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DARWIN REVIEW

The biomechanics of seed germination

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Abstract

From a biomechanical perspective, the completion of seed (and fruit) germination depends on the balance of two opposing forces: the growth potential of the embryonic axis (radicle–hypocotyl growth zone) and the restraint of the seed-covering layers (endosperm, testa, and pericarp). The diverse seed tissues are composite materials which differ in their dynamic properties based on their distinct cell wall composition and water uptake capacities. The biomechanics of embryo cell growth during seed germination depend on irreversible cell wall loosening followed by water uptake due to the decreasing turgor, and this leads to embryo elongation and eventually radicle emergence. Endosperm weakening as a prerequisite for radicle emergence is a widespread phenomenon among angiosperms. Research into the biochemistry and biomechanics of endosperm weakening has demonstrated that the reduction in puncture force of a seed's micropylar endosperm is environmentally and hormonally regulated and involves tissue-specific expression of cell wall remodelling proteins such as expansins, diverse hydrolases, and the production of directly acting apoplastic reactive oxygen. The endosperm-weakening biomechanics and its underlying cell wall biochemistry differ between the micropylar (ME) and chalazal (CE) endosperm domains. In the ME, they involve cell wall loosening, cell separation, and programmed cell death to provide decreased and localized ME tissue resistance, autolysis, and finally the formation of an ME hole required for radicle emergence. Future work will further unravel the molecular mechanisms, environmental regulation, and evolution of the diverse biomechanical cell wall changes underpinning the control of germination by endosperm weakening.

Key words: Apoplastic reactive oxygen species, biological materials, embryo growth potential, endosperm weakening, germination, puncture force, seed biomechanics.

Introduction

All living organisms and processes are bound by the laws of physics and chemistry. Understanding these fundamental mechanisms is key to elucidating the roles of biological materials and structures in life. Plant biomechanics has risen to a topical, multidisciplinary, and expanding field of science (Niklas *et al.*, 2006; Moulia, 2013). The application of new techniques previously only used in material science are leading to new advances and insights in biological materials (Ebenstein and Pruitt, 2006; Cranford and Buehler, 2010; Walters *et al.*, 2010). The mechanical properties of plants are an interplay of cell wall, whole cell, tissue, and organ properties, and are highly dependent on water content (Jeronimidis, 1980; Fratzl and Weinkamer, 2007; Vogler *et al.*, 2015). A plant's life cycle depends on biomechanics at several stages. Starting with the fertilization and the mechanics of pollen tube formation (Gossot and Geitmann, 2007; Zonia and Munnik, 2009) up

to the seed or fruit propagation (Witztum and Schulgasser, 1995; Nathan *et al.*, 2002; Elbaum and Abraham, 2014; Hofhuis *et al.*, 2016). The vulnerable and complex process of seed germination also depends on decisive and specific changes in tissue and cell properties. By definition, seed germination starts with the uptake of water by the quiescent, dry seed followed by the elongation of the embryonic axis (Bewley, 1997*b*). This usually culminates in the rupture of the covering layers and emergence of the radicle, generally considered as the completion of germination (Finch-Savage and Leubner-Metzger, 2006). From a mechanical point of view, the germination process can be seen as an interplay between two opposing forces: the growth potential of the embryo and the restraining force of the seed covering layers. While the physiological, biochemical, and molecular mechanisms of seed germination have been summarized in numerous reviews (see, for example, Bewley, 1997*b*; Koornneef *et al.*, 2002; Finch-Savage and Leubner-Metzger, 2006; Nonogaki, 2006;Linkies and Leubner-Metzger, 2012;Yan *et al.*, 2014), integrated works in which an interdisciplinary effort has been made to combine them with methods from biophysics, engineering, and mathematical sciences are rare, as are reviews from the biomechanical perspective (Welbaum *et al.*, 1998; Schopfer, 2006). In this review we are focusing on seeds as a biomaterial and provide a view on germination mechanisms from a mechanical perspective.

Biological materials

Biological materials and structures are normally composites which are mainly made up from polymeric fibres embedded in a protein matrix (Vincent and Currey, 1980; Wainwright *et al.*, 1982; Vincent, 1990). Considering these weak individual building blocks, it is striking that many biological systems exhibit mechanical properties beyond what can be achieved using the same synthetic materials (Srinivasan *et al.*, 1991; Vincent, 1992; Chen *et al.*, 2008). Plant cell walls consist of cellulose, hemicellulose, pectin, lignin, and protein. This rigid structure, together with the osmotic characteristics of the protoplast, governs the mechanical properties of cells, tissues, and organs (Brett and Waldron, 1996; Cosgrove, 2005). In contrast to this, animal tissue protoplasts are in most cases not surrounded by such a rigid compartment (Vincent and Wegst, 2004; Meyers *et al.*, 2008). It is not so much the material properties of the individual components determining the mechanical behaviour but rather their specific arrangement within a structure. Also, based on the fibre orientations and the amount of the constituents, the mechanical properties of the various material systems or structures are different (Wegst and Ashby, 2004; Burgert, 2006). The exceptional mechanical performance of biological materials resides in their hierarchical organization at multiple levels, from the molecular to the macroscopic scale (Gordon *et al.*, 1980; Jeronimidis and Atkins, 1995; Mann and Weiner, 1999; Aizenberg *et al.*, 2005; Currey, 2005; Rüggeberg *et al.*, 2010; Gibson, 2012). Wood, for example, is one of the most widely distributed high-performance materials with a specific strength comparable with steel (Gordon *et al.*, 1980). Its optimization is achieved by the arrangement of components on at least five structural levels: integral (geometrical make up of axes), macroscopic (tissue structure), microscopic (cell structure), ultrastructural (cell wall structure), and biochemical (cell wall components) (Jeronimidis, 1980). As shown by Ji and Gao (2004) and Gao *et al.* (2003), the smallest hierarchical level is on the nanoscale and intricately linked to higher levels.

Materials respond to external stresses. Engineers describe the mechanical behaviour of materials by loading a sample and measuring the force and displacement of the material as it deforms. This results in force–displacement curves, which can be converted into typical stress–strain curves. These stress–strain curves have several regions of interest and reveal several of the properties of a material (Figs 1, 2A). Stress (or pressure) is defined as the force per area, and strain (or deformation) is defined as the amount of elongation or contraction (increase or decrease in length) caused by the stress.

Stress $\sigma = \frac{F}{A}$ (where F is the force and A is the cross-section)

Strain $\varepsilon = \frac{\Delta L}{L}$ (where ΔL is the change in length and L is the original length)

Some characteristic responses that materials exhibit are shown in Fig. 1 and are defined as follows. (i) Elastic behaviour: recoverable deformation; stress is proportional to strain. Deformation occurs instantly and the material returns to its original shape after the load is removed. For an ideal elastic material, no energy is lost during the loading and unloading.

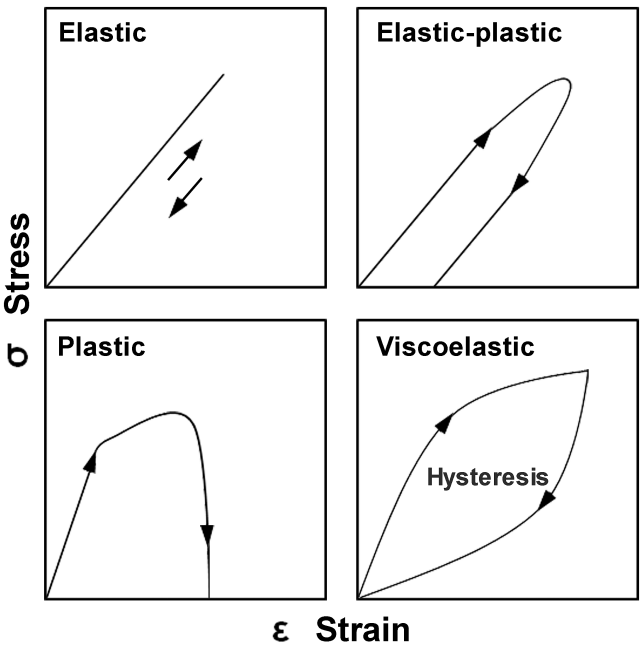

Fig. 1. Stress–strain curves illustrating different types of material behaviour. For an elastic behaviour, loading and unloading paths coincide (no energy lost). Elastic–plastic materials undergo a non-reversible plastic deformation after a threshold is reached, while the unloading includes elastic elements. Plastic materials undergo a non-reversible deformation. Energy is lost during the deformation and corresponds to the area underneath the curve. Viscoelastic materials show a time-dependent behaviour and dissipate energy during loading/unloading. The amount of energy absorbed by the material is equal to the area between the loading and unloading curve (hysteresis).

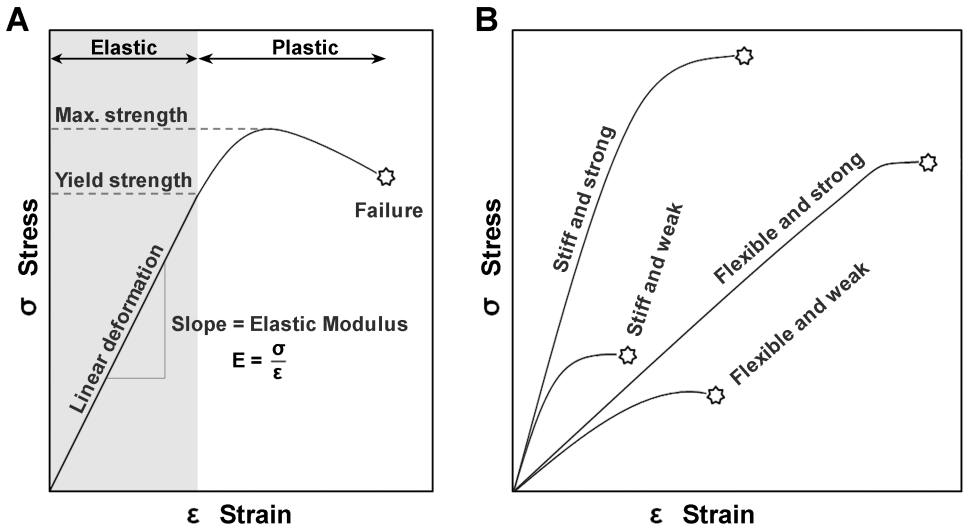

Fig. 2. Schematic diagram showing typical stress–strain curves. (A) The material exhibits an elastic and plastic region. Several key parameters can be derived from the diagram: Elastic modulus E, yield strength (point of elastic limit), and the maximum strength of the material. (B) Typical curves for stiff, strong, weak, or flexible materials.

(ii) Plastic behaviour: non-recoverable deformation; plastic deformation occurs after a certain threshold (yield stress) is reached. An increase in strain leads to a non-linear change in load. (iii) Viscoelastic behaviour: time-dependent deformation; the word viscoelasticity originates from viscosity and elasticity. The rate of deformation is a function of the stresses. That means the deformation depends on how quickly a load is applied. Viscoelastic materials will return to their original shapes after a certain amount of time after the load is removed.

Biological materials are structurally complex and show a complex mechanical behaviour in response to external loading (Fratzl and Weinkamer, 2007; Speck and Burgert, 2011). Most biological materials (if not all) show a viscoelastic behaviour to a greater or lesser extent (Sasaki, 2012). They do have a viscous component and do show time-dependent behaviour. Therefore, the strain or loading rate (change in strain or stress with respect to time) needs to be taken into account. The higher the strain or loading rate, the larger a peak strain/stress will be. Another characteristic a viscoelastic material can possess is creep. Creep is a slow plastic (permanent) deformation that occurs when a constant load is applied over time. Most biological materials operate within the elastic region under normal loading conditions. Furthermore, biological materials are anisotropic. This means that the mechanical properties differ for different directions of loading. Wood, for example, does behave differently if tested along or perpendicular to the grain (Salmén, 2004; Burgert, 2006). The same holds true for diverse seed or fruit coats.

Figure 2 shows stress–strain diagrams, which enable us to derive several key parameters of the tested material. Typically, materials exhibit an initial linear stress–strain response where the slope corresponds to the elastic modulus E (or stiffness) of the material. A flexible material is characterizd by a low elastic modulus, whereas a high elastic modulus correlates to a stiff material. If a test were stopped within the linear (elastic) region, the material would return to its initial shape. At higher forces, above a certain threshold, the elastic limit (yield point) is reached and plastic deformation occurs. Another important variable obtained from the stress–strain curve is the maximum strength of the material under a load such as tension, compression, torsion, or bending. The area underneath the curve corresponds to the energy absorbed by the material and equals the toughness. Stiffness and strength are often used by biologists in the wrong context as they describe very different characteristics of a material. A material can be stiff but weak (e.g. a cookie) or flexible but strong (e.g. leather) (Fig. 2B). An excellent overview of the mechanical properties of materials and their failure is given by Mattheck (2004).

Combining the perspectives of both biologists and material scientists on structure and mechanics is a timely approach to advance our understanding of plants as well as providing new insights on biomaterials. Recent examples of this combined approach include the application of engineering tools to describe seed deterioration and the extension of established material property charts to include seeds (Fig. 3) (Walters *et al.*, 2010). The idea of material property charts was coined by Ashby and compares mechanical properties by plotting one property against another (Ashby, 1989; Ashby *et al.*, 1995; Wegst and Ashby, 2004). They are a sophisticated graphical way of presenting and comparing material property data. Two properties are plotted; one on each axis of the graph, while common combinations are, for example, strength versus density, modulus versus density, modulus versus strength, and fracture toughness versus modulus. Figure 3 illustrates schematically a material property chart where the elastic modulus (E) is plotted against the density (ρ) (Ashby *et al.*, 1995). The scales are logarithmic, showing a wide range of materials on just one chart. For the comparison of different materials, the material indices E/ρ, E1/2/ρ, and E1/3/ρ are plotted onto the figure as guidelines for minimum mass design. Materials which lie on a line perform equally, those above the line are better with respect to lightweight structures, and those below are worse. It is observable that biological materials are relatively light materials with low density yet providing a relatively high elastic modulus. According to Walters *et al.* (2010), the elastic modulus of seeds varies

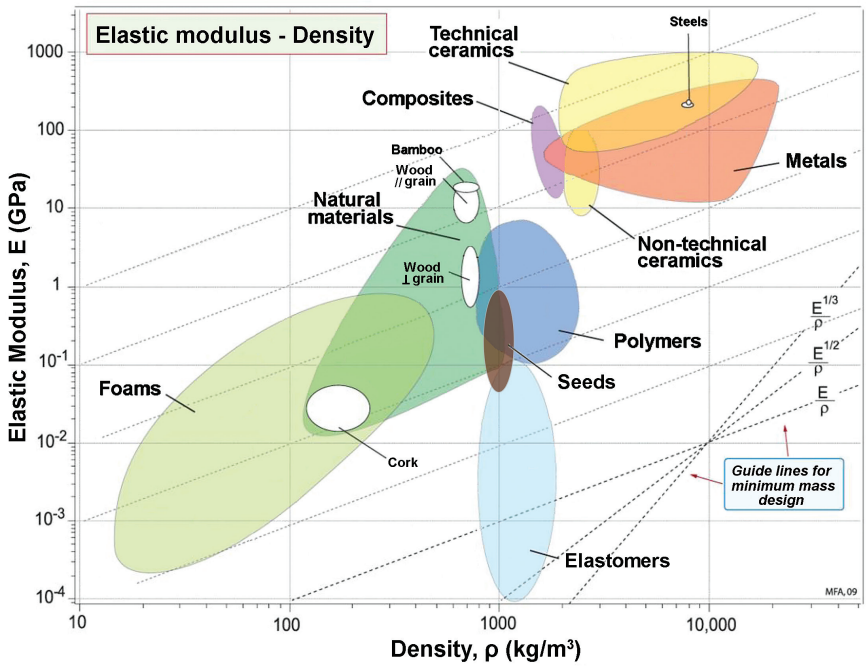

Fig. 3. Material property chart plotting Young's modulus E against density ρ. The heavy envelopes enclose data for a given class of material. The guidelines of constant E/ρ, $E^{1/2}/\rho$, and $E^{1/3}/\rho$ allow identification of structurally efficient materials which are light and stiff (after Ashby, 2007; Ashby *et al.*, 2013; copyright Elsevier, reprinted with permission). Properties for seeds inserted as determined by Walters *et al.* (2010).

by one order of magnitude, and depends on the species and environmental factors. The material density is centred near 1000 kg m^{-3}. The elastic modulus within the seed material family lies within the range of polymers and foams and other natural materials wherever the density is similar to wood, polymers, and elastomers (Fig. 3) (Walters *et al.*, 2010).

Biophysical aspects of seed germination

Seeds, and in many cases also seed-harbouring fruits, evolved as the typical dispersal and propagation units of the angiosperms and gymnosperms (Linkies *et al.*, 2010). Structurally distinct seed and embryo types have been defined (Martin, 1946; Baskin and Baskin, 2014) and their distinct compartments and tissues serve important roles during germination and seedling establishment. In the mature seeds of most angiosperm species, the diploid embryo is enclosed by one or more seed-covering layers. These coverings typically consist of a more or less abundant living triploid endosperm and a diploid dead maternal testa (seed coat) which both play key roles in the control of germination (de Mason *et al.*, 1983; Lisboa *et al.*, 2006; Finch-Savage and Leubner-Metzger, 2006; Buckeridge, 2010; Weitbrecht *et al.*, 2011; Yan *et al.*, 2014). In cases where dry fruits are dispersed, the seed is in addition encased by pericarp (fruit coat) layers (Psaras, 1984; Hermann *et al.*, 2007; Olsen, 2004).

Mechanical properties of whole seeds or parts of seeds have mainly been examined in food science, especially the fracture toughness, impact damage, and tensile and compression strength. Measurements have mainly been carried out with seeds or fruits of beans (Bartsch *et al.*, 1986; Bay *et al.*, 1996; Fahloul *et al.*, 1996; Ogunjimi *et al.*, 2002; Altuntas and Yıldız, 2007; Ozturk *et al.*, 2009; Davies and Zibokere, 2011; Shahbazi *et al.*, 2011), olives (Georget *et al.*, 2001; Kılıçkan and Güner, 2008), walnuts (Altuntas and Özkan, 2008; Altuntas and Erkol, 2011), sunflower (Gupta and Das, 2000), cumin (Saiedirad *et al.*, 2008), and wheat (Mabille *et al.*, 2001). In large parts, these measurements determined the influence of different moisture contents on the mechanical properties. In summary, there is a general trend that an increase in moisture content causes a decrease in fracture toughness and the major mechanical entities and associated features which control seed germination are the properties of the seed/fruit coats, the endosperm weakening, and the embryo growth potential. In papaya (*Carica papaya*), cracking of the seed coat is the first visible sign during germination, and is followed by endosperm rupture. Seed coat removal has been shown to overcome seed dormancy, while germination-stimulating treatments (heat shock) and germination-inhibiting treatments (abscisic acid) did not alter the seed coat mechanics (Webster *et al.*, 2016).

The outer seed coverings consist mostly of dead tissues (testa and pericarp) and represent the seed's interface with the external environment. Their roles include protecting the embryo against adverse ambient conditions. In addition, they serve a mechanical purpose in coat-imposed seed dormancy to control germination timing (Werker, 1980; Kelly *et al.*, 1992; Bewley, 1997*b*; Debeaujon *et al.*, 2000). In many species, a living layer of more or less abundant endosperm is interposed between these dead outer tissues and the embryo (Meier and Reid, 1982; Buckeridge *et al.*, 2000; Finch-Savage and Leubner-Metzger, 2006; Yan *et al.*, 2014). In addition to providing mechanical restraint, coat-associated mechanisms of the endosperm, testa, and/or pericarp are to control or even prevent water uptake, to interfere with leaching of inhibitors of embryo elongation such as abscisic acid (ABA), or

gaseous exchanges which may cause oxygen deficiency within the embryo (see, for example, Coumans *et al.*, 1976; Santos and Pereira, 1989; Kelly *et al.*, 1992; Bewley and Black, 1994; Koornneef *et al.*, 2002; Manz *et al.*, 2005; Finch-Savage and Leubner-Metzger, 2006; Müller *et al.*, 2006; Nonogaki, 2006; Weitbrecht *et al.* 2011). It has, for example, been shown for *Lepidium sativum* seeds prior to testa/endosperm rupture that the testa and endosperm interfere with oxygen uptake required for ethylene production (Linkies *et al.*, 2009). The same is true for sugar beet fruits where the pericarp confers the major restraint (Hermann *et al.*, 2007). For these non-dormant (ND) seeds and fruits, as well as for those from the physiological (PD), morphological (MD), and morphophysiological (MPD) dormancy class (Willis *et al.*, 2014), the covering layers also regulate the speed and spatial pattern of water uptake by imbibition, but for a block to imbibition it requires the hardseededness of physically dormant (PY) seeds.

Water-impermeable outer coverings blocking water uptake are the hallmark of seeds with PY (Finch-Savage and Leubner-Metzger, 2006; Baskin and Baskin, 2014; Willis *et al.*, 2014). The water impermeability of many legume (Fabaceae) seed coats is due the presence of one or more palisade layers of lignified malphigian cells (macrosclerids) tightly packed together and impregnated with water-repellent phenolic and suberin-like substances (Gama-Arachchige *et al.*, 2013; Smýkal *et al.*, 2014). *KNOX4*, a class II KNOTTED-like homeobox gene, and *GmHs1-1*, a gene encoding a calcineurin-like protein, were identified to control water impermeability, hardseededness, and PY in *Medicago truncatula* and soybean seeds, respectively (Sun *et al.*, 2015; Chai *et al.*, 2016). Depending on the species and habitat, various environmental factors are known to release PY with a predictable seasonal timing by making the seeds water permeable. An anatomical structure in the impermeable layer(s) of PY seeds has the role of the 'water-gap'. In legume seeds, the water-gap is a specialized area near the hilum termed the lens (Baskin and Baskin, 2014; Smýkal *et al.*, 2014). The water-gap is closed at seed maturity and is irreversibly opened when PY is released by appropriate environmental triggers. Water-gaps act as environmental signal detectors with a mechanical mechanism which includes a pre-determined breaking point. Lens opening in many legume seeds requires a sequence of two temperature regimes, first chilling and then low alternating temperatures (Baskin, 2003). Water-gap opening in *Ipomoea* spp. seeds is associated with mechanical rupture processes involving the hilar pad (Baskin and Baskin, 2014). For *I. lacunosa* germinating in hot wet conditions, this involves pressure generated by a combination of trapped water vapour and heat which dislodges the hilar pad, whereas for *I. hederaceae* germinating in hot dry conditions this involves shrinking of the hilar pad by the dry heat. In both cases, the generated mechanical stress results in material micro-fractures and thereby water permeability. PY breaking and opening of the micropylar water-gap of *Geranium carolinianum* seeds is initiated by cold temperature causing differential mechanical tensile stress of the palisade and subpalisade layers (Gama-Arachchige *et al.*, 2013). The stronger shrinkage of the metastable (weak) palisade layer leads to micro-cracks. Water uptake through these micro-cracks results in layer separation due to the tension and stronger expansion of the palisade layer. This in turn leads to the formation of a blister which activates a pre-formed hinged valve at the adjacent micropyle. Dislodgement of the hinged valve reveals the water-gap and eventually leads to tearing off the palisade layer covering along the water-gap margin. Species with water-impermeable seed or fruit coats evolved independently in 18 plant families (Baskin and Baskin, 2014). PY is associated with the potential to confer high longevity, but, in contrast to PD cycling, its release is irreversible and leads to water uptake and embryo expansion growth.

Biomechanics of embryo growth

Plant cells possess a rigid cell wall which, together with the turgor pressure from water uptake into the vacuole, provides stability to the plant. In order to grow, the plant cells need to expand in a controlled manner. A good overview on the process is given in a review by Cosgrove (2005). The primary cell walls of plants are presumably a non-linear viscoelastic material which can expand plastically (Niklas, 1992; Schopfer, 2006). The irreversible cell expansion is produced by creating a driving force for water uptake by decreasing the turgor through stress relaxation in the cell wall (Fry, 2004; Schopfer, 2006). Upon cell wall loosening, the polymers in the cell wall move apart from each other (creep) and allow expansion growth of the cell due to water influx into the vacuole. Candidates proposed to be involved in the cell wall loosening include expansins (Cosgrove, 2000*a*, *b*), xyloglucan endotransglycolases/hydrolases (Fry *et al.*, 1992; Van Sandt *et al.*, 2007), endo-(1,4)-β-D-glucanases (Nicol *et al.*, 1998; Inukai *et al.*, 2012), as well as apoplastic reactive oxygen species (aROS) (Schopfer, 2001; Schopfer *et al.*, 2002; Müller *et al.*, 2009). Upon imbibition of a quiescent seed, the low water potential ('dry' state) causes rapid water uptake driven by the matrix potential (Schopfer, 2006; Weitbrecht *et al.*, 2011). The osmotic water uptake eventually leads to a turgid state, to the activation of the metabolism, and to cell expansion growth in the embryo axis (Voegele *et al.*, 2012). Specific embryo growth zones have been identified (Sliwinska *et al.*, 2009; Bassel *et al.*, 2014). While this cell expansion growth is associated with endoreduplication, only the cell growth but not cell division is required for the embryo to complete germination through radicle emergence (Sliwinska *et al.*, 2009; Weitbrecht *et al.*, 2011; Oracz *et al.*, 2012). In order to complete germination, the embryo growth potential must increase and exceed the restraint (Ni and Bradford, 1993; Bewley, 1997*a*; Nonogaki, 2006; Nonogaki *et al.*, 2007). The mechanism by which this occurs is through an increase in the embryo cell wall extensibility which enables plastic rather than merely elastic wall extension, and by simultaneously decreasing the restraints of the embryo-covering layers (Fig. 4). These changes are inhibited by ABA which thereby lowers the embryo growth potential and cell expansion growth (Liptay and Schopfer, 1983; Schopfer and Plachy, 1985; da Silva *et al.*, 2008) and inhibits the restraint weakening of the endosperm (Toorop *et al.*, 2000; Müller *et al.*, 2006; Linkies and Leubner-Metzger, 2012). Similar biochemical mechanisms in the cell walls of micropylar endosperms are also

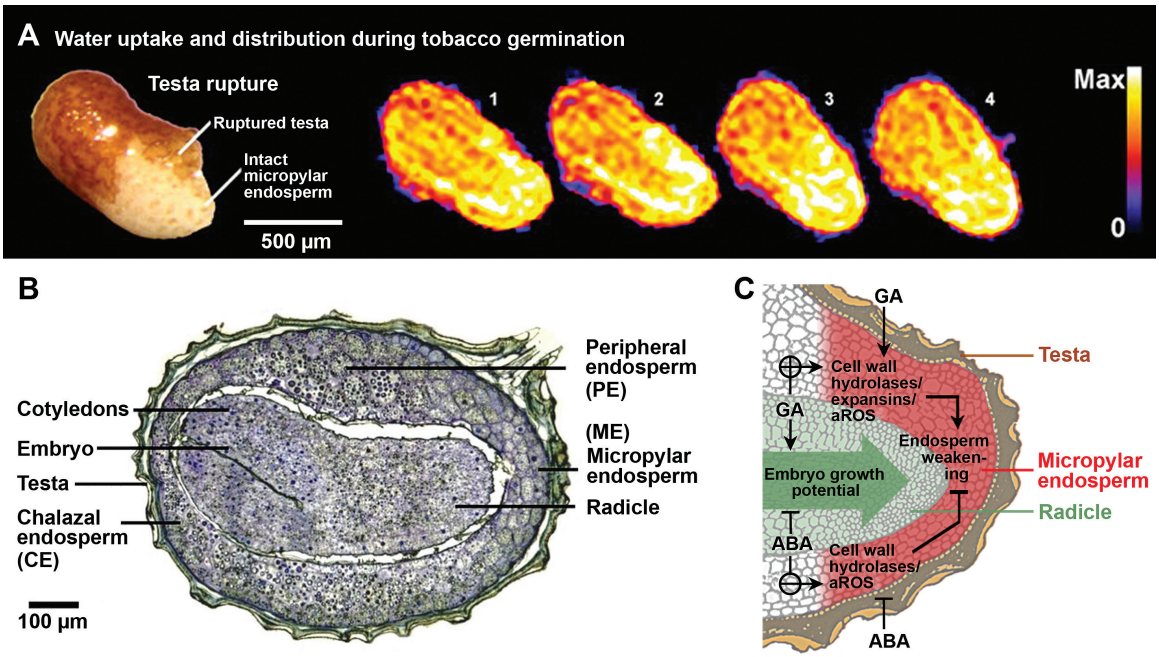

Fig. 4. (A) Non-invasive *in vivo* ^{1}H-NMR microimaging analysis of water uptake and distribution during tobacco seed germination. The spatial distribution of proton mobility within the seed tissues is visualized by false colours [relative scales from zero (0, black) to maximum signal strength (max, white)]. Microimages of the testa rupture stage are shown with a resolution of ~30 µm (after Manz *et al.*, 2005; copyrighted by the American Society of Plant Biologists and reprinted with permission). (B) Seed structure of tobacco (*Nicotiana tabacum*) (after Lee *et al.*, 2012; copyrighted by the American Society of Plant Biologists and reprinted with permission.). (C) Schematic of the micropylar endosperm (ME) and the radicle tip of a tobacco seed. Gibberellins (GAs) promote the induction of cell wall hydrolases, expansins, and apoplastic reactive oxygen species (aROS), thereby promoting endosperm weakening and endosperm rupture. Abscisic acid (ABA) inhibits the induction of cell wall hydrolases and aROS, thereby inhibiting endosperm weakening and endosperm rupture. GA promotes and ABA inhibits the embryo growth potential.

underpinning endosperm weakening required for endosperm rupture during germination. However, cell separation (disrupting cell adhesion) and localized programmed cell death (PCD) are additional features of endosperm weakening (Bethke *et al.*, 2007; Morris *et al.*, 2011).

A biomechanical approach to the evolution of endosperm weakening mechanisms across seed types and angiosperm phylogeny

The evolution of the internal morphology of mature seeds with embryo and endosperm properties as well as their relative size ratios has been reviewed elsewhere (Finch-Savage and Leubner-Metzger, 2006; Nonogaki, 2006; Linkies *et al.*, 2010; Baskin and Baskin, 2014; Willis *et al.*, 2014; Yan *et al.*, 2014). These reviews link the abundance and roles of endosperm in mature seeds to biochemical and molecular mechanisms during dormancy and germination. It is beyond the scope of this review to integrate all these findings and to provide another historical overview about what biomechanical mechanisms were proposed, for example, from spatiotemporal expression patterns of specific cell wall hydrolases in the micropylar endosperm during germination. Therefore, and since the biochemical and molecular mechanisms of endosperm weakening have been summarized in numerous reviews (see, for example, Bewley, 1997*b*; Koornneef *et al.*, 2002; Finch-Savage and Leubner-Metzger, 2006; Nonogaki, 2006; Linkies and Leubner-Metzger, 2012; Yan *et al.*, 2014), our biomechanical approach in this review is to focus primarily on these seed systems where direct evidence for endosperm weakening was obtained by puncture force analysis. The puncture force refers to the maximum strength of the tissue (cf. Fig. 2). There is a general evolutionary trend from high to low endosperm abundance in mature seeds (Finch-Savage and Leubner-Metzger, 2006; Baskin and Baskin, 2014), and we therefore summarize the biomechanical state-of-the-art separately for each of the major phylogenetic clades.

Seed with a tiny embryo (underdeveloped in terms of size) embedded in abundant living endosperm tissue is proposed to be ancestral and associated with the MPD and MD classes of seed dormancy (Baskin and Baskin, 2014; Willis *et al.*, 2014). This type of seed is indeed more abundant in the basal angiosperms and the basal eudicots, especially when compared with the Rosid clade. No direct biomechanical evidence using the puncture force method has been obtained for endosperm weakening in MD/MPD seeds. There is, however, solid biochemical, microscopic, and physiological evidence, for example, from *Trollius* (MPD, Ranunculaceae, basal eudicots) and celery (MD, Apiaceae, Asterid clade) seeds that embryo growth during imbibition is associated with dissolving the endosperm prior to the completion of germination (Jacobsen and Pressman, 1979; Hepher and Roberts, 1985). Willis *et al.* (2014) discuss the different hypotheses of how PD and ND seeds may have evolved from these ancestral seed types with abundant endosperm. Table 1 summarizes the PD and ND seed systems

for which direct biomechanical evidence for endosperm weakening was obtained by the puncture force method. Considering these system, it is possible to compare seeds with thick and thin endosperm within the Asterid clade, as well as seeds with thin endosperm between the Asterid and Rosid clade. The general evolutionary trend from high to low endosperm abundance in mature seeds is evident between these two clades (Finch-Savage and Leubner-Metzger, 2006; Baskin and Baskin, 2014) and will allow future identification of the evolutionarily conserved and ancestral mechanisms of endosperm weakening.

Endosperm weakening in Asterid clade seeds and fruits

In the case of endosperm-limited germination, the endosperm acts, at least in part, as a mechanical barrier for radicle protrusion (Linkies and Leubner-Metzger, 2012). It has been reported for many species that a decline in the mechanical resistance of the micropylar endosperm (the endosperm covering the radicle tip) appears to be a prerequisite for radicle protrusion (Table 1 and associated references). From a mechanistic point of view, seed germination is determined by the interaction of two antagonistic forces: the increase of the embryo growth potential and the decrease in the resistance of the covering layers (Fig. 4). The direct evidence for the endosperm weakening (PF↓ in Table 1) has been obtained by puncture force measurements; that is, the direct quantification of the force needed for puncturing the micropylar endosperm by a metal probe (Fig. 5). This was first achieved with larger seeds from the Asterid clade (Table 1), and was only recently accomplished with tiny (<1 mm length) tobacco (*Nicotiana tabacum*, Solanaceae) seeds (Lee *et al.*, 2012).

Table 1. *Summary of species and closely related species in the major angiosperm clades where direct evidence for endosperm weakening was reported via puncture force experiments*

Rosid clade:		
Cucurbitaceae	*Cucumis*	PF↓ (perisperm)
	Welbaum *et al.*, 1995; Yim and Bradford, 1998; Welbaum, 1999	
Brassicaceae	*Lepidium*	PF↓ GA↓ Ethylene↓ ACC↓ ABA↑ *OH↓
	Müller *et al.*, 2006, 2009; Linkies *et al.*, 2009; Graeber *et al.*, 2010; Morris *et al.*, 2011; Oracz *et al.*, 2012; Voegele *et al.*, 2012; Graeber *et al.*, 2014	
	Arabidopsis	
	Bethke *et al.*, 2007; Creff *et al.*, 2015; Fourquin *et al.*, 2016	
Asterid clade:		
Oleaceae	*Syringa*	PF↓
	Junttila, 1973	
	Fraxinus	PF↓ GA↓
	Finch-Savage and Clay, 1997	
Solanaceae	*Solanum*	PF↓ GA↓ ABA↑ Priming↓
	Groot and Karssen, 1987; Groot *et al.*, 1988; Groot and Karssen, 1992; Chen and Bradford, 2000; Toorop *et al.*, 2000; Wu *et al.*, 2001; Pinto *et al.*, 2007; Anese *et al.*, 2011	
	Capsicum	PF↓ GA↓
	Watkins and Cantliffe, 1983; Petruzzelli *et al.*, 2003	
	Datura	
	Arana *et al.*, 2005; 2007	
	Nicotiana	PF↓
	Leubner-Metzger, 2003; Lee *et al.*, 2012	
	Petunia	
	Petruzzelli *et al.*, 2003	
Rubiaceae	*Coffea*	PF↓ GA↓ ABA↑
	da Silva *et al.*, 2004, 2005	
	Genipa	PF↓ ABA↑
	Queiroz *et al.*, 2012	
Asteraceae	*Lactuca*	PF↓ GA↓ *OH↓ Etephon↓
	Chen *et al.*, 2016; Tao and Khan, 1979; Zhang *et al.*, 2014	
Monocots:		
Iridaceae	*Iris*	PF↓
	Blumenthal *et al.*, 1986	
Poaceae	*Triticum*	PF↓ GA↓ ABA↑
	Benech-Arnold, 2004; J. Hourston *et al.*, unpublished	

PF↓=endosperm weakening (EW); GA↓=EW promoted by GA; Ethylene↓=EW promoted by ethylene; ACC↓ or ethephon↓=EW promoted by ACC or ethephon (via conversion to ethylene); ABA↑=EW inhibited by ABA; *OH↓ EW promoted by apoplastic reactive oxygen species (aROS)

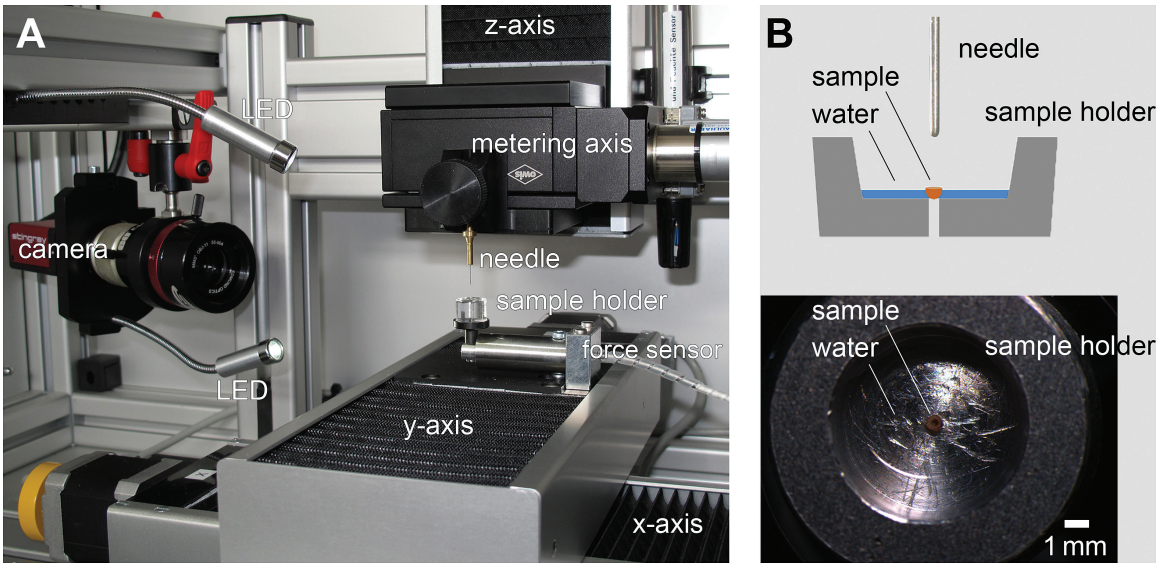

Fig. 5. Puncture force device to measure endosperm weakening. (A) Example of a custom-made puncture force machine consisting of a force and displacement (metering axis) sensor, a camera, LED lights, and an *xy* positioning stage. A measuring tip (needle) with chosen tip diameters/geometry is driven into the sample while force and displacement are recorded. (B) Example of a sample holder for tobacco seeds (schematic and photograph). Tobacco seeds were cut in half and the embryo and testa removed, which left the empty but intact endosperm into which the metal probe could be lowered. Delicate material is kept hydrated by adding water to the sample holder.

The majority of direct puncture force measurements of endosperm resistance and weakening have been carried out in the Asterid clade; examples include tomato (*Solanum lycopersicum*; Groot and Karssen, 1987; Toorop *et al.* 2000), *Solanum lycocarpum* (Pinto *et al.*, 2007), and coffee (da Silva *et al.*, 2004, 2005). The endosperm weakening in these species has been shown to be biphasic. The first phase of the endosperm weakening occurs irrespective of ABA, while the second phase of the weakening process is sensitive to ABA (Fig. 6A). If the micropylar endosperm is isolated from tomato seeds prior to the onset of the weakening (at 3 h), a further 24 h or longer incubation only results in weakening of the tissue if the incubation medium contains gibberellin (GA) (Groot and Karssen, 1992) or if the isolated endosperms are co-incubated with wild-type tomato embryos (Groot and Karssen, 1987). Furthermore, work on gibberellin-deficient mutants provides evidence that GA facilitates germination by weakening the mechanical restraint of the micropylar endosperm (Fig. 6A) (Groot and Karssen, 1987; Groot *et al.*, 1988). Seeds of the GA-deficient tomato mutant *gib1* (*S. lycopersicum* Mill.) do not germinate in the absence of exogenous GA, but the radicle does emerge if the endospermic tissue above the radicle tip is removed (Groot and Karssen, 1987). Similarly it has been shown in several tomato lines that the inhibition of germination by ABA or other stress factors can be abolished by removing the mechanical constraint from the radicle tip (Liptay and Schopfer, 1983). Also, while endosperm weakening and germination of after-ripened tomato seeds completes within 2–3 d, it is not induced in freshly harvested dormant tomato seeds (Groot and Karssen, 1992). In contrast to these dormant wild-type tomato seeds, ABA-deficient *sit*^{w} tomato seeds are non-dormant, and germinate even in the freshly harvested state in association with endosperm weakening. A visible distinction between testa and endosperm rupture is not possible during the germination of tomato seeds and therefore almost all of the biomechanical work in this species is in fact carried out by measuring the puncture force of the micropylar endosperm plus testa. Manual removal of the testa demonstrates that the micropylar endosperm confers ~80% of the total puncture force (Groot and Karssen, 1987). Interestingly, *sit*^{w} tomato seeds are not only non-dormant, but also have a thinner testa when compared with the wild type (Groot and Karssen, 1992; Hilhorst and Downie, 1995).

Cell wall modification, especially the observed physical and microscopic changes in the endosperm cell walls, are considered to be a major player in controlling the weakening process (Groot *et al.*, 1988; Nonogaki *et al.*, 1998; Toorop *et al.*, 2000). The endosperm weakening is associated with cell wall hydrolysis (Watkins *et al.*, 1985; Sánchez *et al.*, 1990). In tomato, several enzymes and proteins with spatiotemporal association with the weakening process have been identified, including endo-ß-1,4-mannanase (Bewley, 1997*a*; Groot *et al.*, 1988; Nonogaki *et al.*, 1998, 2000; Toorop *et al.*, 2000), polygalacturonase (Sitrit *et al.*, 1999), ß-1,4-glucanase (Bradford *et al.*, 2000), ß-1,3-glucanase and chitinase (Wu *et al.*, 2001), and xyloglucan endotransglycosylase/hydrolase (Chen *et al.*, 2002). A battery of cell wall-modifying proteins have therefore been proposed to cause the actual decrease in micropylar endosperm resistance, but the field has not yet evolved to assign a specific biochemical cell wall modification to a specific change or quantified contribution to the resultant change in the endosperm's mechanical properties. These biochemical and molecular mechanisms of endosperm weakening have been summarized in detail elsewhere (see, for example, Bewley, 1997*b*; Hilhorst *et al.*, 1998; Koornneef *et al.*, 2002; Finch-Savage and Leubner-Metzger, 2006; Nonogaki *et al.*, 2007; Linkies and Leubner-Metzger, 2012).

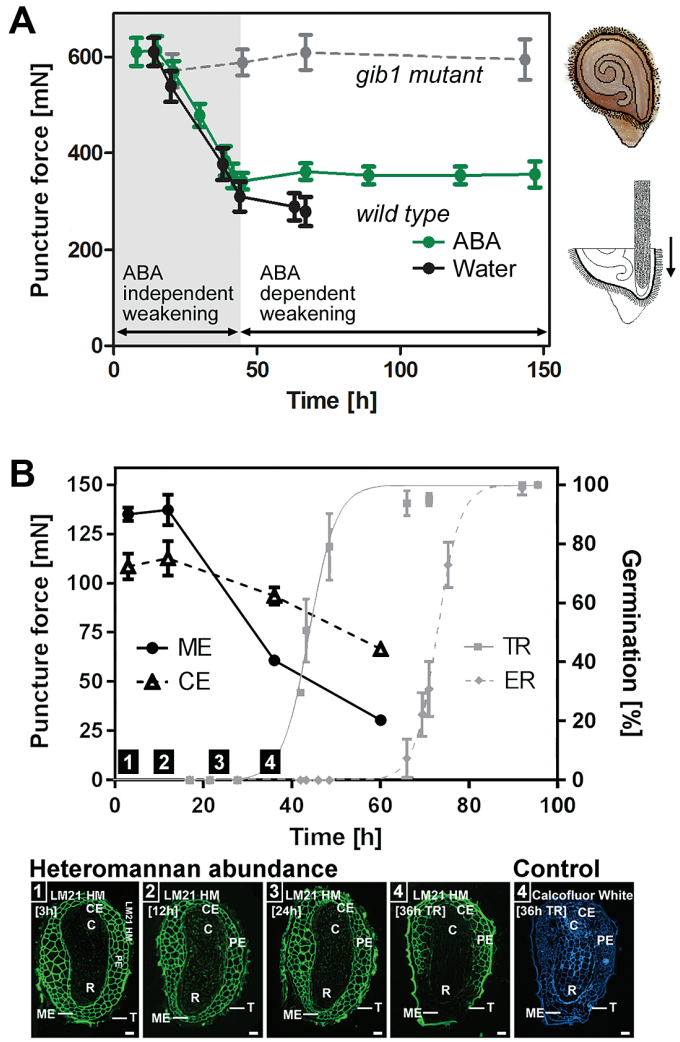

Fig. 6. Endosperm weakening in the Solanaceae (thick endosperm) (A) *Solanum lycopersicum*. The required puncture force of wild-type (WT) seeds in water or 10 µM ABA and *gib1* seeds in water is shown over time. Germination onset of WT seeds in water is at 60 h. The endosperm weakening is biphasic as the force for the WT drops in water and ABA drastically within the first 36 h of imbibition. Afterwards weakening is inhibited by ABA. GA-deficient mutants (*gib1*) show no endosperm weakening. Error bars indicate the SEM [modified from Toorop *et al.* (2000). The second step of the biphasic endosperm cap weakening that mediates tomato (*Lycopersicon esculentum*) seed germination is under control of ABA. Journal of Experimental Botany 51, 1371–1379. Published by Oxford University Press on behalf of the Society for Experimental Biology and Groot and Karssen (1987). Planta, Gibberellins regulate seed germination in tomato by endosperm weakening: a study with gibberellin-deficient mutants, 171, 1987, 525–531, Groot SP and Karsesen CM, with permission of Springer]. (B) *Nicotiana tabacum*. The micropylar (ME) and chalazal (CE) endosperm weakening and rupture of seeds (germination kinetics) are shown over time. The weakening was determined by measuring the tissue resistance via puncture force measurements at the times indicated. Testa rupture (TR) begins at 28 h, and endosperm rupture (ER) at 60 h, respectively. Error bars indicate the SEM. *In situ* localization of cell wall epitopes in longitudinal sections of tobacco seeds. LM21 HM binds to abundant heteromannans in the endosperm. The immunolabelling of germinating tobacco seeds with LM21 HM revealed a specific degradation of heteromannan (HM) at the micropylar endosperm (ME) after testa rupture. Calcofluor White is a non-specific fluorochrome that binds to cellulose in cell walls and was used as control. R, radicle; C, cotyledons; T, testa; PE peripheral endosperm; Scale bars=50 mm. Modified from Lee *et al.* (2012); copyrighted by the American Society of Plant Biologists and reprinted with permission.

The softening of, what is commonly, mannan- (ß-1,4-linked poly-mannose derivates) enriched cell walls is essential in the life cycle of many seeds including tomato (Rodríguez-Gacio *et al.*, 2012) and tobacco (Reid *et al.*, 2003). Mature tobacco seeds exhibit 3–5 layers of rather thick-walled living endosperm cells (Fig. 4B) rich in galactomannan with a very low degree of galactose. The tobacco endosperm is enclosed by a thin testa, which consists of an outer layer of dead cells and a living inner parenchyma layer (Avery, 1933; Leubner-Metzger, 2003). Rupture of the testa (TR) and the endosperm (ER) are temporally well separated successive events during the germination of tobacco seeds (Arcila and Mohapatra, 1983; Leubner-Metzger *et al.*, 1995). The testa rupture starts near the funiculus and progresses along the ridges of the testa, leaving a dome-shaped endosperm structure covering the radicle. Tobacco is not only the smallest seed for which endosperm weakening was directly quantified by the puncture force method (Lee *et al.*, 2012), but also the smallest seed for which the spatiotemporal patterns of water uptake were investigated by ^{1}H-NMR microimaging (Manz *et al.*, 2005). This non-destructive method revealed a non-uniform water uptake and distribution as the micropylar end of the seed is the major entry point of water. Micropylar endosperm and the radicle show the highest water content in the TR stage prior to ER (Fig. 4A). The spatial analysis even revealed that already prior to TR, these compartments have a significantly higher water content compared with the non-micropylar endosperm and the cotyledons. It is therefore obvious to assume that the processes associated with the tobacco seed's late TR stage also include biomechanical and biochemical cell wall alterations.

To investigate the underpinning biomechanical mechanisms of tobacco endosperm weakening, comparative puncture force analyses of the micropylar endosperm (ME) and the chalazal endosperm (CE) were conducted (Lee *et al.*, 2012). To achieve this with such a tiny seed as tobacco, a thin needle and a special sample holder filled with water are required (Fig. 5B). Figure 6 shows that TR is associated with a significant decrease in ME resistance which coincides with TR. A further decrease in ME resistance was just prior to ER. Most strikingly, this TR-associated endosperm weakening was most pronounced in the ME, with a fast ~100 mN decrease in the tissue resistance. In contrast to the ME, there was no appreciable weakening in the CE associated with TR, and the slow decrease in CE resistance just prior to ER was considerably smaller (<50 mN) (Fig. 6B). The major conclusion from this is that the mature tobacco seed exhibits an endosperm polarity in which the ME and CE have distinct roles: the CE does not weaken as dramatically as the ME and consequently can serve as an 'anchor' or 'holding structure' for the embryo to support the elongation growth directed towards the micropylar seed end. The ME weakens, at least partially, by biochemical cell wall changes, allowing enhanced water uptake into the embryonic axis growth zone cells, also allowing ER and radicle protrusion at a defined location, namely at the weakened ME (Fig. 6). The ME weakening is therefore a key biomechanical and biochemical process which controls tobacco germination timing.

In agreement with this conclusion, microscopic studies showed that storage reserves are degraded in the ME cells prior to ER and to radicle protrusion (Arcila and Mohapatra, 1983; Leubner-Metzger *et al.*, 1995). The microscopy also shows that the endospermic hole, which is always formed at the micropylar end of the germinating tobacco seed, has a smooth outline probably resulting from biochemical tissue dissolution rather than the pushing action of the protruding radicle. These processes leading to ER and radicle emergence require transcription and translation (Arcila and Mohapatra, 1992). The endosperm cell walls of solanaceous seeds are known to be rich in mannan (β-1,4-linked D-mannose) and heteromannans (gluco- and galactomannans, glucose or galactose α-1,6-linked to the main β-1,4-mannan chain) (Bewley, 1997*a*; Reid *et al.*, 2003; Buckeridge, 2010; Morris *et al.*, 2011; Lee *et al.*, 2012; Rodríguez-Gacio *et al.*, 2012). These cell wall mannans are rigidity- and mechanical strength-conferring cross-linking hemicellulosic matrix polysaccharides. In some species they serve as endosperm storage reserves, and due to their viscosity and solubility in water may also have roles during seed imbibition. In *Solanum* spp. seeds (Table 1), the second step of the biphasic ME weakening is controlled by ABA and is associated with endo-β-1,4-manannase accumulation in the ME (Nonogaki *et al.*, 2000; Toorop *et al.*, 2000; Gong and Derek Bewley, 2007; Pinto *et al.*, 2007). The hypothesis that hydrolytic enzyme accumulation in the ME is required for endosperm weakening and radicle protrusion was first proposed by Ikuma and Thimann (1963). Over 70% of the tobacco seed galactomannan can be solubilized from the endosperm cell walls by the action of pure endo-β-1,4-manannase (Reid *et al.*, 2003). Tobacco endosperm monosaccharide linkage analysis of neutral sugars shows that ~65% are heteromannans (>90% of these constitute β-1,4-mannan linkages) (Lee *et al.*, 2012). *In situ* localization of heteromannan cell wall epitopes by immunofluorescence microscopy using a specific antibody demonstrated that heteromannan was specifically degraded in the ME at TR, but not at earlier time points and not in the CE (Fig. 6). This spatiotemporal heteromannan degradation pattern in the ME cell walls suggests that endo-β-1,4-manannase accumulation in the ME contributes to the ME weakening during tobacco seed germination (Fig. 6). Other cell wall hydrolases, including endo-β-1,3-glucanase, were also proposed to contribute to tobacco ME weakening (Leubner-Metzger *et al.*, 1995; Leubner-Metzger and Meins, 2000; Manz *et al.*, 2005). To study endosperm weakening further, tobacco is an ideal Asterid system due to the separate TR and ER, and because it has abundant endosperm and a straight embryo, which make it structurally a typical and simple system with a clearly expressed endosperm polarity.

In lettuce (*Lactuca sativa*, Asteraceae) fruits, the embryo is completely enclosed by a living endosperm composed of 2–3 cell layers which is a mechanical constraint to embryo growth and the completion of germination (Ikuma and Thimann, 1963; Halmer *et al.*, 1975; Bewley, 1997*a*). In the intact lettuce fruit (achene), the embryo and endosperm are enclosed by a testa (seed coat) and pericarp (fruit coat) covering (Fig. 7). Lettuce ME and CE cell walls differ considerably in their composition. Indirect biomechanical measurements showed that lettuce endosperm weakening precedes endosperm rupture in the light, but not in darkness (photoinhibition), and GA treatment can replace the light to induce endosperm weakening (Tao and Khan, 1979). To conduct the biomechanical work on lettuce, these authors used an indirect measurement method of the forces, namely by calculating them as the difference between puncturing embryo plus endosperm and embryo alone, perpendicular to the seed axis of radicle elongation. As a technical advance, Zhang *et al.* (2014) provided a new method to measure solely the endosperm using adhesive tape to hold the soft and delicate endosperm tissue in place (Fig. 7B, C). A decrease in the ME puncture force was evident in association with ER while the CE did not weaken (Zhang *et al.*, 2014). Further to this, ABA inhibits and ethylene promotes the lettuce endosperm weakening and ER (Fig. 7C) (Zhang *et al.*, 2014; Chen *et al.*, 2016).

A crucial role for hormonal regulation of endosperm weakening and cell wall remodelling during lettuce germination in light and temperature responses was established (Bewley, 1997*a*; Huo *et al.*, 2013; Chen *et al.*, 2016). The endosperm weakening precedes the completion of lettuce germination by typical ER and radicle emergence (Fig. 7A). If the endosperm weakening is inhibited by treatment of lettuce seeds with sodium dichloroisocyanurate (SDIC), the embryo expands but cannot protrude through the endosperm (Pavlišta and Haber, 1970). Thus the embryo starts to buckle within its hull and may eventually germinate despite an atypical ER (Fig. 7A). Lettuce endosperm cell walls contain L-arabinofuranose, and evidence was provided to propose that α-L-arabinofuranosidase accumulates and causes the endosperm weakening during lettuce germination (Zhang *et al.*, 2014; Liu *et al.*, 2015). SDIC treatment inhibited the enzyme accumulation in association with inhibited endosperm weakening. SDIC was also instructive to establish a role for aROS in lettuce endosperm weakening as well as in lettuce embryo expansion growth (Zhang *et al.*, 2014). Further to this, the accumulation of cellulase activity in the lettuce ME and its regulation by ABA and ethylene was proposed to play a role in both processes (Zhang *et al.*, 2014; Chen *et al.*, 2016). The current findings from various endospermic species from the Asterid clade (Table 1) therefore support the view that endosperm weakening resulting in a decreased ME resistance as quantified by puncture force analysis is mediated through the combined or successive action of several cell wall-modifying hydrolases, transgycolases, expansins, and directly acting aROS. While biochemical mechanisms mediating cell wall loosening such as aROS seem to be shared between embryo expansion growth and endosperm weakening, the differences in cell wall composition and the spatiotemporal accumulation patterns of specific cell wall-modifying proteins or aROS may provide in addition cell separation as a hallmarks of the endosperm weakening process (Bethke *et al.*, 2007 Morris *et al.*, 2011; Lee *et al.*, 2012).

Endosperm weakening in Rosid clade seeds

An increase in the relative embryo to seed ratio is evident as a general evolutionary trend in the Rosids when compared with

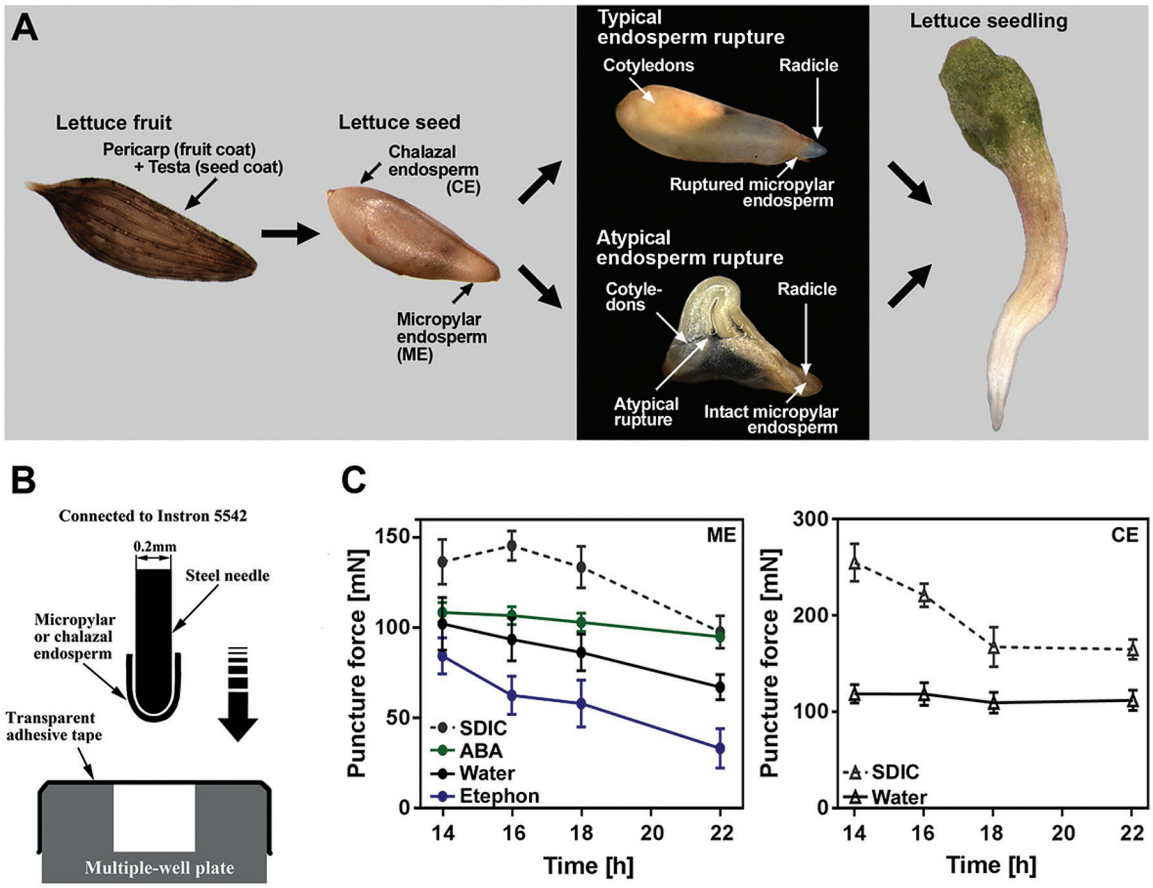

Fig. 7. Endosperm weakening and germination in the Solanaceae (thin endosperm): Lettuce (*Lactuca sativa*). (A) Lettuce fruit/seed morphology, endosperm rupture, and seedling growth. Typical and atypical endosperm rupture (buckling) is shown. Typically the endosperm is ruptured at the micropylar end of the endosperm. Rarely or if endosperm weakening is prevented, lettuce shows atypical endosperm rupture. (B) Puncture force method for lettuce. The lettuce endosperm is placed on top of a thin steel needle and is lowered (punctured) through adhesive tape. (C) The endosperm weakening of the micropylar and the chalazal endosperm is shown versus time. The micropylar endosperm (ME) shows a weakening during germination. The force to rupture the ME is lowered by the addition of ethephon, an ethylene-releasing compound, and the weakening is inhibited by ABA. The chalazal endosperm (CE) shows a higher resistance compared with the ME and does not appreciably weaken (water). Treatment with sodium dichloroisocyanurate (SDIC) causes an initial CE stiffening which is weakened during imbibition. Note that SDIC treatment is associated with the inhibition of ME weakening and with embryo buckling. Error bars indicate the SEM. B and C modified from Yu Zhang *et al*. Involvement of reactive oxygen species in endosperm cap weakening and embryo elongation growth during lettuce seed germination. Journal of Experimental Botany (2014) 65 (12): 3189–3200. Published by Oxford University Press on behalf of the Society for Experimental Biology online here: http://jxb.oxfordjournals.org/content/65/12/3189; and Chen *et al.* 2016. Abscisic acid and ethephon regulation of cellulase in the endosperm cap and radicle during lettuce seed germination. Journal of Integrative Plant Biology 58, 859–869 with permission from Wiley.

the Asterids and the basal angiosperms (Finch-Savage and Leubner-Metzger, 2006; Baskin and Baskin, 2014). It is therefore an interesting question whether endosperm weakening as described in the previous section for Asterid seeds is also widespread and has conserved role(s) also in endospermic Rosid seeds. Puncture force measurements showed that weakening of the thin perisperm–endosperm envelope of muskmelon (*Cucumis melo*, Cucurbitaceae) seeds is evident prior to radicle protrusion (Table 1) (Welbaum *et al.*, 1995, 1998). Possible roles for endo-β-1,4-mannanase and β-1,3-glucanase in mediating this weakening were proposed. Callose deposition is responsible for the apoplastic semi-permeability of the *Cucumis* perisperm–endosperm envelope and may determine the solute and ABA permeability (Yim and Bradford, 1998; Amritphale *et al.*, 2005, 2010).

Endospermic legume (Fabaceae) seeds such as fenugreek (*Trigonella foenum-graecum*) and clover (*Trifolium* spp.) are extremely hard in the mature dry state due to extensive galactomannan deposits within the cell walls of their endosperms (Bewley, 1997*a*). This galactomannan serves a dual purpose; it regulates the seed water balance during germination by becoming mucilaginous during imbibition and is subsequently mobilized to fuel seedling growth (Reid and Bewley, 1979). This mobilization is achieved by the secretion of hydrolases including endo-β-1,4-mannanase and α-galactosidase from the outermost living aleurone layer of the otherwise dead endosperm (Dirk *et al.*, 1999; Gong *et al.*, 2005), and is a process controlled by ABA and ethylene (Buckeridge, 2010). Radicle emergence preceded the accumulation of endo-β-1,4-mannanase activity which excludes their role in endosperm weakening of these endospermic legumes. Cell wall thickenings were mainly present in the lateral endosperm, but mostly absent in the micropylar endosperm of fenugreek. Gong *et al.* (2005) therefore concluded that in many endospermic legumes the micropylar endosperm presents a lower physical constraint, and hence a structure

predisposed to permit radicle protrusion. In contrast, for the endospermic legume wand riverhemp (*Sesbania virgata*), results by Lisboa *et al.* (2006) suggest that in addition to regulating seed water uptake, the galactomannan degradation in the micropylar endosperm is required for weakening and radicle protrusion. The roles of galactomannans and other cell wall polysaccharides in legume seed endosperms is a focus of ongoing research as summarized by Buckeridge (2010). This should in future also include seed biomechanics, as for none of the endospermic legume seeds was the proposed endosperm weakening directly demonstrated using the puncture force method. With this abundant biochemical knowledge, these endospermic legume seeds may indeed provide excellent systems for studying the biomechanics of endosperm weakening in Rosid seeds.

With tomato, tobacco, lettuce, coffee, and other species, several systems for endosperm weakening have been established in the Asterids clade for which the tissue weakening has been directly demonstrated by the puncture force method (Table 1). In contrast to this, in the Rosid clade, besides the perisperm–endosperm weakening in Cucurbitaceae seeds (Table 1), garden cress (*Lepidium sativum*, Brassicaceae) has emerged as an established system for Rosid endosperm weakening (Linkies and Leubner-Metzger, 2012). There is in addition plenty of indirect evidence in strong support of the view that endosperm weakening is a widespread phenomenon in the Rosid clade and also, for example, crucial during *Arabidopsis thaliana* seed germination (Müller *et al.*, 2006; Penfield *et al.*, 2006; Bethke *et al.*, 2007; Yang *et al.*, 2008; Linkies *et al.*, 2009; Denay *et al.*, 2014; Scheler *et al.*, 2015). This includes microscopically visible early reserve breakdown in the ME including vacuolation of protein storage vacuoles which is promoted by GA and inhibited by ABA (Bethke *et al.*, 2007), altered seed germination and dormancy responses of mutants and transgenic lines (Debeaujon *et al.*, 2000; Bentsink and Koornneef, 2008; Denay *et al.*, 2014), as well as local cell separation at the site of radicle protrusion in the *A. thaliana* ME (Bethke *et al.*, 2007). Scarification ('embryo rescue') by removing the testa and endosperm results in embryo growth from dormant *A. thaliana* seeds (Graeber *et al.*, 2014). Figure 8 shows that the endosperm is sufficient to prevent germination when the testa is removed from dormant *A. thaliana* seeds (Bethke *et al.*, 2007). Treatment with dormancy-releasing compounds induces endosperm rupture and radicle emergence (Fig. 8D). This demonstrates that the PD of *A. thaliana* seeds is coat dormancy imposed by the endosperm (Bethke *et al.*, 2007) and the testa (Debeaujon *et al.*, 2000). Both species, *A. thaliana* and *L. sativum*, have, like lettuce, a thin living endosperm encasing the embryo, its one and 2–3 cell layers, respectively (Müller *et al.*, 2006; Bethke *et al.*, 2007). Besides seed size, a major difference between the two species is that while *A. thaliana* seeds have PD, *L. sativum* belong to the ND class of seed dormancy (Willis *et al.*, 2014). Overexpression of the *A. thaliana* dormancy gene DOG1 resulted in establishing PD in transgenic *L. sativum* seeds (DOG1-OE in Fig. 8). This PD of DOG1-OE *L. sativum* seeds is coat dormancy imposed by the altered endosperm; the excised embryos grow and exhibit no difference in their embryo growth potential when compared with the wild type (Graeber *et al.*, 2014). The physiological coat dormancy of DOG1-OE *L. sativum* and *A. thaliana* is therefore imposed by a block to induce endosperm weakening as the actual downstream mechanism to prevent radicle emergence (Fig. 8). It is known from earlier biomechanical work with ND *L. sativum* seeds (Müller *et al.*, 2006) that early during imbibition an embryo signal is necessary and sufficient to induce *L. sativum* endosperm weakening. Upstream signalling by GA is consistent with the importance of seed compartment interactions in the control of germination timing (Müller *et al.*, 2006; Nonogaki, 2006; Yan *et al.*, 2014). The endosperm weakening in ND *L. sativum* wild-type seeds has roles in regulating the speed, uniformity, and response of seed germination towards environmental cues.

For *L. sativum* (Morris *et al.*, 2011) and *Lactuca sativa* (Dutta *et al.*, 1994), incubation of weakening-induced isolated endosperms leads to hormonally regulated cell wall autolysis and eventually a hole may form in the ME. The possible relationship of the cell wall autolysis to endosperm weakening is supported by its hormonal regulation, and for the cell wall autolysis it is clear that transcription and translation are both required (Morris *et al.*, 2011). Due to the larger size, direct measurements of different seed compartments by the puncture force method are possible with *L. sativum* seeds, while direct puncture force measurements of the closely related tiny Arabidopsis seed have not yet been achieved. Direct biomechanical measurement of *L. sativum* endosperm weakening by the puncture force method demonstrated that an early signal from the embryo is required to induce it (Müller *et al.*, 2006). When MEs were isolated very early during imbibition—prior to their induction (for *L. sativum* before 5 h)—they did not weaken. When, however, 8 h-isolated MEs were incubated further, the weakening, hole formation, and autolysis proceeded in an organ-autonomous process (Müller *et al.*, 2006; Linkies *et al.*, 2009; Morris *et al.*, 2011). Further experimentation has shown that in isolated *L. sativum* MEs, GA can replace the embryo signal, that *de novo* GA biosynthesis occurs in the endosperm, and that the weakening is regulated, at least in part, by the GA/ABA ratio. Treatment of seeds with ABA caused a delayed onset and slower rate of ME weakening. The ER of seeds without and with ABA treatment exhibited a very similar relationship to the decreasing ME puncture force (Linkies *et al.*, 2009). While the absolute puncture force values differed by a factor of two between the ME resistances of two *L. sativum* cultivars at 8 h, a similar ~2-fold relative reduction in the resistance was evident at 18 h, and this ME weakening was in both cases inhibited by ABA (Graeber *et al.*, 2010). Like GA, ethylene also promotes *L. sativum* ME weakening and counteracts the ABA inhibition. Ethylene signalling is required, and during the late phase of germination the oxygen-requiring production of ethylene from its precursor 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC oxidase (ACO) activity accumulation enhances the progression of ER (Linkies *et al.*, 2009) These findings for the hormonal regulation of *L. sativum* ME weakening are summarized in Fig. 8E and in a review by Linkies and Leubner-Metzger (2012).

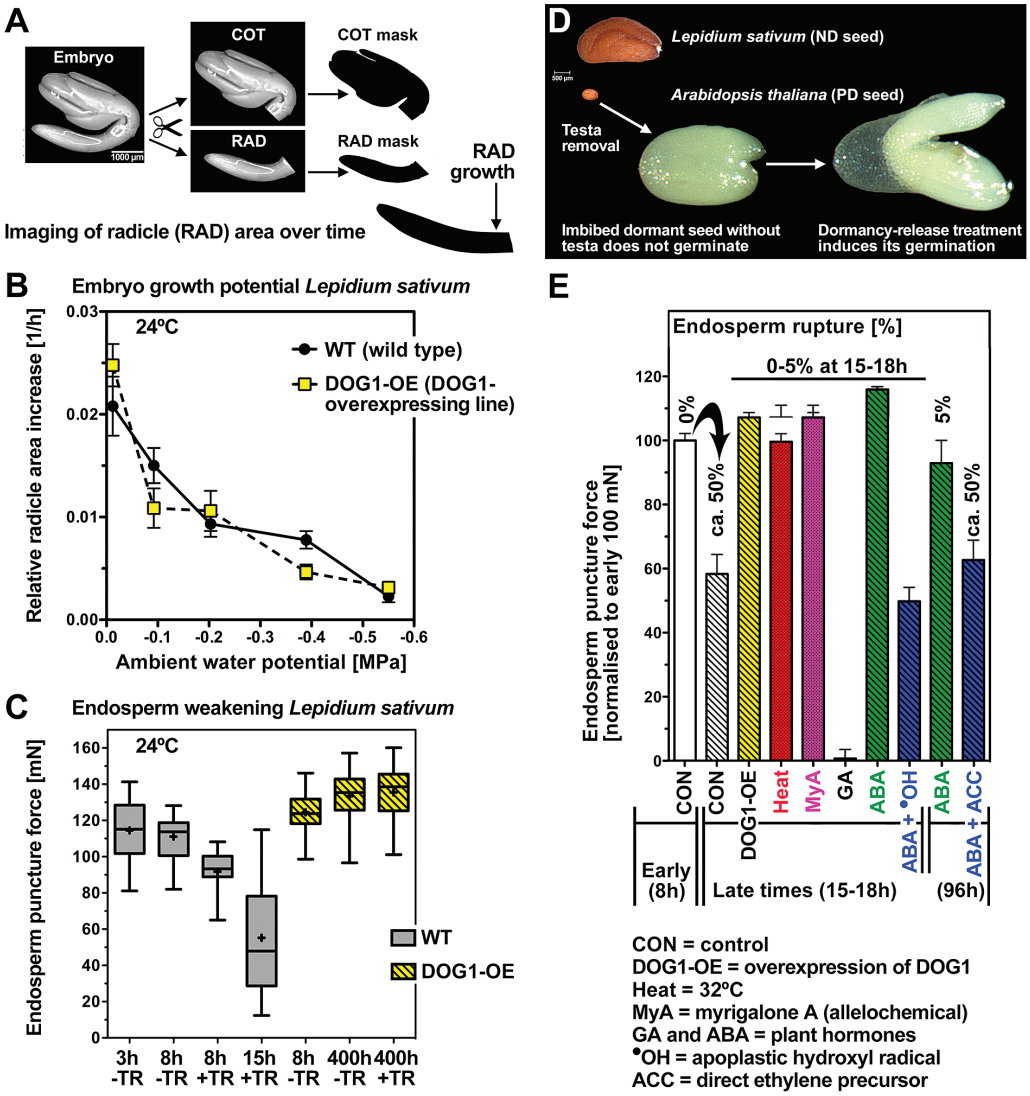

Fig. 8. Coat-imposed dormancy and control of Brassicaceae germination timing by the endosperm. (A) Image analysis of *Lepidium sativum* embryo growth (after Voegele *et al.*, 2012. Embryo growth, testa permeability, and endosperm weakening are major targets for the environmentally regulated inhibition of *Lepidium sativum* seed germination by myrigalone A. Journal of Experimental Botany 63, 5337–5350. Published by Oxford University Press on behalf of the Society for Experimental Biology). (B) Embryo growth potential and (C) micropylar endosperm weakening of *L. sativum* wild type and a transgenic line overexpressing the DOG1 dormancy gene (DOG1-OE; after Graeber *et al.*, 2014. DELAY OF GERMINATION 1 mediates a conserved coat-dormancy mechanism for the temperature- and gibberellin-dependent control of seed germination. Proceedings of the National Academy of Sciences, USA 111, E3571–E3580, with permission). (D) Endosperm-mediated coat dormancy of *Arabidopsis thaliana* seeds revealed by testa removal (after Bethke *et al.*, 2007 copyrighted by the American Society of Plant Biologists and reprinted with permission). (E) Summary of control of *L. sativum* germination timing by micropylar endosperm weakening. Note that *L. sativum* wild type seeds are non-dormant, but that DOG1-OE establishes physiological dormancy mediated by the inhibition of endosperm weakening. The regulation of *L. sativum* wild-type seed endosperm weakening by abiotic (temperature) and biotic (allelochemical) factors as well as by hormones and apoplastic reactive oxygen species is presented. Error bars indicate the SEM.

The endosperm cell wall composition of the Brassicaceae *L. sativum* and *A. thaliana* indicated conserved architectures, with cellulose, unesterified homogalacturonan, and arabinan being major components (Lee *et al.*, 2012). In contrast to the endosperm of Solanaceae seeds which are rich in heteromannans (~65% in tobacco), the endosperm of *L. sativum* contains only 3.5% heteromannans (Lee *et al.*, 2012). Despite the low heteromannan content, regulated endo-β-1,4-mannanase gene orthologue expression was evident in the endosperm of *L. sativum* and *A. thaliana*, and together with the knockout-mutants is in agreement with roles during germination (Iglesias-Fernández *et al.*, 2011; Morris *et al.*, 2011). The spatiotemporal regulation of their gene expression and possible roles in *L. sativum* and *A. thaliana* endosperm weakening of cell wall-remodelling proteins targeting the cellulose microfibrils or the matrix polysaccharides in which they are embedded, namely hemicelluloses and pectins, is described in detail in Morris *et al.* (2011) and Scheler *et al.* (2015). Recent work by Graeber *et al.* (2014) shows that GA metabolism itself and the expression of GA-regulated cell wall-remodelling genes including expansins and xyloglucan endotransglycolases/hydrolases are severely altered in DOG1-OE *L. sativum* seeds (Fig. 8). DOG1 overexpression did not result in an altered embryo

growth potential, but blocked ME weakening in a temperature-dependent manner.

That the endosperm is a mediator of communication between the embryo and its environment has been summarized by Yan *et al.* (2014). In *L. sativum*, DOG1 exerts its temperature-dependent control of germination timing exclusively via the control of ME weakening: in DOG1-OE *L. sativum*, the weakening occurs at 18 ºC, but is inhibited at 24 ºC (Graeber *et al.*, 2014). Interestingly, thermoinhibition of wild-type *L. sativum* seeds is also mediated by inhibiting ME weakening (Fig. 8E). In addition to temperature as an abiotic environmental cue, biotic environmental cues such as the allelochemical myrigalone A (MyA) also exert germination-inhibiting effects, at least in part, by inhibiting ME weakening (Fig. 8E). As for DOG1 overexpression, MyA has the seed's GA metabolism as a target (Oracz *et al.*, 2012; Voegele *et al.*, 2012). In addition to this, MyA also interferes with the production of aROS required to mediate embryo expansion growth and ME weakening. Figure 9 shows that aROS is produced in the growth zone (hypocotyl/radicle) of the *L. sativum* embryo and this production is inhibited by ABA and promoted by GA and ethylene (Linkies *et al.*, 2009; Müller *et al.*, 2009). While ABA inhibits the ME weakening, the artificial production of aROS in the presence of ABA caused endosperm weakening (Figs 8E, 9). Müller *et al.* (2009) showed that aROS-mediated germination is caused by direct scissoring of cell wall polysaccharides. Distinct and tissue-specific target polysaccharides were evident, and the hormonally regulated aROS production serves important roles in embryo expansion growth and in ME weakening.

In summary, for the hormonal regulation of the biomechanically quantified eudicot endosperm weakening, it appears that it is similar in Asterid and Rosid seeds with respect to its promotion by GA and ethylene (Table 1). For seeds with thin endosperm such as lettuce (Asterids) and cress (Rosids), the ABA inhibition also appears to be conserved, but so far there is no evidence for a biphasic weakening process in the Rosid seeds, as was described for Asterid seeds with thick endosperm (tomato and coffee). The endospermic legume (Rosids) seeds have thicker endosperm and may provide excellent systems to study this question.

Biomechanics of cereal grain endosperm weakening and germination

A mature cereal grain is a single-seeded fruit (caryopsis) with several major compartments and bran tissues (Fath *et al.*, 2000; Burton and Fincher, 2014; Domínguez and Cejudo, 2014). The highly differentiated embryo is, with its scutellum, in direct proximity to the large starchy endosperm storage compartment (dead tissue) which is encased by the aleurone layer (living endosperm tissue) and the dead bran layers (testa and pericarp tissues). *In vivo* ^{1}H-NMR microimaging during cereal grain imbibition suggests several preferred pathways for water uptake which include the micropyle as an opening, the embryo and scutellum as water distribution organs, and parts of the bran layers which allow fast water uptake during the very early phases of wheat imbibition (Rathjen *et al.*, 2009). The ratio between the hormones ABA (inhibiting) and GA (promoting) control germination and post-germination reserve mobilization of cereal grains in which GA serves as a signal produced by the embryo to induce the aleurone layer to express and/or secrete hydrolytic enzymes into the starchy endosperm (Fath *et al.*, 2000; Burton and Fincher, 2014; Domínguez and Cejudo, 2014). In agreement with this role, the cereal aleurone is a living tissue layer of the wheat grain, but undergoes PCD during germination and seedling establishment. Tensile tests have been carried out to determine the mechanical properties of the various wheat grain bran layers (Antoine *et al.*, 2003). In agreement with these observations and the PCD of the aleurone layer during germination and starch mobilization, we recently showed by puncture force

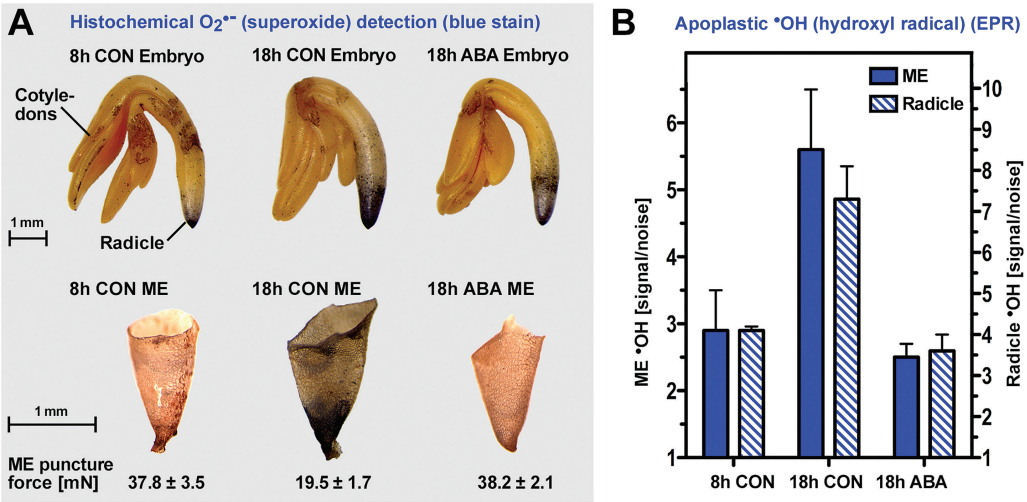

Fig. 9. Accumulation of apoplastic reactive oxygen species (aROS) during *Lepidium sativum* germination (adapted from Müller *et al.* 2009 copyrighted by the American Society of Plant Biologists and reprinted with permission). (A) Apoplastic superoxide ($O_2^{\bullet-}$) in the embryos and the micropylar endosperm of seeds imbibed in continuous white light. NBT (nitroblue tetrazolium) histostaining shows production of apoplastic $O_2^{\bullet-}$. (B) In vivo detection of apoplastic ·OH production in the micropylar endosperm (ME) and the radicle of *L. sativum* during seed germination without and with ABA added. Note the different scales of the *y*-axes for the ME and the radicle.

measurements that GA treatment of isolated aleurone layers promotes the weakening of this living endosperm tissue, while GA does not affect the dead intermediate (testa and inner pericarp) layers of wheat grains (J. Hourston *et al.*, unpublished). Novel tools are required to investigate further the biomechanical changes of cereal grain tissues including the coleorhiza covering the radicle for which a similar ABA-regulated role for dormancy and germination timing as for the eudicot seed ME has been proposed (Millar *et al.*, 2006).

Mechanosensing in seeds

Sensing mechanical forces to control gene expression, tissue growth, and fate is an essential part of plant life (Monshausen and Haswell, 2013). We propose that seeds constitute an excellent system for studying mechanosensing due to the striking interactions between seed-covering layers and the distinct fates leading either to growth (embryo) or to death (ME) of tissues. Mechanical signalling involved in seed coat expansion has been postulated by Creff *et al.* (2015). Their study with *A. thaliana* seeds showed that mechanical stress exerted by the embryo and endosperm is perceived in a mechanosensitive layer in the seed coat. Recently nano-indentation has been used to measure the stiffness of the endosperm of developing *A. thaliana* seeds (Fourquin *et al.*, 2016). A stiffer endosperm was found in *zou* mutants compared with wild-type seeds, and embryo growth was inhibited as the stiff covering layer presumably prevents its expansion (Yang *et al.*, 2008; Fourquin *et al.*, 2016). In agreement with the postulation of these mechanosensitive tissues is the 'touch' gene hypothesis (Monshausen and Gilroy, 2009; Nonogaki, 2013) stating that the induction of ME gene expression is caused by the pushing force of the elongating radicle. This could be in an interplay with their hormonal regulation. Among the 'touch' genes are those encoding cell wall-remodelling proteins such as expansins. Direct evidence for the ME mechanosensing and signalling of this gene induction in seeds is, however, still lacking. Furthermore, seed osmosensing and signalling and its interplay with plant hormones might play a key role during germination, as the water uptake and the water content play major roles in seed germination for the mechanical properties of cell walls. The combination of molecular and biomechanical work is promising to unravel the underpinning mechanisms of the germination process and the endosperm weakening. Unravelling the complex regulation of seed germination and its molecular basis to understand the cell wall-related changes in tissue mechanics in manifold species and with integrative approaches is needed to gain a comprehensive view on the germination process. Despite a strong enthusiasm to understand the vital process of seed germination, there are still open questions (Nonogaki *et al.*, 2010). The acquired evidence reveals that endosperm weakening involves evolutionarily conserved as well as species-specific molecular, biochemical, and biomechanical mechanisms. These mechanisms have the endosperm cell wall properties as target and strongly suggest that further integrative and interdisciplinary studies with several seeds from distinct phylogenetic clades are required. The consideration of crop seeds in these future studies is of utmost relevance to seed industry. It also extends the investigations of the biomechanical seed properties of the natural seed 'coats' to artificial seed 'coats' and the mechanical properties of pellet materials.

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References

Aizenberg J, Weaver JC, Thanawala MS, Sundar VC, Morse DE, Fratzl P. 2005. Skeleton of *Euplectella* sp.: structural hierarchy from the nanoscale to the macroscale. Science **309,** 275–278.

Altuntas E, Erkol M. 2011. The effects of moisture content, compression speeds, and axes on mechanical properties of walnut cultivars. Food and Bioprocess Technology **4,** 1288–1295.

Altuntas E, Özkan Y. 2008. Physical and mechanical properties of some walnut (*Juglans regia* L.) cultivars. International Journal of Food Engineering **4,** 1556–3758.

Altuntas E, Yıldız M. 2007. Effect of moisture content on some physical and mechanical properties of faba bean (*Vicia faba* L.) grains. Journal of Food Engineering **78,** 174–183.

Amritphale D, Ramakrishna P, Singh B, Sharma SK. 2010. Solute permeation across the apoplastic barrier in the perisperm–endosperm envelope in cucumber seeds. Planta **231,** 1483–1494.

Amritphale D, Yoneyama K, Takeuchi Y, Ramakrishna P, Kusumoto D. 2005. The modulating effect of the perisperm–endosperm envelope on ABA-inhibition of seed germination in cucumber. Journal of Experimental Botany **56,** 2173–2181.

Anese S, da Silva EAA, Davide AC, Rocha Faria JM, Soares GCM, Matos ACB, Toorop PE. 2011. Seed priming improves endosperm weakening, germination, and subsequent seedling development of *Solanum lycocarpum* St. Hil. Seed Science and Technology **39,** 125–139.

Antoine C, Peyron S, Mabille F, Lapierre C, Bouchet B, Abecassis J, Rouau X. 2003. Individual contribution of grain outer layers and their cell wall structure to the mechanical properties of wheat bran. Journal of Agricultural and Food Chemistry **51,** 2026–2033.

Arana MV, Burgin MJ, de Miguel LC, Sánchez RA. 2007. The very-low-fluence and high-irradiance responses of the phytochromes have antagonistic effects on germination, mannan-degrading activities, and DfGA3ox transcript levels in Datura ferox seeds. Journal of Experimental Botany **58,** 3997–4004.

Arana MV, de Miguel LC, Sánchez RA. 2006. A phytochrome-dependent embryonic factor modulates gibberellin responses in the embryo and micropylar endosperm of *Datura ferox* seeds. Planta **223,** 847–857.

Arcila J, Mohapatra SC. 1983. Development of tobacco seedling. 2. Morphogenesis during radicle protrusion. Tobacco Science **27,** 35–40.

Arcila J, Mohapatra SC. 1992. Effect of protein synthesis inhibitors on tobacco seed germination and seedling emergence. Journal of Plant Physiology **139,** 460–466.

Ashby MF. 1989. On the engineering properties of materials. Acta Metallurgica **37,** 1273–1293.

Ashby MF. 2007. Materials selection in mechanical design. Heidelberg: Spektrum-Akademischer Verlag.

Ashby MF, Gibson LJ, Wegst U, Olive R. 1995. The mechanical properties of natural materials. I. Material property charts. Proceedings: Mathematical and Physical Sciences **450,** 123–140.

Ashby MF, Shercliff H, Cebon D. 2013. Materials: engineering, science, processing and design, North American edn. Amsterdam: Elsevier Science.

Avery GSJ. 1933. Structure and germination of tobacco seed and the developmental anatomy of the seedling plant. American Journal of Botany **20,** 309–327.

Bartsch JA, Haugh GC, Athow KL, Peart RM. 1986. Impact damage to soybean seed. American Society of Agricultural and Biological Engineers **29,** 0582–0586.

Baskin CC. 2003. Breaking physical dormancy in seeds—focussing on the lens. New Phytologist **158,** 227–238.

Baskin CC, Baskin JM. 2014. Seeds: ecology, biogeography, and, evolution of dormancy and germination. Amsterdam: Elsevier Science.

Bassel GW, Stamm P, Mosca G, Barbier de Reuille P, Gibbs DJ, Winter R, Janka A, Holdsworth MJ, Smith RS. 2014. Mechanical constraints imposed by 3D cellular geometry and arrangement modulate growth patterns in the Arabidopsis embryo. Proceedings of the National Academy of Sciences, USA **111,** 8685–8690.

Bay APM, Bourne MC, Taylor AG. 1996. Effect of moisture content on compressive strength of whole snap bean (*Phaseolus vulgaris* L.) seeds and separated cotyledons. International Journal of Food Science and Technology **31,** 327–331.

Benech-Arnold RL. 2004. Inception, maintenance, and termination of dormancy in grain crops: physiology, genetics, and environmental control. In: Benech-Arnold RL, Sanchez RA, eds. Handbook of seed physiology: applications to agriculture. New York: Food Product Press and The Haworth Reference Press, 169–198.

Bentsink L, Koornneef M. 2008. Seed dormancy and germination. Arabidopsis Book **6,** e0119.

Bethke PC, Libourel IG, Aoyama N, Chung YY, Still DW, Jones RL. 2007. The Arabidopsis aleurone layer responds to nitric oxide, gibberellin, and abscisic acid and is sufficient and necessary for seed dormancy. Plant Physiology **143,** 1173–1188.

Bewley JD. 1997*a*. Breaking down the walls—a role for endo-ß-mannanase in release from seed dormancy? Trends in Plant Science **2,** 464–469.

Bewley JD. 1997*b*. Seed germination and dormancy. The Plant Cell **9,** 1055–1066.

Bewley JD, Black M. 1994. Seeds—physiology of development and germination. New York: Plenum Press.

Blumenthal A, Lerner HR, Werker E, Poljakoff-Mayber A. 1986. Germination preventing mechanisms in Iris seeds. Annals of Botany **58,** 551–561.

Bradford KJ, Chen F, Cooley MB, *et al*. 2000. Gene expression prior to radicle emergence in imbibed tomato seeds. In: Black M, Bradford KJ, Vázquez-Ramos J, eds. Seed biology: advances and applications. Wallingford, UK: CABI Publishers, 231–252.

Brett CT, Waldron K. 1996. Physiology and biochemistry of plant cell walls. Berlin: Springer.

Buckeridge MS. 2010. Seed cell wall storage polysaccharides: models to understand cell wall biosynthesis and degradation. Plant Physiology **154,** 1017–1023.

Buckeridge MS, dosSantos HP, Tine MAS. 2000. Mobilisation of storage cell wall polysaccharides in seeds. Plant Physiology and Biochemistry **38,** 141–156.

Burgert I. 2006. Exploring the micromechanical design of plant cell walls. American Journal of Botany **93,** 1391–1401.

Burton RA, Fincher GB. 2014. Evolution and development of cell walls in cereal grains. Frontiers in Plant Science **5,** 456.

Chai M, Zhou C, Molina I, Fu C, Nakashima J, Li G, Zhang W, Park J, Tang Y, Jiang Q, Wang Z-Y. 2016. A class II KNOX gene, KNOX4, controls seed physical dormancy. Proceedings of the National Academy of Sciences, USA **113,** 6997–7002.

Chen B, Ma J, Xu Z, Wang X. 2016. Abscisic acid and ethephon regulation of cellulase in the endosperm cap and radicle during lettuce seed germination. Journal of Integrative Plant Biology **58,** 859–869.

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

Chen F, Nonogaki H, Bradford KJ. 2002. A gibberellin-regulated xyloglucan endotransglycosylase gene is expressed in the endosperm cap during tomato seed germination. Journal of Experimental Botany **53,** 215–223.

Chen PY, Lin AY, Lin YS, Seki Y, Stokes AG, Peyras J, Olevsky EA, Meyers MA, McKittrick J. 2008. Structure and mechanical properties of selected biological materials. Journal of the Mechanical Behavior of Biomedical Materials **1,** 208–226.

Cosgrove DJ. 2000*a*. Expansive growth of plant cell walls. Plant Physiology and Biochemistry **38,** 109–124.

Cosgrove DJ. 2000*b*. Loosening of plant cell walls by expansins. Nature **407,** 321–326.

Cosgrove DJ. 2005. Growth of the plant cell wall. Nature Reviews. Molecular Cell Biology **6,** 850–861.

Coumans M, Come D, Gaspar T. 1976. Stabilized dormancy in sugarbeet fruits. I. Seed coats as a physicochemical barrier to oxygen. Botanical Gazette **137,** 274–278.

Cranford S, Buehler MJ. 2010. Materiomics: biological protein materials, from nano to macro. Nanotechnology, Science and Applications **3,** 127–148.

Creff A, Brocard L, Ingram G. 2015. A mechanically sensitive cell layer regulates the physical properties of the Arabidopsis seed coat. Nature Communications **6,** 6382.

Currey JD. 2005. Materials science. Hierarchies in biomineral structures. Science **309,** 253–254.

da Silva EA, Toorop PE, Nijsse J, Bewley JD, Hilhorst HW. 2005. Exogenous gibberellins inhibit coffee (*Coffea arabica* cv. Rubi) seed germination and cause cell death in the embryo. Journal of Experimental Botany **56,** 1029–1038.

da Silva EA, Toorop PE, van Aelst AC, Hilhorst HW. 2004. Abscisic acid controls embryo growth potential and endosperm cap weakening during coffee (*Coffea arabica* cv. Rubi) seed germination. Planta **220,** 251–261.

da Silva EA, Toorop PE, Van Lammeren AA, Hilhorst HW. 2008. ABA inhibits embryo cell expansion and early cell division events during coffee (*Coffea arabica* 'Rubi') seed germination. Annals of Botany **102,** 425–433.

Davies RM, Zibokere DS. 2011. Effects of moisture content on some physical and mechanical properties of three varieties of cowpea (*Vigna unguiculata* L. Walp.) Agricultural Engineering International **13,** 1–8.

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

de Mason DA, Sexton R, Reid JSG. 1983. Structure, composition and physiological state of the endosperm of *Phoenix dactylifera* L. Annals of Botany **52,** 71–80.

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

Dirk LMA, Vanderkrol AR, Vreugdenhil D, Hilhorst HWM, Bewley JD. 1999. Galactomannan, soluble sugar and starch mobilization following germination of *Trigonella foenum-graecum* seeds. Plant Physiology and Biochemistry **37,** 41–50.

Domínguez F, Cejudo FJ. 2014. Programmed cell death (PCD): an essential process of cereal seed development and germination. Frontiers in Plant Science **5,** 366.

Dutta S, Bradford KJ, Nevins DJ. 1994. Cell-wall autohydrolysis in isolated endosperms of lettuce (*Lactuca sativa* L.). Plant Physiology **104,** 623–628.

Ebenstein DM, Pruitt LA. 2006. Nanoindentation of biological materials. Nano Today **1,** 26–33.

Elbaum R, Abraham Y. 2014. Insights into the microstructures of hygroscopic movement in plant seed dispersal. Plant Science **223,** 124–133.

Fahloul D, Scanlon MG, Dushnicky LG, Symons SJ. 1996. The fracture toughness of pea testa in relation to temperature abuse during frozen storage. Food Research International **29,** 791–797.

Fath A, Bethke P, Lonsdale J, Meza-Romero R, Jones R. 2000. Programmed cell death in cereal aleurone. Plant Molecular Biology **44,** 255–266.

Finch-Savage WE, Clay HA. 1997. The influence of embryo restraint during dormancy loss and germination of *Fraxinus excelsior* seeds. In:

Ellis RH, Black M, Murdoch AJ, Hong TD, eds. Basic and applied aspects of seed biology. Dordrecht, The Netherlands: Kluwer Academic Publishers, 245–253.

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

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

Fratzl P, Weinkamer R. 2007. Nature's hierarchical materials. Progress in Materials Science **52,** 1263–1334.

Fry SC. 2004. Primary cell wall metabolism: tracking the careers of wall polymers in living plant cells. New Phytologist **161,** 641–675.

Fry SC, Smith RC, Renwick KF, Martin DJ, Hodge SK, Matthews KJ. 1992. Xyloglucan endotransglycosylase, a new wall-loosening enzyme activity from plants. Biochemical Journal **282,** 821–828.

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

Gao H, Ji B, Jaeger IL, Arzt E, Fratzl P. 2003. Materials become insensitive to flaws at nanoscale: lessons from nature. Proceedings of the National Academy of Sciences, USA **100,** 5597–5600.

Georget DMR, Smith AC, Waldron KW. 2001. Effect of ripening on the mechanical properties of Portuguese and Spanish varieties of olive (*Olea europaea* L.). Journal of the Science of Food and Agriculture **81,** 448–454.

Gibson LJ. 2012. The hierarchical structure and mechanics of plant materials. Journal of the Royal Society, Interface **9,** 2749–2766.

Gong X, Bassel GW, Wang A, Greenwood JS, Bewley JD. 2005. The emergence of embryos from hard seeds is related to the structure of the cell walls of the micropylar endosperm, and not to endo-beta-mannanase activity. Annals of Botany **96,** 1165–1173.

Gong X, Derek Bewley J. 2007. Sorting out the LeMANs: endo-β-mannanase genes and their encoded proteins in tomato. Seed Science Research **17,** 143–154.

Gordon JE, Jeronimidis G, Richardson MOW. 1980. Composites with high work of fracture. Philosophical Transactions of the Royal Society A: Mathematical and Physical Sciences **294,** 545–550.

Gossot O, Geitmann A. 2007. Pollen tube growth: coping with mechanical obstacles involves the cytoskeleton. Planta **226,** 405–416.

Graeber K, Linkies A, Müller K, Wunchova A, Rott A, Leubner-Metzger G. 2010. Cross-species approaches to seed dormancy and germination: conservation and biodiversity of ABA-regulated mechanisms and the Brassicaceae DOG1 genes. Plant Molecular Biology **73,** 67–87.

Graeber K, Linkies A, Steinbrecher T, *et al*. 2014. DELAY OF GERMINATION 1 mediates a conserved coat-dormancy mechanism for the temperature- and gibberellin-dependent control of seed germination. Proceedings of the National Academy of Sciences, USA **111,** E3571–E3580.

Groot SP, Karssen CM. 1987. Gibberellins regulate seed germination in tomato by endosperm weakening: a study with gibberellin-deficient mutants. Planta **171,** 525–531.

Groot SP, Kieliszewska-Rokicka B, Vermeer E, Karssen CM. 1988. Gibberellin-induced hydrolysis of endosperm cell walls in gibberellin-deficient tomato seeds prior to radicle protrusion. Planta **174,** 500–504.

Groot SP, Karssen CM. 1992. Dormancy and germination of abscisic acid-deficient tomato seeds: studies with the sitiens mutant. Plant Physiology **99,** 952–958.

Gupta RK, Das SK. 2000. Fracture resistance of sunflower seed and kernel to compressive loading. Journal of Food Engineering **46,** 1–8.

Halmer P, Bewley JD, Thorpe TA. 1975. Enzyme to break down lettuce endosperm cell wall during gibberellin- and light-induced germination. Nature **258,** 716–718.

Hepher A, Roberts JA. 1985. The control of seed germination in *Trollius ledebouri*: the breaking of dormancy. Planta **166,** 314–320.

Hermann K, Meinhard J, Dobrev P, Linkies A, Pesek B, Hess B, Machácková I, Fischer U, Leubner-Metzger G. 2007. 1-Aminocyclopropane-1-carboxylic acid and abscisic acid during the germination of sugar beet (*Beta vulgaris* L.): a comparative study of fruits and seeds. Journal of Experimental Botany **58,** 3047–3060.

Hilhorst HWM, Downie B. 1995. Primary dormancy in tomato (Lycopersicon esculentum cv. Moneymaker): studies with the sitiens mutant. Journal of Experimental Botany **47,** 89–97.

Hilhorst HWM, Groot SPC, Bino RJ. 1998. The tomato seed as a model system to study seed development and germination. Acta Botanica Neerlandica **47,** 169–183.

Hofhuis H, Moulton D, Lessinnes T, *et al*. 2016. Morphomechanical innovation drives explosive seed dispersal. Cell **166,** 222–233.

Huo H, Dahal P, Kunusoth K, McCallum CM, Bradford KJ. 2013. Expression of 9-cis-EPOXYCAROTENOID DIOXYGENASE4 is essential for thermoinhibition of lettuce seed germination but not for seed development or stress tolerance. The Plant Cell **25,** 884–900.

Iglesias-Fernández R, Rodríguez-Gacio MC, Barrero-Sicilia C, Carbonero P, Matilla A. 2011. Three endo-β-mannanase genes expressed in the micropylar endosperm and in the radicle influence germination of *Arabidopsis thaliana* seeds. Planta **233,** 25–36.

Ikuma H, Thimann KV. 1963. The role of the seed-coats in germination of photosensitive lettuce seeds. Plant and Cell Physiology **4,** 169–185.

Inukai Y, Sakamoto T, Morinaka Y, *et al*. 2012. Root growth inhibiting, a rice endo-1,4-β-D-glucanase, regulates cell wall loosening and is essential for root elongation. Journal of Plant Growth Regulation **31,** 373–381.

Jacobsen JV, Pressman E. 1979. A structural study of germination in celery (*Apium graveolens* L.) seed with emphasis on endosperm breakdown. Planta **144,** 241–248.

Jeronimidis G. 1980. Wood, one of nature's challenging composites. Symposia of the Society for Experimental Biology **34,** 169–182.

Jeronimidis G, Atkins AG. 1995. Mechanics of biological materials and structures: Nature's lessons for the engineer. Proceedings of the Institution of Mechanical Engineers. Part C. Mechanical Engineering Science **209,** 221–235.

Ji B, Gao H. 2004. Mechanical properties of nanostructure of biological materials. Journal of the Mechanics and Physics of Solids **52,** 1963–1990.

Junttila O. 1973. The mechanism of low temperature dormancy in mature seeds of Syringa species. Physiologia Plantarum **29,** 256–263.

Kelly KM, van Staden J, Bell WE. 1992. Seed coat structure and dormancy. Plant Growth Regulation **11,** 201–209.

Kılıçkan A, Güner M. 2008. Physical properties and mechanical behavior of olive fruits (*Olea europaea* L.) under compression loading. Journal of Food Engineering **87,** 222–228.

Koornneef M, Bentsink L, Hilhorst H. 2002. Seed dormancy and germination. Current Opinion in Plant Biology **5,** 33–36.

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

Leubner-Metzger G. 2003. Functions and regulation of ß-1,3-glucanase during seed germination, dormancy release and after-ripening. Seed Science Research **13,** 17–34.

Leubner-Metzger G, Frundt C, Vogeli-Lange R, Meins F Jr. 1995. Class I [beta]-1,3-glucanases in the endosperm of tobacco during germination. Plant Physiology **109,** 751–759.

Leubner-Metzger G, Meins F Jr. 2000. Sense transformation reveals a novel role for class I beta-1, 3-glucanase in tobacco seed germination. The Plant Journal **23,** 215–221.

Linkies A, Graeber K, Knight C, Leubner-Metzger G. 2010. The evolution of seeds. New Phytologist **186,** 817–831.

Linkies A, Leubner-Metzger G. 2012. Beyond gibberellins and abscisic acid: how ethylene and jasmonates control seed germination. Plant Cell Reports **31,** 253–270.

Linkies A, Müller K, Morris K, *et al*. 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.

Liptay A, Schopfer P. 1983. Effect of water stress, seed coat restraint, and abscisic acid upon different germination capabilities of two tomato lines at low temperature. Plant Physiology **73,** 935–938.

Lisboa CGS, Tonini PP, Tiné MAS, Buckeridge MS. 2006. Endo-ß-mannanase from the endosperm of seeds of *Sesbania virgata* (Cav.) Pers. (Leguminosae): purification, characterisation and its dual role in germination and early seedling growth. Brazilian Journal of Plant Physiology **18,** 269–280.

Liu C, Li L, Chen B, Wang X. 2015. Suppression of α-L-arabinofuranosidase in the endosperm and atypical germination of lettuce seeds induced by sodium dichloroisocyanurate. Acta Physiologiae Plantarum **37,** 1–7.

Mabille F, Gril J, Abecassis J. 2001. Mechanical properties of wheat seed coats. Cereal Chemistry Journal **78,** 231–235.

Mann S, Weiner S. 1999. Biomineralization: structural questions at all length scales. Journal of Structural Biology **126,** 179–181.

Manz B, Müller K, Kucera B, Volke F, Leubner-Metzger G. 2005. Water uptake and distribution in germinating tobacco seeds investigated in vivo by nuclear magnetic resonance imaging. Plant Physiology **138,** 1538–1551.

Martin AC. 1946. The comparative internal morphology of seeds. American Midland Naturalist **36,** 513–660.

Mattheck C. 2004. The face of failure in nature and engineering. Forschungszentrum Karlsruhe.

Meier H, Reid JSG. 1982. Reserve polysaccharides other than starch in higher plants. In: Loewus FA, Tanner W, eds. Plant carbohydrates I: intracellular carbohydrates. Berlin: Springer, 418–471.

Meyers MA, Chen P-Y, Lin AY-M, Seki Y. 2008. Biological materials: structure and mechanical properties. Progress in Materials Science **53,** 1–206.

Millar AA, Jacobsen JV, Ross JJ, Helliwell CA, Poole AT, Scofield G, Reid JB, Gubler F. 2006. Seed dormancy and ABA metabolism in Arabidopsis and barley: the role of ABA 8'-hydroxylase. The Plant Journal **45,** 942–954.

Monshausen GB, Gilroy S. 2009. Feeling green: mechanosensing in plants. Trends in Cell Biology **19,** 228–235.

Monshausen GB, Haswell ES. 2013. A force of nature: molecular mechanisms of mechanoperception in plants. Journal of Experimental Botany **64,** 4663–4680.

Morris K, Linkies A, Müller K, Oracz K, Wang X, Lynn JR, Leubner-Metzger G, Finch-Savage WE. 2011. Regulation of seed germination in the close Arabidopsis relative *Lepidium sativum*: a global tissue-specific transcript analysis. Plant Physiology **155,** 1851–1870.

Moulia B. 2013. Plant biomechanics and mechanobiology are convergent paths to flourishing interdisciplinary research. Journal of Experimental Botany **64,** 4617–4633.

Müller K, Linkies A, Vreeburg RA, Fry SC, Krieger-Liszkay A, Leubner-Metzger G. 2009. In vivo cell wall loosening by hydroxyl radicals during cress seed germination and elongation growth. Plant Physiology **150,** 1855–1865.

Müller K, Tintelnot S, 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 and Cell Physiology **47,** 864–877.

Nathan R, Katul GG, Horn HS, Thomas SM, Oren R, Avissar R, Pacala SW, Levin SA. 2002. Mechanisms of long-distance dispersal of seeds by wind. Nature **418,** 409–413.

Ni BR, Bradford KJ. 1993. Germination and dormancy of abscisic acid- and gibberellin-deficient mutant tomato (*Lycopersicon esculentum*) seeds (sensitivity of germination to abscisic acid, gibberellin, and water potential). Plant Physiology **101,** 607–617.

Nicol F, His I, Jauneau A, Vernhettes S, Canut H, Höfte H. 1998. A plasma membrane-bound putative endo-1,4-beta-D-glucanase is required for normal wall assembly and cell elongation in Arabidopsis. EMBO Journal **17,** 5563–5576.

Niklas KJ. 1992. Plant biomechanics, an engineering approach to plant form and function. Chicago: University of Chicago Press.

Niklas KJ, Spatz HC, Vincent J. 2006. Plant biomechanics: an overview and prospectus. American Journal of Botany **93,** 1369–1378.

Nonogaki H. 2006. Seed germination—the biochemical and molecular mechanisms. Breeding Science **56,** 93–105.

Nonogaki H. 2013. TOUCH ME—'touch' genes in the micropylar endosperm. Seed Science Research **23,** 217–221.

Nonogaki H, Bassel GW, Bewley JD. 2010. Germination—still a mystery. Plant Science **179,** 574–581.

Nonogaki H, Chen F, Bradford KJ. 2007. Mechanisms and genes involved in germination sensu stricto. In: Bradford KJ, Nonogaki H, eds. Seed development, dormancy and germination. Oxford: Blackwell Publishing Ltd, 264–304.

Nonogaki H, Gee OH, Bradford KJ. 2000. A germination-specific endo-beta-mannanase gene is expressed in the micropylar endosperm cap of tomato seeds. Plant Physiology **123,** 1235–1246.

Nonogaki H, Nomaguchi M, Okumoto N, Kaneko Y, Matsushima H, Morohashi Y. 1998. Temporal and spatial pattern of the biochemical activation of the endosperm during and following imbibition of tomato seeds. Physiologia Plantarum **102,** 236–242.

Ogunjimi LAO, Aviara NA, Aregbesola OA. 2002. Some engineering properties of locust bean seed. Journal of Food Engineering **55,** 95–99.

Olsen OA. 2004. Nuclear endosperm development in cereals and *Arabidopsis thaliana*. The Plant Cell **16 Suppl,** S214–S227.

Oracz K, Voegele A, Tarkowská D, Jacquemoud D, Turecková V, Urbanová T, Strnad M, Sliwinska E, Leubner-Metzger G. 2012. 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 **53,** 81–95.

Ozturk I, Kara M, Yildiz C, Ercisli S. 2009. Physico-mechanical seed properties of the common Turkish bean (*Phaseolus vulgaris*) cultivars 'Hinis' and 'Ispir'. New Zealand Journal of Crop and Horticultural Science **37,** 41–50.

Pavlista AD, Haber AH. 1970. Embryo expansion without protrusion in lettuce seeds. Plant Physiology **46,** 636–637.

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

Petruzzelli L, Müller K, Hermann K, Leubner-Metzger G. 2003. Distinct expression patterns of ß-1,3-glucanases and chitinases during the germination of Solanaceous seeds. Seed Science Research **13,** 139–153.

Pinto LV, da Silva EA, Davide AC, De Jesus VA, Toorop PE, Hilhorst HW. 2007. Mechanism and control of *Solanum lycocarpum* seed germination. Annals of Botany **100,** 1175–1187.

Psaras G. 1984. On the structure of lettuce (*Lactuca sativa* L.) endosperm during germination. Annals of Botany **54,** 187–194.

Queiroz SE, da Silva EA, Davide AC, José AC, Silva AT, Fraiz AC, Faria JM, Hilhorst HW. 2012. Mechanism and control of *Genipa americana* seed germination. Physiologia Plantarum **144,** 263–276.

Rathjen JR, Strounina EV, Mares DJ. 2009. Water movement into dormant and non-dormant wheat (*Triticum aestivum* L.) grains. Journal of Experimental Botany **60,** 1619–1631.

Reid JSG, Bewley D. 1979. A dual role for the endosperm and its galactomannan reserves in the germinative physiology of fenugreek (*Trigonella foenum-graecum* L.), an endospermic leguminous seed. Planta **147,** 145–150.

Reid JS, Edwards ME, Dickson CA, Scott C, Gidley MJ. 2003. Tobacco transgenic lines that express fenugreek galactomannan galactosyltransferase constitutively have structurally altered galactomannans in their seed endosperm cell walls. Plant Physiology **131,** 1487–1495.

Rodríguez-Gacio MdC, Iglesias-Fernández R, Carbonero P, Matilla ÁJ. 2012. Softening-up mannan-rich cell walls. Journal of Experimental Botany **63,** 3976–3988.

Rüggeberg M, Burgert I, Speck T. 2010. Structural and mechanical design of tissue interfaces in the giant reed *Arundo donax*. Journal of the Royal Society, Interface **7,** 499–506.

Saiedirad MH, Tabatabaeefar A, Borghei A, Mirsalehi M, Badii F, Varnamkhasti MG. 2008. Effects of moisture content, seed size, loading rate and seed orientation on force and energy required for fracturing cumin seed (*Cuminum cyminum* Linn.) under quasi-static loading. Journal of Food Engineering **86,** 565–572.

Salmén L. 2004. Micromechanical understanding of the cell-wall structure. Comptes Rendus Biologies **327,** 873–880.

Sánchez RA, Sunell L, Labavitch JM, Bonner BA. 1990. Changes in the endosperm cell walls of two Datura species before radicle protrusion. Plant Physiology **93,** 89–97.

Santos DSB, Pereira MFA. 1989. Restrictions of the tegument to the germination of *Beta vulgaris* L. seeds. Seed Science and Technology **17,** 601–612.

Sasaki N. 2012. Viscoelastic properties of biological materials. In: de Vicente J, ed. Viscoelasticity—from theory to biological applications. InTech, 99–122.

Scheler C, Weitbrecht K, Pearce SP, *et al*. 2015. Promotion of testa rupture during garden cress germination involves seed compartment-specific expression and activity of pectin methylesterases. Plant Physiology **167,** 200–215.

Schopfer P. 2001. Hydroxyl radical-induced cell-wall loosening in vitro and in vivo: implications for the control of elongation growth. The Plant Journal **28,** 679–688.

Schopfer P. 2006. Biomechanics of plant growth. American Journal of Botany **93,** 1415–1425.

Schopfer P, Liszkay A, Bechtold M, Frahry G, Wagner A. 2002. Evidence that hydroxyl radicals mediate auxin-induced extension growth. Planta **214,** 821–828.

Schopfer P, Plachy C. 1985. Control of seed germination by abscisic acid: III. Effect on embryo growth potential (minimum turgor pressure) and growth coefficient (cell wall extensibility) in *Brassica napus* L. Plant Physiology **77,** 676–686.

Shahbazi F, Saffar A, Analooei M. 2011. Mechanical damage to navy beans as affected by moisture content, impact velocity and seed orientation. Quality Assurance and Safety of Crops and Foods **3,** 205–211.

Sitrit Y, Hadfield KA, Bennett AB, Bradford KJ, Downie AB. 1999. Expression of a polygalacturonase associated with tomato seed germination. Plant Physiology **121,** 419–428.

Sliwinska E, Bassel GW, Bewley JD. 2009. 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. Journal of Experimental Botany **60,** 3587–3594.

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

Speck T, Burgert I. 2011. Plant stems: functional design and mechanics. Annual Review of Materials Research **41,** 169–193.

Srinivasan AV, Haritos GK, Hedberg FL. 1991. Biomimetics: advancing man-made materials through guidance from nature. Applied Mechanics Reviews **44,** 463–481.

Sun L, Miao Z, Cai C, *et al*. 2015. GmHs1-1, encoding a calcineurin-like protein, controls hard-seededness in soybean. Nature Genetics **47,** 939–943.

Tao KL, Khan AA. 1979. Changes in the strength of lettuce endosperm during germination. Plant Physiology **63,** 126–128.

Toorop PE, van Aelst AC, Hilhorst HW. 2000. The second step of the biphasic endosperm cap weakening that mediates tomato (*Lycopersicon esculentum*) seed germination is under control of ABA. Journal of Experimental Botany **51,** 1371–1379.

Van Sandt VS, Suslov D, Verbelen JP, Vissenberg K. 2007. Xyloglucan endotransglucosylase activity loosens a plant cell wall. Annals of Botany **100,** 1467–1473.

Vincent JFV. 1990. Structural biomaterials. Princeton, NJ: Princeton University Press.

Vincent JFV. 1992. Plants. In: Vincent JFV, ed. Biomechanics—material: a practical approach. Oxford: IRL Press, 165–191.

Vincent JFV, Currey JD, **eds.** 1980. The mechanical properties of biological materials. 34th Symposium of the Society for Experimental Biology. Cambridge: Cambridge University Press.

Vincent JF, Wegst UG. 2004. Design and mechanical properties of insect cuticle. Arthropod Structure and Development **33,** 187–199.

Voegele A, Graeber K, Oracz K, Tarkowská D, Jacquemoud D, Tureèková V, Urbanová T, Strnad M, Leubner-Metzger G. 2012. Embryo growth, testa permeability, and endosperm weakening are major targets for the environmentally regulated inhibition of *Lepidium sativum* seed germination by myrigalone A. Journal of Experimental Botany **63,** 5337–5350.

Vogler H, Felekis D, Nelson BJ, Grossniklaus U. 2015. Measuring the mechanical properties of plant cell walls. Plants (Basel) **4,** 167–182.

Wainwright SA, Biggs WD, Currey JD, Gosline JM. 1982. Mechanical design in organisms. Princeton, NJ: Princeton University Press.

Walters C, Ballesteros D, Vertucci VA. 2010. Structural mechanics of seed deterioration: standing the test of time. Plant Science **179,** 565–573.

Watkins JT, Cantliffe DJ. 1983. Mechanical resistance of the seed coat and endosperm during germination of *Capsicum annuum* at low temperature. Plant Physiology **72,** 146–150.

Watkins JT, Cantliffe DJ, Huber DJ, Nell TA. 1985. Gibberellic acid stimulated degradation of endosperm in pepper. Journal of the American Society for Horticultural Science **110,** 61–65.

Webster RE, Waterworth WM, Stuppy W, West CE, Ennos R, Bray CM, Pritchard HW. 2016. Biomechanical, biochemical, and morphological mechanisms of heat shock-mediated germination in *Carica papaya* L. seed. Journal of Experimental Botany (in press).

Wegst UGK, Ashby MF. 2004. The mechanical efficiency of natural materials. Philosophical Magazine **84,** 2167–2186.

Weitbrecht K, Müller K, Leubner-Metzger G. 2011. First off the mark: early seed germination. Journal of Experimental Botany **62,** 3289–3309.

Welbaum GE. 1999. Cucurbit seed development and production. HortTechnology **9,** 341–348.

Welbaum GE, Bradford KJ, Yim K-O, Booth DT, Oluoch MO. 1998. Biophysical, physiogical and biochemical processes regulating seed germination. Seed Science Research **8,** 161–172.

Welbaum GE, Muthui WJ, Wilson JH, Grayson RL, Fell RD. 1995. Weakening of muskmelon perisperm envelope tissue during germination. Journal of Experimental Botany **46,** 391–400.

Werker E. 1980. Seed dormancy as explained by the anatomy of embryo envelopes. Israel Journal of Botany **29,** 22–44.

Willis CG, Baskin CC, Baskin JM, Auld JR, Venable DL, Cavender-Bares J, Donohue K, Rubio de Casas R; NESCent Germination Working Group. 2014. The evolution of seed dormancy: environmental cues, evolutionary hubs, and diversification of the seed plants. New Phytologist **203,** 300–309.

Witztum A, Schulgasser K. 1995. Sees dispersal ballistics in *Blepharis ciliaris*. Israel Journal of Plant Sciences **43,** 147–150.

Wu CT, Leubner-Metzger G, Meins F Jr, Bradford KJ. 2001. Class I beta-1,3-glucanase and chitinase are expressed in the micropylar endosperm of tomato seeds prior to radicle emergence. Plant Physiology **126,** 1299–1313.

Yan D, Duermeyer L, Leoveanu C, Nambara E. 2014. The functions of the endosperm during seed germination. Plant and Cell Physiology **55,** 1521–1533.

Yang S, Johnston N, Talideh E, Mitchell S, Jeffree C, Goodrich J, Ingram G. 2008. The endosperm-specific ZHOUPI gene of *Arabidopsis thaliana* regulates endosperm breakdown and embryonic epidermal development. Development **135,** 3501–3509.

Yim KO, Bradford KJ. 1998. Callose deposition is responsible for apoplastic semipermeability of the endosperm envelope of muskmelon seeds. Plant Physiology **118,** 83–90.

Zhang Y, Chen B, Xu Z, Shi Z, Chen S, Huang X, Chen J, Wang X. 2014. Involvement of reactive oxygen species in endosperm cap weakening and embryo elongation growth during lettuce seed germination. Journal of Experimental Botany **65,** 3189–3200.

Zonia L, Munnik T. 2009. Uncovering hidden treasures in pollen tube growth mechanics. Trends in Plant Science **14,** 318–327.