMT2,* were investigated for their role in *O. sativa* seed longevity. Additionally, two chromosome segment substitution lines of *Z. mays*, X178 and I178, were analyzed by transcriptome sequencing before and after giving accelerated aging (AA) treatment to the seeds [93]. The integration of transcriptome data with QTL mapping identified 13 differentially expressed genes (DEGs) involved directly or indirectly in the regulation of seed longevity in *Z. mays*. The omics approaches are widely used to study seed quality and longevity. However, only a few omics studies are available to understand seed vigor in crops like millets, *S. bicolor*, and *H. vulgare*. These crops are gaining importance in both developing and developed countries because of their nutritional quality and ability to grow under stress conditions. Therefore, timely studies are imperative to understand the traits regulating seed quality and longevity in these crops.

Proteomics

Proteomics is also being considered as an important technique to understand the factors regulating seed

longevity. For example, the abundance pattern of proteins in two *T. aestivum* genotypes showed 93 and 105 differential protein spots in both genotypes, respectively; they were majorly involved in defence responses [94]. The results unraveled the mechanisms involved in seed longevity in *T. aestivum*. In *Z. mays*, the proteomic analysis identified 28 proteins with differential expression [86]. They detected many small heat shock proteins, late embryogenesis abundant (LEA) proteins, and antioxidant enzymes with increased expression, indicating their essential role in regulating seed longevity. The study concluded that the expression of proteins such as LEA was decreased in *Z. mays* seeds with low vigor. Hence, increased expression of LEA could enhance seed longevity in *Z. mays*. Another group identified 16 differentially expressed proteins in *Z. mays* in response to aging, emphasizing the proteomic changes that occurred during seed deterioration [95]. Similarly, Zhang et al. [96] studied the *O. sativa* seed aging through proteomics approach and identified differential expression of the protein in embryo and endosperm. Further, genes regulating seed longevity in *H. vulgare* were identified using transcriptomics and proteomics analyses of two parental lines and the L94 NILs [97]. The study identified UDP-glycosyltransferase (MLOC_11661.1) and NADP-dependent malic enzyme (MLOC_35785.1) as candidate genes in the QTLs associated with seed longevity in *H. vulgare*. *T. aestivum* seeds treated with artificial aging (stored at 45 °C with 50% relative humidity) and hydro-priming were analyzed by the isobaric tandem mass tag labeling (TMT) proteomic approach to identify proteins with differential expression during seed aging and priming [98]. The study reported proteins such as: oleosin, hemoglobin 1, non-specific lipid transfer proteins and agglutinin, which might be considered novel markers involved in seed deterioration [98]. Proteomic and carbonylation profiling at the critical node of *O. sativa* seed aging showed decreased abundance of proteins related to defense signaling, antioxidant, and heat shock protein systems, indicating that reduced functioning of these pathways accelerates the aging process [99]. Similarly, seed storability of two genotypes of *T. aestivum* (storage-tolerant and -sensitive) was studied by proteomic approach [94]. The study showed significant activation of: the defense system, mobilization of storage proteins during germination in storage tolerant genotype, suggesting that storage-tolerant seeds have superior potential to trigger these defense pathways [94]. Recently, Isobaric tags for relative and absolute quantitation (iTRAQ) based analysis of storage-tolerant (TRI23248) and -sensitive (TRI10230) *T. aestivum* genotypes was performed at the critical node (CN) of seed viability before and after artificial aging (AA) treatments [100]. The study reported enrichment of redox homeostasis-related pathways in the tolerant genotype of *T. aestivum*, suggesting its better ability to survive storage conditions. Altogether, these results provided comprehensive knowledge about the molecular mechanisms of seed longevity.

Metabolomics

Metabolomics is widely used for studying the dynamics of metabolic responses in seeds to understand seed longevity and viability. A mass spectrometry-based metabolomics approach was performed to identify the significant metabolite changes during seed storage [101]. These metabolic perturbations during *O. sativa* seed storage were persistent with proteomics data. The results concluded that higher raffinose content in *O. sativa* seeds during storage could be positively linked with seed longevity [101]. Recently, another group demonstrated the metabolite profiling of *O. sativa* spikelets at the grain filling stage and showed the effect of water limitation on metabolite accumulation [102]. The study revealed that the accumulation of secondary metabolites was associated with enhanced stress tolerance in *O. sativa*, thereby influencing longevity by reducing the microbial load during harvesting. Similarly, Lee et al. [52] identified metabolite variation between two indica cultivars of *O. sativa* differing in seed longevity. The analysis showed a higher accumulation of flavonoids (quercetin-3-arabinoside and kaempferide), amino acids (cysteine derivatives), and sugars (glucose) in *O. sativa* grain variety having higher seed longevity, indicating a crucial role of these metabolites in enhancing longevity during storage. Besides, metabolites like thiamine monophosphate and harmaline are accumulated more in short-lived *O. sativa*, supporting their potential role in seed deterioration during storage [60]. The study also identified SNP markers linked with flavonols and inferred that these markers could be used to enhance seed longevity in crops. In addition, galactinol is also an important biomarker for seed longevity [103]. Further, biochemical profiling of *Z. mays* seeds showed better longevity of seeds with higher content of phosphorus and sugars [104]. An equivalent omics approach to metabolomics that is widely utilized to study seed longevity is metabonomics, which involves the quantitative assessment of dynamic metabolic responses to genetic editing.

Lipidomics and glycomics are widely used to understand the entire complement of lipids and cellular glycans (carbohydrates and sugars), respectively, in seeds.

Lipidomics identified lipids and glycolipids associated with seed vigor and deterioration in *Arabidopsis* [105]. Whereas, the estimation of sugar changes in *Z. mays* embryo showed alterations in soluble carbohydrates during accelerated aging, suggesting the role of sugars in seed deterioration and decline in seed vigor [106]. The advanced glycomics approach can accelerate analogous studies in other crops.

Phenomics

High-throughput phenotyping provides significant data for several important traits, such as seed longevity on a large germplasm group. Currently, advanced computer algorithms and machine learning approaches have developed image- and sensor-based phenotyping methods [107]. These machine learning-based phenomics approaches have been successfully used to assess and predict seed longevity in *Brassica napus* [108]. The high-throughput seed phenotyping enables precise monitoring of seed germination and survival curves. These phenotyping approaches can be successfully applied to evaluate seed germination in a large population under optimal storage conditions [109]. Although the application of phenomics approaches in understanding the seed longevity of cereals is not yet available, their integration with genomics could assist in delivering crops with desired seed longevity.

The superior genes for seed longevity traits can be identified by two current approaches, pangenomics or genome-wide association studies (GWAS). Pangenomes assist in identifying the required gene involved in regulating a trait, such as seed longevity, then CRISPR-Cas9 based editing of that gene followed by cross-breeding can lead to the generation of the improved trait in cultivars [110]. The platforms like speed breeding have reduced the cost of breeding programs by completing six generations per year for cereal crops, thereby accelerating trait improvement [111]. Another genomics-based breeding method known as Genomic selection (GS) predicts and calculates genomic estimated breeding values (GEBVs) of lines for their selection before phenotyping in the field, hence accelerating the breeding cycle and complementing speed breeding programs [110]. The integration of these genomics gains with phenotypic selection improves the efficacy of breeding programs. For example, genomic and phenotypic selection of *T. aestivum* lines for grain yield increased the selection accuracy and reduced the breeding cycle [112]. Implementation of these approaches targeting seed longevity traits could accelerate the process of developing crops with improved storability. Decoding the genetic basis of seed longevity by employing comparative *-omics* analyses provides significant knowledge about underlying genetic variation. This knowledge can be utilized in QTL and GWAS studies to develop markers linked with seed longevity traits. The availability of reference genome data from various cereals and the comparative studies with other crops by pan-genome approach can contribute to an in-depth understanding of gene function and involved pathways for seed longevity. The long-term storage experiments integrated with -omics approaches can provide significant genetic determinants regulating seed longevity. Subsequently, the genomics-based speed breeding and high-throughput phenotyping methods can hasten the development of crops with improved seed longevity. Together, these approaches could accelerate single-gene manipulations in cereals to improve seed longevity. Additionally, these omics approaches could also assist in understanding the effects of genetic manipulations of seed vigor associated genetic determinants on other seed traits such as: yield, nutritional value, health risk as food and ethical issues of GMO seeds. In summary, these advanced omics approaches could help extract genetic variation of seed longevity traits in the wide germplasm of cereal crops. Consequently, integrative approach including pangenomics, system biology, and genome editing provides a new route to develop crops with enhanced seed longevity.

Approaches to circumvent the storage issues

An in-depth investigation of seed maturation and germination is crucial for understanding seed quality and longevity during post-harvest storage. Multi-omics, system biology, and various mapping techniques have unveiled different molecular markers regulating seed longevity. In addition to this, epigenomics is a novel field to exploit the genetic mechanisms of seed quality traits [113]. Molecular markers identified by these approaches are utilized to develop crops with better seed viability and longevity. Applying omics approaches in agriculture could be more significant and efficient by linking them with modern-day phenomics. Better phenotyping while developing new varieties improve the efficiency of breeding [114]. Conventional breeding and biotechnological approaches are efficiently used in producing crops with higher yield and stress tolerance. Physical priming is being practiced to improve seed longevity [115]. However, GM technology of crop improvement plays a significant role in facing the fundamental challenges of maintaining seed longevity in

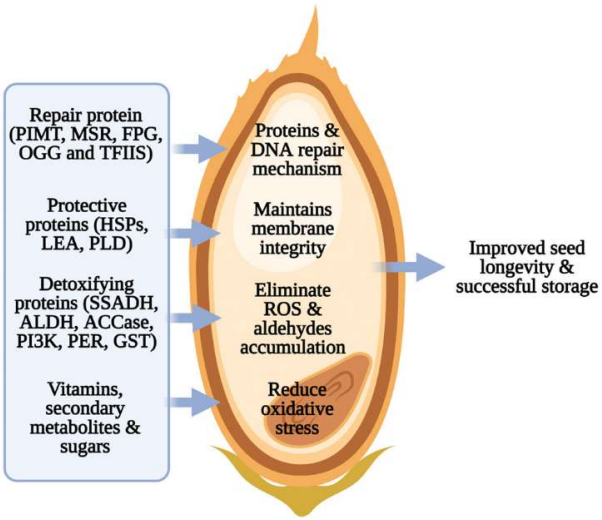

Figure 4. Potential candidates involved in improving seed longevity. PIMT: Protein-L-isoaspartate (D-aspartate) O-methyltransferase; MSR: Methionine sulfoxide reductases; FPG: formamidopyrimidine [fapy]-DNA glycosylase; OGG: 8-Oxoguanine glycosylase; TFIIS: Transcription elongation factor S-II; HSP: Heat shock protein; LEA: Late embryogenesis abundant proteins; PLD: Phospholipase D1; SSADH: Succinic semialdehyde dehydrogenase; ALDH: Aldehyde dehydrogenase; ACCase: Acetyl-CoA carboxylase; PI3K: Phosphatidylinositol 3-kinase; PER: Peroxiredoxin; GST: Glutathione transferases.

crops. In addition, storage conditions should be accessed to ensure the long-term conservation of seeds to preserve biodiversity and food security [116].

The molecular mechanisms underpinning the longevity of grains have been unraveled by advanced molecular studies (Figure 4). As discussed earlier, the *OsPIMT* gene was identified to be involved in repair activities and played an important role in enhancing seed longevity [92,117]. The *PIMT* proteins catalyze the conversions of abnormal proteins into normal, thus repairing various proteins working in anti-aging pathways and reducing the deleterious factors responsible for degrading the seed quality and longevity [118]. These functions of *PIMT* proteins in the enhancement of seed longevity were extended to other important crop species. For example, the expression of *OsPIMT* isoforms in the embryo of transgenic *O. sativa* reduces the isoaspartic acid (IsoAsp) and ROS accumulation in seeds, hence improving the seed vigor [119]. In addition, lipid peroxidation in seeds is also a major factor affecting seed longevity and viability. The lipid peroxidation also leads to ROS accumulation and various deleterious products leading to seed deterioration [120,121]. Detoxication of these harmful products might enhance the seed quality and longevity by inhibiting grain deterioration. For example, overexpression of the aldo-ketoreductase (AKR1) enzyme in *O. sativa* improves the viability and longevity during storage [122]. Similarly, reduction in lipid peroxidation enzymes such as phospholipase D also enhances the seed integrity and thus longevity. *OsLOX2* RNAi lines of *O. sativa* showed enhanced seed longevity during storage compared to the *OsLOX2* overexpressing line [123,124]. Further, Ma et al. [125] reduced the *Os*LOX activity by performing targeted mutagenesis using transcription activator-like effector nucleases (TALENs) in *O. sativa*, leading to improved seed longevity. Similarly, phospholipase D (PLD) activity also has a detrimental effect on seed viability [105]; hence it can be targeted to improve seed longevity by generating knockdown lines. Further, many detoxification proteins, such as: OsPI3K, OsACCase, and OsALDH7, were identified to regulate *O. sativa* grain longevity [126–128]. The translational control of germination was studied in *Z. mays*, leading to the identification of *eIF(iso)4E* and *eIF4E,* having a role in seed vigor [129]. As mentioned above, cellular detoxification is important to enhance seed viability. The detoxification process is also regulated by antioxidants, such as: glutathione (GSH), vitamin E, and ascorbic acid [130,131]. Increasing the expressions of these antioxidants in embryos of all major and minor cereals could help in improving the seed: viability, longevity, and tolerance to various stresses during storage. The studies mentioned above have either reduced the activity of proteins involved in seed deterioration or enhanced the expression of a protein that detoxifies deleterious products. Overall, studies suggested that these proteins affect seed longevity, as the accumulation of deleterious products in seeds during storage adversely affects both nutritional status and viability. Genetic transformations are more beneficial in maintaining the nutritional level and longevity of grains, as excessive physical treatments before storage cause: changes in texture, organic properties, and nutrient loss leading to the deterioration of grain quality and viability. Additionally, QTLs of seed longevity have been identified in many crops, including *O. sativa*, *T. aestivum*, *Z. mays*, *H. vulgare*, *S. bicolor*, and millets [56,59,60,70,72,81,85,90,132,133]. The putative candidate genes linked to these QTLs with a role in seed longevity were identified, which can be targeted to improve the seed longevity by genome editing tools, such as: CRISPR/Cas. In the current OMICS era, extensive data is available on genetic factors regulating seed longevity in almost all cereals. However, further work has to be done in minor cereals, such as millets, *S. bicolor*, *H. vulgare*, and *Avena sativa*.

Unexplored modules in this research and roadmap to address the gaps

Today, the availability of various advanced omics tools provides us a platform to better understand the

molecular mechanism of seed longevity. These approaches revolutionized crop breeding and made it possible to explore complex traits like seed longevity for crop improvement. Although the cereal crops studied so far maintain their viability during short-term storage due to their ability to tolerate desiccation, the viability is lost during long-term storage [134]. Further, it is equally important to enhance seed longevity and stress tolerance, as the stress tolerance will not compensate for the failure of seed germination, which adversely affects grain production and food security.

The candidate genes identified in the above-mentioned omics studies have been used as targets for seed longevity improvement. For example, the function of *PIMT* genes in regulating seed viability and quality was employed by many scientists in various important crops. Petla et al. [119] developed transgenic *O. sativa* with overexpression of the *OsPIMT* gene, leading to improved longevity during storage. Such studies indicated that developing GMO seeds of other cereal crops with higher expression of *PIMT* is equally feasible. Similarly, overexpression of aldo-ketoreductase also increases seed vigor by reducing ROS accumulation in seeds [122]. While these genes enhance seed longevity through repair mechanisms, other genes, such as LEA and HSP, use a protective antioxidant mechanism to enhance seed vigor and viability in *Z. mays* [86]. Several detoxification proteins, such as 2-Cys Prx BAS1, TPX, GST, GLO, SOD4, and CAT3, having a role in seed longevity, have also been identified in *Z. mays* [82,86]. Similar genes can be studied in crops such as millets, *S. bicolor*, and *H. vulgare*, which can grow under stress conditions due to the presence of higher antioxidant activity. Also, metabolomics and proteomics approaches identified various metabolites and proteins, respectively, with a crucial role in seed vigor [60,95,96,101,102]. Though the omics tools identified seed longevity-associated genetic determinants, their actual utilization to improve seed longevity through genetic engineering is still unexplored. This creates a research gap that opens the possibility to explore these candidates in enhancing the seed storability of cereal crops. Notably, other candidate genes in seeds having an important role in regulating seed viability and longevity through different mechanisms are still unexplored in cereals. For example, heat shock proteins (HSPs) play positive roles in enhancing seed longevity [135,136]. Overexpression of *HaHSFA9* from *Helianthus annuus* in *Nicotiana tabacum* seeds enhanced the seed vigor very efficiently [135,137]. Studies on different HSPs identified through proteomic analysis in cereal grains are being used as candidates for genetic improvement of seed longevity. Similarly, defense-related proteins are also linked with seed longevity, as these proteins protect seeds during a dry state [67]. The proteomic studies of cereal grains showed differential expression of various defense-related genes during seed aging [95,96,101]. All these genes are putative candidates for genetic engineering and breeding programs to improve seed longevity. Though these molecular players are well studied in non-crop plants such as *Arabidopsis* and *Medicago truncatula*, they are unexplored in cereal crops. Therefore, the mechanism underlying the regulation of seed longevity in cereal grains might be an interesting domain to explore for seed vigor improvement. In addition, the most concerned area in improving the seed longevity of cereal grains might be the effects of overexpressed or knockout genes on their: nutritional value, public health risk, and ethics of genetically improved crops. Further, QTLs and candidate genes detected using various mapping studies could be employed in breeding programs through marker-assisted selection (MAS), marker-assisted recurrent selection (MARS), and genomic selection (GS) to improve the expression of the selected trait [138].

The new generation techniques are now available to allow precise genome editing in targeted genes to develop genetically modified crops. It is imperative to understand their detailed mechanisms and effects on crop quality. Genome editing is a perfect tool to hasten the process of developing cultivars with superior alleles. CRISPR/Cas-based genome editing could be an excellent tool for editing the genome directly to achieve improved seed longevity. This technique is best suited for targeted functional dissection of various genes, whose expression causes seed deterioration during long-term storage. For example, raffinose biosynthesis and *PLD* activity, which are detrimental to seed longevity, can be manipulated by this technique to improve seed viability. The advances in biotechnological approaches at a greater pace enabled genome editing utilization for design-based crop improvement by targeting important agronomic traits, including seed longevity. CRISPR-mediated promoter editing is a novel approach that can potentially disrupt the targeted gene expression completely [139]. Interestingly, advanced genome editing tools are widely used for the manipulation of epigenetic factors in a site-specific manner. Kang et al. [140] reported the modulation of methylation at specific CpG site using CRISPR/Cas9 system, leading to the enhanced expression of the targeted gene. Epigenetic factors are crucial regulators of: seed germination, dormancy, maturation, and after-ripening events [141].

On account of their crucial role in regulating gene expression, understanding epigenetic events in seeds is

a novel area of interest among researchers. Lu et al. [142] demonstrated the epigenetic regulation of ABA levels in seeds. ABA is a key regulator of various mechanisms related to seed longevity; therefore, epigenetic regulation of its level might correlate with changes in seed dormancy and longevity. Plitta-Michalak et al. [143] analyzed the epigenetic integrity of *Pyrus communis* during cryogenic and conventional storage. Similarly, the epigenetic stability of *S. cereale* seeds during storage was assessed by Methylation Sensitive Amplified Polymorphism (MSAP), which concluded the correlation between epigenetic instability and seed aging [144]. Additionally, reduced viability due to aging of *Quercus robur* seeds was highly correlated with a decline in 5-methylcytosine (m^5C), suggesting the importance of epigenetic factors in regulating seed viability [145]. Storage-induced alterations in methylation states of *Mentha aquatica* seeds were identified, implicating the effect of aging and storage on epigenetic profile [146]. However, a deeper understanding of epigenetic events regulating seed longevity is not well explored in cereals. Therefore, a similar approach is required to have a comprehensive knowledge of its mechanism. Crops such as: *O. sativa*, *T. aestivum*, and *Z. mays* are well studied to understand the mechanism of seed longevity, and some extent of success has been attained in developing improved seeds with improved longevity. Although other cereals like millets: *S. bicolor*, *H. vulgare*, and *A. sativa* are gaining popularity due to their nutritional quality, their seed viability traits remain elusive. These crops provide vast opportunities to explore genes regulating seed longevity mechanisms, followed by their utilization in enhancing seed vigor through genetic engineering. Today, climatic changes, increasing population, and consumer demands for good quality food compel researchers to study the detailed mechanisms of seed viability and longevity in these cereal crops. Several traits have already been identified in this direction so far, and many more have to be explored. In addition, understanding the genetic and molecular mechanisms of seed longevity is crucial for the conserved accession of cereal crops. Improved seed longevity will reduce the regeneration intervals for stored seeds and enhance the acceptability of repropagation success to secure the long-term conservation of high-quality seeds.

Conclusions

Food security is challenged by various factors, including climate change, decreased agricultural lands due to rapid urbanization, and an ever-growing population. Improving the crop yield by conventional and next-generation breeding programs is an important aspect employed by researchers to enhance food production. However, traditional breeding programs often preclude seed longevity, resulting in massive crop loss due to seed deterioration after harvest. The global population widely consumes cereals, and farmers constantly require good quality seeds to maintain the yield and quality every year. Additionally, seeds reserve the complete genetic information of a plant, and they are the best delivery system for crop improvement. The loss in seed viability during storage occurs due to various factors, including the moisture content of seeds, temperature, and relative humidity of storage conditions. Besides improvement in storage facilities to assure seed viability safety, enhancing seed longevity by manipulating genetic factors holds significant potential to effectively maintain the seed viability for a longer duration. Manipulation of identified candidate genes for seed longevity improvement of cereals dramatically influences global food security. With the application of modern genetic tools and molecular breeding approaches, it is possible to implement next-generation breeding effectively for complex traits like seed quality and improve the seed longevity of these crops. In the future, reverse genetics coupled with advanced gene-editing tools, such as the CRISPR/Cas system, will provide a promising way to utilize longevity traits in the breeding programs of major and minor cereal crops. Most of our knowledge about seed longevity comes from studies in Arabidopsis and a few major cereals. However, the regulatory mechanisms of seed longevity might not be conserved in all cereal crops, thereby opening opportunities to understand the mechanism of seed longevity in other cereals. Altogether, the next-generation omics tools and modern breeding programs have great potential to study and improve seed longevity in cereal crops. The improved varieties with better quality and longevity traits can ensure food and nutritional security for the ever-growing climatic changes and human population.

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