Chapter 3

# Signaling Patterns of Reactive Oxygen Species and Phytohormones During Transition Period of Quiescent Seeds into Metabolically Active Organisms

Prabhakaran Soundararajan,
Abinaya Manivannan and Byoung Ryong Jeong

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64789

**Abstract**

Dormancy and germination of seeds are determined by various factors such as vitality, genotype, hardness, and other environmental cues, such as moisture, air, temperature, and light. Metabolic activity of seeds varies between the quiescent and imbibition state. In the dry state, longevity of a seed is determined by the reactive oxygen species (ROS) such as lipid peroxyl radical (LOO•) and lipid hydroperoxide (LOOH) that are generated nonenzymatically due to lipid peroxidation (LPO). During rehydration phase, enormous amount of ROS, such as superoxide ($O_2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl (•OH) radicals, are generated from the metabolically active compartments such as mitochondria, chloroplasts, and peroxisomes. The progressive conditional, temporal, and spatial distribution of ROS is tightly controlled by the effective antioxidant system that leads to the successful germination of seeds and this phenomenon is defined as 'oxidation window.' Gibberellins (GAs) and abscisic acid (ABA) are the key phytohormones involved in the germination/dormancy. Former promotes germination, whereas the latter induces dormancy. Genes involved in the synthesis and signaling of GA, such as *gibberellin 3-β-dioxygenase* (*GA3ox*), *GA20ox*, and *GA-insensitive dwarf* (*GID*), are responsible for the conversion of GA from an inactive to a bioactive form. On the other hand, DELLA, an important protein family acting as the repressors for GA-regulating genes, is activated by ABA. Function of genes, such as *SLEEPY*, *PICKLE*, SPINDLY, *SECRET AGENT*, *AMYLASE*, *GAMYB*, and *LEAFY*, are interrelated with the GA/ABA metabolism. By inducing the ubiquitin-26S proteolysis pathway, GA overcomes the DELLA-mediated effects on germination. The E3 ubiquitin ligase $SCF^{SLY1}$ (skp1-cullin-F-box-$Rbx1^{SLY1}$) complex was reported to be involved in the degradation of DELLA proteins. Additionally, cell differentiation and elongation process sustained by the ROS were also linked with the ethylene, brassinosteroids, and auxins. Hence, this chapter provides the heuristic framework on the phenomenon of

systemic cross-talk between the ROS and phytohormones during the transition period of quiescent seeds into the metabolically active organisms.

**Keywords:** dormancy, germination, metabolism, oxygen radicals, signaling, mechanism

## 1. Introduction

After pollination (double fertilization), the typical diploid embryos are covered by the triploid endosperm and the diploid testa. The triploid endosperm consists of nutritive tissues and living cells, while the diploid testa includes seed coat, maternal tissue, and dead cells. Seeds are the vital component allowing embryo dispersion and its consequent development into mature plants [1]. The seeds of monocots and dicots differ in their structure and method of emergence [1, 2]. However, here we comprehensively focus on the signaling pattern of the reactive oxygen species (ROS) and its interaction during the germination and dormancy condition.

Although the dispersal of seeds is absolutely dependent on various cues such as vitality, genotype, hardness, moisture, air, temperature, light, and duration of seed storage, endosperm weakening is one of the key factors that determine the protrusion of radicle. The term "coat-associated dormancy" refers to the mechanical constraint that can impair germination, while "embryo dormancy" is characterized by the embryo failure to develop [1–3].

After imbibition, the weakening of endosperm is dependent on the gas exchange/respiration. Loosening of the endosperm was suggested to be influenced by the proper localization of ROS and its fine regulation by the antioxidant systems [4, 5]. The proper reduction/oxidation of the ROS (redox homeostasis) plays a key role in the transition from quiescence to active state [6]. Gibberellins (GAs) are involved in the promotion of the endosperm weakening and, on the other hand, abscisic acid (ABA) at least partly inhibits this process either directly or indirectly [2, 3, 7]. Induction/inhibition of the genes responsible for the endosperm weakening is controlled by the ROS-GA-ABA [7–11]. Substantially, cross-talk of other phytohormones, such as ethylene, brassinosteroids and auxins, with GA and ABA were reported to be inevitable for the seed development [12].

Although research in seed biology has reached significant advancements, apparent continuum still lies in the mechanisms underlying germination and dormancy, which needs to be disclosed. In this chapter, the heuristic network on cross-talk between the ROS and phytohormones involved in the release and/or induction of dormancy has been discussed.

## 2. Seed Respiration

To fulfill the higher energy requirement during the transition period (from quiescent to active state) of the seed, cellular respiration is rapid, high, and synchronized with the mitochondrial

http://dx.doi.org/10.5772/64789

activity [13]. According to Law et al., proteins required for the biogenesis of mitochondria were already present in the dried seeds and this process is activated upon imbibition. Although the import of mitochondrial proteins is highly required for the biogenesis, amount of ATP consumption by mitochondria is limited as compared with other processes. On the other hand, amount of ATP required by the mitochondria for the protein import is lesser than the energy required for protein synthesis [14]. In the transition period, oxygen ($O_2$) released by the respiration governs the internal communications between the cell organelles and the rapid cell division and expansion [15]. The excessive generation of ROS is extremely harmful to the cells. Although the $O_2^-$ has very limited half-life (2 μs), the reduction in $O_2^-$ (superoxide dismutation) results in the production of hydrogen peroxide ($H_2O_2$). Hydrogen peroxide can travel long distance and reaches the target as its half-life was determined to be about 1 ms. Other free radicals formed during the enzymatic reduction of $O_2^-$ and $H_2O_2$ is •OH [16]. The formation of •OH radical is mediated by iron in the Haber-Weiss and Fenton reactions. The uncoupled electrons present in the ROS cross-react with other essential metabolites or cellular components, affecting the normal cell physiology. However, a proper antioxidant system detoxifies the excessively generated free radicals and leads to the nondormant phenotype [16]. Detailed mechanisms on the involvement of ROS in seed germination are discussed below.

## 3. Oxidation Window

During the embryogenesis and the seed-filling process, seeds possessed maximum water content [17]. Subsequently, dramatic water loss takes place in the postmaturation stage [2]. According to the recent reports, ROS do not play a detrimental role during development under controlled conditions [18]. The ROS-mediated signaling is majorly involved in the endosperm weakening, mobilization of seed reserves, programmed cell death (PCD), and also protection against the pathogens [4]. Hence, it can be ascertained that the ROS cannot be simply considered as a hazardous material. Controlled production of ROS and ROS-related molecular interactions represent key factors in various central components of plant biology [16]. In the nondormant seeds, $O_2^-$ and $H_2O_2$ radicals were uniformly distributed within the radicle, while in dormant seeds irregular patterns were observed. Only seeds with a proper redox homeostasis display nondormant phenotype [7, 11]. Success of the seed germination is apparently associated with the equilibrium between the ROS and its scavenging antioxidant system [1, 4–6]. Uncontrolled generation of ROS is extremely harmful and can lead to several lethal effects. Meanwhile, the tight control over the ROS helps in various developmental processes including germination. This process is generally termed as 'oxidation window' [19].

### 3.1. Quiescent seed

Quiescent seeds, characterized by low moisture content (5–15%), do not possess active metabolism. During the late embryogenic state, seeds are actively involved in the storage of reserves, while enzymatic activities are gradually decreased. However, during the storage, lipid peroxidation (LPO) occurring on the polyunsaturated fatty acids (PUFA) in the cell

membrane constantly releases the ROS [2, 3]. Longevity of seeds depends on the free radicals generated by the LPO. In the dried condition, ROS are released from polyunsaturated fatty acids by LPO [4]. The free radicals focused on the H-atom in the methyl group of lipids. The single cleavage leads to the release of LOO•, and the double cleavage leads to the release of LOOH [16]. Depending on the aging extremity, ROS affects the viability of the seed. Most of the enzymatic activities are arrested in the dried state of the seed. Damages caused by LPO cannot be retained during the transition from a quiescent to an active state [19, 20]. From the epigenetic study of Nakabayashi et al., it can be suggested that more than 12,000 stored mRNA species or transcripts were detected in the desiccated seeds of *Arabidopsis*. This number is almost a half of the whole genes present in *Arabidopsis*. Moreover, promoters of the highly expressed genes overrepresented the abscisic acid-responsive elements (ABREs) containing motif ACGT that are sufficient for the ABA-induced transcription [4, 21]. During the increase in a desiccation rate, the accumulations of late embryogenesis abundant (LEA) proteins are also increased to enhance the tolerance against water loss. Among various LEA proteins identified, group-2 LEA-dehydrins are highly involved for the desiccation tolerance [22]. Jiang and Kermode reported that nondormant and dormant phenotypes of the seeds were defined by their desiccation tolerance level. Seed storage proteins play important roles during the dehydration processes. If the desiccation process is imposed prematurely or deterioration takes place and then synthesis of storage proteins will be terminated. Consequently, seeds become more sensitive to stress and lose their vigors [23]. The processes of maturation drying are associated with the ability of seed for germination.

### 3.2. Imbibed seed

In general, germination normally begins with the imbibition of seeds by 70–80% of water. El-Maarouf-Bouteau and Bailly reported that high levels of ROS are accumulated during the imbibition phase [5]. This might be due to the resumption of metabolically active sites such as mitochondria, chloroplasts, peroxisomes, glyoxysomes, and plasma membranes. The mitochondrial electron transfer chain (ETC) was considered as a primary source for the ROS ($O_2^{-}$). Foyer et al. reported that 2–3% of oxygen from the mitochondria was the source of $O_2^{-}$ and $H_2O_2$. In addition, chloroplast, a vital site for photosynthesis, and ETCs from the photosystems, such as PSI and PSII, produce $O_2^{-}$, $^1O_2$, and $H_2O_2$. Meanwhile, the mobilizations of the lipids stored in the embryo carried out by the glycolate oxidase are another source of $O_2^{-}$ and $H_2O_2$. Due to the catabolism of lipids and purines in the glyoxysomes and peroxisomes, the release of $O_2^{-}$, $H_2O_2$, and nitric oxide (NO) is inevitable [24]. The $H_2O_2$ is majorly released in the peroxisomes during the conversion of glyoxylate catalyzed by the glyoxylate oxidase. Subsequently, fatty acid β-oxidation by the flavin oxidase generated the •OH and NO. Meanwhile in the peroxisome, xanthine conversion to uric acid, catalyzed by the xanthine oxidase, releases enormous amount of $O_2^{-}$. Recent attention on the cell-wall-dependent peroxidases, oxalate oxidases and NADPH oxidases, and their involvement in the transfer of electrons indicate the plasma membrane to be another important site for the ROS synthesis [25]. The NADPH oxidase, amine oxidase, chytochrome p450, cell wall peroxidase, and germin-like oxalate oxidases disperse the $H_2O_2$ from cell to cell [4, 24].

http://dx.doi.org/10.5772/64789

The hydrated state of seeds allows the longer shelf life $H_2O_2$ to reach the targets distant from the production sites [16]. As mentioned earlier during the unfavorable condition, ROS lead to the breakdown of essential macromolecules such as lipids, nucleic acids, proteins, and other deleterious activities [19]. In the favorable condition, the ROS stimulates the mobilization of reserves and selectively interact with the targets by oxidation. This oxidation triggers a gene-specific signaling pathways and also activates the transcription factors (TFs) either directly or indirectly [15, 19, 20]. The cleavage of cell wall-polymers of endosperm can be correlated with the over-expression of cell wall-peroxidases [26]. During the putative shift from the desiccation to the germination state, exogenous application of optimal $H_2O_2$ increased the regulation of 113 genes and decreased the regulation of 62 genes in *Arabidopsis* [27]. Initial imbibition conditions determine the fate of the subsequent metabolic pathways that are required to complete seed germination.

### 3.3. Temporal and spatial regulation of ROS accumulation

The metabolically active sites are the source of ROS. As the range and action of ROS are limited by diffusion, ROS production source determines its molecular mobility and viscosity [28]. The rate of metabolic activity and the source of ROS production govern the process of seed development. Leymarie et al. reported that after imbibition, the ROS are first localized in the cytoplasm followed by the nucleus and lastly in the cell wall [29]. In the cytoplasm, ROS modulates the redox homeostasis which triggers the protein oxidation and mRNA synthesis is the first sign of seed germination process [30–32]. Antioxidant systems are concordantly involved in maintaining the ROS level. The fine tuning of the ROS is achieved by the direct or indirect interaction with the transcription factors of the genes responsible for the redox status. Finally, the NADPH oxidase located in the cell wall helps in cell-to-cell propagation [33]. In the dormant phenotype, ROS production is high and also scattered. In the dormant phenotype, the ROS is properly diffused from cytoplasm to nucleus and cell wall [19]. The role of ROS (either beneficial or deleterious) is dependent on its distribution. Therefore, the temporal and spatial accumulations of the ROS are inevitable for proper germination [15, 29].

### 3.4. Protein carbonylation

Seed vigor is mainly affected by the protein oxidation process such as carbonylation and decarbonylation. Protein carbonylation is the oxidation of proteins caused by the ROS, especially on the side chains of lysine, arginine, proline, and threonine [34]. Decrease in the carbonylation of proteins is known as decarbonylation [35]. Activation on the oxidation phase of pentose phosphate pathway (oxPPP) modulates the carbonylation of proteins. Modulation of redox potential in the glycolysis and oxPPP were observed during the release of dormancy [36]. The interaction or signaling of the ROS determines or fine tunes various translation and posttranslation processes during the seed development. Job et al. reported that in the dry seed, proteins, such as 12S-cruciferin subunits, aldose reductase and the LEA, undergo carbonylation. After imbibition, protein carbonylation specifically targets glycolytic enzymes, mitochondrial ATP synthase, chloroplastic ribulose carboxylase large chain, aldose reductase, methionine synthase, translation factors, and molecular chaperones [37]. The NADPH-oxidase

also known as respiration burst oxidase homolog (rboh) plays an important role in the transfer of electrons from cytosolic NADPH or NADH to apoplastic oxygen and posttranslational modifications of proteins. In Arabidopsis, *AtrbohD* mutant showed reduced superoxide production and protein carbonylation in dry seeds. However, after imbibition the protein, oxidation level of *AtrbohD* mutant was slightly higher than wild [38]. The posttranslational modifications, especially mRNA oxidations, are governed by the ABA [32].

### 3.5. Antioxidant enzymes

As mentioned earlier, improper desiccation as well as storage increases the LPO and affects seed vigor. Increased production of ROS from the metabolically active sites during the transition from a quiescent to imbibition state could possibly cause stress. The deleterious effects of the ROS can be overcome by the proper antioxidant system. Both enzymatic and nonenzymatic antioxidants play a vital role in the maintenance of level of the ROS. Rather than complete alleviation, proper activation of antioxidant enzymes directs the ROS to the signaling process [24–27]. Muller et al. reported that ROS are important components in the endosperm weakening [26]. Exogenous application of $H_2O_2$ or menadione (to generate superoxide) to 3-day old maize seedlings enhances tolerance against the chilling stress [39]. Meanwhile, Pulido et al. found that nuclear localization of peroxiredoxin and thioredoxin prevents nucleic acids from oxidative damage occurring during the maturation and germination in wheat seeds [40]. The detoxification of $H_2O_2$ in the seed filling is catalyzed by the isoforms of catalase (CAT). The isoform CAT3 is involved highly in the early postpollination, whereas CAT1 and CAT2 isoforms play a crucial role during the seed development [41, 42]. Recently, Leymarie et al. clearly demonstrated the necessity of the ROS and the antioxidant enzymes for successful germination using mutant seeds. In the *Arabidopsis cat2-1* mutant, intracellular $H_2O_2$ and redox perturbation were increased. In case of the *vte1-1* mutant lacking a gene that encodes the tocopherol cyclase, an increase in the redox active biosynthetic intermediate was observed [29]. Tocopherol is generally involved in the protection of lipids from oxidation. Tocopherols, also called vitamin E, functions as terminators of a PUFA recyclable chain reaction. The tocopheroxyl radical can be recycled back to the tocopherol by the reaction of ascorbate or other antioxidants [43]. The lack of the *vte1-1* function in the seeds releases lipid peroxy radicals. Although the *cat2-1* and *vte1-1* affect the seed germination to a certain level, plants lacking the *rbohD* gene can successfully complete germination (with time delay). The *rbohD* gene is involved in the conversion of the $O_2$ and NADPH to form superoxide and plays a vital role in the cell-to-cell propagation of the ROS and generation of the •OH. The •OH is essential for the cell wall loosening of endosperm [29].

### 3.6. Nonenzymatic antioxidants

Nonenzymatic antioxidants that actively participate in the ROS equilibrium are ascorbate, glutathione, and preoxiredoxins [44–47]. Low moisture content decreases the molecular mobility and the accessibility of substrates for the catalysis of antioxidant enzymes [44]. Ascorbate plays a major role in the progression of cell cycle, cell growth, hormonal signaling pathways, and embryogenesis. Ascorbate content of the seed decreased the $H_2O_2$ by increasing

http://dx.doi.org/10.5772/64789

the peroxiredoxins [45]. Nonenzymatic antioxidants also determine the protection of cells against the ROS, particularly at the desiccation stage. Tocopherol is involved in the prevention of membrane damages by the LPO during a prolonged seed storage [4]. Peroxiredoxins protect the nuclear integrity and prevent against the oxidative damages of DNA under high levels of •OH radicals [46]. Involvement of the ascorbate-glutathione cycle alone in the seeds could be another vast area, which needs to be discussed separately.

### 3.7. Interplay between ROS, GA, and ABA

It has been proven that an inhibitor of the ROS, sodium benzoate, decreases the germination rate of the seed [47]. Diphenylene iodonium (DPI), an inhibitor of NADPH oxidase, also affects the germination rate [29]. On the other hand, methylviologen, involved in the release of superoxide from the mitochondrial respiratory chain breaks the seed dormancy [10]. Capacity of seeds to germinate or remain dormant is determined by the two important phytohormones such as GA (dormancy release) and/or ABA (dormancy induction). Bailly et al. reported that GA and ABA are interlinked with the ROS and the scavenging capacity of antioxidant enzymes [19]. Generally, GA is mainly used for the dormancy release, while ABA induces the dormancy. The GA is involved in the stimulation of •OH production, especially in the radicle, and it also downregulates the enzymes involved in the ROS detoxification. Contrastingly, ABA inhibits the Fenton reaction, where the iron (II) is oxidized by the $H_2O_2$ to form the iron (III) and the release of •OH [38]. The processes of seed germination and dormancy are linked with ROS accumulation [48]. The productions of $H_2O_2$ in the sunflower are higher in the germinating seeds than the dormant seeds [5]. Similar results have been observed by comparing the dormant and nondormant seeds of many plants, such as *Arabidopsis* [29], *Triticum aestivum* [49] and *Pisum sativum* L. [50]. Moreover, the $H_2O_2$ stimulates the signaling cascade which induces the expression of specific genes [24]. In addition to $H_2O_2$, accumulation of other ROS species such as $O_2^-$ and •OH has also been observed in various plant species [50–53]. Bazin et al. mentioned that the germination of sunflower seeds was associated with the mRNA oxidation. The oxidation level of mRNA was higher in dormant seeds as compared with the nondormant seeds [32]. Genes such as *GA3ox1* and *GA3ox2* are involved in the synthesis of active GA [54]. Ishibashi et al. observed the induction of $H_2O_2$ in the aleurone layer by the GA in *Hordeum vulgare*, whereas ABA suppresses the production of $H_2O_2$. Furthermore, exogenous addition of $H_2O_2$ degrades Slender1 (SLN1), a well-known repressor of GA. Due to the induction of $\alpha$-amylase ($\alpha$-amy) and GAMyb, we can consider that $H_2O_2$ acts as an antagonist to ABA [55]. Cross-talk of the ROS with the phytohormones was reported to be mediated by the influx and efflux of $Ca^{2+}$ ions [56, 57]. Bethke and Jones stated that $H_2O_2$ was involved in the programmed cell death [58]. Contrastingly, ABA increased the tolerance against the PCD [59]. Recent report suggested that the NO is also involved as a signaling messenger during the seed germination and dormancy process [60].

### 3.8. Protection against pathogens

The release of the ROS in the seeds during the development period protects the seeds against pathogens. It also induces the systemic-acquired resistance (SAR) and PCD. Especially when

the ROS is mobile toward the seed coats, aleurone layers, and embryonic axis, the attack of microorganism is prevented by the induction of SAR and PCD [19, 61]. Briefly, the plasma membrane NADPH oxidase produces $O_2^-$, which is converted into $H_2O_2$ by SOD during the infection. Subsequently, $H_2O_2$ induces a hypersensitive reaction which leads to PCD of the infected cell. Eventually, $H_2O_2$ can also directly affect the pathogens [62, 63]. The main categories of genes involved in the $H_2O_2$ induction are related to defense, transcription, signaling (e.g., phosphatases, kinases), and importantly ROS synthesis and degradation. Perturbation of endosperm for the radicle emergence leads to the induction of defense-related genes. It helps to protect the newly germinating seeds from the pathogens [64]. During the seed germination process, lower concentrations of the ROS are involved in the cell signaling process, whereas higher concentrations trigger the PCD to facilitate the radical protrusion [65].

### 3.9. Endosperm weakening

Proteolytic cleavage of cell wall polymers is induced by the hydrolases such as mannose, glucanase, and cellulose. The scission of polysaccharides is a vital step to determine the radicle emergence. According to Muller et al., •OH accumulation is the main factor, which influences endosperm weakening by the breakdown of H-bonds in the cell wall-polysaccharide required for the radicle protrusion. Generally, •OH is extremely reactive and is considered as the most aggressive form of oxygenated derivatives [26]. Uncontrolled ROS accumulation affects the

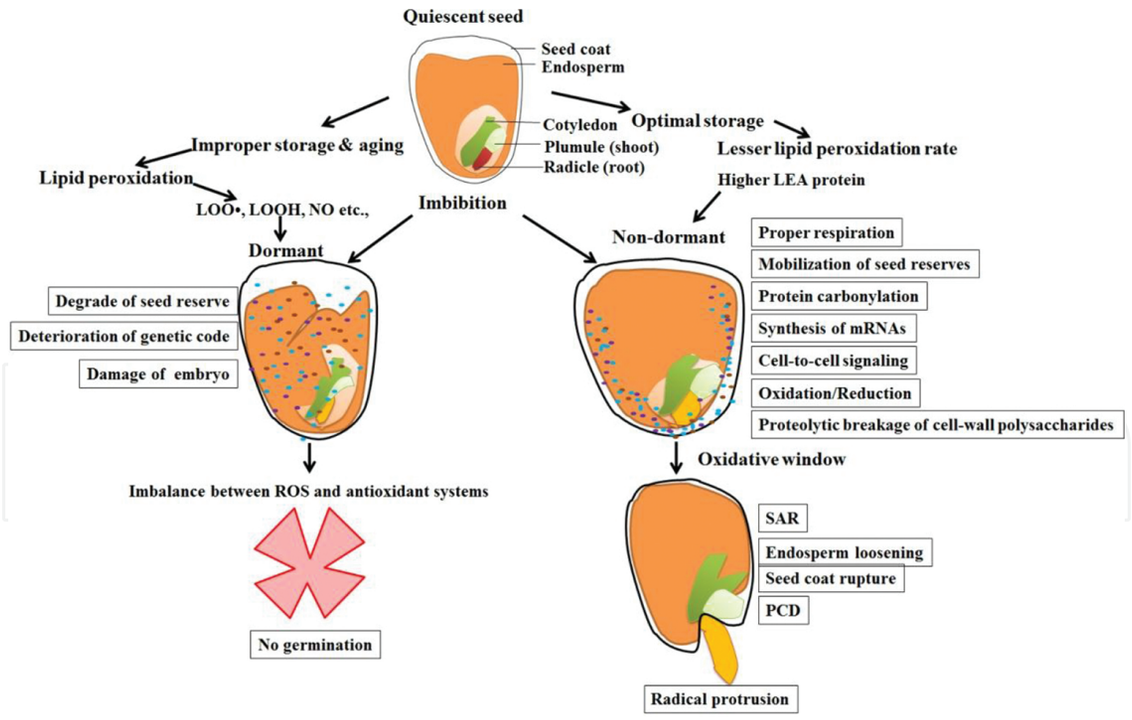


**Figure 1.** Schematic representation of the involvement of the reactive oxygen species (ROS) in the quiescent seed for the nondormancy and dormancy conditions. Dots represent the accumulation of ROS, blue for superoxide ($O_2^-$), brown for hydrogen peroxide ($H_2O_2$), and purple for hydroxyl radical (•OH). The LEA, late embryogenesis-abundant proteins; SAR, systemic acquired resistance; PCD, programmed cell death; LOO•, lipid peroxyl radical; LOOH, lipid hydroperoxide.

http://dx.doi.org/10.5772/64789

integrity of DNA, causing changes at the sequence level that impair proper seed germination and could be able to change the genetic code of the seeds. The $O_2^-$ and $H_2O_2$ seem to be less reactive toward nucleic acid as compared to •OH [38]. However, cellular dysfunctions caused by ROS accumulation can be prevented by the antioxidants [40].

Oxidative damages caused by excess ROS are irreversible. The progressive conditional, temporal, and spatial distribution of the ROS tightly controlled by the antioxidant system leading to seed germination are also defined as 'oxidation window' (**Figure 1**).

## 4. Molecular Network of Gibberellins and Abscisic Acid in Germination/ Dormancy

As mentioned earlier, germination and dormancy release of seeds are governed by the GAs and ABA. According to previous reports, although there are many factors involved in seed germination, GA and ABA biosyntheses have been considered as an important internal factor for the release as well as the induction of dormancy [9]. Metabolisms of ABA and GA is always interrelated [2]. Seo et al. reported that *Arabidopsis aba2-2* (ABA inducer) mutant showed a higher level of GA biosynthesis as compared to the wild type. Contrastingly in the *gai* (GA insensitive) mutant, i.e., GA repressor, synthesis of ABA was higher, and also the degradation of ABA was lesser as than the wild type [11]. Antagonistic effect of GA and ABA is also cross-linked with other phytohormones such as ethylene, brassinosteriods, and auxins [9].

### 4.1. Dormancy breakage

Gibberellins are majorly responsible for the breakdown of dormancy [54]. However, in the later phase of embryogenesis, i.e., during the maturation drying, GA production must be reduced and ABA synthesis is upregulated for the proper maturation and to preserve seed vigor. During the imbibition stage, the endogenous $GA_1$ is released from the viable nondormant embryo to its endosperm. It increases the activities of several enzymes, such as amylase, proteases, ribonucleases, and β-glucanase, which induce the hydrolytic cleavage in the aleurone layer [66]. Moreover, hydrolytic enzymes are also involved in the transcription, transportation of metabolites, and PCD. Along with the GA biosynthesis, genes encoding for various functions are either overlapped or attributed toward the germination, and this process is controlled by the GA [66, 67]. Moreover, genes encoding gibberellin 3-oxidase (*GA3ox*) (GA biosynthesis) and the soluble GA receptor (*GA-insensitive dwarf*, *GID*) are vital for the induction of seed germination. During the germination, the *GA3ox2* is involved in the fast phase of GA synthesis by catalyzing the conversion of GA from an inactive to a bioactive form. In the GA synthesis, genes, such as *gibberellin 3-β-dioxygenase 1* (*GA3ox1*) and *gibberellin 20-β-dioxygenase 1* (*GA20ox1*), play as the positive regulators and *GA 2-oxidase*, especially *GA2ox1*, is involved in the negative regulation [67]. The *GA2ox1* gene is engaged with the catabolism of GA. The *Leafy cotyledon 1 (LEC1)* encodes for the katanin p60 subunit, which promotes the cell elongation in the microfibrill. Kroj et al. reported that the *lec2* and *fusca3* (*fus3*) directly influence the expression of the *GA3ox2* by binding on its *2S3* promoter region, particularly on the RY *cis*-

element motif. The RY motif also regulates the expression of genes encoding seed storage proteins (*SSP*) such as 2S albumins and 12S globulins. Four genes, such as *abi3* (ABA insensitive), *lec1*, *lec2* and *fus3*, are responsible for the conditional dormancy of embryos and are regulated by the ABA for proper maturation [68].

Higher expressions of cell wall-remodeling enzymes (CWRE) is associated with the radicle protrusion. Endo-β-mannanase, β-1,3-glucanase, expansin, xyloglucan endo-transglycosylase, pectin methylesterase, polygalacturonase, and galactanase are the notable major enzymes involved in the cell-wall modification [12]. In *Arabidopsis*, the application of GA induced the expression of the *extension-like gene 1* (*epr1*). The *epr1* is involved in the strengthening of micropylar endosperm cell wall to elongate the radicle of seeds [69]. Cell-wall-associated gene expression under the imbibition is preferentially linked with ABA and GAs in the endosperm weakening. During the rehydration, increased oil bodies and protein storage vacuole packed in cells start to mobilize from the endosperm cells to radicle tip. Penfeild et al. reported that lipids and proteins produced in the endosperm following the imbibition are higher than those produced in the embryo [70]. Consequently, the proteolytic activities are also at higher rates. The GA suppresses the activation of DELLA proteins to enhance the process of seed germination [66, 67, 71]. The E3 ubiquitin ligase SCF (skp1-cullin-F-box-Rbx) complex was reported to be involved in the degradation of DELLA proteins via the ubiquitin-26S proteasome pathway [72]. Perception of GA and its signal transduction determines various other mechanisms in the plant along with the seed germination (**Figure 2**).

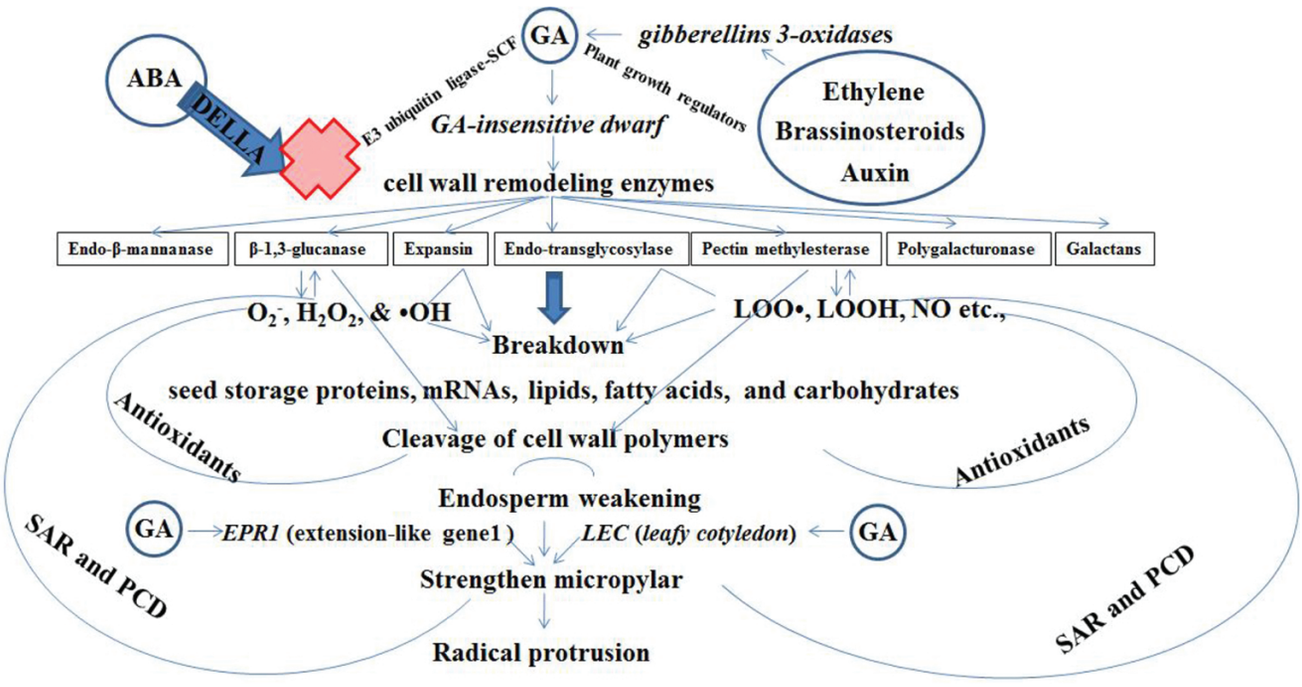


**Figure 2.** Simplified framework of the dormancy release mediated by GA in concordant with the ROS. Interactions between the genes are described in the text.

http://dx.doi.org/10.5772/64789

### 4.2. Dormancy induction

Enhanced dormancy occurred when the ABA content was increased in the *Arabidopsis* seeds due to the overexpression of ABA biosynthesis genes [73]. *Abscisic acid biosynthesis gene* (*aba2*), *9-cis-epoxycarotenoid dioxygenase* (*NCED*s), *Glc-insensitive1* (*GIN1*), and *short-chain dehydrogenase/reductase1* (*SDR1*) are the major genes regulating the synthesis of ABA [74]. Increase in the seed dormancy is also associated with the expression levels of the gene *delay of germination1 (DOG1*). Among the four regulatory genes, *abi3* and *fus3* are able to form the feedback loops. The *Lec1* and *lec2* mutants failed to express the *abi3* and *fus3*, suggesting the strong underlying molecular network between the regulators [75].

The ABA was involved in the vacuolation process to inhibit the endosperm loosening rather than the lipid breakdown [70]. Recently, it was found that the loss of function of the gene coding for E3 ligase ABI3-interacting protein 2 (AIP2) and ABI3-binding factor protein (AFP) leads to the ABA insensitivity and the nondormant phenotype even in the presence of ABA. Major receptors of the ABA are pyrabactin resistance1 (PYR1), PYR1-like protein (PYL), and regulatory components of ABA receptors (RCAR). Those loci encoding the main players in the ABA metabolism are also associated with the RNA translation and metabolism, protein-degradation pathways, and phosphatase components of the signaling pathways [69–75].

### 4.3. DELLA proteins

The DELLA proteins [GA insensitive (GAI), repressor of ga1-3 (RGA), and RGA-like proteins (RGL 1-3)] belong to the subfamily of plant-specific Glycyl-TRNA synthetase (GRAS) proteins. The name of GRAS proteins was derived from the initially identified members such as *G*AI, *RGA*, and SCARECROW (*S*CR) [76]. Potential of GA was repressed by the DELLA-domain containing members such as GAI, RGA, and RGL 1-3. Among them RGL2 has the major influence on seed dormancy. Most of DELLA proteins act as the repressors of the GA biosynthesis [55]. Seeds of DELLA mutants show the symptoms of irregular shape and proportion, especially in the protruded radicle [77].

Recent research found that the GA signal inactivated the functional domain of DELLA protein. The GA induced repression of the RGA through protein degradation rather than the blocking of translational process [71]. The deletion of the conserved motif VHYNP present in the DELLA region or the region between the VHYNP-DELLA, releases the dormancy by enhancing GA metabolism. Additionally, GA-dependent degradation of proteins is also associated with *SLENDER RICE 1* (*SLR1*), especially S/T/V, a regulatory region. However, in the wild type the function of the *SLR1* was not clearly distinct from the mutant. On the other hand, phosphorylation of SCF-E3 ligase and the ubiquitin-26S leads to the proteasome pathway of *SLEEPY1* (*SLY1*) and *GA-INSENSITIVE DWARF2* (*GID2*). Bioactive GA mainly focuses on the inactivation of RGA, GAI, and RGL 1-3 during the seed germination process [78, 79].

### 4.4. *DWARF*, *SLEEPY*, *PICKLE*, *SPY*, and *SECRET AGENT*

The presence of GA at higher levels makes the plant thin and watery. Contrastingly, lack of GA-biosynthetic genes or lesser amount of endogenous GA produces the thicker leaves with

dwarf shoots. It was previously reported that GA-unresponsive dwarf phenotypes were observed in mutants such as *drwaf1* (*d1*) and *gid2* in rice, and *sly1* in *Arabidopsis* [80, 81]. The *GID1* and *SLY1* encoding for the F-box proteins are the subunit of the SCF complex belonging to the E3 ligase [78]. Genes (*SLR*/*RGA*) involved in the repression of GA were controlled by *GID2* and *SLY1* [82].

The *PICKLE* (*PKL*), an important positive regulator involved in the control of radicle protrusion, encodes a CHD3 chromatin remodeling factor. The *PKL* was reported to be actively involved in the later stage of seed germination, particularly on the root differentiation. Additionally, expression of the *PKL* was reported to be higher under an abiotic stress condition [83]. The gene *SPINDLY* (*SPY*) encodes the *O*-linked-*N*-acetyl glucosamine transferees (OGT), which negatively regulates the GA signaling (*GA1-3*) pathway [84]. The *SPY* decreases the GA effect by the suppression of the *SLR1* [85]. It is worthy mentioning that *GA1-3*, a precursor, is involved in the first step of the GA synthesis. Another gene, *SECRET AGENT* (*SEC*) which also encodes the *OGT* gene, found in *Arabidopsis*, did not show obvious phenotype alteration in the *sec* mutant (single mutant), whereas the mutant with both *spy* and *sec* (double mutant) showed the lethal effect in the gamete and also deeply affected the seed development. This double mutant could result from the alteration of not only the GA, but also the cytokine pathway [86]. The increased expression of tetratrico peptide repeats (TPRs) in *Arabidopsis* and *Petunia*, resulted with the repression of the *SPY*. The TRPs could either block directly by forming the inactive heterodimers or indirectly via proteins interacting with the *SPY* [87].

### 4.5. *AMYLASE*, *GAMYB*, and *LEAFY*

The enzyme amylase plays an important role in the hydrolysis of endosperm starch into usable sugars. This provides the necessary energy for the emergence of radicle. Plants possess both alpha ($\alpha$)- and beta ($\beta$)-amylases. The expression of $\alpha$-amylase in the aleurone layer is induced by GA. Activation of the *α-amy1* gene is mediated by GA-responsive elements (GARE) along with the C/TCTTTT and TATCCAT [66, 78]. For the *α-amy2* gene, along with the factors required for *α-amy1*, the BOX1/O2S-like elements are required [88]. The KGM, a Ser-Thr kinase, could repress the *α-amy1* by blocking the expression of the *HyGAMYB* [89]. Translation and stability of the *GAMYB* plays a major role for GA signaling. Meanwhile, interaction of novel zinc finger protein HRT (***H*** *ordeum ordeum* **r**epressor of **t**ranscription) with the *GARE* is able to repress the *α-amy2* gene expression [90]. After the inhibition for 12 h with $GA_4$, the *Arabidopsis ga1-3* mutant showed that 138 genes were upregulated and 120 genes were downregulated. The 20% of the upregulated genes possessed the TAACAAA-like sequences, indicating the importance of GARE in the cleavage of endosperm [91]. The *LEAFY* genes in the shoot apex are linked with the *GAMYB*-like genes. The *GAMYB* gene is also present in the anthers and expressed on the epidermis, endothecium, middle layer, and tapetum in the initial stages of development [92]. The GA activates the $Ca^{2+}$ signaling for the synthesis of hydrolases. Decrease in the suppression of the *SLENDER1* (*SLN1*) increased the cytosolic $Ca^{2+}$ level. The ABA inhibits the hydrolase by blocking the *sln1*, which directly affects the $\alpha$-amy. By increasing the $Ca^{2+}$, GA activates the hydrolases via calmodulin signaling for successful emergence of the radicle [57–59, 82] (**Figure 3**).

http://dx.doi.org/10.5772/64789

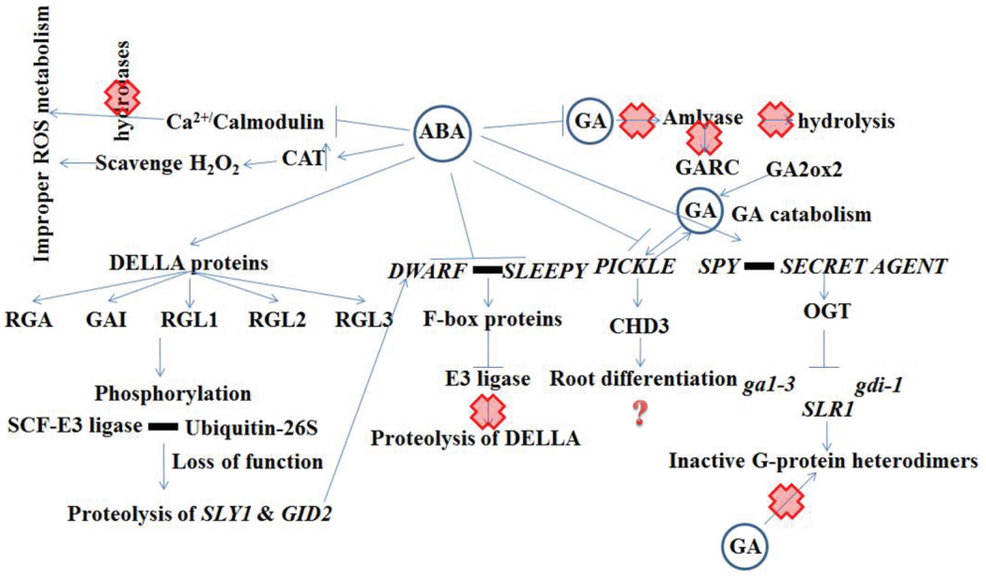


**Figure 3.** Heuristic networks on the dormancy induction by ABA. Interactions between the genes are described in the text. Arrows represent induction, barred lines represent inhibition, boxes represent sharing similar functions, question marks represent not clear, and crosses represent blocked or reduced synthesis. ABA, abscisic acid; $Ca^{2+}$, calcium; CAT, catalase; *GAI, GA insensitive*; *RGA, repressor of ga1-3*; *RGL, RGA-like protein*; *GID, gibberellin-insensitive dwarf*; *SPY, spindly*; *OGT, O-linked N-acetylglucosamine trasnferase* and CHD3 functions as chromatin remodeling factor.

## 5. Role of Other Phytohormones in Seed Germination/Dormancy

### 5.1. Ethylene

Ethylene was reported to be involved in various metabolisms such as dormancy breakage, root induction, defense against pathogens, and signaling [93]. Precisely, 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase), a precursor of ethylene synthesis, is required for higher synthesis of $H_2O_2$. Mutant lacking the GA synthesis gene (*gal-1*) possessed lower ethylene levels. Meanwhile, the exogenous supply of ethylene induced seed germination. The *ETR1* (ethylene receptor) was reported to be involved in the activation of serine/threonine kinase to overcome the dormancy in the *abi1-1* mutant [94]. In the straightway, mutants of *ein2* (*ethylene insensitive2*) and *etr1* (*ethylene receptor1*) showed higher degree of dormancy than the wild type. Especially, increase of dormancy ratio in the *ein2* mutant is directly proportional to the increased ABA content in the seeds. Meanwhile, decrease in the root ethylene content also decreased the ABA level [95].

### 5.2. Brassinosteroids

Brassinosteroids are well known for their functions in the cell elongation, cell cycle, and various other metabolisms. They are involved in the enhanced expression of *GA5*, a GA biosynthesis gene [96]. In the mutant of GA biosynthetic gene, *ga1*, and GA-insensitive mutant, *sly1*, application of brassinosteroids partially improved the seed germination under a light-

deprived condition [97]. Meanwhile, brassinosteroid receptor mutants such as *det2* (*deettiolated 2*) and *bri1* (*brassinosteroid insensitive*) were more sensitive to the ABA. Consequently, synthesis of GAs was also deeply affected [98]. This result signifies the importance of brassinosteriods in the GA synthesis for successful seed germination.

### 5.3. Auxin

Auxins are generally known for their roles in the root induction. Ogawa et al. reported upregulation of a number of auxin biosynthetic genes and genes encoding for auxin-carrying proteins in response to exogenous $GA_4$ application [91]. The GA was well known to promote the auxin synthesis and the transportation of ethylene. Chiwocha et al. (2005) evidenced that the interaction of ethylene biosynthetic genes with the auxin signaling genes such as *axr1* and *axr2* was mediated by GA [99]. The BIG-gene, named due to its large size, encodes the calossin/pushover protein involved in the efflux transportation of auxin [100]. Repressor of RGA proteins by the GA can be delayed by the attenuating auxin transportation or signaling [101]. Contrastingly, recent study in *Arabidopsis* by Lui et al. observed that the mutants of auxin receptors or biosynthesis genes showed the dramatic release of seed dormancy. This auxin-mediated seed dormancy was coordinated with ABA signaling [102]. Both GA and ABA have strong influence on auxins during germination and dormancy, respectively. This kind of cross-talk between the hormones helps in the flexibility of the embryo/seeds in response to the environmental stimuli.

## 6. Conclusions

During the developmental stage of embryos into the vigorous photoautrotropic organisms, numerous metabolic processes are activated and they include oxidation of proteins, cellular structural changes, and synthesis of macromolecules. The cascade of metabolic process ceases with the development of the radicle governed by the well-directed ROS accumulation. Interlinked relation between the GA and ABA aids in the proper development of the embryo, seed filling, desiccation tolerance, imbibition, hydrolysis, temporal and spatial distribution of ROS, proteolysis, and radicle protrusion. The recent evidences suggest that ABA-GA cross-talk with other phytohormones, such as ethylene, brassinosteroids and auxin, could play a vital role in the development of the seed. The important components other than the free radicals such as $O_2^-$, $H_2O_2$, and •OH pertaining to the seed potential is the NO. Tapping of the NO linked with the GA-ABA and their responses to the light and temperature could be one of the interesting areas getting more attention on the seed research.

## Acknowledgements

Prabhakaran Soundararajan and Abinaya Manivannan were supported by a scholarship from the BK21 Plus Program, the Ministry of Education, Republic of Korea.

http://dx.doi.org/10.5772/64789

## Author details

Prabhakaran Soundararajan[1], Abinaya Manivannan[1] and Byoung Ryong Jeong[1,2,3*]

*Address all correspondence to: brjeong@gmail.com

1 Division of Applied Life Science (BK21 Plus), Graduate School, Gyeongsang National University, Jinju, Republic of Korea

2 Institute of Agriculture and Life Science, Gyeongsang National University, Jinju, Republic of Korea

3 Research Institute of Life Science, Gyeongsang National University, Jinju, Republic of Korea

## References

[1] Bewley JD, Black M. Seeds: Physiology of Development and Germination. 3rd ed. New York: Plenum Press; 1994.

[2] Bewley JD. Seed germination and dormancy. Plant Cell. 1997; 9:1055–1066.

[3] Lang GA. Plant Dormancy. Oxford, UK: CAB International; 1996.

[4] Bailly C. Active oxygen species and antioxidants in seed biology. Seed Science Research. 2004; 14:93–107.

[5] El-Maarouf-Bouteau H, Bailly C. Oxidative signaling seed germination and dormancy. Plant Signaling and Behavior. 2008; 3:175–182.

[6] Considine MJ, Foyer CH. Redox regulation of plant development. Antioxidants and Redox Signaling. 2014; 21:1305–1326.

[7] Koornneef M, Karssen CM. Seed dormancy and germination. In: Meyerowitz EM, Somerville CR, editors. Arabidopsis. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1994. pp. 313–334.

[8] Karssen CM. Hormonal regulation of seed development, dormancy, and germination studied by genetic control. In: Kigel J, Galili J, editors. Seed Development and Germination. New York: Marcel Dekker; 1995. pp. 333–350.

[9] Brady SM, McCourt P. Hormone cross-talk in seed dormancy. Journal of Plant Growth Regulation. 2003; 22:25–31.

[10] Bahin E, Bailly C, Sotta B, Kranner I, Corbineau F, Leymarie J. Crosstalk between reactive oxygen species and hormonal signalling pathways regulates grain dormancy in barley. Plant Cell Environment. 2011; 34:980–993.

[11] Seo M, Hanada A, Kuwahara A, Endo A, Okamoto M, Yamauchi Y, North H, Marion-Poll A, Sun TP, Koshiba T, et al. Regulation of hormone metabolism in Arabidopsis seeds: phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellins metabolism. Plant Journal. 2006; 48:354–366.

[12] Kucera B, Cohn MA, Leubner-Metzger G. Plant hormone interactions during seed dormancy release and germination. Seed Science Research. 2005; 15:281–307.

[13] Law SR, Narsai R, Taylor NL, Delannoy E, Carrie C, Giraud E, Millar AH, Small I, Whelan J. Nucleotide and RNA metabolism prime translational initiation in the earliest events of mitochondrial biogenesis during *Arabidopsis* germination. Plant Physiology. 2012; 158:1610–1627.

[14] Law SR, Narsai R, Whelan J. Mitochondrial biogenesis in plants during seed germination. Mitochondrion. 2014; 19:214–221.

[15] Meitha K, Konnerup D, Colmer TD, Considine JA, Foyer CH, Considine MJ. Spatio-temporal relief from hypoxia and production of reactive oxygen species during bud burst in grapevine (*Vitis vinifera*). Annals of Botany. 2015.

[16] Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. 3rd ed. New York: Oxford University Press; 1999.

[17] Cui K, Xing G, Liu X, Xing G, Wang Y. Effect of hydrogen peroxide on somatic embryogenesis of *Lycium barbarum* L. Plant Science. 1999; 146:9–16.

[18] Kärkönen A, Kuchitsu K. Reactive oxygen species in cell wall metabolism and development in plants. Phytochemistry. 2015; 112:22–32.

[19] Bailly C, El-Maarouf-Bouteau H, Corbineau F. From intracellular signaling networks to cell death: the dual role of reactive oxygen species in seed physiology. Comptes Rendus Biologies. 2008; 331:806–814.

[20] Beckman KB, Ames BN. Oxidants, antioxidants, and aging. In: Scandalios JG, editor. Oxidative Stress and the Molecular Biology of Antioxidant Defenses. New York: Cold Spring Harbor Laboratory Press; 1997. pp. 201–246.

[21] Nakabayashi K, Okamoto M, Koshiba T, Kamiya Y, Nambara E. Genome-wide profiling of stored mRNA in Arabidopsis thaliana seed germination: epigenetic and genetic regulation of transcription in seed. The Plant Journal. 2005; 41:697–709.

[22] Han B, Hughes DW, Galau GA, Bewley JD, Kermode AR. Changes in late embryogenesis abundant (LEA) messenger RNAs and dehydrins during maturation and premature drying of *Ricinus communis* L. seeds. Planta. 1996; 201:27–35.

[23] Jiang L, Kermode AR. Role of desiccation in the termination of expression of genes for storage proteins. Seed Science Research. 1994; 4:149–173.

http://dx.doi.org/10.5772/64789

[24] Foyer CH, Noctor G. Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiologia Plantarum. 2003; 119:355–364.

[25] Corpas FJ, Barroso JB, del Rio LA. Peroxisomes as a source of reactive oxygen species and nitric oxide signal molecules in plant cells. Trends in Plant Science. 2001; 6:145–150.

[26] Müller K, Heß B, Leubner-Metzger G. A role for reactive oxygen species in endosperm weakening. Seeds: Biology, Development and Ecology. 2007; 2:87–95.

[27] Desikan R, Mackerness SAH, Hancock JT, Neill SJ. Regulation of the *Arabidopsis* transcriptome by oxidative stress. Plant Physiology. 2001; 127:159–172.

[28] Walters C. Understanding the mechanisms and kinetics of seed aging. Seed Science Research. 1998; 8:223–244.

[29] Leymarie J, Vitkauskaité G, Hoang HH, Gendreau E, Chazoule V, Meimoun P, Corbineau F, El-Maarouf-Bouteau H, Bailly C. Role of reactive oxygen species in the regulation of Arabidopsis seed dormancy. Plant and Cell Physiology. 2012; 1:96–106.

[30] Dietz K, Jacquot J, Harris G. Hubs and bottlenecks in plant molecular signalling networks. New Phytology. 2010; 188:919–938.

[31] Oracz K, El-Maarouf-Bouteau H, Kranner I, Bogatek R, Corbineau F, Bailly C. The mechanisms involved in seed dormancy alleviation by hydrogen cyanide unravel the role of reactive oxygen species as key factors of cellular signaling during germination. Plant Physiology. 2009; 150:494–505.

[32] Bazin J, Langlade N, Vincourt P, Arribat S, Balzergue S, El-Maarouf-Bouteau H, et al. Targeted mRNA oxidation regulates sunflower seed dormancy alleviation during dry after-ripening. The Plant Cell. 2011; 23:2196–2208.

[33] Chandrakuntal K, Shah AK, Thomas NM, Karthika V, Laloraya M, Kumar PG, et al. Blue light exposure targets NADPH oxidase to plasma membrane and nucleus in wheat coleoptiles. Journal of Plant Physiology. 2010; 29:232–241.

[34] Nyström, T. Role of oxidative carbonylation in protein quality control and senescence. The EMBO Journal. 2005; 24:1311–1317.

[35] Wong CM, Marcocci L, Liu LL, Suzuk YJ. Cell signaling by protein carbonylation and decarbonylation. Antioxidants and Redox Signaling. 2010; 12:393–404.

[36] Barba-Espín G, Diaz-Vivancos P, Job D, Belghazi M, Job C, Hernandes JA. Understanding the role of $H_2O_2$ during pea seed germination: a combined proteomic and hormone profiling approach. Plant Cell & Environment. 2011; 34:1907–1919.

[37] Job C, Rajjou L, Lovigny Y, Belghazi M, Job D. Patterns of protein oxidation in Arabidopsis seeds and during germination. 2005; 138:790–802.

[38] Muller K, Carstens AC, Linkies A, Torres MA, Leubner-Metzger G. The NADPH-oxidase *AtrbohB* plays a role in Arabidopsis seed after-ripening. New Phytology. 2009; 184:885–897.

[39] Prasad TK, Anderson, MD, Martin, BA, Stewart, CR. Evidence for chilling-induced oxidative stress in maize seedlings and a regulatory role for hydrogen peroxide. The Plant Cell. 1994; 6:65–74.

[40] Pulido P, Cazalis R, Cejudo FJ. An antioxidant redox system in the nucleus of wheat seed cells suffering oxidative stress. The Plant Journal. 2009; 57:132–145.

[41] Queval G, Issakidis-Bourguet E, Hoeberichts FA, Vandorpe M, Gakie`re B, Vanacker H, et al. Conditional oxidative stress responses in the Arabidopsis photorespiratory mutant cat2 demonstrate that redox state is a key modulator of daylengthdependent gene expression, and define photoperiod as a crucial factor in the regulation of $H_2O_2$-induced cell death. The Plant Journal. 2007; 52:640–657.

[42] Guan LQM, Scandalios JG. Catalase gene expression in response to auxin-mediated developmental signals. Physiologia Plantarum. 2002; 114:288–295.

[43] Mene-Saffrane L, Jones AD, DellaPenna D. Plastochromanol-8 and tocopherols are essential lipid-soluble antioxidants during seed desiccation and quiescence in *Arabidopsis*. Proceedings of the National Academy of Sciences USA. 2010; 107:17815–17820.

[44] Bailly C, Benamar A, Corbineau F, Côme D. Changes in malondialdehyde content and in superoxide dismutase, catalase and glutathione reductase activities in sunflower seeds as related to deterioration during accelerated aging. Physiologia Plantarum. 1996; 97:104–110.

[45] De Tullio MC, Arrigoni O. The ascorbic acid system in seeds: to protect and to serve. Seed Science Research. 2003; 13:249–260.

[46] Aalen RB. Peroxiredoxin antioxidants in seed physiology. Seed Science Research. 1999; 9:285–295.

[47] Hung KT, Kao CH. Nitric oxide acts as an antioxidant and delays methyl jasmonate-induced senescence of rice leaves. Journal of Plant Physiology. 2004; 161:43–52.

[48] Gomes MP, Garcia QS. Reactive oxygen species and seed germination. Biologia. 2013; 68:351–357.

[49] Caliskan M, Cuming AC. Spatial specificity of $H_2O_2$-generating oxalate oxidase gene expression during wheat embryo germination. The Plant Journal. 1998; 15:165–171.

[50] Wojtyla Ł, Garnczarska M, Zalewski T, Bednarski W, Ratajczak L, Jurga S. A comparative study of water distribution, free radical production and activation of antioxidative metabolism in germinating pea seeds. Journal of Plant Physiology. 2006; 163:1207–1220.

[51] Gidrol X, Lin WS, Degousee N, Yip SF, Kush A. Accumulation of reactive oxygen species and oxidation of cytokinin in germinating soybean seeds. European Journal of Biochemistry. 1994; 224:21–28.

[52] Liu X, Xing D, Li L, Zhang L. Rapid determination of seed vigor based on the level of superoxide generation during early imbibition. Photochemical Photobiological Science. 2007; 6:767–774.

[53] Schopfer P, Plachy C, Frahry G. Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid. Plant Physiology. 2001; 125:1591–1602.53.

[54] Yamaguchi S. Gibberellin metabolism and its regulation. Annual Review of Plant Biology. 2008; 59:225–251.

[55] Ishibashi Y, Tawaratsumida T, Kondo K, et al. Reactive oxygen species are involved in gibberellin/abscisic acid signaling in barley aleurone cells. Plant Physiology. 2012; 158:1705–1714.

[56] Bush DS, Biswas AK, Jones RL. Hormonal regulation of $Ca^{2+}$ transport in the endomembrane system of the barley aleurone. Planta. 1993; 189:507–515.

[57] Bethke PC, Jones RL. $Ca^{2+}$-calmodulin modulates ion channel activity in storage protein vacuoles of barley aleurone cells. Plant Cell. 1994; 6:277–285.

[58] Bethke PC, Jones RL. Cell death of barley aleurone protoplasts is mediated by reactive oxygen species. Plant Journal. 2001; 25:19–29.

[59] Fath A, Bethke PC, Jones RL. Enzymes that scavenge reactive oxygen species are down-regulated prior to gibberellic acid-induced programmed cell death in barley aleurone. Plant Physiology. 2001; 126:156–166.

[60] Sanz L, Albertos P, Mateos I, et al. Nitric oxide (NO) and phytohormones crosstalk during early plant development. Journal of Experimental Botany. 2015; 66:2857–2868.

[61] Stacy RAP, Murnthe E, Steinum T, Sharma B, Aalen RB. A peroxiredoxin antioxidant is encoded by a dormant- related gene, *Perl*, expressed during late development in the aleurone and embryo of barley grains. Plant Molecular Biology. 1996; 31:1205–1216.61.

[62] Kumar SJ, Prasad SR, Banerjee R, Thammineni C. Seed birth to death: dual functions of reactive oxygen species in seed physiology. Annals of Botany. 2015.

[63] Levine A, Tenkanen R, Dixon R, Lamb C. $H_2O_2$ from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell. 1994; 79:583–593.

[64] Foyer CH, Noctor G. Redox homeostasis and antioxidant signalling: a metabolic interface between stress perception and physiological responses. Plant Cell. 2005; 17:1866–1875.

[65] Shaban M, Abasalt RA, Ghorban DM, Tahmine B. Review on dual role of reactive oxygen species in seed physiology and germination. International Journal of Agriculture and Crop Sciences. 2013; 5:2390–2393.

[66] Jacobsen JV, Gubler F, Chandler PM. Gibberellin action in germinating cereal grains. In: Davies PJ, editor. Plant Hormones: Physiology, Biochemistry, and Molecular Biology. Dordrecht: Kluwer Acad. 1995. pp. 164–193.

[67] Olszewski N, Sun TP, Gubler F. Gibberellin signaling: biosynthesis, catabolism, and response pathways. Plant Cell Supplement. 2002; S61–S80.

[68] Raz V, Bergervoet JHW, Koornneef M. Sequential steps for developmental arrest in Arabidopsis seeds. Development. 2001; 128:243–252.

[69] Dubreucq B, Berger N, Vincent E, Boisson M, Pelletier G, Caboche M, Lepiniec L. The *Arabidopsis AtEPR1* extensin-like gene is specifically expressed in endosperm during seed germination. The Plant Journal. 2000; 23:643–652.

[70] Penfield S, Rylott EL, Gilday AD, Graham S, Larson TR, Graham IA. Reserve mobilization in the *Arabidopsis* endosperm fuels hypocotyls elongation in the dark, is independent of abscisic acid, and requires phosphoenolpyruvate carboxykinase 1. Plant Cell. 2004; 16:2705–2718.

[71] Holdsworth MJ, Bentsink L, Soppe WJ. Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. New Phytologist. 2008; 179(1):33–54.

[72] McGinnis KM, Thomas SG, Soule JD, Strader LC, Zale JM, Sun TP, Steber CM. The Arabidopsis SLEEPY1 gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. Plant Cell. 2003; 15:1120–1130.

[73] Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, Asami T, Hirai N, Koshiba T, Kamiya Y, Nambara E. The *Arabidopsis* cytochrome P450CYP707A encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. EMBO Journal. 2004; 23:1647–1656.

[74] Nambara E, Marion-Poll A. ABA action and interactions in seeds. Trends in Plant Science. 2003; 8:213–217.

[75] To A, Valon C, Savino G, Guilleminot J, Devic M, Giraudat J, Parcy F. A network of local and redundant gene regulation governs Arabidopsis seed maturation. Plant Cell. 2006; 18:1642–1651.

[76] Pysh LD, Wysocka-Diller JW, Camilleri C, Bouchez D, Benfey PN. The GRAS gene family in Arabidopsis: sequence characterization and basic expression analysis of the SCARECROW-LIKE genes. The Plant Journal. 1999; 18:111–119.

[77] Cao DN, Hussain A, Cheng H, Peng JR. Loss of function of four DELLA genes leads to light- and gibberellin-independent seed germination in Arabidopsis. Planta. 2005; 223:105–113.

[78] Sun TP, Gubler F. Molecular mechanism of gibberellin signaling in plants. Annual Review of Plant Biology. 2004; 55:197–223.

[79] Penfield S, Gilday AD, Halliday KJ, Graham IA. DELLA-mediated cotyledon expansion breaks coat-imposed seed dormancy. Current Biology. 2006; 16:2366–2370.

[80] Mitsunaga S, Tashiro T, Yamaguchi J. Identification and characterization of gibberellin-insensitive mutants selected from among dwarf mutants of rice. Theoretical and Applied Genetics. 1994; 87:705–712.

[81] Steber CM, Cooney S, McCourt P. Isolation of the GA-response mutant sly1 as a suppressor of ABI1-1 in *Arabidopsis thaliana*. Genetics. 1998; 149:509–521.

[82] Vierstra RD. The ubiquitin/26S proteasome pathway, the complex last chapter in the life of many plant proteins. Trends in Plant Science. 2003; 8:135–142.

[83] Ogas J, Kaufmann S, Henderson J, Somerville C. PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in Arabidopsis. Proceedings of the National Academy of Sciences USA. 1999; 96:13839–13844.

[84] Jacobsen SE, Binkowski KA, Olszewski NE. SPINDLY, a tetratricopeptide repeat protein involved in gibberellin signal transduction in Arabidopsis. Proceedings of the National Academy of Sciences USA. 1996; 93:92–96.

[85] Swain SM, Tseng T-S, Olszewski NE. Altered expression of SPINDLY affects gibberellin response and plant development. Plant Physiology. 2001; 126:1174–1185.

[86] Hartwek LM, Scott CL, Olszewski NE. Two O-Linked N-acetylglucosamine transferase genes of *Arabidopsis thaliana* L. Heynh. have overlapping functions necessary for gamete and seed development. Genetics. 2002; 161:1279–1291.

[87] Izhaki A, Swain SM, Tseng T-S, Borochov A, Olszewski NE, Weiss D. The role of SPY and its TPR domain in the regulation of gibberellin action throughout the life cycle of *Petunia hybrida* plants. The Plant Journal. 2001; 28:181–190.

[88] Gubler F, Kalla R, Roberts J, Jacobsen JV. Gibberellin-regulated expression of a myb gene in barley aleurone cells: evidence for Myb transactivation of ahigh-pI $\alpha$-amylase gene promoter. Plant Cell. 1995; 7:1879–1891.

[89] Woodger FJ, Gubler F, Pogson BJ, Jacobsen JV. A Mak-like kinase is a repressor of GAMYB in barley aleurone. The Plant Journal. 2003; 33:707–717.

[90] Raventos D, Meier C, Mattsson O, Jensen AB, Mundy J. Fusion genetic analysis of gibberellin signaling mutants. The Plant Journal. 2000; 22:427–438.

[91] Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y, Yamaguchi S. Gibberellin biosynthesis and response during *Arabidopsis* seed germination. Plant Cell. 2003; 15:1591–1604.

[92] Gocal GFW, Sheldon CC, Gubler F, Moritz T, Bagnall DB, et al. GAMYB-like genes, flowering and gibberellins signaling in *Arabidopsis*. Plant Physiology. 2001; 127:1682–1693.

[93] Bleecker AB, Esch JJ, Hall AE, Rodriguez FI, Binder BM. The ethylene-receptor family from Arabidopsis: structure and function. Philosophical Transactions of the Royal Society of London B: Biological Science. 1998; 353:1405–1412.

[94] Beaudoin N, Serizet C, Gosti F, Giraudat J. Interactions between abscisic acid and ethylene signalling cascades. Plant Cell. 2000; 12:1103–1105.

[95] Ghassemian M, Nambara E, Cutler S, Kawaide H, Kamiya Y, McCourt P. Regulation of abscisic acid signalling by the ethylene response pathway in Arabidopsis. Plant Cell. 2000; 12:1117–1126.

[96] Bouquin T, Meier C, Foster R, Nielse ME, Mundy J. Control of specific gene expression by gibberellin and brassinosteroid. Plant Physiology. 2001; 127:450–458.

[97] Clouse SD, Sasse JM. Brassinosteroids: essential regulators of plant growth and development. Annual Review of Plant Physiology and Plant Molecular Biology. 1998; 49:427–451.

[98] Steber CM, McCourt P. A role for brassinosteroids in germination in Arabidopsis. Plant Physiology. 2001; 125:763–769.

[99] Chiwocha SD, Cutler AJ, Abrams SR, Ambrose SJ, Yang J, Ross AR, Kermode AR. The *etr1-2* mutation in Arabidopsis thaliana affects the abscisic acid, auxin, cytokinin and gibberellin metabolic pathways during maintenance of seed dormancy, moist-chilling and germination. The Plant Journal. 2005; 42:35–48.

[100] Kanyuka K, Praekelt U, Franklin KA, Billingham OE, Hooley R, et al. Mutations in the huge Arabidopsis gene BIG affect a range of hormone and light responses. The Plant Journal. 2003; 35:57–70.

[101] Fu X, Harberd NP. Auxin promotes Arabidopsis root growth by modulating gibberellin response. Nature. 2003; 421:740–743.

[102] Liu X, Zhang H, Zhao Y, Feng Z, Li Q, Yang HQ, Luan S, Li JM, He ZH. Auxin controls seed dormancy through stimulation of abscisic acid signaling by inducing ARF-mediated ABI3 activation in Arabidopsis. Proceedings of the National Academy of Sciences. 2013; 110:15485–15490.