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Biology of Seed Vigor in the Light of *-omics* Tools

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Abstract

Seed vigor is a major agronomic trait measurable by seed longevity in storage, germination capacity, and seedling growth in the field. Seed vigor has potential to significantly elevate crop resilience to biotic and abiotic stresses. That is important for crop yields enhancement and other enterprises that involve seeds like plant breeding, research and education, germplasm conservation and the seed trade. With the availability of high precision *-omics* tools for biological research, lots of investigations are undertaken globally to answer the physiological questions underlying seed germination and invigoration. The increasing *-omics* datasets constitute important resources for the delivery of new seed vigor markers and advancing new seed vigor manipulation opportunities. There is need to regularly update the knowledge generated from these investigations for the scientific improvement of seed vigor. Thus, this chapter highlights the biological backgrounds involved in the development of seed vigor traits in the light of modern *-omics* tools. The chapter is sectioned into; 1. Attributes of seed vigor and the *-omics* sciences; 2. State of *-omics*-based knowledge on underlying mechanisms of seed vigor; 3. Future perspectives of *-omics* application to genetic engineering of seed vigor with an insight to the latest technique of genome editing, the CRISPR-Cas9 technology.

Keywords: seed vigor, seed aging, seed priming, molecular mechanisms, *-omics* application

1. Introduction

Seeds constitute the basic biological input for crop production. The most important potential seed attribute directly affecting crop productivity is the seed vigor, because a good crop stand establishment is required to deliver the genetic and yield potentials of the seed. Thus, seed vigor had been a target trait of economic and ecologic values in crop improvement projects since the green revolution era [1]. Scientific manipulations to innate seed vigor of crops can be a key to increase crop yields per unit area because it can improve crop resilience against

climate change effects and biotic impediments to crop yields. More importantly, it will promote low-input agriculture by minimizing crop production inputs e.g. fertilizers, pesticides etc. that increases the environmental footprints of agriculture. However, seed vigor is a complex trait for genetic manipulation since it involves multiple physiological parameters and metabolic events of water uptake by the mature dry seeds to produce morphological events of radicle protrusion (germination) and seedling growth and development [2, 3]. Hence, exploring the fundamental biological processes underlying the trait at the various levels of these events is of intense scientific research interest.

In many recent seed biology literature, seed vigor studies engage modern biology tools using seed deterioration/invigoration experimental models. In the context of seed deterioration, seed vigor is often measured as seed longevity in storage which is relevant to seed conservation and seed bank operation efforts [4, 5]. The processes involved in the loss of seed vigor during seed deterioration elucidate the complex biological phenomenon of seed vigor. Thus, studies on the mechanism of seed vigor involve storing seeds under conditions that accelerate aging, e.g. the accelerated aging (AA) test [6] or the controlled deterioration (CD) tests to simulate the seed aging or seed vigor loss processes. In the context of seed invigoration, seed vigor has been widely studied towards increasing the performance of commercial seed lots. Seed priming is one of the most acknowledged technology-based seed invigoration treatments, which is mainly controlled imbibition of the seeds followed by dehydration back to their initial water content [7–9]. Priming treatments are hypothesized as kick-starting physiological and biochemical processes of seed germination, thus giving the treated seeds, a head-start which should increase resilience, reduce the time it takes seeds to sprout and elicit uniformity of seedling growth. All these can be achieved because primed seeds sort of “memorize” the metabolic signals they acquired from the priming process when stimulated to germinate later [10]. Many studies have proved the phenotypic advantages of priming in terms of early germination, seedling vigor [11] and stress tolerance [12]. The metabolic mechanisms underpinning priming are still been actively investigated, providing veritable data resources for scientific improvement of seed vigor. As will be discussed later in this chapter, many of the published reports suggest that cellular repair, detoxification and induction of protective proteins are the mechanisms underlying the seed vigor process [3, 13–16].

The advances in the science of biology brought a new phrase termed the *-omics* [17]. The *-omics* tools are essentially a hybrid of biological technologies encompassing liquid chromatography-mass spectrometry (LC-MS) and next-generation sequencing (NGS) [18]. They include all genomic and post-genomic approaches which in recent years have been further contributing to identifying genes and understanding their functions [19]. *-Omics* serves as an informal suffix to prefixes of specific biological fields of study such as genomics for genetics *-omics*, proteomics for protein *-omics*, etc. (**Figure 1**). In general, all *-omics* science aim at the collective characterization and quantification of pools of biological molecules that translate into the structure, function, and dynamics of living organisms [19].

With reference to seed vigor, several *-omics* dissections have been reported [20]. Here is a quick run of definitions of key *-omics* sciences that have been applied to seed vigor. *Genomics*, which is the study of the genome of an organism, have been severally used to dissect genomic

regions that have significant genetic effects on seed vigor of many crops [21]. *Proteomics* is the study of the entire complement of proteins, modifications made to a particular set of proteins and their functions in the cell. Unlike the genome, which is fixed for most cells, the proteome is known to be dynamic, changing with internal or external (environmental) stimuli. The proteomics of seed vigor of many plant species have been widely reported [22]. Transcriptome involves all set of RNA molecules—mRNA, rRNA, tRNA, and other non-coding RNAs, produced in one or a population of cells, which is important in gene expression. *Transcriptomics* of seed vigor traits has been studied; Soeda et al. [12] used the microarrays technique to dissect the expression of seed vigor related genes during the priming of *Brassica oleracea* seed lots while Dinkova et al. [23] used the RNA-Seq, method to study translation initiation factors during maize seed germination. *Metabolomics* is the scientific study of chemical processes and metabolites (chemical fingerprinting). A related scientific branch to metabolomics that is well researched for seed vigor traits is *Metabonomics*, the quantitative measurement of the dynamic, multi-parametric metabolic responses to pathological and physiological stimuli or genetic modifications. Examples of metabolomic and metabonomic dissections of seed vigor are the investigations of metabolic pathways and signaling involved in seed vigor. Lipidome is the entire complement of cellular lipids, and *lipidomics* has been involved in studies of lipids and glycolipids and lipid metabolites during seed deterioration or invigoration [24]. *Glycomics* is the analyses of the glycome—collection of cellular glycans (sugars and carbohydrates) of an organism. A glycomics study that associated seed vigor with sugar metabolites involved has helped to identify carbohydrate and sugar biomarkers for seed vigor [25]. Most of the published studies utilized one or more genomic tools, which explains why so much seed vigor -omics references has been categorized under genomics (**Figure 1**).

With the advent of genomic and post-genomic technologies, high-throughput analyses of molecular profiles have been implemented at the protein, RNA and metabolite levels to dissect the biological processes involved in seed vigor development. The aim of this chapter is to review advances in seed vigor studies in the light of new high precision -omics tools. In this

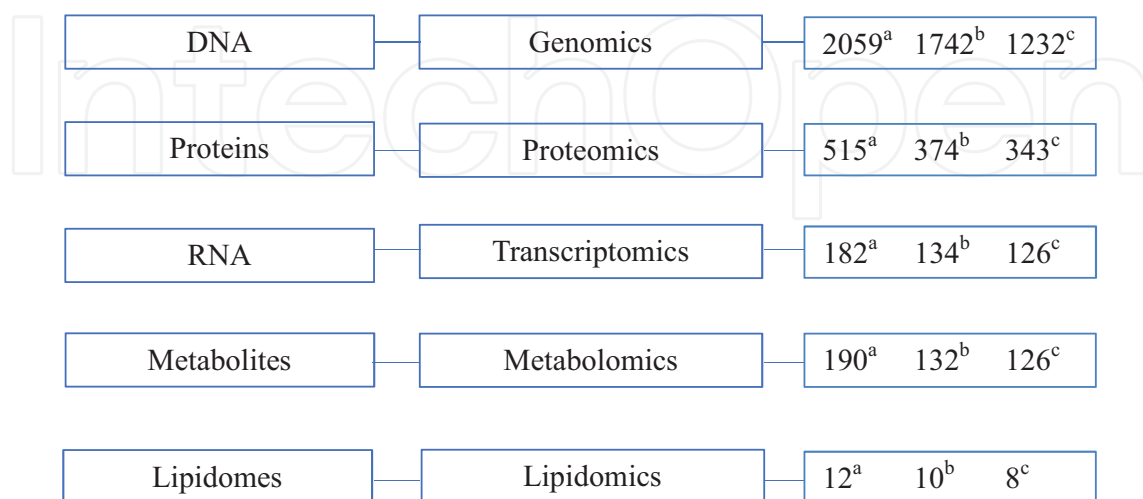


Figure 1. References of -omics sciences reported on seed vigor related traits in popular library databases as at July 2017. (a) Science Citation Index (expanded web of science), (b) ProQuest, and (c) PubMed.

chapter, I will be discussing some of the major advances made with specific *-omics* technologies towards dissecting the complex seed vigor traits in various crop plant species. I will also focus on the direction to which the current advances in seed vigor *-omics* might be pointing the seed industry in the near future. In discussing all the sections, I will pay attention to seed vigor reports from the experimental perspectives of seed deterioration (aging) and seed invigoration (priming).

2. *-Omics* technologies for seed aging/priming

2.1. Development of seed quality biomarkers

Rajjou et al. [20] argued that characterizing biomarkers of seed vigor as a component of breeding programs is an important strategy for producing seeds of the highest possible quality, particularly under the environmental stresses occasioned by climate change and increasing world population. Thus developing bio-markers for seed aging and vigor loss traits are the first notable achievements of various seed vigor *-omics* projects. **Table 1** summarizes key *-omics*-based biomarkers of seed aging and/or vigor that have gained significant attention in the seed research community.

Advances in *-omics* sciences have made the acquisition of seed aging signals feasible so that a number of sensitive and effective biomarkers has been developed to identify aging signals and evaluate aging status [26]. Fu et al. [27] summarized *-omics* based biomarkers for seed aging signals of 17 species and classified them into six categories namely: molecular, biochemical, physiological, metabolic, mitochondrial and morphological signals (**Table 1**). Catusse et al. [15] used comparative proteomics to identify 18 proteins during seed priming, germination and aging. Bentsink et al. [28] showed that the *DOG1* gene that controls seed dormancy in *Arabidopsis* is also a biomarker for seed longevity since the mutations within the *DOG1* gene, specifically were associated with a seed longevity phenotype. Prieto-Dapena et al. [29] found that transgenic *Arabidopsis* seeds that over-accumulate a heat stress transcription factor exhibit a heat shock protein (*HSP*) biomarker for enhanced longevity. Whereas Devaiah et al. [24] reported a high level of a membrane lipid-hydrolyzing phospholipase-D (*PLD α 1*) as a biomarker for reduced seed longevity. Kranner et al. [30] proposed the concept of half-cell reduction potential ($E_{GSSG/2GSH}$) of glutathione (*GSH*) an antioxidant that scavenges ROS which increases to more oxidizing values during viability loss as biomarker signaling cascades that trigger cell death. Nagel et al. [5] engaged the *GSH* redox method as a biomarker for seed aging in barley. The activity of the protein L-isoaspartyl methyltransferase (*PIMT*), an enzyme repairing abnormal L-isoaspartyl residues in aging proteins of *Arabidopsis* is increasingly becoming a common biomarker in *-omics* both seed deterioration (aging) and seed invigoration (priming) studies in a number of crop plants [13, 14, 16, 22, 31] (**Table 1**).

Reports on seed priming-based *-omics* experiments have also been sources of seed vigor biomarkers. Chen et al. [32] reported protein profiling of spinach (*Spinacia oleracea* cv. Bloomsdale) seeds during priming with -0.6 MPa PEG at 15°C . The results showed two groups of proteins: Type I *i.e.* 37 and 35-kDa proteins which are major proteins in unprimed seeds gradually

Crop	Biomarkers	Description	Reference
Seed aging studies			
Arabidopsis	<i>DOG1</i>	Delay of germination	Bentsink et al. [28]
Arabidopsis	<i>HSFA9</i>	Heat stress transcription factor	Prieto-Dapena et al. [29]
Arabidopsis	<i>PLDα1</i>	Phospholipase D-alpha (membrane lipid enzyme)	Devaiah et al. [24]
Arabidopsis	<i>PIMT1</i>	l-Isoaspartyl methyltransferase (Repair)	Ogé et al. [22]
Maize	<i>eIF4E/ eIF(iso)4E</i>	Translation initiation factor	Dinkova et al. [23]
Chickpea	<i>PIMT2</i>	l-Isoaspartyl methyltransferase (Repair)	Verma et al. [13]
Barley	<i>GSH</i>	Glutathione di-sulfide (antioxidant)	Nagel et al. [5]
Seed priming studies			
Brassica	<i>AT5G06760</i>	Type 1 LEAs	Soeda et al. [12]
Spinach	LEAs	Stress tolerance	Chen et al. [32]
Maize/Spinach	<i>18S</i>	HKG	Chen et al. [33]
Rice	<i>PIMT1</i>	l-Isoaspartyl methyltransferase (Repair)	Wei et al. [16]
Rice	<i>PIMT2</i>	l-Isoaspartyl methyltransferase (Repair)	Petla et al. [14]

Table 1. Biomarkers of that have gained attention in -omics' dissection of seed aging/vigor.

depleting as priming progresses, and Type II such as ~20 kDa doublets proteins having an accumulation patterns opposite to Type I during priming. The depletion and/or accumulation of Type I and Type II proteins during germination constitute biomarkers for seed vigor behaviors for the species. The type I proteins seed vigor biomarkers were seed maturation proteins, such as late embryogenesis abundant (LEA) or dormancy related proteins (e.g. short-chain dehydrogenase). The depletion of these proteins during germination and priming was also reported to mark seed vigor genes at transcription and translation levels in *B. oleracea* [12].

Chen et al. [33] identified a set of transcriptomic biomarkers for seed germination and vigor from quantitative real-time polymerase chain reaction (qRT-PCR) gene expression studies during germination of maize and spinach seeds. Since seed germination involves seeds transiting from dry and physiologically inactive state to hydrated and active state, the expression of house-keeping reference genes (HKG) may alter during the transition. From the study, the HKGs identified as valid reference genes and hence seed vigor biomarkers were *Actdf*, *UBQ*, *β tub*, *18S*, *Act*, and *GAPDH*. The HKG *18S* notably maintained stability through the transition state and was stable for both maize and spinach.

As the biomarker capabilities of *PIMT* genes has been reported for seed aging studies, likewise some seed priming studies have reported *PIMT* as biomarkers for seed vigor [16, 34]. The potential importance of *PIMT* as one key candidate seed vigor biomarker will be discussed later in this chapter.

2.2. -Omics of regulatory mechanisms for seed aging/priming

Studies from the '70s and '80s identified the roles of regulatory hormones like ABA and GAs in seed germination control through mutations in Arabidopsis [35, 36]. With the advent of -omics methodologies, a significant in-depth understanding of regulatory mechanisms signaling seed deterioration and invigoration has been gained and applies to crops [37, 34]. Changes in specific sequences of highly polymorphic genetic markers in aging rice [38], tomatoes [39] and wheat seeds [40] provide hints of molecular hints on genetic influences behind seed aging.

Scanning through recent molecular studies on seed deterioration or invigoration, the mechanisms regulating seed vigor can be summarized into three systems: repair, protection, and detoxification systems [3, 23, 34, 41–44]. Research advances in manipulation of the cellular repair system for seed invigoration has been more pronounced, often intertwining with detoxification system research. The most apparently forward-looking cellular repair studies came out of the search for mechanisms underpinning the extra-ordinary longevity of sacred lotus (*Nelumbo nucifera*) seeds, which was found to be due to the repair activities of abnormal L-isoaspartyl residues accumulated in proteins during seed aging by *PIMT* in Arabidopsis [31]. *PIMT* combats protein mis-folding resulting from L-isoaspartyl formation by catalyzing the conversion of abnormal L-isoaspartyl residues to their normal L-aspartyl forms thus repairing an enzyme system which likely works with other anti-aging pathways to eliminate deleterious protein products and enable successful seedling establishment in the phenotypes [22, 31]. Studies of the role of *PIMT* in seed invigoration and longevity enhancement has extended to other crops, most of them reporting similar results. An immuno-localization study on rice concluded that the distinct *OsPIMT* isoform expression in embryo and aleurone layers of transgenic rice revealed its role in the restriction of deleterious isoAsp and age-induced ROS accumulation to improve seed vigor and longevity [14]. *PIMT* and *PIMT2* contains two genes (*At3g48330* and *At5g50240*) encoding protein-L-isoaspartate methyltransferase located on chromosome 5 and produces two proteins differing by three amino acids reported in Arabidopsis [22, 31], chickpea [13] and rice [16, 14]. The activities of two important *PIMT* coding genes (*At3g48330* and *At5g50240*) are gradually forming the bedrock for seed aging -omics and genetic engineering of seed vigor in many crop species [13, 22, 34].

Several -omics studies have generated information on the protective regulatory mechanism of seed aging/vigor. Protein and enzymes regulatory systems that are active in structural, membrane and genomic integrity were engaged in most of the studies that were attempting to dissect the protective system for seed invigoration [24, 29, 45]. Transcriptomics analysis of *de-novo* protein synthesis during priming-enhanced seed germination had shown the expression of aquaporins (AQPs) in abundance [46, 47]. AQPs are plasma membrane proteins known to regulate water transport, since they are mostly expressed in hydrated seeds. AQPs are either plasma membrane intrinsic proteins (-PIPs) or tonoplast intrinsic proteins (-TIPs) serving as water channels in membranes that control cell-to-cell water movement, plant cell expansion and organ development. The expression of four spinach (*S. oleracea*) AQP coding genes (SoPIP1;1, SoPIP1;2, SoPIP2;1, and So δ TIP) during osmopriming and germination under chilling drought and optimal conditions were investigated by [46]. The up-regulation of the four genes within 2–4 days of priming (phase II-imbibition) suggests that these proteins

are essential for radicle protrusion and subsequent progress of seed germination vigor. The expression of vacuolar aquaporin genes increases a thousand times after the initiation of cell elongation in both orthodox and recalcitrant seeds [47]. During priming of *Beta vulgaris* L. (sugarbeet) seeds, Catusse et al. [15] used comparative proteomics to reveal 18 proteins exhibiting up-regulation during priming and down-regulation during aging and up-regulation again upon priming of the aged seeds. In the study, six translation initiation factors were found among the proteins exhibiting the highest levels of up-regulation upon priming the aged seeds, highlighting the roles of stored mRNAs and *de-novo* synthesized mRNAs in seed vigor protection regulatory mechanism. Proteomic analysis of sugarbeet seeds led to the identification of 758 proteins whose metabolic status in seed longevity protection can be inferred and reconstructed in further details [15]. Dinkova et al. [23] streamlined the translational control of seed germination in maize using the ratio of two cap binding proteins (*eIF(iso)4E* to *eIF4E*) in the corresponding eIF4F complex, *eIF(iso)4E* being more abundant in dry seeds and both cap-binding proteins being present at similar levels following 24-hour seed imbibition. Furthermore, Prieto-Dapena et al. [29] found that over-accumulation of heat stress transcription factor (HSPs) enhanced seed longevity in transgenic Arabidopsis seeds. Regente et al. [41] reported regulation of phospholipid accumulation in extracellular fluids of sunflower during priming and seed germination. Devaiah et al. [24] reported that the ablation of the gene for a membrane lipid-hydrolyzing phospholipase D (*PLD α 1*) in Arabidopsis enhanced seed germination and oil stability after storage or exposure of seeds to adverse conditions. The *PLD α 1*-deficient seeds exhibited a smaller loss of unsaturated fatty acids and lower accumulation of lipid peroxides than did wild-type seeds. However, *PLD α 1*-knockdown seeds were more tolerant of aging than were *PLD α 1*-knockout seeds. The results demonstrate the *PLD α 1* plays an important role in seed deterioration and aging in Arabidopsis. A high level of *PLD α 1* is detrimental to seed quality, and attenuation of *PLD α 1* expression has the potential to improve oil stability, seed quality and seed longevity.

Cellular detoxification mechanisms have been widely viewed as an important mechanism for seed invigoration. Several gene activities have been identified that controls these mechanisms [48, 49]. Detoxification genes/proteins that scavenge ROS are the most investigated system for mechanism for seed vigor enhancement. For example, Nagel et al. [5] linked seed aging to genetic backgrounds that regulate the production of ROS-scavenging antioxidants which are known to detoxify aging cells to enhance vigor in a similar fashion to cellular repair mechanisms. Antioxidants such as glutathione (GSH), tocochromanols and ascorbic acid scavenge ROS [42]. Decreases in the antioxidant capacity of GHS under continuous accelerated aging stress increase ROS, shifting the antioxidant redox state towards more oxidizing conditions. In agreement with this concept, the glutathione half-cell reduction potential ($EGSSG/2GSH$) increases to oxidizing values during viability loss, which is assumed to initiate further signaling cascades that trigger cell death [30]. The accumulation of oxidative damage in seeds was correlated with seed vigor loss [26]. At the molecular level, the process of carbonylation, in other words, increased protein oxidation often induces loss of functional properties of target seed proteins or enzymes thus increasing their susceptibility to proteolysis. Since the presence of ROS attacks proteins by oxidizing them, the important role of antioxidant systems through detoxification and protection of upstream mechanisms to maintain seed vigor is underscored.

2.3. Mapping the genes controlling seed aging/vigor

The use of molecular markers in modern plant breeding to increase selection efficiency through mapping genes to specific traits of interest was made possible by *-omics* precision tools. For many simply inherited traits of economic importance, fine-mapping and tagging with closely linked or gene-specific markers is straightforward simple. However, seedling vigor in crop plants is a complex quantitative trait under the control of large genotype and environment (G×E) effects. Hence, the advent of genomics tools for mapping and analyzing quantitative trait loci (QTL) is a major breakthrough for breeding seed vigor traits and gene identification for further experimentation. From many seed deterioration experiments, QTLs of seed longevity traits like LD₅₀ in *Arabidopsis* seeds [21], germination of aged wheat seeds [48, 49] and half-life (P₅₀) of aging barley seeds [50] have been mapped and linked to various genes. For the germination vigor of seeds, several seed priming experiments have found QTLs for germination of maize seeds [51] and QTLs for 30 vigor traits of rice seeds [52] to mention a few. These studies also provided useful information on chromosome regions and putative genes controlling various seed vigor traits in different crops.

QTL work on seed vigor began with the pioneering work of Clercx et al. [21] on the model plant *Arabidopsis*, where QTL mapping was used to identify the loci controlling various aspects of seed longevity during storage and germination. Genotyping a recombinant inbred line population with 65 PCR-based markers and seed LD₅₀ of phenotypic marker *erecta*, they identified three QTLs affecting seed longevity after controlled deterioration on chromosomes 1, 3, and 4 for *Arabidopsis*. Nagel et al. [50] also reported large QTL effects associated with seed half-life (P₅₀) on chromosomes 5 and 7 in a doubled haploid mapping population of barley. Han et al. [51] found 65 QTLs in two maize populations mapped using single-nucleotide polymorphism (SNP) markers to four seed vigor traits under four germination treatment conditions. Integrating the QTLs into 18 meta-QTLs (mQTLs), 23 candidate genes associating with seed vigor phenotype coincides with 13 mQTLs controlling protein metabolism and the glycolytic pathway. They reported four seed vigor hotspots on chromosome regions for mQTL2, mQTL3-2, mQTL3-4, and mQTL5-2 with large QTL effects under various germination environments. There are a number of recent QTL studies on seed vigor of rice [52, 53]. Singh et al. [52] reported seed germination capacity of primed rice seeds derived from 253 BC₃F₄ lines of crosses between Swarna and Moroberekan, phenotyped for early vigor and genotyped with 194 SNP markers. They identified six seed vigor genomic regions on chromosomes 3, 4, 5, and 6 [52]. Two of the QTL regions namely chr3 (*id3001701-id300833*) and chr5 (*wd5002636-id5001470*) were identified and tagged QTL hotspots because they were expressed consistently in field and glasshouse conditions. In the chr3 hotspot, most of QTLs identified for early vigor-related traits were *qEV*_{3.1'}, *qEUE*_{3.1'}, *qSHL*_{3.1'}, *qSL*_{3.1'}, *qSFW*_{3.1'}, *qTFW*_{3.1'}, *qRDW*_{3.1} associated with early vigor, early uniform emergence, shoot length, stem length, shoot fresh weight, total fresh weight and root dry weight respectively. The QTL hotspot on chr5 includes almost similar seed vigor traits as the first hotspot except total fresh weight and root dry weight but includes seed dry weight (*qSDW*_{5.1}) and total dry weight (*qTDW*_{5.1}).

From these QTL regions identified in the brief review above, putative candidate genes associated with many seed vigor traits in the hotspot QTL regions have been published for crops like

wheat [35], maize [38] and rice [40]. Besides, Carrera et al. [54] used gene expression profiling -omics method to produce a list of candidate genes that signify seed germination which was used to produce TAGGIT, a spreadsheet based seed specific gene ontology that describes the seed germination signature. Other seed specific genomic resources for seed vigor are: PageMan/MapMan package which visualizes transcriptome changes in *Arabidopsis* [55] seeds during germination, and SeedNet which describes transcriptional interactions for seed vigor regulation [56].

3. Future perspectives for seed vigor improvement through -Omics results

This review has highlighted key advances provided by various -omics platforms for the dissection of the complex trait called seed vigor. With the current advances in -omics applications to seed vigor biomarkers, understanding of the regulatory mechanisms, gene mapping to traits, and genomic database resources for seed vigor, a unique platform for genetic manipulation of seed traits is emerging. We are moving towards a revolution of crop production that explores the complex traits of seed vigor for enhanced productivity in the face of environmental challenges, increasing human population and rising intensity of costs and land resources for food production.

The work of Xu et al. [31] on *PIMT* encoded genes (*PIMT1* and *PIMT2*), which display distinct expression patterns but similar biochemical properties of repairing IsoAsp accumulation in seed proteins is blazing a trail which researchers have validated for a number of crops [14, 16, 22]. For example, Rajjou et al. [20] confirmed that transgenic *Arabidopsis* seeds over-expressing *NnMT2a* and *NnMT3* displayed a remarkably improved resistance to accelerated aging treatment, indicating their significant roles in seed germination vigor. Wei et al. [16] worked on one of the two *PIMT* genes from rice (*Oryza sativa* L.) and found that over-expression of *OsPIMT1* in transgenic rice seeds reduced the accumulation of isoAsp-containing protein in embryos, and increased embryo viability. Petla et al. [14] also reported that transgenic rice constitutively over-expressing *OsPIMT1* and *OsPIMT2* exhibited improved seed vigor and longevity. These data indicated that engineering *OsPIMT*-related seed longevity improvement is a feasible option for producing enhanced vigor GMO seeds through target-gene methods. A way forward from understanding the clear role of *PIMT* in seed vigor improvement is the application of these findings for genetic engineering of *PIMT* towards improving seed vigor. Wu et al. [34] summarized current knowledge on *PIMT* gene modifications, specific genetic engineering methodologies and their outcomes for seed vigor improvement in three different crops (**Table 2**). Altering *PIMT* accumulation in seeds shows various effects of physiological significance in the various studies opening opportunities for genetically manipulating seed vigor. While *PIMT* offer opportunity for producing high vigor seeds through the repair mechanism, other candidate genes utilizing alternative strategies to producing seed vigor phenotypes are also waiting to be explored. Examples are the LEA and HSP proteins that use the protective gene mechanism, the detoxification mechanism that uses the ROS scavenging gene action [43] and the AQP water uptake mechanism to enhance seed vigor [46]. This leaves a wide research gap that are indeed opportunities to explore towards mapping and genetic engineering of these classes of proteins already identified as implicated in enhancing inherent seed vigor. The obvious research questions raised

from this review are whether other single-gene manipulations methods can also produce such effects as *PIMT*. Other research concerns might be the investigation of the effects of enhanced expression of seed vigor genes/proteins on other seed traits like nutrient value, potential health risk as food and feed and ethical issues of GMO seeds for innate vigor.

Since 2013, newer *-omics* tools that allow genome-editing and gene targeting are poised to contain ethical concerns of GMOs because of its capacity for precise modulation of traits of interest with unprecedented control and efficiency. A set of techniques called clustered, regularly interspaced, short palindromic repeat (CRISPR) technology capable of making precise targeted changes in the genome of living cells appeared recently [57], and can be the next great opportunity for genetic manipulation of seed vigor. Coming out of this is the CRISPR-Cas9 which is the latest borderline technology based on a bacterial CRISPR-associated protein-9 nuclease (Cas9) from *Streptococcus pyogenes* [58]. This has already been successfully used to target important genes in many cell lines and organisms. The simplicity of this method lends it to wide applications in biology, currently, it is possible to introduce single point mutations (deletions or insertions) in a target DNA with a guide RNA (gRNA) [59]; and induce large genomic re-arrangements, such as inversions or translocations with a pair of gRNA-directed

Species	Methodology	Outcome	Reference
<i>Arabidopsis thaliana</i>	T-DNA insertion line with increased <i>PIMT1</i> expression and transgenic lines with altered <i>PIMT1</i> expression	The physiological role of <i>AtPIMT1</i> in seed vigor and longevity has been established in <i>Arabidopsis</i> . The higher <i>PIMT1</i> amount in <i>pimt1-1</i> seeds correlates with lower isoAsp accumulation <i>in vivo</i> and increases both seed longevity and germination vigor, and <i>vice versa</i>	Ogé et al. [22]
<i>Cicer arietinum</i>	Seed-specific over-expression of <i>CaPIMT1</i> and <i>CaPIMT2</i> in <i>Arabidopsis</i>	The role of <i>CaPIMT2</i> in seed vigor and longevity has been elucidated <i>CaPIMT2</i> enhances seed vigor and longevity by repairing abnormal isoAsp in the seed nuclear proteome	Verma et al. [13]
<i>O. sativa</i>	Overexpressing <i>OsPIMT1</i> lines and <i>OsPIMT1</i> RNAi lines	The role of <i>OsPIMT1</i> in seed vigor and longevity has been elucidated Germination % after 21 days of CDT, overexpressing <i>OsPIMT1</i> transgenic seeds, increased 9–15%; <i>OsPIMT1</i> RNAi lines, rapid loss of germination.	Wei et al. [16]
<i>O. sativa</i>	Transgenic rice and <i>Arabidopsis</i> lines with altered expression of <i>OsPIMT1</i> and <i>OsPIMT2</i>	Transgenic rice and <i>Arabidopsis</i> lines with altered expression of <i>OsPIMT1</i> and <i>OsPIMT2</i> Germination % after 4 days of CDT, control seeds, 8% (maximum); <i>OsPIMT1</i> , <i>OsPIMT2</i> , and <i>OsPIMT2</i> transformed seeds, 43–48%.	Petla et al. [14]

Table 2. Seed vigor outcomes of different *PIMT* gene alteration methodologies from different reference sources in various crop species [34].

Cas9 nucleases [60]. Proteins can also be targeted for transcriptional regulation using dCas9 version of the CRISPR-Cas9 system [57].

For seed vigor improvement, CRISPR-Cas9 can be used to manipulate gene functions that directly regulate DNA repair pathways like nucleotide and base excision repair, the non-homologous end joining and homologous recombination all of which play notable roles in seed vigor development [3]. Furthermore, the capacity of CRISPR-Cas9 to enable rapid genome-wide study of gene function by generating large gRNA libraries for genomic screening offer opportunity for large scale deployment of precision *-omics* technology for genetic engineering of seed vigor. Of particular relevance of the new technology to crop breeding is the possibility of removing targeted gene constructs by conventional breeding in subsequent generations of the modified plants, thus addressing concerns of GMO contaminations.

4. Conclusion

On a global scale, modern agriculture is currently pressurized to achieve food security with limited arable land due to the changing climate and increasing global population. For increases in crop yields with reduced inputs. This paper reviews the state of the art *-omics* results on seed vigor and offers insights towards up-scaling the laboratory results to field productivity. From the reviews, several biological studies that dissect seed vigor traits in crops are discussed narrowing down to few *-omics* approaches offering possibilities for genetically improving seed vigor by plant breeding. Availability of numerous candidate genes and/or proteins along with enormous seed-specific genomic libraries and high-precision *-omics* techniques like CRISPR-Cas9 constitute new resources for drastic improvement of the trait. One strategy mentioned in this review is genetic manipulation of a number of genes controlling cellular repair, protection, detoxification and enhanced membrane integrity in crops. In the near future, studies on reverse genetics coupled with high precision genetic engineering tools, will lead the way to breeding high vigor phenotypes of many crops on large-scale. The results are expected to produce exciting *-omics* contributions to the advancement of crop yields with less environmental damages when these techniques are up-scaled for agricultural applications. Application to important cereals such as wheat, rice, and maize may have a dramatic impact on global food security.

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