CellPress
PARTNER JOURNAL

Molecular Plant
Review Article

Two Faces of One Seed: Hormonal Regulation of Dormancy and Germination

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http://dx.doi.org/10.1016/j.molp.2015.08.010

ABSTRACT

Seed plants have evolved to maintain the dormancy of freshly matured seeds until the appropriate time for germination. Seed dormancy and germination are distinct physiological processes, and the transition from dormancy to germination is not only a critical developmental step in the life cycle of plants but is also important for agricultural production. These processes are precisely regulated by diverse endogenous hormones and environmental cues. Although ABA (abscisic acid) and GAs (gibberellins) are known to be the primary phytohormones that antagonistically regulate seed dormancy, recent findings demonstrate that another phytohormone, auxin, is also critical for inducing and maintaining seed dormancy, and therefore might act as a key protector of seed dormancy. In this review, we summarize our current understanding of the sophisticated molecular networks involving the critical roles of phytohormones in regulating seed dormancy and germination, in which AP2-domain-containing transcription factors play key roles. We also discuss the interactions (crosstalk) of diverse hormonal signals in seed dormancy and germination, focusing on the ABA/GA balance that constitutes the central node.

Keywords: seed dormancy, germination, ABA, GA, auxin, crosstalk

Shu K., Liu X.-d., Xie Q., and He Z.-h. (2016). Two Faces of One Seed: Hormonal Regulation of Dormancy and Germination. Mol. Plant. **9**, 34–45.

INTRODUCTION

Seed dormancy is crucial to plant survival and ensures that seeds germinate only when environmental conditions are optimal. It thus is an adaptive trait in numerous seed-plant species, enabling wild plants to survive under stressful conditions in nature (Finkelstein et al., 2008). Most crops have been domesticated from wild species and show decreased levels of seed dormancy compared with their wild relatives, which ensures higher emergence rates after sowing (Lenser and Theissen, 2013; Meyer and Purugganan, 2013). However, the inappropriate loss or release of seed dormancy results in the rapid germination of freshly matured seeds or even pre-harvest sprouting (vivipary) in crops (Figure 1), causing substantial losses in yield and quality in agricultural production in addition to problems including post-harvest management and subsequent industrial utilization (Simsek et al., 2014).

Induction, maintenance, and thereafter release of seed dormancy are important physiological processes in seed plants. The ecological significance of seed dormancy includes preventing germination out of season, and consequently decreasing competition within species and ensuring plant survival under stressful conditions. As a complex and mysterious biological question, seed dormancy has attracted increasing attention from multi-disciplinary researchers, including plant biologists, crop geneticists, breeders, and food scientists. Nevertheless, it remains ‘‘one of the least understood phenomena in seed biology’’ (Finkelstein et al., 2008), despite considerable progress over past decades (Graeber et al., 2012; Rajjou et al., 2012). In this review, we summarize the mechanisms underlying the regulation of seed dormancy and germination, and focus on the emerging findings concerning the phytohormone network controlling this transition, mostly from studies with the model plant *Arabidopsis thaliana*.

Published by the Molecular Plant Shanghai Editorial Office in association with Cell Press, an imprint of Elsevier Inc., on behalf of CSPB and IPPE, SIBS, CAS.

Figure 1. Representative Image of the Pre-harvest Sprouting Phenotype of Rice in the Field.
Pre-harvest sprouting of crops often occurs when mature plants encounter prolonged rainfall and high humidity during the harvest season, which decreases yields and grain quality and also causes problems in industrial process. Red arrows indicate sprouting seeds on panicles.

DISTINCT PROCESSES OF SEED DORMANCY AND GERMINATION

Seed dormancy and germination has been studied intensively and extensively in the past; however, what constitutes seed dormancy at the molecular level remains largely unknown. Here, we attempt to address this question from a new viewpoint based on recent progress.

Seed dormancy ensures that seeds germinate at the appropriate time. Therefore, during maturation, the embryo must be kept in a quiescent state, mobilizing almost no stored nutrients and undergoing no cell division or elongation. In this quiescent state, germination-promoted genes are not actively expressed. Therefore, the radicle does not penetrate the testa and endosperm. It is now widely recognized that the chromatin structure determines gene expression and thereby regulates multitudinous developmental processes. In recent years, many genes associated with chromatin remodeling have been reported to regulate seed dormancy and germination (Liu et al., 2007; Saez et al., 2008; Wang et al., 2011a; Cho et al., 2012; Zheng et al., 2012). Emerging evidence shows that ABA (abscisic acid) is also involved in chromatin remodeling (Chinnusamy et al., 2008). For example, the histone methyltransferase gene *KYP/SUVH4* is repressed by ABA (Zheng et al., 2012), while histone acetyltransferase *HvGNAT/MYST* is induced by ABA (Papaefthimiou et al., 2010), and the epigenetic regulators *HUB1* and *RDO2* are strikingly up-regulated during the induction of seed dormancy (Liu et al., 2011). These investigations indicated that the epigenetic regulatory-related genes possess key roles during seed maturation, which thereafter affect the seed dormancy establishment process (Figure 2).

We propose that subsequently, during the germination process, seed dormancy may be related to a characteristic chromatin structure in certain regions of chromosomes in the seed, where germination-promoted genes cannot be activated even in the presence of related transcription factors because their binding sites are unavailable due to steric hindrance, with phytohormones also involved in this process. In contrast, dormancy release leading to germination is a process in which the chromatin structure is modified by cold stratification or after-ripening treatments, making the germination-promoted genes available for transcription, resulting in cell elongation and division, seed coat and endosperm rupture, and finally emergence of the radicle when conditions are favorable.

Although dormancy is established during seed maturation, whereas exogenous ABA application (or even maternal ABA in the plant during seed development) only inhibits seed germination but fails to induce seed dormancy; only ABA synthesized by the seed can establish dormancy (Kucera et al., 2005). Thus, the differently localized ABA in plant tissues possesses distinct effects on seed dormancy or germination. In addition, ABI5 is an important positive regulator in the ABA-signaling pathway, and its loss-of-function mutant *abi5* is insensitive to ABA-mediated inhibition of seed germination; however, *abi5* does not show altered seed dormancy (Finkelstein, 1994; Brocard-Gifford et al., 2003; Finkelstein et al., 2008). Furthermore, DOG1 (Delay of Germination 1) is a key player in the induction and maintenance of seed dormancy, but ABA sensitivity is unchanged in *dog1* (Nakabayashi et al., 2012). A new study demonstrated that DOG1 mediates a conserved coat-dormancy mechanism including the temperature- and gibberellin (GA)-dependent pathways (Graeber et al., 2014). Subsequent studies suggested the importance of epigenetic regulation for DOG1. Histone demethylases LDL1 (LYSINESPECIFIC DEMETHYLASE LIKE 1) and LDL2 repress seed dormancy by regulating *DOG1* (Zhao et al., 2015), and chromatin remodeling of DOG1 is also involved in dormancy cycling (Footitt et al., 2015). Furthermore, the histone methyltransferases KRYPTONITE (KYP)/SUVH4 and SUVH5 repress *DOG1* and *ABI3* transcription during seed maturation (Zheng et al., 2012) (Table 1). These studies demonstrated that the DOG1-mediated regulation pathway might be distinct from the ABA and/or GA pathway (Figure 2). These observations suggest that distinct signaling pathways may be adopted in the regulation of seed dormancy and seed germination.

ABA AND GA, THE MAJOR DETERMINANTS: NEWLY EMERGING EVIDENCE

It is widely recognized that ABA and GA are the primary hormones that antagonistically regulate seed dormancy and germination (Gubler et al., 2005; Finkelstein et al., 2008; Graeber et al., 2012; Hoang et al., 2014; Lee et al., 2015a). During seed maturation, endogenous ABA accumulates in the seed, inducing and maintaining seed dormancy and thus preventing

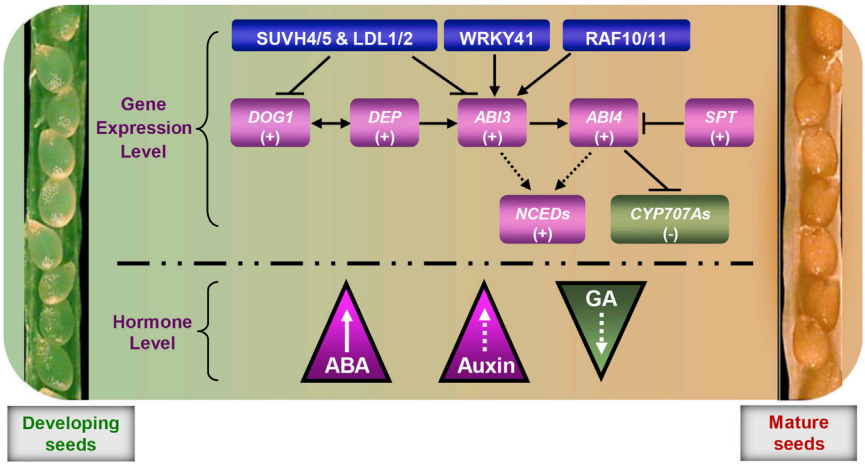

Figure 2. Changes in Accumulation of Key Hormones and Expression of Key Regulators during Seed Maturation.

Several key regulators are involved during the seed maturation process, and constitute a complex network. At the gene expression level, transcription levels of the two important ABA catabolic genes *CYP707A1* and *CYP707A3* are down-regulated, while the ABA biosynthesis genes including the *NCEDs* genes are up-regulated, by ABI4 and other regulators, thus ABA accumulates to initiate dormancy. The other key dormancy-controlling regulator genes, including *ABI3*, *ABI4*, *DOG1*, *DEP*, and *SPT*, are activated during seed maturation to induce and maintain primary seed dormancy, and some of these genes interact with each other to regulate seed dormancy levels. Among them, SUVH4, SUVH5, LDL1, and LDL2 negatively regulate *DOG1* and *ABI3* transcription, while WRKY41 and RAF10/11 directly control *ABI3* expression. At the phytohormone level, ABA accumulates and seed dormancy is initiated, established, and maintained during seed development. However, the genetics of whether auxin biosynthesis is up-regulated and GA biosynthesis is down-regulated is not yet understood. The active lines with upward arrows indicate the change of ABA level, while dashed lines indicate the changes of auxin and GA level. The symbol (+) indicates the elevated transcription level, while (−) indicates the decreased expression level during seed maturation. The black arrows and bars indicate the positive and negative regulatory roles, respectively.

vivipary (Figure 2). In contrast, before the onset of the germination process the endogenous ABA level in the seed is down-regulated, while the GA content is up-regulated with imbibition and stratification treatments.

ABA is a major inducer and protector of seed dormancy (Vaistij et al., 2013). Seeds of typical ABA-deficient mutants germinate faster than the wild-type (Frey et al., 2011), and transgenic plants constitutively expressing the ABA biosynthesis gene maintain deep seed dormancy (Martinez-Andujar et al., 2011; Nonogaki et al., 2014). Conversely, ABA catabolism mutants accumulate high ABA levels and thus cause hyperdormancy in seeds (Matakiadis et al., 2009) (Table 1). In addition to ABA biogenesis, the ABA-signaling-dependent pathway also affects seed dormancy. During the seed germination process, ABA signaling must be desensitized, whereby the membrane-associated transcription factor peptidases S1P (Site-1 Protease) and S2P, process the bZIP17 protein from the endoplasmic reticulum (ER) to the Golgi and then to nucleus; and subsequently, the activated bZIP17 regulates the downstream transcription of ABA-signaling negative regulators (Zhou et al., 2015a). ABA acts through the PYR/PYL/RCAR–PP2C–SnRKs signaling cascade (Cutler et al., 2010; Hubbard et al., 2010). The PP2C proteins, ABI1 and ABI2, bind to the ABA receptors to inhibit signaling. Their dominant-negative mutants *abi1-1* and *abi2-1* show reduced seed dormancy due to the failure of interaction between the mutated proteins and receptors (Ma, 2009; Park et al., 2009). Another PP2C protein, HONSU, also acts as a negative regulator of seed dormancy by concurrently inhibiting ABA signaling and activating GA signaling (Kim et al., 2013), suggesting that HONSU is a key factor in mediating the ABA and GA crosstalk concerning seed dormancy. Unexpectedly, a newly identified PP2C gene, *RDO5* (*Reduced Dormancy 5*), shows the strongly reduced seed dormancy phenotype, but its ABA sensitivity and content remain unchanged (Xiang et al., 2014). Further genetic and bioinformatics analysis demonstrated that RDO5 regulates seed dormancy through mediating the transcription of the PUF family of RNA binding genes, *APUM9* (*Arabidopsis PUMILIO 9*) and *APUM11* (Xiang et al., 2014). This evidence suggests that the RDO5-mediated regulation pathway is distinct from the ABA-signaling pathway, and further detailed investigation is needed.

As the major downstream component of ABA signaling, ABI3 is a main regulator of seed dormancy and germination (Bentsink and Koornneef, 2008). *ABI3* expression is regulated by *DEP* (*DESPIERTO*), which is involved in ABA sensitivity during seed development, and *dep* seeds show complete dormancy loss (Barrero et al., 2010). WRKY41 regulates *Arabidopsis* seed dormancy also through directly controlling *ABI3* transcription during seed maturation and germination (Ding et al., 2014). Another key component in the ABA-signaling pathway, ABI4, was also described as a positive regulator of primary seed dormancy (Shu et al., 2013). Subsequent studies demonstrated that MYB96, the ABA-responsive R2R3-type MYB transcription factor, positively regulates seed dormancy and negatively regulates germination through mediating expression of *ABI4* and ABA biogenesis genes, including *NCED2* and *NCED6* (Lee et al., 2015a, 2015b) (Table 1). Furthermore, a study showed that calcium also regulates seed germination by affecting *ABI4* transcription that controls ABA signaling (Kong et al., 2015). These studies demonstrated the key regulatory roles of positive regulators in ABA signaling during the transition from seed dormancy to germination.

Although ABI5 has no effect on seed dormancy and does not affect dormancy level (Finkelstein, 1994), this transcription factor negatively regulates seed germination (Piskurewicz et al., 2008; Kanai et al., 2010), suggesting the distinct signaling pathways for ABA-mediated seed dormancy and ABA-inhibited seed germination discussed above. A recent study showed that the MAP3K (mitogen-activated protein kinase kinase kinase) genes, *RAF10* and *RAF11*, regulate seed dormancy by affecting *ABI3* and *ABI5* transcription (Lee et al., 2015c). At post-transcription level, PKS5 (SOS2-like Protein Kinase 5, also known as CIPK11 or SnRK3.22) phosphorylates the special residue

Gene name	Dormancy level of mutant	General description of genes	References
ABI3	Decreased	Positively regulates ABA signaling and represses seed germination	Finkelstein, 1994
ABI5	Not changed	Positively regulates ABA signaling and represses seed germination	Brocard-Gifford et al., 2003; Finkelstein, 1994; Finkelstein et al., 2008
ABI4	Decreased	Positively regulates ABA signaling and represses seed germination	Shu et al., 2013; Kong et al., 2015
NCED5	Decreased	ABA biosynthesis gene, and the ABA content is decreased	Frey et al., 2011
CYP707A1/2	Enhanced	ABA-inactivated gene, and ABI4 negatively regulates its transcription	Millar et al., 2006; Matakiadis et al., 2009; Shu et al., 2013
GA1/2	Enhanced	GA biosynthesis genes, and the GA content is decreased in mutants	Lee et al., 2002
GA2oxs	Decreased	GA-inactivated genes, and the GA content is up-regulated in mutants	Yamauchi et al., 2007
RGL2/SPY	Enhanced	GA signaling is blocked in mutants	Jacobsen and Olszewski, 1993; Lee et al., 2002
MYB96	Decreased	Decreases *ABI4* and some ABA biogenesis gene transcription	Lee et al., 2015a, 2015b
DOG1	Enhanced	ABA sensitivity of *dog1* seeds is unchanged	Nakabayashi et al., 2012; Graeber et al., 2014
SUVH4/SUVH5	Enhanced	Repress *DOG1* and *ABI3* transcription	Zheng et al., 2012
LDL1/LDL2	Enhanced	Repress seed dormancy by negatively regulating *DOG1*	Zhao et al., 2015
WRKY41	Decreased	Directly promotes *ABI3* transcription	Ding et al., 2014
RAF10/RAF11	Decreased	Directly enhances *ABI3* transcription	Lee et al., 2015c
DEP	Decreased	Promotes *ABI3* transcription	Barrero et al., 2010
SPT	Decreased in *Ler*, while enhanced in *Col* background	Opposite roles in *Ler* and *Col* ecotypes	Belmonte et al., 2013; Vaistij et al., 2013
ARF10/ARF16	Decreased	Directly promote *ABI3* transcription	Liu et al., 2013b
BIN2	Not mentioned	Phosphorylates and stabilizes ABI5 to enhancing ABA signaling	Hu and Yu, 2014
PKS5	Not mentioned	Phosphorylates ABI5 (Ser42) and controls transcription of ABA-responsive genes	Zhou et al., 2015b
HONSU	Enhanced	As a PP2C protein, and impairs ABA signaling	Kim et al., 2013
RDO5	Enhanced	ABA sensitivity and content remain unchanged	Xiang et al., 2014
ABI1/2	Decreased	Dominant-negative mutants, and thus the mutated proteins cannot interact with ABA receptors	Ma, 2009; Park et al., 2009
CHO1	Decreased	Acts upstream on ABI4 genetically	Yamagishi et al., 2009; Yano et al., 2009
OsAP2-39	Decreased	Promotes *OsNCED1* and *OsEUI*, and thus enhances ABA biogenesis and impairs GA accumulation	Yaish et al., 2010
DDF1	Decreased	Directly promotes *GA2ox7* and thus decreases GA content	Magome et al., 2008

Table 1. Key Genes Involved in Seed Dormancy and Germination.

(Ser42) in ABI5 and controls transcription of ABA-responsive genes and, consequently, precisely regulates ABA signaling and the germination process (Zhou et al., 2015b) (Table 1). Altogether, the endogenous ABA level and ABA signaling positively regulate seed dormancy and therefore negatively regulate seed germination, and some key genes are involved in these physiological processes (Figure 2).

Another key phytohormone, GA, breaks dormancy and stimulates germination by antagonistically suppressing ABA-triggered seed dormancy (Gubler et al., 2005; Graeber et al., 2012). High GA levels or GA signaling promote seed germination, possibly from the secretion of hydrolytic enzymes to weaken seed testa structure (Holdsworth et al., 2008), but the detailed mechanisms, especially in *Arabidopsis*, are largely

unknown. GA-deficient mutants, such as *ga1* and *ga2*, show strong seed dormancy and fail to germinate without exogenous GA treatment (Lee et al., 2002; Shu et al., 2013). In contrast, mutants defective in GA2-oxidases (GA2ox), which deactivate bioactive GA, show decreased seed dormancy (Yamauchi et al., 2007). Similarly, mutations in DELLA genes including *RGL2* (*RGA-LIKE2*) and *SPY* (*SPINDLY*), negative regulators of the GA-signaling pathway, can rescue the non-germination phenotype of *ga1* (Jacobsen and Olszewski, 1993; Lee et al., 2002). Furthermore, DELLAs also maintain the seed embryo in a quiescent state by restricting cell-cycle progression through repression of the activities of TCP14 (Teosinte branched1/Cycloidea/Proliferating cell factor) and TCP15 (Resentini et al., 2015), further supporting the ''quiescent state'' hypothesis of the embryo described above.

KEY ROLES OF AP2 DOMAIN-CONTAINING TRANSCRIPTION FACTOR IN SEED DORMANCY REGULATION

The ABA/GA balance determines the fate of a seed: high endogenous ABA and low GA levels result in deep seed dormancy and low emergence, while low ABA and high GA levels induce preharvest sprouting. Therefore, the ABA/GA balance must be strictly regulated. There are two major aspects of the ABA/GA balance: the balance of hormone levels and the balance of the signaling cascades. The question arises: in the ABA–GA interaction, which is cause and which is effect? It has been reported that ABA is involved in the suppression of GA biogenesis (Seo et al., 2006), and GA also negatively regulates ABA biogenesis during seed germination (Shu et al., 2013; Oh et al., 2007). Therefore, ABA and GA may interact as both cause and effect during this process. However, the molecular mechanisms involved in precisely controlling the ABA/GA balance were largely unknown up to now, with AP2 domain-containing transcription factors found to possess the pivotal roles.

Numerous previous studies demonstrated that ABI4 is a versatile factor that regulates many signaling pathways, including the responses to ABA, glucose, sucrose, ethylene (ET), and salt stress (Wind et al., 2013). Interestingly, ABI4 also positively regulates ABA catabolism genes, but negatively affects GA biogenesis genes; thus, the loss of *ABI4* function increases the expression of GA biosynthesis genes but decreases the expression of GA-inactivation genes, together leading to decreased primary seed dormancy in *abi4* (Shu et al., 2013). As an AP2 domain-containing transcription factor, ABI4 directly binds to the promoters of *CYP707A1* and *CYP707A2*, which function in ABA catabolism, subsequently promoting ABA accumulation. However, no direct targeting of GA metabolism genes by ABI4 has been detected so far, suggesting that ABI4 may not bind directly to the promoters of GA biogenesis genes but may recruit or activate an additional seed-specific transcription factor to repress the transcription of GA biogenesis genes. Nevertheless, in sorghum, SbABI4 and SbABI5 can directly bind to the promoter of *SbGA2ox3*, likely activating its expression and affecting seed dormancy (Cantoro et al., 2013). Further investigations of ABI4-repressed GA signaling may identify the missing link in the ABI4–GA signaling crosstalk.

It is noted that *ABI4* transcription is regulated by the transcription factor SPT (SPATULA), which is also a key factor in seed dormancy regulation pathways; its role depends on the ecotype background (Vaistij et al., 2013); furthermore, the expression of *SPT* is increased during seed maturation (Belmonte et al., 2013), suggesting that the *SPT–ABI4* module takes the key role during dormancy establishment and maintenance (Figure 2). Similarly, another AP2 domain-containing transcription factor, CHO1 (CHOTTO1), positively regulates seed dormancy and, more interestingly, acts upstream on ABI4 genetically (Yamagishi et al., 2009; Yano et al., 2009). In rice, a model monocot, the AP2 domain-containing transcription factor OsAP2-39, directly promotes transcription of the ABA biosynthesis gene *OsNCED1* and expression of the GA-inactivating gene *OsEUI* (*ELONGATED UPPERMOST INTERNODE*), thus enhancing ABA biogenesis and impairing GA accumulation (Yaish et al., 2010). Consequently, the transgenic overexpression of *OsAP2-39* leads to increased seed dormancy (Yaish et al., 2010). These phenotypes have been documented in GA-deficient mutants (Richter et al., 2013), indicating that OsAP2-39 plays a pivotal role in regulating the ABA/GA biogenesis balance. DDF1 (DELAYED FLOWERING 1), a further player and another AP2-class transcription factor, directly promotes transcription of the GA-inactivation gene *GA2ox7*, and thus remarkably decreases endogenous GA content (Magome et al., 2008). The next player, EBE (ERF BUD ENHANCER), also an AP2 domain-containing transcription factor, has been shown to positively regulate seed dormancy (Mehrnia et al., 2013). Altogether, these AP2-containing transcription factors negatively regulate GA biogenesis while positively regulating ABA biogenesis.

Consequently, we propose that the AP2 domain plays a critical but cryptic role in the dual regulation of ABA and GA biogenesis in fine-tuning seed dormancy and germination (Figure 2). It is speculated that a DNA motif may be among these regulators and may possess undiscovered functions regarding seed dormancy regulation, especially for the ABA/GA balance concerning biogenesis and/or signaling levels. Furthermore, because these genes positively regulate endogenous ABA and decrease GA levels, transgenic overexpression lines show deep dormancy levels and other undesirable agronomic traits, given that the optimal endogenous hormone levels are essential to normal plant development. Consequently, the negative regulation of these transcription factors (ABI4, DDF1, OsAP2-39, and CHO1) is important for normal seed dormancy, and these factors must be strictly regulated at the mRNA and protein levels by these unknown negative regulators. Finally, further screening for suppressors of these mutants (e.g. *abi4*, *ddf1*, *osap2-39*, and *cho1*) might provide important information about the genetic network of the AP2 family in seed dormancy and germination.

AUXIN: A NEW MASTER PLAYER IN SEED DORMANCY

The phytohormone auxin is involved in almost all aspects of plant development and in response to a multitude of environmental cues (Zhao, 2010). Previously, auxin alone was not considered a key regulator of seed germination, although it participates in crosstalk with ABA (Wang et al., 2011a). Exogenous auxin

application suppresses seed germination under high salinity (Park et al., 2011), indicating that this hormone plays an important role in seed dormancy and germination in response to environmental stimuli. Earlier studies revealed that IAA (indole-3-acetic acid) can delay seed germination and inhibit pre-harvest sprouting in wheat (Ramaih et al., 2003); ABA represses embryonic axis elongation during seed germination also by potentiating auxin signaling (Belin et al., 2009); and a next study suggested that after-ripening treatment-mediated dormancy release is associated with decreased seed sensitivity to auxin (Liu et al., 2013a). All these observations imply that auxin may play a role in regulating seed dormancy and germination.

Emerging genetic data show that auxin protects and strictly regulates seed dormancy alongside ABA (Liu et al., 2013b). Evidence for this conclusion is provided by the dormancy variation among seeds with altered auxin synthesis genes. Auxin-overproducing transgenic *iaaM-OX* seeds show higher IAA levels compared with wild-type seeds, while *yuc1/yuc6* seeds show lower IAA content. Consistently, *iaaM-OX* seeds exhibit strong seed dormancy, while *yuc1/yuc6* seeds show the opposite phenotype. Phenotypic analysis demonstrated that nearly all auxin-signaling mutants, including *tir1/afb3* and *tir1/afb2*, show a decreased seed dormancy level. These observations reveal a close positive correlation between auxin content/signaling and seed dormancy, as also found for ABA.

What is the mechanism by which auxin controls seed dormancy? Detailed genetic and biochemical evidence shows that ABI3 is required for auxin-mediated seed dormancy and germination. When auxin levels are low, the auxin-responsive transcription factors *ARF10* and *ARF16* are repressed by AXR2/3. Thus, the expression of *ABI3* cannot be activated by ARF10/ARF16, and seed dormancy cannot be maintained. In contrast, when auxin levels are high, ARF10 and ARF16 are released to activate *ABI3* transcription, and seed dormancy is maintained. Since ARF10 and ARF16 likely do not directly bind to the *ABI3* promoter (Liu et al., 2013b), they may recruit or activate an additional seed-specific transcription factor(s) to stimulate *ABI3* expression; thus, further screening of dormancy mutants is needed to identify the missing link in the *ARF10/16–ABI3* signaling cascade. In summary, auxin affects ABA signaling to achieve its physiological effect (Liu et al., 2013b). Whether auxin also affects *ABI4* and *ABI5* is a worthwhile project in future studies.

In addition, many endogenous and environmental signals can also affect auxin content and distribution, thus shaping plant development (Vanneste and Friml, 2009). This poses an interesting question: do the same signals affect auxin biogenesis or signaling to regulate seed dormancy during seed development?

DIVERSE REGULATORS: OTHER PHYTOHORMONES INVOLVED IN SEED DORMANCY AND GERMINATION

In addition to ABA, GA, and auxin, nearly all other phytohormones are also likely involved in modulation of seed dormancy and germination, including ethylene (ET), brassinosteroids (BRs), jasmonic acid (JA), salicylic acid (SA), cytokinins (CTKs), and strigolactones (SLs).

ET breaks seed dormancy and promotes seed germination by counteracting the effect of ABA (Arc et al., 2013b; Corbineau et al., 2014). Mutations in positive regulators of the ET signaling pathway result in deep dormancy, while the negative regulator *ctr1* (*Constitutive Triple Response 1*) seeds germinate more rapidly (Subbiah and Reddy, 2010). Several studies have demonstrated that ET negatively affects ABA biogenesis and signaling (Cheng et al., 2009; Linkies et al., 2009). Previous studies showed that ET may affect seed germination through an ABA/GA-independent pathway (Linkies and Leubner-Metzger, 2012), and ABA and ET regulate seed dormancy by the antagonistic effect, which is mediated by key factors, such as *SNL1* (*SIN3-LIKE1*) and *SNL2* (Wang et al., 2013), suggesting a diversification of seed dormancy regulation mechanisms during evolutionary history. Interestingly, a recent study showed that the ET receptors ETR1 (Ethylene Response 1) and ETR2 possess contrasting roles for ABA biosynthesis during seed germination under salt-stress conditions, which may be independent of ET signaling (Wilson et al., 2014). However, whether and how ET affects GA biogenesis and signaling regarding seed dormancy and germination is largely unknown so far.

During seed germination, BR-deficient or BR-signaling mutants show stronger responses to ABA compared with wild-type seeds, indicating that BR overcomes the inhibitory effect of ABA on germination (Steber and McCourt, 2001). BR was found to promote seed germination in opposition to ABA partly through an MFT (MOTHER OF FT AND TFL1)-mediated pathway, which forms a negative feedback loop to modulate ABA signaling (Xi and Yu, 2010; Xi et al., 2010). A further elegant study demonstrated that BIN2 (Brassinosteroid Insensitive 2), a key repressor of the BR-signaling pathway, phosphorylates and stabilizes ABI5 protein to mediate ABA signaling during seed germination, whereby BR treatment represses the BIN2–ABI5 interaction, thus antagonizing ABA-mediated inhibition (Hu and Yu, 2014). However, the detailed mechanisms underlying the BR–GA crosstalk are elusive. For example, does BR induce GA biogenesis or enhance GA signaling during germination? More research on the effect of BR on seed dormancy is clearly needed.

SA is a plant hormone mainly associated with various defense pathways. Circumstantial evidence suggests that SA also regulates seed germination as a bifunctional modulator. SA inhibits germination by inhibiting the expression of GA-induced α-amylase genes under normal growth conditions (Xie et al., 2007). However, it promotes germination under high salinity via another pathway that reduces oxidative damage (Lee et al., 2010b). CTKs promote seed germination by antagonizing ABA, specifically by down-regulating *ABI5* transcription (Wang et al., 2011b). Further study demonstrated that CTKs antagonize ABA signaling by inducing ABI5 protein degradation (Guan et al., 2014). These observations highlight the importance of *ABI5* at both mRNA and protein levels, and ABI5 is the pivot involved in CTK–ABA crosstalk. It is noteworthy that although CTKs have positive effects on germination, CTK-receptor mutants exhibit lower dormancy levels compared with wild-type seeds (Riefler et al., 2006). These inverse effects suggest that there are distinct pathways in the CTK-mediated seed germination

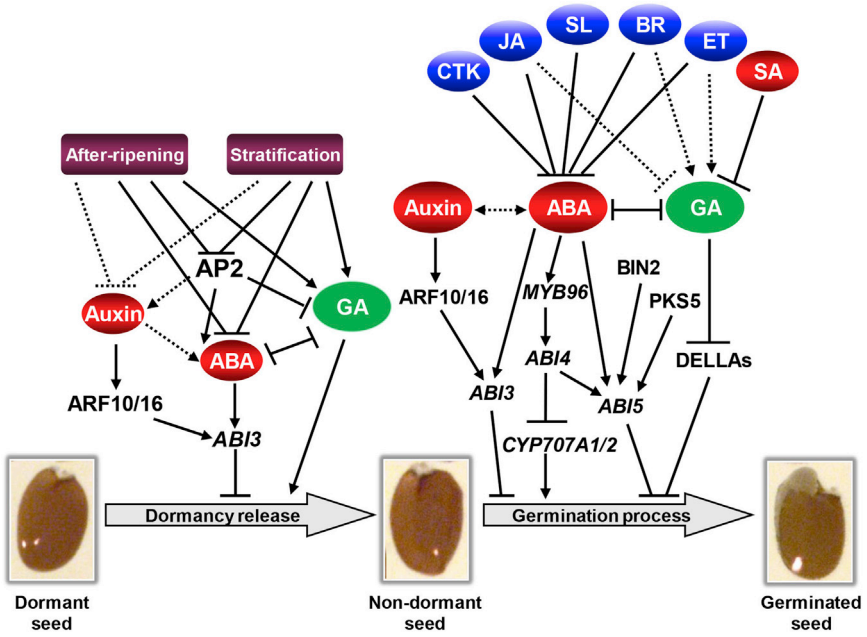

Figure 3. Preliminary Network of Phytohormone Functions in Seed Dormancy and Germination.

Dormancy release and germination of the seed are two separate but continuous phases. Freshly matured seeds are dormant and contain high levels of ABA and probably auxin, and low GA contents, resulting from changes in hormone biogenesis during seed development, as described in Figure 2. As the first phase in seed germination, after-ripening or stratification treatments break seed dormancy (dormancy release) by regulating ABA, GA, and auxin biogenesis and/or signals. These three hormones may interact to precisely control seed dormancy. In particular, ABA and auxin positively regulate seed dormancy in an interdependent manner, with auxin promoting *ABI3* transcription. Furthermore, AP2 domain-containing transcription factors, including ABI4, DDF1, OsAP2-39, and CHO1, positively regulate seed dormancy by promoting ABA biogenesis and repressing GA biogenesis/accumulation. The remaining question is whether AP2 domain-containing transcription factors also regulate auxin biogenesis and/or signaling. After seed dormancy is broken, non-dormant seeds initiate germination in the second phase. Different hormones affect this process by regulating the ABA/GA balance at either the biogenesis or signaling levels. The transcription factors ARFs, MYB96, ABI3, ABI4, and ABI5, the downstream target genes including *CYP707A1* and *CYP707A2*, and the GA-signaling negative regulators DELLAs play key roles in this process. Being a key factor, ABI5 was regulated precisely at transcription and post-transcription levels (ABI4 enhancing its expression while BIN2 and PKS5 phosphorylate ABI5). As the final step of seed germination, GA induced, but ABA inhibited, the rupture of the seed coat and enabled the radicle to penetrate the coat and complete emergence. The ABA/GA balance is the core determinant node in both steps. Arrows indicate positive regulation and bars indicate negative regulation.

regulation pathway. In future investigations, whether CTK affects GA biogenesis and/or signaling during the transition from dormancy to germination will be a pertinent topic.

Exogenous JA application delays seed germination (Nambara et al., 2010), indicating that JA has an inhibitory effect on the germination process. Interestingly, however, JA separately represses the transcription of ABA biosynthesis genes and promotes ABA-inactivating genes (Jacobsen et al., 2013), suggesting an antagonistic effect between JA and ABA. Consistent with this hypothesis, *coi1-16* and *jar1*, two JA-signaling mutants, show an ABA hypersensitive phenotype during germination (Fernandez-Arbaizar et al., 2012). However, it remains unclear why the role of JA in seed germination is sometimes contradictory. SLs are a small class of carotenoid-derived compounds that regulate many aspects of plant development, through the signaling pathway with D53 (*DWARF 53*) as a repressor (Umehara et al., 2008; Jiang et al., 2013; Zhou et al., 2013). SLs are host-derived germination stimulants for the seeds of parasitic weeds (Cook et al., 1966). They also trigger seed germination in other species, evidently by reducing the ABA/GA ratio (Toh et al., 2012). Furthermore, some key components in the SL signaling pathway affect seed germination, including *SMAX1* (*Suppressor of More Axillary Growth2 1*) in *Arabidopsis* (Stanga et al., 2013) and *OsD53* (Jiang et al., 2013; Zhou et al., 2013), which is the homolog of *SMAX1* in rice. However, the precise regulatory mechanisms underlying SLs need further investigation.

In summary, these plant hormones, including ET, BRs, JA, SA, CTKs, and SLs, regulate seed dormancy and germination, most likely by mediating the ABA/GA balance, although the interactions among these hormones and GA need further investigation, and some known detailed mechanisms are only the tip of the iceberg. In addition to these phytohormones, other small molecular compounds, including ROS (reactive oxygen species) and NO (nitric oxide), are involved in regulating seed dormancy and germination. ROS and NO synergistically break seed dormancy and probably act upstream of ABA (Bykova et al., 2011; Arc et al., 2013a). Thus, hormones and signaling compounds precisely regulate seed dormancy and germination through an integrated network of interactions with the ABA/GA balance as the central node (Figure 3).

In addition to phytohormones, various environmental cues determine the appropriate timing for seed germination, also by mediating the ABA/GA balance. Light is a major environmental factor during seed germination, increasing the expression of GA anabolic genes, *GA3ox1* and *GA3ox2*, and repressing the expression of *GA2ox2*, a GA catabolism gene (Cho et al., 2012). Previous studies demonstrated that blue light represses seed germination through enhancing the transcription of ABA biosynthetic genes and impairing the expression of ABA catabolic genes (Gubler et al., 2008; Barrero et al., 2014). After-ripening can also break seed dormancy, which negatively regulates ABA biogenesis (Figure 2). The transcription level of the ABA catabolism gene *CYP707A2* increases following after-ripening (Millar et al., 2006). Temperature is another environmental factor that influences seed dormancy both during seed maturation and in the soil by regulating the ABA/GA biogenesis balance (Footitt et al., 2011; Kendall et al., 2011). Temperature variation during seed maturation affects

primary seed dormancy by regulating coat permeability, which is a regulatory mechanism distinct from ABA/GA pathways (MacGregor et al., 2015). In addition, although the detailed mechanisms underlying the pre-harvest sprouting phenotype of *TaMFT*-RNAi plants are elusive, the homolog of *TaMFT* in *Arabidopsis*, *MFT*, is a pivotal factor that fine-tunes the ABA/GA-signaling balance (Xi et al., 2010; Nakamura et al., 2011). Consequently, seed dormancy is the integrated result of endogenous and environmental factors that regulate the ABA/GA balance, in either hormone accumulations or hormone signaling cascades.

CONCLUDING REMARKS AND PERSPECTIVES

Owing to forward- and reverse-genetic approaches in the model plant *Arabidopsis* and recent advances primarily in rice, rapid progress has been achieved in the field of seed dormancy and germination. Although certain key factors that regulate this important transition have been identified, and we know that plant hormones regulate seed dormancy and germination through a complex network (Figure 3), several open questions remain to be addressed.

First, the germination process includes two sequential steps: rupture of the seed coat and emergence of the radicle. Previous studies demonstrated that in cereal grains the starch granule deposition, hydrolase activity, and protein catabolism are important to seed germination, and thus the transcription level of genes encoding α-amylases are key determinants (Hong et al., 2012; Shaik et al., 2014). However, in *Arabidopsis* the precise molecular mechanisms underlying the rupture of the seed coat and endosperm processes remain largely unknown. Consequently, further detailed screening of the key genes involved in both of these stages is worthwhile.

Second, ABA is the key inducer of seed dormancy, and ABA represses GA biogenesis. Nevertheless, we still know little about changes in GA biogenesis during seed maturation (Figure 2). With the development of hormone detection assays, we can precisely investigate the amount of phytohormones in different tissues, even in single cells (Chen et al., 2011), which allows investigation of the kinetics of GA biogenesis over a time course during seed dormancy establishment.

Third, as the central node of seed dormancy and germination, where within the seed are the molecular activities of ABA and GA localized? Are ABA and GA synthesized de novo at these two sites? Pioneering studies developed a ''seed coat bedding'' assay, which was employed to demonstrate that ABA is synthesized de novo in the seed coat in an RGL2-dependent manner and thus represses germination of the embryo (Lee et al., 2010a; Lee and Lopez-Molina, 2013). Subsequently, where in the seed is GA biogenesis located?

Fourth, the possible increase in auxin levels during seed maturation raises an important question: what are the molecular mechanisms monitoring the auxin pathway during seed development? The key auxin biosynthesis genes *YUC1*, *YUC2*, and *YUC6* reach peak levels during the later stages of seed development (Liu et al., 2013b), suggesting that auxin biosynthesis may be enhanced during seed maturation. It will be interesting to investigate how the *YUC* genes are activated to fine-tune auxin biosynthesis during seed maturation.

Fifth, because ABA and auxin act synergistically to positively regulate seed dormancy, GA and auxin therefore may antagonistically function in seed dormancy. However, the detailed mechanisms underlying these synergistic and antagonistic effects are also largely elusive at the molecular level, including the precise interactions among ABIs, DOG1, DEP1, SPT (Figures 2 and 3), and downstream targets of these transcription factors, which directly function in seed germination.

Finally, given that environmental cues, such as light and temperature (Lim et al., 2014), regulate seed dormancy and germination through the ABA/GA biogenesis and signaling pathways, it is quite possible that environmental factors also affect auxin and other hormone pathways during seed germination. In this field, epigenetic effects are of particular interest because both hormonal and environmental cues are involved in epigenetic modifications. Breakthroughs concerning these regulatory mechanisms will provide a more complete network of hormone-mediated seed dormancy and germination, in addition to new solutions for controlling pre-harvest sprouting in crops.

FUNDING

This work was supported by the Natural Science Foundation of China (Grants 90817102, 91317308 and 91117018) and China Postdoctoral Science Foundation funded project (2014M552377).

ACKNOWLEDGMENTS

We thank Dr. Chengcai Chu and Dr. Pengfei Wang of the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, for providing the pictures of pre-harvest sprouting rice and *Arabidopsis* siliques. We apologize to colleagues whose work could not be discussed and cited because of space limitations. No conflict of interest declared.

Received: June 18, 2015
Revised: August 10, 2015
Accepted: August 13, 2015
Published: September 4, 2015

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