Chapter 3

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

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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

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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].

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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

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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 α-amylase (α-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

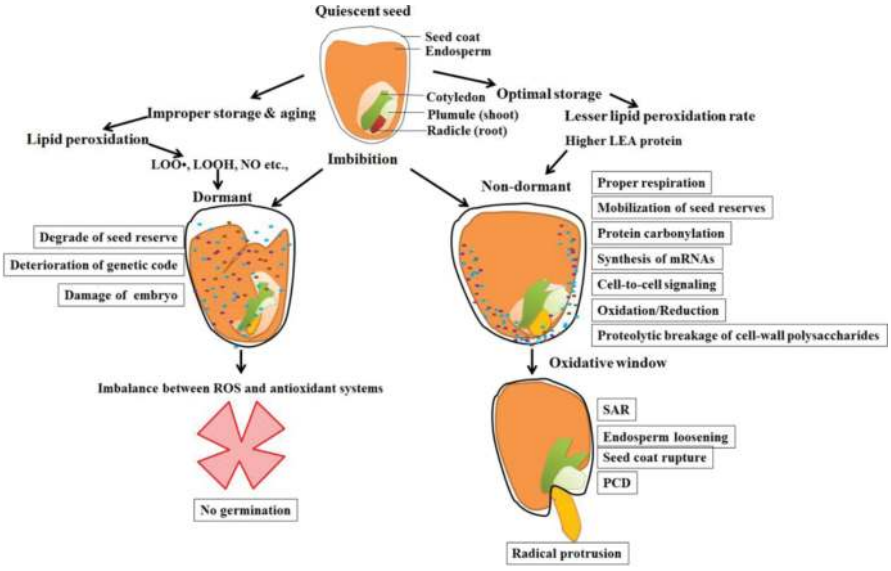

Figure 1. Schematic representation of the involvement of the reactive oxygen species (ROS) in the quiescent seed for the nondormancy and dormancy conditions. Dots represent the accumulation of ROS, blue for superoxide (O_2^-), brown for hydrogen peroxide (H_2O_2), and purple for hydroxyl radical (•OH).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

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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**).

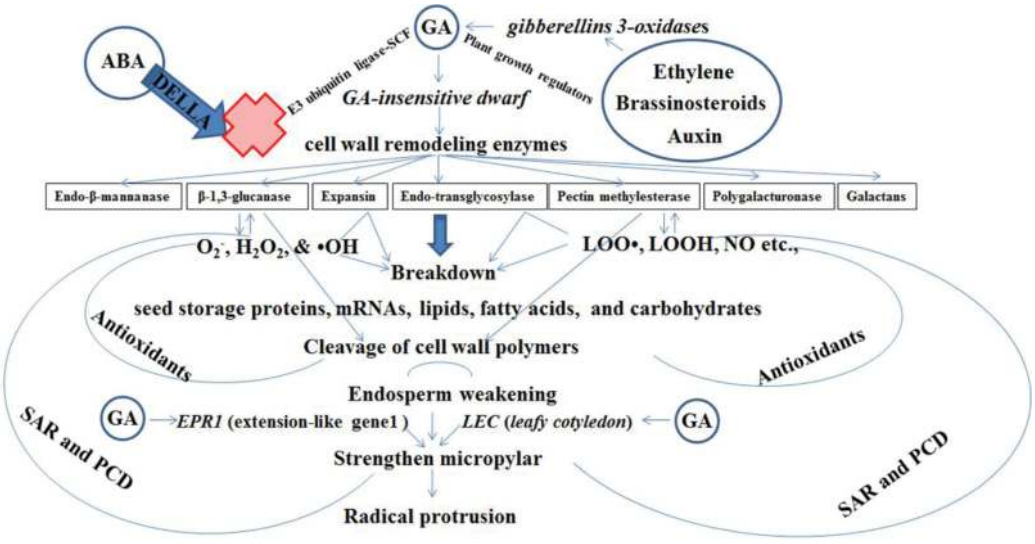

Figure 2. Simplified framework of the dormancy release mediated by GA in concordant with the ROS. Interactions between the genes are described in the text.

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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 (*SCR*) [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 (α)- and beta (β)-amylases. The expression of α-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 α-amy. By increasing the Ca^{2+}, GA activates the hydrolases via calmodulin signaling for successful emergence of the radicle [57–59, 82] (**Figure 3**).

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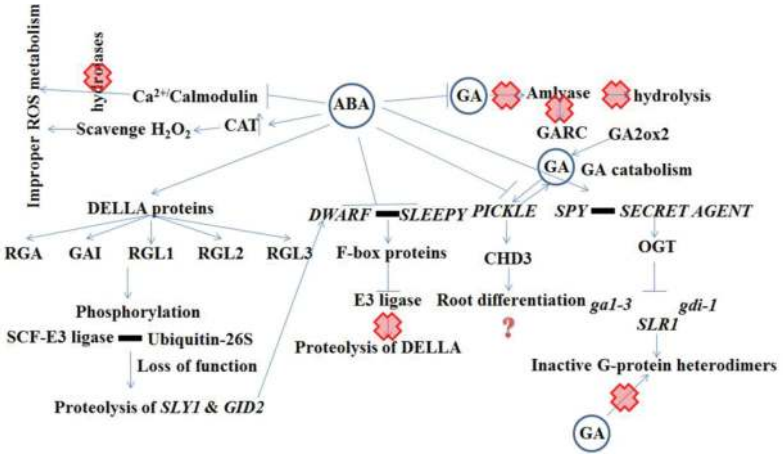

Figure 3. Heuristic networks on the dormancy induction by ABA. Interactions between the genes are described in the text. Arrows represent induction, barred lines represent inhibition, boxes represent sharing similar functions, question marks represent not clear, and crosses represent blocked or reduced 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.

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