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Review

Teaching an Old Dog a New Trick: Multifaceted Strategies to Control Primary Seed Germination by DELAY OF GERMINATION 1 (DOG1)

Ping Li*, Huanhuan Ni, Songbei Ying, Jiali Wei and Xiangyang Hu

Shanghai Key Laboratory of Bio-Energy Crops, School of Life Sciences, Shanghai University, Shanghai, 200444, China
*Corresponding Author: Ping Li. Email: liping80@shu.edu.cn
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Abstract: Primary seed dormancy is a critical trait for sustainable agricultural development, ensuring seed germination under favorable conditions. The induction, maintenance and release of seed dormancy is a complicated physiological process that is strictly controlled by a variety of endogenous signals and environmental factors. In *Arabidopsis*, *DOG1* (*DELAY OF GERMINATION 1*) is identified as the main quantitative trait locus (QTL) of seed dormancy, which contributes to deep dormancy in the Cvi ecotype. In recent years, considerable progress has been made to elucidate the molecular regulatory mechanism by which *DOG1* controls seed dormancy. In this review, we describe a series of findings on the role of DOG1 in controlling primary seed dormancy, ranging from transcriptional and posttranslational regulation, epigenetic modification, and protein stability to biochemical functions during environmental perception and adaptation. We propose that the DOG1-based regulatory network is a good model for elucidating the basic biological mechanism governing seed dormancy and providing valuable information to genetically engineer crops for quality improvement.

Keywords: Seed Dormancy; *DOG1*; ABA; GA

1 Introduction

Seed dormancy (physiological dormancy) refers to the inability of a viable seed to germinate under conditions conducive to embryo germination, which is usually initiated by either the embryo or the surrounding endosperm tissues [1]. Dormancy is a quantitative trait locus in which the depth of seed dormancy determines the time of seed germination. Primary dormancy is induced during the seed maturation phase. Dormancy release occurs primarily through seed postripening or cold stratification treatments [2,3]. Seed dormancy plays an important role in the evolution and adaptation of seed plants to environments. Although the biological significance of seed dormancy is clear, the molecular mechanism of seed dormancy induction, maintenance and mitigation has not been elucidated. Various seed tissues, such as seed coat, endosperm and embryo, contribute to dormancy, which is a highly complex trait determined by genetic and environmental factors [4-6]. Among these factors, hormone regulation may be an important mechanism for seed plant dormancy. Abscisic acid (ABA) and gibberellin (GA) are the main factors regulating dormancy and germination. ABA induced and maintained seed dormancy, while GA broke it. Changes in the ABA/GA balance and sensitivity among seed ABA/GA constitute the core

regulatory mechanism for maintaining and releasing seed dormancy. Other endogenous signals, such as reactive oxygen species (ROS) and environmental factors (such as temperature), regulate seed dormancy, which is at least partly mediated by the balance of ABA and GA [5,7-9].

The genetic variation for seed dormancy is notably large [10]. *DOG1* (*DELAY OF GERMI-NATION 1*) is the main quantitative locus underlying the natural genetic variation in Arabidopsis accessions. This gene was first identified between the high dormancy Cvi accession and the low dormancy Ler accession. *DOG1* is one of the main genes leading to abnormal dormancy of Cvi [7,10,11]. *DOG1* encodes a protein of unknown function that is conserved in plant species [2,12]. The genetic role of *DOG1* in seed dormancy and the significance of its expression in environmental perception and adaptation have been fully demonstrated [3,7,13-16]. However, for a long time, the biochemical function and role of its conserved dormancy mechanism in phylogeny had not been elucidated. In recent years, there have been notable breakthroughs in the biochemical function of *DOG1* in seed dormancy. However, these findings also give rise to many new questions. Therefore, it is necessary to elucidate the molecular mechanism by which *DOG1* controls seed dormancy.

2 Function of DOG1 in Primary Seed Dormancy

DOG1 is the main regulator of seed dormancy. *DOG1 is* mainly expressed in seeds. This gene is also expressed in vascular tissues of embryos, hypocotyls of early seedlings and cotyledons. The encoding protein is located in the nucleus [7,17]. The levels of *DOG1* mRNA and protein are closely related to the degree of seed dormancy in freshly harvested seeds, but the level of DOG1 protein in after-ripened seeds is not [7,18]. The *DOG1* transcripts were detected at 10 DAP after pollination and accumulated during the seed ripening period; there was a high accumulation peak at 16 days after pollination (DAP), and the transcripts disappeared during imbibition [3]. However, low temperature stratification (used to remove primary dormancy) did not induce *DOG1* transcriptional downregulation during the imbibition process. Therefore, the time of seed dormancy release during seed ripening is mainly determined by the level of DOG1 proteins. However, freshly harvested seeds have higher levels of DOG1 proteins that accumulate during seed maturity, and protein levels remain relatively high, even in imbibed seeds, at 12 weeks after ripening [3]. In after-ripened seeds, it is speculated that the dormancy of seeds is determined by the chemical properties of DOG1 proteins, not by their quantity. The activity of DOG1 proteins may be lost during seed storage, probably because of modifications and transformation into a nonfunctional form, which promotes seed germination [3].

In addition, *DOG1* participates in both seed dormancy and seed lifespan. The expression of some mature-related genes (including desiccation tolerance-related genes) was altered in *dog1-1* mutants. *DOG1* and *ABI3* (*ABSCISIC ACID INSENSITIVE3*) interact genetically and participate in seed maturation together. It is speculated that *DOG1* may have evolved at first as a seed ripening regulator and later became the main regulator of seed dormancy [19,20]. DOG1 also mediates the adaptation of plants to drought stress. The high-level expression of *DOG1* can enhance the drought resistance of *A. thaliana*, while inactivation of *DOG1* is sensitive to drought conditions. However, it is clear that the role of *DOG1* in the environmental adaptation of mature plants is independent of the gene's role in seed dormancy regulation [21].

3 Regulation of DOG1 Expression at Multiple Layers

Seed dormancy is initiated at the seed maturation stage. Four transcription factors, LEC1 (LEAFY COTYLEDON1), ABI3, FUS3 (FUSCA3) and LEC2 (LEAFY COTYLEDON 2) (abbreviated as the *LAFL* genes), play an important role in seed maturation and the transition from seed to seedling. The main function of *LAFLs* is to maintain high ABA levels, which will promote dormancy and inhibit seed germination. When one of these genes is mutated, dehydration tolerance will be reduced, and dormancy will be lost. *DOG1* is induced during seed maturation, and its expression is indirectly dependent on *LAFLs* [22]. To summarize, the expression level of *DOG1* is regulated by such means as alternative splicing, selective polyadenylation, histone modification, and *cis*- and anti-noncoding RNA [7,23-26].

3.1 Cis-element Within the DOG1 Promoter

The seed dormancy of *A. thaliana* varies with geographical distribution. Differences in *DOG1* expression among Arabidopsis accessions lead to geographic variations in dormancy and germination, revealing the local adaptation of DOG1 in *A. thaliana* [27,28]. As early as 2006, when DOG1 was first identified, Bentsink *et al*. noted that the dormancy variation between Cvi with high seed dormancy and Ler with low seed dormancy is caused by polymorphisms of *cis*-regulatory regions and coding regions of the *DOG1* gene, leading to a significant expression difference of *DOG1* alleles between Cvi et al. [7]. However, no relationship between polymorphism and dormancy was found at that time. The latest study revealed that LEC1 induced the expression of *bZIP67*; the dormancy of *bZIP67* mutants decreased, and *bZIP67* overexpression led to the deepening of dormancy. Further studies indicated that bZIP67 helps to establish the primary dormancy of seeds by directly binding to GBL and RYL-CIS of the *DOG1* promoter to activate its expression during maturation. This regulation process is also affected by the polymorphism of the *DOG1* promoter from Cvi and Ler, which contain different indels between GBL and TSS. It has been confirmed that these different indels directly affect the ability of bZIP67 to transactivate DOG1 transcription *in vivo*, which may explain the difference in *DOG1* transcription levels in both Cvi and Ler accessions. However, it is not clear how indels affect the expression of *DOG1* [26].

3.2 Alternative Splicing

There are five transcript variants (α, β, γ, δ, ε) of *DOG1*, encoding three protein isoforms (β, γ and ε encode the same protein) [7]. All DOG1 proteins are localized in the nucleus. *DOG1*- ε is the main form in developing seeds. The *dog1* mutant seeds expressing single *DOG1* transcript variants driven by the endogenous *DOG1* promoter did not complement the phenotype. However, the simultaneous expression of two or more different *DOG1* transcript variants can lead to the accumulation of DOG1 proteins and an increase in seed dormancy. The combination of *DOG1*- β and *DOG1*- α or *DOG1*- δ was more dormant than that of *DOG1*- α and *DOG1*- δ alone. Interestingly, transgenic plants constitutively expressing (with 35 s promoter) *DOG1*- α, *DOG1*- β and *DOG1*- δ complemented the phenotype, suggesting that each *DOG1* subtype has the biochemical function of seed dormancy. In addition, DOG1 protein variants can interact, which is necessary for DOG1 functions. However, the exact mechanism governing this interaction is not clear [29]. Nishimura et al. [30] found that *DOG1*- δ transcripts become relatively abundant in the late stage of seed ripening compared with *DOG1*- β, suggesting that the ratio change of these two transcripts may be related to the accumulation of DOG1 proteins.

3.3 Alternative Polyadenylation

DOG1 transcripts are regulated by alternative polyadenylation in *Arabidopsis*. As a result, there are two transcript products, short *DOG1* (*shDOD1*, mainly *DOG1*- *ε*), which ends at the proximal transcription termination site (PTT), and long *DOG1* (*lgDOG1*, mainly *DOG1*- α, β, γ and δ), whose transcription extends to distal transcription termination (dTT) [31]. The preference for polyadenylation PTTs can increase the expression of *shDOG1*, while the selection of dTTs can enhance the expression of *lgDOG1*. The deletion mutation of *CPL1* (C-terminal domain (CTD) phosphatase-like 1) will lead to an increase in *shDOG1* transcripts and dormancy due to the preferred selection of PTTs. On the other hand, the *fy2* (flowering time control protein) mutation promotes the priority selection of DTTs, resulting in an increase in the expression of *lgDOG1* with a decrease in dormancy. At the seed ripening stage, *shDOG1* and *lgDOG1* could restore the dormancy phenotype of *dog1-2*, indicating that *shDOG1* and *lgDOG1* are positive and negative regulators of seed dormancy, respectively [18,31,32].

3.4 Noncoding RNA Regulation

The expression of *DOG1* may also be regulated by *cis*-acting antisense transcripts. Fedak et al. [18] found that the 3' region of *DOG1* contains a promoter of the noncoding antisense RNA *asDOG1*, which

originates close to the *DOG1* proximal polyadenylation site (pTTs). The promoter initiates independently to produce *asDOG1* transcripts. The expression levels of *asDOG1* and *DOG1* transcripts were different. Further study revealed that *asDOG1* strongly suppressed the expression of *DOG1* during seed maturation. However, the expression of *DOG1* and *asDOG1* decreased during seed imbibition, indicating that the function of *asDOG1* may be limited during seed maturation [18,32]. Kowalczyk et al. [24] found that upregulation of the *lgDOG1* isoform resulted in decreased *asDOG1* levels in the *fy-2* mutant compared with Col-0. Further research also showed that *lgDOG1* repressed *asDOG1* expression through gene chromatin remodeling (*H2Bubq*). The expression of *asDOG1* in the *hub1-5* mutant was significantly upregulated, indirectly supporting this viewpoint [24,31,33]. The inhibition of *DOG1* by *asDOG1* indicates that *LgDOG1* and *asDOG1* participate in the regulation of *DOG1* expression together. If *lgDOG1* and *asDOG1* inhibit the production of each other, *fy-2* will show an increase in dormancy, rather than a decrease in dormancy; the *cpl1-9* mutant will show a lower dormancy decline, rather than a stronger dormancy. Therefore, more details are needed to clarify the biological significance mechanism [25]. In addition, *DOG1* was also reported to participate in the regulation of the expression of noncoding RNA to regulate seed dormancy. Huo et al. [12] showed that *DOG1* regulated seed germination and flowering time by affecting the production of miR156 and miR172.

3.5 Transcriptional Prolongation and Chromatin Remodeling

Saunders et al. [34] showed that the regulation of transcriptional efficiency may be one of the core mechanisms of seed dormancy. The transcriptional efficiency of a gene depends on the recruitment of RNA polymerase II to the DNA template and transcriptional elongation after binding to DNA. The whole process may also be affected by chromatin modifications, such as histone ubiquitin, methylation and acetylation, leading to the regulation of seed dormancy [23,34]. *RDO2* encodes TFIIS (a transcriptional extension factor). The dormancy of the *rdo2* mutant decreased [35]. Another independent experiment suggested that the dormancy of *TFIIS* mutants was reduced due to the decrease in *DOG1* expression during seed maturation. Seed dormancy was enhanced by introducing *DOG1* into the *TFIIs* mutant [36], suggesting that it is regulated by the transcription extension efficiency of *DOG1*.

Another mutant, *rdo4* (*hub1*), also showed a low level of dormancy. *RDO4* encodes *HUB1* (an H2B ubiquitin 1 gene, histone monoubiquitination 1) that participates in the monoubiquitination of histone H2B, methylation of H3K4 and H3K79 and activation of gene expression [33]. Further studies showed that HUB1 interacted with transcriptional elongation-related PAF1C (RNA Polymerase II Associated Factor-1 Complex). It has also been suggested that transcriptional lengthening and histone ubiquitination may be associated with the mechanism of seed dormancy [23]. Interestingly, the expression of *DOG1* was found to be downregulated in *rdo2* and *rdo4* [35]. However, transgenic *hub1-2* generated by introducing *DOG1*-Cvi had only a moderate dormancy level, suggesting that although *HUB1* acts as an upstream regulator of *DOG1*, it may not be the sole factor controlling the expression of *DOG1* [24,34]. *DOG1* also participates in seed dormancy through epigenetic regulation of chromatin remodeling. On the one hand, *DOG1* is involved in seed dormancy regulation through the chromatin remodeling-related factor *KYP/SUVH4* (KYP methyltransferase), which will lead to gene H3K9me2 modification. The seed dormancy of *kyp-2* was enhanced, and *DOG1* and *ABI3* expression were upregulated in *kyp-2*, suggesting that *KYP/SUVH4* may be associated with DNA methylation of *DOG1* and *ABI3* and has a negative effect on seed dormancy [37]. On the other hand, Zhao et al. [38] found that H3K4 histone demethylases *LDL1* and *LDL2* (LYSINE SPECIFIC DEMETHYLASE-LIKE) had redundancy functions in regulating seed dormancy, and the double mutant *ldl1ldl2* showed enhanced seed dormancy, mainly by regulating the expression levels of *DOG1*, *ABA2* and *ABI3* [38]. In addition, Footitt et al. [39] showed that the change in the seed dormancy level was related to inhibition markers (H3K27me3) and activation markers (H3K4me3) of *DOG1*. Under light conditions, H3K27me3 markers accumulated rapidly on *DOG1* chromatin, while H3K4me3 markers gradually disappeared. DNA methylation of heterochromatin

labeling H3K9me2 and CHG could also be associated with *DOG1* chromatin, and the whole process is regulated by temperature [39]. Recent studies have shown that the chromatin remodeling factor *PKL* (PICKLE), which physically interacts with clock-related protein *LUX* (LUX ARRYTHMO), regulates seed dormancy. *pkl* shows an enhanced dormancy phenotype. Further studies revealed that *LUX* could directly bind to *DOG1* and recruit *PKL* to affect the level of H3K27me3 of *DOG1*. *LUX* could suppress the expression of *DOG1* and promote seed germination. The recruitment of *PKL* in the *DOG1* chromatin region depends on *LUX*. Therefore, the biological clock can directly regulate the expression of *DOG1* and participate in seed dormancy. Interestingly, the binding site of *LUX* and *DOG1* is close to the transcriptional initiation site of the noncoding antisense *asDOG1* sequence, and the transcription of *asDOG1* in *pkl-1* is also downregulated. It is speculated that *LUX* may directly regulate the expression of *asDOG1* and suppress the expression of *DOG1* [40].

3.6 DOG1 Protein Stability

Posttranslational modification of PTMs also plays an important role in the regulation of seed development and maturation [41]. Nakabayashi et al. [3] showed that the activity of DOG1 proteins in postripening seeds was changed compared with fresh seeds, indicating that DOG1 could be modified. Reactive oxygen species (ROS) may be involved in the activity transition of DOG1 proteins during ripening. Because DOG1 can bind to heme, which acts as an oxygen and nitric oxide sensor, it is possible that the protein modification of DOG1 could be associated with PTMS regulation, such as cysteine oxidation and S-nitrosylation modification [30].

In addition, Miatton et al. [42] screened a DOG1 interacting protein, PP2AA/PDF1, a Ser/Thr phosphatase 2A. *PDF1* and *DOG1* were coexpressed during seed ripening. *PDF1* is a negative regulator of seed dormancy since *pdf1-1* dormancy is enhanced. *pdf1-1dog1* showed the same phenotype as *dog1-2*, indicating that the *dog1* mutation is epistatic to *PDF1*. Interestingly, the transcriptional level of *DOG1* in the *pdf1-1* mutant did not change, suggesting that PDF1 may affect the protein level of DOG1. Further studies indicated that *PDF1* regulated seed dormancy by regulating the dephosphorylation of the DOG1 protein [42]. Recently, Footitt et al. [43] found that during a dormancy cycle, the expression level of *PDF1* was similar to that of *ANAC060*. *ANAC060* was previously considered to be the QTL related to seed dormancy (*DOG6*) [20]. Moreover, *ANAC060* decreased the sensitivity of ABA, and its expression was induced by the glucose-ABA signal cascade [44]. Interestingly, during a dormancy cycle, the ratio change patterns of *PDF1*, *ANAC060* and *AHG1* to *DOG1* as a negative regulation in dormancy are similar. At present, except for *ANAC060*, both *PDF1* and *AHG1* have direct relationships with *DOG1* [42,43,45]. It can be speculated that *ANAC060* will tend to suppress the regulation of hormone balance, influencing *DOG1* to participate in seed dormancy. The details need to be further investigated.

4 DOG1 and Hormone Regulation

It is well-known that the hormonal balance between abscisic acid (ABA) and gibberellin (GA) is associated with the regulation of seed germination and dormancy in response to environmental signals. The balance of ABA/GA is regulated by mutual antagonism of ABA and GA [46]. The dormant seed state is induced and maintained by ABA and is released by GA. Other hormones (ethylene, jasmonic acid and auxin) also play a role in seed germination regulation [47,48].

4.1 DOG1 and ABA

Bentsink et al. [7] reported that *DOG1*-Cvi/*aba1-3* had no dormancy phenotype, and in *dog1* mutants, the level of ABA decreased, and the level of GA increased, indicating that the function of *DOG1* depends on ABA. However, further study indicated that DOG1 proteins were still maintained at a high level without ABA. Moreover, compared with *cyp707a2-1*, the seeds of *dog1-2*/*cyp707a2-1* showed lower dormancy, but their ABA contents were similar, indicating that *DOG1* and ABA are necessary for inducing seed

dormancy, and the function of *DOG1* was independent of ABA. *DOG1* could be used as a timer for seed dormancy release [3,7]. In addition, it was reported that glucose induced the expression of Cvi-*DOG1*. *DOG1* is involved in the ABA-mediated glucose signaling pathway, of which *ABI4* is an important component [51]. Shu et al. [50] showed that *ABI4* is also considered to be a positive regulator of dormancy. Whether *DOG1* and *ABI4* are associated with seed dormancy regulation needs to be further studied [50]. It has also been proposed that the regulation of *DOG1* expression from ABA is at least partly mediated by the transcription factors ABI3 and ABI5 [51]. In short, the relationship between *DOG1* and ABA metabolism and signal transduction merits further study.

Recently, notable breakthroughs have been made in research on *DOG1* and ABA regulation. It has been confirmed that *DOG1* participates in a unique ABA signaling pathway that mediates seed dormancy [30]. *DOG1* was determined to interact with *AHG1* and *AHG3*, two negative regulators of ABA signaling and seed dormancy. The functional deletion mutation of *AHG1* (*ahg1-5*) and *AHG3* (*ahg3-2*) enhanced seed dormancy. *dog1-2/ahg1-5/ahg3-2* showed deeper dormancy, indicating that *AHG1* and *AHG3* are located downstream of *DOG1*. Further studies showed that DOG1 directly binds to AHG1 and AHG3 and suppresses their activities to enhance the sensitivity of ABA in association with seed dormancy. DOG1/AHG1 is independent of AHG3 and other PP2C pathways to downregulate ABA signaling [30]. It was also found that DOG1 can bind to heme [45], whose binding is not necessary for the DOG1-AHG1 interaction, but both interactions are essential for the function of *DOG1 in vivo* [25,30]. Since heme-binding proteins are oxygen and nitric oxide sensors [52], ROS and NO counteract ABA signaling regulation of seed dormancy, and it is possible that DOG1 binding to heme may regulate the levels of ROS and NO in seeds involved in downstream regulation, such as ABI5 protein stability affecting seed dormancy. The DOG1-heme complex may have a negative effect on heme transfer and heme lyase activity in mitochondria, limiting the production of total cytochrome c and increasing the sensitivity of ABA in seeds [53]. NO is considered to be the common regulator of ABA and ethylene. NO is produced rapidly after seed imbibition, promoting germination by inducing CYP707A2 expression and stimulating the production of ethylene [25,32]. Whether this process is involved in the regulation of *DOG1* warrants further research.

4.2 DOG1 and GA

DOG1 expression and dormancy induction during seed maturation are regulated not only by ABA but also by GA. In contrast to ABA, GA negatively regulates dormancy by releasing coat-mediated seed dormancy [54]. During the postripening process, the transcriptional level of *GA3ox2* has a 40-fold increase, while *GA2ox1* (GA inactivating enzyme) is highly expressed in Cvi [7]. Compared with the wild type, the level of *GA2ox6* in *dog1* was decreased by 10-fold, suggesting that *DOG1* may indirectly participate in the catabolism of GA. The transcriptional level of *DOG1* in *della* was decreased, indicating that GA also had a certain effect on the expression of *DOG1* [55]. In addition, Graeber et al. [9] found that DOG1 is associated with the temperature-dependent regulation of GA metabolites. The expression of the *GA20ox* gene is regulated by *DOG1* in a temperature-dependent manner, which is consistent with the temperature-dependent accumulation of GA metabolites catalyzed by GA20ox.

4.3 DOG1 and Ethylene

Ethylene can promote seed germination. During seed germination, ABA usually suppresses weakening of endosperm caps, while ethylene promotes weakening of micropyle endosperm by inducing the expression of CWRP (Cell Wall Remodeling Proteins) or ROS [51]. Therefore, it can be speculated that ethylene may play an important role in counteracting the ABA effect involved in seed dormancy regulation. It has been shown that ethylene can promote seed germination by degrading ERF, which mediates active oxygen and nitrogen signals in seeds [22]. Moreover, NO is associated with the regulation of ABA and ethylene [41, 56]. Therefore, ethylene, ABA and NO may regulate seed dormancy with an important node. Whether

DOG1 is associated with this process is an interesting question. The latest study revealed that *DOG1* plays a role downstream of ethylene receptor *ETR1* and ethylene response factor *ERF12*. *ERF12* recruits the cosuppressor *TPL* (TOPLESS) to form a complex that binds to the *DOG1* promoter DRE/CRT element to suppress *DOG1* expression and regulate seed dormancy [17].

5 DOG1 Expression in Response to Environmental Signals

5.1 DOG1 and Temperature

The primary dormancy depth of seeds is determined by heredity and environment before and after shedding. The environmental impact of mother plants on the whole life cycle has a significant impact on progeny seed dormancy, which will affect the development and metabolism of fruit and testa during seed development and maturity [22]. In addition, seeds are also affected by various environmental factors, including temperature, moisture, oxygen and light [20]. Temperature is an important environmental factor that controls the induction and release of seed dormancy.

Temperature in the process of seed ripening determines the depth of seed original dormancy. Generally, low temperature during seed ripening leads to deep primary dormancy, while high temperature leads to shallow dormancy [3]. Footitt et al. [39] showed that the expression of *DOG1* responds to the seed ripening environment and is closely related to dormancy. When the mother plant matures at low temperature, it shows stronger dormancy than the seeds matured at warmer temperature. The expression of *DOG1* was negatively correlated with soil temperature and was associated with changes in chromatin levels [39]. Thus, *DOG1* is thought to be the temperature sensor that determines the degree of seed dormancy [3,9]. The dormancy of Cvi is alleviated by exposure to higher temperatures, while the situation of annual ecological Bur, Col-0 and Ler in summer is the opposite, and the increase in temperature accelerates the induction of their secondary dormancy [3,55,57-59]. After cold induction, the expression of *NCED4*, *NCED6* and *GA2ox2* was upregulated, while the expression of *CYP707A2* was downregulated, suggesting that *DOG1* mediated seed dormancy at low temperature by promoting the biosynthesis of ABA and the catabolism of GA [3,46,55]. Bryant et al. [26] reported that low temperature can increase the protein abundance of *bZIP67* that activates the expression of *DOG1*, which could explain how low temperature enhances *DOG1* expression and promotes dormancy.

5.2 DOG1 and Reactive Oxygen Signal

ROS play an important role in seed germination. Oxidation can break seed dormancy. Some studies have suggested that ABA suppresses ROS production during seed imbibition, and ROS decrease may be key to suppressing seed germination by ABA [62]. During seed maturation, the level of DOG1 protein was not decreased with the removal of dormancy, indicating that its activity is changed, which could be associated with the oxidation level [3,61]. The latest study revealed that an increase in the oxidation level of seeds during dry storage was indeed associated with a decrease in DOG1 activity and dormancy [45]. ROS were also found in dry seeds and imbibed seeds, suggesting that ROS may activate seed germination through GA signaling [64]. Increased ROS in seeds reacting with all proteins in cells may lead to changes in dormancy-related enzyme activities and their binding mechanisms [3,5,61]. It will be of considerable significance to further study the function of ROS-*DOG1* and their relationship with hormones in the transition from maturity to pregermination. Therefore, in-depth analysis of the function of specific carbonylation proteins during seed dormancy and germination may be helpful to reveal the function of ROS involved in the regulation of DOG1 proteins.

6 Function of DOG1 Homology in Dormancy

In *A. thaliana*, in addition to *DOG1*, there are four other *DOG1*-like genes (*DOG2* to 4) that belong to a function-unknown gene family. The sequence similarities of *DOGL1*, *DOGL2*, and *DOGL3* compared with *DOG1* were 54.3%, 43.1% and 39.3%, respectively. Only 23.4% sequence similarity between *DOGL4* and

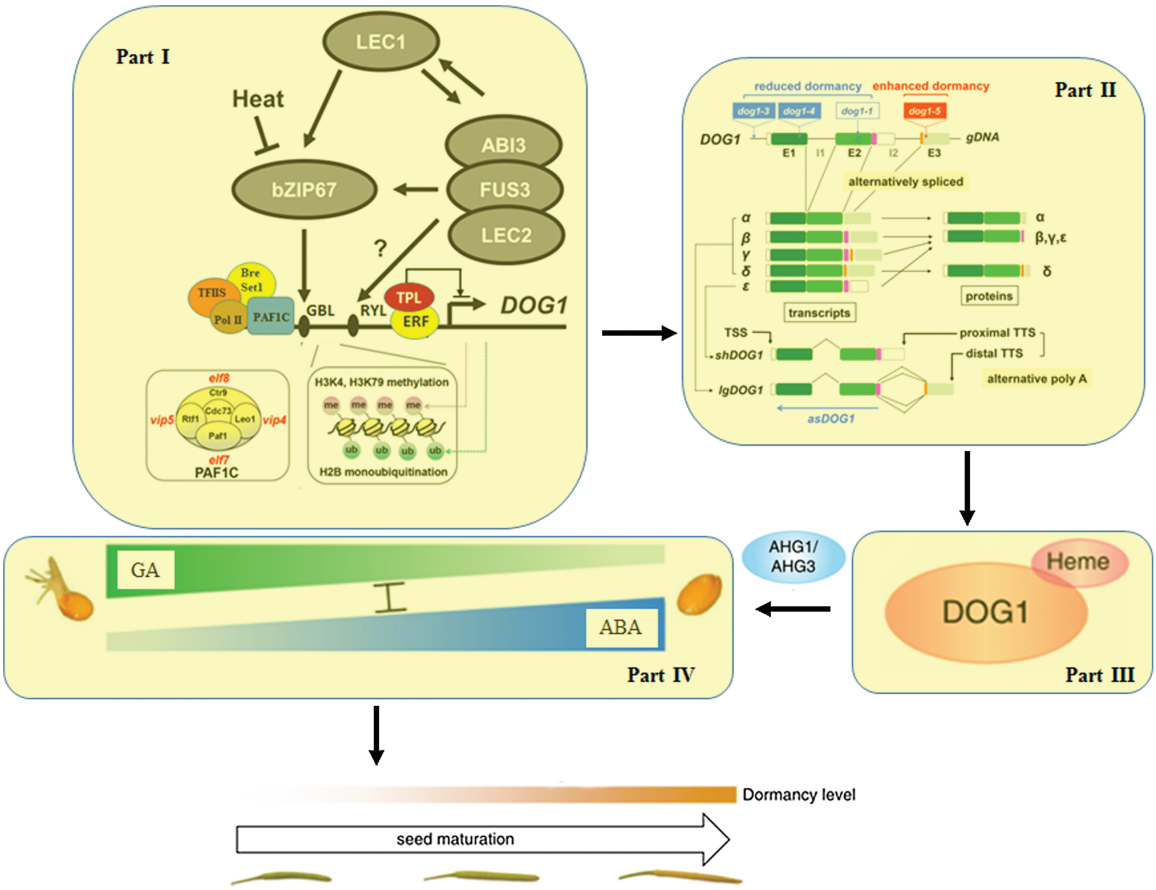

Figure 1: Possible model to illustrate how primary seed dormancy is strictly controlled by DOG1 at different levels. Among these levels, Part I showed how DOG1 is regulated by several transcription factors, such as bZIP67 or epigenetic factors; Part II shows the alternative splicing regulation of DOG1; Part III shows the interaction of DOG1 with Heme and AHGs; and Part V shows the dynamic change of GA and ABA, ultimately inducing primary seed dormancy

DOG1 was found [7]. There was no difference in the dormancy phenotype among *dogl1*, *dogl2* and *dogl3*. However, the seeds overexpressing *DOGL3* are highly sensitive to ABA, which may be associated with dormancy. *dogl4* showed enhanced seed dormancy. It has been suggested that *DOGL4* is a negative regulator of seed dormancy, and its function is different from that of *DOG1* [30,63]. At present, evolution analyses of *DOG1* family proteins is still puzzling, and further comprehensive evaluation is needed.

The protein with the highest similarity (42%) to AtDOG1 is wheat HBP-1b (histone gene binding protein-1b) [7]. There is low similarity (approximately 25%) between AtDOG1 and OsDOG1. Ectopic expression of these *OsDOG1*-like genes in *Arabidopsis* delayed seed germination [64]. Therefore, future studies on the function of *DOG1* homologous genes in different species may be helpful to explain the origin and evolution of seed dormancy mechanisms.

7 Conclusion

DOG1 is the main dormancy regulator, and the mechanism of seed dormancy has been thoroughly characterized. In this review, we integrate different regulatory pathways, as shown in Fig. 1, to illustrate

the regulatory mechanism of DOG1 at different levels, including transcriptional, posttranscriptional, protein modification and epigenetic regulation. However, some phenomena regarding the role and mechanism of *DOG1* in seed ripening and environmental adaptation have not been fully elucidated. Future studies can focus on the expression and regulatory mechanism of *DOG1* in the seed dormancy cycle, including such processes as the protein modification of DOG1 during seed ripening and its modulation with environmental signals (temperature and ROS). Future research may also investigate such phenomena as the relationship between the expression of *DOG1* in different ecotypes and dormancy and the function of *DOG1* in the integration of oxidative imprinting in the early imbibition process. Additionally, it will be significant to study the role of DOG1 in controlling seed dormancy under environmental stress conditions, such as high light intensity, drought, photoperiod and nutrition level. Overall, a DOG1-based network is a good model for elucidating the regulatory mechanism governing seed dormancy in plants and to provide valuable information for crop breeding.

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