ANNUAL REVIEWS

Annual Review of Plant Biology

Parental and Environmental Control of Seed Dormancy in *Arabidopsis thaliana*

Mayumi Iwasaki,[1,*] Steven Penfield,[2,*] and Luis Lopez-Molina[1,3,*]

[1]Department of Botany and Plant Biology, University of Geneva, Geneva, Switzerland; email: Luis.LopezMolina@unige.ch

[2]Department of Crop Genetics, John Innes Centre, Norwich Research Park, Norwich, United Kingdom

[3]Institute of Genetics and Genomics in Geneva (iGE3), University of Geneva, Geneva, Switzerland

Annu. Rev. Plant Biol. 2022. 73:355–78

First published as a Review in Advance on February 9, 2022

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

https://doi.org/10.1146/annurev-arplant-102820-090750

*These authors contributed equally to this article

Keywords

seed dormancy, endosperm, abscisic acid, DOG1, epigenetics, temperature

Abstract

Seed dormancy—the absence of seed germination under favorable germination conditions—is a plant trait that evolved to enhance seedling survival by avoiding germination under unsuitable environmental conditions. In *Arabidopsis*, dormancy levels are influenced by the seed coat composition, while the endosperm is essential to repress seed germination of dormant seeds upon their imbibition. Recent research has shown that the mother plant modulates its progeny seed dormancy in response to seasonal temperature changes by changing specific aspects of seed coat and endosperm development. This process involves genomic imprinting by means of epigenetic marks deposited in the seed progeny and regulators previously known to regulate flowering time. This review discusses and summarizes these discoveries and provides an update on our present understanding of the role of DOG1 and abscisic acid, two key contributors to dormancy.

Contents

1. INTRODUCTION 356
2. *ARABIDOPSIS* SEED DORMANCY 358
   2.1. The *Arabidopsis thaliana* Seed 358
   2.2. The Central Role of Abscisic Acid and the Endosperm 359
3. THE ROLE OF DOG1 IN PROMOTING DORMANCY 361
   3.1. *DOG1* Gene Expression Regulation and Product Function 361
   3.2. *DOG1*: Unresolved Questions 362
4. MATERNAL AND ENVIRONMENTAL CONTROL OF SEED DORMANCY 365
   4.1. Regulation of Seed Dormancy by the Maternal Environment 365
   4.2. Genomic Imprinting Is Involved in Maternal Inheritance of Seed Dormancy 365
   4.3. Noncanonical RNA-Directed DNA Methylation Mediates Cold Temperature–Induced Seed Dormancy 369
   4.4. Maternal Environmental Control of Seed Dormancy Mediated by Flowering Time Genes 370
   4.5. Additional Cases of Epigenetic Regulation of Seed Dormancy 370

1. INTRODUCTION

Plant terrestrialization, the evolutionary process that produced a phototrophic lineage on land from algal ancestors, started around 540 million years ago (Mya) and changed the Earth's landscape while exerting a profound influence on animal evolution and human history. Seeds, produced by gymnosperms and angiosperms, initiated their evolution ~350 Mya (Late Devonian). Nevertheless, the full extent of land plants' colonization and influence on terrestrial animal life would not have been realized without a dramatic step forward during the Cretaceous (~140 Mya). This was the time when angiosperms spread rapidly around the Earth and underwent an astonishing diversification. It coincided with the appearance in the fossil record of numerous types of seed shapes and sizes (53). There is little doubt that the seed is among the major innovations that drove land colonization by gymnosperms and angiosperms (52, 53, 79).

Gymnosperms: group of plants that produce exposed, nonenclosed seeds

Angiosperms: flowering plants that arose ~140–250 Mya and became the dominant plant group among terrestrial plants; produce seeds enclosed by mature ovaries or fruits

Gametophyte: haploid sexual phase of plants that arises from mitotic divisions of a haploid spore and produces gametes

Unlike in nonseed plants, the female gametophyte of seed-bearing plants is physically associated with the mother plant by means of a specialized tissue, the ovule, where it is protected and nourished. Furthermore, the male gametophyte (pollen) protects the male gametes while enabling the fertilization of distant female gametes. Unlike nonseed plants, the pollen tube delivers the male gametes directly to the female gametes, thus avoiding the need for external water for fertilization. Seeds arise from the fertilized ovule, which harbors and protects the developing embryo. Upon completion of embryogenesis, embryos undergo a maturation program in which they enter a highly resistant, metabolically inert, desiccated state. Seed longevity is illustrated by a 2,000-year-old Judean date palm seed, unburied from the palace of Herod the Great in Israel, that sprouted and produced Methuselah, a male palm tree that produces functional pollen (129). Thus, seed-bearing land plants achieved less-hazardous reproduction, expanded the physical range where fertilization could take place, and enclosed their embryos in highly resilient capsules, enabling colonization of new habitats.

Seeds can be dormant, a trait whereby the embryo-to-seedling transition is withheld even under favorable conditions. Dormancy provides seeds with an opportunity to be dispersed away from their mother plant, influencing a given species' distribution while enabling plants to experiment with a wider variety of environments, thus promoting diversification (36, 148). In addition, dormancy is important for seasonal plant behavior and thus influences the environment in which other plant traits are expressed (36).

Abscisic acid (ABA): isoprenoid plant hormone that promotes seed maturation and plays a key role in repressing dormant seed germination

Different kinds of dormancy have been reported in different seed-bearing plants. On the basis of a classification by Nikolaeva et al. (107), Baskin & Baskin (9) introduced a system of five classes of dormancy: morphological, physiological, morphophysiological (combination of morphological and physiological), physical, and combinational (combination of physiological and physical). Physiological dormancy is the form of dormancy most frequently found in gymnosperm and angiosperm species (9). Nondeep physiological dormancy is the most common physiological dormancy type in which dormancy requires seed integrity (i.e., the seed's covering layers are necessary to prevent embryonic growth upon seed imbibition) (70, 112). It also requires the phytohormone abscisic acid (ABA), which blocks embryonic growth upon seed imbibition (71). Physiological dormancy is highly labile; in other words, final dormancy levels in mature seeds vary according to aspects of the maternal environment during seed development, such as nutrient availability (e.g., nitrate) and physical parameters (e.g., temperature) (48). Nondeep physiological dormancy observed in *Arabidopsis thaliana*, where it is best characterized, is the focus of this review and is hereafter referred to as primary dormancy or simply dormancy.

Dormancy is established during seed development within the mother plant. An individual seed may benefit from germinating in the close environment of its mother, since that environment proved to be beneficial for seed production. Nevertheless, as suggested by theoretical research by Hamilton & May (51), it is expected that the mother plant will promote diversity in the properties of its seed progeny, including in their individual seed dormancy levels, so as to enhance their dispersion potential, enabling them to find new favorable locations while avoiding competition among siblings for local resources (123, 141). Seed dormancy is also influenced by the variable environmental conditions experienced by the mother plant, especially temperature changes (137). This review describes recent developments in the maternal and environmental control of seed dormancy.

Once isolated from their mother plants, seeds start losing dormancy in their dry state, a poorly understood process referred to as dry after-ripening, or else lose their dormancy after exposure to certain environmental conditions, such as imbibition under cold temperatures (cold stratification). For a given batch of seeds produced by a given mother plant, the days of seed dry storage required to reach 50% of germination (DSDS50), contingent on the particular environment used for the germination test, define the dormancy of the seed batch (4). Dormancy levels of the seed progeny vary among species and, indeed, among *Arabidopsis* accessions, consistent with the adaptive function of seed dormancy. Once seeds lose dormancy, they may be exposed to prolonged unfavorable conditions. Such exposure can induce a state, referred to as secondary dormancy, wherein seeds no longer germinate when exposed to favorable conditions. Secondary dormancy is less studied than dormancy and has recently been reviewed elsewhere (15).

A major difficulty in studying seed dormancy is that dormancy is functionally defined, as there is no test to assess whether a dry seed is dormant or not (i.e., no test predicting whether a seed will germinate under conditions otherwise favorable for germination). One must therefore distinguish the mechanism of after-ripening present in dry seeds (referred to as the dry seed mechanism) from the mechanism that blocks germination, namely the sustained activation of ABA signaling by means of sustained ABA accumulation upon seed imbibition [i.e., ABA-dependent germination arrest program (**Figure 1**)]. Clearly, the dry seed mechanism must interact with the

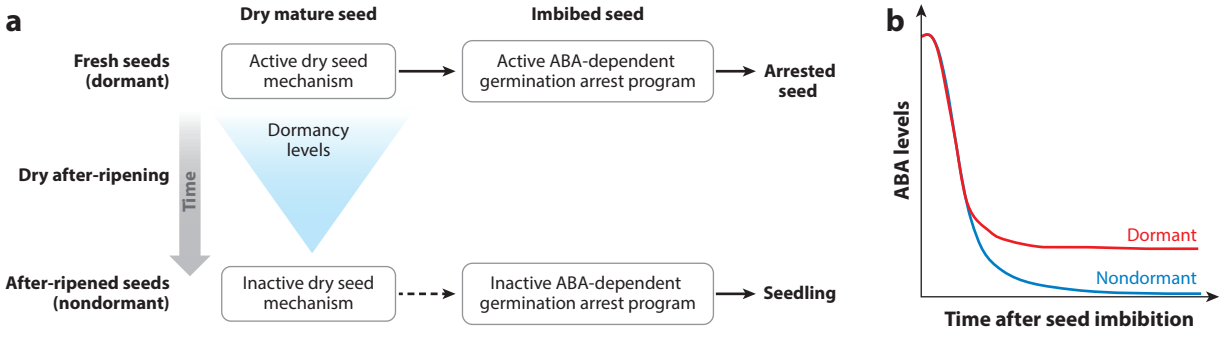

Figure 1

(*a*) Model describing the interaction between the time-dependent dormancy mechanism present in dry seeds and the abscisic acid (ABA)-dependent germination arrest program present in imbibed seeds. Newly produced seeds (fresh seeds) have an active time-dependent dormancy mechanism able to activate the ABA-dependent germination arrest program upon imbibition. As seeds age (dry after-ripening), dormancy levels stored in seeds decrease and the time-dependent dormancy mechanism progressively loses the capacity to activate the ABA-dependent germination arrest program. ABA levels present in dormant and nondormant dry seeds drop sharply within 12 h upon imbibition but remain elevated over time in dormant seeds. (*b*) Representative curves of ABA levels in seeds over 72 h upon dry seed imbibition.

ABA-dependent germination arrest program upon imbibition (**Figure 1*a***). Over time, the dry seed mechanism loses the capacity to activate the ABA-dependent germination arrest program and becomes inactive (**Figure 1*a***).

The dry seed mechanism may be an intrinsic part of the ABA-dependent germination arrest program, but it may also be unrelated. The germination behavior of a seed reflects the capacity of the dry seed mechanism to activate the ABA-dependent germination arrest program. Therefore, one cannot ascertain whether changes in such behavior, resulting from mutations or changes in environmental cues, reflects a perturbation of the dry seed mechanism or a perturbation of the ABA-dependent germination arrest program.

2. *ARABIDOPSIS* SEED DORMANCY

2.1. The *Arabidopsis thaliana* Seed

In *Arabidopsis*, the unfertilized ovule consists of a seven-celled female gametophyte, of the *Polygonum* type present in approximately 70% of angiosperms, that is completely surrounded by inner and outer integuments except for the micropylar opening (39). The pollen tube enters the micropylar opening and delivers two sperm cells in close proximity to the female gametes: the haploid egg and homodiploid central cells. Gamete fusion, which produces the diploid zygote and the triploid endosperm, initiates seed development by triggering specific developmental programs of the three genetically distinct tissues composing the seed: integuments, endosperm, and embryo (**Figure 2**). The maternal integuments differentiate and die to produce the seed coat of the mature seed (**Figure 2**). The endosperm proliferates alongside the embryo and serves as a nourishing tissue for the embryo. *Arabidopsis* is a nonpersistent endosperm species, and its endosperm is gradually consumed by the embryo after embryogenesis as the embryo enters the maturation phase, where it accumulates nutrients, expands in size, and eventually desiccates (**Figure 2**). In the mature seed, food is stored mainly in the embryo, whereas the endosperm persists as a single layer of cells surrounding the embryo (**Figure 2**).

Seed dormancy is established during maturation, and repression of germination of dormant seeds upon imbibition critically require the endosperm (see Section 2.2). The maternal seed coat

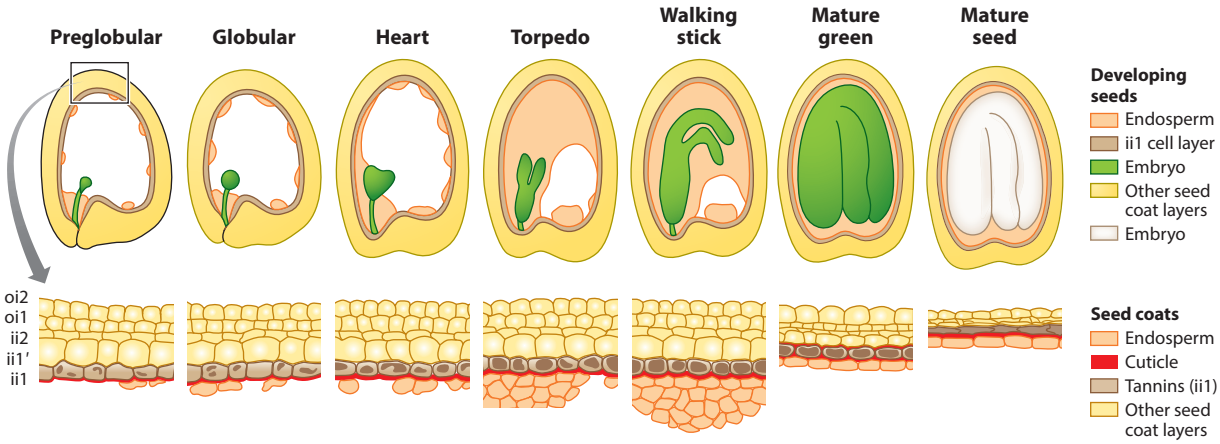

Figure 2

Seed developmental stages in *Arabidopsis thaliana*, showing the different seed coat cell layers. The illustrations are not to scale, allowing the tannins and endosperm-associated cuticle to be visualized. Abbreviations: ii1, inner integument 1 layer; ii1′, inner integument 1′ layer; ii2, inner integument 2 layer; oi1, outer integument 1 layer; oi2, outer integument 2 layer. Figure adapted from Reference 84 (CC BY 4.0).

plays an important role: The inner integument layer 1 accumulates proanthocyanidins (tannins), a type of flavonoid, and *transparent testa* (*tt*) mutants, deficient in proanthocyanidin synthesis, have low dormancy (33) (**Figure 2**). Tannins are antioxidants, and their absence in the seed coat likely promotes the release of dormancy by accelerating oxidation in seeds or by increasing seed permeability to oxygen. Accordingly, *tt* mutant seeds also have low seed viability (32). Furthermore, the inner integument layer 1 produces a cuticle and tannic cell walls tightly associated with the endosperm that limit seed oxidation and promote dormancy (30, 35, 84) (**Figure 2**).

The final seed dormancy levels depend on environmental factors during seed development, especially cold temperatures, which increase final seed dormancy levels. Seed development is delayed and is poorly characterized under cold temperatures. Whereas the anatomy of the mother plant can be modified in response to environmental cues, that of the final seed remains mostly unchanged by them. Therefore, cold must act on the functions pertaining to seed dormancy of the seed coat [e.g., its tannin content (88)] and endosperm or on the capacity of the embryo to respond to these functions, or both. Concrete cases are discussed in Section 4.

2.2. The Central Role of Abscisic Acid and the Endosperm

In *Arabidopsis*, high ABA levels first appear in seeds at the onset of seed maturation and are largely maternal in origin (63). Maternal ABA is gradually replaced by ABA synthesized by zygotic tissues as maturation progresses, and this zygote-derived ABA is more important for maintenance of primary dormancy before and after imbibition. These conclusions were confirmed by a later study (62). ABA levels fall upon dry seed imbibition irrespective of seed dormancy levels; however, repression of seed germination in imbibed dormant seeds is due to sustained high ABA accumulation over time (3, 74) (**Figure 1*b***). ABA accumulation upon imbibition activates ABA signaling to block seed germination and maintain the embryonic state (67). This process includes stimulation of the expression of *ABI3* and *ABI5*, which encode a B3 transcription factor and a basic leucine-zipper (bZIP) transcription factor, respectively, promoting the seed maturation protective program (82, 83). Notably, it also leads to expression of *LEA* genes, which encode osmotolerance proteins, and

Tannin: member of a group of phenolic compounds and a type of flavonoid that accumulates in the *Arabidopsis* seed coat and has antioxidant properties

the blockade of food store consumption in the embryo by blocking triacylglycerol catabolism (83, 118). ABA signaling involves the PYR/PYL/RCAR family of ABA receptors, group A type 2C protein phosphatases (PP2Cs), and Snf1-related protein kinases, group 2 (SnRK2s). ABA signals by binding to PYR/PYL/RCAR, enabling the sequestration of PP2Cs by direct interaction with them (87, 116). In turn, this sequestration enables SnRK2 activation through autophosphorylation, as SnRK2s are inhibited by PP2C-dependent dephosphorylation in the absence of ABA (139, 142). Activated SnRK2s phosphorylate downstream targets, such as ABI5, that repress seed germination (121). Detailed reviews of ABA signaling can be found elsewhere (29, 125).

ABA-INSENSITIVE 5 (ABI5): basic leucine-zipper transcription factor that represses seed germination in response to ABA

Snf1-related protein kinase family, group 2 (SnRK2): family of plant-specific serine/threonine kinases that play a major role in plant responses to ABA

DELAY OF GERMINATION (DOG1): protein promoting dormancy

Gibberellic acid (GA): isoprenoid plant hormone that promotes seed germination

DELLAs: members of the GRAS family of transcriptional regulators that lack a DNA-binding domain and are key negative regulators of GA signaling

ABA synthesized by both the endosperm and the embryo contribute to repress seed germination. However, in the *Arabidopsis* mature seed, the endosperm is essential to enable seed dormancy because its removal triggers the growth of the embryo even in the most dormant accessions (13, 74). Upon dormant seed imbibition, the endosperm releases ABA toward the embryo, likely through AtABCG exporters and importers in the endosperm and embryo, respectively (61). The endosperm of dormant seeds releases markedly more ABA than does that of nondormant seeds (74). The induction of ABA synthesis in nondormant seeds and even in *tt* mutants is sufficient to impose dormancy, showing that seed coat integrity ultimately affects dormancy via the ABA status of seeds (89).

Recent research has improved our understanding of how ABA signaling is regulated in seed dormancy, and this review focuses on the dormancy positive regulator DOG1 (see Section 3). However, although ABA signaling is undoubtedly strengthened in dormant seeds, how dormant and nondormant seeds regulate the level of ABA in the first place is virtually unknown. Maintenance of high ABA levels upon dormant seed imbibition is the combined result of initial ABA levels present in dry seeds and the rate of ABA catabolism and de novo synthesis. Numerous reports have provided evidence that, indeed, all these processes influence seed germination and therefore dormancy (e.g., 130).

The hormone gibberellic acid (GA) is usually described as an antagonist of ABA for the control of seed germination (80). Indeed, inhibition of GA synthesis in nondormant seeds blocks germination by promoting the accumulation of DELLA factors such as RGL2, which among the five *Arabidopsis* DELLA factors plays a major role in repressing germination (75). In turn, DELLA factors promote ABA accumulation in seeds, and evidence strongly suggests that endosperms lacking DELLA factors release less ABA (74, 121). Mutant seeds lacking DELLA factors are completely nondormant, even when set at cool temperatures (65). However, in highly dormant seeds, exogenous GA fails to downregulate RGL2 protein levels and does not markedly promote seed germination (74), perhaps because ABA can also raise DELLA protein levels independently of GA activity (1, 118, 121, 122). Therefore, dormancy could be a state of defective GA-mediated DELLA factor degradation, which triggers constitutive high ABA accumulation in seeds.

The cytochrome P450 ABA 8′-hydroxylases CYP707A1–CYP707A4 play a predominant role in ABA catabolism and, therefore, in determining ABA levels (72). ABA accumulated during seed maturation is catabolized upon imbibition in proportion to the expression of *CYP707A2*, and *cyp707a2* seeds have high ABA levels and are hyperdormant (95). *CYP707A2* expression peaks 3–6 h after imbibition in nondormant seeds, but this expression peak is absent in dormant seeds (95, 102). This timing is associated with a peak in the expression of morning-expressed gene *CCA1* in the circadian clock, which is also prevented in dormant seeds (119). Interestingly, *CCA1* couples nitrate signaling to the clock, and nitrate also strongly induces *CYP707A2* expression, suggesting that nitrate-derived signals induce ABA catabolism in nondormant seeds (91). In general, multiple environmental signals that regulate germination or dormancy appear to act by regulating *CYP707A2* shortly after imbibition, suggesting that *CYP707A2* expression is an early marker of future germination. *CYP707A2* and circadian responses to nitrate are regulated by a common

mechanism via NIN-Like Protein 8 (144, 153). Both *CYP707A1* and *CYP707A2* were identified as potential candidate genes for primary dormancy quantitative trait loci (QTLs) in a cross between accessions from Northern and Southern Europe (124). *CYP707A1* and *CYP707A3* are expressed mainly during the middle stages of seed development, and their expression is stimulated by the histone H3K27me3 demethylase RELATIVE OF EARLY FLOWERING 6 (REF6). Accordingly, *ref6* mutant seeds have enhanced ABA and dormancy levels (REF6 is discussed further in Section 4.2) (17, 131).

Fundamental questions in seed dormancy research are (*a*) the mechanism that leads to sustained high ABA levels in dormant seeds upon their imbibition and how it is established, (*b*) how dormancy levels are regulated during seed maturation, and (*c*) how the dry seed mechanism loses its activity as seeds age, for instance, during dry after-ripening or in response to dormancy-breaking treatments. These questions remain mostly unanswered.

3. THE ROLE OF DOG1 IN PROMOTING DORMANCY

3.1. *DOG1* Gene Expression Regulation and Product Function

Together with ABA, DOG1 is an important factor promoting seed dormancy in *Arabidopsis*. Numerous publications referring directly or indirectly to *DOG1* have recently been reviewed elsewhere (42, 112, 113). In this section, we focus on unresolved questions regarding the potential relationship of DOG1 with the dry seed dormancy mechanism and the ABA-dependent germination arrest program.

DOG1 encodes an α-helical protein of unknown function (12, 108). *DOG1* mRNA expression is enriched in seeds, where it is first detected 9 days after pollination (DAP), around the torpedo stage, and peaks during the mature green stage, around 16 DAP, before decreasing during late maturation stages (12, 99). Upon seed imbibition, *DOG1* mRNA levels drop to undetectable levels within 2 days, irrespective of dry seed age (12, 101). At the protein level, DOG1 accumulation during seed development follows that of *DOG1* mRNA accumulation during the rising phase; however, DOG1 protein levels remain constant beyond 16 DAP up to the dry seed stage, suggesting either that *DOG1* mRNA translation is stimulated during late maturation stages or that the protein is very stable (101). Consistent with the latter idea, DOG1 protein levels 48 h after imbibition are similar to those in dry seeds despite the disappearance of *DOG1* mRNA levels (101). How *DOG1* expression is regulated in seeds remains unclear. The transcription factor bZIP67 activates *DOG1* expression during seed maturation (14). In imbibed seeds, *DOG1* expression is not induced by ABA (41), but ABA does induce *DOG1* in vegetative tissues during drought via inhibition of antisense *DOG1* transcription (154). Studies have shown that *DOG1* transcript levels in dry seeds are highly dependent on the temperature during seed maturation, leading to the hypothesis that DOG1 is important for temperature-responsive seed dormancy (21, 65). Presently, how temperature mediates this change in *DOG1* mRNA levels is unknown. Bryant et al. (14) provided evidence that *DOG1* transcription is upregulated by bZIP67 specifically at low temperatures. However, Chen et al. (20) showed that *DOG1* transcript levels peak at approximately the same level at different temperatures but that at cool temperatures *DOG1* mRNA is too stable to decay prior to quiescence, leading to higher levels of stored DOG1 in mature dry seeds.

DOG1 interacts with itself (100). The self-binding requires a small, four-amino-acid domain at amino acids 13–16. Three main haplotypes were identified at these amino acids (100). The Col-0 type (D-SY) and Sei-0 type (D-RY) haplotypes have a single amino acid deletion that leads to very weak self-binding, whereas the Ler/Cvi type (ECCY) leads to strong self-binding. Experiments with transgenic plants expressing *DOG1* with different haplotypes provided evidence that DOG1 self-binding capacity is important for DOG1 promotion of seed dormancy (100).

DOG1 interacts with AHG1 and AHG3, two group A PP2Cs. *abg1* and *abg3* have strong hypersensitive germination arrest responses to ABA; therefore, AHG1 and AHG3 are negative regulators of ABA signaling (105, 108, 109). Interestingly, PYL/RCARs repress the activity of AHG3 in the presence of ABA but do not, or at least not as efficiently, repress that of AHG1, which lacks a key tryptophane residue involved in the binding of PYR/PYL/RCARs with group A PP2Cs (5, 138). AHG1 may therefore be a phosphatase that negatively regulates ABA responses even in the presence of ABA. For this reason, DOG1 has been proposed to promote dormancy by enhancing receptor-dependent ABA signaling (through interaction with AHG3) but also independently of PYR/PYL/RCARs (through interaction with AHG1).

3.2. *DOG1*: Unresolved Questions

So far, in vitro evidence has offered partial evidence regarding the capacity of DOG1 to inhibit PP2C activity. Indeed, Née et al. (105) did not detect decreased PP2C activity in the presence of DOG1 in vitro, whereas Nishimura et al. (108) reported weak inhibition. Therefore, whether DOG1 inhibits PP2C activity in vivo, perhaps with other factors, remains to be fully investigated. Furthermore, Nishimura et al. found that DOG1 amino acids 13–18 are required for DOG1 binding with AHG1. This domain also contains the amino acids needed for DOG1 self-binding. Nishimura et al. (108) used DOG1 from Col-0, which lacks strong self-binding capacity, whereas Née et al. (105) used DOG1 from Cvi, which has strong self-binding activity but also binds AHG1. Thus, the potential link between the capability of DOG1 to self-bind and its capacity to interact with AHG1 in vivo remains to be understood.

Recombinant DOG1 also binds bacterial heme independently of its interaction with AHG1 (104, 108). Strikingly, the DOG1 heme-binding site is essential to promote dormancy in transgenic plants overexpressing DOG1 (108). Heme-binding proteins can function as sensors for oxygen and nitric oxide (77). A prevailing view invokes accumulation of oxidative events in dry seeds as the process releasing dormancy during dry after-ripening (106). Thus, DOG1 could serve as a redox sensor to integrate oxidative events during seed development and/or after-ripening to regulate seed dormancy. Consistent with this notion, DOG1 isoelectric focusing changes during after-ripening (101). However, it remains to be understood how redox sensing by DOG1 is mechanistically integrated with its function to regulate ABA signaling through its interaction with PP2Cs.

It also remains to be understood at what developmental stage DOG1 functions to promote dormancy—that is, whether DOG1 function regulates the manufacture of the dry seed mechanism during seed development, whether it is an intrinsic component of the dry seed mechanism, or whether it is a component of the ABA-dependent germination arrest program activated upon seed imbibition (**Figure 3**). DOG1 could potentially act in all stages.

By virtue of its capacity to enhance ABA signaling, DOG1 could influence seed maturation, which is heavily regulated by ABA, and therefore the manufacture of the dry seed mechanism, including its initial dormancy levels (**Figure 3**). Consistent with this possibility, Dekkers et al. (34) showed that *DOG1* regulates the expression of hundreds of genes during seed maturation, including that of *ABI5*, and genetically interacts with *ABI3* to promote seed maturation. DOG1, by virtue of its potential redox-sensing function, could be an intrinsic part of the dry seed mechanism. However, available evidence indicates that DOG1 is not essential to establish dormancy. Indeed, *dog1* mutant seeds produced under cold temperatures exhibit dormancy, although at lower levels than wild-type seeds (65). Furthermore, available DSDS50 values in *Arabidopsis* accessions show that accessions with nonfunctional *DOG1* (i.e., lacking self-binding ability) can be as dormant as accessions with a functional *DOG1* (6, 90) (**Figure 4**).

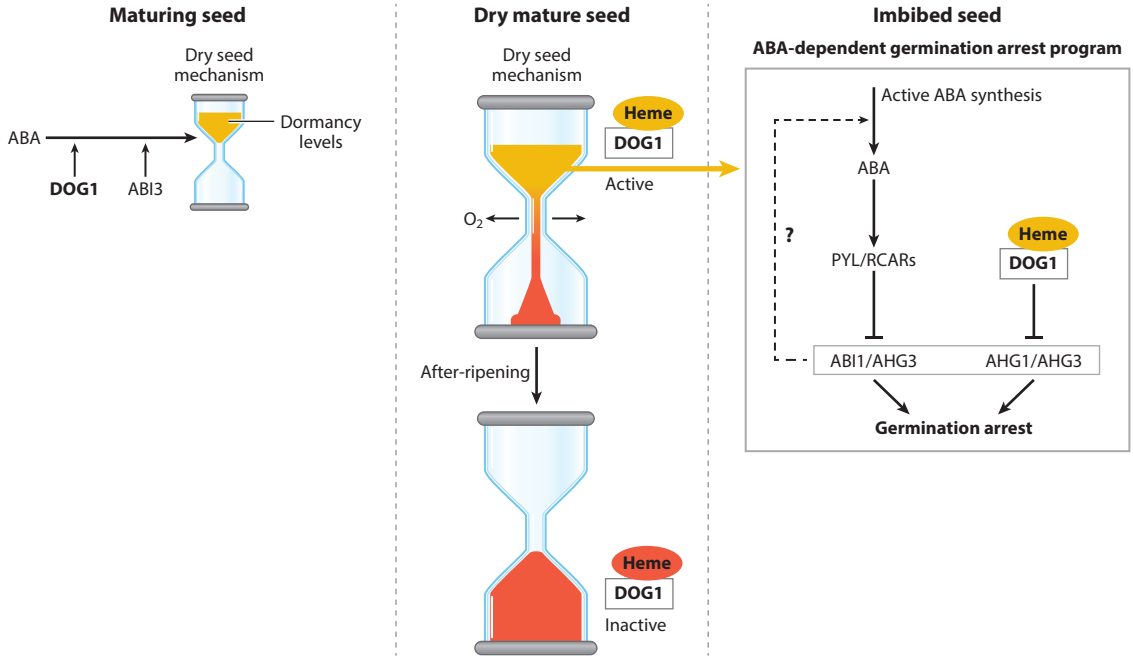

Figure 3

Model describing how DOG1 could promote dormancy during seed maturation, in the dry seed, and upon seed imbibition. During maturation, DOG1 could promote, together with ABI3, the manufacture of the time-dependent dormancy mechanism (*sand clock*) operating in dry seeds including its initial dormancy levels (*yellow sand*). In the dry seeds, dormancy levels gradually diminish, a phenomenon most likely driven by oxidative events. Heme-bound DOG1 could serve as an O_2 (oxidation) sensor. In absence of O_2 binding to heme, heme-bound DOG1 is active so as to enhance abscisic acid (ABA) signaling upon seed imbibition. Therefore, the amount of heme unbound to O_2 would determine dormancy levels. Dormancy levels would diminish upon binding of O_2 to heme (*red sand*) and would render heme-bound DOG1 inactive. ABA levels in dry seeds influence ABA signaling responses upon imbibition. However, crucially, dormant seeds maintain high ABA levels upon imbibition in a poorly understood manner. High ABA levels could be the result of enhanced ABA signaling mediated by heme-bound DOG1.

Upon imbibition, DOG1 could promote germination arrest by stimulating ABA signaling. However, dormant seeds can repress their germination for weeks upon seed imbibition. This raises a question about the nature of the regulation of DOG1 accumulation and activity over extended periods of time upon seed imbibition. DOG1 protein levels beyond 48 h following imbibition have not been reported, but if secondary dormancy is induced, then *DOG1* transcription reinitiates (16), and presumably *DOG1* protein levels rise again because DOG1 is necessary for secondary dormancy induction in *Arabidopsis* (44). If DOG1 activity is indeed linked to a redox-sensing function during seed after-ripening, then the DOG1 protein ought to be extremely stable to enable germination arrest for extended periods of time upon seed imbibition, unless an additional unknown mechanism is invoked.

Expression of *DOG1* under endosperm-specific promoters such as *FIS2* or *ZHOUPI* is sufficient to induce primary dormancy in *dog1* mutant seeds (101). Expression of *DOG1* in the embryo is less effective but does induce some primary dormancy, so DOG1 appears to act in both compartments during either seed maturation or imbibition. Overexpression of *DOG1* in *Lepidium sativum* inhibits endosperm weakening upon imbibition and delays germination (47), suggesting that *DOG1* exerts physiological activity if expressed in endosperm tissues. The endosperm is an essential source of ABA that implements dormancy upon seed imbibition, and whether DOG1

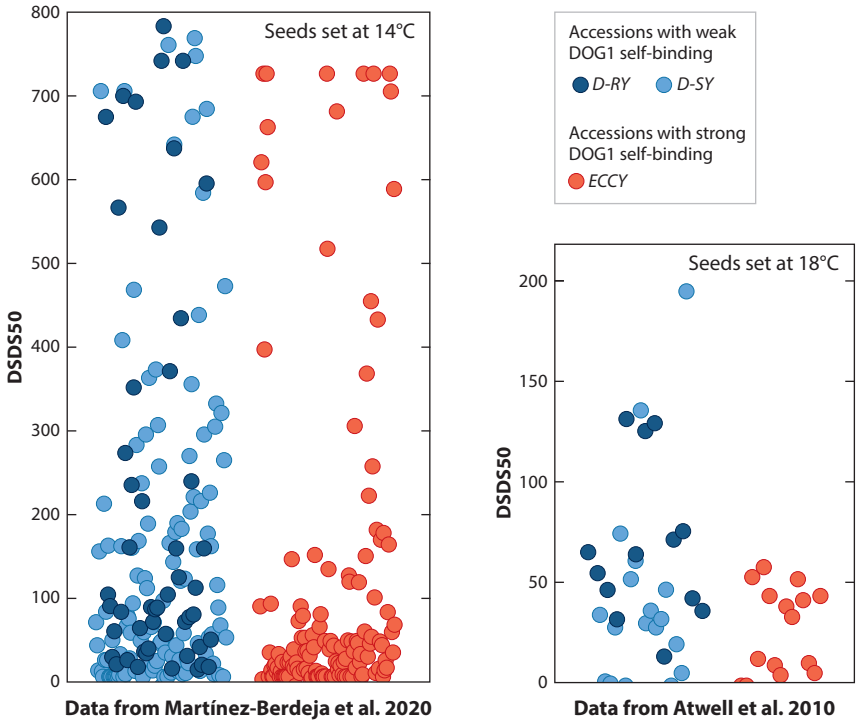

Figure 4

Dormancy levels among different *Arabidopsis* accessions. Days of seed dry storage required to reach 50% of germination (DSDS50) in different *Arabidopsis* accessions. Different haplotypes for the DOG1 amino acid sequence at the self-binding domain are indicated by different colors. The *ECCY* haplotype shows strong self-binding, whereas the *D-RY* and *D-SY* show weak self-binding (100). Given the rather large number of accessions, we spread them horizontally for clarity. DSDS50 data are from Martínez-Berdeja et al. (2020; Reference 90) (*left*) and Atwell et al. (2010; Reference 6) (*right*). Note that the temperatures during seed set differ between the two data sets, which affects final DSDS50 values.

promotes the release of ABA by the endosperm is unknown. DOG1 could stimulate ABA signaling in the embryo, while the dry seed mechanism could control release of ABA by the endosperm.

Lastly, the role of DOG1 needs to be understood in a wider phenological, environmental, and populational context. A genome-wide association study determined that *DOG1* self-binding haplotypes are associated with seed-chilling responses (i.e., the extent to which a seed from a given accession is able to germinate in response to chilling upon imbibition), rather than DSDS50 (90). This could be because DOG1 has a specific role in low-temperature responses or because chilling just happens to reduce dormancy to the ideal level for separation of accessions with different *DOG1* haplotypes. In a study by Martínez-Berdeja et al. (90), seeds were matured at low temperatures (14°C), which increased seed dormancy levels even in *dog1* mutants (65). Kerdaffrec et al. (66) showed that four single-nucleotide polymorphisms in the *DOG1* region, including one 21 kb upstream of the *DOG1* gene, are strong predictors of primary dormancy in Swedish accessions, and they identified a putative loss-of-function haplotype of *DOG1* common in accessions from northern Sweden. The molecular basis of this loss of function remains unclear. Low-dormancy *DOG1* haplotypes may be common in northern Sweden because the low growth temperature can generate sufficient primary dormancy, even in the absence of DOG1, to populate the soil seed bank.

In buried seeds, *DOG1* transcript levels follow an annual cycle, with high expression in winter and low expression during summer (43). But studies of germination timing in a Cvi × Bur-0 recombinant inbred population showed that, while a QTL for primary dormancy mapped to *DOG1*, QTLs for germination timing mapped elsewhere (45). Therefore, the genes that regulate seasonal germination timing remain to be uncovered.

4. MATERNAL AND ENVIRONMENTAL CONTROL OF SEED DORMANCY

4.1. Regulation of Seed Dormancy by the Maternal Environment

Multiple environmental signals during seed development, including temperature, photoperiod, light levels, and nutritional status, are known to affect *Arabidopsis* seed dormancy, with temperature being the dominant signal (54, 91, 133). These signals affect the final levels of ABA in mature dry seeds; for example, low temperatures and drought increase ABA levels (11, 65), whereas increasing nitrate supply to the mother plant reduces the ABA content and dormancy levels of its seed progeny (91). It is important to remember that seeds of low-dormancy accessions, such as Col-0, set in warm conditions show dormancy early during seed maturation, but that this primary dormancy is lost prior to shedding (63). In cold temperatures, ABA accumulates in seeds as in warm temperatures, but ABA catabolism is slower relative to synthesis such that more ABA remains at maturity, especially in the endosperm (20). Thus, at lower temperatures seeds do not exit primary dormancy prior to shedding. This effect of temperature is associated with lower expression of *CYP707A* genes during seed maturation (20). *CYP707A1* and *CYP707A3* appear to be especially important for determining the ABA level in mature seeds (114).

Arabidopsis populations show a strong tendency toward the winter-annual habit in the far north of Europe with low primary dormancy (66), while in Southern Europe they have higher dormancy levels and an increased preference for summer-annual behavior (96). The Col-0 accession from Central Europe can grow as a summer or winter annual, with artificial Col-0 soil seed banks showing both autumn and spring germination windows (137). Col-0 seed set from winter annuals in the field in York, United Kingdom, has dormancy levels equivalent to seed set at around 15°C in the laboratory, and modeling shows that 15°C is the favored mean seed set temperature in winter annual Col-0 (137). In contrast, Ler seed set in autumn from summer sowings has stronger primary dormancy than seed set at other times of year (31). Thus, seed dormancy is increased by seed set outside of summer, and genetic control of high dormancy levels is more important in warmer climates, where warm temperatures would otherwise promote too low a level of primary seed dormancy to maintain a soil seed bank.

4.2. Genomic Imprinting Is Involved in Maternal Inheritance of Seed Dormancy

Maternal inheritance of seed dormancy levels can be observed in F1 hybrid seeds generated by reciprocally crossing some *Arabidopsis* accessions with different dormancy levels. For example, the low-dormancy accession Col-0 pollinated with the high-dormancy accession Cvi produces hybrid seeds with lower dormancy than do hybrid seeds produced by the reciprocal cross. Similar maternal effects are observed in F1 hybrids arising from reciprocal crosses between Cvi and the low-dormancy accession C24 (120). These maternal effects could result from the maternal seed coat of the hybrid seeds and/or from genomic imprinting taking place in the endosperm.

Genomic imprinting is the preferential expression of a given parental allele over the other. This phenomenon is observed in mammals and seed plants, and in both cases, it occurs in tissues nourishing the embryo, the placenta, and the endosperm (10). In *Arabidopsis*, genomic imprinting had been studied mostly in the endosperm during seed development rather than in mature seeds.

DNA methylation: process by which methyl groups are added to DNA; typically acts to repress gene transcription

Piskurewicz et al. (120) identified a set of imprinted genes in the endosperm of mature hybrid seeds upon their imbibition. The vast majority of these genes are maternally expressed genes (MEGs). Interestingly, for many MEGs and paternally expressed genes (PEGs), imprinted expression is observed either only in the endosperm of dormant seeds or only in the endosperm of nondormant seeds, indicating that imprinted gene expression programs are adjusted according to the dormancy levels of the seed.

This is the case for *ALLANTOINASE* (*ALN*), which is a MEG only in dormant seeds. *ALN* encodes allantoin amidohydrolase, which participates in plant purine metabolism by converting allantoin to allantoate. In addition to its housekeeping function, allantoin plays a role in abiotic stress tolerance via activation of ABA metabolism (145). A mutation in *ALN* increases ABA levels, and *aln* mutant seeds are more dormant compared with wild-type seeds (120, 145). Furthermore, Piskurewicz et al. (120) showed that endospermic *ALN* expression from the maternal alleles, but not the paternal allele, regulates dormancy levels in seeds.

These results show that genomic imprinting in the endosperm can implement maternal inheritance of seed dormancy levels. Dozens of MEGs identified in the mature endosperm by Piskurewicz et al. are involved or potentially involved in regulating seed germination. Thus, given that single-nucleotide polymorphisms or other variations in MEG DNA sequences could alter gene function among *Arabidopsis* accessions, genomic imprinting might contribute to the maternal inheritance of seed dormancy levels in hybrid seeds arising from crosses between different accessions.

Various hypotheses have been proposed for the evolutionary driving force behind genomic imprinting. Haig's (49) kinship theory, which is the most widely accepted, stipulates that genomic imprinting evolved as a result of a parental conflict, such as over the distribution of food among progeny. In the case of plants, a given mother plant may bear seeds from different fathers. In Haig's view, the interest of the father is to maximize the food resources allocated to its descendants, while that of the mother is to distribute evenly those same food resources (50, 69, 147). Consistent with this view, some imprinted genes have been found to be involved in the control of seed size or nutrient uptake and allocation (10).

In most plant species, including *Arabidopsis*, most seeds are dispersed at relatively short distances (149) and are expected to germinate in the mother's close vicinity. If a seed inherits different alleles controlling dormancy, it would be beneficial for both parents to silence the paternal allele so as to confer maternal inheritance of seed dormancy, since most seeds are likely to grow under the local environmental conditions to which the mother plant is adapted, which is a scenario analogous to that invoked in the maternal-offspring coadaptation theory proposed by Wolf & Hager (150). However, it is also possible that imprinting is a random process related with silencing of transposable elements (see below), and imprinted expression of many genes is maintained without any adaptive selection. Further studies are needed to investigate the functions and natural variation of imprinted genes in relation to seed dormancy.

In both mammals and plants, two epigenetic repressive marks are involved in the regulation of genomic imprinting: DNA methylation and H3K27 trimethylation (H3K27me3) (10, 127). In plants, DNA methylation occurs at cytosines in all sequence contexts (CG, CHG, and CHH, where H stands for adenine, cytosine, or thymine), in contrast to mammals, where DNA methylation occurs almost exclusively in a CG sequence context. Before fertilization, the DNA glycosylase DEMETER (DME) is active in the central cell and removes methylated cytosines in any sequence context from specific loci through base-excision repair. The resulting hypomethylated central cell genome is inherited in the endosperm after fertilization, resulting in asymmetric DNA methylation between the maternal and paternal genomes (10, 24). DME preferentially targets transposable elements in euchromatic regions, leading to imprinted expression of nearby genes (56, 151).

Different DNA methyltransferases maintain DNA methylation for each sequence context. DNA METHYLTRANSFERASE 1 (MET1), CHROMOMETHYLTRANSFERASE3 (CMT3), and CHROMOMETHYLTRANSFERASE2 (CMT2) are responsible for maintenance of CG, CHG, and CHH methylation, respectively. In addition to those methyltransferases, DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2) mediates de novo methylation in all contexts through the RNA-directed DNA methylation (RdDM) pathway (40). MET1 is required for imprinting by maintaining paternal allele CG methylation during DNA replication (60). Furthermore, RdDM is required for paternal allele silencing at several loci (143).

Canonical RdDM involves the plant-specific RNA polymerases IV and V. Initial transcripts produced by polymerase IV from heterochromatic loci are converted to double-stranded RNAs by RNA-dependent RNA polymerase 2 (RDR2); the resulting double-stranded RNAs are then cleaved by DICER-LIKE 3 (DCL3) into 24-nt siRNAs. siRNAs are loaded into ARGONAUTE 4 (AGO4), forming an AGO4-siRNA complex. Transcripts generated by polymerase V function as a scaffold RNA enabling AGO4-siRNA complex recognition through sequence complementarity. The AGO4-siRNA complex further recruits DRM2, resulting in DNA methylation at the target region transcribed by polymerase V (93). Several alternative RdDM pathways, called noncanonical RdDM, can direct RdDM but without involving polymerase IV-RDR2-DCL3 (27).

ALN genomic imprinting involves DNA methylation established by noncanonical RdDM in the *ALN* promoter region, where the paternal allele has higher levels of CHH methylation compared with the maternal allele (57). The methylated region of the *ALN* promoter contains a transposable element belonging to the *AthPOGON1* family, which is known to be targeted by noncanonical RdDM involving RDR6 and AGO6 (RDR6-RdDM) (94). Indeed, CHH methylation of the *ALN* promoter does not necessitate polymerase IV. CHH methylation levels in the central cell are low in both wild-type and *dme* mutants, indicating that the asymmetric CHH methylation between maternal and paternal *ALN* alleles is not due to demethylation by DME in the central cell. CHH methylation of the *ALN* promoter is observed in sperm cells, endosperm, and embryo, but not in seedlings. While canonical RdDM functions ubiquitously, RDR6-RdDM functions in a tissue-specific manner due to the tissue-specific expression of *AGO6*. *AGO6* is expressed in mature pollen and developing seeds, where CHH methylation of the *ALN* promoter is observed. These results suggest that paternal allele methylation of *ALN* promoter is induced in the male germ cell lineage, which is maintained in the mature seed endosperm (**Figure 5**) (57).

DOG1-LIKE 4 (*DOGL4*) is one of five *DOG1-LIKE* (*DOGL*) genes. Both *DOG1* and *DOGL4* are expressed in developing and maturing seeds. *DOGL4* is necessary to induce the expression of numerous genes encoding seed storage proteins (128). In contrast to DOG1, which positively regulates seed dormancy, DOGL4 negatively regulates seed dormancy (157). *DOGL4* is a partially imprinted gene, which shows preferential expression from the maternal allele. Imprinting of *DOGL4* requires DNA methylation of the paternal allele in the −1 kb region of the *DOGL4* promoter, which is mediated by canonical RdDM. DNA methylation of the *DOGL4* promoter in both maternal and paternal alleles is not substantially increased in *dme* mutants, suggesting that imprinting of *DOGL4* does not require DME activity. In addition to *DME*, three other DNA glycosylase genes in *Arabidopsis* exist: *ROS1*, *DEMETER-like 2* (*DML2*), and *DEMETER-like 3* (*DML3*). While *DME* is preferentially expressed in the central cell and the pollen vegetative cell, *ROS1*, *DML2*, and *DML3* are ubiquitously expressed (2, 24, 46, 115). Mutations in *ROS1* result in increased DNA methylation in the promoter of the *DOGL4* paternal allele and in decreased *DOGL4* paternal allele expression, suggesting that ROS1 protects the paternal allele from hypermethylation and complete silencing. Mutations in *DML2* or *DML3* do not affect the DNA methylation levels in the *DOGL4* promoter, indicating that only ROS1 is involved in regulating *DOGL4* DNA demethylation. *ros1* mutant seeds have higher dormancy levels than do wild-type seeds, and

RNA-directed DNA methylation (RdDM): major epigenetic pathway in plants that mediates DNA methylation

Canonical RdDM: best-characterized RdDM; preferentially targets regions that are already DNA methylated

Noncanonical RdDM: recently identified RdDM; involved in DNA methylation at new target loci

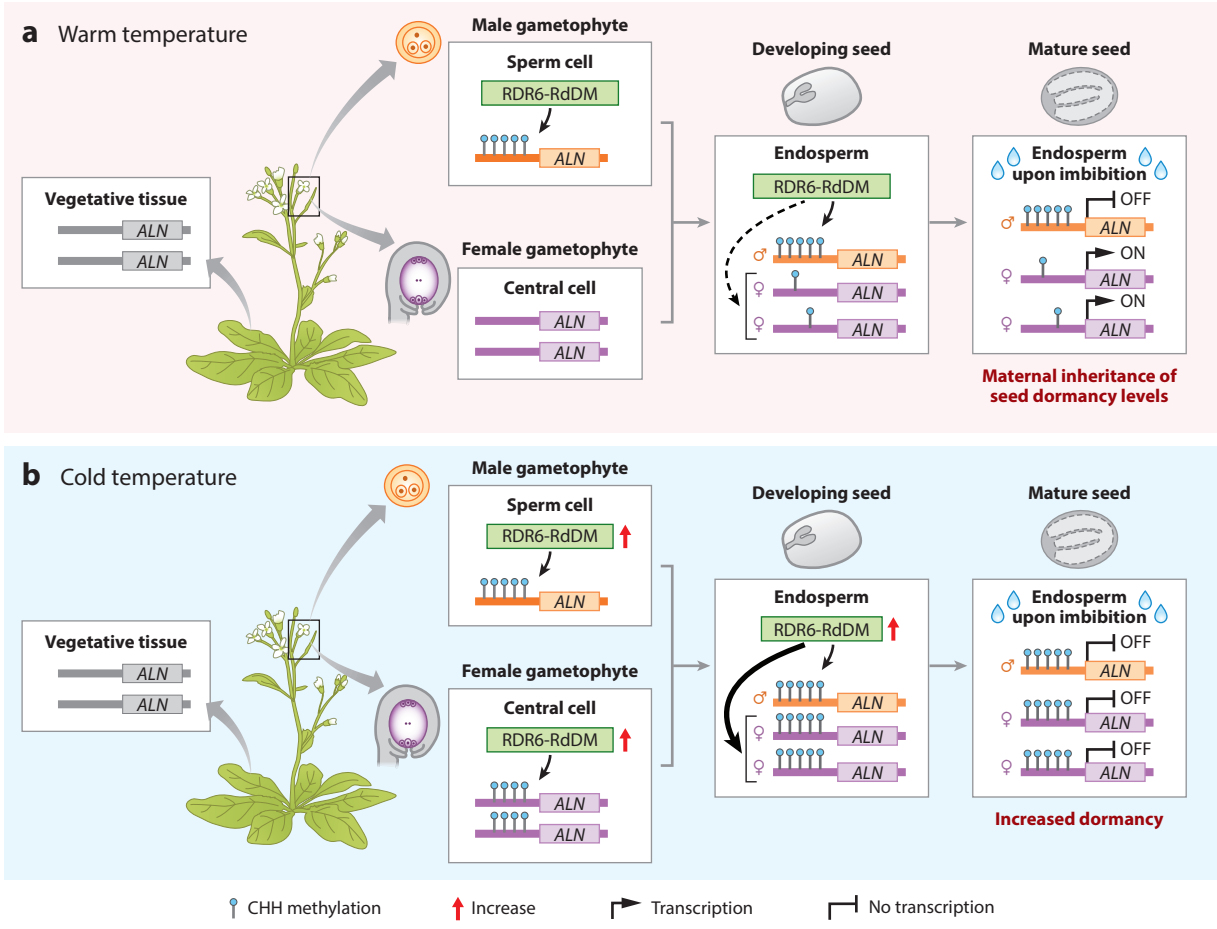

Figure 5

Epigenetic mechanisms for maternal and environmental control of seed dormancy through *ALN*. (*a*) Under warm temperatures, CHH methylation levels in the *ALN* promoter are low in vegetative tissues. During gametogenesis, CHH methylation is induced in sperm cells by noncanonical RNA-dependent RNA polymerase 6–RNA-directed DNA methylation (RDR6-RdDM). CHH methylation in the paternal allele is maintained during seed development; subsequently, in mature seeds, only the *ALN* maternal allele is expressed upon seed imbibition. (*b*) Under cold temperatures, RDR6-RdDM activity is enhanced in prefertilization ovular tissues and postfertilization ovular tissues, increasing CHH methylation of the *ALN* promoter in mature seeds and repressing *ALN* expression. In turn, suppression of *ALN* expression promotes seed dormancy. Adapted from Reference 57 (CC BY 4.0).

transgenic lines overexpressing *DOG4L* in *ros1* mutants show lower dormancy than *ros1* mutants, suggesting that ROS1 negatively regulates seed dormancy through *DOGL4* demethylation (157).

H3K27me3 is another epigenetic repressive mark involved in imprinted gene expression. H3K27me3 imprinting marks are mediated by Fertilization-Independent Seed–Polycomb Repressive Complex 2 (FIS-PRC2), consisting of MEDEA (MEA), FERTILIZATION-INDEPENDENT ENDOSPERM (FIE), FIS2, and MULTICOPY SUPPRESSOR OF IRA1 (MSI1), in central cells and are maintained after fertilization (10, 127). Studies in *Arabidopsis*, maize, and rice indicate that PEGs tend be more closely associated with H3K27me3 marks than MEGs (10, 37, 55, 68, 98, 126, 127, 155). Furthermore, in *Arabidopsis*, significant numbers of PEGs are associated with two additional repressive epigenetic marks, H3K9me2 and CHG methylation (97).

Recently, Sato et al. (131) showed that genes bearing H3K27me3, H3K9me2, and CHG methylation marks have a significant tendency to have their paternal allele preferentially expressed during germination, whereas genes bearing the single H3K27me3 mark have their maternal allele preferentially expressed. The expression of many genes with the single H3K27me3 mark is induced during germination but not in mutants lacking REF6, a Jumonji C (JmjC) domain–containing histone demethylase, while the expression of genes with the triple H3K27me3, H3K9me2, and CHG methylation marks is kept suppressed. This finding led Sato et al. (131) to propose that REF6 is able to activate genes with a single H3K27me3 mark on their maternal allele during germination in the endosperm, but not genes with the triple marks.

Earlier studies had shown that *ref6* mutants display hypermethylation of H3K27me3 in several hundred genes in seedlings (28, 76, 85). *ref6* mutants show divergent developmental phenotypes including enhanced seed dormancy, which is associated with increased ABA content in seeds. Chen et al. (17) showed that enhanced seed dormancy and ABA levels in *ref6* mutants are due mainly to the reduced expression of two ABA catabolism genes, *CYP707A1* and *CYP707A3*. REF6 directly binds to *CYP707A1* and *CYP707A3*, and their H3K27me3 levels in *ref6* mutants are higher than in wild type in developing seeds. This finding suggests that, during seed development, REF6 directly promotes the expression of *CYP707A1* and *CYP707A3* through demethylation of H3K27me3, thus leading to low ABA levels and reduced seed dormancy. Sato et al. (131) showed that REF6 activates the expression of genes involved in ethylene signaling, which is known to release dormancy by counteracting ABA responses. Therefore, REF6 appears to regulate seed dormancy in different developmental stages. Whether genes targeted by REF6 participate in the maternal inheritance of seed dormancy needs to be investigated.

4.3 Noncanonical RNA-Directed DNA Methylation Mediates Cold Temperature–Induced Seed Dormancy

Wild-type seeds produced under cold temperatures have higher final seed dormancy levels. Cold stimulates CHH methylation in the *ALN* promoter, which is associated with suppression of *ALN* expression upon seed imbibition. As for *ALN* imprinting, CHH methylation induced by cold also involves RDR6-RdDM. This might result from increased AGO6 accumulation and, therefore, activity under cold temperatures in developing seeds (57).

Under cold temperatures, transgenic lines overexpressing *ALN* produced seeds with low dormancy levels relative to wild-type seeds, suggesting that ABA homeostasis regulated by ALN is important for seed dormancy. Mutant seeds lacking RDR6-RdDM, such as *drm2*, *nrpe1*, *ago6*, and *rdr6* mutants, in which cold-induced CHH methylation of *ALN* promoter is not observed, are less dormant compared with wild type when produced under cold temperatures. Furthermore, *drm2 aln* seeds produced under cold temperatures have dormancy levels similar to those of wild-type seeds. These observations suggest that cold-induced CHH methylation of the *ALN* promoter participates in cold-induced seed dormancy (**Figure 5**). Interestingly, cold-induced CHH methylation overrides genomic imprinting as both parental alleles become hypermethylated.

Cold temperatures increase CHH methylation in seed tissues, endosperm, and embryo but not in other tissues, such as leaves or flowers, probably due to tissue-specific activity of RDR6-RdDM. Higher levels of CHH methylation in the embryo are lost in seedlings after germination as a result of the combination of cell divisions and absence of *AGO6* expression in seedlings (57).

In plants, CG methylation patterns are stably maintained throughout mitotic or meiotic cell divisions, except in a few tissues, such as the endosperm and the vegetative cell of the pollen grain (73, 92, 132). In contrast, CHH methylation patterns are subject to dynamical changes during seed development, including in response to environmental cues such as cold temperatures (38, 64, 134,

135, 146). In most cases, CHH methylation changes induced by environmental cues are transient. There are different resetting mechanisms of epigenetic changes induced by environmental cues. It has been proposed that the resetting mechanism enables the next plant generation to adapt to new environmental conditions (23, 25, 26, 58). In the case of *ALN*, cold-induced CHH methylation would be a mechanism allowing the seed to keep information about past cold temperatures in order to optimize seed germination timing (dormancy); however, this information is not maintained (see above) after germination, allowing optimal gene expression once again in the next generation (57).

4.4. Maternal Environmental Control of Seed Dormancy Mediated by Flowering Time Genes

The same genetic network that controls winter bud dormancy in plants also facilitates maternal environmental control of seed dormancy. Seeds in a carpel or fruit can be viewed as a specialized bud, with temperature responses in zygotic tissues coordinated with temperature-regulation of the properties of the surrounding seed coat, pericarp, or fruit. Thus, the reduction in seed dormancy caused by warmth is closely mirrored by changes in the fruit, with warm temperatures accelerating fruit development and increasing pod shattering (78). The activity of the maternal pathway has been revealed in *Arabidopsis* by assessing the effect of changes in temperature before fertilization of the seed (7, 18). Changes in ambient temperature or vernalization status can modify the expression of *FLOWERING LOCUS C* (*FLC*) in the mother plant. However, any silencing of the paternal *FLC* allele via H3K27me3 is eliminated during male gamete development (86), while the maternal allele carries a temperature memory at least until the onset of seed maturation. *FLC* can affect both proanthocyanidin synthesis in the seed coat and ABA signaling–related gene expression in the seed (18, 22). Maternal *FLOWERING LOCUS T* (*FT*) also affects seed dormancy, with *ft* mutants showing a dormancy increase that requires the downstream activity of maternal *FLC* (19). Although *FT* is highly expressed in fruits, the FT protein does not appear to move to the seed to control dormancy, as was first hypothesized (18), because while maternal FT reduces seed dormancy, direct expression of *FT* in seeds increases it (20). Instead, *FT* expression in the seed itself likely interferes with the activity of the seed-specific FT homolog MOTHER OF FT AND TFL1 (MFT). MFT increases dormancy in response to low temperature experienced by the seed (103). Interestingly *MFT* is regulated by the PIF family transcription factor SPATULA (SPT), which is stabilized at the protein level by cool temperatures (136, 140); however, it remains unclear whether SPT mediates the temperature regulation of *MFT* in *Arabidopsis* seeds. *MFT* is expressed in the endosperm, regulates hormone responses in imbibed seeds in response to light signals (8, 152), and acts during seed maturation to increase primary dormancy.

4.5. Additional Cases of Epigenetic Regulation of Seed Dormancy

The regulation of seed dormancy through distinct posttranslational modifications of histones has been further identified. Additional epigenetic mechanisms regulating seed dormancy have been described. However, whether these epigenetic regulations involve parental or environmental control of seed dormancy is covered elsewhere (110, 111) and remains to be elucidated.

4.5.1. H2B monoubiquitination. The *reduced dormancy4* (*rdo4*) mutant was isolated by a gamma-irradiation mutagenesis screen. *rdo4* mutants display pleiotropic phenotypes in the adult plants as well as reduced seed dormancy (117). Liu et al. (81) cloned *RDO4*, which was then renamed *HISTONE MONOUBIQUITINATION1* (*HUB1*). Mutations in *HUB2*, a homolog of *HUB1*, also cause reduced seed dormancy. *HUB1* and *HUB2* encode RING E3 ligases responsible for histone H2B monoubiquitination, which is generally associated with activation of

transcription. The expression of several genes regulating dormancy, such as *DOG1*, *ATS2*, *NCED9*, *PER1*, and *CYP707A2*, is significantly decreased in freshly harvested *hub1* seeds, suggesting that *HUB1* regulates seed dormancy by targeting those genes, either directly or indirectly (81).

4.5.2. H3K9 methylation. The *kryptonite* (*kyp*) mutant was originally isolated as a suppressor of silencing of the *SUPERMAN* locus. *KYP*, also known as *SUVH4*, encodes a histone methyltransferase required for H3K9 methylation, which is generally associated with transcriptional suppression (59). *kyp* mutants show enhanced seed dormancy levels and increased germination sensitivity to ABA and paclobutrazol, an inhibitor of GA biosynthesis. The expression of several dormancy-related genes, including *DOG1* and *ABI3*, is increased in *kyp* mutant seeds. *dog1 kyp* and *hub1 kyp* mutants show reduced dormancy similar to that of *dog1* or *hub1* single mutants, suggesting that *KYP/ SUVH4* regulates seed dormancy through the same pathway as *DOG1* and *HUB1* (156).

SUMMARY POINTS

1. Seed dormancy is an important adaptive trait determining the onset of the embryo-to-seedling transition.
2. Repression of seed germination upon imbibition of dormant seeds critically requires release of abscisic acid (ABA) from the endosperm.
3. A dry seed mechanism controls ABA metabolism upon seed imbibition according to dry seed dormancy levels.
4. DOG1 promotes dormancy, likely by interfering with ABA signaling.
5. Cold temperatures promote seed dormancy by modifying seed coat properties and epigenetic marks.
6. Maternal inheritance of seed dormancy levels involves paternal allele silencing through RNA-directed DNA methylation (RdDM).
7. Temperature control of flowering time has evolved to regulate seed dormancy.

FUTURE ISSUES

1. The mechanism that reduces dormancy levels in dry seeds remains elusive.
2. Oxidation events taking place in mature seeds are widely regarded as underlying the release of dormancy, but their exact nature is unknown.
3. How temperature affects ABA homeostasis during seed development needs to be better understood.
4. The mechanism maintaining high ABA levels in dormant seeds upon their imbibition remains to be understood.
5. Elucidating how environmental factors regulate the final anatomy and composition of the seed coat (e.g., its apoplastic barriers) is important for a better understanding of seed dormancy.
6. Despite substantial progress in understanding the function of DOG1, it remains unclear when DOG1 is important to promote dormancy.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Pedro Rodriguez, Eiji Nambara, Hiroyuki Nonogaki, Wim Soppe, and Noriyuki Nishimura for helpful discussions.

LITERATURE CITED

1. Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, et al. 2006. Integration of plant responses to environmentally activated phytohormonal signals. *Science* 311:91–94
2. Agius F, Kapoor A, Zhu JK. 2006. Role of the *Arabidopsis* DNA glycosylase/lyase ROS1 in active DNA demethylation. *PNAS* 103:11796–801
3. **Ali-Rachedi S, Bouinot D, Wagner MH, Bonnet M, Sotta B, et al. 2004. Changes in endogenous abscisic acid levels during dormancy release and maintenance of mature seeds: studies with the Cape Verde Islands ecotype, the dormant model of *Arabidopsis thaliana*. *Planta* 219:479–88**
4. Alonso-Blanco C, Bentsink L, Hanhart CJ, Blankestijn-de Vries H, Koornneef M. 2003. Analysis of natural allelic variation at seed dormancy loci of *Arabidopsis thaliana*. *Genetics* 164:711–29
5. Antoni R, Gonzalez-Guzman M, Rodriguez L, Rodrigues A, Pizzio GA, Rodriguez PL. 2012. Selective inhibition of clade A phosphatases type 2C by PYR/PYL/RCAR abscisic acid receptors. *Plant Physiol.* 158:970–80
6. Atwell S, Huang YS, Vilhjálmsson BJ, Willems G, Horton M, et al. 2010. Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. *Nature* 465:627–31
7. Auge GA, Blair LK, Neville H, Donohue K. 2017. Maternal vernalization and vernalization-pathway genes influence progeny seed germination. *New Phytol.* 216:388–400
8. Barros-Galvão T, Dave A, Cole A, Harvey D, Langer S, et al. 2019. *cis*-12-Oxo-phytodienoic acid represses Arabidopsis seed germination in shade conditions. *J. Exp. Bot.* 70:5919–27
9. Baskin JM, Baskin CC. 2004. A classification system for seed dormancy. *Seed Sci. Res.* 14:1–16
10. Batista RA, Köhler C. 2020. Genomic imprinting in plants—revisiting existing models. *Gene Dev.* 34:24–36
11. Benech RLA, Fenner M, Edwards PJ. 1991. Changes in germinability, ABA content and ABA embryonic sensitivity in developing seeds of *Sorghum bicolor* (L.) Moench. induced by water stress during grain filling. *New Phytol.* 118:339–47
12. Bentsink L, Jowett J, Hanhart CJ, Koornneef M. 2006. Cloning of *DOG1*, a quantitative trait locus controlling seed dormancy in *Arabidopsis*. *PNAS* 103:17042–47
13. **Bethke PC, Libourel IG, Aoyama N, Chung YY, Still DW, Jones RL. 2007. The *Arabidopsis* aleurone layer responds to nitric oxide, gibberellin, and abscisic acid and is sufficient and necessary for seed dormancy. *Plant Physiol.* 143:1173–88**
14. Bryant FM, Hughes D, Hassani-Pak K, Eastmond PJ. 2019. Basic LEUCINE ZIPPER TRANSCRIPTION FACTOR67 transactivates *DELAY OF GERMINATION1* to establish primary seed dormancy in *Arabidopsis*. *Plant Cell* 31:1276–88
15. Buijs G. 2020. A perspective on secondary seed dormancy in *Arabidopsis thaliana*. *Plants* 9:749
16. Cadman CS, Toorop PE, Hilhorst HW, Finch-Savage WE. 2006. Gene expression profiles of *Arabidopsis* Cvi seeds during dormancy cycling indicate a common underlying dormancy control mechanism. *Plant J.* 46:805–22
17. Chen HH, Tong JH, Fu W, Liang ZW, Ruan JX, et al. 2020. The H3K27me3 demethylase RELATIVE OF EARLY FLOWERING6 suppresses seed dormancy by inducing abscisic acid catabolism. *Plant Physiol.* 184:1969–78
18. **Chen M, MacGregor DR, Dave A, Florance H, Moore K, et al. 2014. Maternal temperature history activates Flowering Locus T in fruits to control progeny dormancy according to time of year. *PNAS* 111:18787–92**

3. Showed that dormant seeds sustain high abscisic acid levels upon seed imbibition.

13. Showed that the endosperm is necessary to repress dormant seed germination upon imbibition.

18. Showed that temperature during seed set regulates seed coat tannin content through FT.

19. Chen M, Penfield S. 2018. Feedback regulation of COOLAIR expression controls seed dormancy and flowering time. *Science* 360:1014–17
20. Chen X, Yoong FY, O'Neill CM, Penfield S. 2021. Temperature during seed maturation controls seed vigour through ABA breakdown in the endosperm and causes a passive effect on *DOG1* mRNA levels during entry into quiescence. *New Phytol.* 232:1311–22
21. Chiang GC, Bartsch M, Barua D, Nakabayashi K, Debieu M, et al. 2011. *DOG1* expression is predicted by the seed-maturation environment and contributes to geographical variation in germination in *Arabidopsis thaliana*. *Mol. Ecol.* 20:3336–49
22. Chiang GC, Barua D, Kramer EM, Amasino RM, Donohue K. 2009. Major flowering time gene, *FLOWERING LOCUS C*, regulates seed germination in *Arabidopsis thaliana*. *PNAS* 106:11661–66
23. Choi J, Hyun Y, Kang MJ, In Yun H, Yun JY, et al. 2009. Resetting and regulation of *FLOWERING LOCUS C* expression during *Arabidopsis* reproductive development. *Plant J.* 57:918–31
24. Choi Y, Gehring M, Johnson L, Hannon M, Harada JJ, et al. 2002. DEMETER, a DNA glycosylase domain protein, is required for endosperm gene imprinting and seed viability in *Arabidopsis*. *Cell* 110:33–42
25. Crevillen P, Yang H, Cui X, Greeff C, Trick M, et al. 2014. Epigenetic reprogramming that prevents transgenerational inheritance of the vernalized state. *Nature* 515:587–90
26. Crisp PA, Ganguly D, Eichten SR, Borevitz JO, Pogson BJ. 2016. Reconsidering plant memory: intersections between stress recovery, RNA turnover, and epigenetics. *Sci. Adv.* 2:e1501340
27. Cuerda-Gil D, Slotkin RK. 2016. Non-canonical RNA-directed DNA methylation. *Nat. Plants* 2:16163
28. Cui X, Lu FL, Qiu Q, Zhou B, Gu LF, et al. 2016. REF6 recognizes a specific DNA sequence to demethylate H3K27me3 and regulate organ boundary formation in *Arabidopsis*. *Nat. Genet.* 48:694–99
29. Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR. 2010. Abscisic acid: emergence of a core signaling network. *Annu. Rev. Plant Biol.* 61:651–79
30. De Giorgi J, Piskurewicz U, Loubéry S, Utz-Pugin A, Bailly C, et al. 2015. An endosperm-associated cuticle is required for *Arabidopsis* seed viability, dormancy and early control of germination. *PLOS Genet.* 11:e1005708
31. de Souza Vidigal D, He H, Hilhorst HWM, Willems LAJ, Bentsink L. 2020. *Arabidopsis* in the wild-the effect of seasons on seed performance. *Plants* 9:576
32. Debeaujon I, Leon-Kloosterziel KM, Koornneef M. 2000. Influence of the testa on seed dormancy, germination, and longevity in *Arabidopsis*. *Plant Physiol.* 122:403–14
33. Debeaujon I, Lepiniec L, Pourcel L, Routaboul J-M. 2007. Seed coat development and dormancy. In *Seed Development, Dormancy and Germination*, ed. K Bradford, H Nonogaki, pp. 25–43. Oxford, UK: Blackwell
34. Dekkers BJ, He H, Hanson J, Willems LA, Jamar DC, et al. 2016. The *Arabidopsis DELAY OF GERMINATION 1* gene affects *ABSCISIC ACID INSENSITIVE 5* (*ABI5*) expression and genetically interacts with ABI3 during *Arabidopsis* seed development. *Plant J.* 85:451–65
35. Demonsais L, Utz-Pugin A, Loubéry S, Lopez-Molina L. 2020. Identification of tannic cell walls at the outer surface of the endosperm upon *Arabidopsis* seed coat rupture. *Plant J.* 104:567–80
36. Donohue K, Rubio de Casas R, Burghardt L, Kovach K, Willis CG. 2010. Germination, postgermination adaptation, and species ecological ranges. *Annu. Rev. Ecol. Evol. Syst.* 41:293–319
37. Du M, Luo M, Zhang R, Finnegan EJ, Koltunow AM. 2014. Imprinting in rice: the role of DNA and histone methylation in modulating parent-of-origin specific expression and determining transcript start sites. *Plant J.* 79:232–42
38. Dubin MJ, Zhang P, Meng D, Remigereau MS, Osborne EJ, et al. 2015. DNA methylation in *Arabidopsis* has a genetic basis and shows evidence of local adaptation. *eLife* 4:e05255
39. Erbasol Serbes I, Palovaara J, Gross-Hardt R. 2019. Development and function of the flowering plant female gametophyte. *Curr. Top. Dev. Biol.* 131:401–34
40. Erdmann RM, Picard CL. 2020. RNA-directed DNA methylation. *PLOS Genet.* 16:e1009034
41. Fedak H, Palusinska M, Krzyczmonik K, Brzezniak L, Yatusevich R, et al. 2016. Control of seed dormancy in *Arabidopsis* by a *cis*-acting noncoding antisense transcript. *PNAS* 113:E7846–55
42. Finch-Savage WE, Footitt S. 2017. Seed dormancy cycling and the regulation of dormancy mechanisms to time germination in variable field environments. *J. Exp. Bot.* 68:843–56

32. Revealed the importance of flavonoid pigments to promote seed dormancy.

34. Showed that DOG1 promotes various seed maturation processes in addition to promoting dormancy.

43. Footitt S, Douterelo-Soler I, Clay H, Finch-Savage WE. 2011. Dormancy cycling in *Arabidopsis* seeds is controlled by seasonally distinct hormone-signaling pathways. *PNAS* 108:20236–41
44. Footitt S, Olcer-Footitt H, Hambidge AJ, Finch-Savage WE. 2017. A laboratory simulation of *Arabidopsis* seed dormancy cycling provides new insight into its regulation by clock genes and the dormancy-related genes *DOG1*, *MFT*, *CIPK23* and *PHYA*. *Plant Cell Environ.* 40:1474–86
45. Footitt S, Walley PG, Lynn JR, Hambidge AJ, Penfield S, Finch-Savage WE. 2020. Trait analysis reveals *DOG1* determines initial depth of seed dormancy, but not changes during dormancy cycling that result in seedling emergence timing. *New Phytol.* 225:2035–47
46. Gong ZH, Morales-Ruiz T, Ariza RR, Roldan-Arjona T, David L, Zhu JK. 2002. *ROS1*, a repressor of transcriptional gene silencing in *Arabidopsis*, encodes a DNA glycosylase/lyase. *Cell* 111:803–14
47. Graeber K, Linkies A, Steinbrecher T, Mummenhoff K, Tarkowska D, et al. 2014. *DELAY OF GERMINATION 1* mediates a conserved coat-dormancy mechanism for the temperature- and gibberellin-dependent control of seed germination. *PNAS* 111:E3571–80
48. Graeber K, Nakabayashi K, Miatton E, Leubner-Metzger G, Soppe WJ. 2012. Molecular mechanisms of seed dormancy. *Plant Cell Environ.* 35:1769–86
49. Haig D. 2014. Coadaptation and conflict, misconception and muddle, in the evolution of genomic imprinting. *Heredity* 113:96–103
50. Haig D, Westoby M. 1989. Parent-specific gene-expression and the triploid endosperm. *Am. Nat.* 134:147–55
51. Hamilton WD, May RM. 1977. Dispersal in stable habitats. *Nature* 269:578–81
52. Hanson T. 2015. *The Triumph of Seeds: How Grains, Nuts, Kernels, Pulses, and Pips Conquered the Plant Kingdom and Shaped Human History*. New York: Basic
53. Harris LW, Davies TJ. 2016. A complete fossil-calibrated phylogeny of seed plant families as a tool for comparative analyses: testing the 'time for speciation' hypothesis. *PLOS ONE* 11:e0162907
54. He H, Willems LA, Batushansky A, Fait A, Hanson J, et al. 2016. Effects of parental temperature and nitrate on seed performance are reflected by partly overlapping genetic and metabolic pathways. *Plant Cell Physiol.* 57:473–87
55. Hsieh TF, Shin JY, Uzawa R, Silva P, Cohen S, et al. 2011. Regulation of imprinted gene expression in *Arabidopsis* endosperm. *PNAS* 108:1755–62
56. Ibarra CA, Feng XQ, Schoft VK, Hsieh TF, Uzawa R, et al. 2012. Active DNA demethylation in plant companion cells reinforces transposon methylation in gametes. *Science* 337:1360–64

57. Identified a cold-induced RdDM pathway in seeds regulating seed dormancy.

57. Iwasaki M, Hyvärinen L, Piskurewicz U, Lopez-Molina L. 2019. Non-canonical RNA-directed DNA methylation participates in maternal and environmental control of seed dormancy. *eLife* 8:e37434

58. Iwasaki M, Paszkowski J. 2014. Identification of genes preventing transgenerational transmission of stress-induced epigenetic states. *PNAS* 111:8547–52
59. Jackson JP, Lindroth AM, Cao XF, Jacobsen SE. 2002. Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature* 416:556–60
60. Jullien PE, Kinoshita T, Ohad N, Berger F. 2006. Maintenance of DNA methylation during the *Arabidopsis* life cycle is essential for parental imprinting. *Plant Cell* 18:1360–72
61. Kang J, Yim S, Choi H, Kim A, Lee KP, et al. 2015. Abscisic acid transporters cooperate to control seed germination. *Nat. Commun.* 6:8113
62. Kanno Y, Jikumaru Y, Hanada A, Nambara E, Abrams SR, et al. 2010. Comprehensive hormone profiling in developing *Arabidopsis* seeds: examination of the site of ABA biosynthesis, ABA transport and hormone interactions. *Plant Cell Physiol.* 51:1988–2001
63. Karssen CM, Brinkhorst-van der Swan DLC, Breekland AE, Koornneef M. 1983. Induction of dormancy during seed development by endogenous abscisic acid: studies on abscisic acid deficient genotypes of *Arabidopsis thaliana* (L.) Heynh. *Planta* 157:158–65
64. Kawakatsu T, Nery JR, Castanon R, Ecker JR. 2017. Dynamic DNA methylation reconfiguration during seed development and germination. *Genome Biol.* 18:171
65. Kendall SL, Hellwege A, Marriot P, Whalley C, Graham IA, Penfield S. 2011. Induction of dormancy in *Arabidopsis* summer annuals requires parallel regulation of *DOG1* and hormone metabolism by low temperature and CBF transcription factors. *Plant Cell* 23:2568–80

66. Kerdaffrec E, Filiault DL, Korte A, Sasaki E, Nizhynska V, et al. 2016. Multiple alleles at a single locus control seed dormancy in Swedish *Arabidopsis*. *eLife* 5:e22502
67. Kinoshita N, Berr A, Belin C, Chappuis R, Nishizawa NK, Lopez-Molina L. 2010. Identification of *growth insensitive to ABA3* (*gia3*), a recessive mutation affecting ABA signaling for the control of early post-germination growth in *Arabidopsis thaliana*. *Plant Cell Physiol.* 51:239–51
68. Klosinska M, Picard CL, Gehring M. 2016. Conserved imprinting associated with unique epigenetic signatures in the *Arabidopsis* genus. *Nat. Plants* 2:16145
69. Köhler C, Weinhofer-Molisch I. 2010. Mechanisms and evolution of genomic imprinting in plants. *Heredity* 105:57–63
70. Koornneef M, Bentsink L, Hilhorst H. 2002. Seed dormancy and germination. *Curr. Opin. Plant Biol.* 5:33–36
71. Kucera B, Alan Cohn M, Leubner-Metzger G. 2005. Plant hormone interactions during seed dormancy release and germination. *Seed Sci. Res.* 15:281–307
72. Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, et al. 2004. The *Arabidopsis* cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. *EMBO J.* 23:1647–56
73. Law JA, Jacobsen SE. 2010. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat. Rev. Genet.* 11:204–20
74. Lee KP, Piskurewicz U, Tureckova V, Strnad M, Lopez-Molina L. 2010. A seed coat bedding assay shows that *RGL2*-dependent release of abscisic acid by the endosperm controls embryo growth in *Arabidopsis* dormant seeds. *PNAS* 107:19108–13
75. Lee S, Cheng H, King KE, Wang W, He Y, et al. 2002. Gibberellin regulates *Arabidopsis* seed germination via *RGL2*, a GAI/RGA-like gene whose expression is up-regulated following imbibition. *Genes Dev.* 16:646–58
76. Li CL, Gu LF, Gao L, Chen C, Wei CQ, et al. 2016. Concerted genomic targeting of H3K27 demethylase REF6 and chromatin-remodeling ATPase BRM in *Arabidopsis*. *Nat. Genet.* 48:687–93
77. Li T, Bonkovsky HL, Guo JT. 2011. Structural analysis of heme proteins: implications for design and prediction. *BMC Struct. Biol.* 11:13
78. Li XR, Deb J, Kumar SV, Ostergaard L. 2018. Temperature modulates tissue-specification program to control fruit dehiscence in Brassicaceae. *Mol. Plant* 11:598–606
79. Linkies A, Graeber K, Knight C, Leubner-Metzger G. 2010. The evolution of seeds. *New Phytol.* 186:817–31
80. Liu X, Hou X. 2018. Antagonistic regulation of ABA and GA in metabolism and signaling pathways. *Front. Plant Sci.* 9:251
81. Liu YX, Koornneef M, Soppe WJJ. 2007. The absence of histone H2B monoubiquitination in the *Arabidopsis hub1* (*rdo4*) mutant reveals a role for chromatin remodeling in seed dormancy. *Plant Cell* 19:433–44
82. Lopez-Molina L, Mongrand S, Chua NH. 2001. A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in *Arabidopsis*. *PNAS* 98:4782–87
83. Lopez-Molina L, Mongrand S, McLachlin DT, Chait BT, Chua NH. 2002. ABI5 acts downstream of ABI3 to execute an ABA-dependent growth arrest during germination. *Plant J.* 32:317–28
84. Loubéry S, De Giorgi J, Utz-Pugin A, Demonsais L, Lopez-Molina L. 2018. A maternally deposited endosperm cuticle contributes to the physiological defects of transparent testa seeds. *Plant Physiol.* 177:1218–33
85. Lu FL, Cui X, Zhang SB, Jenuwein T, Cao XF. 2011. Arabidopsis REF6 is a histone H3 lysine 27 demethylase. *Nat. Genet.* 43:715–U144
86. Luo X, Ou Y, Li R, He Y. 2020. Maternal transmission of the epigenetic 'memory of winter cold' in *Arabidopsis*. *Nat. Plants* 6:1211–18
87. Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, et al. 2009. Regulators of PP2C phosphatase activity function as abscisic acid sensors. *Science* 324:1064–68
88. MacGregor DR, Kendall SL, Florance H, Fedi F, Moore K, et al. 2015. Seed production temperature regulation of primary dormancy occurs through control of seed coat phenylpropanoid metabolism. *New Phytol.* 205:642–52

74. Showed that the endosperm of dormant seeds releases abscisic acid upon seed imbibition.

89. Martinez-Andújar C, Ordiz MI, Huang Z, Nonogaki M, Beachy RN, Nonogaki H. 2011. Induction of 9-*cis*-epoxycarotenoid dioxygenase in *Arabidopsis thaliana* seeds enhances seed dormancy. *PNAS* 108:17225–29
90. Martínez-Berdeja A, Stitzer MC, Taylor MA, Okada M, Ezcurra E, et al. 2020. Functional variants of *DOG1* control seed chilling responses and variation in seasonal life-history strategies in *Arabidopsis thaliana*. *PNAS* 117:2526–34
91. Matakiadis T, Alboresi A, Jikumaru Y, Tatematsu K, Pichon O, et al. 2009. The *Arabidopsis* abscisic acid catabolic gene *CYP707A2* plays a key role in nitrate control of seed dormancy. *Plant Physiol.* 149:949–60
92. Mathieu O, Reinders J, Caikovski M, Smathajitt C, Paszkowski J. 2007. Transgenerational stability of the *Arabidopsis* epigenome is coordinated by CG methylation. *Cell* 130:851–62
93. Matzke MA, Kanno T, Matzke AJ. 2015. RNA-directed DNA methylation: the evolution of a complex epigenetic pathway in flowering plants. *Annu. Rev. Plant Biol.* 66:243–67
94. McCue AD, Panda K, Nuthikattu S, Choudury SG, Thomas EN, Slotkin RK. 2015. ARGONAUTE 6 bridges transposable element mRNA-derived siRNAs to the establishment of DNA methylation. *EMBO J.* 34:20–35
95. Millar AA, Jacobsen JV, Ross JJ, Helliwell CA, Poole AT, et al. 2006. Seed dormancy and ABA metabolism in *Arabidopsis* and barley: the role of ABA 8′-hydroxylase. *Plant J.* 45:942–54
96. Montesinos-Navarro A, Pico FX, Tonsor SJ. 2012. Clinal variation in seed traits influencing life cycle timing in *Arabidopsis thaliana*. *Evolution* 66:3417–31
97. Moreno-Romero J, Del Toro-De León G, Yadav VK, Santos-González J, Köhler C. 2019. Epigenetic signatures associated with imprinted paternally expressed genes in the *Arabidopsis* endosperm. *Genome Biol.* 20:41
98. Moreno-Romero J, Jiang H, Santos-González J, Köhler C. 2016. Parental epigenetic asymmetry of PRC2-mediated histone modifications in the *Arabidopsis* endosperm. *EMBO J.* 35:1298–311
99. Mortensen SA, Sonderkaer M, Lynggaard C, Grasser M, Nielsen KL, Grasser KD. 2011. Reduced expression of the *DOG1* gene in *Arabidopsis* mutant seeds lacking the transcript elongation factor TFIIS. *FEBS Lett.* 585:1929–33
100. Nakabayashi K, Bartsch M, Ding J, Soppe WJ. 2015. Seed dormancy in *Arabidopsis* requires self-binding ability of DOG1 protein and the presence of multiple isoforms generated by alternative splicing. *PLOS Genet.* 11:e1005737
101. Nakabayashi K, Bartsch M, Xiang Y, Miatton E, Pellengahr S, et al. 2012. The time required for dormancy release in *Arabidopsis* is determined by DELAY OF GERMINATION1 protein levels in freshly harvested seeds. *Plant Cell* 24:2826–38
102. Nakabayashi K, Okamoto M, Koshiba T, Kamiya Y, Nambara E. 2005. Genome-wide profiling of stored mRNA in *Arabidopsis thaliana* seed germination: epigenetic and genetic regulation of transcription in seed. *Plant J.* 41:697–709
103. Nakamura S, Abe F, Kawahigashi H, Nakazono K, Tagiri A, et al. 2011. A wheat homolog of MOTHER OF FT AND TFL1 acts in the regulation of germination. *Plant Cell* 23:3215–29
104. Née G. 2014. *The identification of heme and flavin as cofactors of the dormancy protein DOG1*. Paper presented at 5th NIBB-MPIPZ-TLL Symposium: Horizons in Plant Biology, Cologne, Ger., Nov. 25

105. Showed that DOG1 interacts with AHG1 and AHG3, indicating that it regulates abscisic acid signaling.

105. Nee G, Kramer K, Nakabayashi K, Yuan B, Xiang Y, et al. 2017. DELAY OF GERMINATION1 requires PP2C phosphatases of the ABA signalling pathway to control seed dormancy. *Nat. Commun.* 8:72
106. Nee G, Xiang Y, Soppe WJ. 2017. The release of dormancy, a wake-up call for seeds to germinate. *Curr. Opin. Plant Biol.* 35:8–14
107. Nikolaeva MG, Rasumova MV, Gladkova VN. 1985. *Reference Book on Dormant Seed Germination*. Leningrad: Nauka

108. Showed that DOG1 binds heme, indicating a possible redox-sensing function.

108. Nishimura N, Tsuchiya W, Moresco JJ, Hayashi Y, Satoh K, et al. 2018. Control of seed dormancy and germination by DOG1-AHG1 PP2C phosphatase complex via binding to heme. *Nat. Commun.* 9:2132
109. Nishimura N, Yoshida T, Kitahata N, Asami T, Shinozaki K, Hirayama T. 2007. *ABA-Hypersensitive Germination1* encodes a protein phosphatase 2C, an essential component of abscisic acid signaling in *Arabidopsis* seed. *Plant J.* 50:935–49

110. Nonogaki H. 2014. Seed dormancy and germination—emerging mechanisms and new hypotheses. *Front. Plant Sci.* 5:233
111. Nonogaki H. 2017. Seed biology updates—highlights and new discoveries in seed dormancy and germination research. *Front. Plant Sci.* 8:524
112. Nonogaki H. 2019. Seed germination and dormancy: the classic story, new puzzles, and evolution. *J. Integr. Plant Biol.* 61:541–63
113. Nonogaki H. 2020. A repressor complex silencing ABA signaling in seeds? *J. Exp. Bot.* 71:2847–53
114. Okamoto M, Kuwahara A, Seo M, Kushiro T, Asami T, et al. 2006. *CYP707A1* and *CYP707A2*, which encode abscisic acid 8′-hydroxylases, are indispensable for proper control of seed dormancy and germination in *Arabidopsis*. *Plant Physiol.* 141:97–107
115. Ortega-Galisteo AP, Morales-Ruiz T, Ariza RR, Roldan-Arjona T. 2008. *Arabidopsis* DEMETER-LIKE proteins DML2 and DML3 are required for appropriate distribution of DNA methylation marks. *Plant Mol. Biol.* 67:671–81
116. Park SY, Fung P, Nishimura N, Jensen DR, Fujii H, et al. 2009. Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science* 324:1068–71
117. Peeters AJM, Blankestijn-de Vries H, Hanhart CJ, Leon-Kloosterziel KM, Zeevaart JAD, Koornneef M. 2002. Characterization of mutants with reduced seed dormancy at two novel *rdo* loci and a further characterization of *rdo1* and *rdo2* in *Arabidopsis*. *Physiol. Plant.* 115:604–12
118. Penfield S, Gilday AD, Halliday KJ, Graham IA. 2006. DELLA-mediated cotyledon expansion breaks coat-imposed seed dormancy. *Curr. Biol.* 16:2366–70
119. Penfield S, Hall A. 2009. A role for multiple circadian clock genes in the response to signals that break seed dormancy in *Arabidopsis*. *Plant Cell* 21:1722–32
120. Piskurewicz U, Iwasaki M, Susaki D, Megies C, Kinoshita T, Lopez-Molina L. 2016. Dormancy-specific imprinting underlies maternal inheritance of seed dormancy in *Arabidopsis thaliana*. *eLife* 5:e19573
121. Piskurewicz U, Jikumaru Y, Kinoshita N, Nambara E, Kamiya Y, Lopez-Molina L. 2008. The gibberellic acid signaling repressor RGL2 inhibits *Arabidopsis* seed germination by stimulating abscisic acid synthesis and ABI5 activity. *Plant Cell* 20:2729–45
122. Piskurewicz U, Tureckova V, Lacombe E, Lopez-Molina L. 2009. Far-red light inhibits germination through DELLA-dependent stimulation of ABA synthesis and ABI3 activity. *EMBO J.* 28:2259–71
123. Poisot T, Bever JD, Nemri A, Thrall PH, Hochberg ME. 2011. A conceptual framework for the evolution of ecological specialisation. *Ecol. Lett.* 14:841–51
124. Postma FM, Agren J. 2015. Maternal environment affects the genetic basis of seed dormancy in *Arabidopsis thaliana*. *Mol. Ecol.* 24:785–97
125. Raghavendra AS, Gonugunta VK, Christmann A, Grill E. 2010. ABA perception and signalling. *Trends Plant Sci.* 15:395–401
126. Rodrigues JA, Ruan R, Nishimura T, Sharma MK, Sharma R, et al. 2013. Imprinted expression of genes and small RNA is associated with localized hypomethylation of the maternal genome in rice endosperm. *PNAS* 110:7934–39
127. Rodrigues JA, Zilberman D. 2015. Evolution and function of genomic imprinting in plants. *Genes Dev.* 29:2517–31
128. Sall K, Dekkers BJW, Nonogaki M, Katsuragawa Y, Koyari R, et al. 2019. DELAY OF GERMINATION 1-LIKE 4 acts as an inducer of seed reserve accumulation. *Plant J.* 100:7–19
129. Sallon S, Solowey E, Cohen Y, Korchinsky R, Egli M, et al. 2008. Germination, genetics, and growth of an ancient date seed. *Science* 320:1464
130. Sano N, Marion-Poll A. 2021. ABA metabolism and homeostasis in seed dormancy and germination. *Int. J. Mol. Sci.* 22:5069
131. Sato H, Santos-González J, Köhler C. 2021. Combinations of maternal-specific repressive epigenetic marks in the endosperm control seed dormancy. *eLife* 10:e64593
132. Saze H, Mittelsten Scheid O, Paszkowski J. 2003. Maintenance of CpG methylation is essential for epigenetic inheritance during plant gametogenesis. *Nat. Genet.* 34:65–69
133. Schmuths H, Bachmann K, Weber WE, Horres R, Hoffmann MH. 2006. Effects of preconditioning and temperature during germination of 73 natural accessions of *Arabidopsis thaliana*. *Ann. Bot.* 97:623–34

134. Secco D, Wang C, Shou H, Schultz MD, Chiarenza S, et al. 2015. Stress induced gene expression drives transient DNA methylation changes at adjacent repetitive elements. *eLife* 4:e09343
135. Secco D, Whelan J, Rouached H, Lister R. 2017. Nutrient stress-induced chromatin changes in plants. *Curr. Opin. Plant Biol.* 39:1–7
136. Sidaway-Lee K, Josse EM, Brown A, Gan Y, Halliday KJ, et al. 2010. SPATULA links daytime temperature and plant growth rate. *Curr. Biol.* 20:1493–97
137. Springthorpe V, Penfield S. 2015. Flowering time and seed dormancy control use external coincidence to generate life history strategy. *eLife* 4:e05557
138. Tischer SV, Wunschel C, Papacek M, Kleigrewe K, Hofmann T, et al. 2017. Combinatorial interaction network of abscisic acid receptors and coreceptors from *Arabidopsis thaliana*. *PNAS* 114:10280–85
139. Umezawa T, Sugiyama N, Mizoguchi M, Hayashi S, Myouga F, et al. 2009. Type 2C protein phosphatases directly regulate abscisic acid–activated protein kinases in *Arabidopsis*. *PNAS* 106:17588–93
140. Vaistij FE, Barros-Galvão T, Cole AF, Gilday AD, He Z, et al. 2018. *MOTHER-OF-FT-AND-TFL1* represses seed germination under far-red light by modulating phytohormone responses in *Arabidopsis thaliana*. *PNAS* 115:8442–47
141. Venable DL. 1989. Modeling the evolutionary ecology of seed banks. In *Ecology of Soil Seed Banks*, ed. MA Leck, VT Parker, RL Simpson, pp. 67–87. San Diego, CA: Academic
142. Vlad F, Rubio S, Rodrigues A, Sirichandra C, Belin C, et al. 2009. Protein phosphatases 2C regulate the activation of the Snf1-related kinase OST1 by abscisic acid in *Arabidopsis*. *Plant Cell* 21:3170–84
143. Vu TM, Nakamura M, Calarco JP, Susaki D, Lim PQ, et al. 2013. RNA-directed DNA methylation regulates parental genomic imprinting at several loci in *Arabidopsis*. *Development* 140:2953–60
144. Walker L, Boddington C, Jenkins D, Wang Y, Gronlund JT, et al. 2017. Changes in gene expression in space and time orchestrate environmentally mediated shaping of root architecture. *Plant Cell* 29:2393–412
145. Watanabe S, Matsumoto M, Hakomori Y, Takagi H, Shimada H, Sakamoto A. 2014. The purine metabolite allantoin enhances abiotic stress tolerance through synergistic activation of abscisic acid metabolism. *Plant Cell Environ*. 37:1022–36
146. Wibowo A, Becker C, Marconi G, Durr J, Price J, et al. 2016. Hyperosmotic stress memory in *Arabidopsis* is mediated by distinct epigenetically labile sites in the genome and is restricted in the male germline by DNA glycosylase activity. *eLife* 5:e13546
147. Wilkins JF, Haig D. 2003. What good is genomic imprinting: the function of parent-specific gene expression. *Nat. Rev. Genet*. 4:359–68
148. Willis CG, Baskin CC, Baskin JM, Auld JR, Venable DL, et al. 2014. The evolution of seed dormancy: environmental cues, evolutionary hubs, and diversification of the seed plants. *New Phytol.* 203:300–9
149. Willson MF. 1993. Dispersal mode, seed shadows, and colonization patterns. *Vegetatio* 108:261–80
150. Wolf JB, Hager R. 2006. A maternal-offspring coadaptation theory for the evolution of genomic imprinting. *PLOS Biol.* 4:2238–43
151. Wolff P, Weinhofer I, Seguin J, Roszak P, Beisel C, et al. 2011. High-resolution analysis of parent-of-origin allelic expression in the *Arabidopsis* endosperm. *PLOS Genet.* 7:e1002126
152. Xi W, Yu H. 2010. *MOTHER OF FT AND TFL1* regulates seed germination and fertility relevant to the brassinosteroid signaling pathway. *Plant Signal. Behav.* 5(10):1315–17
153. Yan D, Easwaran V, Chau V, Okamoto M, Ierullo M, et al. 2016. NIN-like protein 8 is a master regulator of nitrate-promoted seed germination in *Arabidopsis*. *Nat. Commun.* 7:13179
154. Yatusevich R, Fedak H, Ciesielski A, Krzyczmonik K, Kulik A, et al. 2017. Antisense transcription represses *Arabidopsis* seed dormancy QTL *DOG1* to regulate drought tolerance. *EMBO Rep.* 18:2186–96
155. Zhang M, Xie SJ, Dong XM, Zhao X, Zeng B, et al. 2014. Genome-wide high resolution parental-specific DNA and histone methylation maps uncover patterns of imprinting regulation in maize. *Genome Res.* 24:167–76
156. Zheng J, Chen FY, Wang Z, Cao H, Li XY, et al. 2012. A novel role for histone methyltransferase KYP/SUVH4 in the control of *Arabidopsis* primary seed dormancy. *New Phytol.* 193:605–16
157. Zhu HF, Xie WX, Xu DC, Miki D, Tang K, et al. 2018. DNA demethylase *ROS1* negatively regulates the imprinting of *DOGL4* and seed dormancy in *Arabidopsis thaliana*. *PNAS* 115:E9962–70