Advances in the molecular regulation of seed germination in plants

Jia Zhao¹, Yongqi He¹, Hongsheng Zhang²* and Zhoufei Wang¹*

¹ Laboratory of Seed Science and Technology, Guangdong Key Laboratory of Plant Molecular Breeding, Key Laboratory for Enhancing Resource Use Efficiency of Crops in South China, Ministry of Agriculture and Rural Affairs, South China Agricultural University, Guangzhou 510642, China
² State Key Laboratory of Crop Genetics & Germplasm Enhancement application, Jiangsu Collaborative Innovation Center for Modern Crop Production, Nanjing Agricultural University, Nanjing 210095, China

* Corresponding authors, E-mail: hszhang@njau.edu.cn; wangzf@scau.edu.cn

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Abstract
Seed germination is a key process in the life cycle of seed plants. The initiation of seed germination requires the activity of specific internal signaling molecules, such as hormones and reactive oxygen species (ROS), and is dependent on external environmental factors, such as water, temperature, and light. Seed germination is a complex trait that is regulated by multiple factors, including transcript, protein, and metabolite levels. This review highlights current knowledge relating to the regulatory roles of hormones, ROS, small RNAs, epigenetic modifications, post-translational modifications, and environmental cues on seed germination, mainly focusing on Arabidopsis and rice. The review on the molecular regulation of seed germination contributes to the improvement of crop seed quality using bio-breeding approaches.

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Introduction
Seeds are vital for angiosperm and gymnosperm survival and dispersion¹. Seed germination is the basis for crop production. Seed germination refers to the process via which viable seeds transition from a dormant state to an active physiological one, leading subsequently to the development of a plant with normal roots, stems, and leaves under appropriate conditions²−⁴. Physiologically, seed germination is characterized by the absorption of water, which initiates the metabolism of reserves and energy in seeds. Molecularly, the expression of certain genes is activated, which instigates a series of reactions that lead to embryo growth⁵.

Seed germination is influenced by internal and external factors⁶,⁷. Hormones such as abscisic acid (ABA), gibberellin (GA), auxin (AUX), brassinosteroid (BR), cytokinin (CTK), ethylene (ET), and jasmonate (JA) are the key internal factors regulating seed germination⁸−¹⁵. Post-translational modifications (PTMs), such as phosphorylation¹⁶ and ubiquitylation¹⁷, are critical for seed germination, directly or indirectly affecting protein localization, stability, and activity¹⁸,¹⁹. Meanwhile, external factors, such as light, temperature, and water, are the main signals that seeds can perceive for determining the timing of seed germination²⁰−²². Here, the current knowledge relating to the regulatory roles of hormones, reactive oxygen species (ROS), small RNAs, epigenetic modifications, PTMs, and environmental cues in seed germination concentrating primarily on Arabidopsis and rice were summarized.

Seed dormancy and germination

Seed dormancy
Seed dormancy is a temporary intrinsic block to germination even under favorable environmental conditions²³. Seed dormancy is established during seed maturation²³ and seed dormancy is gradually released during after-ripening or stratification stages²⁴. Seed dormancy is an effective way to regulate the optimal spatiotemporal distribution of seed germination and seedling formation²⁵. The transition between dormancy and germination is mainly precisely regulated by endogenous hormones ABA and GA, in which ABA positively regulates dormancy induction and maintenance, while GA promotes seed germination²⁶. Other plant hormones, such as auxin, JA, salicylic acid (SA), and CTKs, are involved in seed dormancy and germination via the ABA or GA pathways²⁷.

Seed germination
Seed germination begins with imbibition, and can be divided into three phases, namely, a rapid imbibition phase (Phase I), a lag phase (Phase II), and a phase in which active water uptake is resumed (Phase III)²⁸. In Phase I of seed germination, the seed absorbs water rapidly, which immediately initiates the repair of cellular structures, such as cell organelles, and the reactivation of biochemical processes, such as enzyme activities²⁹. When hydration levels exceed 60%, seeds enter the lag phase (Phase II), in which metabolism becomes active and the seed enters a new physiological state. Embryonic cells grow rapidly, and active substances, such as sugars and amino acids, accumulate in large amounts, while cell wall acidification promotes the loosening of cell wall polymers³⁰,³¹. Concomitantly, H⁺-ATPase activity is enhanced, which further promotes seed water absorption (Phase III) and weakens the restrictions on the development of embryonic tissues (such as endosperm), ultimately causing embryonic axis elongation, the breaking of radicle through the seed coat, and the completion of germination³¹. During seed germination, seed nutrients, such as lipids, proteins, and starch, are decomposed and utilized to maintain the early growth of the seedling until it reaches autotrophy³². Although the characteristics of seed germination have been...
widely investigated, the key events that determine seed germination remain unclear.

**Hormones regulate seed germination**

**ABA signaling pathway**

The hormone ABA promotes seed dormancy and inhibits germination\[^{32,33}\]. Several genes regulate seed germination by influencing ABA content (Fig. 1). The RNA-binding protein RZ-1 and polycomb repressor complex 2 (PRC2) can synergistically silence the expression of ABA biosynthesis enzyme gene 9-CIS-EPOXYPHYTOL DIOXYGENASE 6 (NCED6), thereby promoting seed germination in Arabidopsis, but promote seed dormancy in rice\[^{34}\]. The bHLH transcription factors Seed Dormancy 6 (SD6) and INDUCER OF CBF EXPRESSION2 (ICE2) directly regulate the expression of the ABA degradation gene ABABOX3, while OsbHLH048 of rice directly regulates the expression of the ABA synthesis gene NCED2 during rice seed germination\[^{35}\].

ABSCISIC ACID INSENSITIVE5 (ABI5) is a key component of the ABA signaling pathway during seed germination (Fig. 1). The VQ motif (FxxxFQxxTG) proteins VQ18 and VQ26 interact with ABI5 and negatively modulate its transcriptional activity, thereby promoting Arabidopsis seed germination\[^{36}\]. The histone-binding protein ENAP1 regulates H3K9 acetylation, which mediates the positive feedback regulation of ABI5 and inhibits Arabidopsis seed germination\[^{39}\]. INDUCER OF CBF EXPRESSION1 (ICE1) interacts with ABI5 and negatively regulates the response to ABA during seed germination in Arabidopsis; and ICE1 also interacts with and antagonizes the activities of DELLA proteins, which are positive regulators of ABA signaling. Thus, ICE1 establishes appropriate ABA signaling by counter-acting ABI5 and DELLA proteins activity\[^{40}\]. ABI5 also interacts with the circadian clock proteins PSEUDO-RESPONSE REGULATORS 5 (PRR5) and PRR7, thereby stimulating ABA signaling and inhibiting seed germination in Arabidopsis\[^{41}\]. C-type Cyclin1; 1 (CycC1; 1), another interacting partner of ABI5, inhibits the transcription-promoting activity of ABI5 by occupying the promoters of ABI5 target genes, thereby stimulating seed germination in Arabidopsis\[^{42}\].

**GA signaling pathway**

The components of GA signaling pathway include the GA receptor GIBBERELLIN INSENSITIVE DWARF1 (GID1), the DELLA proteins, and the F-box proteins GID2, SLEEPY 1 (SLY1) in Arabidopsis\[^{43}\]. The GA-receptor GID1 perceives bioactive GA and undergoes conformational changes that enable the interaction between GID1 and DELLAAs in Arabidopsis\[^{44}\]. When GA is present in large quantities, GA binds to a nuclear receptor GID1A and form a complex, which promotes the ubiquitination and degradation of DELLA proteins mediated by the F-box ubiquitin ligase SLY1 and then promotes seed germination\[^{45}\]. Mutations in the SLY1 lead to increased seed dormancy, and a triple knockout of AtGID1 leads to germination failure (Fig. 1)\[^{46}\].

In Arabidopsis, the DELLA subfamily of GRAS regulatory genes consists of GA INSENSITIVE (GAI), REPRESSOR OF ga1-3 (RGA), RGA-LIKE1 (RGL1), RGL2, and RGL3\[^{47}\]. In which, RGL2 functions as main signaling intermediate involved in GA-mediated seed germination\[^{48}\]. RGL2 negatively regulates IAA functions as main signaling intermediate involved in GA-mediated seed germination\[^{48}\]. RGL2 negatively regulates
seed germination in response to GA, and that RGL1, GAI, and RGA do not[49]. However, the function of RGL2 can be enhanced by GAI, RGA, and RGL1[50]. A mutation in RGL2 can rescue the non-germinating phenotype of the gibberellin-deficient mutant[51]. CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) positively regulates seed germination by directly interacted with RGL2[48], increasing the expression of downstream regulators (such as GASA6 and EXP1) of seed germination[53]. Meanwhile, GAI and RGA can also be degraded via the COP1/SUPPRESSOR OF PHYA-105 (SPA) complex[52]. In Arabidopsis, SMA1 interacts with the DELLA proteins RGL1 and RGL3, thus enhances the transcriptional activity of SMAX1 and inhibits GA biosynthesis key enzyme GIBBERELLIN 3-oxidase 2 (GA3ox2) gene expression, which inhibit seed germination under weak light conditions[53].

In rice, the DELLA protein is identified as SLENDER RICE1 (SLR1), which has significant homology with RHT-1Da in wheat, D8 in maize, and GAI and RGA in Arabidopsis[54]. When GA is present, the GA-GID1-SLR1 complex is formation, which facilitates the degradation of SLR1, and then the released GAMYB from SLR1 promote the gene expression of α-amylase[27]. In addition, stress-associated protein 8 (OsSAP8) interacts with lesion simulating disease 1-like 1 (OsLSDL1) and OsZIP58 to reduce the binding of OsZIP58 to the GA biosynthesis gene KAURENE OXIDASE 2 (KO2) promoter, which promotes the biosynthesis of GA and, consequently, the activation of amylase expression and seed germination[55]. GERMINATION DEFECTIVE 1 (OsGID1) binds to the promoter of the LEC2/FUS3-like gene OsSFL1 and activates its expression, which represses the expression of GA 2-oxidase 3 (GA2ox3) and induces that of GA2oxox1, OsGA2oxox2, and OsGA3ox2, thereby influencing seed germination in rice[56].

Other hormones

BR and ethylene pathways also promote seed germination in plants[57,58]. It has been found that blocking BR signaling delays seed germination and inhibits embryonic growth. BRASSINAZOLE-RESISTANT 1 (BZR1), a key regulatory factor in the BR signaling pathway, upregulates α-amylase expression by binding to the promoter of alpha-Amylase 3D (R Amy3D), thus influencing starch degradation in the endosperm and subsequently promoting seed germination[59]. Similarly, seed germination is promoted by the ethylene pathway in plants[58]. The production of ethylene occurs immediately after seed imbition and increases as germination progresses. Moreover, the peak of ethylene release coincides with the emergence of the radicle through the seed coat[60–62]. The direct precursor of ethylene, 1-aminoacyclopropane-1-carboxylic acid (ACC), promotes seed germination in many species, such as lettuce, sunflower, chrysanthemum, chickpea, amaranth, and beet (Fig. 1)[63–67]. Low temperature, GA, nitric oxide, and hydrogen cyanide (HCN) treatments can all increase ethylene production and promote seed germination[67,50,51]. In Arabidopsis, the ethylene-insensitive mutants Aetetr1 and Aetin2 show delayed seed germination[64]. The Arabidopsis ethylene-responsive factor ERF12 can bind to the promoter of the key dormancy gene DELAY OF GERMINATION 1 (DOG1) and recruit the transcriptional co-repressor TOPLESS (TPL), which inhibits DOG1 expression and promotes seed germination[68].

In contrast to BR and ethylene, seed germination is inhibited by jasmonic acid (JA) and its derivatives[69,70]. The application of exogenous JA or methyl jasmonate (MeJA) can inhibit seed germination, as can their precursor 12-oxo-phytodienoic acid (OPDA)[71]. Interestingly, auxin both stimulates and inhibits seed germination in plants, depending on its concentration[72]. For instance, at high concentrations (0.3 to 1 μM indole-3-acetic acid (IAA)), auxin inhibits seed germination in Arabidopsis[73,74], whereas at low concentrations (0.03 to 3 nM IAA), the opposite is observed[75]. Recent research has shown that exogenous auxin and JA synergistically enhance the ABA-induced delay in seed germination. Auxin Response Factor10 (ARF10) and ARF16 positively mediate JA-increased ABA responses, and this process is mainly dependent on AB15 (Fig. 1)[76]. In general, the regulatory roles of signaling pathways associated with other hormones except ABA and GA, such as ETH, BR, JA, and auxin, on seed germination remains unclear.

Hormone interactions

The crosstalk among hormones plays an important role in seed germination in plants (Fig. 1). For example, in wheat, JA can suppress the ABA biosynthesis genes, TanCED1 and TanCED2, and thereby promote seed germination[77]. Several JAZ repressors stimulate seed germination by interacting with ABI3 and inhibit its transcription as well as that of AB15[78]. OsPKS improves seed germination by increasing the GA/ABA ratio[79]. Similarly, BR promotes seed germination by antagonizing ABA signaling through a feedback loop mediated by MOTHER OF FT AND TFL1 (MFT)[80]. BRINSENSITIVE1 (BRI1)-EMS-SUPPRESSOR1 (BES1), a BR signaling component, interacts with ABI3 and inhibits its binding to the promoter region of target genes, resulting in a decrease in their expression levels and the promotion of seed germination in Arabidopsis[80]. In the presence of ABA, the protein kinase BRASSINOSTEROID-INSENSITIVE2 (BIN2), another constituent of the BR signaling pathway, interacts with ABI3 and stabilizes it through phosphorylation, thus positively regulating ABA signaling and inhibiting seed germination[90]. Ethylene can antagonize the effect of ABA on endosperm weakening and seed coat rupture, which consequently stimulates seed germination without affecting ABA levels[81]. ABI4 can directly bind to the promoters of the ethylene biosynthesis genes ACC SYNTHASE2 (ACS2) and ACS5, resulting in reduced ethylene production and the suppression of seed germination[82]. The indole-3-acetic acid glucosyltransferase gene of rice (OsiAGLU) positively regulates seed germination by reducing IAA and ABA contents and OsABI3/5 expression[83]. AUXIN RESISTANT 1 (AUX1) is required for ABA-mediated inhibition of seed germination and AtAUX1 loss-of-function mutants display an enhanced ABA-resistant phenotype[84].

Studies have also shown that BR and auxin regulate seed germination in a manner involving GA metabolism or the GA signaling pathway (Fig. 1). For example, GA and BR can synergistically induce the degradation of the key gluten protein-encoding gene GLU1A2, thereby promoting seed germination in rice[85]. Rice LATE EMBRYOGENESIS ABUNDANT 33 (LEA33) affects seed germination possibly by reducing BR accumulation and enhancing GA biosynthesis[86]. Two components of the BR signaling pathway, the basic helix-loop-helix transcription factors HBI1 and BEE2, can directly regulate the gene expression of GA-stimulated Arabidopsis 6 (GAS6), which promotes seed coat and endosperm breakage for seed germination[87]. Moreover, exogenous auxin treatment represses soybean seed germination by positively mediating ABA and negatively
regulating GA biosynthesis\(^\text{[88]}\). The expression of the auxin transporters AUX1, PIN-FORMED 2 (PIN2), and PIN7 are highly upregulated in ga1 mutant seeds following treatment with GA\(^\text{[89]}\). These findings underscore the importance of hormone crosstalk on seed germination in plants. However, an in-depth understanding of the crosstalk among JA, BR, ethylene, and auxin is still lacking.

**Reactive oxygen species regulate seed germination**

**Roles of ROS in seed germination**

Reactive oxygen species (ROS) are a class of highly active oxygen-containing molecules or ions that mainly include superoxide anions (\(\text{O}_2^•−\)), hydrogen peroxide (\(\text{H}_2\text{O}_2\)), singlet oxygen (\(\text{O}_2(\text{Singlet})\)), and hydroxyl radicals (\(\text{OH}^•\)). In dry seeds, ROS are mainly generated by lipid autooxidation, whereas following imbibition, they are primarily produced by enzymatic reaction\(^\text{[90]}\). ROS have a dual role in seed physiology. Low levels of ROS stimulate seed germination, while excessive ROS accumulation causes oxidative damage and inhibits seed germination\(^\text{[91]}\). ROS promote seed germination by contributing to cell wall loosening, endosperm weakening, and radicle and root elongation\(^\text{[92−97]}\). Rice polyamine oxidase 5 (OsPAOS) oxidizes spermine and generates \(\text{H}_2\text{O}_2\), which promotes mesocotyl cell elongation during seed germination\(^\text{[98]}\). Cotton HSP24.7 enhances the release of ROS from mitochondria, which leads to the degradation of key components within the endosperm membrane and reduces its strength for seed germination\(^\text{[100]}\). Generally, within a certain concentration range, known as the ‘oxidative window’, ROS promote seed germination, while the opposite is observed at concentrations that deviate from this window\(^\text{[101]}\). Although the appropriate ROS concentrations that contribute to seed germination have been reported for camphor, wheat, soybean, barley, and pea\(^\text{[101−103]}\), the oxidative window for the promotion of seed germination remains unclear for most crops.

**Interactions between ROS and hormones regulate seed germination**

Interactions between ROS and hormones such as GA and ABA play important roles in seed germination in plants. Exogenous GA treatment can induce ROS production and promote seed germination in wild oat (\(\text{Avena fatua}\)) and Chinese flowering cabbage (\(\text{Brassica parachinensis}\))\(^\text{[104,105]}\). Exogenous \(\text{H}_2\text{O}_2\) can enhance the expression of kaurenoic acid oxidase 1 (\(\text{KAO1}\)) and \(\text{HvGA2ox3}\), thereby promoting GA synthesis and seed germination in barley\(^\text{[106]}\). Similarly, \(\text{H}_2\text{O}_2\) can enhance the GA-induced expression of the expansin gene \(\text{HvExpA1}\) and the GA biosynthesis gene \(\text{HvGA2ox1}\), as well as inhibit the expression of the GA catabolic gene \(\text{HvGA2ox3}\), thereby promoting GA accumulation and, consequently, seed germination in barley\(^\text{[92]}\). In \(\text{Arabidopsis}\), exogenous \(\text{H}_2\text{O}_2\) treatment can activate the expression of the GA synthesis-related genes \(\text{GA3ox}\) and \(\text{GA2ox}\) and the ABA metabolism-related gene \(\text{CYT707A}\), which enhances GA synthesis and ABA metabolism and improves seed germination\(^\text{[107]}\). In tomato, \(\text{H}_2\text{O}_2\) enhances germination capacity by upregulating the expression of the GA biosynthesis gene \(\text{GA3ox1}\) as well as that of the ABA catabolism gene \(\text{ABA}^8\)-hydroxylase (\(\text{ABA}^8\text{ox}\))\(^\text{[108]}\). \(\text{H}_2\text{O}_2\) regulates barley seed germination by influencing the activity of an ABA catabolic enzyme, and consequently, ABA content in seed embryos\(^\text{[109]}\). Meanwhile, \(\text{H}_2\text{O}_2\) can suppress the phosphatase activity of \(\text{ABI1}\) and \(\text{ABI2}\), thus inhibiting seed germination in \(\text{Arabidopsis}\)\(^\text{[109,110]}\). \(\text{ABI4}\), another major constituent in ABA signaling, modulates ROS metabolism during seed germination under salt stress by directly combining with \(\text{RboHD}\) and \(\text{Vitamin C Defective 2 (VTC2)}\), key genes in ROS production and scavenging\(^\text{[112]}\). Similarly, \(\text{ABIS}\) can modify ROS homeostasis by inducing \(\text{CATALASE 1 (CAT1)}\) expression and, consequently, catalase activity\(^\text{[113]}\). Furthermore, exogenous \(\text{H}_2\text{O}_2\) can induce ethylene biosynthesis, which promotes seed germination in soybean\(^\text{[102]}\). Exogenous ethylene positively regulates seed germination in sunflower by activating NADPH oxidase, which leads to ROS accumulation in the embryonic axis\(^\text{[60]}\). However, whether crosstalk between ROS and other hormones such as GA, BR, or auxin also exerts regulatory effects on seed germination requires further investigation.

**Internal regulatory factors regulate seed germination**

**Epigenetic modifications**

**Small RNAs**

MicroRNAs (miRNAs) are a class of small, non-coding RNAs, approximately 20 to 24 nucleotides in length\(^\text{[114,115]}\). They can influence gene expression at the transcriptional level via the methylation of target genes or at the post-transcriptional level by promoting target mRNA degradation or inhibiting target mRNA translation\(^\text{[116]}\). Several hormone-related signaling pathways are controlled by miRNAs during seed germination in plants (\text{Fig. 2})\(^\text{[20]}\). In \(\text{Arabidopsis}\), the overexpression of \(\text{miR159}\) can inhibit the transcription of the ABA response factors \(\text{MYB33}\) and \(\text{MYB101}\), resulting in reduced sensitivity to ABA during seed germination. \(\text{MiR159}\) has also been reported to regulate seed germination by regulating the mRNA level of \(\text{GAMYB}\), and thus modulating GA signaling\(^\text{[117]}\). \(\text{MiR9678}\) regulates seed germination via its effects on ABA/GA signaling pathways in wheat\(^\text{[118]}\). Meanwhile, \(\text{miR160}\) was shown to participate in seed germination by negatively regulating the auxin response factor \(\text{ARF10}\) in \(\text{Arabidopsis}\)\(^\text{[74]}\). In addition, rice \(\text{miR393}\) negatively mediates coleoptile elongation under flooded conditions by regulating the expression of the auxin receptor-encoding genes \(\text{OsTIR1}\) and \(\text{OsAFB2}\)\(^\text{[119]}\). MiRNAs have also been found to affect seed germination by regulating epigenetic factors. For example, \(\text{miR402}\) regulates seed germination under stress conditions by targeting the mRNA of \(\text{DML3}\), a DNA demethylase, and promoting its degradation\(^\text{[120]}\). Overall, only a few small RNAs that participate in seed germination in crops have been identified to date. However, given their biological importance, the application of small RNAs for the improvement of seed germination deserves further investigation.

**Genomic imprinting**

Genomic imprinting refers that one parent allele is silenced while the other parent allele remains active, which caused by the asymmetric DNA methylation between parental alleles, including maternally expressed genes (MEGs), or paternally expressed genes (PEGs)\(^\text{[121]}\). In \(\text{Arabidopsis}\), DNA methylation is an important imprinting for many MEGs\(^\text{[122]}\). Trimethylation of histone H3 on lysine 27 (\(\text{H3K27me3}\)), catalyzed by the PRC2, is an important epigenetic mark involved in the regulation of
some imprinted genes in the endosperm[123]. Many genes marked by single H3K27me3 have been found to be induced during seed germination[124]. In Arabidopsis, H3K27me3 is catalyzed by histone methyltransferases[125]. Arabidopsis endosperms are targeted by the H3K27me3 demethylase REF6 and became activated during germination[124]. Additionally, AtREF6 can directly targets ABA catabolizing enzymes CYP707A1 and CYP707A3, which contributes to suppress seed dormancy (Fig. 2)[126]. DOGL4 is an imprinted gene in Arabidopsis endosperm, and it negatively affects seed dormancy. DNA demethylase ROS1 negatively regulates DOGL4 imprinting via demethylation of the DOGL4 promoter on the paternal allele, and ROS1 regulates seed dormancy by controlling DOGL4 expression[127]. In castor bean, imprinted genes showed dynamic expression characteristics at different stages of endosperm, mainly involved in endosperm development and storage material accumulation, and MEGs and PEGs had obvious functional differentiation. It showed that imprinted genes persisted in germinated endosperm and participated in seed germination[128]. Overall, only a few genomic imprinting genes that participate in seed germination in crops have been identified to date.

Other modifications

Epigenetic modifications, including methylation, demethylation, deacetylation have also been reported to be involved in the regulation of seed germination in plants[129] (Fig. 2). H3K27me3 plays a key role in regulating gene repression and cell fate specificiation. Relative of Early Flowering 6 (REF6) mediates the demethylation of H3K27, which helps to activate gene transcription and promote seed germination in Arabidopsis[126]. The EARLY FLOWERING IN SHORT DAYS (EFS) gene encodes a H3K4 and H3K36 methyltransferase that inhibits seed germination in Arabidopsis by directly binding to the promoter of PHYTOCHROME-INTERACTING FACTOR 1 (PIF1) and increasing the levels of H3K36me2 and H3K36me3 at the binding sites, thus upregulating PIF1 expression[131]. The histone mark reader Early Bolting in Short Days (EBS) is recruited by the transcription factor Agamous-Like67 (AGL67) to H3K4me3 at the promoter of the gene encoding the zinc-finger protein SOMNUS (SOM), thereby epigenetically activating SOM expression and suppressing seed germination under high-temperature conditions[132]. The histone deacetylase HDA15 is recruited by the bHLH transcription factor PIF1 to the promoters of hormone signaling-related genes and inhibits their expression by reducing H3 acetylation levels. Additionally, HDA15 was shown to negatively regulate phytochrome B (PhyB)-dependent seed germination under dark conditions[133].

Several studies have demonstrated that epigenetic factors regulate seed germination via their effects on hormone-related metabolism and signaling pathways (Fig. 2). The non-coding RNA HIDDEN TREASURE 1 (HID1) promotes PhyB-dependent seed germination by directly inhibiting the expression of N breeze, which encodes the rate-limiting enzyme in ABA biosynthesis in Arabidopsis[134]. Switch/sucrose non-fermentable (SWI2/SNF2) chromatin remodeling ATPase BRAHMA (BRM) directly represses the expression of ABI5 and the loss of function of BRM results in ABA hypersensitivity during seed germination[135]. The JmjC domain-containing demethylase JMJ17 participates in the response to ABA during seed germination in Arabidopsis by co-regulating WRKY DNA-BINDING PROTEIN 40 (WRKY40), HYPOCOTYL5 (HYS), and ABI5[136]. The
expression and ABA biosynthesis, and also negatively regulates NCED1 [145] ABF4 during seed germination. In addition, MAPK11 phosphorylates and stimulates the type 2C protein phosphatase calcium-dependent protein kinase CPK12 of Arabidopsis. The signal and inhibits seed germination in [150]. The signal and inhibits seed germination in

Protein kinases CARK1 and CARK6 interact with and phosphorylate ABI5 and act antagonistically with SnRK2 to regulate the protein phosphatases FyPP1 and FyPP2 directly dephosphorylate ABI5, which activates the expression of downstream genes involved in seed germination in Arabidopsis [148]. Similarly, the protein phosphatases FyP1 and FyP2 directly dephosphorylate ABI5 and act antagonistically with SnRK2 to regulate ABA responses in seed germination [149]. The receptor-like protein kinases CARK1 and CARK6 interact with and phosphorylate the ABA receptors RAR11–14, which enhances the ABA signal and inhibits seed germination in Arabidopsis [150]. The calcium-dependent protein kinase CPK12 of Arabidopsis phosphorylates and stimulates the type 2C protein phosphatase ABI2, a negative regulator of ABA signaling, while also phosphorylating two ABA-responsive transcription factors, ABF1 and ABF4, during seed germination [151]. In addition, MAPK11 positively influences ABA signaling by upregulating both NCED1 expression and ABA biosynthesis, and also negatively regulates seed germination by influencing the phosphorylation status of SnRK2.2 in tomato [152]. Totally, most relevant studies have reported that phosphorylation plays an important role in the ABA signaling pathway-mediated regulation of seed germination. However, whether phosphorylation is also involved in the regulation of seed germination in other signaling pathways needs to be further investigated.

Post-translational modifications

Phosphorylation

Protein phosphorylation, which refers to the transfer of a phosphate group from adenosine triphosphate (ATP) to a specific amino acid residue in a substrate protein by protein kinases (PKs) [143], is widely involved in the regulation of seed germination [142]. Four types of kinases—sucrose non-fermenting 1-related protein kinases (SnRks) [143], mitogen-activated protein kinases (MAPKs) [137,144], calcium-dependent protein kinases (CDPKs) [145], and receptor-like kinases (RLKs) [146] have been widely shown to play significant roles in seed dormancy and germination. Studies have reported that phosphorylation related to seed germination mainly affects the ABA signaling pathway (Fig. 2). For example, the binding of ABA to its receptors PYR1/PYL/RCAR activates SnRK2s by inhibiting the phosphatase activity of PP2Cs. The activated SnRK2s subsequently phosphorylate ABI5 and promote its stability, thereby inhibiting seed germination [147]. The Glycogen Synthase Kinase 3-like kinase BRASSINOSTEROID INSENSITIVE2 (BIN2) enhances ABA signaling by phosphorylating ABI5 during seed germination in Arabidopsis [9]. The Arabidopsis RAV (Related to ABI3/VP1) transcription factor RAV1 is phosphorylated by SnRK2.2, SnRK2.3, and SnRK2.6, leading to an increase in the expression levels of ABI3, ABI4, and ABI5 during seed germination and early seedling development [143]. SOS2-LIKE PROTEIN KINASES (PKSS) phosphorylates ABI5, which activates the expression of downstream genes involved in seed germination in Arabidopsis [148]. Similarly, the protein phosphatases FyP1 and FyP2 directly dephosphorylate ABI5 and act antagonistically with SnRK2 to regulate ABA responses in seed germination [149]. The receptor-like protein kinases CARK1 and CARK6 interact with and phosphorylate the ABA receptors RAR11–14, which enhances the ABA signal and inhibits seed germination in Arabidopsis [150]. The calcium-dependent protein kinase CPK12 of Arabidopsis phosphorylates and stimulates the type 2C protein phosphatase ABI2, a negative regulator of ABA signaling, while also phosphorylating two ABA-responsive transcription factors, ABF1 and ABF4, during seed germination [151]. In addition, MAPK11 positively influences ABA signaling by upregulating both NCED1 expression and ABA biosynthesis, and also negatively regulates

Environmental factors that regulate seed germination

Light signals

Light is an important environmental factor that regulates seed germination [19,21,160]. Phytochromes (Prs) are key photoreceptors that regulate responses to light and are responsible for initiating between 10% and 30% of the transcriptional cascades of the entire transcriptome [163]. Under red light illumination, the inactive form, Pr, is transformed into the biologically active form, Pfr, thus promoting seed germination; however, Pr is converted into Pr under far-red light conditions, leading to the inhibition of seed germination [164]. In Arabidopsis, there are five phytochrome proteins—PhyA, PhyB, PhyC, PhyD, and PhyE—with PhyB playing a dominant role in light-mediated seed germination (Fig. 3) [153,165]. The bHLH transcription factor PIF1 plays an important role in phytochrome-mediated seed germination [20,166]. The F-Box protein Cold Temperature-Germinating 10 (CTG10) of Arabidopsis can sense light signals. PIF1 and
CTG10 coexist under dark conditions; however, after exposure to light, CTG10 helps reduce PIF1 levels, thereby promoting seed germination[167].

Exogenous light signals regulate seed germination mainly via the ABA and GA pathways (Fig. 3). For example, PhyA and PhyB mediate ABA and GA synthesis and catabolism by binding to PIF1 and inhibiting SOM activation, thus exerting a regulatory effect on seed germination[168]. In darkness, PIFs directly bind to the promoter of the key transcription factor ABI5 and activate its transcription, thereby positively regulating the ABA signaling pathway[169]. PIF1 can repress the expression of GA biosynthetic genes (GA3ox1 and GA3ox2) and activate that of a GA catabolic gene (GA2ox) in PhyA- and PhyB-dependent seed germination, which blocks GA degradation and increases GA biosynthesis[22,170]. PIF1 interacts with REVILLE1 (RVE1), and, together, they synergistically regulate the expression of multiple genes in the ABA and GA pathways that are involved in PhyB-mediated seed germination[22]. Mutations in the AP2/ERF transcription factors ERF55 and ERF58 result in stronger light dependency during seed germination by influencing ABA and GA levels in Arabidopsis[171]. MFT is a key negative regulatory factor for seed germination; the expression of the MFT gene is promoted by far-red light through the PIF1/SOM/ABI5/DELLA pathway but is inhibited by red light through the transcription factor SPATULA (SPT)[172]. MFT affects the levels of the JA precursor oxylipin cis-12-oxo-phytodienoic acid (OPDA) and ABA under shading conditions, which inhibits seed germination[173]. Light signals regulate seed germination mainly by influencing ABA and GA signaling pathways; however, whether they also influence seed germination via other signaling pathways is not known.

**Temperature**

Temperature is another important factor affecting seed germination. Both too-high and too-low temperatures inhibit or delay seed germination by disrupting a variety of molecular and physiological processes[174]. Under low-temperature conditions, seeds experience reduced water absorption, protein degradation, carbohydrate metabolism, and energy production but an increase in ABA synthesis, which delays seed germination[175]. When seeds are exposed to heat stress, meanwhile, ROS, malondialdehyde, antioxidant enzyme, and ABA levels are increased, which suppresses seed germination[176]. Molecular analysis indicated that ABI3, ABI5, and DELLA target the promoter of the transcription factor SOM and activate its transcription, which suppresses seed germination under high temperatures (Fig. 3)[177]. Similarly, the epigenetic factor Powdery mildew resistance (PWR) interacts with ABI3 and activates SOM transcription through epigenetic modifications, leading to the suppression of seed germination under high-temperature conditions[178]. A mitochondrial heat shock protein GhHSP24.7 regulates seed germination in response to temperature in cotton. GhHSP24.7 promotes seed germination under both high and low-temperature conditions by inducing ROS production, thereby accelerating endosperm breakdown[179]. Heat shock protein 70-16 (HSP70-16) and voltage-dependent anion channel 3 (VDAC3)
jointly suppress seed germination by promoting ABA flow from the endosperm to the embryo under low-temperature conditions\[179\].

**Water**

Imbibition is the first step in seed germination, and water movement thus plays a crucial role in the breaking of dormancy\[180–182\]. Plant aquaporins (AOPs), including plasma membrane intrinsic protein (PIPs), tonoplasma intrinsic proteins (TIPs), nodulin 26-like MIPs (NIPs), and small and basic intrinsic proteins (SIPs), are membrane channels that mediate intracellular water movements. Most PIPs and TIPs are water-selective channel proteins\[183–186\]. In pea, PsPIP1;1, PsPIP2;1, and PsTIP1;1 are expressed in germinating seeds. PsPIP1;1 plays a role in water absorption during seed imbibition, while PsPIP2;1, possibly together with PsPIP1;1, may be involved in the release of phloem water from the seed coat symplast (Fig. 3)\[187\]. TIP3;1 and TIP3;2 have previously been implicated in water or solute transport during seed germination under water stress conditions in *Arabidopsis*\[185\]. *Sesuvium portulacastrum* SpAQP1 promotes seed germination and root growth in transgenic tobacco and increases salt tolerance by increasing the activities of antioxidative enzymes\[188\]. Hydration is the key trigger for the initiation of germination. Recent investigations have shown that the *Arabidopsis* prion-like protein FLOE1 undergoes phase separation upon hydration, which allows the embryo to sense water stress, and hence regulate the best time for seed germination under unfavorable environments\[189,190\]. Overall, the mechanisms that determine plant seed germination under drought or submergence conditions remain unclear and require further investigation.

**Perspectives**

Seed germination is a key determinant of crop production and final yield. An in-depth understanding of the internal and external regulatory factors that determine seed germination is crucial for achieving high crop yields. In this review, we summarized the findings relating to molecular mechanisms involved in seed germination in plants. Studies on seed germination to date have mainly focused on *Arabidopsis* and rice, and relatively little is known about this process in crops such as maize, wheat, and barely. This situation needs to be addressed in the future. Seed germination is a complex trait that is determined by both genetic and environmental factors. Abiotic stresses, such as drought, flooding, salt, heat, and cold, are the most common adverse environmental conditions affecting seed germination in plants. Further unraveling the molecular mechanisms of seed germination under stress conditions is needed in the future.

The hormones ABA and GA are the key regulators of seed germination. Other hormones involved in this process, such as AUX, CTK, JA, and BR, in addition to ROS, function mainly through the ABA and GA signaling pathways. However, how to precisely control the levels and distribution of hormones and ROS in germinating seeds remains unclear. It is well known that there is crosstalk among hormones and ROS involved in seed germination; nevertheless, the detailed molecular mechanisms underlying their synergistic and antagonistic relationships remain to be resolved. Additionally, it would be interesting to reveal whether other as yet unidentified molecules, besides hormones and ROS, are involved in seed germination in plants. The recent rapid development of omics technologies provides important tools for the identification of factors involved in seed germination at the transcript, protein, and metabolite levels\[197\]. To date, the functional validation of newly identified regulators of seed germination is lacking.

Seed germination is regulated by extremely complex signaling networks. The endosperm not only constitutes a source of nutrients but also controls seed germination by actively secreting signals. The molecular mechanisms underlying how cell-to-cell communication coordinates seed germination and the role of the endosperm in seed germination also require further investigation\[176\]. Environmental factors such as light, temperature, and humidity play crucial roles in the regulation of seed germination in plants, but how seeds sense these environmental factors remains incompletely understood. Recent studies have indicated that environmental factors regulate seed germination mainly via the ABA pathway, and whether other hormones (AUX, CTK, JA, BR) and/or ROS-related pathways are also involved needs to be further analyzed. Abiotic stresses are the predominant environmental determinants of crop productivity worldwide. Despite this, the mechanisms that regulate crop seed germination under stress conditions such as drought, flooding, salt, heat, and cold have not been studied in depth. A combination of molecular, organismal, and ecological studies will reveal the mechanisms of seed germination with direct implications for the design of elite crops in the face of climate change.

**Author contributions**

The authors confirm contribution to the paper as follows: study conception and design: Wang Z, Zhang H; data collection: Zhao J, He Y; draft manuscript preparation: Wang Z, Zhao J. All authors reviewed the results and approved the final version of the manuscript.

**Data availability**

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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