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Tissue and cellular mechanics of seeds

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Distinct plant seed/fruit structures evolved to support reproduction and dispersal in distinct environments. Appropriate biomechanical properties and interactions of the various seed compartments are indispensable to plant survival. Most seeds are dispersed in a dry state generated during seed development/maturation for which novel aspects of endosperm–embryo interaction were discovered. The various layers covering the embryo of a mature seed define the patterns of water uptake during germination. Their biomechanical weakening together with embryo cell expansion is mediated by cell wall remodelling to facilitate radicle protrusion. Recent work with different species has revealed mechanisms underpinning specific embryo growth zones. Abiotic and biotic factors were shown to release different types of seed and fruit coat-mediated constraints to water uptake and germination.

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Current Opinion in Genetics & Development 2018, **51**:1–10

This review comes from a themed issue on **Developmental mechanisms, patterning and evolution**

Edited by **Lance A Davidson and Gwyneth C Ingram**

https://doi.org/10.1016/j.gde.2018.03.001

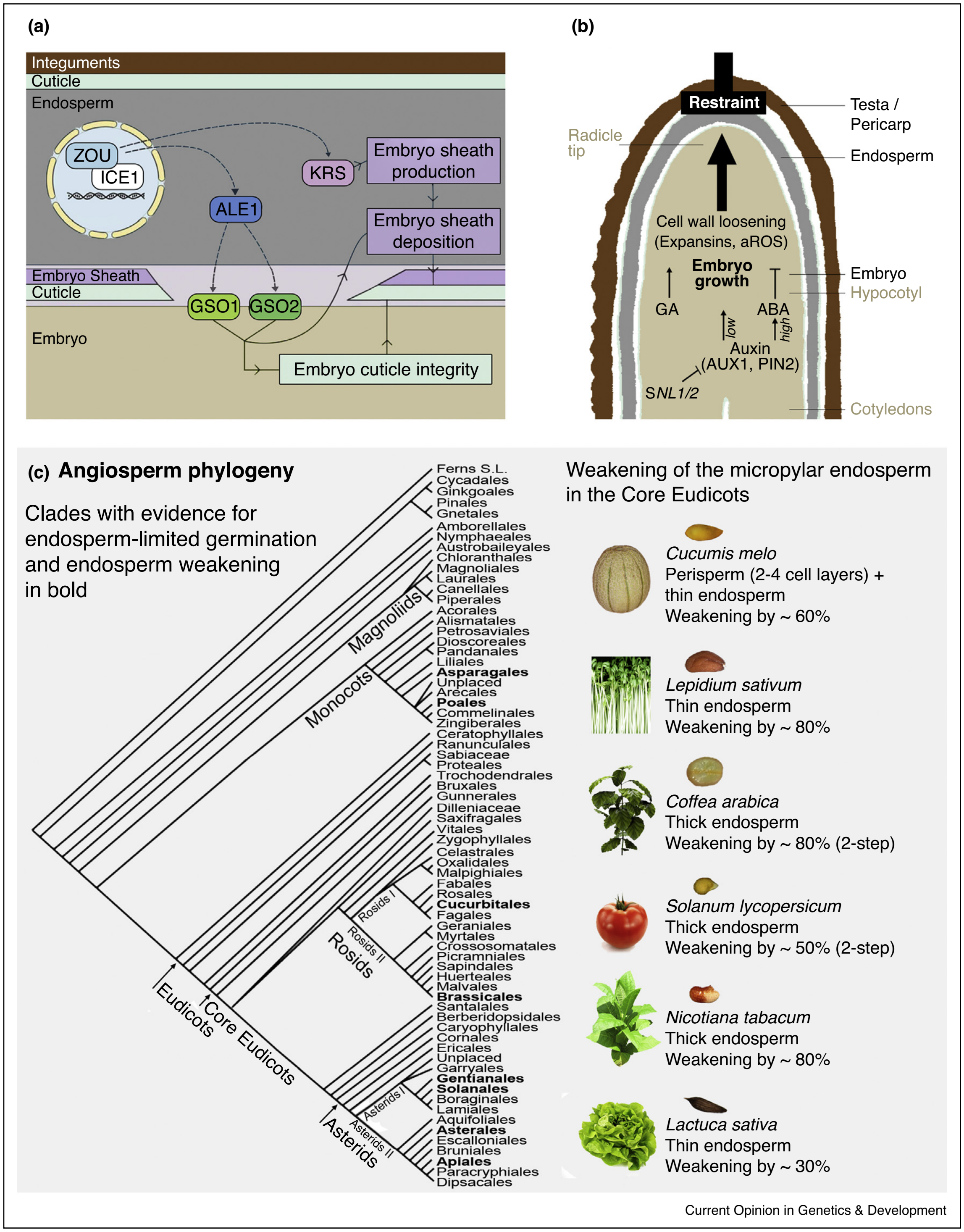
Introduction

Seed germination is a complex process and we need to understand the underlying molecular, hormonal and mechanical aspects. At maturity, a typical angiosperm diaspore (seed/fruit) consists of an embryo and its 'coats', which might include living endosperm, dead testa (seed coat derived from the outer integument) and pericarp (fruit coat). Mechanical interplay between different seed compartments is a crucial factor from seed development to the completion of germination. New insights into the mechanical interactions between embryo and endosperm during seed development have revealed a key role of the transcription factor ZHOUPI [1••]. The endosperm, seed and fruit coats are a major mechanical constraint to embryo expansion. Germination is triggered by the uptake of water into the 'dry' seed and new techniques made it possible to track the water entering a seed with a holistic *in vivo* approach [2•]. Plant cell wall loosening and weakening of the various 'coats' mediate embryo growth and radicle protrusion. These processes are under the control of hormones including gibberellins (GA), abscisic acid (ABA), ethylene, jasmonates, nitric oxide and auxin [3••,4,5,5a]. Advanced imaging enables 3D monitoring of embryo growth *in planta* to reveal distinct spatiotemporal patterns on the cellular level and the influence of plant hormones [6••]. Fruit coats can confer dormancy by hindering water-uptake or by acting directly as a mechanical constraint [7••]. Seed biology is a major research topic of importance for food security and climate change [5,8,9]. Plant biomechanics can be defined as the study of the structure and function of plant systems (such as seeds) by the application of concepts and methods from mechanics; for terms and definitions see [9a]. Integrating molecular, epigenetic, morphological and biophysical aspects are key to advance our understanding of the complex process of seed germination.

The mechanics of embryo and endosperm interaction during seed development

All events during seed development and seed germination are finely tuned and coordinated. A seed is formed from a fertilised ovule and consists in the mature stage of seed coat, embryo and a food storage compartment. Food storage can either be in the cotyledons (embryonic leaves, e.g. *Pisum sativum*) or the endosperm, the nutritive tissue of most angiosperms [5]. The triploid endosperm arises from the fusion of a paternal sperm nucleus with two maternal polar nuclei, while the second paternal sperm nucleus fuses with the egg cell nucleus forming the embryo (double fertilisation) [10,11]. Endosperm development differs among species but in principal it can be divided in formation, cellularisation, differentiation, maturation and cell death. Endosperm formation and expansion is the major stimulus driving the early seed growth, which is restricted by the outer seed coat. It has been shown that seed turgor, leading to seed expansion is generated by the endosperm [12]. Auxin is necessary for normal endosperm and seed coat development [13]. During *Arabidopsis thaliana* seed development the endosperm undergoes programmed cell death to make space for the growing embryo [1••,11]. This endosperm breakdown is mediated by the transcription factors ZHOUPI and ICE1 (Figure 1a) [1••,14]. ZHOUPI triggers cell death by regulating cell wall remodelling proteins (CWRP), altering the mechanical properties of the endosperm and thus allowing the embryo to compress the surrounding endospermic tissue. *ZHOUPI* is exclusively expressed in the endosperm and is also involved in the

Figure 1



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The mechanics of embryo and endosperm interaction during seed development and germination. **(a)** A model for ZOU-dependent sheath formation during early seed development, after [16•] ©ASPB. ZOU regulates expression of ALE1 and KRS. ALE1 and the GSO1/GSO2 kinases ensure normal embryo cuticle formation. KRS is necessary for the production of an embryo sheath that surrounds the embryo. The mechanical and molecular interplay between endosperm and embryo is essential for normal seed development. **(b)** Schematic of the interplay between the growing embryonic axis and the surrounding seed 'coats' (endosperm and where applicable testa and/or pericarp) in a mature seed during germination. Gibberellins (GAs) promote cell wall loosening, thereby facilitating endosperm weakening. Abscisic acid (ABA) inhibits this process. Auxin influences the germination in a dose-dependent matter. Low concentrations promote germination and AUX1 is a key factor downstream of SNL1 and SNL2 [3]. High concentrations inhibit germination via an ABA–auxin interaction [46]. Elongation of the embryonic axis requires auxin formation of an embryonic cuticle [15]. An embryonic sheath and cuticle (Figure 1a) which form on the surface of the embryo as it starts to grow are required for normal seed growth. This depends on the ZHOUPI-mediated expression of the peptide KERBEROS [16•]. ZHOUPI is therefore required for both normal formation of the embryo sheath and for endosperm–embryo separation. Seeds of the *zou-4* mutant are up to 3× stiffer than wild-type, probably due to increased turgor. It has been proposed that the mechanical stress from the expanding embryo and endosperm is perceived by a mechanosensitive cell layer in the seed coat [17]. The mature *Arabidopsis* seed has a single cell endosperm layer which plays a vital role in the control of germination [10,18,19]. An endosperm-associated cuticle is required for *Arabidopsis* seed germination [20], and phenolic compounds during seed production affects seed coat biophysical properties [9]. The amount of endosperm in mature seeds varies enormously between species. Many species, for example, tobacco (*Nicotiana tabacum*), have abundant endosperm though the evolutionary trend is towards endospermless seeds (e.g. legumes) [5].

The mechanics of endosperm weakening across angiosperm phylogeny

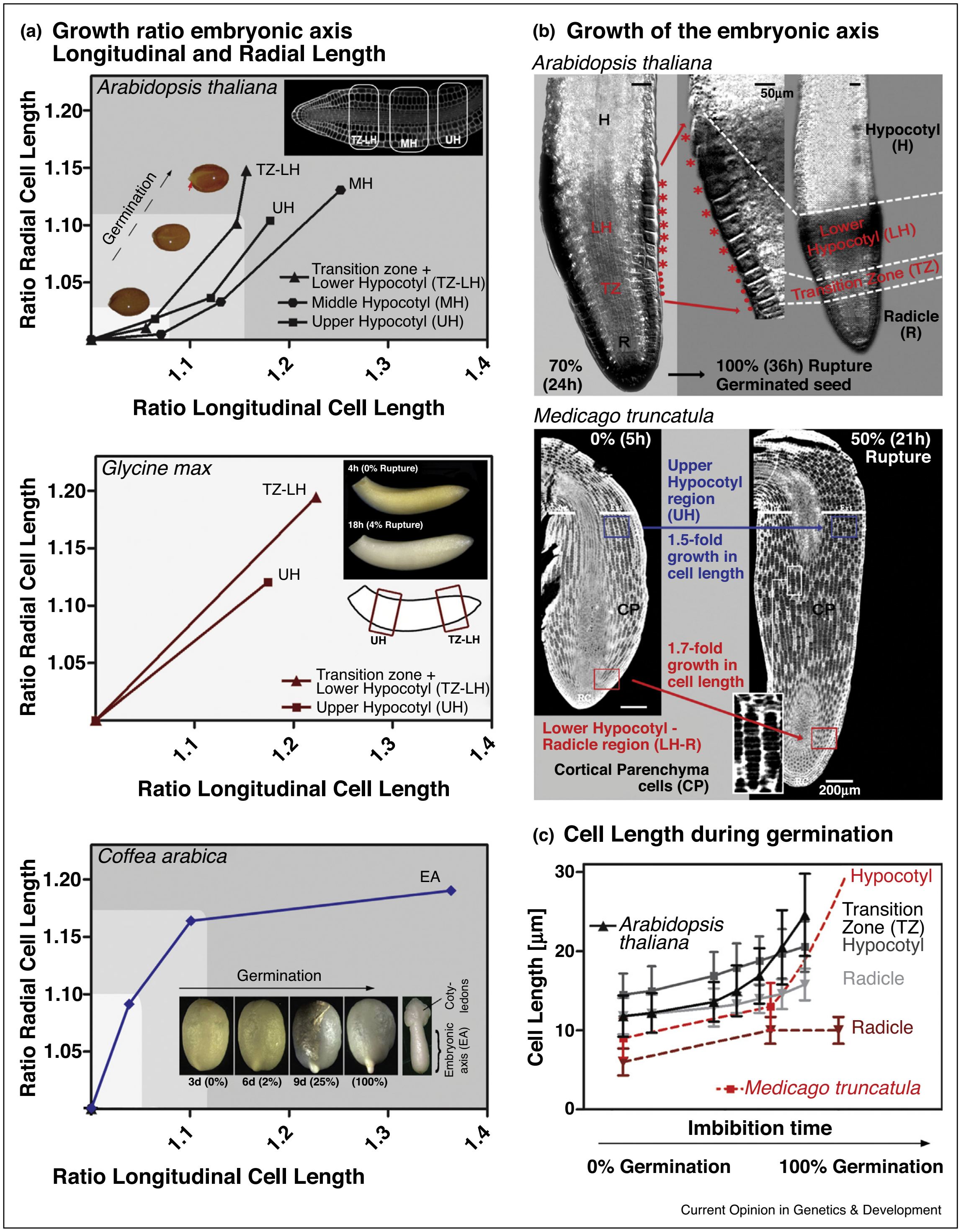
In the mature seed, the mechanical balance between endosperm and embryo remains pivotal for the control of seed germination [4,10]. There is evidence for 'endosperm-limited' germination across the angiosperm phylogenetic tree (Figure 1c). The endosperm encasing the embryo acts as a mechanical barrier for radicle (embryonic root) protrusion. The tissue resistance of the endosperm decreases before completion of germination (endosperm weakening). Established model systems are mainly within the Asteraceae and Brassicaceae. Some species, for example, coffee and tomato, show a two-step weakening process; the first phase ABA-independent and the second phase inhibited by ABA. Germination and endosperm weakening is under hormonal control (Figure 1b) and involve CWRPs. β-Endo-mannanases are active in the endosperm in several species, including tomato, tobacco and *Arabidopsis* [21,22]. Changes in CWRPs seem to be directly linked to the germination process. In tobacco endosperm weakening is associated with a degradation in heteromannan [23]. The *Arabidopsis* endosperm produces ABA which serves as a signal to inhibit embryo growth and endosperm weakening [8,23a]. An early signal from the embryo is required to induce endosperm weakening, and this signal can be replaced by GA [5,8,23a]. Consistent with the importance of GA in endosperm weakening, the embryos of GA-deficient *Arabidopsis* and tomato grow if the endosperm and testa covering the radicle is removed (without a requirement for GA addition). Further to this, puncture-force measurements of GA-deficient tomato seeds demonstrate that the endosperm weakening is blocked. The mechanical resistance of the *Arabidopsis* endosperm has not been measured, but the published evidence (summarised in [4,8,9,23a]) led to the conclusion that in *Arabidopsis* the endosperm and testa both act as a physical barriers to embryo growth and germination, and that in dormant seeds the endosperm weakening is blocked. In agreement with this, puncture-force measurements with *Lepidium sativum* seeds overexpressing the dormancy gene *DOG1* (*DELAY OF GERMINATION1*) demonstrated that their endosperm weakening is indeed blocked, while the embryo growth is not affected [24]. Hormone measurements demonstrated that GA levels were elevated and ABA levels not altered in these seeds, excluding that the GA-ABA ratios caused the DOG1-mediated block to endosperm weakening. We conclude from these findings that for future research a wider range of species with dormant and non-dormant seeds, their specific endosperm cell wall structures during germination and the link to environmental cues are of major interest.

Water uptake, cell walls and the mechanical properties of growing cells

Seed germination starts with the uptake of water and ends with the emergence of the radicle through the surrounding tissues [5,19,25]. Uptake is triphasic, with phase I (imbibition) being characterised by rapid water uptake and cell swelling from their dry state. This is followed by a plateau phase II (metabolic activation) and a further increase in water uptake (phase III) associated with radicle protrusion (completion of germination). Non-destructive imaging showed that water uptake often starts near the micropyle, a canal in the seed coat through which the pollen tube passed during fertilisation [4]. A recent study combined MRI and other imaging tools [2•] to provide a holistic *in vivo* approach to visualise water uptake. They propose that an endospermic lipid gap in oilseed rape seeds channels water towards the radicle. The tissue architecture of seeds seems to guide the water and predetermines germination events. Unravelling how water uptake is controlled and linking other aspects of

(Figure 1 Legend Continued) transport via AUX1 and PIN2. **(c)** Endosperm-controlled seed germination across the angiosperm phylogeny. Clades with experimental evidence for endosperm-limited germination and endosperm weakening are in bold. Examples including the approx. decrease in tissue resistance (measured by puncture-force experiments) from the core Eudicots are given. Some species exhibit a two-phase weakening, where the first phase is ABA-independent and the second phase is ABA-dependent. For references see [4,5]. Angiosperm seed evolution depicted in a phylogenetic tree after Stevens, P. F. Angiosperm Phylogeny Website; Version 14, 2017. *Abbreviations:* ZOU, transcription factor ZHOUPI [14]; ICE1, transcription factor INDUCER OF C-REPEAT BINDING FACTOR EXPRESSION 1 [14]; ALE1, subtilisin-like serine protease ABNORMAL LEAF-SHAPE 1 [16•]; GSO1/GSO2, receptor-like kinases GASSHO1/2 [16•]; KRS, peptide KERBEROS [16•]; AUX1, carrier protein AUXIN RESISTANT 1 [3]; SNL1/2, histone deacetylase-binding factors SIN3-LIKE 1/2 [3]; PIN2, auxin efflux carrier PIN-FORMED 2 [3].

Figure 2



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Mechanisms and patterns of embryo cell growth during germination. **(a)** Relative changes in the dimensions of the cells of the embryonic axis during the germination of *Arabidopsis thaliana* (after [6••]), soybean (*Glycine max*, after [38•]) and coffee (*Coffea arabica*, after [32]). For *Arabidopsis*, ratios between longitudinal and radial length are given for the outer cortex at 16 hours (before test rupture), 25 hours (testa rupture) and 32 hours (just germinated) compared to 3 hours imbibition (unexpanded embryo). For *Glycine max*, ratios are given for the outer cortex cells (cell layers 3 and 5) at 18 hours (4% germination) compared to 4 hours imbibition (unexpanded embryo). For coffee the embryonic axis was divided into ten equal parts and cell growth occurred evenly in these ten regions. Cell size was measured in the outer cortex (cell layer 4). Ratios are given at 6 days (2% germination), 9 days (25% germination) and a germinated seed compared to 3 days (unexpanded embryo). An isolated embryo (6 days) depicting the cotyledons and the embryonic axis. **(b)** Cell growth of the embryonic axis of *Arabidopsis* (after [39]) and *Medicago* seed germination (dormancy, viability) will remain a major topic for future research.

The plant cell wall is a rigid multi-layered structure that gives the cell its shape and form. It has to be strong enough to withstand the forces generated by the internal turgor pressure but must also allow cell growth [4,26–28]. Turgor pressure exerts a homogenous, multidirectional force onto the cell wall. Cell expansion involves selective wall loosening that leads to cell wall creep [26]. The direction(s) and extent of this irreversible cell growth depend on local wall structure. Cell expansion is a mechanical process facilitated by cell wall loosening, stress relaxation and wall extension driven by water uptake. Key players involved are CWRPs including cellulases, pectin- and xyloglucan-modifying enzymes, as well as apoplastic (i.e. in the cell-wall of the plant cell, not in the protoplast) reactive oxygen species (aROS) [4,21,26–29]. As the load-bearing polymers in the cell wall loosen the internal turgor pressure decreases. This drives further water uptake into the cell until the turgor pressure and the restraint from the surrounding cell wall is balanced again. A current model of the plant cell wall predicts 'biomechanical hotspots' (site of close contact between the cellulose microfibrils) that are responsible for wall extensibility [27]. Recently nano-scale movements of microfibrils have been directly observed via atomic force microscopy [30].

The mechanical mechanisms and patterns of embryo cell growth during germination

Typically, a mature embryo consists of the shoot apical meristem, cotyledons (embryonic leaves), hypocotyl (stem-like embryonic axis below the cotyledons) and the radicle (embryonic root). To complete germination 'coat' weakening and cell expansion/elongation in the embryonic axis are required [4,31–33]. Cell division is neither required nor evident during the germination of Brassicaceae, Fabaceae and species from other families [19,31,34]. Cell expansion occurs in specific growth zones and is associated with endoreduplication [35,36]. Recent work employed advanced 3D imaging to reveal the spatiotemporal patterns of the embryo cell growth Brassicaceae and Fabaceae seeds [6••,37,38•]. Defined regions, namely hypocotyl, transition zone (TZ) and radicle were identified in the growing *Arabidopsis* embryo [31]. Figure 2 shows that cell expansion during the early phase of *Arabidopsis* seed germination is not primarily localised to the radicle tip itself, but to the hypocotyl-radicle TZ and the lower-hypocotyl (LH). This expansion provides radial and longitudinal growth leading to testa rupture [6••,31,37]. A gradient of GA-mediated gene expression emanating from the radicle combined with 3D cellular geometries define this first growth module in the TZ-LH. During late *Arabidopsis* seed germination mainly longitudinal cell expansion mediated by the HB5 transcription factor in the middle region of the hypocotyl provides embryo elongation growth (Figure 2). This second growth module in the mid/upper-hypocotyl drives the embryo elongation required for the completion of *Arabidopsis* germination by radicle protrusion [6••]. This second growth module is associated with ABA-signalling via the HRS1 transcription factor expressed in the LH [39]. Several publications use the term TZ for the region where the radicle and the LH are joined together, but it must be used with care as TZ is not clearly defined. Only some publications [31,39] clearly assigned specific cells in the *Arabidopsis* embryo. Further to this, when the term is used in publications with other species (e.g. [38•]), the comparison may have additional limitations.

In agreement with the spatiotemporal pattern found for *Arabidopsis*, ABA-sensitive coffee embryo cell expansion during early germination is in the radial and longitudinal direction, but is mainly longitudinal during late germination (Figure 2a) [32]. Radial and longitudinal expansion of cells in legume (*Glycine max* and *Medicago truncatula*) embryonic axes during early germination is in both, the TZ-LH and the upper-hypocotyl (UH) region (Figure 2a) and is inhibited by ABA [34,38•]. It therefore seems that key aspects of the TZ-LH cell expansion module are evolutionarily conserved. However, in contrast to *Arabidopsis*, in the legume embryonic axes the early cell expansion in the LH, UH and maybe within the entire hypocotyl seems of roughly equal importance (Figure 2). In contrast to the two-step germination of *Arabidopsis*

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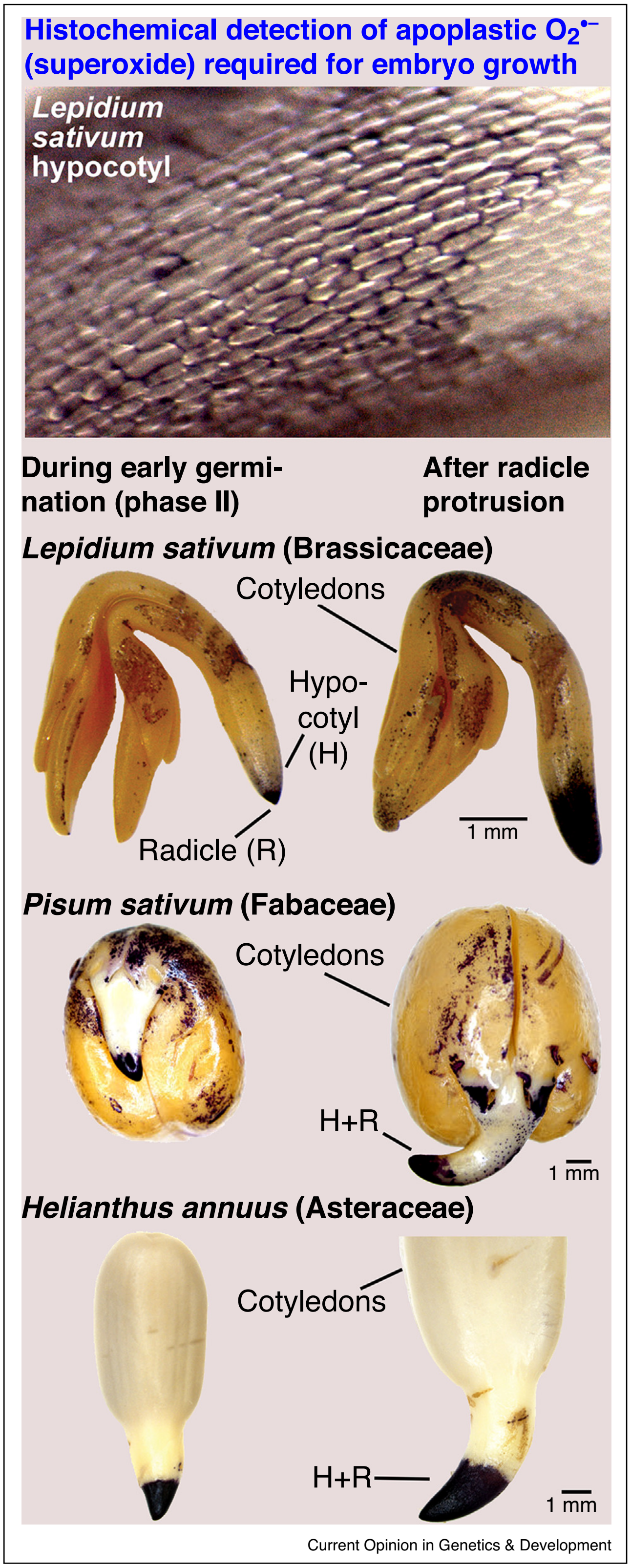
truncatula (after [34]). The *Arabidopsis* TZ-LH is labelled with either dots or asterisks respectively with comparisons shown at 24 hours imbibition (70% germination) and 36 hours (100% germination). Scale bars = 50 μm. For *Medicago* the embryonic axis at 5 hours (0% germination) and a just germinated sample at 21 hours (50% germination) is shown. Cell length measured in the cortical parenchyma. **(c)** Cell Length in longitudinal direction during germination of *Arabidopsis* (extracted from [31]) and *Medicago truncatula* (after [34]). For *Arabidopsis* the length was measured at 22 hours, 27 hours, 38 hours (beginning of testa rupture), 42 hours, 46 hours (beginning of endosperm rupture), 50 hours and 54 hours of imbibition. For *Medicago* cell length was measured at 5 hours (0% germination) and 21 hours (50% germination) in the cortical parenchyma.
Details: (a) *Arabidopsis* [6••]: ©American Society of Plant Biologists). Transition zone + lower hypocotyl (TZ-LH) equals cell position 9–13, middle hypocotyl (MH) cell position 16-23 and upper hypocotyl (UH) cell position 26–30. *Glycine max* [38•] ©2017 Oxford University Press. Initial cell sizes (*l*/*r*) were 27.0/13.2 μm (TZ-LH) and 28.9/14.6 μm (H). *Coffea arabica* [32], Da Silva *et al.* 'ABA inhibits embryo cell expansion and early cell division events during coffee (*Coffea arabica* 'Rubi') seed germination', Annals of Botany, 2008, 102, 3, 428-429, by permission of Oxford University Press. Initial cell sizes (*l*/*r*) were 29.1/23.3 μm. (b) *Arabidopsis* [39] ©*PLOS ONE*. *Medicago truncatula* [34] reprinted from 'ABA-mediated inhibition of germination is related to the inhibition of genes encoding cell-wall biosynthetic and architecture: Modifying enzymes and structural proteins in *Medicago truncatula* embryo axis', 2/1, Gimeno-Gilles *et al.*, 108–119, ©2009, with permission from Elsevier. (c) *Arabidopsis* (extracted from [31]). The radicle equals cell position 1–8, the TZ cell position 9–13 and the hypocotyl cell position 14–37 along the embryonic axis. *Medicago truncatula* (after [34], see above).

Figure 3



The accumulation of apoplastic superoxide ($O_2^{\bullet-}$) in the embryonic axes during and after the completion of seed germination is localised to the lower hypocotyl-radicle growth zone. Histochemical superoxide detection in *Lepidium sativum* micrographs (from [35], Oracz *et al.*, 'Myrigalone A inhibits *Lepidium sativum* seed germination by interference with gibberellin metabolism and apoplastic superoxide production required for embryo extension growth and endosperm rupture', Plant and Cell Physiology, 2012, 53, 1, 81–95, by permission of Oxford University Press), embryos (from [33], www.plantphysiol.org, ©ASPB), and in pea and sunflower embryos (from [36], Rewers & Sliwinska, 'Endoreduplication in the germinating embryo and young seedling is related to the type of seedling establishment but is not coupled with superoxide radical accumulation', J Exp Bot, 2014, 65, 15, 4385–96, by permission of Oxford University Press).

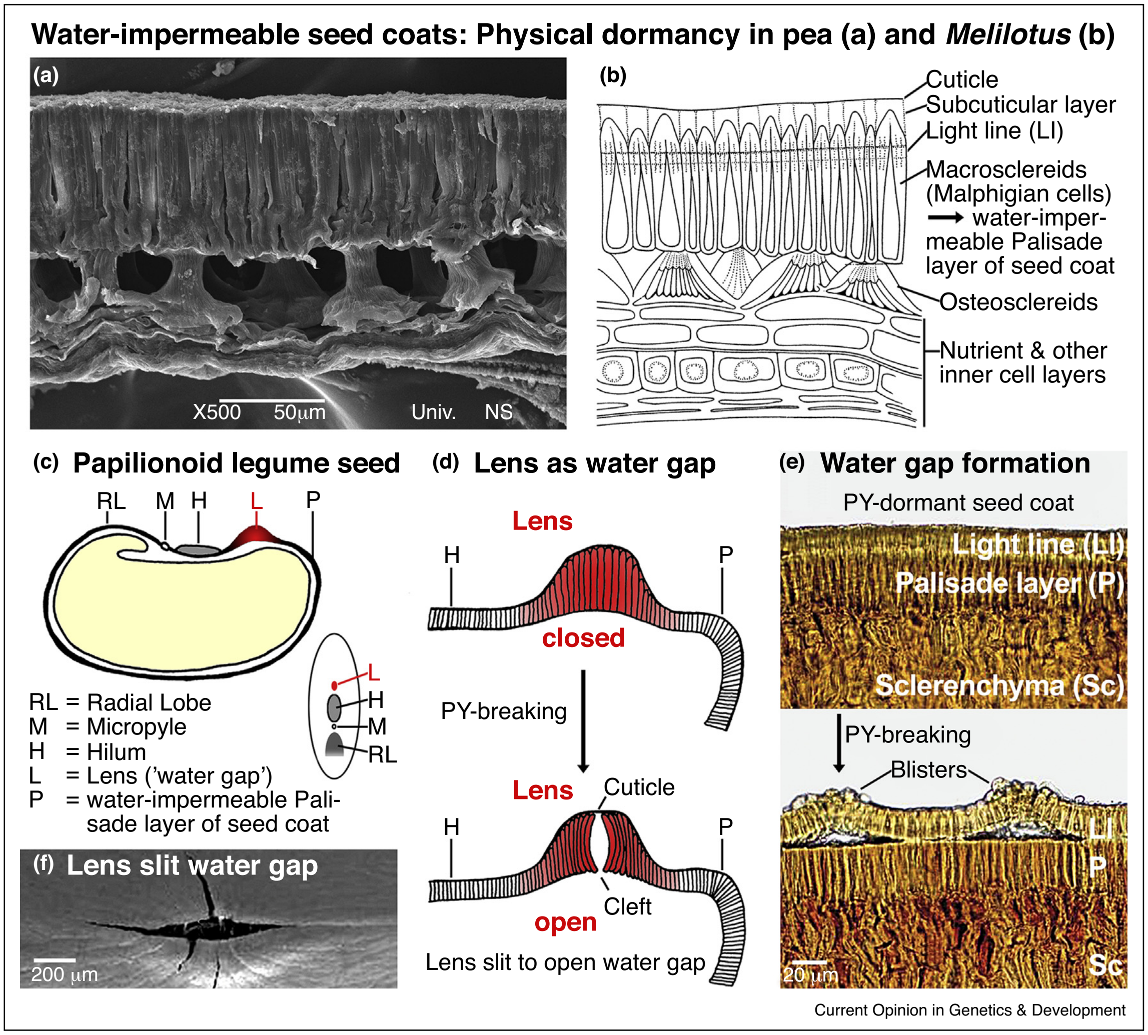
seeds, for these non-endospermic legume seeds only testa rupture is necessary to permit the completion of germination by radicle protrusion [5]. Late and post-germination cell expansion of *M. truncatula* and coffee is almost exclusively in the longitudinal direction in either the entire hypocotyl or in *M. truncatula* exclusively the UH [32,34].

Consistent with a role in embryo elongation during germination, aROS is produced in cell walls of growing embryos (Figure 3) [33,35,36]. This aROS production is hormonally controlled, it intensifies during germination and is associated with the growth module in the TZ-LH (Figure 3). However, histological aROS staining appears to be absent from the mid/upper-hypocotyl (Figure 3). This does not exclude aROS-mediated expansion by hydroxyl radicals [28,29,33], but suggest distinct mechanisms for the second (mainly longitudinal) growth module. These may include GA-regulated and ABA-regulated expansin gene expression during late germination in association with the ABA-sensitive longitudinal cell expansion [6••,19,34,40,41]. Xyloglucan remodelling by alpha-xylosidase alters *Arabidopsis* cell wall properties during germination, and the mechanical changes were proposed to influence the GA/ABA metabolism [42•,43•]. Other hormones and histone modifications also control embryo elongation and endosperm weakening [44,45]. Altered histone acetylation, auxin biosynthesis and expression of auxin transporters caused altered *Arabidopsis* radicle growth and seed germination (Figure 1b) [3••,46].

The role of seed/fruit coats as biophysical constraints to germination

Physical dormancy (PY) is caused by water-impermeable cell layers in the seed coat (testa) or fruit coat (pericarp) which along with sealed chalaza and micropyle openings block water uptake completely [4,47,48]. An overview of function and structure of the seed and fruit coat can be found in [48a]. The water-impermeability of many legume seed coats is due to palisade layer(s) of lignified macrosclerids (Figure 4) impregnated with water-repellent phenolic and suberin-like substances [47–51]. Genes controlling PY, seed coat palisade layer and cuticle development have been identified in soybean [52••] and *M. truncatula* [53••]. The breaking of PY involves opening of

Figure 4



Water-impermeable seed coats and water gap formation as hallmarks of physical dormancy (PY). The PY of pea **(a)**, *Melilotus alba* **(b)**, and other legume seeds is caused by water-impermeable palisade layers in the seed coat. Structures in the scanning electron micrograph of the pea seed coat section (from [49], ©2017 by the Serbian Biological Society) are as in the drawing of the *M. alba* seed coat (modified from Hamly 1932, www.seedbiology.eu). **(c)** Structures of a typical Papilionoid legume seed including the lens (from [50], ©John Wiley & Sons, Baskin 2003, 'Breaking physical dormancy in seeds — focussing on the lens', New Phytologist). **(d)** In dormant seeds with the lens is closed. The breaking of PY by defined temperature-moisture conditions involving lens (water gap) opening (from [50], see above). **(e)** Water gap opening by defined temperature-moisture conditions is caused by mechanical mechanisms which involve blister formation of seed coat layers of *Koelreuteria paniculata* (from [48], Gama-Arachchige *et al.*, 'Identification and characterization of ten new water gaps in seeds and fruits with physical dormancy and classification of water-gap complexes', Annals of Botany, 2013, 112, 1, 69-84, by permission of Oxford University Press). **(f)** Different types of water gap complexes are described and species-specific opening mechanisms include a lens slit as water gap of *Cladrastis kentukea* (from [48], see above).

water-gap structures which act as environmental signal detectors [47,48,50], for example, the lens in legume seeds (Figure 4). Species-specific temperature-dependent and moisture-dependent environmental cues trigger physical mechanisms to irreversibly open the water gap (Figure 4).

Mechanical dormancy imposed by water-permeable hard seed/fruit coats employs a different mechanism to prevent germination [7••,54,55]. The hard coats allow water uptake by the embryo to a certain extent, but impose a mechanical constraint to the full water uptake required for the completion of germination. The examples are fruit valves of *Lepidium didymum* whose distinct fruit fracture biomechanics are proposed to have contributed to its global distribution [7••]. Its pericarp-imposed mechanical dormancy is released by fruit-associated common fungi, which weaken predetermined breaking zones by degrading the non-lignified pericarp layers. Likewise in palm seeds, removing parts of the fruit coat releases dormancy, but their mechanical properties did not differ between dormant and non-dormant varieties [56]. In *Grevillea* *juniperina* micropylar parts of the seed coat facilitate dormancy while this specific region in the seed coat seems to be softer than other regions [57]. Papaya seeds provide another example of a biomechanical restraint to germination conferred by the seed coat [58•].

Conclusions

Recent 3D imaging and biomechanical studies emphasize the importance of mechanics in advancing our understanding of seed compartment interactions during seed development and germination [1••,2•,4,6••,7••]. Recent publications also highlight two other aspects which bring a more holistic understanding of seeds. First, the integration of mechanical and molecular findings is achievable for example, [12,17,24,35,42•], which require quantitative spatiotemporal analyses which cover the entire developmental process. Second, conclusions from work across the plant phylogeny with different species and distinct seed types provide insight into the evolution of the seed trait [16•,23,33,38•,58•]. These findings reveal that even generally important processes such as embryonic axis growth, involve conserved as well as species-specific mechanisms, and that the diversity of mechanical mechanisms is best studied across a wide range of species. Beyond interactions between seed compartments, interactions with the abiotic and biotic environment were uncovered. The evolved mechanical mechanisms for seed adaptation are therefore complex and future work should aim to further unveil how seeds conquered the world.

Data Availability Statement

All data presented or analysed are included in this published article or are available from the resources cited as described in detail in the figure legends.

Conflict of interest statement

Nothing declared.

Acknowledgements

A sincere thank you to James E Hourston for his excellent proofreading of the manuscript. This work was supported by the Biotechnology and Biological Sciences Research Council (BBSRC) to G.L.-M. and T.S. [grant number BB/M000583/1].

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. •• Fourquin C, Beauzamy L, Chamot S, Creff A, Goodrich J, Boudaoud A, Ingram G: **Mechanical stress mediated by both endosperm softening and embryo growth underlies endosperm elimination in *Arabidopsis* seeds**. *Development* 2016, **143**:3300-3305.

This publication is an essential breakthrough in the understanding of the embryo–endosperm interaction during seed development.

2. • Munz E, Rolletschek H, Oeltze-Jafra S, Fuchs J, Guendel A, Neuberger T, Ortleb S, Jakob PM, Borisjuk L: **A functional imaging study of germinating oilseed rape seed**. *New Phytol* 2017, **216**:1181-1190.

This publication reports an important advance in visualising the key structures in the seed which facilitate water uptake by using magnetic resonance imaging (MRI).

3. •• Wang Z, Chen F, Li X, Cao H, Ding M, Zhang C, Zuo J, Xu C, Xu J, Deng X *et al.*: ***Arabidopsis* seed germination speed is controlled by SNL histone deacetylase-binding factor-mediated regulation of AUX1**. *Nat Commun* 2016, **7**:13412.

This publication provides for the first time a integrated model that links histone deacetylation to seed dormancy and germination.

4. Steinbrecher T, Leubner-Metzger G: **The biomechanics of seed germination**. *J Exp Bot* 2017, **68**:765-783.

5. Finch-Savage WE, Leubner-Metzger G: **Seed dormancy and the control of germination**. *New Phytol* 2006, **171**:501-523.

5(a). Corbineau F, Xia Q, Bailly C, El-Maarouf-Bouteau H: **Ethylene, a key factor in the regulation of seed dormancy**. *Front Plant Sci* 2014, **5**:539.

6. •• Stamm P, Topham AT, Mukhtar NK, Jackson M, Tome D, Beynon JL, Bassel GW: **The transcription factor ATHB5 affects GA-mediated plasticity in hypocotyl cell growth during seed germination**. *Plant Physiol* 2017, **173**:907-917.

This publication is a very important read and highlights the spatiotemporal cell expansion mechanisms and its plasticity in *Arabidopsis* using a state-of-the art 3D imaging technique.

7. •• Sperber K, Steinbrecher T, Graeber K, Scherer G, Clausing S, Wiegand N, Hourston JE, Kurre R, Leubner-Metzger G, Mummenhoff K: **Fruit fracture biomechanics and the release of *Lepidium didymum* pericarp-imposed mechanical dormancy by fungi**. *Nat Commun* 2017, **8**:1868.

This publication highlights the mechanical interplay between fruit coat and embryo. A solely mechanical dormancy and its underlying functional morphology is described for the first time.

8. Nonogaki H: **Seed biology updates — highlights and new discoveries in seed dormancy and germination research**. *Front Plant Sci* 2017, **8**:524.

9. Penfield S, MacGregor DR: **Effects of environmental variation during seed production on seed dormancy and germination**. *J Exp Bot* 2017, **68**:819-825.

9(a). Moulia B: **Plant biomechanics and mechanobiology are convergent paths to flourishing interdisciplinary research**. *J Exp Bot* 2013, **64**:4617-4633.

10. Yan D, Duermeyer L, Leoveanu C, Nambara E: **The functions of the endosperm during seed germination**. *Plant Cell Physiol* 2014, **55**:1521-1533.

11. Ingram GC: **Dying to live: cell elimination as a developmental strategy in angiosperm seeds**. *J Exp Bot* 2017, **68**:785-796.

12. Beauzamy L, Fourquin C, Dubrulle N, Boursiac Y, Boudaoud A, Ingram G: **Endosperm turgor pressure decreases during early *Arabidopsis* seed development**. *Development* 2016, **143**:3295-3299.

13. Figueiredo DD, Batista RA, Roszak PJ, Hennig L, Köhler C: **Auxin production in the endosperm drives seed coat development in *Arabidopsis***. *Elife* 2016, **5**:20542.

14. Denay G, Creff A, Moussu S, Wagnon P, Thévenin J, Gérentes M-F, Chambrier P, Dubreucq B, Ingram G: **Endosperm breakdown in *Arabidopsis* requires heterodimers of the basic helix–loop–helix proteins ZHOUPI and INDUCER OF CBP EXPRESSION 1**. *Development* 2014, **141**:1222-1227.

15. Xing Q, Creff A, Waters A, Tanaka H, Goodrich J, Ingram GC: **ZHOUPI controls embryonic cuticle formation via a signalling pathway involving the subtilisin protease ABNORMAL LEAF-SHAPE1 and the receptor kinases GASSHO1 and GASSHO2**. *Development* 2013, **140**:770-779.

16. • Moussu S, Doll NM, Chamot S, Brocard L, Creff A, Fourquin C, Widiez T, Nimchuk ZL, Ingram G: **ZHOUPI and KERBEROS mediate embryo/endosperm separation by promoting the formation of an extracuticular sheath at the embryo surface**. *Plant Cell* 2017, **29**:1642-1656.

This publication makes a major contribution to our understanding of early embryo growth during early seed development and the interactions and signalling between seed compartments.

17. Creff A, Brocard L, Ingram G: **A mechanically sensitive cell layer regulates the physical properties of the *Arabidopsis* seed coat**. *Nat Commun* 2015, **6**:7382.

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

19. Weitbrecht K, Müller K, Leubner-Metzger G: **First off the mark: early seed germination**. *J Exp Bot* 2011, **62**:3289-3309.

20. De Giorgi J, Piskurewicz U, Loubery S, Utz-Pugin A, Bailly C, Mène-Saffrané L, Lopez-Molina L: **An endosperm-associated cuticle is required for *Arabidopsis* seed viability, dormancy and early control of germination**. *PLoS Genet* 2015, **11**: e1005708.

21. Gómez-Maqueo X, Gamboa-deBuen A: **The dynamics of plant cell wall in muro modifications and its physiological implications on seed germination**. In *New Challenges in Seed Biology — Basic and Translational Research Driving Seed Technology*. Edited by Araújo S, Balestrazzi A:. InTech; 2016 http://dx.doi.org/10.5772/64085.

22. Rodríguez-Gacio MdC, Iglesias-Fernández R, Carbonero P, Matilla ÁJ: **Softening-up mannan-rich cell walls**. *J Exp Bot* 2012, **63**:3976-3988.

23. Lee KJD, Dekkers BJW, Steinbrecher T, Walsh CT, Bacic A, Bentsink L, Leubner-Metzger G, Knox JP: **Distinct cell wall architectures in seed endosperms in representatives of the Brassicaceae and Solanaceae**. *Plant Physiol* 2012, **160**:1551-1566.

23(a). Chahtane H, Kim W, Lopez-Molina L: **Primary seed dormancy: a temporally multilayered riddle waiting to be unlocked**. *J Exp Bot* 2017, **68**:857-869.

24. Graeber K, Linkies A, Steinbrecher T, Mummenhoff K, Tarkowská D, Turečková V, Ignatz M, Sperber K, Voegele A, de Jong H *et al.*: **DELAY OF GERMINATION 1 mediates a conserved coat-dormancy mechanism for the temperature- and gibberellin-dependent control of seed germination**. *Proc Natl Acad Sci U S A* 2014, **111**:E3571-E3580.

25. Nonogaki H: **Seed dormancy and germination — emerging mechanisms and new hypotheses**. *Front Plant Sci* 2014, **5**:233.

26. Cosgrove DJ: **Catalysts of plant cell wall loosening**. *F1000Research* 2016, **5** F1000 Faculty Rev-1119.

27. Park Y, Cosgrove D: **A revised architecture of primary cell walls based on biomechanical changes induced by substrate-specific endoglucanases**. *Plant Physiol* 2012, **158**:1933-1943.

28. Schopfer P: **Biomechanics of plant growth**. *Am J Bot* 2006, **93**:1415-1425.

29. Richards SL, Wilkins KA, Swarbreck SM, Anderson AA, Habib N, Smith AG, McAinsh M, Davies JM: **The hydroxyl radical in plants: from seed to seed**. *J Exp Bot* 2015, **66**:37-46.

30. Zhang T, Vavylonis D, Durachko DM, Cosgrove DJ: **Nanoscale movements of cellulose microfibrils in primary cell walls**. *Nat Plants* 2017, **3**:17056.

31. Sliwinska E, Bassel GW, Bewley JD: **Germination of *Arabidopsis thaliana* seeds is not completed as a result of elongation of the radicle but of the adjacent transition zone and lower hypocotyl**. *J Exp Bot* 2009, **60**:3587-3594.

32. da Silva EAA, Toorop PE, Van Lammeren AAM, Hilhorst HWM: **ABA inhibits embryo cell expansion and early cell division events during coffee (*Coffea arabica* 'Rubi') seed germination**. *Ann Bot* 2008, **102**:425-433.

33. Müller K, Linkies A, Vreeburg RAM, Fry SC, Krieger-Liszkay A, Leubner-Metzger G: ***In vivo* cell wall loosening by hydroxyl radicals during cress (*Lepidium sativum* L.) seed germination and elongation growth**. *Plant Physiol* 2009, **150**:1855-1865.

34. Gimeno-Gilles C, Lelievre E, Viau L, Malik-Ghulam M, Ricoult C, Niebel A, Leduc N, Limami AM: **ABA-mediated inhibition of germination is related to the inhibition of genes encoding cell-wall biosynthetic and architecture: modifying enzymes and structural proteins in *Medicago truncatula* embryo axis**. *Mol Plant* 2009, **2**:108-119.

35. Oracz K, Voegele A, Tarkowská D, Jacquemoud D, Turečková V, Urbanová T, Strnad M, Sliwinska E, Leubner-Metzger G: **Myrigalone A inhibits *Lepidium sativum* seed germination by interference with gibberellin metabolism and apoplastic superoxide production required for embryo extension growth and endosperm rupture**. *Plant Cell Physiol* 2012, **53**:81-95.

36. Rewers M, Sliwinska E: **Endoreduplication in the germinating embryo and young seedling is related to the type of seedling establishment but is not coupled with superoxide radical accumulation**. *J Exp Bot* 2014, **65**:4385-4396.

37. Bassel GW, Stamm P, Mosca G, Barbier de Reuille P, Gibbs DJ, Winter R, Janka A, Holdsworth MJ, Smith RS: **Mechanical constraints imposed by 3D cellular geometry and arrangement modulate growth patterns in the *Arabidopsis* embryo**. *Proc Natl Acad Sci U S A* 2014, **111**:8685-8690.

38. • Souza NM, Topham AT, Bassel GW: **Quantitative analysis of the 3D cell shape changes driving soybean germination**. *J Exp Bot* 2017, **68**:1531-1537.

This publication outlines the plasticity in the cell growth patterns in a legume seed. Cell type-specific anisotropy during germination is monitored for individual cells using 3D-imaging.

39. Wu C, Feng J, Wang R, Liu H, Yang H, Rodriguez PL, Qin H, Liu X, Wang D: **HRS1 acts as a negative regulator of abscisic acid signaling to promote timely germination of *Arabidopsis* seeds**. *PLoS ONE* 2012, **7**:e35764.

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

41. de Farias ET, da Silva EAA, Toorop PE, Bewley JD, Hilhorst HWM: **Expression studies in the embryo and in the micropylar endosperm of germinating coffee (*Coffea arabica* cv. Rubi) seeds**. *Plant Growth Regul* 2015, **75**:575-581.

42. • Shigeyama T, Watanabe A, Tokuchi K, Toh S, Sakurai N, Shibuya N, Kawakami N: **alpha-Xylosidase plays essential roles in xyloglucan remodelling, maintenance of cell wall integrity, and seed germination in *Arabidopsis thaliana***. *J Exp Bot* 2016, **67**:5615-5629.

This publication together with [43•] integrates *XYL1* gene mediated xyloglucan remodelling with the corresponding alterations in cell wall mechanics and seed germination.

43. • Sechet J, Frey A, Effroy-Cuzzi D, Berger A, Perreau F, Cueff G, Charif D, Rajjou L, Mouille G, North HM *et al.*: **Xyloglucan metabolism differentially impacts the cell wall characteristics of the endosperm and embryo during *Arabidopsis* seed germination**. *Plant Physiol* 2016, **170**:1367-1380.

See annotation to Ref. [42•].

44. Footitt S, Müller K, Kermode A, Finch-Savage W: **Seed dormancy cycling in *Arabidopsis*: chromatin remodelling and regulation of DOG1 in response to seasonal environmental signals**. *Plant J* 2015, **81**:413-425.

45. Müller K, Bouyer D, Schnittger A, Kermode AR: **Evolutionarily conserved histone methylation dynamics during seed life-cycle transitions**. *PLoS ONE* 2012, **7**:e51532.

46. Belin C, Megies C, Hauserova E, Lopez-Molina L: **Abscisic acid represses growth of the *Arabidopsis* embryonic axis after germination by enhancing auxin signaling**. *Plant Cell* 2009, **21**:2253-2268.

47. Smýkal P, Vernoud V, Blair MW, Soukup A, Thompson RD: **The role of the testa during development and in establishment of dormancy of the legume seed**. *Front Plant Sci* 2014, **5**:351.

48. Gama-Arachchige NS, Baskin JM, Geneve RL, Baskin CC: **Identification and characterization of ten new water gaps in seeds and fruits with physical dormancy and classification of water-gap complexes**. *Ann Bot* 2013, **112**:69-84.

48(a). Radchuk V, Borisjuk L: **Physical, metabolic and developmental functions of the seed coat**. *Front Plant Sci* 2014, **5**:510.

49. Lazarević JM, Zorić L, Karagć Đ, Miloević B, Karanović D, Milić D, Tepić A, Luković J: **Anatomical and micromorphological characteristics of the seed coat of field pea (*Pisum sativum* L.) genotypes in relation to cracks and damage of seeds**. *Arch Biol Sci* 2017, **69**:503-512.

50. Baskin CC: **Breaking physical dormancy in seeds — focussing on the lens**. *New Phytol* 2003, **158**:227-238.

51. Robles-Diaz E, Flores J, Yanez-Espinosa L: **Paths of water entry and structures involved in the breaking of seed dormancy of *Lupinus***. *J Plant Physiol* 2016, **192**:75-80.

52. •• Sun L, Miao Z, Cai C, Zhang D, Zhao M, Wu Y, Zhang X, Swarm SA, Zhou L, Zhang ZJ *et al.*: **GmHs1-1, encoding a calcineurin-like protein, controls hard-seededness in soybean**. *Nat Genet* 2015, **47**:939-943.

See annotation to Ref. [53••].

53. •• Chai M, Zhou C, Molina I, Fu C, Nakashima J, Li G, Zhang W, Park J, Tang Y, Jiang Q *et al.*: **A class II KNOX gene, KNOX4, controls seed physical dormancy**. *Proc Natl Acad Sci U S A* 2016, **113**:6997-7002.

This publication together with [52] describes the identification of master regulators for the water-impermeability of the seed coat to confer physical dormancy.

54. Orozco-Segovia A, Marquez-Guzman J, Sanchez-Coronado M, Gamboa de Buen A, Baskin J, Baskin C: **Seed anatomy and water uptake in relation to seed dormancy in *Opuntia tomentosa* (Cactaceae, Opuntioideae)**. *Ann Bot* 2007, **99**:581-592.

55. Cousens R, Young K, Tadayyon A: **The role of the persistent fruit wall in seed water regulation in *Raphanus raphanistrum* (Brassicaceae)**. *Ann Bot* 2010, **105**:101-108.

56. Dias DS, Ribeiro L, Sérgio Nascimento Lopes P, Munné-Bosch S, Souza Garcia Q: **Hormonal profile and the role of cell expansion in the germination control of Cerrado biome palm seeds**. *Plant Physiol Biochem* 2017, **118**:168-177.

57. Briggs CL, Morris EC, Stone G: **Micropylar seed coat restraint and embryonic response to heat shock and smoke control seed dormancy in *Grevillea juniperina***. *Seed Sci Res* 2016, **26**:111-123.

58. • Webster R, Waterworth W, Stuppy W, West C, Ennos R, Bray C, Pritchard H: **Biomechanical, biochemical, and morphological mechanisms of heat shock-mediated germination in *Carica papaya* seed**. *J Exp Bot* 2016, **67**:6373-6384.

This publication provides insights into the mechanisms underpinning heat shock-mediated seed coat weakening and its dependence on *de novo* protein synthesis.