Control of seed coat rupture by ABA-INSENSITIVE 5 in *Arabidopsis thaliana*

Thiago Barros-Galvão, Fabián E. Vaistij, Ian A. Graham\*

Centre for Novel Agricultural Products, Department of Biology, University of York,

York YO10 5DD, United Kingdom

\*Corresponding author: email, ian.graham@york.ac.uk; tel., +44 (0) 1904 328750

*Short title: ABA blocks seed coat rupture of nicked seeds via ABI5*

**Abstract**

In Arabidopsis, seed germination is a biphasic process involving rupture of the seed coat followed by emergence of the radicle through the micropylar endosperm. Embryo expansion results in seed coat rupture and removal of seed coat imposed dormancy with DELLA proteins blocking embryo expansion in the absence of GA. Exogenous ABA treatment does not block seed coat rupture but does block radicle emergence. We used this limited effect of exogenous ABA to further investigate the mechanism by which it blocks the onset of germination marked by seed coat rupture. We show that physical nicking of the seed coat results in exogenous ABA treatment blocking both seed coat and endosperm rupture and this block requires the transcription factors ABI3 and ABI5, but not ABI4. Furthermore, we show that the repression of expression of several *EXPANSIN* genes (*EXPA1*, *EXPA2*, *EXPA3*, *EXPA9* and *EXPA20*) by exogenous ABA requires ABI5. We conclude that ABI5 plays an important role in the ABA mediated repression of germination through prevention of seed coat rupture and propose that this involves EXPANSIN related control of cell wall loosening.

Key words: ABA; ABI5; EXPANSIN; Nicking; Testa rupture.

**Introduction**

Seed germination is a complex developmental process that marks the beginning of the life cycle of a higher plant. For over forty years, seed biologists have understood that the balance between the inhibitory actions of abscisic acid (ABA) and the stimulating actions of gibberellins (GA) is the primary determinant of seed dormancy and germination (Luckwill, 1952; Karssen and Laçka, 1985; Footitt et al., 2011; Rajjou et al., 2012). In coat-imposed dormancy species, germination can be achieved via simple removal of or damage to the seed coat. Previous work has shown that the seed coat may restrict germination by controlling permeability to either water, oxygen or germination inhibitors that leach from the seed (Edwards, 1968; Wyatt, 1977; Corbineau & Côme, 1993). Arabidopsis seeds have a single endosperm cell layer between the embryo and the seed coat, and endosperm-imposed dormancy prevails over embryo- and seed coat-imposed dormancy with the phytohormones GA and ABA regulating germination in a tissue specific manner (Penfield et al., 2004; Lee et al., 2010). ABA is biosynthesized mainly in the endosperm of dormant seeds and transported to the embryo in order to repress embryo expansion and therefore germination (Lee et al., 2010; Kang et al., 2015). However, Arabidopsis seeds still display some degree of coat-imposed dormancy, and alterations in seed coat development or pigmentation often result in reduced dormancy (Shirley et al., 1995; Debeaujon et al., 2000, 2003).

Upon imbibition, GA promotes Arabidopsis germination processes and ABA inhibits them (Müller et al., 2006; Piskurewicz et al., 2008). Arabidopsis seeds germinate in a two-step process that involves longitudinal splitting of the seed coat along the embryo axis followed by emergence of the radicle through the micropylar endosperm (Liu et al., 2005; Müller et al., 2006). Both these events require embryo expansion, with the latter generally considered as completion of seed germination. Under certain germination conditions or genetic backgrounds radicle emergence through both the endosperm and seed coat can occur prior to longitudinal splitting of the seed coat (Piskurewicz et al., 2008; Piskurewicz et al., 2009; Lee et al., 2012) demonstrating that control of the two phases of germination can be separated and therefore may be under separate control.

GA induces germination via destabilization of the DELLA proteins, which are negative regulators of GA-inducible genes (Lee et al., 2002; McGinnis et al., 2003; Tyler et al., 2004; Cao et al., 2005). The requirement for exogenous GA to promote seed coat rupture and germination in the *ga1-3* background, which is disrupted in GA biosynthesis, can be compensated for by loss of function mutations in the DELLA genes (e.g. *ga1-3 rgl2-1 gai-t6 rga-t2*) in non-dormant seeds (Piskurewicz et al., 2009). Curiously, exogenously applied ABA has been shown to repress endosperm rupture but not seed coat rupture (Müller, et al., 2006; Linkies et al., 2009; Piskurewicz et al., 2009). However, under low GA conditions induced by paclobutrazol, *aba1*-6 mutant seeds exhibit seed coat rupture suggesting that endogenous ABA synthesis is still somehow required in the repression of both endosperm and seed coat rupture (De Giorgi et al., 2015).

ABA-INSENSITIVE 3 (ABI3), ABI4 and ABI5 encode B3-, AP2-, and bZIP-type transcription factors, respectively, which are important for the ABA responses during seed maturation, seed germination and seedling establishment. ABI3 is a seed- specific regulator that plays a crucial role in all three of these seed stages (Giraudat et al., 1992; Parcy et al., 1997; Nambara et al., 2000; Finkelstein et al., 2002; Lopez- Molina et al., 2002; Delmas et al., 2013). ABI4 has been shown to play a role in controlling oil mobilization in the embryo of germinating seeds (Penfield et al., 2006b) while ABI5 has been shown to control germination downstream of ABI3 by regulating GA and ABA responses (Lopez-Molina et al., 2001; Lopez-Molina et al., 2002; Lee et al., 2012).

Analyzing the expression pattern of ABI transcription factors during seed germination in the presence of ABA, we have previously observed that ABI3 is expressed ubiquitously throughout the seed, including the endosperm, while ABI4 is restricted to the embryo (Penfield et al., 2006b). Both ABI3 and ABI5 are expressed throughout the embryo but unlike ABI3, ABI5 expression is confined to the micropylar region of the endosperm through which the radicle emerges (Penfield et al., 2006b). This region of the endosperm plays an important role in the regulation of seed germination and in many species is characterized by a distinct gene expression pattern and cell wall composition compared with the lateral endosperm (Bewley, 1997).

Remarkably, GA biosynthesis pathway genes (*GA3ox1* and *GA3ox2*) can be traced to the cortex and endodermis cells in the hypocotyl transition zone that exhibits early expansion to complete germination, suggesting that GA triggers germination by specific cell expansion (Yamaguchi et al., 2001; Topham et al., 2017). Additionally, GA induces genes encoding cell-wall-modifying enzymes such as expansins (EXPAs), which constitute one of the four molecular mechanisms of cell wall loosening in plants (Cosgrove, 2005). Expansins are encoded by a multigene family and are very conserved among higher plants (Shcherban et al., 1995; Hutchison et al., 1999; Cosgrove, 2005). Expansins are thought to play multiple roles during the life cycle of higher plants, including in seed germination (Chen and Bradford, 2000; Chen et al., 2001; Yan et al., 2014; Marowa et al., 2016).

We have previously shown that cotyledon expansion precedes radicle protrusion during Arabidopsis seed germination and that this process is mediated by DELLA-protein growth repressors and ABA (Penfield et al., 2006a). Given that seed coat rupture requires expansion of the embryo, we set out to investigate the contribution of the ABI3, ABI4 and ABI5 transcription factors in the repression of seed coat rupture in *A. thaliana* seeds.

**Methods**

*Plant materials and growth conditions*

All transgenic lines used in this study were described previously: *abi3-5* (*abi3*) (Ooms et al., 1993); *abi4-1* (*abi4*) (Penfield et al., 2006b); *abi5-7* (*abi5*) (Nambara et al., 2002); *ga1-3* (*ga1*) (Piskurewicz et al., 2009); *ga1-3 rgl2-1 gai-t6 rga-t2* (*ga1 della3*) (Piskurewicz et al., 2009). *abi4* and *abi5* were in Columbia (Col) background, while *abi3*, *ga1* and *ga1 della3* were in Landsberg erecta (Ler). Plants were grown in a greenhouse supplemented with artificial light to give a photoperiod of 16 h light at a temperature of 20-22 °C. Seeds were harvested when plants had stopped flowering and siliques had started to dehisce, and size sieved using a sieve with mesh size 250 μm. All seeds used in this study were after-ripened, i.e. seeds had lost primary dormancy.

*Germination assays*

Sterilized seeds were plated on medium containing 0.9 % (w/v) water agar. Plates were supplemented with ABA (Sigma-Aldrich) according to the germination condition examined. Percentage of seed coat rupture and radicle protrusion was scored after five days of imbibition. Nicking was performed in seeds after 1 hour of imbibition using a needle to pucture the seed according to Fig. 1A. Between 50 and 100 seeds were used to check seed coat rupture and radical emergence and repeated at least two times. Differences between results stated in the text are statistically significant as determined by Student’s t-test (P≤0.01). Pictures of seeds were taken using a GXMXTL3 stereo microscope (GT Vision) coupled with a GXCAM-HICHROME Camera (GT Vision).

*Real-Time PCR gene expression assays*

Total RNA was extracted from three biological replicates of frozen seed tissue (200 nicked seeds were used for each replicate) as described previously (Vaistij et al. 2013). cDNA synthesis was performed using standard methods. qPCR was performed using iQ SYBR Green Supermix and the MyiQ Real-Time PCR detection system (Bio- Rad) according to the manufacturer's instructions. The primer sequences used in this study are described in Supplementary Table 1, expression of UBQ11 was used for normalization.

**Results and Discussion**

*Exogenous ABA blocks seed coat rupture of nicked seeds*

Nicking is a common procedure used in both dormant and GA-deficient seeds to promote germination. In Arabidopsis, nicking disrupts the integrity of both the seed coat and the endosperm (Fig. 1A). In non-dormant seeds, as expected, disruption of the endosperm did not affect the appearance of radicle protrusion (Fig. 1B, bottom panels). This finding is consistent with the fact that the role of the endosperm is to synthesize and continuously release ABA towards the embryo to block germination of dormant seeds (Lee et al., 2010; Kang et al., 2015). But because nicking disrupts the integrity of the seed coat, we questioned whether or not nicking would also affect the appearance of seed coat rupture during germination; i.e. display radicle protrusion without prior seed coat rupture. For the purpose of this work, we only considered as non-artificial seed coat rupture (not promoted by puncturing) those longitudinal openings that were at least a third the size of the seeds. Nicking was performed in non-dormant Col seeds after 1 hour of imbibition using a needle to puncture the chalazal region in order to create a continuum between the embryo and external media (Fig. 1A). Intact Col seeds showed seed coat and endosperm rupture after 29 and 42 hours of imbibition respectively (Fig. 1B, top panels), while Col nicked seeds showed seed coat and endosperm rupture after 24 and 29 hours of imbibition respectively (Fig. 1B, bottom panels). Thus seed coat nicking does not affect the appearance of seed coat rupture per se, but it does decrease the post imbibition period to both seed coat rupture and radicle protrusion compared to intact seeds.

In tetrazolium uptake assays, several reports have shown that defects in the seed coat properties are associated with less dormancy, more permeability to tetrazolium salts and more sensibility to exogenous ABA (Debeaujon et al., 2000; Beisson et al., 2007; Vishwanath et al., 2013; MacGregor et al., 2015). De Giorgi et al. (2015) reported a thick cutin-containing cuticular layer inside the seed coat which surrounds whole endosperm of Arabidopsis seeds. These authors also showed that toluidine blue staining of embryos only occurs when this cuticule is disrupted. Hence, we hypothesized that the embryo is inaccessible to exogenous ABA and this is why ABA treatment does not block seed coat rupture in non-dormant seeds. To investigate this, we combined seed coat nicking and exogenous ABA treatment for 120 hours after imbibition. Consistent with previous work (Müller et al., 2006), 10 μM ABA-treated intact wild-type seeds all showed seed coat rupture, but no radicle protrusion (Fig. 2B, C). Following seed coat nicking, 10 μM ABA efficiently blocked seed coat rupture of both Ler and Col seeds (Fig. 2B, C, E and F). At 5 μM ABA nicked Col seeds showed a decreased frequency of seed coat rupture (Sup. Fig. 1). These results demonstrate that seed coat nicking results in seed coat rupture becoming sensitive to exogenous ABA, presumably because the nicking results in the ABA accessing the embryo and blocking expansion. In the case of exogenous ABA, we propose that the cuticle prevents ABA uptake until the embryo expands and causes seed coat rupture, after which ABA then is taken up and blocks any further expansion of the radicle and endosperm rupture. Nicking of the seed coat will disrupt the cuticle, allowing immediate uptake of ABA and blockage of embryo expansion seed coat rupture and radicle emergence. This explanation is consistent with our previous demonstration that cotyledon expansion is necessary for seed coat rupture (Penfield et al., 2006a).

*ABI5 represses seed coat rupture of nicked seeds in the presence of exogenous ABA*

Previous studies have shown that DELLA loss of function mutations lead to an induction of seed coat and endosperm rupture in Arabidopsis under low GA conditions (Cao et al., 2005; Piskurewicz et al., 2009). We previously reported that GA and the DELLA proteins regulate cotyledon expansion prior to endosperm rupture (Penfield et al., 2006a). Consistent with these reports, we found that the *della3* mutant rescues the *ga1* phenotype by showing seed coat rupture but not radicle emergence through the endosperm of intact seeds treated with ABA (Fig. 2A). However, when nicked, only a few percentage of the *ga1 della3* seeds showed seed coat rupture and about 30 % showed radicle protrusion without prior visible seed coat rupture in the presence of ABA, while *ga1* seeds remained quiescent (Fig. 2A and D). The fact that DELLA proteins, mainly RGL2, promote ABA synthesis (Lee et al., 2010) could explain the leaky seed coat rupture and radicle protrusion observed in the *ga1 della3* nicked-seeds. Taken together, these results suggest that DELLA proteins are at least partly involved in the ABA-dependent repression of seed coat rupture and radicle protrusion during germination.

To establish if the ABA-insensitive transcription factors are involved in the response of nicked seeds to exogenous ABA, we used loss of function *abi3*, *abi4* and *abi5* mutants. Consistent with previous reports (Ooms et al., 1993; Finkelstein, 1994; Bies-Etheve et al., 1999), we found that intact *abi3*, *abi4* and *abi5* seeds are insensitive to exogenous application of ABA in comparison with their respective wild types (Fig. 2B and C). ABA-insensitive mutants *abi1-1*, *abi2-1*, and *abi3-4* show a large cotyledon phenotype compared to their corresponding wild-types (Penfield et al., 2006a). We did not observe seed coat rupture in either intact or nicked seeds of *abi3* in the presence of exogenous ABA, although seeds clearly showed radicle protrusion (Fig. 2B and E). ABI3 is a critical component of the network involved in many processes during seed development including the positive regulation of FUSCA3, a transcriptional factor that bears similarity to the B3 region of the ABI3-like proteins, which directly represses TRANSPARENT TESTA GLABRA1 (TTG1) (Tsuchiya et al., 2004; Delmas et al., 2013; Chen et al., 2015). TTG1 also causes the transparent testa phenotypes, which includes deficiency in pigment accumulation in the seed coat (Debeaujon et al., 2000; Sagasser et al., 2002; Chen et al., 2015). It is possible that altered composition of *abi3* seed coats renders them more resistant to rupture compared to wild type when the radicle protrudes.

Nicked seeds of *abi4* do not show seed coat rupture in the presence of exogenous ABA (Fig. 2C and F), which is consistent with our previous findings that the events leading to germination in the endosperm of *abi4* seeds are completely blocked under concentrations of PAC or ABA inhibitory to wild-type seeds (Penfield et al., 2006b). Bossi et al. (2009) demonstrated that ABI4 acts as a transcriptional activator of the expression of ABI5 through direct binding to CE1-like *cis*-acting elements in the promoter of ABI5; while Lopez-Molina et al. (2002) have shown that ABI5 acts downstream of ABI3 in order to repress germination in the presence of ABA. Thus, it is possible that, in the *abi4* mutant background, an early exposure to ABA promoted by nicking blocks seed coat rupture via the ABI3/ABI5 pathway described by Lopez-Molina et al. (2002).

As expected, *abi5* nicked seeds are insensitive to exogenous ABA showing more than 80 % seed coat rupture (Fig. 2C and F). In contrast to these findings Piskurewicz et al. (2008 and 2009) indicate that ABI5 is not involved in the repression of seed coat rupture under low GA conditions. However, this rather contradictory result may be due to the fact that GA is required to promote seed coat rupture.

*ABI5 regulates expression of EXPANSINs in nicked seeds treated with ABA*

Piskurewicz and others (2008 and 2009) reported that RGL2, RGA and GAI are responsible for repression of seed coat rupture under low GA conditions, while Lee et al. (2012) have shown that ABI5 directly regulates the expression of *RGL2*, *RGA* and *GAI* under FR conditions. However, we did not observe any differences in the transcript levels of *RGL2*, *RGA* or *GAI* between Col and *abi5* nicked seeds in the presence of ABA (Fig. 3).

A recent study showed that seeds of an endosperm-specific expansin mutant (*exp2*) are delayed in germination in comparison with the wild type (Yan et al., 2014). In addition to this, using the β-glucuronidase (GUS) assay with a pAtEXP2:GUS construct these authors observed GUS staining in the radicle prior to germination. In an earlier study, a tomato expansin, LeEXPA4, was found to be involved in the control of germination by contributing to cell wall loosening in the micropylar endosperm (Chen and Bradford, 2000). Interestingly, in the presence of ABA, *ABI5* expression is limited to the micropylar region of the endosperm, but also expressed in the embryo (Penfield et al., 2006b), which led us to speculate that ABI5 is involved in the repression of genes associated with cell wall loosening in the micropylar region of the endosperm through which the radicle must emerge for germination to occur.

Publically available transcriptomic data (vseed.nottingham.ac.uk) was used to select 7 expansins genes that are highly expressed in imbibed Arabidopsis seeds for comparative expression analysis in *abi5* and wild type. Remarkably, *EXPANSIN 1* (*EXPA1*), *EXPA2*, *EXPA3*, *EXPA9*, and *EXPA20* expression were all increased in *abi5* nicked seeds compared with Col in the presence of ABA, while no significant changes were observed in the expression of *EXPA8* and *EXPA10* (Fig. 3). These results support a role for ABI5 in repressing the expression of expansins which in turn could lead to decreased embryo expansion and failure of the seed coat to rupture in the presence of ABA. It will be interesting to establish if the same suite of expansin genes are altered in an ABI5 dependent manner in the micropylar region of the endosperm and in the embryo in response to ABA.

*Conclusion*

Combining the data presented here with the current understanding of how seed coat rupture is regulated, we propose that ABI5 plays a key role integrating ABA and GA responses in addition to DELLA proteins to control embryo expansion. Previous reports have established that control of seed coat rupture involves modulation of GA and ABA responses mediated by DELLA proteins, largely by RGL2 (Piskurewicz et al., 2008; Piskurewicz et al., 2009; De Giorgi et al., 2015). In the present work we have shown that, when exogenous ABA is readily available to the embryo as a consequence of nicking, ABI5 is required to repress seed coat rupture. Our data further indicate that ABI5 may act via repression of EXPANSIN genes, which are associated with cell wall loosening, thus affecting embryo expansion.

**Acknowledgements**

TBG was funded by the Brazilian Government Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Program. IAG received support from the UK Biotechnology and Biological Sciences Research Council (grant BB/J00216X/1) and The Garfield Weston Foundation. We thank George Bassel for helpful discussion, and Luis Lopez-Molina for donating *abi5-7* and *ga1-3 rgl2-1 gai-t6 rga-t2* seeds.

**References**

**Beisson F, Li Y, Bonaventure G, Pollard M and Ohlrogge JB** (2007) The ac-yltransferase GPAT5 is required for the synthesis of suberin in seed coat and root of Arabidopsis. Plant Cell **19**, 351-368.

**Bewley JD** (1997) Breaking down the walls – A role for endo-b-mannanase in release from seed dormancy? Trends Plant Science **12**, 464-469.

**Bies-Etheve N, da Silva Conceicao A, Giraudat J, Koornneef M, Léon-Kloosterziel K, Valon C and Delseny M** (1999) Importance of the B2 domain of the Arabidopsis ABI3 protein for Em and 2S albumin gene regulation. Plant Molecular Biology **40**, 1045-1054.

**Bossi F, Cordoba E, Dupré P, Mendoza MS, Román CS and León P** (2009) The Arabidopsis ABA-INSENSITIVE (ABI) 4 factor acts as a central transcription activator of the expression of its own gene, and for the induction of ABI5 and SBE2.2 genes during sugar signaling. Plant Journal **59**, 359-374.

**Cao D, Hussain A, Cheng H and Peng J** (2005) Loss of function of four DELLA genes leads to light- and gibberellin- independent seed germination in Arabidopsis. Planta **223**, 105-113.

**Chen F and Bradford KJ** (2000) Expression of an expansin is associated with endosperm weakening during tomato seed germination. Plant Physiology **124**, 1265-1274.

**Chen F, Dahal P and Bradford KJ** (2001) Two tomato expansin genes show divergent expression and localization in embryos during seed development and germination. Plant Physiology **127**, 928-936.

**Chen M, Zhang B, Li C, Kulaveerasingam H, Chew FT and Yu H** (2015) TRANSPARENT TESTA GLABRA1 Regulates the Accumulation of Seed Storage Reserves in Arabidopsis. Plant Physiology **169**, 391-402.

**Corbineau F and Côme D** (1993) The concept of dormancy in cereal seeds. Proceedings of the 4th International Workshop on seeds, basic and applied aspects of seedbiology, July 1993, Angers, France.

**Cosgrove DJ** (2005) Growth of the plant cell wall. Nature Reviews Molecular Cell Biology, **11**, 850-861.

**Debeaujon I, Léon-Kloosterziel KM and Koornneef M** (2000) Influence of the testa on seed dormancy, germination, and longevity in Arabidopsis. Plant Physiology **122**, 403-414.

**Debeaujon I, Nesi N, Perez P, Devic M, Grandjean O, Caboche M and Lepiniec L** (2003) Proanthocyanidin-accumulating cells in Arabidopsis testa: regulation of differentiation and role in seed development. Plant Cell **15**, 2514-2531.

**De Giorgi J, Piskurewicz U, Loubery S, Utz-Pugin A, Bailly C, Mène-Saffrané L and Lopez-Molina L** (2015) An Endosperm-Associated Cuticle Is Required for Arabidopsis Seed Viability, Dormancy and Early Control of Germination. PLoS Genetics **12**, e1005708.

**Delmas F, Sankaranarayanan S, Deb S, Widdup E, Bournonville C, Bollier N, Northey JG, McCourt P and Samuel MA** (2013) ABI3 controls embryo degreening through Mendel's I locus. Proceedings of the National Academy of Sciences of the USA **40**, E3888-E3894.

**Edwards MM** (1968) Dormancy in seeds of Charlock: III. Occurrence and mode of action of an inhibitor associated with dormancy. Journal of Experimental Botany **19**, 601-610.

**Finkelstein RR, Gampalab SSL and Rock CD** (2002) Abscisic acid signaling in seeds and seedlings. The Plant Cell **14**, S15-S45.

**Finkelstein RR** (1994) Mutations at two new Arabidopsis ABA response loci are similar to the abi3 mutations. Plant Journal **5**, 765-771.

**Footitt S, Douterelo-Soler I, Clay H and Finch-Savage WE** (2011) Dormancy cycling in Arabidopsis seeds is controlled by seasonally distinct hormone-signaling pathways. Proceedings of the National Academy of Sciences of the USA **50**, 20236-20241.

**Giraudat J, Hauge BM, Valon C, Smalle J, Parcy F and Goodman HM** (1992) Isolation of the Arabidopsis ABI3 gene by positional cloning. The Plant Cell **10**, 1251-1261.

**Hutchison KW, Singer PB, McInnis S, Diaz-Sala C and Greenwood MS** (1999) Expansins are conserved in conifers and expressed in hypocotyls in response to exogenous auxin. Plant Physiology **120**, 827-832.

**Kang J, Yim S, Choi H, Kim A, Lee KP, Lopez-Molina L, Martinoia E and Lee Y** (2015) Abscisic acid transporters cooperate to control seed germination. Nature Communications **6**, 1-10.

**Karssen CM and Laçka E** (1985) A revision of the hormone balance theory of seed dormancy: studies on gibberellin and/or abscisic acid-deficient mutants of *Arabidopsis thaliana*, pp. 315-323 in Bopp, M. (Ed.) *Plant growth substances*. Heidelberg, Berlin, Springer.

**Lee S, Cheng H, King KE, Wang W, He Y, Hussain A, Lo J, Harberd NP and Peng J** (2002) Gibberellin regulates Arabidopsis seed germination via RGL2, a GAI/RGA-like gene whose expression is up-regulated following imbibition. Genes & Development **16**, 646-658.

**Lee KP, Piskurewicz U, Turecková V, Strnad M and Lopez-Molina L** (2010) A seed coat bedding assay shows that RGL2-dependent release of abscisic acid by the endosperm controls embryo growth in Arabidopsis dormant seeds. Proceedings of the National Academy of Sciences of the USA **44**, 19108-19113.

**Lee KP, Piskurewicz U, Turečková V, Carat S, Chappuis R, Strnad M, Fankhauser C and Lopez-Molina L** (2012) Spatially and genetically distinct control of seed germination by phytochromes A and B. Genes & Development **17**, 1984-1996.

**Linkies A, Müller K, Morris K, Turecková V, Wenk M, Cadman CS, Corbineau F, Strnad M, Lynn JR, Finch-Savage WE and Leubner-Metzger G** (2009) Ethylene interacts with abscisic acid to regulate endosperm rupture during germination: a comparative approach using Lepidium sativum and *Arabidopsis thaliana*. The Plant Cell **21**, 3803-3822.

**Liu PP, Koizuka N, Homrichhausen TM, Hewitt JR, Martin RC and Nonogaki H** (2005) Large-scale screening of Arabidopsis enhancer-trap lines for seed germination-associated genes. Plant Journal **41**, 936-944.

**Lopez-Molina L, Mongrand S and Chua NH** (2001) A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in Arabidopsis. Proceedings of the National Academy of Sciences of the USA **98**, 4782-4787.

**Lopez-Molina L, Mongrand S, McLachlin DT, Chait BT and Chua NH** (2002) ABI5 acts downstream of ABI3 to execute an ABA dependent growth arrest during germination. The Plant Journal **32**, 317-328.

**Luckwill LC** (1952) Growth-inhibiting and growth-promoting substances in relation to the dormancy and after-ripening of apple seeds. Journal of Horticultural Science **1**, 53-67.

**MacGregor DR, Kendall SL, Florance H, Fedi F, Moore K, Paszkiewicz K, Smirnoff N and Penfield S** (2015) Seed production temperature regulation of primary dormancy occurs through control of seed coat phenylpropanoid metabolism. New Phytologist **205**, 642-652.

**Marowa P, Ding A and Kong Y** (2016) Expansins: roles in plant growth and potential applications in crop improvement. Plant Cell Reports **35**, 949-965.

**McGinnis KM, Thomas SG, Soule JD, Strader LC, Zale JM, Sun TP and Steber CM** (2003) The Arabidopsis SLEEPY1 gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. Plant Cell **15**, 1120-1130.

**Müller K, Tintelnot S and Leubner-Metzger G** (2006) 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 Physiology **47**, 864-877.

**Nambara E, Hayama R, Tsuchiya Y, Nishimura M, Kawaide H, Kamiya Y and Naito S** (2000) The role of ABI3 and FUS3 loci in Arabidopsis thaliana on phase transition from late embryo development to germination. Developmental Biology **220**, 412-423.

**Nambara E, Suzuki M, Abrams S, McCarty DR, Kamiya Y and McCourt P** (2002) A screen for genes that function in abscisic acid signaling in Arabidopsis thaliana. Genetics **161**, 1247-1255.

**Ooms JJJ, Léon-Kloosterziel KM, Bartels D, Koornneef M and Karssen CM** (1993) Acquisition of desiccation tolerance and longevity in seeds of *Arabidopsis thaliana*: a comparative study using abscisic acid-insensitive abi3 mutants. Plant Physiology **102**, 1185-1191

**Parcy F, Valon C, Kohara A, Misera S and Giraudat J** (1997) The ABSCISIC ACID-INSENSITIVE3, FUSCA3, and LEAFY COTYLEDON1 loci act in concert to control multiple aspects of Arabidopsis seed development. The Plant Cell **9**, 1265-1277.

**Penfield S, Rylott ER, Gilday AD, Graham S, Larson TR and Graham IA** (2004) Reserve mobilization in the Arabidopsis endosperm fuels hypocotyl elongation in the dark, is independent of abscisic acid and requires PHOSPHOENOLPYRUVATE CARBOXYKINASE1. The Plant Cell **16**, 2705-2718.

**Penfield S, Gilday AD, Halliday KJ and Graham IA** (2006) (a) DELLA-Mediated Cotyledon Expansion Breaks Coat-Imposed Seed Dormancy. Current Biology **16**, 2366-2370.

**Penfield S, Li Y, Gilday AD, Graham S and Graham IA** (2006) (b) Arabidopsis ABA INSENSITIVE4 regulates lipid mobilization in the embryo and reveals repression of seed germination by the endosperm. The Plant Cell **18**, 1887-1899.

**Piskurewicz U, Jikumaru Y, Kinoshita N, Nambara E, Kamiya Y and Lopez-Molina L** (2008) The gibberellic acid signaling repressor RGL2 inhibits Arabidopsis seed germination by stimulating abscisic acid synthesis and ABI5 activity. The Plant Cell **20**, 2729-2745.

**Piskurewicz U, Turecková V, Lacombe E and Lopez-Molina L** (2009) Far-red light inhibits germination through DELLA-dependent stimulation of ABA synthesis and ABI3 activity. EMBO Journal **28**, 2259-2271.

**Rajjou L, Duval M, Gallardo K, Catusse J, Bally J, Job C and Job D** (2012) Seed germination and vigor. Annual Reviews of Plant Biology **63**, 507-533.

**Sagasser M, Lu GH, Hahlbrock K and Weisshaar B** (2002) A. thaliana TRANSPARENT TESTA 1 is involved in seed coat development and defines the WIP subfamily of plant zinc finger proteins. Genes & Development **16**, 138-149.

**Shcherban TY, Shi J, Durachko DM, Guiltinan MJ, McQueen-Mason SJ, Shieh M and Cosgrove DJ** (1995) Molecular cloning and sequence analysis of expansins–a highly conserved, multigene family of proteins that mediate cell wall extension in plants. Proceedings of the National Academy of Sciences of the USA **92**, 9245-9249.

**Shirley BW, Kubasek WL, Storz G, Bruggemann E, Koornneef M, Ausubel FM and Goodman HM** (1995) Analysis of Arabidopsis mutants deficient in flavonoid biosynthesis. Plant Journal **8**, 659-671.

**Topham AT, Taylor RE, Yan D, Nambara E, Johnston IG and Bassel GW**

(2017) Temperature variability is integrated by a spatially embedded decision-making center to break dormancy in Arabidopsis seeds. Proceedings of the National Academy of Sciences of the USA **114**, 6629-6634.

**Tsuchiya Y, Nambara E, Naito S and McCourt P** (2004) The FUS3 transcription factor functions through the epidermal regulator TTG1 during embryogenesis in Arabidopsis. Plant Journal **37**, 73-81.

**Tyler L, Thomas SG, Hu J, Dill A, Alonso JM, Ecker JR and Sun TP** (2004) Della proteins and gibberellin-regulated seed germination and floral development in Arabidopsis. Plant Physiology **135**, 1008-1019.

**Vaistij FE, Gan Y, Penfield S, Gilday AD, Dave A, He Z, Josse EM, Choi G, Halliday KJ and Graham IA** (2013) Differential control of seed primary dormancy in Arabidopsis ecotypes by the transcription factor SPATULA. Proceedings of the National Academy of Sciences of the USA **110**, 10866-10871.

**Vishwanath SJ, Kosma DK, Pulsifer IP, Scandola S, Pascal S, Joubès J, Dittrich-Domergue F, Lessire R, Rowland O and Domergue F** (2013) Suberin-associated fatty alcohols in Arabidopsis: distributions in roots and contributions to seed coat barrier properties. Plant Physiology **163**, 1118-1132.

**Wyatt JE** (1977) Seed coat and water absorption properties of seed of near-isogenic snap bean lines differing in seed coat color. Journal of the American Society for Horticultural Science **102**, 478-480.

**Yamaguchi S, Kamiya Y and Sun T** (2001) Distinct cell-specific expression patterns of early and late gibberellin biosynthetic genes during Arabidopsis seed germination. Plant Journal **28**, 443-453.

**Yan A, Wu M, Yan L, Hu R, Ali I and Gan Y** (2014) AtEXP2 is involved in seed germination and abiotic stress response in Arabidopsis. PLoS One **9**, e85208.