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Review

Desiccation tolerance as the basis of long-term seed viability

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Abstract: Desiccation tolerance appeared as the key adaptation feature of photoautotrophic organisms for survival in terrestrial habitats. During the further evolution, vascular plants developed complex anatomy structures and molecular mechanisms to maintain the hydrated state of cell environment, which essentially increased their ability to sustain water deficit and dehydration. However, the role of the genes encoding the mechanisms behind this adaptive feature in the higher vascular plants is restricted to the dehydration protection of spores, seeds and pollen, whereas the mature vegetative stages became sensitive to desiccation. During maturation, orthodox seeds lose up to 95% of their water and successfully enter dormancy. This feature allows seeds maintaining their viability even under strongly fluctuating environmental conditions. The mechanisms behind the desiccation tolerance are activated at the late seed maturation stage and are associated with the accumulation of late embryogenesis abundant proteins (LEA proteins), small heat shock proteins (sHSP), non-reducing oligosaccharides, and antioxidants of different chemical nature. The main regulators of maturation and desiccation tolerance onset are abscisic acid and protein DOG1, which control the network of transcription factors, among which are LEC1, LEC2, FUS3, ABI3, ABI5, AGL67, PLATZ1, PLATZ2. This network is complemented by epigenetic regulation of gene expression by methylation of DNA, post-translational modifications of histones and chromatin remodeling impact on seed desiccation tolerance and longevity. Moreover, orthodox seeds are able to maintain desiccation tolerance during germination up to the stage of radicle protrusion. This time point is critical in the process of seed development, as the seeds lose desiccation tolerance at this moment.

Keywords: abscisic acid, after-ripening, desiccation tolerance, dormancy, germination, gibberellins, LAFL, seeds, viability

1. Introduction

The ability to survive after desiccation appeared as a crucial evolutionary step, which allowed the first plants to colonize the terrestrial habitat [1]. Rapid mobilization of tolerance mechanisms during desiccation and subsequent re-hydration is common for the so-called poikilohydric plants – bryophytes, some algae and ferns, as well as a small group of angiosperm plants [2]. Although this feature was mostly lost during the further evolution of terrestrial flora, more than 60 species of spore vascular plants and about 140 species of angiosperms were identified as tolerant to desiccation [3]. However, due to their slow growth, geographic distribution of such plants is limited. Phylogenesis of seed plants is characterized by growing complexity of anatomy structures, which, on one hand, essentially restrict water loss from the plant surface (cuticle, endodermis, stomata), on the other – makes its transport within the plant more efficient (tracheary elements) [1,2]. Unfortunately, the restriction in water penetration across the plant barrier structures limited its transport in both directions, i.e. the increasing ability to retain water turned to be accompanied with compromised viability under severe dehydration conditions. Thus, the role of the genes, which encoded adaptation

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of the whole organism to water loss at earlier steps of plant evolution, in higher vascular plants is restricted to the protection of spores, seeds and pollen from dehydration. This situation represents the principal ecological paradox: the plants, which are sensitive to even minimal dehydration and dramatically reduce their productivity under drought conditions, are able to tolerate almost complete loss of water without noticeable damage at the stage of seed.

In respect to desiccation tolerance (DT), the seeds of vascular plants can be classified into orthodox and recalcitrant types [4–6]. The maturation of the orthodox seeds is accompanied with a water loss up to 5 – 10% w/w, which allows them sustaining unfavorable environmental conditions, such as extremely high and low temperatures and drought. In contrast, water loss during maturation is not characteristic for recalcitrant seeds. These seeds are sensitive to dehydration and, similarly to the tissues of vascular plants, are damaged during strong desiccation. Thus, the viability of dormant recalcitrant seeds is highly dependent on environmental conditions. Due to this fact, they are widely represented only in tropical forests. Some authors believe that the plant species with recalcitrant seeds evolved from ancestors with orthodox seeds as a result of adaptation to wet climate with constant rains, where immediate germination upon separation from the parental plant would be evolutionary advantageous [1]. Besides these two contrasting seed development strategies, so-called “intermediate" plant species, the mature seeds of which are able to sustain slight dehydration, are also known [1,5,6]. The differences in dehydration tolerance of orthodox and recalcitrant seeds are important for the long-term storage of the former in gene banks [7].

In orthodox seeds, the mechanisms behind the onset of desiccation tolerance are activated at the final stages of maturation [8]. The loss of this principle feature accompanies seed germination and corresponds to the moment of radicle protrusion [4,9]. Although the phenomenon of drought tolerance is being intensively studied in plants, the molecular mechanisms behind the onset and loss of desiccation tolerance during seed maturation and germination, respectively, remain mostly unknown. To some extent, these mechanisms can be approached by deeper evaluation of the molecular events behind the physiological and biochemical adaptation in so-called resurrection plants, which acquired desiccation tolerance as a secondary adaptation during the evolution, and, therefore, do not lose the viability of vegetative organs after drying/rehydration cycles [3,10]. Resurrection plants are generally small and can grow on soils with a thin fertile layer (e.g., on rock outcrops). It is assumed that the molecular mechanisms underlying desiccation tolerance of resurrection plants and mature orthodox seeds are essentially similar [4].

2. Mechanisms behind the onset of desiccation tolerance during seed maturation

In general, the process of seed formation comprises two principal sequential steps – development of embryo and seed maturation, which, in turn can be sub-divided in early, middle and late maturation stages. During the embryo development, its axial structures are formed, tissue-specific cell differentiation starts, and the main plant organs are defined [11,12]. During the early seed maturation stage, the storage compounds (proteins, fats, and carbohydrates) are intensively accumulated [13]. At the late seed maturation stage such important events as transition to dormancy and onset of desiccation tolerance occur [8]. The molecular basis of the desiccation tolerance of the orthodox seeds is accumulation of such compounds as late embryogenesis abundant (LEA) proteins, small heat shock proteins (sHSP), non-reducing oligosaccharides of the raffinose group (RFO) [1,3,8,14] and such low molecular weight antioxidants as glutathione [15], tocopherols [16] and carotenoids [17] (Fig. 1A).

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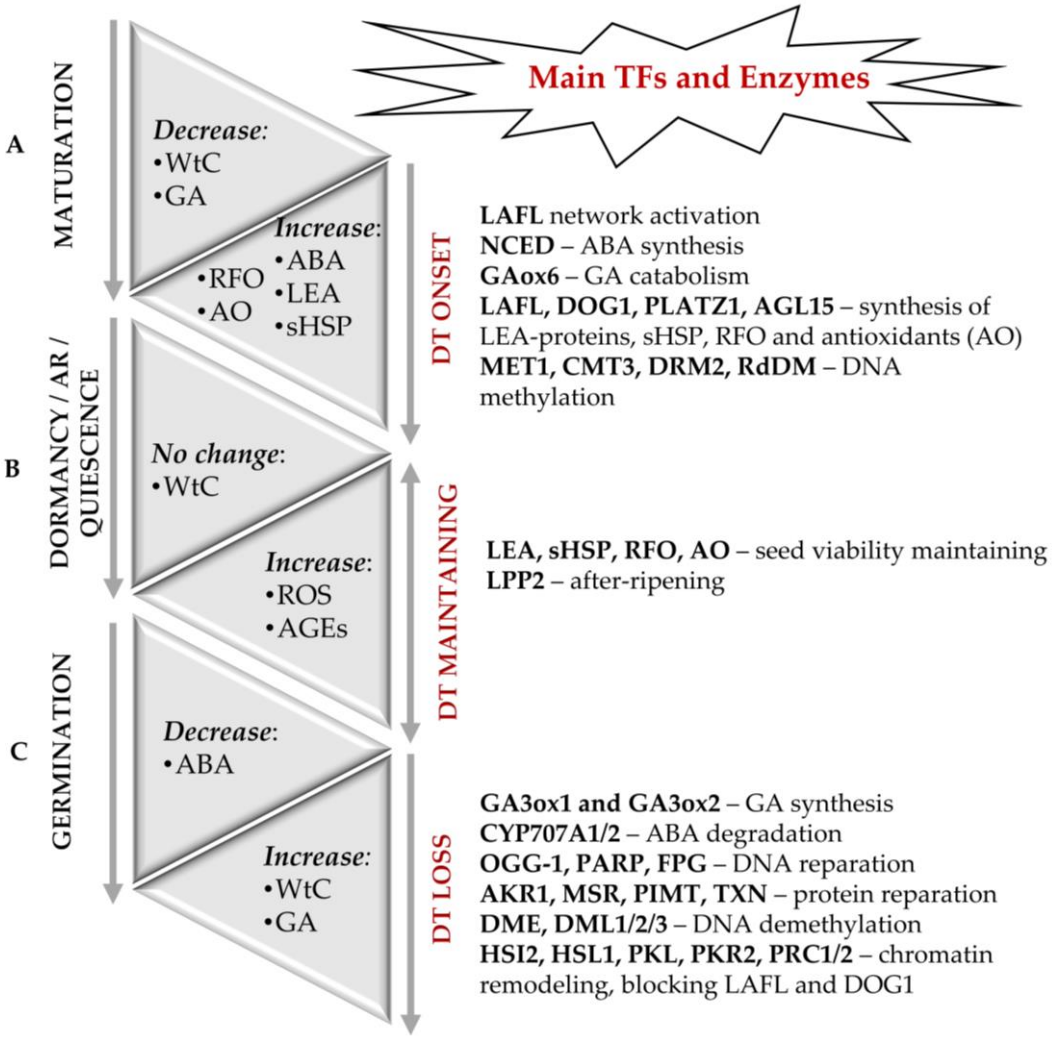

Figure 1. Molecular events accompanying the onset and loss of desiccation tolerance (DT) in the orthodox seeds. (A) The onset of DT and seed dormancy is defined during the late maturation stage and associated with a sharp decrease in water content (WtC). The molecular basis of DT is the accumulation of LEA proteins, sHSP, RFO, and antioxidants. Regulation of seed maturation relies on the ratio ABA/GA along with LAFL – the network of the transcription factors LEC1, ABI3, FUS3 and LEC2, as well as by DOG1, DOG4 and DOGL4 proteins. In addition to the transcription factors of LAFL network as major regulators of desiccation tolerance, PLATZ 1, PLATZ2, and AGL67 can also be involved. Epigenetic modifications such as changes in DNA methylation and histone post-translational modifications are also crucial for the DT onset. **(B)** Long-term storage can result in enhanced damage of dormant seeds due to non-enzymatic modifications of proteins with ROS and glycation agents. After-ripening (AR) results in termination of dormancy and switch of seeds to a quiescent state, in which they can germinate when favorable environmental conditions occur. **(C)** The transition from quiescent seeds to germination is associated with rapid water uptake, hydration of macromolecules, repair of membranes, proteins, and nuclear acids, as well as accumulation of H_2O_2 as a secondary messenger. The activity of the catabolic enzymes involved in the oxidative degradation of ABA increases and the level of major active GA, on the contrary, increases. The moment as of the radicle protrusion occurs, the program controlled by the LAFL is getting blocked and seeds lose desiccation tolerance. Modifications of chromatin, which involve PRC1 and PRC2, as well as PKL and PKR2 proteins, represent the key mechanism behind the LAFL suppression.

Late embryogenesis abundant (LEA) proteins

LEA proteins play the key role in sustaining of seed viability [18–21]. These polypeptides accumulate in seeds during the late maturation stage, i.e. they are present in dry seeds and disappear after germination [19]. In the promoters of the Arabidopsis genes encoding LEA proteins *cis*-elements sensitive to abscisic acid **(**ABA), drought and low temperatures were found [18]. Chen *et al* reported

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an increase in the potato *LEA* gene expression in response to drought, salinity, extremely high and low temperatures [22].

LEA-proteins are characterized by high glycine (Gly) contents, low amount (or even lack) of cysteine (Cys) and tryptophan (Trp) residues, and predominance of such amino acid residues as alanine (Ala), glutamate (Glu), lysine/arginine (Lys/Arg) and threonine (Thr) [23]. Due to this structure, they are stable in a broad temperature range and are highly hydrated. During cell dehydration, LEA-proteins act as chaperons, i.e. impact on structural stabilization of other proteins and cell membranes by intensive hydrogen bond formation, they stabilize denatured proteins and promote their refolding [19]. LEA proteins were shown to sequester ionic compounds, accumulating during cell dehydration, and protect membrane proteins and enzymes from the deleterious effects of increased salt concentrations [19]. Although the LEA proteins were detected in all cell compartments, they are predominantly localized in cytoplasm[24]. The most of the LEA-proteins adopt unordered, randomly coiled structure in aqueous solutions [19]. However, dehydration results in their re-folding into the structures with higher percentage of amphiphylic α-helix. Flexible structural elements (such as polyproline II helices), which enhance binding to DNA, RNA or other proteins, are also characteristic for LEA proteins [18,19].

Altogether, 51 genes encoding LEA proteins were identified in Arabidopsis genome [18]. According to the Pfam protein domain database, they can be distributed in eight families: Dehydrins (PF00257), LEA-1 (PF03760), LEA-2 (PF03168), LEA-3 (PF03242), LEA-4 (PF02987), LEA-5 (PF00477), LEA-6 (PF10714) and seed maturation protein (SMP, PF04927) [18,20]. LEA proteins, as well as some heat shock proteins, belong to the group of so-called moonlighting proteins, which are characterized with several physiologically relevant functions [20,25]. The expression level of *LEA* genes is much higher in seeds than in vegetative organs of plants. Indeed, in total, 21 of 51 Arabidopsis genes encoding LEA proteins are expressed only in seeds and are directly involved in the control of their formation [18]. This tendency is even more pronounced in *Camellia sinensis*: 39 among 48 *LEA* genes detected in this plant might play an important role in seed maturation [26].

The largest group of LEA-proteins is represented by the LEA-2 family [18,20,26]. The proteins representing this family are featured with higher content of hydrophobic amino acids and conservative three-dimensional structure. *Dehydrins* represents the most studied group of LEA proteins, and comprises the family of universal protective molecules involved in plant responses to various abiotic stressors. Binding and structural stabilization of cellular biopolymers by dehydrins relies on their homo- and hetero- complexes, which are often multimeric [27]. In agreement with the fact, that decrease in cellular levels of dehydrins can enhance sensitivity of plant tissues to desiccation [2], the orthodox, intermediate and recalcitrant seeds have been shown to have different dehydrin composition and contents [28].

Heat shock proteins (HSPs)

Activation of the HSP system represents one of the most universal responses of living organisms to stressors. Based on their molecular weights, eukaryotic HSPs can be distributed in five classes: Hsp100, Hsp90, Hsp70, Hsp60, Hsp40 and small HSPs (sHSP).

Among the whole HSP superfamily, sHSPs represent a large and heterogeneous (in comparison to the other four) group of proteins with molecular weight of 12-43 kDa and are characterized with a highly conservative C-terminal α-crystalline domain containing 80-100 amino acid residues [29]. Similarly to LEA proteins, sHSPs are accumulated at the late seed maturation stage and are present in their native state in dry seeds [8,29,30]. In seeds, sHSP promote folding of newly synthesized proteins, protection against oxidation, as well as refolding of proteins with damaged tertiary structure [8,29–31]. One of the most important features of sHSPs is their ability to form large oligomeric complexes (100-1000 kDa), the size of which can increase up to 5000 kDa under stress conditions [29]. Apparently, only relatively large oligomers have high chaperone activity, i.e. able to interact with damaged or misfolded proteins and stabilize their structure [29].

The Arabidopsis family of heat stress transcription factors (HSFs) comprises 21 members in three classes (A, B, and C) [32]. Kotak *et al*. analyzed the performance of HSFA9 which is expressed in

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Arabidopsis seed exclusively at the late maturation stage. The authors demonstrated that the expression of HSFA9 is regulated by the seed-specific transcription factor ABI3. Indeed, the ABI3 knockout lines lack detectable levels of HSFA9 transcripts and proteins. On the other hand, ectopic expression of ABI3 ensured the accumulation of HSFA9 in transgenic plantlets in response to application of ABA. Furthermore, it was shown that ABI3 could activate the HSFA9 promoter, whereas HSFA9, in turn, proved to be a potent activator of some HSP genes[33]. In addition, analogously to the genes of LEA proteins, expression of HSPs, can be mediated by the key dormancy regulator DELAY OF GERMINATION (DOG1) [34]. Moreover, expression of several seed HSP genes was shown to be induced by osmotic stress. For example, dehydration triggers expression of HaHSP17.6 and HaHSP17.9 genes in the seeds of sunflower (*Helianthus annuus*), and the levels of corresponding mRNAs correlated with the degree of the water loss [29]. The embryogenesis of soybean was reported to be accompanied with accumulation of specific HSP transcripts, and the levels of individual mRNAs positively correlated with seed longevity [14]. It was shown recently, that expression of 41 *RcsHSP* genes encoding cytoplasmic, mitochondrial and microsomal α-crystalline domain-containing HSPs, accompanied maturation of castor bean (*Ricinus communis)* seeds [35]. Kaur *et al*. reported an essential increase in the abundance of the OsHSP18.2 transcripts in rice seeds at the late maturation stage [31]. Thereby, the expression levels significantly increased after artificial aging, but dramatically decreased after beginning of germination. It was shown further that expression of OsHSP18.2 improved seed vigor and longevity by reducing the deleterious ROS accumulation in stored seeds [31].

Non-reducing carbohydrates

Accumulation of non-reducing carbohydrates (primarily sucrose, raffinose and stachyose), which stabilize the structure of membranes and cytoplasmic proteins by replacing water molecules, is one of the most important strategies of seed survival during desiccation. In contrast, the presence of reducing monosaccharides negatively correlates with seed longevity [8]. It can be explained by enhanced glycation of cellular proteins with reducing carbohydrates [36], their phosphorylated derivatives [37] and carbonyl products of sugar degradation, which are mostly represented by α-dicarbonyl compounds (3-deoxyglucosone, glyoxal, and methylglyoxal) [38]. This process is known, to be affected by environmental stressors and ageing [39,40].

The patterns of soluble sugars change essentially at the late seed maturation stages: hexoses (glucose and fructose, as well as their phosphorylated derivatives) gradually disappear, whereas non-reducing oligosaccharides accumulate [8,41]. During dehydration, oligosaccharides and LEA proteins replace water as a hydrogen-bonding partner of the phosphate groups in the polar heads of phospholipids [42]. This results in the transition of the liquid-crystalline state of membranes to the gel phase, which is accompanied with a decrease in lipid mobility due to stronger van der Waals interactions between lipid heads after the loss of their hydrate shells [42,43]. The cytoplasm of dehydrated cells becomes "glassy" without the transition of cytoplasm to the solid state. Thereby, the viscosity of cytoplasm increases, the diffusion of water and oxygen is suppressed and the rates of all possible chemical reactions are dramatically reduced [5,42,43]. Due to these shifts in the physicochemical properties of multiple cellular structures, orthodox seeds are able to maintain viability for decades [30].

3. Epigenetic regulation of desiccation tolerance

Epigenetics is the field of biology, specifically addressing heritable (both via mitosis- and meiosis-related mechanisms) changes in genome, which are not accompanied with any changes in DNA sequence [44,45]. Such epigenetic changes mainly involve DNA methylation at cytosine residues, post-translational covalent modifications of histones (e.g. acetylation, methylation and ubiquitylation), and synthesis of small RNAs [44,45].

DNA methylation is involved in both negative and positive regulation of gene expression, representing thereby one of the most well-characterized epigenetic regulatory mechanisms in plant development [46]. It ensures genome stability by inactivating potentially dangerous elements -

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transposons and foreign DNA sequences [47]. On the other hand, plants use DNA methylation to maintain genome plasticity, which allows efficient adaptation to changing environmental conditions [46,48]. This modification underlies genomic (parental) imprinting, cell differentiation, embryo formation, as well as seed maturation and germination [46,49,50]. It is assumed, that DNA methylation also plays an important role in the formation of desiccation tolerance at the late seed maturation stage [51,52].

DNA methylation is an ubiquitous covalent modification of cytosines in DNA sequence and can be of either *maintenance* or *de novo* type [48,53]. In all eukaryotic organisms, the modification sites are typically localized in the CG consensus, although in plants, methylation can also occur at the cytosines localized in the CHG and CHH consensus sequences, where H is any nucleotide excepting G [46]. The methylation reaction is catalyzed by site-specific DNA cytosine methyltransferases and yields 5-methylcytosine residues in the DNA chain [46]. In the case of *de novo* methylation, the substrate is a non-methylated DNA molecule. Maintenance methylation assumes modification of newly synthesized DNA strands, complementary to the parent DNA, to preserve the existing patterns of cytosine methylation.

Plants have at least three families of DNA methyltransferases, which are represented, respectively, by (*i*) methyltransferases 1 (MET1), (*ii*) chromomethylase 3 (CMT3) and (*iii*) domain rearranged methyltransferases 2 (DRM2). MET1 is involved in maintenance methylation at CG sites, whereas CMT3 catalyzes maintenance methylation at CHG and CHH sites [46]. The methyltransferase DRM2, which catalyzes *de novo* methylation, is expressed in all organs and tissues of the plant organism. The recognition of target methylation sites relies on small interfering RNAs complementary to the target DNA loci. After recognition and interaction with the corresponding loci, the RNA-directed DNA methylation (RdDM) occurs at CG and, to a less extent, at CHG and CHH sites [53]. Methylation of CHH sites via the RdDM mechanism was demonstrated during seed formation and at the late maturation stages [50].

During seed formation, DNA methylation at the CG and CHG sites remains, in general, stable, whereas the levels of mCHH methylation noticeably increase throughout the whole process of seed development, and later on gradually decrease during seed germination [50,53]. The genome regions, hypomethylated during the whole plant life cycle, are largely enriched in genes encoding transcription factors, as well as other genes playing a critical role in seed formation– e.g. those encoding storage proteins and enzymes of fatty acid metabolism [54]. Mature embryos are characterized by a higher level of genome methylation compared to the embryos at the early stages of development and seedlings, mainly due to the high level of methylation at the CHH sites.

An *et al*. studied DNA methylation in the developing seeds of soybean (*Glycine max*) and identified 40, 66, and 2136 genes containing differentially methylated regions at the CG, CHG, and CHH sites, respectively [55]. Methylation was detected in 66, 45 and 9% of the CG, CHG and CHH sites, respectively. Thereby, the CHH methylation levels increased during seed maturation from 6% to 11%, whereas the expression of the genes, containing the CHH consensus, was mostly reduced. These genes were predominantly associated with DNA replication and cell division [55].

Seed desiccation at the late maturation stage also initiates methylation of nuclear DNA [51,56], which is often associated with long-term repression of gene transcription [46]. Michalak *et al.* addressed the effect of desiccation on the orthodox seeds of wild pear (*Pyrus communis* L.) [56]. The authors found an increase in the overall DNA methylation levels immediately after completion of seed maturation, which was detectable during two years afterwards [56]. However, recalcitrant maple seeds (*Acer pseudoplatanus*), which were dried in the presence of silica gel from 47.7 to 13.9 – 35.0%, showed decreased 5'-methylcytosine contents in the embryonic axes and cotyledons by 27 and 37%, respectively [51]. Apparently, an increase in the levels of DNA methylation during desiccation is characteristic only for orthodox seeds.

Despite the cytosine methylation is a relatively stable epigenetic modification, it is dynamically controlled by enzymatic demethylation [57]. It is accomplished by the excision of methylated nucleotides and subsequent insertion of a non-methylated cytosine [58]. The following enzymes are involved in demethylation of plant DNA: demeter (DME), repressor of silencing 1 (ROS1)/demeter-

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like 1 (DML1), DML2 and DML3 [59]. The DME protein is required for the imprinting of the paternal or maternal alleles of certain genes, while ROS1, DML2 and DML3 are active in vegetative tissues. ROS1 mediates RdDM-dependent and RdDM-independent methylation and can prevent the spread of DNA methylation from transposable elements to protein-coding genes.

It is important to note, that besides DNA methylation, epigenetic regulation of gene expression can also rely on covalent post-translational modifications (PTMs) of histones and chromatin remodeling [48]. Such PTMs of histones as acetylation, methylation, and ubiquitination play an important role in the regulation of seed formation and dormancy [46,60,61]. Small interfering RNAs and/or long non-coding RNAs can also trigger epigenetic changes in dormant or germinating seeds. For example, Huo *et al.* found that the regulatory effects of DOG1 on seed dormancy, at least partly, are mediated by miRNA regulated pathways. Thus, DOG1 can regulate seed dormancy and flowering in lettuce (*Lactuca sativa* L) and Arabidopsis via alteration in the levels of miR156 and miR172. Suppression of LsDOG1 enabled seed germination at elevated temperatures and promoted early lettuce flowering in association with reduced miR156 and increased miR172 levels. In Arabidopsis, higher miR156 levels (due to over-expression of the MIR156 gene) enhanced seed dormancy and delayed flowering [62].

4. Seed longevity in the context of dormancy and after-ripening

Seed longevity

Seed longevity (also referred to as storability and seed life span) is one of the principal target parameters in modern agriculture in the context of global climate change and is critical for plant biodiversity conservation [7,63,64]. Seed longevity is usually defined as the maximal possible (in terms of viability) period of time from the completion of full maturation of the seed on the mother plant till the initiation of its germination [1]. The longevity of seeds depends on their structure and chemical composition, as well as on the environmental conditions of their development [7,63,65]. Thus, seed longevity is influenced by the mother plant at the late maturation stage [64]. In general, higher longevity of seeds can be considered as one of the adaptive mechanisms, which allows plant populations to spread in time and space [8]. In different plant species it varies from several years to decades, however, seeds of some species can sustain viability for hundreds and even thousands of years [63]. Indeed, the seeds of the palm *Phoenix dactylifera* L., excavated from a Herodian fortress and dated as 2000 years old, can serve as an example [66].

In the beginning of this decade, Nguyen *et. al*. identified five loci of quantitative traits of seed longevity (*Germination Ability After Storage, GAAS1-5*) in Arabidopsis inbred lines [67]. The *GAAS* loci were shown to be co-localized with seed dormancy locus *DOG*. The detailed expression analysis of the quantitative trait loci *GAAS5* and *DOG1* revealed that deep seed dormancy correlates with low seed longevity and *vice versa*. However, Dekker *et al.* showed that the seeds of *dog1-1* mutants exhibit a reduced lifespan and lower expression of *LEA* and *sHSP* genes [34]. The mechanisms responsible for the seed longevity are likely to correlate with the mechanisms underlying regulation of their dormancy, but the nature of such interaction has not been revealed yet.

Seed dormancy

Seed dormancy is typically referred to as the physiological state of normally developed viable seeds with a temporal germination block, duration of which is independent from environmental conditions [68–70]. The main factors involved in the regulation of onset and termination of dormancy, are DOG proteins and the ratio of ABA and gibberellins (GA), specifically GA1, GA3 and GA4 [60,71,72]. Seed dormancy can rely on different mechanisms and may be associated with incomplete morphological development of embryos (morphological dormancy), hardening of seed coat (which prevents imbibition - physical dormancy) and/or low GA level (physiological dormancy) [69,70]. Physiological dormancy can be terminated by incubation of imbibition seeds at decreased (0-10°C) or increased (>15°C) temperatures, or by treatment of seeds with GA, karrikins, nitrates, or inhibitors of ABA biosynthesis [72]. As a result, the balance of ABA and GA is shifted towards the higher levels

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of the latter. This leads to the activation of GA-related signaling pathways involved in control of seed germination. In the natural way, the termination of seed dormancy triggered by so-called after-ripening (AR), i.e. storage under dry conditions [72] (Fig. 1B).

Seed after-ripening

Seed after-ripening is a complex process, which still remains to a great extent under-explored. It is triggered by the separation of the seed from the mother plant and is accompanied with gradual increase of its germination potential [72–74]. AR is characterized by a decrease in ABA levels and reduced seed sensitivity to this hormone in parallel to the increase in GA levels [75,76]. This process is enhanced by improvement of tissue oxygen supply and increase of storage temperature, whereas even a minimal increase in seed water contents suppresses AR [77,78]. After completion of this developmental phase, seeds become able to germinate under a broad range of temperature and lighting conditions [79]. It is important to note, that the depth of seed dormancy (i.e. the time required for its termination) is defined not only genetically, but also by the environmental conditions of seed development and maturation on the mother plant (temperature, nutrient and water supply) [80].

Already at the end of the last decade, Carrera *et al*. identified the main groups of *Arabidopsis* genes, demonstrating differential expression patterns upon dry storage of seeds [75]. In that work, the core gene sets, positively or negatively regulated by dry storage, were identified. Each set included the gene encoding repression or activation of ABA function: *LIPID PHOSPHATE PHOSPHATASE* 2 (*LPP*2) and *ABA DEFICIENT1* (*ABA*1), respectively. The set of the genes, down-regulated by AR, included *ABA*1 involved in ABA synthesis. On the other hand, the set, up-regulated under AR conditions comprised LPP2. Recently, Ishikawa *et al.* identified 1730 phosphopeptides in a large-scale liquid chromatography-mass spectrometry (LC-MS)-based analysis of ABA-related phosphoproteome of barley seed embryos [76]. Analysis of phosphoproteome of the embryos isolated from the freshly harvested seeds and those after AR, showed significant differences in the patterns of ABA-responsive phosphosites. The obtained results were supported by peptide motif analysis which suggested activation of a new set of protein kinases at the after-ripening stage.

Unfortunately, to date, it remains unclear how these complex regulatory events can occur in dehydrated seed tissues. One possible explanation could be differential distribution of water between seed structures and individual biomolecules within them. The assessment of H_2O_2 proton mobility in tobacco seeds by nuclear magnetic resonance (NMR) supports this hypothesis, i.e. indicates uneven distribution of water molecules in dry seeds [74]. A higher level of proton mobility, indicating a higher content of water and/or oils, is characteristic for the micropillar endosperm layer. Indeed, here occurs the expression of β-1,3-glucanase which hydrolyzes cell wall β-glucans to oligosaccharides [74]. Recently, the hypothesis, postulating reactive oxygen species (ROS) as regulators of seed maturation was proposed [79]. For example, it was assumed, that overproduction of ROS can result in site-specific oxidation of mRNA molecules, along with oxidation and carbonylation of proteins, associated with desiccation/re-hydration-related signaling pathways [80].

Thus, the physiological state of seeds during dormancy, AR and prolonged storage is defined by the water content in their tissues. It means that for successful AR, dehydrated state of seeds is critical: even a small and transient increase in the water content of dormant seeds can interrupt this process and trigger seed damage. Therefore, understanding of the mechanisms behind the survival of seeds after desiccation is one of the central problems of modern seed physiology.

5. Role of ABA and DOG1 in the regulation of seed maturation.

ABA is involved in several key events, accompanying seed maturation: biosynthesis and transport of nutrients, degradation of chlorophylls, dehydration of seed tissues and onset of dormancy [13]. This hormone is also involved in suppression of the genes encoding germination-related proteins: α-amylases, lipase, lipid transfer proteins [8,81,82].

At the early stages of embryogenesis, the developing embryo is controlled by ABA transported from the mother plant tissues, but at the later developmental stages the hormone is produced by the seed itself [71,83,84]. During the seed development, ABA sequentially affects embryo growth in two

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different ways. Thus, in the beginning of embryogenesis, ABA prevents seed abortion and promotes embryo growth. At the final stages of embryogenesis, in contrast, the levels of ABA increase, that results in the block of embryo growth, i.e. in this case, ABA acts as a GA antagonist [83,84]. Once the embryo has been formed, its size begins to increase by cell elongation and due to accumulation of storage compounds. ABA blocks transition of the embryonic cells from the G_1- to the S-phase of the cell cycle, and stimulates transport of monosaccharides, amino acids from mother plant and synthesis of their storage forms – polysaccharides and proteins. At the late maturation stage, metabolic processes are gradually slowed down, the seeds are dehydrated and enter dormancy [68,81]. The regulation of seed maturation mostly relies on the network of transcription factors known as LAFL (LEAFY COTYLEDONS 1 (LEC1), ABA-INSENSITIVE 3 (ABI3), FUSCA 3 (FUS3) and LEAFY COTYLEDONS 2 (LEC2)) [61], as well as DOG1 and DOG4, which are associated with ABA-dependent seed maturation regulators [34,60,85]. These factors control expression of the seed genes involved in the regulation of embryogenesis, suppression of germination, accumulation of storage compounds and onset of desiccation tolerance. The mutants *abi3* and *fus3* are sensitive to dehydration, and the seeds of *fus3* germinate directly on the mother plant [8,71,86,87]. LEC2, FUS3 and ABI3 also regulate expression of the genes encoding the storage proteins – oleosins and globulins. The seeds of *abi3*, *fus3* and *lec2* mutants contain lower amounts of storage proteins and lipids [8,81,82,86]. The transcription factor DELAY OF GERMINATION 1-like 4 (DOGL4), which initiates the expression of about 70 genes specific for seed maturation, including those encoding such storage proteins as albumin, cruciferin and oleosin, is believed to be the main regulator of storage compound accumulation in seeds [85]. In addition, the expression of *DOG*L4 gene is induced by ABA, while the expression of *DOG*1 is independent from ABA [85].

ABA plays a crucial role in the late stages of seed maturation [83]. This hormone was shown to activate the synthesis of LEA-proteins, which are known to increase desiccation tolerance of developing seeds, as well as their tolerance to other adverse environment factors [8,82]. The crucial transcription factors involved in the control of seed maturation, expression of the genes, encoding LEA proteins and metabolism of raffinose family oligosaccharides are ABI3 and ABI5 [34,61,88]. It was shown that ABA-insensitive seeds of Arabidopsis *abi3* and maize *vp1* mutants are unable to accumulate storage proteins, do not acquire desiccation tolerance, do not enter dormancy and are capable of germination on the mother plant (vivipary) [81]. The transcription factors ABI3 and ABI5 are proposed to be involved in regulation of the genes encoding chlorophyll degradation enzymes during seed maturation[8,14,88,89].

Chlorophylls are synthesized during the early embryogenesis, participate in the photochemical photosynthetic reaction sand degrade at the late maturation stage [90–92]. However, this degradation often remains incomplete [93]. For example, mature seeds of the pea (*Pisum sativum* L.) and alfalfa (*Medicago truncatula* L.) *abi5* mutants retain significant amounts of chlorophylls [88]. As ABI5 controls not only the synthesis of LEA-proteins and raffinose family oligosaccharides, but is also involved in regulation of chlorophyll degradation enzymes. Zinsmeister *et al* consider this factor as the most important regulator, affecting the maintenance of seed viability during dormancy [88]. Recently, new major regulators of desiccation tolerance were identified in Arabidopsis seeds in addition to the LAFL network: plant AT-rich sequence- and zinc-binding protein 1 and 2 (PLATZ 1 and 2), and AGAMOUS-like 67 (AGL67) [94]. González-Morales *et al* showed that the over-expression of PLATZ1 and AGL67 or Dehydration-responsive element-binding protein 2D (DREB2D) at least partially compensates the loss of desiccation tolerance in mutant *abi3-5* [94]. The authors assume that in the signal cascade controlling seed desiccation tolerance, transcription factors PLATZ1, PLATZ2 and AGL67 plays after LEC1, ABI3, FUS3 and LEC2.

The completion of embryogenesis and onset of dormancy are controlled by the transcription factors LEC and FUS3, as well as by phytohormones – ABA, GA and ethylene [68,95,96]. Indeed, ABA was found to stimulate LEC1- and FUS3-mediated protein synthesis. Furthermore, FUS3 triggers up-regulation of ABA tissue levels, providing a positive feedback for the processes regulated by ABA and FUS3. LEC1 was shown to activate LEC2 and FUS3, while LEC2 activates LEC1 and FUS3 [71,81,95]. The expression of the genes, encoding GA 3-oxidases 1 and 2 (genes *GA3ox1* and *GA3ox2*,

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respectively), i.e the enzymes involved in conversion of GA precursors into their biologically active forms, is suppressed by FUS3 and LEC2. Besides, LEC2 induces expression of the factor AGL15, which, in turn, activates expression of GA 2-beta-dioxygenase 6 (*GA2ox6)* gene. Recently, Braybrook et al showed that GA is implicated in the repression of *LEC* genes in seedlings, i.e. this regulatory pathway is not suppressed by germination [95].

Several recent studies reported the regulation of seed dormancy via the interaction of ABA- and DOG1-related signaling pathways [60,87,97,98]. Nakabayashi *et al.* proposed that DOG1 and ABA act in mostly parallel pathways involved in regulation of dormancy [99]. These pathways merge downstream at ABI3 or ABI3. The key elements of ABA signaling are represented by the receptor Pyrabactin resistance/PYR-like/Regulatory component of abscisic acid receptor (PYR/PYL/RCAR) and protein phosphatases type 2C (PP2C), encoded by *ABI1* and *ABI2* genes (Fig. 2). PP2C are the negative regulators of sucrose-non-fermenting-related kinases (SnRK2), which mediate the activity of transcription factors ABI3 and ABI5 [71,82,97].

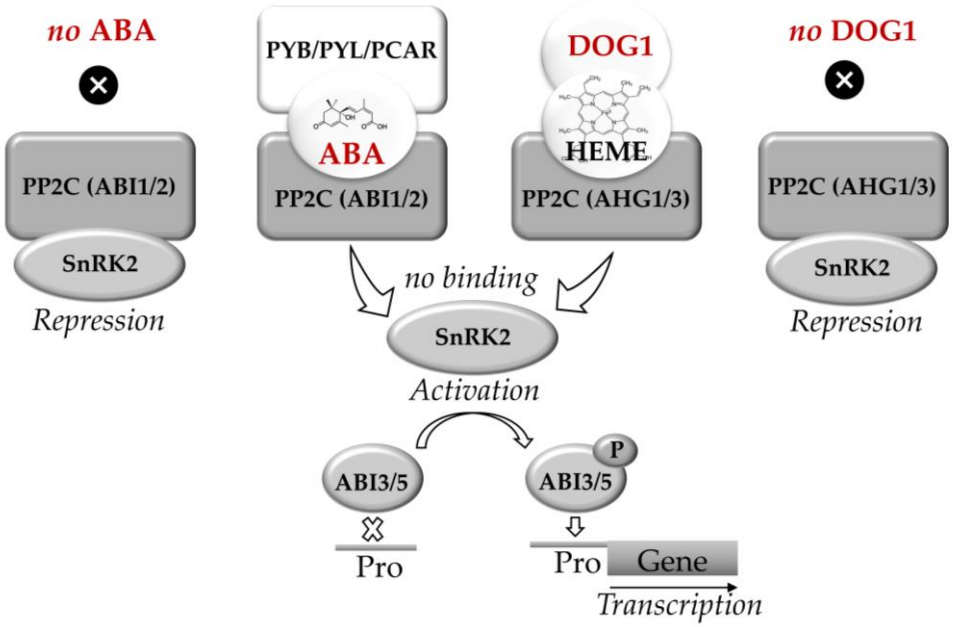

Figure 2. Abscisic acid (ABA) and DELAY OF GERMINATION 1 (DOG1) signaling pathways in seeds. The key elements of ABA signaling are receptor PYR/PYL/RCAR and PP2C encoded by (*ABI*1 and *ABI*2 genes. The key elements of DOG1 signaling are heme molecule and PP2C encoded by *AHG*1 and *AHG*3 genes. Triplex complexes of PCAR-ABA-PP2C and/or DOG1-HEME-PP2C block the binding of PP2C with SnRK2. Active SnRK2 phosphorylates the ABI3 and ABI5 which bind to the promoters (Pro) of ABA-related genes. In seeds the parallel ABA and DOG1 signaling pathways activate the expression of LEA, HSP and RFO synthesis thus regulating the onset of desiccation tolerance and transit to dormancy. Modified from [34,60,82,87,97,98].

Group A PP2C which is a key negative regulator of ABA signaling is represented by two subfamilies, namely ABI1 and ABA Hypersensitive Germination1 (AHG1) [98]. At low ABA levels, PP2C blocks the transduction of the ABA signal by suppressing the SnRK2 and, consequently, blocking the regulatory events mediated by ABI5 and ABI3. In contrast, the increase in ABA levels triggers binding of PP2C to the receptor, forming a triple ABA-RCAR-PP2C complex. This complex can block the phosphatase activity of PP2C. This, in turn, triggers phosphorylation of the transcription factors ABI5 and ABI3 and induces the expression of ABA-dependent genes [96,97].

DOG1 was demonstrated to trigger ABI5/ABI3-mediated expression of *LEA* and *HSP*. This might enhance accumulation of nitrogen-containing compounds and promote completion of seed maturation and subsequent desiccation [34] . The key elements of DOG1 signaling are the protein phosphatases ABA Hypersensitive Germination 1 and 3 (AHG1 and AHG3y), which are the members of the PP2C family and also suppress protein kinases SnRK2 [98]. Furthermore, DOG1 was showed to bind heme. Binding of DOG1 to AHG1 and to heme represent two independent processes, but both

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are essential for DOG1 function. When binding DOG1 to AHG1 and/or AHG3, SnRK2 is released and phosphorylates ABI5. Moreover, DOG1 was shown to affect the expression of multiple genes controlling seed maturation, including genes encoding LEA and HSP proteins and raffinose family oligosaccharides accumulation. This function of DOG1 protein is partially complemented the transcription factor ABI5 [34].

Thus, the signaling pathways regulated by ABA and DOG1 result in the inhibition of PP2C family protein phosphatases, which suppresses the transcription factors responsible for the expression of ABA-dependent genes [87].

6. Mechanisms of seed antioxidant and redox protection.

Seed damages

After completion of AR and termination of dormancy, seeds become ready for germination [72]. However, to ensure meeting the optimal environmental conditions for germination, seeds must maintain viability for a long period of time (months, years, sometimes decades) [60,68]. This phenomenon is primarily underlied by the lack of free water therein and non-specific inhibition, therefore, of all enzymatic activities. Nevertheless, long-term storage, as well as increased humidity and ambient temperature (i.e. higher than the storage optimum) of the environment, can promote deleterious alterations in seed metabolism and related structural changes in seed proteins, which are often referred to as seed damage. This damage is typically manifested by the loss of functions of individual polypeptides, at the molecular level underlied by non-enzymatic modifications of proteins with ROS and carbonyl compounds accompanied with accumulation of oxidation [100,101] and advanced glycoxidation end products (AGEs) [102]. Surprisingly, glycation of seed proteins was addressed only in few studies till now [36,103]. As glycation was recently proposed to be also involved in signaling in plants [104], this aspect needs to be elaborated in more detail. Nevertheless, numerous studies proved that seed damage during storage is caused by the accumulation of ROS, which can react with almost all biological molecules, including lipids, DNA, and proteins [52,79]. Overproduction of ROS (which are to a large extent are free radicals) result in enhancement of lipid peroxidation, which is along with AGEs formation might lead to destruction of cell membranes [64,102,105,106].

Seed redox homeostasis and antioxidant protection

The metabolism of all aerobic organisms, including plants, is based on an array of redox processes, critically important for both catabolic and anabolic pathways [107–109]. Redox reactions in electron transport chains (ETCs) of mitochondria and chloroplasts are characterized with high rates of electron or/and energy transfer, that inevitably lead to the formation of reactive oxygen species (ROS) by electron or energy leakage to O_2 [110], as well as in the enzymatic reactions catalyzed by NADPH oxidases, glycolate oxidases, amine oxidases, and cell-wall-bound peroxidases [111–114]. In parallel to ROS, reactive nitrogen, sulfur and carbonyl species (RNS, RSS and RCS, respectively) [102,103,115–117] can be formed in seeds, although for the physiological role of RSS and RCS only limited information is available [117].

ROS are the products of constitutive plant redox metabolism and are, therefore, continuously generated in plant tissues, being involved in multiple normally occurring in cell physiological processes [118–120]. Thus, ROS generation is one of the earliest responses of plant cells to various biotic and abiotic stresses, as well as internal regulatory events [121]. In the most of cases, superoxide anion radical ($O_2^{\bullet-}$) is produced as a primary ROS type. This intermediate can be easily converted to hydrogen peroxide (H_2O_2) and other peroxides, which are, in turn, readily involved in Fenton reaction, yielding hydroxyl radical ($^{\bullet}OH$) [109,122]. Other chemically active oxygen-containing species are also referred to as ROS, in particular –triplet chlorophyll (^3Chl) and singlet oxygen ($^1O_2$) [52,105,111].

Being a by-products of electron transport in ETCs [123,124], ROS are acting as signals in distinct biological processes, such as growth and development, responses to biotic and abiotic stresses, and

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programmed cell death [111,119], where they are typically function as secondary messengers in signaling networks [114,125,126]. During seed formation, generation of ROS accompanies several developmental steps - maturation, desiccation, dormancy, aging, and germination [111,118,127]. In particular, ROS are involved in the regulation of embryogenesis and seed germination [105,111]. ROS participate in dormancy release by seed dry storage (during after-ripening) and is involved in the perception and transduction of environmental conditions that control germination. The signaling role of ROS in seed germination is likely to occur only when the ROS content is enclosed within values of the so-called "oxidative window" that allows ROS to function as secondary messengers [111,118,120]. The basal level of ROS in cells is essential for supporting cell proliferation and differentiation. Moreover, cell death (which was previously thought to be the outcome of oxidative stress) is now considered to be the result of the ROS-induced programmed process [120].

Under normal conditions, continuous generation of ROS is accompanied with their detoxification by a broad range low-molecular weight and enzyme-based radical scavenging and antioxidant systems [128]. However, when production of ROS overwhelms the activity of these antioxidant protective systems, their tissue contents increase and oxidative stress develops [112,129]. At the molecular level, oxidative stress is manifested by enhanced lipid peroxidation, disruption of membrane integrity, inactivation of enzymes, oxidative degradation of proteins and of nucleic acids along with depletion of the antioxidant pools. Since the hydroxyl radical is extremely short-lived, it is impossible to neutralize it enzymatically [105]. To date, no specific scavengers of $OH^{\cdot}$ are known. The effects of unspecific scavengers (mannitol, sorbitol, dimethyl sulfoxide, thiourea) are mainly dependent on the nature of corresponding ROS precursors of the hydroxyl radical (hydroperoxyl, peroxynitrite, H_2O_2 or superoxide), although the presence of transition metals in solutions and efficiency of their chelation are also important [109].

In contrast to hydroxyl radical, detoxification $O_2^{\bullet-}$, H_2O_2 and $^1O_2$ can be mediated by antioxidant enzymes [109]. The crucial role in the antioxidant protection of seeds is played by superoxide dismutases (Cu-SOD, Zn-SOD, Mn-SOD, Fe-SOD), peroxidases (guaiacol peroxidase, ascorbate peroxidase, glutathione peroxidase), catalase. Additionally, low molecular weight hydrophobic antioxidants tocochromanols (i.e. tocopherols and tocotrienols), carotenoids, as hydrophilic redox metabolism – ascorbate and glutathione, can be involved [16,17,52].

The ROS levels in seeds are also controlled by the enzymes of the ascorbate-glutathione cycle also known as Foyer-Halliwell-Asada pathway [130,131]. The reduced form of ascorbate is able to directly interact with ROS, as well as participate in the reduction of other low molecular weight antioxidants – tocopherols, glutathione. Tocopherols are universal protectors of cell membranes, participating in the quenching of ROS and thus preventing non-enzymatic oxidation of lipids [16,105]. Glutathione can directly act as a ROS-quenching molecule or can serve as an electron donor for enzymes involved in ROS detoxification, for example, glutathione S-transferase [132]. The interaction of glutathione with ROS is accompanied by the oxidation of its sulfhydryl group and conversion to glutathione disulfide. Glutathione reductase, which reduces oxidized glutathione to its sulfhydryl form, is present in dry seeds and is rapidly activated upon hydration [52,130]. The ratio of the reduced and oxidized forms of glutathione is also considered as a marker of seed viability [15,65,132,133]. In addition, tocochromanols can have significant effect on the glutathione content [132,134].

Reactive nitrogen species (RNS) in particular nitric oxide (NO) are the biological messengers, which orchestrate a plethora of plant functions, mainly through post-translational modifications (PTMs) such as S-nitrosylation or tyrosine nitration. Hundreds of proteins have been identified as potential targets of NO-related PTMs [135]. This illustrates the importance of NO as a key signaling molecule tremendously impacting on plant growth, development and senescence [119,136,137]. Indeed, the NO-signaling pathway is involved in regulation of seed germination, growth of pollen tube, root organogenesis, flowering and fruit ripening [138–140]. Similarly to ROS, RNS play an important role in mediating and regulation of plant response to abiotic stress and in plant interactions with ecosystem partners [140,141]. As was shown in the last decade, the exogenous NO can interrupt seed dormancy and promote seed germination [142–144]. These effects clearly indicate that NO is

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involved in modulation of ABA-related signaling pathways, affecting seed dormancy and germination [145,146].

7. Repair of nucleic acids and proteins in germinating seeds

Under the optimal environmental conditions (i.e. humidity, temperature and lighting specific for each species) metabolic processes in seeds are re-activated and their germination is initiated. Successful germination of mature seeds essentially depends on the repair of damage, inevitably accumulated in DNA, RNA, and proteins during the sequential periods of dehydration and rehydration [147]. This repair is triggered by tissue re-hydration, when all required enzymatic systems are reactivated and continuous energy supply is secured by enhancement of aerobic respiration in mitochondria [148,149]. The expression of the genes encoding enzymes involved in the repair of nucleic acids and proteins is strongly up-regulated at the late stages of seed maturation. This ensures fast translation of required enzymes during the imbibition stage [8,30,148].

The repair of damaged DNA in seeds relies on the universal mechanisms, typical for all eukaryotic cells: repair of double-strand breaks by homologous recombination and non-homologous end-joining, nucleotide excision repair, base excision repair and correction of unpaired DNA bases [150]. Oxidative damage of seed DNA is mostly associated with either desaturation of deoxyribose or covalent base modifications [30]. As was shown for Arabidopsis, hydroxylation of guanine yields a potentially mutagenic base 7,8-dihydro-8-oxoguanine (8-oxoG) as the major oxidative modification of seed DNA [151]. This base can form complementary pairs not only with cytosine, but also with adenine, that might result in errors during replication [148,152]. The repair, i.e. removal of damaged bases (in particular, 8-oxoG) from the DNA double helix, typically relies on the base excision repair (BER) system [151]. First, the damaged base is recognized and removed by cleavage of the corresponding N-glycosidic bond by DNA glycosylase [152]. The resulted apurinic/apyrimidinic (AP) sites are the substrates of the 8-oxoguanine DNA glycosylase/lyase, which cleaves the sugar-phosphate backbone of DNA to form a single-strand break, before DNA polymerase beta (POLB) the chain repair [151]. Thus, BER includes the excision of the damaged DNA base, cleavage of the sugar-phosphate backbone at AP sites, clean-up of the resulting DNA ends, gap-filling through DNA synthesis, and DNA ligation [152].

The other types of DNA damage, like double-strand breaks, also require specific repair machinery [148]. These base damages can be repaired by DNA glycosylases - 8-oxoguanine DNA glycosylase/lyase (OGG) and formamidopyrimidine-DNA glycosylase (FPG) [151,153]. After the subsequent cleavage of the sugar-phosphate backbone, the breaks can be restored by DNA ligases [64,148]. The critical role in the repair of dehydration-related DNA damage plays poly (ADP-ribose) polymerase (PARP), which is catalyzing poly-ADP-ribosylation – reversible covalent modification of proteins by a homopolymer chain consisting of ADP-ribose residues [154]. The catalytic activity of PARPs is stimulated by DNA strand breaks. PARP is also involved in transcriptional regulation, formation of mitotic spindle during cell division, intracellular trafficking etc. In plants, activation of PARP superfamily members is the marker of stress responses [154–157]. This enzyme is featured with two catalytic functions: NAD^+-hydrolase and ADP-transferase. The hydrolysis of NAD^+ yields ADP-ribose residue, which is further involved in the synthesis of poly(ADP-ribose). Formation of this polymer is likely to be a signal of DNA damage, which is involved in the recruitment of repair proteins to the damage site [154]. It needs to be taken into account, that poly(ADP-ribose) is a potent glycation agent, which is able to form characteristic Amadori compounds in histones [158]. Taking into account the potential epigenetic role of this modification in humans [159], this aspect needs to be explored in plants, and specifically in seeds, as well.

The protection of mRNAs, which are synthesized in seeds at the late maturation stages and are translated when germination is initiated, plays a critical role in the success of germination [160]. Thereby, their length, secondary structure and specific motifs required for translation, need to be preserved [64]. In mature dry Arabidopsis seeds, about 10,000 individual mRNAs were annotated, whereas in rice this number reached 17,000. These RNA pools were mainly represented by the transcripts of the genes, responsible for primary metabolism and protein synthesis [64,161–163]. Early

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translation of mRNAs, stored during maturation, gives access to a rapid re-onset of their metabolic activity during germination [163]. Among all proteins, stored in seeds during the desiccation period, those involved in translation can be expected to be more amenable to non-enzymatic modification during the period of dehydration, sub-sequent rehydration and germination [162]. In agreement with this, the proteins involved in translation were reported as particular targets of both protection and repair[162,164].

The loss of germination efficiency during natural or artificial ageing is accompanied by decreased contents and compromised integrity of mRNAs, which are highly amenable to oxidative damage by ROS [165]. The alterations in structures of mRNAs might result in translation blocks, whereas the loss of translational activity correlates with decreased seed germination rates [166]. The RNA repair system includes ATP-dependent RNA ligase, nucleotidyl transferases, and enzymes modifying the RNA ends for ligation (phosphatase and kinase) or protection (methylase) [167].

The protection of seed proteins from dehydration-related damage mostly relies on redox mechanisms. In this context, reversible formation of disulfide bonds in proteins plays a key role in protein protection during seed dehydration: disulfide bonding makes them more compact, less inactive, and protects against proteases [168]. Proteins are usually synthesized in a reduced sylfhydryl (SH) form and oxidized to yield more stable disulfide (S-S) form during seed maturation and dehydration [169]. After the completion of germination, the sulfhydryl form of seed proteins is restored by thioredoxins [170]. During seed formation and maturation, these proteins suppress the activities of the enzymes, catalyzing degradation of storage proteins and carbohydrates, and promote their re-activation afterwards - at the stage of germination [170].

Importantly, during storage, seed proteins accumulate spontaneous oxidative, glyco- and lipoxidative non-enzymatic covalent post-translational modifications [36,79,103,104,166,171,172]. Although these modifications are mostly irreversible, oxidation at some amino acid residues is often reversible [147,168]. This reversibility can be underlied with both enzymatic and enzyme-independent mechanisms[162]. For example, Châtelain *et al*. showed that methionine sulfoxide reductase (MSR) reduces methionine sulfoxide to methionine, contributing, thereby, to the maintenance of the dormant seed longevity in the model legume *Medicago truncatula* L. [168]. The loss of protein function can also result from the conversion of aspartate or asparagine residues into abnormal isoaspartic acid by spontaneous covalent modification. These abnormalities can be restored by *L*-isoaspartyl methyl transferase (PIMT) which repairs age-dependently damaged *L*-isoaspartyl and *D*-aspartyl residues in proteins via methylation by their side-chain carboxylic groups. *L*-succinimide, formed after a non-enzymatic rearrangement, can be hydrolyzed yielding *L*-amino acid residues. Over-expression of PIMT1 in Arabidopsis enhanced seed longevity, whereas reduced PIMT1 expression led to loss of seed vigor [67,147]. Long-term and inappropriate storage of seeds might result in enhancement of protein glycation, which is usually referred to as an array of non-enzymatic post-translational modifications formed by reducing sugars and carbonyl products of their degradation [36,79,127,173]. Conversion of carbonyl compounds in corresponding sugars and alcohols can be mediated by the activity of aldo-keto reductase family 1 (AKR1) enzymes [174]. Indeed, the over-expression of AKR1 was recently shown to improve seed longevity in tobacco and rice by detoxifying reactive carbonyl compounds generated during the storage-related aging. Small HSPs and LEA proteins, which ensure correct folding of synthesized proteins, protection from oxidative damage, and restoration of the original structure, can be also involved in repair of protein damages [19,31,162].

8. Loss of desiccation tolerance during seed germination

Based on the dynamics of water uptake and metabolic re-activation, seed germination can be divided into three phases [13,96,175]. Phase I (imbibition) is accompanied by rapid water uptake, hydration of macromolecules and repair of membranes, proteins and DNA damage accumulated during the period of seed storage. Activation of aerobic respiration in mitochondria also starts in this period. The phase II is characterized with a decrease of water uptake and enhanced mobilization of storage compounds and reactivation of metabolic processes, which, in turn, result in activation of

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protein synthesis. The end of the phase II is usually defined as the moment of radicle protrusion, which is characterized by elongation and division of root cells. At this moment, the germination *sensu stricto* ends and the post-germination phase III begins [13]. Interestingly, the orthodox seeds are able to maintain their desiccation tolerance throughout the phases I and II, i.e. germination can be stopped during this period and imbibed seeds can be dried up to the initial dehydration level with a possibility of metabolism reactivation upon following rehydration [4,8,9,176]. This stage of germination, when reversible dehydration/rehydration cycles are still possible without loss of germination efficiency, is often referred to as the "window of desiccation tolerance"[176].

In this context, the transition from the phase II to the phase III (transition from seed to seedling) is the critical moment of the plant ontogenesis, when seeds irreversibly lose their desiccation tolerance [9,149] (Fig. 1C). Apparently, at this moment, the program controlled by the LAFL network is getting blocked [61,176]. The suppression of LAFL expression during seed germination relies on the homeodomain-containing proteins High-level expression of Sugar-Inducible gene 2 (HSI2) and HSI2-like1 (HSL1), which are also called VP1/ABI3-like1 (VAL1) and VP1/ABI3-like2 (VAL2) [61]. Modifications of chromatin, which involve the chromatin remodeling complexes – Polycomp Repressive Complexes 1 AND 2 (PRC1 and PRC2, respectively), as well as the PICKLE (PKL) and PICKLE-RELATED 2 (PKR2) proteins, represent the key mechanism behind the repression of the LAFL transcriptional network by the HSI2 and HSL1 proteins during seed germination. Recently, the chromatin remodeling factor PKL was also shown to block the expression of the *DOG1* gene directly[177].

9. Conclusion

The mature seed is a highly-tolerant organism, which is able to sustain extreme environmental conditions during prolonged storage. According to Chahtane *et al*, seeds transform plants into time and space travelers, which undoubtedly explains the success of angiosperms among terrestrial plants in colonizing numerous habitat [72]. Seed development can be divided in two main stages– embryo development and seed maturation. Early- and middle seed maturation stages are accompanied with accumulation of nutrients. At the late maturation stage, orthodox seeds develop desiccation tolerance, which allows maintaining seed viability after the loss of up to 95% of water and onset of dormancy. Desiccation tolerance allows long-term survival of dormant seeds under varying and often adverse environmental conditions. Thus, desiccation of seeds during maturation is a necessary event in the life cycle of the most of terrestrial plant species. Indeed, it underlies seed longevity, i.e. the ability of seeds to preserve their germination potential during prolonged times without detectable damage. The mechanisms behind the desiccation tolerance mostly rely on LEA proteins, small heat shock proteins, non-reducing oligosaccharides and antioxidants. Interestingly, orthodox seeds are tolerant to desiccation not only during the period of dormancy, but also in the germination phase, up to the moment of embryonic root initiation. Up to this time point, the seeds can be dried without loss of viability and the metabolic processes can be resumed upon sub-sequent re-hydration. The mechanisms behind this fascinating feature of germinating seeds are still not well understood. Therefore, it is necessary to identify genes, underlying the control of seed desiccation tolerance, and to understand the mechanisms behind their blockage during seed to seedling transition. It might provide a new insight in the problem of increasing drought tolerance in the plants developed from orthodox seeds.

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Abbreviations

ABA	Abscisic acid
ABI	ABA-INSENSITIVE
AGEs	Advanced glycoxidation end products
AGL	AGAMOUS-like
AHG	ABA Hypersensitive Germination
AKR	Aldo-ketoreductase
BER	base excision repair
CMT	Chromomethylase
DME	Demeter
DOG	DELAY OF GERMINATION
DOGL	DELAY OF GERMINATION-like
DREB2D	Dehydration-responsive element-binding protein 2D
DT	desiccation tolerance
DRM	Domains rearranged methyltransferase
FPG	Formamidopyrimidine-DNA glycosylase
FUS	FUSCA
GA	Gibberellic acid
GA3ox	Gibberellin 3-oxidase
GAAS	Germination Ability After STORAGE
HsfA	Heat shock transcription factor
HSI2	High-level expression of Sugar-Inducible gene
HSL1	HSI2-like1
HSP	heat shock proteins
LAFL	Network of transcription factors (LEC1, ABI3, FUS3 and LEC2)
LEA	late embryogenesis abundant
LEC	LEAFY COTYLEDONS
LPP	Lipid phosphate phosphatase
MET	Methyltransferase
MSR	Methionine sulfoxide reductase
NCED	9-cis-epoxycarotenoid dioxygenase
OGG 1	8-oxoguanine DNA glycosylase/lyase
PARP	Poly (ADP-ribose) polymerase
PIMT	*L*-isoaspartylmethyl transferase
PKL	PICKLE
PKR	PICKLE-RELATED
PLATZ	Plant AT-rich sequence- and Zinc-binding protein
PRC	Polycomb Repressive Complex
PYR/PYL/RCAR	Pyrabactin resistance/PYR-like/Regulatory component of abscisic acid receptor
RdDM	RNA directed DNA Methylation
RFO	Raffinose family oligosaccharides
ROS	Reactive oxygen species
ROS1	repressor of silencing1
DML1	demeter-like1
SMP	seed maturation protein
SnRK	sucrose-nonfermenting related kinase
TXN	Thioredoxin
VAL	VP1/ABI3-like

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