REVIEW

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Seed dormancy and ABA signaling
The breakthrough goes on

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The seed is an important organ in higher plants, it is an important organ for plant survival and species dispersion. The transition between seed dormancy and germination represents a critical stage in the plant life cycle and it is an important ecological and commercial trait. A dynamic balance of synthesis and catabolism of two antagonistic hormones, abscisic acid (ABA) and giberellins (GAs), controls the equilibrium between seed dormancy and germination. Embryonic ABA plays a central role in induction and maintenance of seed dormancy and also inhibits the transition from embryonic to germination growth. Therefore, the ABA metabolism must be highly regulated at both temporal and spatial levels during phase of dessication tolerance. On the other hand, the ABA levels do not depend exclusively on the seeds because sometimes it becomes a strong sink and imports it from the roots and rhizosphere through the xylem and/or phloem. These events are discussed in depth here. Likewise, the role of some recently characterized genes belonging to seeds of woody species and related to ABA signaling are also included. Finally, although four possible ABA receptors have been reported, not much is known about how they mediate ABA signaling transduction. However, new publications seem to show that almost all these receptors lack several properties to consider them as such.

Introduction

The seed is the dispersal unit emerged in the course of plant evolution. The biology of seeds can be divided in three important phases: development that includes zygotic embryogenesis, dormancy that prevents seeds from germinating under unfavorable conditions and germination (seed emergence). The transition between dormancy and germination represents a critical stage in the life cycle of higher plants and it is an important ecological and commercial trait. Seed germination is regulated by endogenous hormonal cues and external environmental signals such as water, low temperature and light, which influence whether an imbibed seed completes germination or remains dormant (reviewed in refs. 1 and 2). Seed dormancy, a temporary quiescent state that is observed in seeds from many plants species, prevents untimely germination and ensures plant survival by adjusting vegetative development to seasonal changes in the environment.[1,3] A dynamic balance between synthesis and catabolism of the abscisic acid (ABA) and gibberellins (GAs) controls the equilibrium between dormancy and germination.[4] At the molecular level, the ABA/GA balance is in part determined by the antagonistic control of ABA and GA on each other through their reciprocal regulation of the transcription of their metabolic genes.[5-7]

The ABA, derived from epoxycarotenoid cleavage, serves as a plant-specific signal during development and in response to environmental stresses such as cold, drought and high concentrations of salt in the soil (reviewed in ref. 8). The ABA also elicits, among others numerous physiological functions, the closure of stomatal pores to restrict transpiration, adjustment of metabolism to tolerate desiccation and cold temperatures, and inhibition seedlings growth. Likewise, ABA represses germination and is presumed to function to stabilize the dormant state (reviewed in refs. 1, 9–11). ABA, like other hormones, functions through a complex network of signaling pathways where the cell response is initiated by ABA perception which triggers downstream signaling cascades to induce the final physiological effects. Numerous downstream components involved in ABA signal transduction have been identified by genetic approaches (reviewed in refs. 12 and 13). Signaling pathways are usually made of regulatory networks of transcription factors (TFs) which specifically bind short DNA sequences (cis-elements) in the regulatory regions (promoters) of their target genes to regulate their expression levels in response to hormonal/environmental signals.[14] Recently, it was demonstrated that the N-end rule pathway (i.e., one of several proteolytic pathways of ubiquitin system) promotes seed germination in Arabidopsis through removal of ABA sensitivity.[15] In this review, we mainly discuss recent findings that are related with ABA controlled dormancy and signaling at the receptor level.

ABA Biosynthesis Pathway and Temporal and Spatial Expression of its Biosynthetic Genes in Seeds

The knowledge of ABA biosynthesis pathway notably advanced in the past few years (updated in **Fig. 1**). Thus, most intermediates and enzymes involved in its synthesis have been identified and a large number of genes and mutants related to the ABA biosynthetic pathway were also isolated and characterized.[8,16-19] But their regulation has mainly been studied in vegetative

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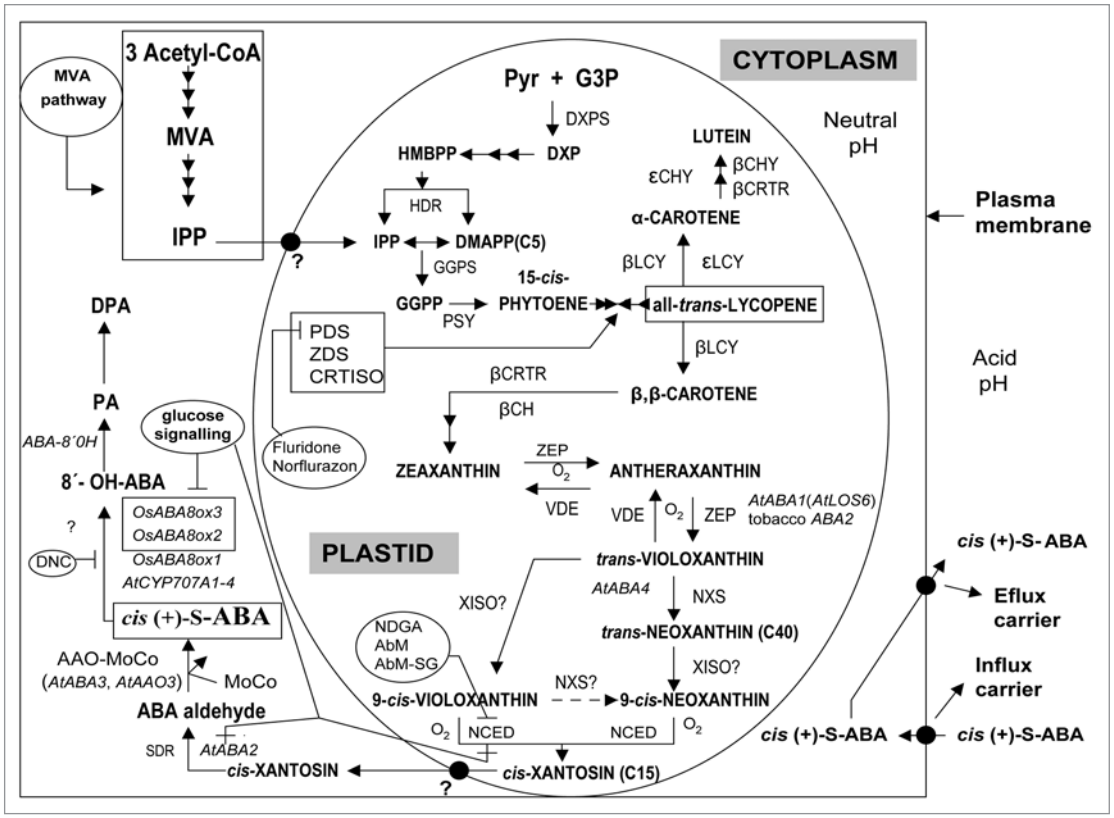

Figure 1. ABA biosynthesis pathway, inhibitors and intracellular compartmentalization in higher plants. AbM, abamine; DMAPP, dimethylallyl di-P; DNC, diniconazole; DPA, dihydrophaseic acid; DXP, 1-deoxy-D-xylulose-5-P; G3P, glyceraldehyde-3-P; GGPP, geranylgeranyl di-P; HMBPP, hydroxymethylbutenyl 4-di-P; IPP, isopentenyl di-P; MVA, mevalonic acid; NDGA, nordihydroguaiaretic acid; PA, phaseic acid; Pyr, piruvate. Involved enzymes: AAO-MoCo, abscisic aldehyde oxidase or MoCo sulfurase; βCH, β-carotene hydroxylase; βCHY and βCRTR, β-ring hydroxylases; εCHY, ε-ring hydroxylase; ABA 8'-hydroxylase (*Hordeum vulgare*, *HvABA8'OH*); CRTISO, carotenoid isomerase; βCRTR, β-ring hydrolase; DXPS, DXP synthase; GGPS, geranylgeranyl diphosphate synthase; HDR, HMBPP reductase; βLCY, lycopene β-cyclase; εLCY, lycopene ε-cyclase; NCED, 9-cis-epoxycarotenoid dioxigenase (*AtNCED1-9*; maize, *VP14*; tomato, *NOT*); NXS, neoxanthin synthase; PDS, phytoene desaturase; PSY, phytoene synthase; SDR, member of short-chain dehydrogenases/reductases family; VDE, violoxanthin de-epoxidase; XISO, xanthophyll cis-*isomerase* (predicted); ZDS, ξ-carotene desaturase; ZEP, zeaxanthin epoxidase.

tissues, often in relation to hydric stress, and expression studies in seeds are still incomplete. ABA is a sexquiterpene derived from oxidative cleavage of phytoene, a C_{40} common precursor of all plant carotenoids which are synthesized in plastids by nuclear-encoded enzymes.[20] The phytoene is synthesized by phytoene-synthase after condensation of two molecules of geranylgeranyl diphosphate (GGPP), a C_{20} formed from isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP). The IPP can be synthesized from mevalonic acid (MVA), via the cytosolic MVA pathway and subsequently sent to the plastid,[21] or alternatively formed from 1-deoxy-D-xylulose 5-phosphate (DXP) which is synthesized in plastid from pyruvate and glyceraldehide 3-phosphate, via the methylerythritol phosphate pathway.[22] The all-trans-lycopene synthesized from phytoene is successively converted in β-carotene and zeaxanthin, the first oxygenated carotenoid precursor of ABA, which produces successively antheraxanthin and trans-violaxanthin mediated by ZEP. The all-trans-violaxanthin is either converted to 9-cis-violoxanthin or to 9'-cis-neoxanthin, both C_{40} carotenoids being cleaved in the plastid to the C_{15} aldehyde xanthosine and a C_{25} compound.[13] The *ABA1*/*ZEP* gene is ubiquitously expressed during *A. thaliana* seed maturation but their expression becomes restricted to the embryo and endosperm during desiccation.[23] Enzyme(s) involved in the conversion of all-trans-violaxanthin to 9-cis-violoxanthin or 9'-cis-neoxanthin are yet to be identified. However, the enzymatic step that catalyzes the all-trans-violoxanthin to the all-trans-neoxanthin is the latest to be solved by positional cloning of the *AtABA4* gene.[24]

The 9-cis-epoxycarotenoid dioxygenase (NCED) catalyzes the oxidative cleavage of the 9-cis-violoxanthin or 9-cis-neoxanthin, synthesized from all-trans-violoxanthin to produce 9-cis-xanthoxin.[25] It is suggested that 9-cis-neoxanthin might be the major substrate in vivo of NCED to produce cis-xantosin, the first cytoplasmic precursor for the catalytic conversion to ABA.[17] *NCED* expression in response to environmental stresses is so rapid that NCED activity is considered the rate-limiting step in ABA biosynthesis. On the other hand, AtCCD1 enzyme catalyzes the oxidative cleavage of the 9,10 (9',10') double bonds of carotenoid substrates as β-carotene.[26] The NCED activity is inhibited by nordihydroguaiaretic acid (NDGA), abamine AbM; competitive inhibitor;[27] and AbM-SG (more potent competitive inhibitor than AbM to reduce ABA accumulation[28]). *NCED*

genes are not regulated by ABA, which indicates that ABA does not have a positive feedback effect on *NCED* gene expression.[29] Although *NCED* genes have been characterized in several species, limited data are available about their expression in seeds.[8,16] Overexpression of *LeNCED* gene in tomato leads to higher ABA level in seeds, increasing seed dormancy and induced expression of bean *PvNCED1* in imbibed tobacco seeds delays seed germination.[30] Differential expression of *AtNCED* members in different tissues and subcellular localization was found,[31] suggesting that a dynamic mobility of ABA precursors and/or ABA to the target sites despite the developing seeds themselves being capable of synthesizing ABA. Similar conclusions were made in the case of AAO family.[32,33] Molecular genetic analyses indicate that different members of *AtNCED* family play distinct roles in the regulation of ABA synthesis during seed development and germination, *AtNCED3* (mainly expressed in the base of seed), *AtNCED5* and *AtNCED6* (both expressed throughout the seed) and *AtNCED9*, contribute to expression in developing seeds with high levels of *AtNCED6* present at an early stage.[31] Transcripts of several members of *AtNCED* family are present in dry seeds.[31] *AtNCED6* gene is expressed specifically in immature endosperm, and *AtNCED9* gene is abundantly expressed in the embryo and endosperm during seed development, playing a major role in ABA synthesis during last steps of zygotic embryogenesis and germination.[34] The *nced6* and *nced9* mutants show reduced ABA contents in dry seeds and radicle emergence of these mutants seeds is not affected by paclobutrazol. Together, the results of Lefebvre et al.[34] suggest that cis-xanthosin synthesis is a prerequisite for the induction of seed dormancy. The *nced3* mutant was identified in a screen for enhanced germination on hypertonic media,[35] concluding these authors that AtNCED3 is involved in germination under hyperosmotic conditions. On the other hand, reduced seed dormancy was only observed in the *nced6 nced9* double mutant. Each member of *NCED* family seems to play a particular role in seeds. Overexpression of bean *PvNCED1* in tobacco[30] and *AtNCED3* in Arabidopsis[36] displays an increased ABA levels in seeds and extended seed dormancy. Similar results were also obtained by the overexpression of *ABI* genes in Arabidopsis (reviewed in ref. 16). Interestingly, unlike other plants with overexpressed *NCEDs*, prolonged delay of seed germination is the only ABA-related phenotypic effect in the *GINCED1* transgenic lines.[37]

However, ABA levels may not only be controlled by NCED because overexpression of zeaxanthin epoxidase (ZEP) in tobacco resulted in increased ABA levels in mature seed and greater seed dormancy.[38] Also, since ABA4 does not convert trans-violoxanthin to its cis isomer, an unknown isomerase is still one of the missing links to the biosynthetic pathway.[39] The 9-cis-xanthosin formation is now considered to be the most important regulatory step in ABA biosynthesis.[30] The cleavage product 9-cis-xanthosin is further processed in the cytosol to the biologically active cis-configuration ABA, via ABA aldehyde, and involving the short-chain dehydrogenase/reductase (SDR)[40] and ABA aldehyde oxidase (AAO; a molybdenum cofactor-requiring enzyme) activities,[16,39] respectively. *AtSDR1* gene is expressed at low levels in seeds and developing embryos and may contribute to maternally derived ABA synthesis.[41] However, reporter gene analysis in Arabidopsis showed strong *AtSDR1* expression in seed funiculus and at the junction of pedicels and young siliques.[41] Taking into account the results from Cheng's group,[41] a possibility exists that in addition to ABA, xanthosin might also be supplied to reproductive organs by vegetative tissues to be further converted into ABA. Since AAO requires the sulfurylated form of a molybdenum cofactor (MoCo) for its activity, mutants defective in MoCo., sulfurase (MOSU) (v.e. *AtABA3*) also result in ABA deficiency.[18,42] Finally, the expression of genes encoding ZEP, SDR, AAO, NCED and MOSU are regulated in organ- and/or stress-specific manners, and theirs transcripts or ABA levels are reduced or eliminated in most mutant allels reflecting the positive feedback of ABA biosynthesis (reviewed in ref. 18).

The committed steps in ABA catabolism are categorized into two types of reactions: hydroxylation and conjugation.[8] Normally, the ABA is converted into a compound hormonally inactive and unestable (i.e. 8'-hydroxy ABA) through the intervention of the ABA 8'-hydroxylase, which is a cytochrome P450 monooxygenase (P450),[43] whose family has four members in Arabidopsis (*AtCYP707A1-4*;[44]). Alternatively, ABA can also be hydroxylated at position C-7'. Recently, an ABA 9'-hydroxylation pathway has been identified.[45] The 8'-hydroxy ABA isomerizes spontaneously to phaseic acid (PA) and is further catabolized to dihydrophaseic acid (DPA) by an unknown soluble reductase enzyme.[43] Both PA and DPA as ABA can be conjugated to compounds of low molecular weight (i.e. UDP-D-glucose to ABA by means a glycosyltransferase activity).[8,46] The decrease in ABA during both barley and Arabidopsis seed imbibition is associated with increase in PA.[47,48]

It is likely that the ABA hydroxylation is involved in seed dormancy. In recent reports, Arabidopsis aldehyde-oxidase3 (AAO3) was shown to be localized abundantly in vascular tissues of roots, hypocotyls and leaves, indicating that the vascular tissue is an important site of ABA biosynthesis in vegetative tissues.[49] On the other hand, AAO_3 is scarcely expressed at early phases of seed development and *AAO3*-mRNA is present in dry viable seeds.[50] However, Seo's works[32,33] were the first to demonstrate the *AtAAO3* expression in seeds. Moreover, the authors concluded that *AtAAO3* is the AAO that plays a major role in ABA biosynthesis in Arabidopsis seeds as well as in leaves.[33] In addition, genes for the short-chain alcohol dehydrogenase/reductase (*AtABA2*) and *AAO3* are also expressed in the vascular tissues of the embryo during the mid-maturation stage. On the other hand, genetic evidence demonstrated that overexpressing *ABA2* in Arabidopsis transgenic plants leads to seed germination delay, and elevated levels of both ABA and dormancy.[51] ABA biosynthesis and degradation in Arabidopsis seeds is localized in the embryo as well as in the endosperm.[34,52] Thus, *CYP707A1* and *CYP707A2* genes have been shown, respectively, to play roles in the reduction of ABA content in the embryo at mid-maturation and in both the embryo and endosperm during late maturation.[52] The high abundance of CYP707A2-mRNA in the dry seeds, and its transient expression pattern during early imbibition (6 h), suggests that ABA degradation in seeds is mainly achieved by the CYP707A2 isoform.[44,53] *CYP707A2* is a single copy gene that displays only subtle phenotypes during

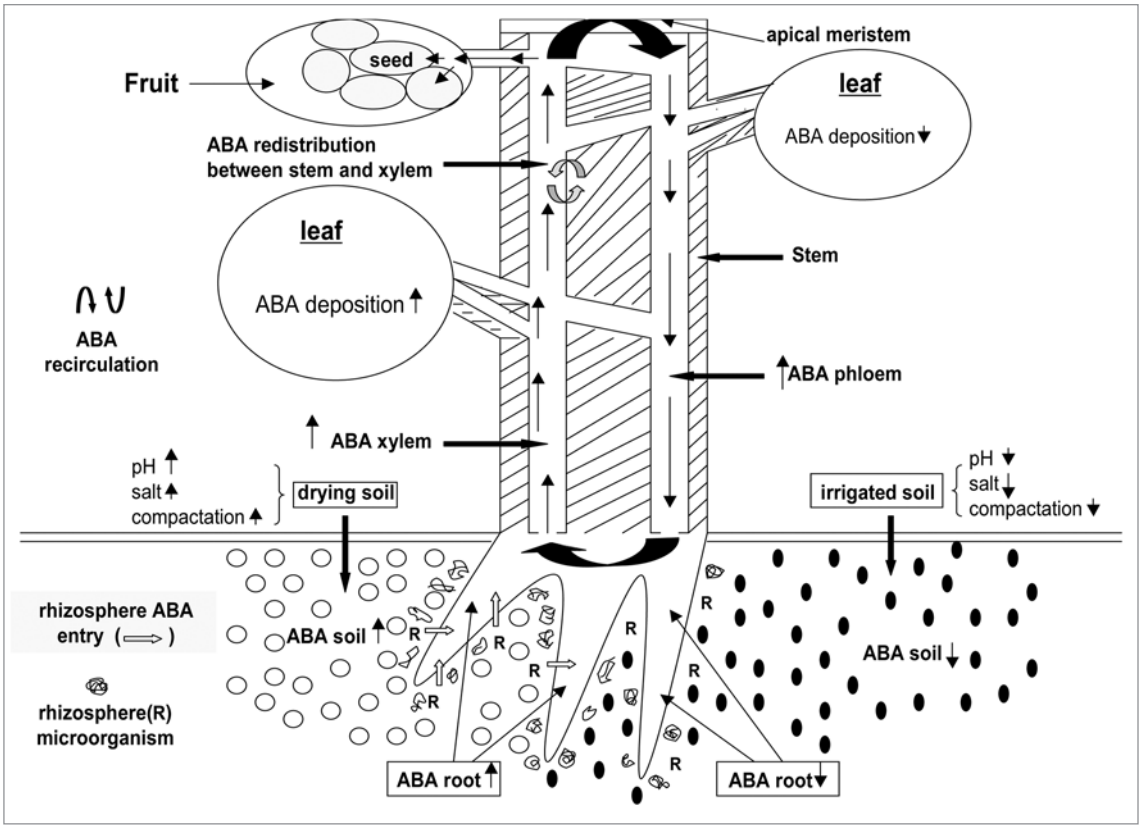

Figure 2. Presence of ABA in rhizosphere, their entry in roots and distribution toward leaves and seeds (ref. 80).

other developmental stages outside the seed which makes it ideal for genetic manipulation. The CYP707A2-mRNA is localized in the radicle tip and the micropylar endosperm during early imbibition, suggesting that the ABA degradation is mediated by the CYP707A2 enzyme expressed in these tissues. It is speculated that endosperm weakening is delayed in the Arabidopsis *cyp707a2* mutant due to impaired ABA degradation and that is, at least in part, the reason for the higher ABA sensitivity of the *cyp707a2* endosperm rupture. By contrast, *CYP707A1* is hardly expressed during zygotic embryogenesis.[44] In short, ABA 8'-hydroxylase family plays a prominent role in regulating endogenous ABA levels during seed development and germination in *A. thaliana*.[52,53] In non dormant *Hodeum vulgare* seeds, it was demonstrated that *HvABA8'OH-1* was expressed strongly and uniformly through the coleorhiza in the region of the primary root tip. These authors conclude that the coleorhiza may be the pivotal tissue in determining whether or not germination occurs.[47] Moreover, *HvNCED2* is responsible for a significant increase in ABA levels during grain development, whereas *HvCYP707A1* is responsible for a rapid subsequent decrease in ABA levels.

The *cyp707a2* mutant accumulates more than sixfold ABA content in dry and imbibed seeds and these exhibits hyperdormancy.[44] On the other hand, the *cyp707a1* mutant accumulated ABA to higher levels in dry seed than *cyp707a2* and *CYP707A1* was expressed predominantly in the vascular tissue in the embryo during mid-maturation and was downregulated during late-maturation.[47] Likewise, these authors demonstrated that both *cyp707a* mutants exhibited enhanced seed dormancy. Taken together, it is suggested that expression of *CYP707As* genes, and mainly *HvCYP707A1*,[54] is controlled by environmental cues or GA,[7,44,55,56] and has also been found to be the major mechanism regulating ABA catabolism in the seeds of bean[57] and barley.[53] Finally, the glucose-induced delay of seed germination is a consequence of an increase in the expression of ABA biosynthesis genes (v.e. *AtABA2* and *AtNCED3*)[58] or suppression of ABA catabolism genes (v.e. *OsABA8ox2* and *OsABA8ox3*)[59] by glucose signaling.

Is Rhizosphere ABA Affecting Plant Growth and Seed Dormancy?

Plant hormones present in soils are believed to play a significant role in root growth and development. However, other functions are not discarded (i.e., rhizosphere microorganisms development, seed-bank physiology, etc.). Microorganisms are considered the primary sources of biologically active substances in soil, although plants may also contribute to the soil pool through root exudation, especially under non-transpiring conditions. At present, there is increasing interest in studies of microorganisms producing phytohormones and hormone-like substances, which determine the formation and development of relationships within natural communities (reviewed in refs. 60 and 61). The ability of plant-associated microorganisms to synthesize some phytohormones is widely known.[62-64] Soil microorganisms can either break down[65]

or produce phytohormones, including ABA.[60,66,67] Direct ABA synthesis (via MVA) is largely found in phytopathogenic fungi.[67] Microbial communities in soil, particularly the rhizosphere (v.e. soil in which the proliferation of microorganisms is induced by the presence of plants roots) (**Fig. 2**), possess great potential to produce a vast range of metabolites that may affect plant growth directly after being taken up by the plant, or indirectly by modifying the soil environment.[68,69] Rhizosphere bacteria confers beneficial effects for the plants such as increased growth or toleration of abiotic stress.[70]

Soil ABA is necessary to maintain an ABA equilibrium between root and rhizosphere. Moreover, ABA is known to be involved in plant-pathogenic fungi interactions, as the level of ABA in the plant determines its susceptibility to phytopathogenic microorganisms.[71,72] Several explanations for this phenomenon have been proposed: plant stimulation of fungal ABA biosynthesis, stimulation of plant ABA biosynthesis by pathogenic fungi, and suppression of metabolic activity of the plant host. Many phytopathogenic fungi synthesize and excrete ABA into the medium.[71,73] There are researchers who believe that the ability of phytopathogens to synthesize ABA may be viewed as a factor of pathogenicity in plant infections.[73] Notable ABA synthesis and accumulation in roots can be observed in hemiparasites such as *Rhinanthus minor*. They release ABA in substantial amounts to rhizosphere solution.[74]

Scarce data exist on ABA levels in soil. However, it is well known that the root is equipped with all the enzymes and precursors that synthesize ABA. A mathematical model predicts that most of the ABA synthesised in the root would move to the soil solution unless the soil water contained at least 1.0 nM ABA at a slightly acid pH.[75] Likewise, simulation also predicted that at pH 5.5 in rhizosphere ABA synthesized in roots under stress would be released into xylem rather than into the soil. The Slovik's group postulated that: (1) ABA is likely to be present in the rhizosphere in the low nanomolar range to prevent dramatic loss of ABA from roots; and (2) in most of the cases, the ABA in the soil solution had a concentration that allowed plants to maintain an equilibrium between external and internal ABA. Taken together, the above predicted data indicates that soil ABA concentrations in the low nanomolar range maintain this equilibrium and prevent dramatic ABA loss to the rhizosphere. Hartung et al.[76] found in a study with different soils that the ABA in rhizosphere solution ranged from 0.6–2.8 nM. This range, predicted with computer simulations, is required in soils in order to prevent ABA release from the root hair zones of plant roots. The highest concentrations of ABA around 4 nM were detected in acid soils where ABA degradation by rhizosphere microorganisms is weak.[76]

By contrast, plants growing under alkaline conditions would become ABA deficient, unless a sufficiently high ABA concentration was present in the rhizosphere solution to re-establish equilibrium conditions.[75,77] Nevertheless, anatomical apoplastic barriers such as Casparian bands in the exodermis could retard ABA loss and uptake.[77-79] ABA retention by roots causes alkalinity tolerance in species which contain these bands, but only in fertilized soils.[77] Legumes suffer severe ABA loss into the alkaline surroundings. The absence of the ABA equilibrium between root and soil could disturb the root-to-shoot signaling processes. In drying soils the ABA concentration increases probably because water is removed. It is not clear how much of this external ABA will be taken up under these conditions because water and solute movement in the soil will slow dramatically as it dries. Localized soil drying around the roots will also restrict the uptake of phytohormones in the soil solution. ABA can be accumulated in fruit and seeds in drying soil because these are relatively alkaline compartments.[80] Recently, it has been shown that growth-promoting rhizobacteria have an impact on ABA flows in plants. Thus, increased amounts of ABA was detected in the shoots of lettuce that were treated with the cytokinin-producing bacterium *Bacillus subtilis*.[63] These authors concluded that locally high cytokinin concentrations induced ABA biosynthesis in the roots and this ABA would be loaded quickly to the xylem vessels. Auxin-producing rhizobacteria should also be able to affect ABA flows. Since IAA is known to induce the ethylene biosynthesis, an impact of auxin-producing rhizobacteria on ABA production and flows may be expected as previously was demonstrated with rhizobacterium *Variovovax paradoxus*, an ABA synthetizing microorganism.[66,81] On the other hand, exogenous ABA supply to plants can promote rather than inhibit plant growth, perhaps by limiting shoot ethylene production (reviewed in ref. 82). Moreover, microorganisms that are able to degrade ABA in the rhizosphere should be able to influence ABA flows. Whether it is possible for such soil microorganisms to influence ABA signaling in plants remains to be shown.

Finally, the impact of soil conditions and rhizosphere microorganisms on ABA signaling in seeds are far to be known. That is, although the presence of ABA in the rhizosphere appears to be beyond doubt, there is no evidences that the soil seed banks may be affected by this and other phytohormones. There are no data about the possible effects of environmental parameters on both rizhosphere ABA levels and the behavior soil seed banks. Possible influences of rizhosphere ABA on seed dormancy maintenance and seed germination can be of great interest, and therefore should be studied. Likewise, although ABA accumulates in all seed tissues, either as a result of biosynthesis in the seed itself or translocation from the mother plant through the phloem, it is far to know if rizhosphere constitutes a source of ABA. A better knowledge of rizhosphere ABA and its transport toward the seed (sink) would be valuable for the understanding of ABA action in seeds. However, the identification of an ABA transporter is still an enigma.

ABA is Not the Only Signal in Establishment and Dormancy Release

The seed is the organ by which angiosperms disperse and propagate and assures the survival and perpetuation of the mother plant.[83] To survive in a particular environment, plants have developed mechanisms that regulate seed germination to coincide with the most appropriate season of the year. One mechanism for proper timing of seed germination is seed dormancy, a genetically and environmentally determined process.[1-4,9-11,84] That is, seed dormancy prevents the adverse environmental conditions, maximizes

the competitive advantages and ensures the establishement of the mother plant.[85,86] Dormancy is the most important altered trait during domestication of wild species[87] and its function is similar between different species. An appropriate balance between dormancy and germination is a desirable trait for the crop industry since too much dormancy can lead to non-uniform germination while too little makes seeds germinate early (pre-harvest sprouting).[54,88] Pre-harvest sprouting, which is very important in cold and humid environments, reduces grain quality and viability and is one of the most significant losses to industry.[85,88]

On the other hand, it is still unclear whether all higher plants have a common molecular mechanism for a trait very well preserved traitsuch as seed dormancy. Seed dormancy appears at the end of the seed maturation, in which the cell cycle ceases, molecular dependence from the mother plant disappears, water content decreases, storage products are synthesized, abscisic acid (ABA) is accumulated (i.e., high ABA to GA ratio[89]), and primary seed dormancy is established,[4,8-11,90,91]. There is a growing body of scientific evidence that the ABA content in the seed must be lowered in order for dormancy to be broken, and that the germination potential of a given seed is determined, at least in part, by hormones ABA and GA.[1,10,92] Thus, the loss of dormancy of many seeds is directly related to the increase in sensitivity to GA[91,93-96] and the ABA/GA ratio is important in the maintenance and loss of seed dormancy.[89] The mutants with altered ABA/GA ratio seem to prove it. The high levels of ABA in imbibited *A. thaliana* ecotype Cvi, which is strongly dormant,[97] decrease when the dormancy is broken.[91] The dormancy state of *A. thaliana* Cvi accession depends of balance between biosynthesis and catabolism of GA and ABA; that is to say, it depends on the endogenous levels of both bio-active hormones.[89,91] The Cadman's group suggests that the key genes to lead to seed germination or dormancy belong to families *NCED* and *CYP707A*. Taking together, a dynamic balance between synthesis and catabolism of these two antagonistic hormones controls the equilibrium between dormancy and germination processes by regulating signaling pathways that modify seed sensitivity to the ambient germination environment (reviewed in refs. 98–100). Recently, an interesting study was published on the alterations in metabolism of ABA and GA induced by after-ripening in dormant barley seeds.[101] However, a crosstalk between ABA and GA with other signals (v.e. ethylene,[98,102-104] reactive oxygen species (ROS),[10,59,104] sugars such as glucose,[10,59] nitrate[105] or calcium-binding protein like calreticulin[106]), must be taken into account to understand the triggering of seed dormancy process.

ABA Production in Seeds and -Omics Involved in Dormancy Signaling

Seed dormancy is a sufficiently complex process to be controlled by a single endogenous or exogenous factor. There are increasing molecular and genetic evidences that indicate strongly that ABA is central to the establisment and maintenance of both primary[1,9-11,96] and secondary[107,108] dormancy. Thus, ABA deficiencies during seed development are associated with the absence of primary dormancy of mature seed, whereas overexpression of ABA synthesis genes increases the ABA content and seed dormancy.[10,48,96,109,110] On the other hand, endogenous ABA levels and expression pattern of genes involved in its biosynthesis change drastically during seed development in response to developmental and environmental cues.[9,16] In addition, the ABA seems to avoid the abortion of the seed and promotes the growth of embryo during zygotic embryogenesis.[41,111] An increase in seed abortion in the pea *lh-2* mutant indicates that GAs is essential for normal seed development. The *lh-2* mutation was shown to be a single base substitution in the *ent*-kaurene oxidase gene.[110] This experiment with *lh-2* mutant is one of many that demonstrate the negative correlation ABA/GAs during seed development. During seed development, ABA is synthesized in tissues of different origins conditioning its physiological action. Thus, the ABA accumulated during mid-maturation is of maternal origin, is related to *FUS3* and *LEC* and involved in the inhibition of precocious germination and processes of seed maturation.[10,90,111]

The ABA de novo synthesized during late-maturation is derived from the zygotic tissues and is essential for desiccation tolerance and induction and maintenance of durable seed dormancy[10] (**Fig. 3**). This synthesized ABA is partially accumulated in dry seed and decreases with seed imbibition. However, the relationship between the ABA content in mature dry seed and the dormancy degree is not yet clear. That is, while the ABA is important to start the dormancy, high ABA levels are not need to maintain it.[9] Endogenous ABA in imbibed seed is maintained at a given level that is correlated with the germination potential of the seed. Thus, de novo synthesis of ABA during the imbibition of dormant mature seeds contributes to dormancy maintenance.[9,85,91,112] In fact, the degree of seed dormancy is correlated with endogenous ABA levels in imbibed seeds rather than in dry seeds in various species, such as Arabidopsis,[91] lettuce,[55] barley,[47] and tobacco (*Nicotiana plumbaginifolia*).[112] By contrast, those seeds have been subjected to an effective treatment for the dormancy rupture (v.e. after-ripening or stratification), still synthesize ABA; but ABA degradation generally predominates over its biosynthesis[8,9,91] (**Fig. 3**). Several studies have shown that the catabolic removal of ABA is essential for the transition dormancy-germination. The increase in ABA catabolism (i.e., oxidation or conjugation;[45]) is associated with completion of seed dormancy of barley, *Pseudotsuga menziesii*, *Cupressus nootkatensis* and yellow-cedar.[47,93,113,114] Thus, mutant seeds deficient in ABA 8'-hydroxilase show increased levels of dormancy.[48] The breaking of dormancy by the most-chilling (cold stratification) is because the cold increased the ABA catabolism.[9] Interestingly, seed dormancy can be eliminated by smoke,[88] and this elimination is accompanied in *Nicotiana attenuata* seeds by a decrease of 8 times in the ABA content.[115]

ABA-like genes appear to have some function in the control of seed dormancy. This feature is supported by studies carried out with orthologue genes (i.e., *Vp1* ortologue of *ABI3*).[1,9,96] Although the intensity of *Vp1* expression and the degree of seed dormancy seems to be related, more data are needed to confirm it.[116] Interestingly, the dormancy state is characterized by the transcription of genes with large presence of ABRE sequences to

which transcription factors TFs-like (i.e., bZIP) bind to regulate the seed dormancy.[1] Transcriptomic studies have demonstrated the existence in dormant seeds of groups of genes that have very plentiful expression (reviewed in ref. 117). This expression is scantily related to environmental conditions, demonstrating that the dormancy process has its own signaling.[89,118] During seed development, environmental factors can significantly influence on the content and sensitivity to ABA of mature seed and alter their dormancy.[86,96,119-122] Several mRNAs present in the dormant stage are also found in dry and imbibited seeds, and these transcrips are consistent with those regulated by ABA or environmental stress.[89] They have been identified in *A. thaliana* 30 genes which expression during dormancy phase was higher than under after-ripening status; these genes can be strong candidates to regulate the seed dormancy.[118] Within this group of 30 genes are included phosphatases (i.e. *ABI1* and *ABI2*), TFs (*ABI3*, *ABI4* and *ABI5*) and genes with unknown functions (i.e. (reviewed in ref. 117) *DOG1*).[12,123] Recently, was evidenced a relationship between changes in chromatine structure and transcriptional control of seed dormancy.[124]

Despite studies of both Cadman and Liu's groups, is currently unknown what is the mechanism by which increases the transcription of genes involved in seed dormancy.[10] Likewise, it was not demonstrated that alterations detected in the dormant state are concomitantly related to transcriptional and translational activities.[2] However, the post-transcriptional seems to be envolved; at least for some genes.[125,126] The delay in breaking of seed dormancy induced by exogenous ABA is associated to regulation of translation at the level of initiation and elongation factors;[106] this fact suggests that the dormant status regulates the ability of seed translation. Chibani et al. (2006) show that de novo synthesized proteins during imbibition are very different in dormant and non-dormant seeds.[127] Moreover, they also demonstrated that although ABA inhibits germination in non-dormant seeds, does not inhibit translation. Likewise, it was observed that the transcriptomes from after-ripened treated with ABA seeds were more similar to after-ripened non-treated ones than to those dormant seeds.[127-129] Only the seeds capable of breaking the dormancy can adquire the ability to reprogram the pattern of protein synthesis during imbibition, allowing completion of the germination process.[127] All results discussed until now, together to those of Müller et al. (2006),[130] suggest that exogenous ABA inhibits germination by a route different from the one of dormancy.

Seed Dormancy in Woody Species: Searching Genes ABA Regulated

Genetic evidences have shown the involvement of three Arabidopsis Ser/Thr protein phosphatases 2C (AtPP2C), named ABI1, ABI2 and PP2CA, as negative regulators of ABA signaling.[131,132] Whereas ABI1 and ABI2 are key regulators in seeds,[133] PP2CA does not appear.[134] However, from the work of Wu et al.[135] in which is demonstrated that ABI1 overexpression does not affect the ABA-signaling pathway, a controversy with regard the role of ABI1 has been created. On the other hand, *ABI3*, *ABI4* and *ABI5* encode different types of transcription factors known to act as positive regulators of ABA-mediated regulation of seed development, germination and early seedling growth.[10,136]

One of the woody species most studied is beechnuts (*Fagus silvatica*). Their seeds have a dormancy maintained by ABA and eliminated by a long stratification (8 weeks) in water at 4°C.[137] Exogenous GA_3 proved to be also efficient in breaking the dormancy and could be substituted for cold treatment. These treatments regulate the expression of some dormancy-related genes (v.e. *FsPk1* and *FsPK2*,[138] *FsERF1*,[137,139] and *FsPP2C1* and *FsPP2C2*.[140,141] The expression of *FsPP2C1*, a functional PP2C from beechnuts, is: (1) specifically induced in seeds upon ABA treatment but not by drought stress, while low temperatures or GAs treatment decrease the level of transcripts; (2) negatively correlated with seed germination; (3) abolished by treatments that break seed dormancy; (4) upregulated by ABA and its expression is correlated with the level of seed dormancy; and (5) seed exclusive.[142] Taking togheter all results, FsPP2C1 is a strong candidate to be a negative regulator of ABA signaling in seeds.[142,143] Likewise, the constitutive expression of *FsPP2C1* confers ABA insensitivity in seeds and, consequently, a reduced degree of seed dormancy.[140] Therefore, the negative regulation of ABA signaling by FsPP2C1 is a factor that contributes to promote the transition from dormancy to germination during early weeks of stratification. Moreover, *FsPP2C2* gene was also isolated and characterized in *Fagus silvatica* seeds.[144] Contrary to *FsPP2C1*,[142] it is probably that ABA regulate *FsPP2C2* expression in a Ca^{+2}-dependent way, which feature is described for the first time in seeds.[144] On the other hand, in Arabidopsis plants overexpressing *FsPP2C2* was demonstrated:[141] (1) an enhanced sensitivity to ABA and a deeper degree of seed dormancy compared to WT seeds; (2) transgenic lines of *35S:FsPP2C2* contain reduced levels of GA associated with altered expression of *GA20ox* and *GA3ox* genes; and (3) FsPP2C2 is localized in the nucleus only in the presence of ABA and strongly supports its involvement in ABA signaling through possibily a reduction of GA biosynthesis, probably affecting GA3-oxidase activity. The above results indicate the existence of potential cross-talk between ABA signaling and GA biosynthesis with a role of FsPP2C2 as a positive regulator of ABA signaling by inhibiting GA biosynthesis. By contrast, HAB1, a protein phosphatase type-2C, seems to play as a negative regulator of ABA signaling.[145] More recently, a proteomic approach was used to analyze mechanisms of dormancy breaking in beechnuts seeds and the participation of ABA and GAs in this process.[106] Most of the ABA-responsive proteins are involved in protein destination, energy metabolism and development. Finally, *CnABI3*, an ABI3/VP1 gene homologue, was cloned from yellow cedar, a conifer that produces seeds that are deeply dormant at maturity.[146] CnABI3 was synthesized exclusively in megagametophyte and embryo of dormant mature and warm stratified seeds, but decline during subsequent moist chilling, a treatment effective in breaking dormancy.

How Many ABA Receptors?

Background. Several biochemical and genetic approaches have permitted the characterization of numerous components envolved

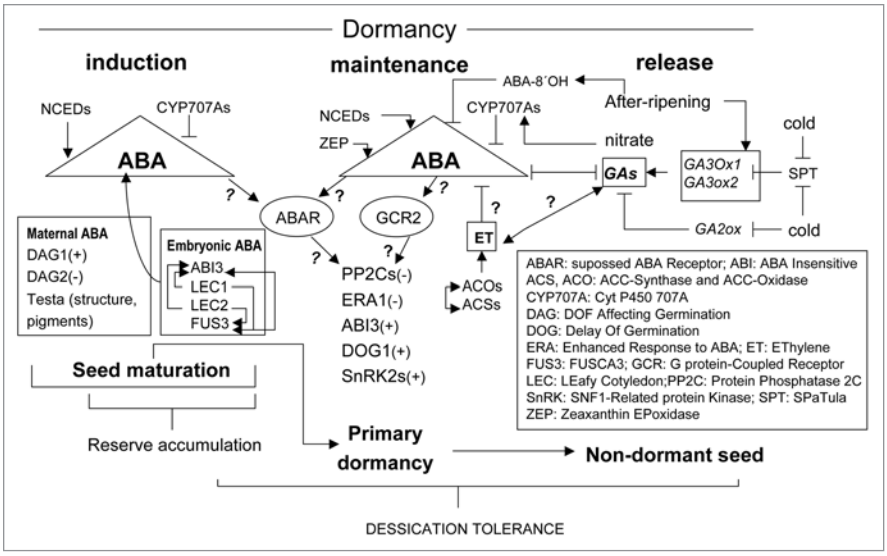

Figure 3. Possible regulation of seed dormancy status by ABA and its interaction with GAs (cross-talk) and environmental factors (mainly, cold and after-ripening). ABA, ET and GAs means relative hormone levels due to action of theirs anabolic and catabolic enzymes. Promotive and repressive effects are shown by arrows and bars, respectively. Interrogation symbol indicates the absence of data to confirm the effect (refs. 2, 10, 187 and 188).

in ABA biosynthesis and signaling.[8,10,110,123,147] However, until the last decade there was a failure in the approach to the identification and characterization of ABA receptors. That is, although proteins that bind ABA have been identified, no strong evidence has been presented to link them to physiological effects of ABA in vivo. At present, four supposed ABA receptors exist in *A. thaliana*: the nuclear flowering-time protein FCA,[148] the plastid-associated Mg-chelatase H subunit (CHLH),[149] a protein originally identified as a membrane-bound G-protein-coupled receptor (GCR2)[124] and, recently, two novel G-protein coupled receptors (GPCRs), GPCR-type G proteins (GTG) 1 and GTG2.[150] This variety of cellular sites for potential ABA perception may be a way of explaining the complexity of its signaling and suggests that multiple receptors exist for ABA. Moreover, biochemical and electrophysiological studies provide evidences for both intracellular (i.e., CHCL and FCA) and extracellular (i.e., GCR2, GTG1 and GTG2) perception of the ABA.[150-152] The most important implications is that ABA acts simultaneously and independently at multiple sites in the cell and evokes different responses at each site.[153]

CHLH (magnesium protoporphyrin-IX CHeLatase H subunit). On the year 2002, a protein (putative ABA receptor; ABAR) with affinity by ABA and involved in stomatal signaling, was isolated from *Vicia faba*.[154] El gen CHLH was identified in Arabidopsis as a homologue of ABAR.[149] ABAR/CHLH encode for the subunit H of Mg-protoporphyrin IX chelatase, an enzyme located in chloroplasts and key component in both chlorophyll biosynthesis and plastid-to-nucleus signaling. *ABAR* is a single-copy gene in the *A. thaliana* genome, is highly conserved in higher plants and shares high sequence similarities to its homologues in bacteria. It is said that CHLH perceives the ABA signal since in seed germination: (1) ABAR binds specifically to ABA; (2) transgenic downregulation of ABAR expression results in a decline in the number of ABA-binding sites and leads to ABA-insensitive phenotypes; (3) ABAR-overexpressing plants have ABA-hypersensitive phenotypes with a elevated number of ABA-binding sites; (4) a LOF mutation in ABAR results in an immature embryo; and (5) a *chlh* mutant that downregulates both *ABAR* expression and ABA-binding activity is an ABA-insensitive mutant like the post-transcriptional gene-silencing RNAi or antisense mutants.[149] CHLH-mRNA is also present in seeds,[149] and *abar-1* seeds are deficient in lipid and mature protein bodies, indicating a possible alteration of late embryonic development.[123] In short, CHLH has been proposed to be an ABA receptor involved in mediating cellular responses to ABA during seed development (**Fig. 3**), germination and post-germination growth and stomatal movement.[149] However, the CHLH-ABA binding assays used were similar to those of the paper retracted on July 14 2008,[147] and therefore further experiments are required to validate the role of CHLH.[155]

FCA (flowering time control protein A). Although ABA-binding protein FCA is predominantly localized to the nucleus, it shares sequence homology at the C-terminus with ABAP1, an in vitro ABA-binding protein that is associated with the plasma-membrane of barley aleurone cells.[156] According to the Razem group, ABAP1 is an ABA-inducible protein, and posseses: (1) high affinity for ^{3}H-(+)-ABA; (2) saturation kinetic and specificity for S-(+)-ABA; and (3) the ability to bind to both natural precursors of ABA i.e., (+)-ABA aldehyde and (+)-ABA alcohol.[156] However, because the difficulty of purifying ABAP1, and the same happens with CHLH-ABA binding, the published results of ABAP1-ABA binding are subjected to criticisms.[156] Like ABAR/CHCL, FCA binds ABA with an interaction that is stereospecific (i.e., FCA binds (+)-ABA but not two of the non-active ABA analogues (-)-ABA and trans-(+)-ABA) and follows receptor kinetics.[148] The FY protein, a RNA 3'-end processing factor, is required to FCA funtion[157] and ABA disrupts the FCA-FY interaction in vitro and FCA function in vivo.[148] Through the use of mutants *abi-1* and *abi-2*, it was demonstrated that FCA and ABI1 proteins are involved in distinct ABA responses.[148] Seeds of mutant *fca-1* were used to examine the posible role of FCA in germination. Because none of the *fca-1* seeds germinated in the presence of ABA, the authors concluded that FCA is not required for Arabidopsis seed germination. FCA is not also required for stomatal opening. Taking one thing with another, FCA has been proposed to be an ABA receptor, which controls ABA-mediated RNA metabolism and flowering.[148]

GCR (G-protein-coupled receptor). In contrast to the ABA intracellular perception, several experiments had suggested that

extracellular perception is critical to achieve ABA functions. That is, ABA receptors localized at cellular periphery must be necessary to recognize external ABA. At the cell surface (i.e., plasma-membrane), the ABA signal was recently proposed to be perceived by GCR2, which acts as an extracellular-ABA receptor and controls all the major responses mediated by ABA (**Fig. 3**), including seed germination.[124] Thus, the T-DNA insertional mutations of *GCR2* causes expression of ABA inducible genes and ABA insensitivity in seeds germination. GCR2 specifically binds with high affinity to natural occurring ABA, but not to the physiologically inactive isomer (trans-ABA). Moreover, GCRC2 interacts physically with GPA1, the only Arabidopsis Gα subunit of trimeric G-protein, and the binding of ABA to GCR2 disrupts the GCR2-GPA1 interaction.[124] On the other hand, it was also shown that LOF *gcr2* exhibits all known ABA defects, and overexpression of GCR2 shows an ABA-hypersensitive phenotype.[158] These authors suggest that *GCR2* and *GCR2*-likes genes (i.e., *GCL1* and *GCL2*) share partial functional redundance,[158] being this fact recently demostrated.[159] Likewise, it was also proved that GCR2, GCL1 and GCL2, the three only members of *GCR2* family in Arabidopsis, are not required for ABA response in seed germination,[159] thus discarding that GCR2 functions as an ABA receptor in this process.[124] Therefore, because it was not discovered any morphological or conditional phenotypes in all *gcr2* mutants, the exact role of *GCR2* in plants remains unknown and possibility that GCR2 is an ABA receptor is at present debatable.[158] However, it was previously demonstrated that the overexpression of *GCR1* abolished seed dormancy[160] and that GCR1 interacts specifically with GPA1 suggesting that GCR1 is a component of an ABA perception and signaling complex.[161] Interestingly, it was also suggested that GCR2 may actually be a member of the bacterial lanthionine synthetase (LanC),[158] and define a new type of "non-classical" ABA-signaling G-protein-coupled receptor (GPCR) required for ABA perception.[162]

GT1 and GT2 (GPCR-type G-proteins). G-proteins are involved in several fundamental growth and developmental processes in higher plants (reviewed in ref. 163). Phenotypic analysis of null mutants were used to demonstrate that G-proteins also modulate the seed germination.[164] GPCR-like proteins exist in plants.[165] In the year 2009, two proteins named GTG1 and GTG2 (GPCR-type G-proteins 1 and 2), were characterized.[150] Both GTG1 and GTG2 proteins: (1) are topologycally similar to GPCRs but with classic GTP-binding/GTPase activity; (2) interact with GPA1; (3) have highly specific ABA binding; (4) posses two different conformations, GTP-bound and GDP-bound; (5) have dependence of the efficiency of ABA-binding on their conformation; (6) are consistent with their proposed role in ABA signaling, Arabidopsis *gtg1/gtg2* double mutants show typical hyposensitivity to ABA, including reduced seed dormancy; and (7) no effect of ABA on expression of *GTG* transcripts, indicating that the role of GTG proteins in ABA signaling is posttranslational.[150] However, these authors report that ABA-binding experiments were carried out with purified protein; but only 1% of the purified GTP proteins were capable of binding ABA.

News, Views and Controversies

The approach of the study of hormone receptors is highly complex. Thus, although it is relatively feasible to isolate and characterize molecules that bind to a hormonal ligand, it is not easy to prove that some of them fulfil the conditions that must have a receptor. Between the end of the second millenium and the beginning of the third, the receptors of several plant hormones have been characterized. Likewise, mutants with an impediment in the hormone-receptor binding were also isolated.[166] By contrast, the speed to get the ABA receptor was rapid but very slippery, and some approaches have ended in failure and others that seemed correct, still need to be strongly confirmed. The use of sequence analysis and bioinformatics approaches appears to be the main weaknesses of developped protocols to confirm a true ABA receptor. To confirm these criticisms, we will refer to very recent manuscripts that do not agree with certain publications listed in section 6.1. In the last two years: (2) Risk et al. (2008)[167] found no evidence to indicate that FCA, a RNA-binding protein reported by Razem et al. (2006) as an ABA receptor,[148] is an ABA receptor; (3) Jones and Sussman (2009) propose that "A ligand binds to its cognate receptor reversibly, saturably, selectively, and with a stoichiometry of one or more molecules of ligand per molecule protein and the binding is usually heat sensitive and affected by proteases";[168] (3) Risk et al. (2009)[167] showed that the putative extracellular ABA receptor GCR2, a protein originally identified by Liu et al. (2007) as a membrane-bound G-protein coupled receptors,[124] does not bind ABA;[155] other researchers have also questioned some results of Liu's work;[162,169,170] (4) McCourt and Creelman (2008) published an interesting review with a title no less interesting and subliminally intentional: "The ABA receptors—we report you decide";[171] and finally, (5) Pandey et al. (2009),[150] with the isolation and characterization of GTG1 and GTG2, novel GPCR proteins, may be closer to the coveted ABA receptor(s). However, some experimental criticisms (i.e., protein purification and ABA-binding experiments) still cast a shadow over the protocole carried out by the Pandey group.

ABA Mutants as Key to Breakthrough

Notable progresses are currently being carried out on dormancy at the transcriptomic,[14,89,118,129] proteomic[127] and metabolomic[172] levels. ABA-mutants with alterations in the degree of seed dormancy and germination provide special tools to approach to the understanding of the mechanisms of dormancy. The first participants loci in the dormancy of *A. thaliana* seeds were identified in the last years of the twentieth century through mutations affecting the ABA biosynthesis and signaling (reviewed in ref. 94). At present, although there are genes that are specifically expressed in dormant seeds, it has not yet been convincingly shown that the products of these genes directly affect inhibiting the germination process or how ABA is involved in it. Some characterized ABA mutants for building-up the ABA signaling pathway related to the seed dormancy appears in an updated **Table 1**.

Table 1. Selected ABA synthesis/catabolism and response genes involved in moduling seed dormancy

Species	Gene/locus	Protein	Mutant/ transgenic line	Effects on dormancy and ABA sensitivity	References
			Synthesis/Catabolism		
Arabidopsis	ABA1	Zeaxanthin epoxidase (ZEP)	*aba1*	Reduced	173–175
	ABA2	Short-chain dehydrogenasereductase (AB-SDR)	*aba2*	Reduced	40, 41, 176
	ABA3	Molybdenum cofactor sulfurase (MCS)	*aba3*	Reduced	42, 173
	AtNCED6 AtNCED9	9-cis Epoxycarotenoid dioxygenase (NCED)	*Atnced6*/*Atnced9* double mutant	Reduced	31, 34
	AAO3	Aldehyde oxidase 3	*Aao3-1*	Slightly reduced	33
	CYP707A2	ABA 8'-hydroxylase	*cyp707a2-1* *cyp707a2-2*	Enhanced	48
	CYP707A1	ABA 8'-hydroxylase	*cyp707a1*	Enhanced	52
Zea mays	VP14	9-cis Epoxycarotenoid dioxygenase (NCED)	*vp14*	Vivipary, Reduced	177
Nicotiana plumbaginifolia	NpABA1	Zeaxanthin epoxidase (ZEP)	*Npaba1*	Reduced	111
	NpABA2		*Npaba2*	Reduced	16
Lycopersicum esculentum	NOT	9-cis Epoxycarotenoid dioxygenase (NCED)	*not*	Reduced	178
			Response		
Arabidopsis	ABI1	PP2CSer/Thr protein phosphatase	*abi1-1*	Reduced; ABA insensitive	179
	ABI2	PP2CSer/Thr protein phosphatase	*abi2-1*	Reduced; ABA insensitive	180
	ABI3	TF specific seeds, B3 domain	*abi3*	Reduced; ABA insensitive	98, 181
	ABI4	TF specific seeds, DREB subfamily A-3 of ERF/APETALA TF	*abi4*	Normal; ABA insensitive	173, 182
	ABI5	TF specific seeds, bZIP	*abi5*	Normal; ABA insensitive	183, 184
	ERA1	Farnesyl transferase	*era1*	Enhanced	179
	ERA3	Farnesyl transferase	*era3*	Enhanced	174
	AHG1	Putative protein phosphatase 2C (PP2C)	*ahg1-1*	Enhanced	126
	SAD1	Sm-like snRNP protein	*sad1*	Enhanced	177
	MARD1	Zinc-finger protein (TF?)	*mard1*	Reduced; ABA insensitive	185
Zea mays	VP1	TF specific seeds, B3 domain	*vp1*	Vivipary or reduced dormancy; ABA insensitive	186

TF, transcription factor.

Conclusions

The ABA is a well-known hormone that participates in the induction and maintenance of seed dormancy. A negative correlation ABA/GAs during seed development was demonstrated. However, the role of ET in this ABA/GAs cross-talk, if it has one, is not clarified. ET, together with GAs, antagonize ABA actions during dormancy induction/termination and germination. In recent years, progress has been made, but a significant number of gaps in ABA signaling still need to be filled. Perhaps the existence of genetic redundancy in plants is one of the culprits. Isolation and characterization of genes in the synthesis and deactivation pathways of ABA were and are key tools to decipher the spatial compartimentalization of ABA and its participation in different kinds of seed dormancy. Besides, the application of molecular genetic tools and the large-scale transcriptome and proteome technologies newly available to seed biology will be powerful aids in developing our understanding of the exact roles of each phytohormone in seed dormancy. Currently, it is unknown the role of DELLA and basic helix-loop-helix (HLH) proteins, negative regulators of GAs signaling, in seed dormancy. Likewise, no information is available about the alleged action of ET on both GAs regulators. On the other hand, as updated in this review, the existing puzzle on ABA receptors is, at present, indecipherable. A high percentage of failure lies in the methodology used to check the binding and specificity in the ABA-receptor complex. It would be interesting to use measures of free-energy to check the binding ABA-ligand, and make sure that the ligand (v.e. a protein) has not undergone changes throughout the experimental procedure. Finally, deep experimentation on the ABA levels in the rhizosphere is needed, and its potential impact on seed dormancy in soil banks must be analyzed. We must not forget that the soil is the natural habitat of the seeds once dispersed from mother plant.

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References

1. Finch-Savage WE, Leubner-Metzger G. Seed dormancy and the control of germination. New Phytol 2006; 171:501-23.
2. Holdsworth MJ, Finch-Savage WE, Grappin P, Job D. Post-genomics dissection of seed dormancy and germination. Trends Plant Sci 2008; 13:7-13.
3. Donohue K, Dorn L, Griffith C, Kim E, Aguilera A, Polisetty CR, et al. The evolutionary ecology of seed germination of *Arabidopsis thaliana*: variable natural selection on germination timing. Evol Int Org J Evol 2005; 59:758-70.
4. Gutierrez L, Van Wuytswinkel O, Castelain M, Bellini C. Combined networks regulating seed maturation. Trends Plant Sci 2007; 12:294-300.
5. Seo M, Hanada A, Kuwahara A, Endo A, Okamoto M, Yamauchi Y, et al. Regulation of hormone metabolism in Arabidopsis seeds: phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellin metabolism. Plant J 2006; 48:354-66.
6. Oh E, Yamaguchi S, Hu J, Yusuke J, Jung B, Paik I, et al. PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the *GAI* and *RGA* promoters in Arabidopsis seeds. Plant Cell 2007; 19:1192-208.
7. Toh S, Imamura A, Watanabe A, Nakabayashi K, Okamoto M, Jikumaru Y, et al. High temperature induced abscisic acid biosynthesis and its role in the inhibition of gibberellin action in Arabidopsis seeds. Plant Physiol 2008; 146:1368-85.
8. Nambara E, Marion-Poll A. Abscisic acid biosynthesis and catabolism. Ann Rev Plant Biol 2005; 56:165-85.
9. Kermode AR. Role of abscisic acid in seed dormancy. J Plant Growth Regul 2005; 24:319-44.
10. Finkelstein RR, Reevers W, Ariizumi T, Steber C. Molecular aspects of seed dormancy. Ann Rev Plant Biol 2008; 59:387-415.
11. Bentsink L, Soppe WJJ. Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. New Phytol 2008; 179:33-54.
12. Himmelbach A, Yang Y, Grill E. Relay and control of abscisic acid signalling. Curr Opin Plant Biol 2003; 6:470-9.
13. Shinozaki K, Yamaguchi-Shinozaki K. Gene networks involved in drought stress response and tolerance. J Exp Bot 2007; 58:221-7.
14. Nakabayashi K, Okamoto M, Koshiba T, Kamiya Y, Nambara E. Genome-wide profiling of stored mRNA in *Arabidopsis thaliana* seed germination: epigenetic and genetic regulation of transcription in seed. Plant J 2005; 41:697-709.
15. Holman TJ, Jones PD, Russell L, Medhurst A, Ubeda Tomás S, Talloji P, et al. The N-end rule pathway promotes seed germination and establishment through removal of ABA sensitivity in Arabidopsis. Proc Natl Acad Sci USA 2009; 106:4549-54.
16. Seo M, Koshiba T. Complex regulation of ABA biosynthesis in plants. Trends Plant Sci 2002; 7:41-8.
17. Schwartz SH, Qin X, Zeevaart JA. Elucidation of the indirect pathway of abscisic acid biosynthesis by mutants, genes and enzymes. Plant Physiol 2003; 131:1591-601.
18. Xiong L, Zhu JK. Regulation of abscisic acid biosynthesis. Plant Physiol 2003; 133:26-36.
19. Marion-Poll A, Leung J. Abscisic acid synthesis, metabolism and signal transduction. In: Hedden P, Thomas SG, eds. Plant Hormone Signalling, Ann Plant Rev, Vol 24. Oxford UK: Blackwell Publishers 2006; 1-35.
20. Fraser PD, Bramley PM. The biosynthesis and nutritional uses of carotenoids. Prog Lipid Res 2004; 43:228-65.
21. Lange BM, Rujan T, Martin W, Croteau R. Isoprenoid biosynthesis: The evolution of two ancian and distinct pathways across genomes. Proc Natl Acad Sci USA 2000; 97:13172-7.
22. Lichtebthaler HK, Schwender J, Disch A, Rohmer M. Biosynthesis of isoprenoids in higher plant chloroplasts proceeds via a mevalonate-independent pathway. FEBS Letts 1997; 400:271-4.
23. Audran C, Liotenberg S, Gonneau M, et al. Localisation and expression of zeaxanthin epoxidase mRNA in Arabidopsis in response to drought stress and during seed development. Aust J Plant Physiol 2001; 28:1161-73.
24. North HM, De Almeida A, Boutin JP, Frey A, To A, Botran L, et al. The Arabidopsis ABA-deficient mutant aba4 demonstrates that the major route for stress-induced ABA accumulation is via neoxanthin isomers. Plant J 2007; 50:810-24.
25. Chernys JT, Zeevaart JAD. Characterization of the 9-cis-epoxycarotenoid dioxygenase gene family and the regulation of abscisic acid biosynthesis in avocado. Plant Physiol 2000; 124:343-53.
26. Schmidt H, Kurtzer R, Elsenreich W, Schwab W. The carotenase AtCCD1 from *A. thaliana* is a dioxygenase. J Biol Chem 2006; 281:9845-51.
27. Han SY, Kitahata N, Sekimata K, Saito T, Kobayashi M, Nakashima K, et al. A novel inhibitor of 9-cis-epoxycarotenoid dioxygenase in abscisic acid biosynthesis in higher plants. Plant Physiol 2004; 135:1574-82.
28. Kitahata N, Han SY, Noji N, Saito T, Kobayashi M, Nakano T, et al. A 9-cis-epoxycarotenoid dioxygenase inhibitor for use in the elucidation of abscisic acid action mechanisms. Bioorg Med Chemi 2006; 14:5555-61.
29. Iuchi S, Kobayashi M, Yamaguchi-Shinozaki K, Shinozaki K. A stress-inducible gene for 9-cis-epoxycarotenoid dioxygenase involved in abscisic acid biosynthesis under water stress in drought-tolerant cowpea. Plant Physiol 2000; 123:553-62.
30. Qin X, Zeevaart JAD. Overexpression of a 9-cis-epoxycarotenoid dioxygenase gene in *Nicotiana plumbaginifolia* increases abscisic acid and phaseic acid levels and enhances drought tolerance. Plant Physiol 2002; 128:544-51.
31. Tan BC, Joseph LM, Deng WT, Liu L, Li QB, Cline K, et al. Molecular characterization of the Arabidopsis 9-cis epoxycarotenoid dioxygenase gene family. Plant J 2003; 35:44-56.
32. Koiwai H, Nakaminami K, Seo M, Mitsuhashi W, Toyomasu T, Koshiba T. Tissue-specific localization of an abscisic acid biosynthetic enzyme, AAO3, in Arabidopsis. Plant Physiol 2004; 134:158-65.
33. Seo M, Aoki H, Koiwai H, Kamiya Y, Nambara E, Koshiba T. Comparative studies on the Arabidopsis aldehyde oxidase (AAO) gene family revealed a major role of *AAO3* in ABA biosynthesis in seeds. Plant Cell Physiol 2004; 45:1694-703.
34. Lefebvre V, North H, Frey A, Sotta B, Seo M, Okamoto M, et al. Functional analysis of Arabidopsis *NCED6* and *NCED9* genes indicates that ABA synthesized in the endosperm is involved in the induction of seed dormancy. Plant J 2006; 45:309-19.
35. Ruggiero B, Koiwa H, Manabe Y, Quist TM, Inan G, Saccardo F, et al. Uncoupling the effects of ABA on plant growth and water relations: analysis of *sto1/nced3*, an ABA-deficient but salt-tolerant mutant in Arabidopsis. Plant Physiol 2004; 136:3134-47.
36. Iuchi S, Kobayashi M, Taji T, Naramoto M, Seki M, Kato T, et al. Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in Arabidopsis. Plant J 2001; 27:325-33.
37. Zhu C, Kauder F, Römer S, Sandmann G. Cloning of two individual cDNAs encoding 9-cis-epoxycarotenoid dioxygenase from *Gentiana lutea*, their tissue-specific expression and physiological effect in transgenic tobacco. J Plant Physiol 2007; 164:195-204.
38. Frey A, Audran C, Marin E, Sotta B, Marion-Poll A. Engineering seed dormancy by the modification of zeaxanthin epoxidase gene expression. Plant Mol Biol 1999; 39:1267-74.
39. Seo M, Peeters AJ, Koiwai H, Oritani T, Marion-Poll A, Zeevaart JA, et al. The Arabidopsis aldehyde oxidase 3 (*AAO3*) gene product catalyzes the final step in abscisic acid biosynthesis in leaves. Proc Natl Acad Sci USA 2000; 97:12908-13.
40. González-Guzmán M, Apostolova N, Bellés JM, Barrero JM, Piqueras P, Ponce MR, et al. The short-chain alcohol dehydrogenase ABA2 catalyzes the conversion of xanthoxin to abscisic aldehyde. Plant Cell 2002; 14:1833-46.
41. Cheng WH, Endo A, Zhou L, Penney J, Chen HC, Arroyo A, et al. A unique short-chain dehydrogenase/reductase in Arabidopsis glucose signaling and abscisic acid biosynthesis and functions. Plant Cell 2002; 14:2723-43.
42. Xiong L, Ishitani M, Lee H, Zhu JK. The Arabidopsis *LOS5/ABA3* locus encodes a molybdenum cofactor sulfurase and modulates cold stress- and osmotic stress-responsive gene expression. Plant Cell 2001; 13:2063-83.
43. Zaharia LI, Walker-Simmon MK, Nicolás C, Abrams SR. Chemistry of abscisic acid, abscisic acid catabolites and analogs. J Plant Growth Regul 2005; 24:274-84.
44. Saito S, Hirai N, Matsumoto C, Ohigashi H, Ohta D, Sakata K, et al. Arabidopsis CYP707As encode (+)-abscisic acid 8'-hydroxylase, a key enzyme in the oxidative catabolism of abscisic acid. Plant Physiol 2004; 134:1439-49.
45. Zhou R, Cutler AJ, Ambrose SJ, Galka MM, Nelson KM, Squires TM, et al. A new abscisic acid catabolic pathway. Plant Physiol 2004; 134:361-9.
46. Lim EK, Doucet CJ, Hou B, Jackson RG, Abrams SR, Bowles DJ. Resolution of (+)-abscisic acid using an Arabidopsis glycosyltransferase. Tetrahedron: Asymmetry 2005; 16:143-7.
47. Jacobsen JV, Pearce DW, Poole AT, Pharis RP, Mander LN. Abscisic acid, phaseic acid and gibberellin contents associated with dormancy and germination in barley. Physiol Plant 2002; 115:428-41.
48. Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, Asami T, et al. The Arabidopsis cytochrome P450 CYP707A encodes ABA 8'-hydroxylases: key enzymes in ABA catabolism. EMBO J 2004; 23:1647-56.
49. Koiwai H, Nakaminami K, Seo M, Mitsuhashi W, Toyomasu T, Koshiba T. Tissue-specific localization of an abscisic acid biosynthetic enzyme, AAO3, in Arabidopsis. Plant Physiol 2004; 134:1697-707.
50. González-Guzmán M, Abia D, Salinas J, Serrano R, Rodríguez PR. Two new alleles of the *abscisic aldehide oxidase 3* gene reveal its role in abscisic acid biosynthesis in seeds. Plant Physiol 2004; 135:325-33.
51. Lin PC, Hwang SG, Endo A, Okamoto M, Koshiba T, Cheng WH. Ectopic expression of ABSCISIC *ACID 2/GLUCOSE INSENSITIVE 1* in Arabidopsis promotes seed dormancy and stress tolerance. Plant Physiol 2007; 143:745-58.

52. Okamoto M, Kuwahara A, Seo M, Kushiro T, Asami T, Hirai N, et al. CYP707A1 and CYP707A2, which encode abscisic acid 8'-hydroxylases, are indispensable for proper control of seed dormancy and germination in Arabidopsis. Plant Physiol 2006; 141:97-107.
53. Millar AA, Jacobsen JV, Ross JJ, Helliwell CA, Poole AT, Scofield G, et al. Seed dormancy and ABA metabolism in Arabidopsis and barley: the role of ABA 8'-hydroxylase. Plant J 2006; 45:942-54.
54. Chono M, Honda I, Shinoda S, Kushiro T, Kamiya Y, Nambara E, et al. Field studies on the regulation of abscisic acid content and germinability during grain development of barley: molecular and chemical analysis of pre-harveswt sprouting. J Exp Bot 2006; 57:2421-34.
55. Gonai T, Kawahara S, Tougou M, Satoh S, Hashiba T, Hirai N, et al. Abscisic acid in the thermoinhibition of lettuce seed germination and enhancement of its catabolism by gibberellin. J Exp Bot 2004; 55:111-8.
56. Yamaguchi S, Ogawa M, Kuwahara A, Hanada A, Kamiya Y, Yamaguchi S. Activation of gibberellin biosynthesis and response pathways by low temperatura during imbibition of *Arabidopsis thaliana* seeds. Plant Cell 2004; 16:367-78.
57. Yang SH, Zeevaart JA. Expression of ABA 8'-hydroxylases in relation to leaf water relations and seed development in bean. Plant J 2006; 47:675-86.
58. Chen Y, Xie H, Liang J, Zhang J. The regulator of G protein signaling proteins involved in sugar and abscisic acid signaling in Arabidopsis seed germination. Plant Physiol 2006; 140:302-10.
59. Zhu G, Ye N, Zhang J. Glucose-induced delay of seed germination in rice is mediated by the suppression of ABA catabolism rather than an enhancement of ABA biosynthesis. Plant Cell Physiol 2009; 50:644-51.
60. Tsavkelova EA, Klimova SYu, Cherdyntseva TA, Netrusov AI. Hormones and hormone-like substances of microorganisms: A review. Appl Biochem Microbiol 2006; 42:229-35.
61. Boeiro L, Perrig D, Masciarelli O, Penna C, Cassán F, Luna U. Phytohormones production by three strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. Appl Microbiol Biotechnol 2007; 74:874-80.
62. Costacurta A, Vanderleyden J. Synthesis of phytohormones by plant-associated bacteria. Crit Rev Microbiol 1995; 21:1-18.
63. Arkhipova TN, Veselov SU, Melentiev AI, Martynenko EV, Kudoyarova GR. Ability of bacterium *Bacillis subtilis* to produce cytokinins and to influence the growth and endogenous hormone content of lettuce plants. Plant Soil 2005; 272:201-9.
64. Frugier F, Kosuta S, Murray JD, Crespi M, Szczyglowski K. Cytoquinin: secret agent of symbiosis. Trends Plant Sci 2008; 13:115-20.
65. Belimov AA, Dodd IC, Hontzeas N, Theobald JC, Safronova VI, Davies WJ. Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase yield of plants grown in drying soil via both local and systemic hormone signalling. New Phytol 2009; 181:413-23.
66. Forchetti G, Masciarelli O, Alemano S, Alvarez D, Abdala G. Endophytic bacteria in sunflower (*Helianthus annuus* L.): isolation, characterization and production of jasmonates and ABA in culture medium. Appl Microbiol Biotechnol 2007; 76:1145-52.
67. Hirai N, Yoshida R, Todoroki Y, Ohigshi H. Biosynthesis of ABA by non-mevalonate pathway in plants and by the mevalonate pathway in fungi. Bios Biotechnol Biochem 2000; 64:1448-54.
68. Dey R, Pal KK, Bhatt DM, Chauhan SM. Growth promotion and yield enhancement of peanut (*Arachis hypogea* L.) by application of plant growth-promoting rhizobacteria. Microbiol Res 2004; 159:371-94.
69. Frankenberger WT, Arshad M. Phytohormones in Soils. Marcel Dekker, New York USA 1995.
70. Yang J, Kloepper JW, Ryu Ch-M. Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 2009; 14:1-4.
71. Audenaert K, De Meyer G, Hofte M. Abscisic Acid determines basal susceptibility of tomato to *Botrytis cinerea* and suppresses salicylic acid-dependent signaling mechanisms. Plant Physiol 2002; 128:491-501.
72. Mauch-Mani B, Mauch F. The role of abscisic acid in plant-pathogen interactions. Curr Opin Plant Biol 2005; 8:409-14.
73. Janitor A. Growth of mycelia of phytopathogenic fungi after application of abscisic acid in vitro conditions. Plant Protect Sci 2002; 38:94-7.
74. Jiang F, Jeschke WD, Hartung W. ABA flows from *Hordeum vulgare* to the hemiparasite *Rhinanthus minor* and the influence of infection on host and parasite ABA relations. J Exp Bot 2004; 55:2323-9.
75. Slovik S, Daeter W, Hartung W. Compartmental distribution and redistribution of ABA in roots as influenced by environmental changes in the rhizosphere. A biomathematical model. J Exp Bot 1995; 46:881-94.
76. Hartung W, Sauter A, Turner NC, Fillery I, Heilmeier H. ABA in soils: what is its function and which factors and mechanisms influence its concentration? Plant Soil 1996; 184:105-10.
77. Degenhardt B, Gimmler H, Hose E, Hartung W. Effect of alkaline and saline substrates on ABA contents, distribution and transport in plant roots. Plant Soil 2000; 225:83-94.
78. Freundl E, Steudle E, Hartung W. Apoplastic transport of abscisic acid across maize roots. The role of the exodermis. Planta 2000; 210:222-31.
79. Hartung W, Sauter A, Hose E. ABA in the xylem: where does it come from, where does it go to? J Exp Bot 2002; 53:27-32.
80. Sauter A, Davies WJ, Hartung W. The long-distance ABA signal in the droughted plant: the fate of the hormone on its way from root to shoot. J Exp Bot 2001; 52:1991-7.
81. Jiang F, Hartung W. Long-distance signalling of ABA: the factors regulating the intensity of the ABA signal. J Exp Bot 2008; 59:37-43.
82. Sharp RE. Interaction with ethylene: changing views on the role of ABA in root and shoot growth responses to water stress. Plant Cell Environ 2002; 25:211-22.
83. Bewley JD. Seed germination and dormancy. Plant Cell 1997; 9:1055-66.
84. Bentsink L, Soppe W, Koornneef M. Genetics aspects of seed dormancy. In: Bradford K, Nonogaki H, eds. Seed Development, Dormancy and Germination. Oxford, UK: Blackwell Publishing 2007; 113-32.
85. Kermode AR. Developmental traits. Germination. In: Klee H, Christou P, eds. Handbook of plant biotechnology. UK: John Wiley & Sons 2004; 673-713.
86. Fenner M, Thompson K. The ecology of seeds. Cambridge University Press, Cambridge RU 2005; 250.
87. Vaughan DA, Balázs E, Heslop-Harrison JS. From Crop Domestication to Super-domestication. Ann Bot 2007; 100:893-901.
88. Gubler F, Millar AA, Jacobsen JV. Dormancy release, ABA and pre-harvest sprouting. Curr Opin Plant Biol 2005; 8:183-7.
89. Cadman CS, Toorop PE, Hilhorst HW, Finch-Savage WE. Gene expression profiles of Arabidopsis Cvi seeds during dormancy cycling indicate a common underlying dormancy control mechanism. Plant J 2006; 46:805-22.
90. Raz V, Bergervoet JH, Koornneef M. Sequential steps for developmental arrest in Arabidopsis seeds. Development 2001; 128:243-52.
91. Ali-Rachedi S, Bouinot D, Wagner MH, Bonnet M, Sotta B, Grappin P, et al. Changes in endogenous abscisic acid levels during dormancy release and maintenance of mature seeds: studies with the Cape Verde Islands ecotype, the dormant model of *Arabidopsis thaliana*. Planta 2004; 219:479-88.
92. Yazaki J, Kikuchi S. The genomic view of genes responsive to the antagonistic phytohormones, abscisisc acid and gibberellin. Vit Horm 2005; 72:1-30.
93. Corbineau F, Bianco J, Garello G, Côme D. Breakage of *Pseudotsuga menziesii* seed dormancy by cold treatment as related to changes in seed ABA sensitivity and ABA levels. Physiol Plant 2002; 114:313-9.
94. Koornneef M, Bentsink L, Hilhorst H. Seed dormancy and germination. Curr Opin Plant Biol 2002; 5:33-6.
95. Chiwocha SD, Cutler AJ, Abrams SR, Ambrose SJ, Yang J, Ross ARS, et al. The *etr1-2* mutation in *Arabidopsis thaliana* affects the abscisic acid, auxin, cytokinin and gibberellin metabolic pathways during maintenance of seed dormancy, moist-chilling and germination. Plant J 2005; 42:35-48.
96. Kucera B, Cohn MA, Leubner-Metzger G. Plant hormone interactions during seed dormancy release and germination. Seed Sci Res 2005; 15:281-307.
97. Alonso-Blanco C, Bentsink L, Hanhart CJ, Blankestijn-de Vries H, Koornneef M. Analysis of natural allelic variation at seed dormancy loci of *Arabidopsis thaliana*. Genetics 2003; 64:711-29.
98. Brady SM, McCourt P. Hormone cross-talk in seed dormancy. J Plant Growth Regul 2003; 22:25-31.
99. Yamaguchi S, Nambara N. Seed development and germination. In: Hedden P, Thomas SG, (Eds.) Plant Hormone Signaling, Blackwell Publishing Ltd, 9600 Garsington Road, Oxford OX4 2DQ UK 2006; 311-38.
100. Yamaguchi S, Kamiya Y, Nambara N. Regulation of ABA and GA levels during seed development and germination in Arabidopsis. In: Bradford K, Nonogaki H, (Eds.) Seed Development, Dormancy and Germination, Blackwell Publishing Ltd, 9600 Garsington Road, Oxford OX4 2DQ UK 2007; 224-47.
101. Gubler F, Hughes T, Waterhouse P, Jacobsen J. Regulation of dormancy in barley by blue light and after-ripening: effects of abscisic acid and gibberellin metaboloism. Plant Physiol 2008; 147:886-96.
102. Dugardeyn J, Vandenbussche F, Van Der Straeten D. To grow or not grow: what can we learn on ethylene-gibberellin cross-talk by in silico gene expression analysis? J Exp Bot 2008; 59:1-16.
103. Matilla AJ, Matilla-Vázquez MA. Involvement of ethylene in seed physiology. Plant Sci 2008; 175:87-97.
104. Cao X, Costa LM, Biderre-Petit C, Kbhaya B, Dey N, Perez P, et al. Abscisic acid and stress signals induce Viviparous 1 expression in seed and vegetative tissues of maiz. Plant Physiol 2007; 143:720-31.
105. Alboresi A, Gestin C, Leydecker M-T, Bedu M, Meyer C, Truong HN. Nitrate, a signal relieving seed dormancy in Arabidopsis. Plant Cell Environm 2005; 28:500-12.
106. Pawlowski TA. Proteomic of european beech (*Fagus silvatica* L.) seed dormancy breaking: influence of abscisic acid and gibberellic acids. Proteomics 2007; 114:482-90.
107. Leymarie J, Bruneaux E, Gibot-Leclerc S, Corbineau F. Identification of transcripts potentially involved in barley seed germination and dormancy using cDNA-AFLP. J Exp Bot 2007; 58:425-37.
108. Leymarie J, Robayo-Romero ME, Gendreau E, Benech-Arnold RL, Corbineau F. Involvement of ABA in induction of secondary dormancy in barley (*Hordeum vulgare* L.) seeds. Plant Cell Physiol 2008; 49:1830-8.
109. Nambara E, Marion-Poll A. ABA action and interactions in seeds. Trends Plant Sci 2003; 8:195-244.
110. Finkelstein RR. The role of hormones during seed development and germination. In: Davies PJ, (ed.) *Plant Hormones-Biosynthesis, signal transduction, action!* Dordrecht, Kluwer Academic 2004; 513-37.
111. Frey A, Godin B, Bonnet M, Sotta B, Marion-Poll A. Maternal synthesis of abscisic acid controls seed development and yield in *Nicotiana plumbaginifolia*. Planta 2004; 218:958-64.
112. Grappin P, Bouinot D, Sotta B, Miginiac E, Jullien M. Control of seed dormancy in *Nicotiana plumbaginifolia*: postimbibition abscisic acid synthesis imposes dormancy maintenance. Planta 2000; 210:279-85.

113. Schmitz N, Abrams SR, Kermode AR. Changes in abscisic acid content and embryo sensitivity to (+)-abscisic acid during termination of dormancy of yellow-cedar seeds. J Exp Bot 2000; 51:1159-62.
114. Schmitz N, Abrams SR, Kermode AR. Changes in ABA turnover and sensitivity that accompany dormancy termination of yellow-cedar (*Chamaecyparis nootkatensis*) seeds. J Exp Bot 2002; 53:89-101.
115. Schwachtje J, Baldwin IT. Smoke exposure alters endogenous gibberellin and abscisic acid pools and gibberellin sensitivity while eliciting germination in the post-fire annual, *Nicotiana attenuate*. Seed Sci Res 2004; 14:51-60.
116. Baumbusch LO, Hughes DW, Galau GA, Jakobse KS. LEC1, FUS3, ABI3 and Em expression reveals no correlation with dormancy in Arabidopsis. J Exp Bot 2004; 55:77-87.
117. Bentsink L, Koornneef M. Seed dormancy and germinatino. The Arabidopsis Book BioOne ASPB 2008; 1-18
118. Finch-Savage WE, Cadman CS, Toorop PE, Lynn JR, Hilhorst HW. Seed dormancy release in Arabidopsis Cvi by dry after-ripening, low temperature, nitrate and light shows common quantitative patterns of gene expression directed by environmentally specific sensing. Plant J 2007; 51:60-78.
119. Benech-Arnold RL, Sánchez RA, Forcella F, Kruk BC, Ghersa CM. Enviromental control of dormancy in weed seed banks in soil. Field Crops Res 2000; 67:105-22.
120. Baskin JM, Baskin CC. A classification system for seed dormancy. Seed Sci Res 2004; 14:1-16.
121. Battla D, Benech-Arnold RL. A predictive model for dormancy loss in *Polygonum aviculare* L. seeds based on changes in population hydrotime parameters. Seed Sci Res 2004; 14:277-86.
122. Oh E, Yamaguchi S, Hu J, Yusuke J, Jung B, Inyup Paik I, et al. PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the *GAI* and *RGA* promoters in Arabidopsis seeds. Plant Cell 2007; 19:1192-208.
123. Finkelstein RR, Srinivas SL, Gampala SSL, Rock CD. Abscisic acid signaling in seeds and seedlings. Plant Cell 2002; 14:15-45.
124. Liu X, Yue Y, Li B, Nie Y, Li W, Wu WH, Ma L. A G protein-coupled receptor is a plasma membrane receptor for the plant hormone abscisic acid. Science 2007; 315:1712-6.
125. Bove J, Lucus P, Godin B, Ogé L, Jullien M, Grappin P. Gene expression analysis by cDNA-AFLP highlights a set of new signalling networks and translational control during seed dormancy breaking in *N. plumbaginifoloia*. Plant Mol Biol 2005; 57:593-612.
126. Nishimura N, Yoshida T, Kitahata N, Asami T, Shinozaki K, Hirayama T. ABA-Hypersensitive Germination 1 encodes a protein phosphatase 2C, an essential component of abscisic acid signalling in Arabidopsis seed. Plant J 2007; 50:935-49.
127. Chibani K, Ali-Rachedi S, Job C, Job D, Jullien M, Grappin P. Proteomic analysis of seed dormancy in Arabidopsis. Plant Physiol 2006; 142:1493-510.
128. Carrera E, Holman T, Medhurst A, Peer W, Schmuths H, Footitt S, et al. Gene expression profiling reveals defined functions of the ABC transporter COMATOSE late in phase II of germination. Plant Physiol 2007; 143:1669-79.
129. Carrera E, Holman T, Medhurst A, Dietrich D, Footitt S, Theodoulou FL, et al. Seed after-ripening is a discrete developmental pathway associated with specific gene networks in Arabidopsis. Plant J 2008; 53:214-24.
130. Müller K, Tintelnot S, Leubner-Metzger G. Endosperm-limited Brassicaceae seed germination: Abscisic acid inhibits embryo-induced endosperm weakening of *Lepidium sativum* (cress) and endosperm rupture of cress and *Arabidopsis thaliana*. Plant Cell Physiol 2006; 47:864-77.
131. Merlot S, Gosti F, Guerrier D, Vavasseur A, Giraudat J. The ABI1 and ABA2 protein phosphatases 2C act in a negative feedback regulatory loop of the abscisic acid pathway. Plant J 2001; 25:295-303.
132. Khun JM, Boisson-Dernier A, Dizon MB, Maktabi MH, Schroeder JI. The protein phosphatase *AtPP2CA* negatively regulates abscisic acid signal transduction in Arabidopsis, and effects of *abh1* on *AtPP2CA* mRNA. Plant Physiol 2006; 140:127-39.
133. Rodríguez PL. ABI2, a second protein phosphatase 2C (PP2C) function in higher plants. Plant Mol Biol 1998; 38:919-27.
134. Tahtiharju S, Palva T. Antisense inhibition of protein phosphatase 2C accelerates cold acclimation in *A. thaliana*. Plant J 2001; 26:461-70.
135. Wu Y, Sánchez JP, López-Molina L, Himmelbach A, Grill E, Chua NH. The *abi1-1* mutation bloks ABA downstream cADPR action. Plant J 2003; 34:307-15.
136. Arroyo A, Bossi F, Finkelstein RR, Leon P. Three gene that affect sugar sensing (abscisic acid insensitive 4, abscisic acid insensitive 5, and constitutive triple response 1) are differentially regulated by glucose in Arabisopsis. Plant Physiol 2003; 133:231-42.
137. Mortensen LC, Rodriguez D, Nicolás G, Eriksen EN, Nicolás C. Decline in a seed-specific ABA responsive glycine rich protein (GRPF1) mRNA may reflect the release of seed dormancy in *Fagus silvatica* during moist prechilling. Seed Sci Res 2004; 14:27-34.
138. Lorenzo O, Rodríguez D, Nicolás C, Nicolás G. Characterization and expression of two protein kinase and an EIN3-like genes, which are regulated by ABA and GA3 in dormant *Fagus silvatica* seeds. In: Black M, Bradford KJ, Vázquez-Ramos J. (Eds) Seed Biology: Advances and applications. CAB International, Wallingford 2000; 329-40.
139. Jiménez JA, Rodríguez D, Calvo AP, Mortensen LCh, Nicolás G, Nicolás C. Expresión of a transcription factor (FsERF1) involved in ethylene signallling during the breaking of dormancy in *Fagus silvatica* seeds. Physiol Plant 2005; 125:373-80.
140. González-García MP, Rodríguez D, Nicolás C, Rodríguez PL, Nicolás G, Lorenzo O. Negative regulation of abscisic acid signaling by the *Fagus silvatica* FsPP2C1 plays a role in seed dormancy regulation and promotion of seed germination. Plant Physiol 2003; 133:135-44.
141. Reyes D, Rodríguez D, González-García MP, Lorenzo O, Nicolás G, García-Martínez JL, et al. Overexpression of a protein phosphatase 2C from beech seeds in Arabisopsis shows phenotypes related to abscisic acid responses and gibberellin biosynthesis. Plant Physiol 2006; 141:1414-24.
142. Lorenzo O, Rodríguez D, Nicolás G, Rodríguez PL, Nicolás C. A new protein phosphatase 2C (FsPP2c1) induced by abscisic acid is specifically expressed in dormant beechnut seeds. Plant Physiol 2001; 125:1949-56.
143. Lorenzo O, Nicolás C, Nicolás G, Rodríguez D. Characterization of a dual plant protein kinase (FsPK1) upregulated by abscisic acid and calcium and specifically expressed in dormant seeds of *Fagus sylvatica* L. Seed Sci Res 2003; 13:261-71.
144. Lorenzo O, Nicolás C, Nicolás G, Rodríguez D. Molecular cloning of a functional protein phosphatase 2C (FsPP2C2) with unusual features and synergistically upregulated by ABA and calcium in dormant seeds of *Fagus sylvatica*. Physiol Plant 2002; 114:482-90.
145. Saez A, Apostolova N, Gonzalez-Guzman M, Gonzalez-Garcia MP, Nicolas C, Lorenzo O, et al. Gain-of-function and loss-of-function phenotypes of the protein phsophatases 2C *HAB1* reveal its role as a negative regulator of abscisic acid signalling. Plant J 2004; 37:354-69.
146. Zeng Y, Raimondi N, Kermode AR. Role of an ABI3 homologue in dormancy maintenance of yellow-cedar seeds and in the activation of storage protein and Em gene promoters. Plant Mol Biol 2003; 51:39-49.
147. Razem FA, Baron K, Hill RD. Turning on gibberellin and abscisic acid signaling. Curr Opin Plant Sci 2006; 9:454-9.
148. Razem FA, El-Kereamy A, Abrams SR, Hill RD. The RNA-binding protein FCA is an abscisic acid receptor. Nature 2006; 439:290-4.
149. Shen YY, Wang XF, Wu FQ, Du SY, Cao Z, Shang Y, et al. The Mg-chelatase H subunit is an abscisic acid receptor. Nature 2006; 443:823-6.
150. Pandey S, Nelson DC, Assmann SM. Two novel GPCR-type G proteins are abscisic acid receptors in Arabidopsis. Cell 2009; 136:136-48.
151. Christmann A, Moes D, Himmerlbach A, Yang Y, Tang Y, Grill E. Integration of abscisic acid signalling into plant responses. Plant Biol 2006; 8:314-25.
152. Verslues PE, Zhu J-KN. New developments in abscisic acid perception and metabolism. Curr Opin Plant Biol 2007; 10:447-52.
153. Hirayama T, Shinozaki K. Perception and transduction of abscisic acid signals: key to the function of the versatile plant hormone ABA. Trend Plant Sci 2007; 12:343-51.
154. Zhang DP, Wu ZY, Li XY, Zhao ZX. Purification and identification of a 42-kilodalton abscisic acid-specific-binding protein from epidermis of broad bean leaves. Plant Physiol 2002; 128:714-25.
155. Risk JM, Day CL, Macknight RC. Re-evaluation of abscisic acid (ABA) binding assays shows that GCR2 does not bind ABA. Plant Physiol 2009; 150:6-11.
156. Razem FA, Luo M, Liu JH, Abrams SR, Hill RD. Purification and characterization of a barley aleurone abscisic acid-binding protein. J Biol Chem 2004; 279:9922-9.
157. Simpson GG, Dijkwel PP, Quesada V, Henderson I, Dean C. FY is an RNA 3'end-processing factor that interacts with FCA to control the Arabisopsis floral transition. Cell 2003; 113:777-87.
158. Gao Y, Zeng Q, Guo J, Cheng J, Ellis BE, Chen J-G. Genetic characterization reveals no role for the reported ABA receptor, GCR2, in ABA control of seed germination and early seedling development in Arabisopsis. Plant J 2007; 52:1001-13.
159. Guo J, Zeng Q, Emami M, Ellis BE, Chen J-G. The *GCR2* gene family is not required for ABA control of seed germination and early seedling development in Arabidopsis. PLoS ONE 2008; 3:2982.
160. Colucci G, Apone F, Alyeshmerni N, Chalmers D, Chrispeels MJ. *GCR1*, the putative Arabidopsis G protein-coupled receptor gene is cell cycle-regulated, and its overexpression abolished seed dormancy and shortens time to flowering. Proc Natl Acad Sci USA 2002; 99:4736-41.
161. Pandey S, Assmann SM. The Arabidopsis putative G protein-coupled receptor GCR1 interacts with the G protein α subunit GPA1 and regulates abscisic acid signaling. Plant Cell 2004; 16:1616-32.
162. Illingworth CJ, Parkes KE, Snell CR, Mullineaux PM, Reynolds CA. Criteria for confirming sequence periodicity identified by Fourier transform analysis: Applicaation to GCRC2, a candidate plant GPCR? Biophys Chem 2008; 133:28-35.
163. Assmann SM. Plant G proteins, phytohormones and plasticity: three questions and a speculation. Sci STKE 2004; 264:20.
164. Pandey S, Chen JG, Jones AM, Assmann SM. G-protein complex mutants are hypersensitive to abscisic acid regulation of germination and postgermination development. Plant Physiol 2006; 141:243-56.
165. Gookin TE, Kim J, Assmann SM. Whole proteome identification of plant candidate G-protein coupled receptors in Arabidopsis, rice and poplar: computational prediction and in-vivo protein coupling. Gen Biol 2008; 8:120.
166. Chow B, McCourt P. Plant Hormone receptors: perception is everything. Genes Dev 2009; 20:1998-2008.
167. Risck JM, Macknight RC, Day CL. FCA does not bind abscisic acid. Nature 2008; 456:5-6.

168. Jones AM, Sussman MR. A binding resolution. Plant Physiol 2009; 150:3-5.
169. Gao Y, Zeng Q, Guo J, Cheng J, Ellis BE, Chen J-G. Genetic characterization reveals no role for the reported ABA receptor, GCR2, in ABA control of seed germination and early seedling development in Arabisopsis. Plant J 2007; 52:1001-13.
170. Guo J, Zeng Q, Emami M, Ellis BE, Chen J-G. The *GCR2* gene family is not required for ABA control of seed germination and early seedling development in Arabidopsis. PLoS ONE 2008; 3:2982.
171. McCourt P, Creelman R. The ABA receptors—we report you decide. Curr Opin Plant Biol 2008; 11:474-8.
172. Fait A, Angelovici R, Less H, Ohad I, Urbanczyk-Wochniak E, Fernie AR, et al. Arabidopsis seed development and germination is associated with temporally distinct metabolic switches. Plant Physiol 2006; 142:839-54.
173. Arenas-Huertero F, Arroyo A, Zhou L, Sheen J, Leon P. Analysis of Arabidopsis glucose insensitive mutants, *gin5* and *gin6*, reveals a central role of the plant hormone ABA in the regulation of plant vegetative development by sugar. Genes Dev 2000; 14:2085-96.
174. Ghassemian M, Nambara E, Cutler S, Kawaide H, Kamiya Y, McCourt P. Regulation of abscisicacid signaling by the ethylene response pathway in Arabidopsis. Plant Cell 2000; 12:1117-26.
175. Xiong L, Lee H, Ishitani M, Zhu JK. Regulation of osmotic stress-responsive gene expression by the LOS6/ABA1 locus in Arabidopsis. J Biol Chem 2002; 277:8588-96.
176. Rook F, Corke F, Card R, Munz G, Smith C, Bevan MW. Impaired sucrose-induction mutants reveal the modulation of sugar-induced starch biosynthetic gene expression by abscisic acid signalling. Plant J 2001; 26:421-33.
177. White CN, Proebsting WM, Hedden P, Rivin CJ. Gibberellins and seed development in maize I. Evidence that gibberellin/abscisic acid balance governs germination versus maturation pathways. Plant Physiol 2000; 122:1081-8.
178. Thompson AJ, Jackson AC, Symonds RC, Mulholland BJ, Dadswell AR, Blake PS, et al. Ectopic expression of a tomato 9-cis-epoxycarotenoiddioxygenase gene causes over-productionof abscisic acid. Plant J 2000; 23:363-74.
179. Beaudoin N, Serizet C, Gosti F, Giraudat J. Interactions between abscisic acid and ethylene signaling cascades. Plant Cell 2000; 12:1103-15.
180. Nambara E, Suzuki M, Abrams S, McCarty DR, Kamiya Y, McCourt P. A screen for genes that function in abscisic acid signaling in *Arabidopsis thaliana*. Genetics 2002; 161:1247-55.
181. Mazzella MA, Arana MV, Staneloni RJ, Perelman S, Rodriguez Batiller MJ, Muschietti J, et al. Phytocrome control of the Arabidopsis transcriptome anticipates seedling exposure to light. Plant Cell 2005; 17:2507-16.
182. Acevedo-Hernandez GJ, León P, Herrera-Estrella LR. Sugar and ABA responsiveness of a minimal RBCS light responsive unit is mediated by direct binding of ABI4. Plant J 2005; 43:506-19.
183. Finkelstein RR, Lynch TJ. The Arabidopsis abscisic acid response gene *ABI5* encodes a basic leucine zipper transcription factor. Plant Cell 2000; 12:599-609.
184. Laby RJ, Kincaid MS, Kim D, Gibson SI. The Arabidopsis sugar-insensitive mutants sis4 and sis5 are defective in abscisic acid synthesis and response. Plant J 2000; 23:587-96.
185. He YH, Gan SS. A novel zinc-finger protein with a proline-rich domain mediates ABA-regulated seed dormancy in Arabidopsis. Plant Mol Biol 2004; 54:1-9.
186. Young TE, Gallie DR. Programmed cell death during endosperm development. Plant Mol Biol 2000; 44:283-301.
187. Iglesias-Fernández R, Angel Matilla AJ. Afterripening alters the gene expression pattern of oxidases involved in the ethylene and gibberellin pathways during the early imbibition of *Sisymbrium officinale* L. seeds. J Exp Bot 2009; 60:1645-65.
188. Matakiadis T, Alboresi A, Jikumaru Y, Tatematsu K, Pichon O, Renou JP, et al. The Arabidopsis abscisic acid catabolic gene *CYP707A2* plays a key role in nitrate control of seed dormancy. Plant Physiol 2009; 149:949-60.