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REVIEW

The pivotal role of abscisic acid signaling during transition from seed maturation to germination

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Abstract Seed maturation and germination are two continuous developmental processes that link two distinct generations in spermatophytes; the precise genetic control of these two processes is, therefore, crucially important for the survival of the next generation. Pieces of experimental evidence accumulated so far indicate that a concerted action of endogenous signals and environmental cues is required to govern these processes. Plant hormone abscisic acid (ABA) has been suggested to play a predominant role in directing seed maturation and maintaining seed dormancy under unfavorable environmental conditions until antagonized by gibberellins (GA) and certain environmental cues to allow the commencement of seed germination when environmental conditions are favorable; therefore, the balance of ABA and GA is a major determinant of the timing of seed germination. Due to the advent of new technologies and system biology approaches, molecular studies are beginning to draw a picture of the sophisticated genetic network that drives seed maturation during the past decade, though the picture is still incomplete and many details are missing. In this review, we summarize recent advances in ABA signaling pathway in the regulation of seed maturation as well as the transition from seed maturation to germination, and highlight the importance of system biology approaches in the study of seed maturation.

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Introduction

Angiosperms reproduce their progeny by producing seed as dispersal unit. Seed contains the whole diploid genome and is capable of developing into an integrated plant. The arrested state of seed ensures survival of the species under unfavorable environment until environmental conditions favor growth of the new generation. Therefore, seed development is very important in connecting two distinct sporophyte generations and sustaining the species (Gutierrez et al. 2007). In *Arabidopsis* and many other dicots, seed development is physically divided into two distinct stages: embryogenesis and maturation (West and Harada 1993; Gutierrez et al. 2007; Sun et al. 2010). Upon the unique double fertilization in angiosperm, embryogenesis initiates with the formation of a diploid zygote and a fertilized triploid central cell. Subsequently, zygote develops into embryo and fertilized central cell develops into endosperm (Sun et al. 2010). Embryogenesis ends at the heart stage, when all embryo structures have been formed and cell division arrests (Raz et al. 2001; Holdsworth et al. 2008). After the seed development process switches into maturation stage, embryo grows to fill the cavity at the expense of endosperm, storage components are accumulated, water content is decreased, seed gradually acquires the ability to survive desiccation, and eventually seed enters into a dormant state which ensures its survival under a range of environmental conditions. After a certain period of storage, seed dormancy level declines significantly and seed becomes sensitive to environmental stimuli; once the environmental conditions are favorable for the growth of

next generation, seed germination occurs (Finkelstein 2010). The development stage from seed maturation to seed germination is tightly controlled by intrinsic phytohormones; among them, ABA plays a crucial role. ABA is a C15 weak acid that appears to be present in all vascular plants. It has been shown that ABA participates in various developmental and physiological processes during plant life cycle, including seed development, seed dormancy and germination, stomatal movement, fruit development, and various biotic and abiotic stress responses (Finkelstein 2013). Besides ABA, nearly all other phytohormones are likely involved in the transition from seed maturation to germination; numerous genes involved in this process have been identified, conferring a very complex and intricate regulatory network which is still not well understood. Inspiringly, system biology approach is starting to shed light on some aspects of the molecular mechanism underlying seed maturation and germination. The various aspects of seed maturation have been extensively discussed previously (Gutierrez et al. 2007; Holdsworth et al. 2008; Angelovici et al. 2010; Finkelstein 2013). In this review, we describe the recent advances on the role of ABA in guiding the developmental events from seed maturation to seed germination, mainly focus on seed dormancy induction, maintenance and release, and discuss the contributions made by system biology approaches on these events.

ABA pathway

ABA metabolism

ABA is a sesquiterpenoid derived from carotenoid, which is produced by successive synthesis from precursor isopentenyl diphosphate to phytoene. In plastid, phytoene is desaturated by phytoene desaturase to produce lycopene. Then, lycopene is cyclized to β-carotene by lycopene β-cyclase. β-Carotene undergoes hydroxylation catalyzed by β-carotene hydroxylases to produce zeaxanthin. Zeaxanthin epoxidase (ZEP), which is encoded by *ABA-DEFICIENT 1* (*ABA1*) in *Arabidopsis*, converts zeaxanthin to violaxanthin. The final plastid-localized step in ABA synthesis is conversion of violaxanthin into 9-*cis*-violaxanthin or 9-*cis*-neoxanthin, which are subsequently subjected to cleavage by 9-*cis*-epoxycarotenoid dioxygenase (NCED) to produce xanthoxin (Finkelstein 2013). This is the first step specific to ABA biosynthesis and the rate-limiting step (Nambara and Marion-Poll 2005). In *Arabidopsis*, genes encoding these NCED enzymes form a multigene family which has nine members, of which only *NCED2*, *NCED3*, *NCED5*, *NCED6*, and *NCED9* are involved in ABA biosynthesis (Schwartz et al. 2003; Tan et al. 2003). After xanthoxin is transported from the plastids to the cytosol, a short chain dehydrogenase/reductase-like (SDR1) enzyme encoding by *ABA2* catalyzes the conversion of xanthoxin into abscisic aldehyde. Loss of function of *ABA2* causes a severe ABA deficiency. The final step of ABA biosynthesis is oxidation of abscisic aldehyde to ABA, which is catalyzed by abscisic aldehyde oxidase (AAO). In *Arabidopsis*, *AAO3* is the major AAO gene involved in the oxidation of abscisic aldehyde. The catalytic activity of aldehyde oxidase requires a sulfurylated form of molybdenum cofactor (MoCo), which is catalyzed by MoCo sulfurase. In *Arabidopsis*, the MoCo sulfurase is encoded by *ABA3*; loss of function of *ABA3* causes ABA deficiency (Xiong et al. 2001). Alternatively, ABA is also produced via hydrolysis of ABA glucose ester (ABA-GE) by two β-glucosidase homologues AtBG1 and AtBG2 in *Arabidopsis* (Lee et al. 2006; Xu et al. 2012).

Endogenous ABA homeostasis is also regulated by the balance between ABA synthesis and catabolism. ABA catabolism leads to the degradation of ABA through two major pathways: ABA hydroxylation pathway and ABA conjugation pathway. ABA hydroxylation at the 8′ position is mediated by the cytochrome P450 (CYP) 707A family. There are four CYP707As (CYP707A1, 2, 3 and 4) in *Arabidopsis*. CYP707A1 and CYP707A3 play important roles in the reduction of ABA content in embryo at mid-maturation stage, whereas CYP707A2 functions in both embryo and endosperm to reduce ABA level from late-maturation to germination (Okamoto et al. 2006). After hydroxylation isomerization, ABA is converted into phaseic acid (PA). PA has a weaker bioactivity compared with ABA. A recent study showed that PA acts as a hormone molecule to regulate plant development and stress responses (Weng et al. 2016). PA is eventually converted into dihydrophaseic acid (DPA), which is biologically inactive. This process is catalyzed by PA reductase (PAR), which is encoded by *ABH2* in *Arabidopsis* (Weng et al. 2016). Another ABA catabolism pathway is the inactivation of ABA by covalent conjugation to another molecule, catalyzed by ABA glucosyl-transferases (UGTs). The most common conjugate form is ABA-GE, which is cleaved by AtBG1. The ABA metabolism pathway is outlined in Fig. 1a.

ABA signaling

The ABA signaling is transmitted via the core ABA signaling complex, which consist of ABA receptors, protein phosphatase 2C (PP2C) and SNF1-related protein kinase 2 (SnRK2). In *Arabidopsis*, there are 14 ABA receptors, including PYRABACTIN RESISTANCE 1 (PYR1), PYR1-LIKE (PYL) 1-13 or RCAR1-RCAR14 (regulatory component of ABA receptor) (Ma et al. 2009; Park et al. 2009), which localize to the cytosol and nucleus. PP2Cs

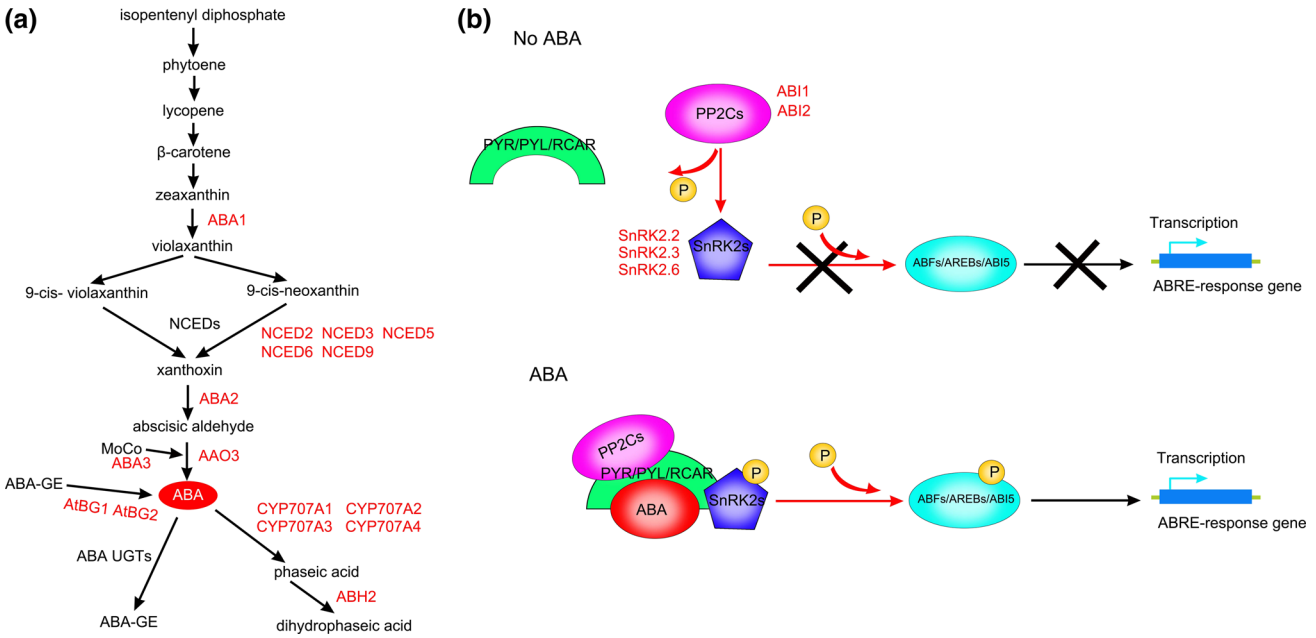


Fig. 1 Schematic representation of ABA metabolic pathway and signaling pathway in *Arabidopsis*. **a** ABA metabolic pathway; **b** ABA signaling pathway. Key regulatory factors are shown in *red*. *NCED* nine-cis-epoxycarotenoid dioxygenase, *CYP707As* cytochrome P450 (CYP) 707A family, *MoCo* molybdenum cofactor, *ABA-GE* ABA glucose ester, *ABA UGTs* ABA glucosyl-transferases, *PP2C* protein phosphatase 2C, *SnRK2* SNF1-related protein kinase 2, *PYR/PYL/RCAR* PYRABACTIN RESISTANCE 1/PYR1-LIKE/Regulatory Component of ABA Receptor, *ABFs* ABRE-binding factors, *AREBs* ABRE-binding proteins

negatively regulate ABA signaling by dephosphorylation of SnRK2s. PP2C family group A contains nine members in *Arabidopsis* (Nishimura et al. 2007), of which *ABA-INSENSTIVE 1 (ABI1)* and *ABI2* are the first identified ABA response loci, *abi1-1* and *abi2-1* exhibit ABA insensitivity in various tissues (Koornneef et al. 1984). SnRK2s family is classified into three subclasses (I, II and III), of which subclass III SnRK2s are the major ABA-activated protein kinases. Subclass III SnRK2s contains three members, SnRK2.2, SnRK2.6 and SnRK2.3. Autophosphorylation of SnRK2s can activate basic leucine zipper (bZIP) transcription factors such as ABA-responsive element (ABRE) binding proteins (AREBs), ABRE-binding factors (ABFs) and ABA-INSENSITIVE 5 (ABI5) to promote ABA-mediated transcriptional regulation (Miyakawa et al. 2013).

Current model for ABA signaling pathway is diagrammed in Fig. 1b. ABA signaling cascade is initiated from the binding of ABA to ABA receptors, which result in conformational changes in PYR/PYL/RCAR, then ABA-bound PYR/PYL/RCAR interact with PP2Cs to form a ternary ABA-responsive complex ABA-ABAR-PP2C, thereby suppresses PP2C-mediated dephosphorylation of SnRK2s, results in the relieve of SnRK2s, which is required to activate ABFs/AREBs/ABI5 transcription factors and lead to subsequent ABRE-dependent gene expression (Miyakawa et al. 2013) (Fig. 1b).

Regulation of seed maturation by ABA signaling

Storage reserve accumulation

Seed maturation begins with a transition phase during which embryo cell division arrests, embryo growth switches to cellular expansion and differentiation, which lead to storage reserves accumulation in embryo (Raz et al. 2001; Finkelstein 2013). At the end of embryogenesis, cell cycle arrest is regulated by ABA (Levi et al. 1993), seed of tomato ABA-deficient mutant contains a significantly higher amount of G_2 cells (4C DNA) during maturation, suggests that cell cycle activity in ABA-deficient seed is less efficiently arrested in G_1 (Liu et al. 1994), a later study demonstrated that ABA induces a cyclin-dependent kinase (CDK) inhibitor genes *ICK1* (Wang et al. 1998) which may function to block G1/S transition during cell cycle (Nasmyth and Hunt 1993). During early seed maturation, storage products begin to accumulate within embryo by consuming nourishing endosperm until the entire seed cavity is filled in *Arabidopsis* (Gutierrez et al. 2007; Sreenivasulu and Wobus 2013). Main storage products include nitrogen (proteins) and carbon storage compounds (lipids), each account for approximately 40% of dry matter at the end of maturation stage (Baud et al. 2002). These proteins and lipids will be used as nitrogen and carbon sources during subsequent germination. Lipids accumulate

in the form of triacylglycerols (TAGs), esters of glycerol and fatty acids. TAGs are stored in cytosolic oil bodies which occupy about 60% of the cell volume in the cotyledons of mature embryo (Mansfield and Briarty 1992; Baud et al. 2002).

Storage reserve accumulation is largely controlled by coordination of ABA signaling and transcriptional regulators (Finkelstein et al. 2002). During this period, ABA accumulation reaches peak level (Finkelstein 2010). Double mutant combining ABA deficiency (*aba1-1* mutant) with ABA insensitivity (*abscisic acid insensitive 3-1* mutant) produces less storage proteins (Meurs et al. 1992) and oil (Bruijn et al. 1997). While, application of exogenous ABA can enhance the accumulation of soluble sugar content and total lipid content in castor bean seeds. In addition, transcriptome profile revealed that many genes involved in lipid and storage protein synthesis are differentially expressed upon ABA treatment (Chandrasekaran et al. 2014). Many storage protein coding genes contain specific cis-acting elements in the promoter region; these specific cis-acting elements are essential for ABA response during seed storage accumulation, suggesting that these storage protein coding genes are regulated by ABA. ABSCISIC ACID INSENSITIVE 3 (ABI3), a positive regulator in ABA signaling, plays an important role in the regulation of some seed storage protein genes such as *At2S3/SEED STORAGE ALBUMIN 3* (*SESA3*) and *CRUCIFERIN C* (*CRC*) during seed maturation, *abi3* mutation strongly affects the accumulation of seed storage proteins (Santos-Mendoza et al. 2008), while ectopic expression of *ABI3* in vegetative tissues can induce expression of seed storage protein coding genes (Kagaya et al. 2005a). *ABI3* is also required in *LEAFY COTYLEDON 1* (*LEC1*)-mediated seed storage protein gene expression (Kagaya et al. 2005b). A genome-wide analysis identified a set of 98 ABI3 target genes in *Arabidopsis*, many of which appeared to be involved in protein and lipid storage accumulation, and most of these ABI3 targets require the presence of ABA for their activation (Mönke et al. 2012). Another positive regulator of ABA signaling, ABSCISIC ACID INSENSITIVE 4 (ABI4), is also involved in lipid biosynthesis. During TAGs accumulation in *Arabidopsis*, acyl-coenzyme A: diacylglycerol acyltransferase1 (DGAT1) is the rate-limiting enzyme. ABI4 binds directly to the CE1-like elements in *DGAT1* promoter to activate *DGAT1* expression (Yang et al. 2011). Similar with *ABI3*, ectopic expression of *ABI4* also causes the induction of seed storage protein genes in vegetative tissues (Söderman et al. 2000).

Seed dormancy induction and maintenance

ABA suppresses germination of mature seed as well as premature seed, as ABA-deficient or insensitive mutants are often viviparous (Finkelstein 2010). During early seed maturation phase, endogenous ABA is first produced in maternal tissues and accumulates to a peak level (Karssen et al. 1983; Frey et al. 2004; Kanno et al. 2010). The accumulation of maternal ABA combines with FUSCA 3 (FUS3) and LEC1/2 to prevent premature germination before embryo growth arrest, because only double mutants combining *lec1/2* or *fus3* with *aba1* or *abi3* show premature germination (Raz et al. 2001). In maize, mutant of *VIVIPAROUS-1* (*VP1*) (ortholog of *Arabidopsis ABI3*) is viviparous due to reduced ABA sensitivity, while other mutants (*vp2*, *vp5*, *vp7*, *vp8*, *vp9*) with defects in either ABA sensitivity or biosynthesis are also viviparous (Robichaud et al. 1979; Finkelstein 2010).

Seed dormancy has been defined as a quiescent state of a viable seed that is incapable to germinate under favorable conditions (Bewley 1997; Finch-Savage and Leubner-Metzger 2006; Bentsink and Koornneef 2008). Seed dormancy controls the timing of germination in response to environmental cues and plays an important role in seed plant evolution and adaptation to climatic changes (Linkies et al. 2010). Seed dormancy induction and establishment rely on ABA signal (Finch-Savage and Leubner-Metzger 2006; Gutierrez et al. 2007). During the late-maturation phase, embryonic-derived ABA accumulates to another peak level (Karssen et al. 1983; Kanno et al. 2010), which is regulated by the dynamic balance between ABA biosynthesis and degradation (Nambara and Marion-Poll 2005; Gutierrez et al. 2007). Many mutants of ABA biosynthesis genes in *Arabidopsis* are non-dormant or show reduced dormancy, such as mutants of *ABA1*, *ABA2*, *ABA3, NCED6* and *NCED9* (Koornneef et al. 1982; Léon-Kloosterziel et al. 1996; Raz et al. 2001; Lefebvre et al. 2006). In *Arabidopsis*, *NCED6* and *NCED9* both exhibit seed-specific expression during seed maturation, *NCED6* is exclusively expressed in endosperm while *NCED9* is expressed in both embryo and endosperm. Genetic study showed that *nced6 nced9* double mutant has reduced seed dormancy (Lefebvre et al. 2006). Consistently, loss-of-function mutants of ABA catabolism genes such as *CYP707A1*, *CYP707A2,* and *CYP707A3* exhibit enhanced seed dormancy (Kushiro et al. 2004; Okamoto et al. 2006; Finkelstein et al. 2008), suggesting a positive correlation between seed dormancy and ABA biosynthesis. This is supported by the observation that deep dormant rice cultivar N22 had high ABA levels during seed development, while non-dormant G46B had lower ABA content (Liu et al. 2014). Recently, a microarray-based gene expression analysis demonstrated that genes involved in ABA signaling were down-regulated in less dormant mutants when compared to strongly dormant cultivar, while genes involved in GA biosynthesis and signaling were up-regulated (Wu et al. 2016), indicating a role of ABA/GA

balance in the control of seed dormancy. Furthermore, increase of ABA levels caused by amplification of ABA biosynthesis gene *NCED* during seed maturation results in unusually deep dormancy which may last for more than 3 months (Martínez-Andújar et al. 2011; Nonogaki et al. 2014).

In addition, deficiency in ABA response also influences seed dormancy. The PP2Cs gain-of-function mutants *abi1-1* and *abi2-1* are ABA-insensitive and show reduced seed dormancy (Koornneef et al. 1984; Finkelstein 1994). Loss-of-function mutant of *HONSU*, another negative regulator of ABA signaling, displays deep seed dormancy, whereas *HONSU*-overexpressing lines show shallow seed dormancy (Kim et al. 2013b). Mutation of *ABI3* causes premature germination after embryos mature in *Arabidopsis* (Raz et al. 2001); Consistent with the role of *ABI3* in *Arabidopsis*, expression level of wheat homologous gene *VP1* is positively correlated with the degree of seed dormancy and embryo sensitivity to ABA (Nakamura and Toyama 2001). Triple mutant of *SnRK2.2*, *SnRK2.3*, and *SnRK2.6*, which play essential roles for phosphorylation of bZIP transcription factors in ABA signaling pathway, exhibits a loss of dormancy, along with global changes of ABA-regulated gene expression (Nakashima et al. 2009). Moreover, ABA-hypersensitive mutant *era1* shows increased dormancy (Cutler et al. 1996; Finkelstein 2010). Recently, more lines of evidence have emerged to support the correlation between ABA sensitivity and seed dormancy, any alteration of ABA sensitivity, directly or indirectly, could cause modification of seed dormancy states (Barrero et al. 2010; Alonso-Ramírez et al. 2011; Di Mauro et al. 2012; Jiang et al. 2012; Schramm et al. 2012a, b; Lee et al. 2015c). Collectively, these observations demonstrate the fundamental role of ABA in the regulation of seed dormancy.

It has been found that seed dormancy is also subjected to epigenetic regulation. Mutant of *FERTILIZATIONINDEPENDENT ENDOSPERM* (*FIE*), a component of polycomb repressive complex 2 (PRC2) which is essential for H3K27me3 deposition, exhibits enhanced dormancy and germination defects, genome-wide transcriptome analysis indicated that enhanced dormancy of *fie* is due to up-regulation of master regulators in seed development and ABA signaling pathway, including LEC2, FUS3, ABI3 and ABI4 (Bouyer et al. 2011). Arabidopsis *KYP/SUVH4* (*KRYPTONITE*) gene, encoding a histone methyltransferase, which is responsible for H3K9me2 deposition (Jackson et al. 2002), negatively regulates ABA sensitivity by repressing *ABI3* and *ABI4* expression, *kyp-2* mutant shows increased seed dormancy and enhanced sensitivity to ABA, whereas *KYP/SUVH4* overexpression lines exhibit reduced dormancy and ABA sensitivity (Zheng et al. 2012).

Combination of ABA signaling with other regulators

Regulation of seed maturation is concerted by the combination of ABA signaling with many transcriptional factors. The best characterized positive regulators of ABA signaling so far are ABI3/VP1, ABI4 and ABI5, which belong to B3-type, APETALA2-domain, and bZIP-domain transcription factor families, respectively (Finkelstein et al. 2002). Another two B3-type transcription factors, FUS3 and LEC2, and HAP3 subunit of the CCAAT-box binding transcription factor LEC1, together with ABI3 have been demonstrated as the four master regulators of seed maturation (Gutierrez et al. 2007). The interactions among these four regulators form a network to control various aspects of seed maturation, including accumulation of storage compounds, cotyledon identity, acquisition of desiccation tolerance, and establishment of seed dormancy (To et al. 2006). As a major component of ABA signaling, *ABI3* is expressed uniformly throughout the cotyledons and embryo axis, LEC2 and FUS3 up-regulate *ABI3* expression, and LEC1 also positively regulates *ABI3* in the cotyledons. Moreover, ABI3 can positively regulate itself, thereby forming a feedback loop which is essential for sustained and uniform expression of *ABI3* in the embryo (To et al. 2006). Although the four genes show different spatial and temporal expression patterns during seed development, their mutants display common defects in seed maturation, such as reduced dormancy and seed storage proteins. Genetic studies have demonstrated that they act redundantly to regulate overlapping subsets of seed-specific genes during seed maturation. Although the *lec1* and *fus3* mutations have little or no effect on ABA sensitivity, double mutant combing *fus3* or *lec1* with *abi3* is at least tenfold less sensitive to ABA than monogenic *abi3*, indicating a potentiation effect of ABI-dependent ABA sensitivity by FUS3 and LEC1 (Finkelstein 2013). In addition, FUS3-mediated induction of *CRC* is completely dependent on ABA (Kagaya et al. 2005a), while in turn FUS3 is also involved in ABA response, as only double mutant combining *fus3* with *aba1* or *abi3* is highly viviparous (Nambara et al. 2000; Raz et al. 2001). A recent study showed that ABI3 contributes more on the control of storage protein content whereas FUS3 contributes more on lipid content, which suggests a complementation of seed reserves production by ABI3, FUS3 and LEC2, and confirms the redundancy among these three regulators during seed storage accumulation (Roscoe et al. 2015).

Besides master regulators of seed maturation, many other regulators are involved in the control of seed maturation in an ABA-dependent manner. Recent study showed that a chromodomain, helicase and DNA-binding CHD1 protein, CHR5, is required to associate with the promoters of *ABI3* and *FUS3* and reduce nucleosome occupancy near

the transcriptional start site to establish active state of *ABI3* and *FUS3*, thereby enhance seed protein accumulation (Shen et al. 2015). Another key component of ABA signaling pathway, ABI4, has also been demonstrated to positively regulate primary seed dormancy. *Arabidopsis abi4* mutant seed is less dormant and germinates more quickly than wild-type; moreover, dry *abi4* seed has lower ABA accumulation and higher GA levels. Further study demonstrated that ABI4 binds directly to promoters of *CYP707A1* and *CYP707A2* to repress theirs expression, thereby promotes ABA accumulation (Shu et al. 2013). ABI5 is another important positive regulator of ABA response; ABI5 can directly bind to ABREs in the promoter region of target genes to regulate ABA-responsive genes in seeds. *abi5* mutant shows decreased sensitivity to ABA-mediated inhibition of seed germination (Finkelstein and Lynch 2000), while ectopic expression of *GhABI5* (*Gladiolus ABI5* homolog) in *Arabidopsis* can rescue the ABA insensitivity of *abi5-5* during dormancy maintenance (Wu et al. 2015). Genetic study suggested that ABI5 acts downstream of ABI3 to regulate ABA response, because *ABI5* expression is greatly reduced in *abi3-1*, and *ABI5* overexpression can rescue ABA insensitivity of *abi3-1* (Lopez-Molina et al. 2002). Both ABI4 and ABI5 can interact genetically with LEC1 and FUS3 to suppress vivipary and control germination sensitivity to ABA (Brocard-Gifford et al. 2003). It is suggested that ABI3, ABI4 and ABI5 function in a combinatorial network and act synergistically to cross-regulate extensive gene expression in seed (Söderman et al. 2000). Due to the advantages of systems biology approaches, genome-wide omics studies have generated enormous amount of data during last decade. Accordingly, a variety of tools and databases have been developed to integrate and interpret these data and to facilitate the predication of unknown genes. The co-expression tool CORNET (CORrelation NETworks; https://bioinformatics.psb.ugent.be/cornet) is a user-friendly tool for data mining and integration in *Arabidopsis*; it facilitates the compilation and construction of gene co-expression network by integration of the massive amount of datasets generated by high-throughput experiments (De Bodt et al. 2012). Using CORNET, we constructed a regulatory network and co-expression network of ABI3/ABI4/ABI5, the key components of ABA signaling, by identifying direct or indirect targets through interpretation of 83 seed microarray experimental datasets, in addition, we integrated the co-expression network with regulatory information generated from AGRIS (*Arabidopsis* Gene Regulatory Information Server) database (Fig. 2). Such a network enables us to have an overview of the regulatory interaction and co-expression of genes involved in *ABI3*, *ABI4* and *ABI5* regulation. As shown in this network, many genes that are co-expressed with *ABI3*, *ABI4*, and *ABI5* are involved in seed storage metabolism, including *OLEO1* (*OLEOSIN 1*), *SESA3*, *SESA5*, *CRU3* (*CRUCIFERIN 3*), *AT3G01570*, *AT5G45920*, and *AT5G14450*, some genes are involved in embryo development (*AGAMOUS-LIKE 15*, *AT1G72100*, *ATMT4A*) and stress response (*AT3G58450*, *AT2G15010*). The regulatory network also implied a crosstalk between ABA response and light signaling, as several key regulatory factors of light signaling are involved in this network, such as PHYTOCHROME INTERACTING FACTOR3-LIKE5 (PIL5), PHYTOCHROME INTERACTING FACTOR 4 (PIF4), FAR-RED ELONGATED HYPOCOTYL3 (FHY3), and FAR-RED IMPAIRED RESPONSE1 (FAR1), these factors were shown to directly activate the transcription of *ABI5* or *ABI3* (Fig. 2). In addition, the co-expression analysis predicted many genes with unknown function are co-expressed with *ABI3*, *ABI4* and *ABI5*. Therefore, a more comprehensive understanding of ABA signaling network requires further functional studies on these network members.

Seed dormancy release and germination

The role of ABA during seed imbibition

After a period of seed storage, seed becomes sensitive to favorable environmental conditions. These environmental cues (e.g., light and cool temperature) can break seed dormancy by repressing ABA activity or altering ABA sensitivity, eventually lead to the onset of seed germination. As the initiation of a new plant generation, seed germination is sustained by a variety of physiological events including energy mobilization and cell wall modification, while ABA acts as an inhibitor of these processes. A whole genome Affymetrix tiling array analysis identified 336 ABA-upregulated genes which were downregulated in *aba2* and upregulated in *cyp707a* triple mutant, and 586 ABA-downregulated genes that were upregulated in *aba2* and downregulated in *cyp707a* triple mutant during seed imbibition. By using MapMan, a tool for the visualization of omics data, we generated a figure which enable us to have a visual overview of the biological involvement of these differentially expression genes. As shown in Fig. 3, ABA-downregulated genes (blue square in Fig. 3a and red square in Fig. 3b) are involved in photosynthesis, cell wall modification and degradation, and Calvin cycle, while ABA-upregulated genes (red square in Fig. 3a and blue square in Fig. 3b) are related to dormancy and seed storage reserves such as LEA proteins and TAGs, suggests that ABA plays an important role in determining whether seeds remain dormant or germinate after imbibition (Okamoto et al. 2010) (Fig. 3).

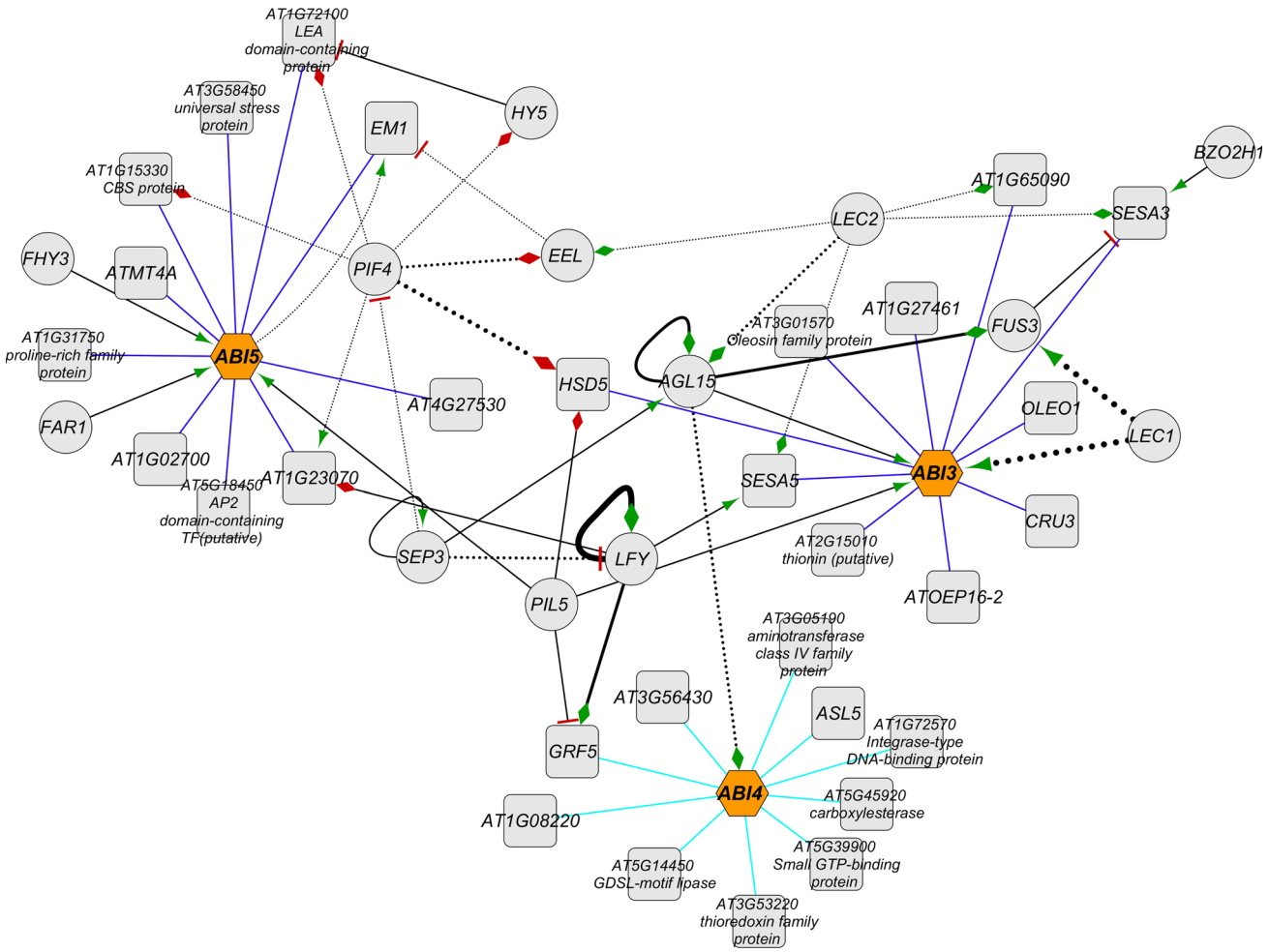
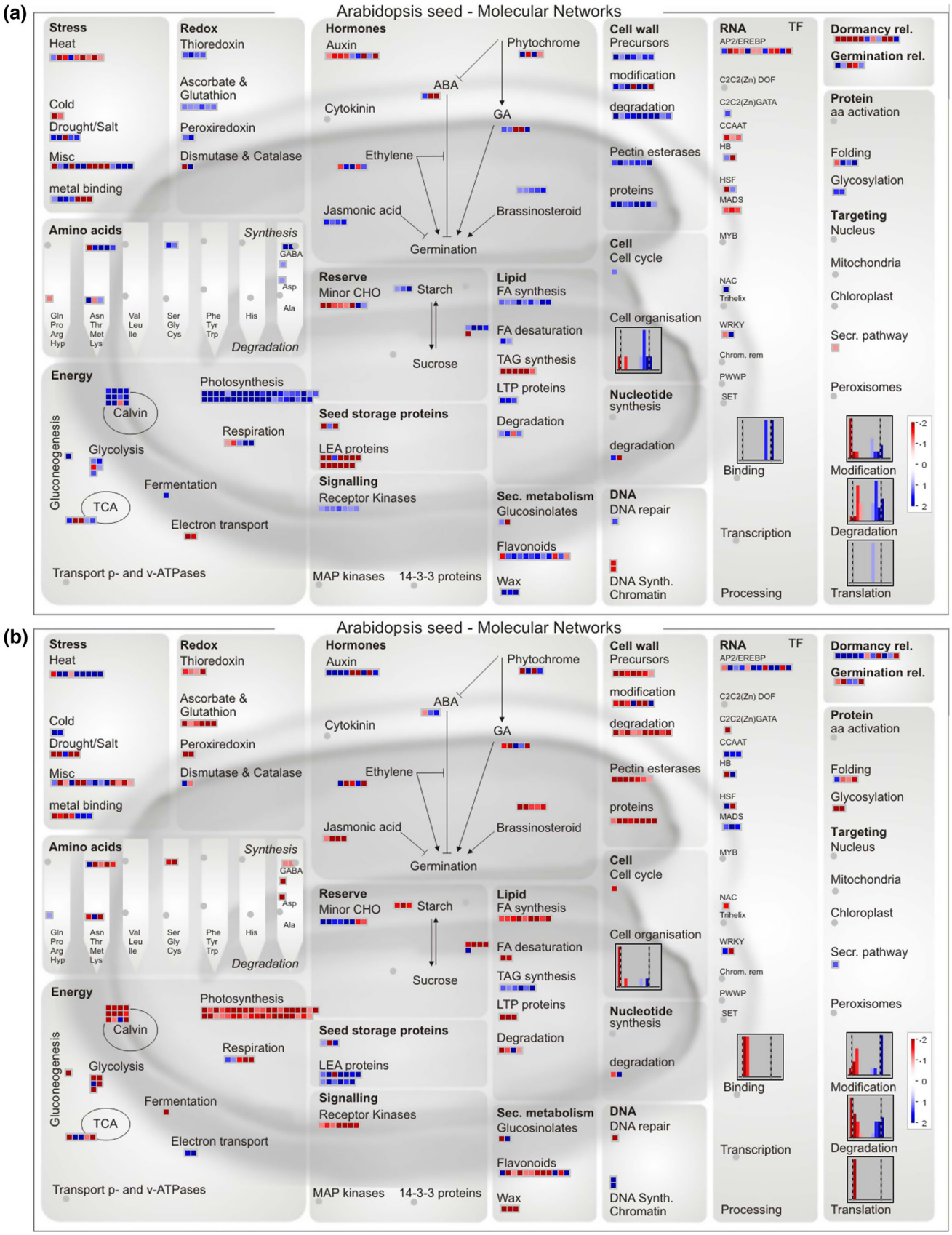


Fig. 2 Co-expression networks of ABI3, ABI4 and ABI5 during seed development in *Arabidopsis*. Correlation between gene expression profiles was calculated by co-expression tool CORNET (De Bodt et al. 2012) based on microarray expression data generated from 83 experiments in seed. AGRIS (*Arabidopsis* Gene Regulatory Information Server) database was used to add regulatory interactions to the network. The co-expression significance is determined by the Pearson's correlation coefficient. *Dark blue lines* represent a positive correlation of 0.9, and *light blue lines* represent a positive correlation of 0.8. *Black lines* indicate confirmed regulatory interaction between transcription factors (TFs), while *dotted black lines* represent predicted interaction. *Triangle arrowheads* mean direct interaction, *diamond arrowheads* mean indirect interaction. *Green arrowheads* indicate activation, *red arrowheads* indicate repression, and *red T-bars* indicate direct repression

During the early stage of germination, ABA contents of seed decrease rapidly within 3–18 h upon imbibition (Nakabayashi et al. 2005; Hermann et al. 2007; Linkies et al. 2009; Preston et al. 2009). The decrease of ABA level is a prerequisite for seed dormancy breaking and germination initiation (Weitbrecht et al. 2011). *CYP707A2* is the major 8′ hydroxylase gene responsible for the decline of ABA level in seed during imbibition, expression of *CYP707A2* reaches to a peak level at 6 h after imbibition (Kushiro et al. 2004). Because seed germination is repressed by endogenous ABA levels and ABA signaling, any modification of ABA levels or ABA response can result in changes in germination potential. Two homeodomain transcription factors, BEL1-LIKE HOMEODOMAIN 1 (BLH1) and KNOTTED1-LIKE HOMEOBOX GENE 3 (KNAT3), synergistically enhance ABA responses through activating *ABI3* expression by binding to its promoter. *blh1* and *knat3* mutants show lower sensitivity to ABA than the wild-type, and exhibit higher germination rate (Kim et al. 2013a). Overexpression of *DOF TRANSCRIPTION FACTOR 6* (*DOF6*), a DOF transcription factor, induces expression of *ABA1* and causes ABA-hypersensitive phenotypes, leading to a delayed germination phenotype (Rueda-Romero et al. 2012). Expression of a putative E3 ubiquitin ligase coding gene *ECERIFERUM9* (*CER9*) resembles that of *ABI5* in seeds. *cer9* mutant is hypersensitive to ABA and has increased ABA levels, which causes delayed germination, suggesting that CER9 is a negative regulator of ABA biosynthesis and signaling during seed germination (Zhao et al. 2014).


(a)
Arabidopsis seed - Molecular Networks
Stress
Heat
Cold
Drought/Salt
Misc
metal binding
Redox
Thioredoxin
Ascorbate & Glutathion
Peroxiredoxin
Dismutase & Catalase
Hormones
Auxin
Phytochrome
ABA
Cytokinin
GA
Ethylene
Jasmonic acid
Brassinosteroid
Germination
Cell wall
Precursors
modification
degradation
Pectin esterases
proteins
Cell
Cell cycle
Cell organisation
Nucleotide
synthesis
degradation
DNA
DNA repair
DNA Synth.
Chromatin
RNA
TF
AP2/EREBP
C2C2(Zn) DOF
C2C2(Zn)GATA
CCAAT
HB
HSF
MADS
MYB
NAC
Trihelix
WRKY
Chrom. rem
PWWP
SET
Binding
Transcription
Processing
Dormancy rel.
Germination rel.
Protein
aa activation
Folding
Glycosylation
Targeting
Nucleus
Mitochondria
Chloroplast
Secr. pathway
Peroxisomes
Modification
Degradation
Translation
Amino acids
Synthesis
Degradation
Gln Pro Arg Hyp
Asn Thr Met Lys
Val Leu Ile
Ser Gly Cys
Phe Tyr Trp
His
GABA
Asp
Ala
Energy
Photosynthesis
Calvin
Respiration
Glycolysis
Gluconeogenesis
Fermentation
TCA
Electron transport
Transport p- and v-ATPases
Reserve
Minor CHO
Starch
Sucrose
Seed storage proteins
LEA proteins
Signalling
Receptor Kinases
MAP kinases
14-3-3 proteins
Lipid
FA synthesis
FA desaturation
TAG synthesis
LTP proteins
Degradation
Sec. metabolism
Glucosinolates
Flavonoids
Wax
(b)
Arabidopsis seed - Molecular Networks

◀**Fig. 3** MapMan seed custom maps display fold changes in transcript levels of ABA-regulated genes between ABA metabolism mutants and wild-type during seed imbibition. **a** *aba2* versus WT; **b** *cyp707a triple* versus WT. Data were collected from a whole genome Affymetrix tiling arrays study (Okamoto et al. 2010) and visualized by the MapMan software (Usadel et al. 2009). *Red* indicated a decrease whereas *blue* indicates an increase of transcript level

Posttranslational modifications of *ABI5* also exert influence on ABA response. A SUMO E3 ligase SIZ1 can attenuate ABA sensitivity during seed germination by sumoylation of *ABI5*, indicating the existence of multiple regulation levels of ABA signaling during seed germination (Miura et al. 2009). Desensitization of ABA signaling is required during seed dormancy release, a membrane-associated transcription factor peptidase, site-2 protease (S2P), has been shown to desensitize ABA signaling through regulating the activation of bZIP17, thereby control the expression of negative regulators of ABA signaling. Loss of function of *S2P* confers high ABA sensitivity during seed germination (Zhou et al. 2015). Recently WRKY transcription factors have been shown to play important roles in ABA response during seed germination. *wrky40*, *wrky18*, and *wrky60* mutants show ABA-hypersensitive phenotypes in ABA-induced inhibition of seed germination, genetic study revealed that WRKY40 negatively regulates ABA response by directly repressing expression of *ABI5* and other ABA-responsive genes (Shang et al. 2010). Whereas, WRKY41 acts collaboratively with ABA to control primary seed dormancy via direct regulation of *ABI3* expression. *wrky41* mutation causes reduced sensitivity to ABA, and displays reduced primary seed dormancy, resembling that of *abi3* mutation (Ding et al. 2014). WRKY6 negatively regulates seed germination by directly repressing *RELATED TO ABI3/VP1* (*RAV1*) expression and subsequently enhancing expression of *ABI3*, *ABI4* and *ABI5* (Huang et al. 2016). RAV1 acts as a negative regulator of ABA response by directly repressing the expression of *ABI3*, *ABI4*, and *ABI5*, *RAV1*-underexpressing lines are more sensitive to ABA during seed germination, in addition, the negative activity of RAV1 in ABA response is inhibited by SnRK2.2, SnRK2.3, and SnRK2.6-mediated phosphorylation of RAV1 (Feng et al. 2014). Besides these key regulators of ABA response, the functions of ABA receptor PYR/PYL/RCAR in seed germination have been implicated recently. Overexpression of *RCAR1, RCAR7/PYL13* or *PYL5* in *Arabidopsis* and *OsPYL/RCAR5* in rice resulted in hypersensitivity phenotypes in ABA-regulated seed germination (Ma et al. 2009; Kim et al. 2012; Fuchs et al. 2014), while double mutant *rcar7 rcar9* and triple mutant *pyr1 pyl1 pyl4*, as well as quadruple mutant *pyr1 pyl1 pyl2 pyl4* exhibit ABA insensitivity (Park et al. 2009; Fuchs et al. 2014). Collectively, these observations demonstrate that ABA is an important regulator of seed germination.

ABA/GA balance determines the timing of seed germination

In fact, ABA does not act solely in controlling seed germination; it has been shown that the timing of germination is determined by the dynamic balance between ABA and GA (Finkelstein et al. 2008; Seo et al. 2009). Release of dormancy is accompanied by decreased ABA levels and increased GA levels, which are contributed by decreased ABA synthesis and enhanced ABA catabolism, increased GA synthesis and decreased GA catabolism, respectively. These dynamic hormone metabolism changes were observed in numerous studies. For example, a cold shock domain (CSD) protein CSP2 negatively regulates seed germination by controlling ABA and GA levels, *CSP2*-overexpressing seeds have higher ABA levels which due to reduced expression of *CYP707A2*; Meanwhile, expression of GA biosynthesis genes *GA20ox* and *GA3ox* are also reduced (Sasaki et al. 2015). Regulators in ABA signaling also play important roles in the control of ABA/GA balance. In *Arabidopsis*, ABI4 promotes ABA accumulation by directly repressing *CYP707A1* and *CYP707A2* (Shu et al. 2013), similarly, in sorghum, grains with higher dormancy level exhibit increased ABA sensitivity and higher expression of *SbABI4* and *SbABI5*. Moreover, either SbABI4 or SbABI5 can interact with promoter of GA catabolic gene *SbGA2ox3* to activate its expression and promote SbGA2ox3 protein accumulation, thereby causes degradation of GA4 and maintains seed dormancy (Cantoro et al. 2013). ABI4 has also been suggested to play a central role in GA/ABA homeostasis and antagonism in post-germination stages by directly activating *NCED6* and *GA2ox7* expression (Shu et al. 2016). Recently, an ABA-responsive R2R3-type MYB transcription factor MYB96 has been shown to act as a central ABA signaling mediator in a variety of physiological processes including seed dormancy and germination (Lee and Seo 2015). During seed germination, MYB96 positively regulates *NCED2*, *NCED5*, *NCED6*, and *NCED9* to promote ABA synthesis, meanwhile, MYB96 represses GA biosynthetic genes *GA3ox1* and *GA20ox1* to reduce GA accumulation (Lee et al. 2015a). Furthermore, MYB96 directly regulates *ABI4* to repress lipid breakdown, a metabolism process which provide the embryo with energy before the initiation of photosynthesis, thereby fine-tune lipid mobilization in embryo to ensure a proper timing of germination (Lee et al. 2015b).

ABA/GA balance is also controlled by mutually antagonistic regulation between ABA and GA.

In ABA-deficient *aba2* mutant, expression of GA biosynthetic genes *GA3ox1* and *GA3ox2* is elevated, GA4 levels are thereby increased. Whereas in ABA over-accumulating *cyp707a2* mutant, expression of GA biosynthetic genes is partially suppressed, suggesting that ABA negatively regulates GA biosynthesis during seed germination (Seo et al. 2009). On the contrary, GA also negatively regulates endogenous ABA biosynthesis. In GA-deficient mutant *ga1*, expression of ABA biosynthetic genes is increased, resulting in the accumulation of higher ABA levels (Oh et al. 2007). Regulators in GA signaling pathway also exert functions on the regulation of ABA biosynthesis, RGA-LIKE2 (RGL2), a key DELLA protein, represses seed germination by positively regulating the expression of *XERICO*, a RING-H2 zinc finger factor that promotes ABA synthesis, resulting in elevated ABA levels, which in turn induce *RGL2* and *ABI5* expression (Piskurewicz et al. 2008). Subsequent study showed that RGL2 is required to maintain high ABA levels in highly dormant C24 seeds in *Arabidopsis* (Lee et al. 2010). Mutant of a F-box protein coding gene *SLEEPY1* exhibits strong seed dormancy phenotype, which is attributed to high levels of ABA accumulation caused by elevated DELLA levels (Ariizumi et al. 2013).

Environmental control of seed germination

Favorable environmental conditions stimulate the breaking of seed dormancy and promote germination. The balance between ABA and GA acts as an integrator of environmental cues to determine appropriate timing of germination. The most important environmental signals in breaking seed dormancy are light and cold temperature. Light promotes GA synthesis by inducing *GA3ox1* and *GA3ox2* expression, partially through repressing PIL5 (Oh et al. 2009). PIL5 is a basic helix-loop-helix (bHLH) transcription factor that inhibits light-dependent seed germination in *Arabidopsis*. PIL5 represses GA biosynthesis and promotes GA degradation by down-regulating *GA3ox1* and *GA3ox2* and up-regulating a GA catabolic gene *GA2ox2*, respectively (Oh et al. 2006). Meanwhile, PIL5 activates the expression of ABA biosynthesis genes (*ABA1*, *NCED6*, and *NCED9*) and represses the expression of ABA catabolic gene *CYP707A2* to promote ABA accumulation (Oh et al. 2007). In addition, PIL5 also directly activates the expression of *GIBBERELLIC ACID INSENSITIVE* (*GAI*), *REPRESSOR OF GA* (*RGA*), *ABI3* and *ABI5*, indicating that PIL5 regulates both metabolism and signaling of GA and ABA (Oh et al. 2007, 2009). Furthermore, genetic studies identified SOMNUS (SOM) as another target of PIL5, *SOM* encodes a CCCH zinc finger protein that negatively regulates light-dependent seed germination (Kim et al. 2008). PIL5 and ABI3 collaboratively activate the expression of *SOM* by directly binding to its promoter in imbibed seeds, suggesting that SOM integrates ABA and light signaling to regulate seed germination (Park et al. 2011) (Fig. 4). Two key components of phytochrome A pathway, FHY3 and FAR1, have been shown to serve as positive regulators of ABA signaling during seed germination. They directly bind to the promoter of *ABI5* and activate its expression, providing new insights into the integration of light and ABA signaling (Tang et al. 2013). A key component of photomorphogenesis, ELONGATED HYPOCOTYL 5 (HY5), has been demonstrated to be involved in the integration of light and ABA signaling during seed germination. HY5 directly binds to the promoter of *ABI5* to promote expression of *ABI5* and ABI5-targeted genes in seeds (Chen et al. 2008). Moreover, a recent study showed that a B-box (BBX) protein BBX21 negatively regulates *ABI5* expression by interfering with HY5 binding to *ABI5* promoter (Xu et al. 2014).

Temperature is another important environmental factor in the control of seed dormancy induction and release. Low temperature during seed maturation induces seed dormancy by promoting ABA biosynthesis and GA catabolism, partially through the actions of DELAY OF GERMINATION1 (DOG1) and FLOWERING LOCUS C (FLC). After cold induction, expression of *NCED4*, *NCED6*, *GA2ox2* is up-regulated, while *CYP707A2* is down-regulated, which are consistent with the changes of ABA/GA balance (Chiang et al. 2009; Footitt et al. 2011; Kendall et al. 2011). On the contrary, cold temperature during seed imbibition stimulates the release of seed dormancy, partially by promoting bioactive GA contents via activating expression of *GA3ox* and *GA20ox* (Yamauchi et al. 2004). A bHLH transcription factor SPATULA (SPT) has been reported to be involved in the seed dormancy release in response to cold stratification in *Arabidopsis*. SPT maintains seed dormancy by repressing transcription of *GA3ox* in imbibed seeds in Col-0 ecotype, further analysis indicated that SPT directly targets *ABI5*, *MOTHER OF FT AND TFL1* (*MFT*) and *RGA*, and indirectly regulates *ABI4* and *RGL3* to mediate seed germination in an ecotype-dependent way (Penfield et al. 2005; Vaistij et al. 2013). In contrast, high temperature inhibits seed germination, partially by activating *SOM* expression. In this case, ABI3, ABI5 and DELLA proteins are required for high temperature-mediated induction of *SOM* (Lim et al. 2013). Collectively, light and temperature exert influence on seed germination either by regulating ABA/GA metabolism or by affecting ABA/GA responses.

Conclusions and future perspectives

Seeds are the foundation of agriculture. High crop yield and quality are the most important breeding goals during agriculture production. Seed size is a crucial determinant of

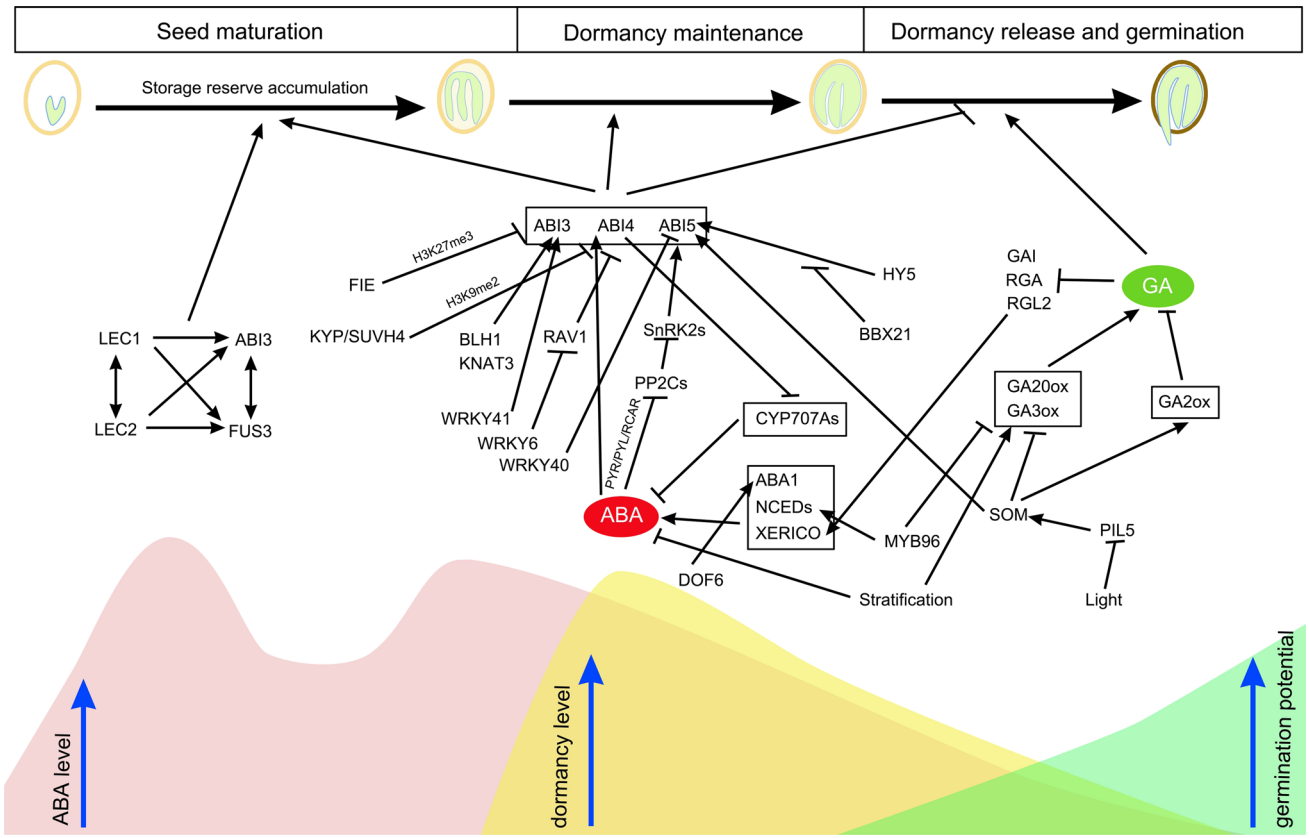


Fig. 4 Simplified model represent interactions among ABA/GA signals and regulatory factors during transition from seed maturation to germination. During seed maturation, there are two peaks of ABA accumulation which derived from maternal tissue and embryonic tissue, respectively. Seed dormancy is induced and maintained during and after the late-maturation phase. After suitable period of storage and environmental stimuli (light and cold stratification), seed dormancy is gradually released and ABA contents of seed decrease rapidly upon imbibition; meanwhile, GA level increases, and eventually leads to seed germination. *Arrows* indicate positive regulation and *T-bars* indicate negative regulation

crop yield, seed dormancy is also an important characteristic related to crop yield and quality, as pre-harvest sprouting lead to the losses in crop yield and decrease in seed vigor. Therefore, understanding the molecular regulation of seed development and germination will be of great significance for crop yield improvement. Developmental processes from seed maturation to seed germination are tightly controlled by a variety of genes and pathways which constitute a complicated genetic network. In this network, ABA has been proved to play a leading role in the regulation of seed maturation (Fig. 4). After seed maturation, a proper timing of germination is necessary for the survival of new generation under changing environmental conditions. This timing of germination is determined by both internal hormonal signals and external environmental cues, and ABA/GA balance acts as the key internal determinant. Besides the antagonistic interaction between ABA and GA, other plant hormones have also been shown to interact with ABA during seed dormancy and germination regulation.

In recent years, numerous studies have greatly advanced our knowledge of seed maturation and germination; however, due to the enormous complexity of seed maturation, the regulatory network driving seed maturation is still incomplete. This complexity of seed maturation is partially contributed by the involvement of numerous genes and multiple gene regulation levels, which include transcriptional, post-transcriptional and translational regulation. Moreover, gene expression profiles within different seed compartments such as embryo and endosperm are distinct, while ABA distribution also varies at these seed compartments, reflecting independent regulatory mechanisms in different seed compartments. Therefore, how these distinct seed compartments perceive different developmental cues and ABA signals to keep pace with each other during seed maturation remain to be an open question.

Fortunately, with the advent of new experimental technologies and system biology approaches, we could be able to integrate information on genome-wide scale to draw a clearer picture of regulatory network of seed maturation, which will enable us to better understand molecular mechanism underlying seed maturation events such as crop

seed filling and help to identify more seed size-related genes. Moreover, the comprehensive information will make it possible to manipulate seed storage metabolism and therefore to engineer for high-yield crop cultivar. In addition, the comparison of co-expression networks between model plants and crop species will contribute to the extension of breeding methodologies to a broad range of crop species and also to the identification of new specific agronomic trait-related genes in different crop species which will greatly benefit crop yield and quality. In future, increasingly accumulated data sets generated by all kinds of omics studies, along with emergence of new advanced analysis tools which facilitate the integration and visualization of these data, will dramatically improve our understanding of seed maturation and finally contribute to an overall picture of regulatory network of seed maturation.

Author contribution statement ZC conceived and initiated the work. AY contributed to the figures. AY and ZC wrote and revised the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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