Chapter 7

The Dynamics of Plant Cell Wall *In Muro* Modifications and its Physiological Implications on Seed Germination

Ximena Gómez-Maqueo and
Alicia Gamboa-deBuen

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64085

Abstract

Seed germination is a complex process in which the embryo, enclosed within the surrounding tissues, must quickly switch from a maturation program to a germination-driven developmental process that will prepare the embryo for seedling growth and establishment. The germination process initiates with water uptake by the dry seed and culminates, usually, with the radicle protrusion. The radicle emergence from the seed is a highly regulated process that involves discrete and coordinated changes in plant cell wall extensibility and rearrangements of its components, among other processes. In this chapter we will review current knowledge of the physiological process of controlled cell separation and expansion, which give the primary cell wall its plastic properties by "loosening" of the main components of the cell wall during seed germination. We will focus on the physiological importance of primary cell wall constitution and modification by the activity *in muro* of a broad variety of cell wall-modifying enzymes that include hydrolases and transglycosylases, as well as non-enzymatic processes such as expansin-mediated loosening during seed germination.

Keywords: cell wall modification, primary cell wall, seed germination

1. Introduction

Seeds constitute a critical stage in the life cycle of embryophytes. In this stage, the plant embryo remains in a quiescent state until the proper conditions of temperature, water availability, and, in some species, light are met in order for the processes of germination and seedling establishment to occur [1, 2]. The mature seed contains the embryo, which is surrounded by the seed coat

(testa) that is derived from the maternal tissues and in some species by one or more layers of storage tissue (endosperm) [2]. Seeds can function as resistance structures. Several mechanisms have evolved, in tight relation with the environment, to ensure the survival of the quiescent embryo [3]. Part of these mechanisms includes the modification of the structure and composition of plant cell walls.

One characteristic feature of plant cells is that they are enclosed in a polysaccharide and protein matrix, denominated as cell wall [4]. Plant cells can have two different types of wall. Primary walls, produced during cytokinesis, are flexible structures that regulate cell growth and shape. The secondary walls are deposited after the cell has achieved its final size and shape, by the inclusion of lignin and other phenolic compounds, thus making the cell wall rigid and usually impermeable. Cell walls have several functions that include the regulation of cell-cell adhesion and abscission, apoplastic transport, mechanical support and maintenance of turgor pressure, and defense against pathogens [2, 5]. In seeds, cell walls are modified in order to generate hard, and in some cases impermeable, coats that protect the embryo from the environmental conditions. Also, seed cell walls can store energy that can be mobilized to feed embryo growth and development. Finally, cell walls regulate the timing of seed germination by fine-tuning the processes of matrix polysaccharide loosening/breakage, as well as the integration of environmental cues with the hormonal and physiological status of the embryo [4, 6]. In this chapter we will focus only on primary cell walls and their importance on seed germination.

2. Seed germination

Seed germination is a physiological process initiated with water uptake and culminating with the emergence of the embryo through its protective tissues, which might include the testa, endosperm, perisperm, or pericarp [2]. The testa and the endosperm rupture must be coordinated with environmental seasonality to facilitate germination in the most favorable conditions [1, 6]. Several mechanisms have evolved to ensure proper synchronization of germination with environmental cues; among these is the interplay of hormonal signaling pathways via abscisic acid (ABA), gibberellins (GA), ethylene, and jasmonates [7–10]. These hormones exert their regulation on germination through different pathways including cell wall remodeling [7, 11].

In the classical model of seed germination described by Bewley et al. [12], the process of germination is divided into three phases, distinguished by the rate of water absorption by the seed tissues. The phase I, or imbibition phase, is characterized by a rapid water uptake rate driven by the difference in water potential between the seed and the environment. In this phase also the reactivation of primary metabolism and DNA repair pathways starts. Next, in phase II or activation phase, the imbibition rate decreases, water content remains stable, and major changes in the metabolic pathways and activation of other cellular processes take place. In this phase the integration of environmental cues with the internal status of the seed that will determine whether or not the seed will enter into the next phase occurs. Finally, in phase III there is another rapid water uptake driven by radicle protrusion and is mainly related to seedling growth. Germination is completed once the radicle has emerged at the onset of phase

http://dx.doi.org/10.5772/64085

III. This triphasic model of imbibition can be applied to all seeds analyzed thus far [12, 13]. The imbibition time needed for completion of germination is highly variable among species and even within seed lots, and it depends on several factors like seed history and environmental conditions experienced by the mother plant at the moment of seed dispersion and during the after-ripening period [12, 14, 15].

It is now generally accepted that radicle protrusion occurs by two nonexclusive processes [2, 13]. The first process involves a decrease in the mechanical resistance of the enclosing tissues, especially in the micropylar region of the testa and endosperm [2, 10]. The second process deals with an increasing growth potential of the embryo, driven by turgor pressure and cellular expansion in the embryonic axis [2, 13]. Most of the knowledge generated about the regulation of radicle protrusion comes from endospermic seeds, where testa and endosperm rupture can occur in two easily distinguishable stages (*Arabidopsis thaliana –Arabidopsis–*, *Chenopodium album*, *Lepidium* sp., *Nicotiana* sp., to mention a few) [2].

In recent years, with the advent of whole genome/transcriptome analysis, it has been possible to study the process of germination with high spatial-temporal resolution. Transcriptomic analysis allows a comprehensive view of seed germination by dissecting "early" or "late" germination processes, the first being the initial response to water and the second corresponding to the interval from the imbibed seed to the radicle protrusion [14, 15]. Also, in endospermic seeds, an important landmark is the distinction between the processes that occur prior to testa rupture and after it that leads to endosperm rupture [8, 16–18].

Several studies demonstrate that the main transcripts, enzymes, and other proteins accumulated in dry seeds participate in primary metabolism, starch and storage protein mobilization, reactive oxygen species (ROS) scavenging, and cell wall synthesis [14, 15]. Aside from providing building blocks to sustain protein production and cell growth, the reactivation of primary metabolism in the early stages of seed germination plays a major role in the generation of the proper redox state to promote the activity of different enzymes and produce energy to support processes essential for radicle protrusion [14, 19].

In *Arabidopsis*, the seed development and maturation programs are regulated by the LAFL transcription factor network (LEAFY COTYLEDON 1 (LEC1) and LEC1-LIKE (L1L), ABA INSENSITIVE 3 (ABI3), FUSCA 3 (FUS3), and LEC2), which activates other downstream transcription factor networks in concerted action of hormone, sugar, and light signalization pathways. Some target genes are involved in ABA, GA, ethylene, brassinoesteroids (BR), auxin, jasmonic acid (JA), and cytokinin (CK) signalization pathways [20]. The ABA signalization pathway participates in the regulatory networks of seed maturation, reserve accumulation, and desiccation tolerance acquisition [21]. GA blocks the LAFL and ABA networks during germination. The degradation of transcripts and enzymes related to seed maturation, which accumulated in the dry seed, has been described to occur in the first 6–12 h of seed imbibition in *Arabidopsis* [22] and within the first 24 h in *rice* and *barley* [14, 23].

Gibberellins play a major role in promoting a myriad of developmental programs, and its antagonistic role in ABA-mediated block of germination has been described [24]. GA stimulates seed germination by enhancing embryo growth; embryos of *Arabidopsis* GA-deficient

mutant seeds exhibit reduced growth rate phenotypes [25]. Also, GA enhances seed germination by overcoming the mechanical restraint to radicle protrusion of the surrounding tissues. In *Solanum lycopersicum* (tomato), GA-deficient embryos (unable to germinate unless incubated with exogenous GA) can grow into dwarf plants when the testa and the endosperm were removed mechanically [26]. This role of GAs in stimulating germination can be linked to the upregulation of several cell wall-modifying proteins (CWMPs) detected in whole-seed *Arabidopsis* transcriptomes of *ga1-3* mutants treated with GA_4 [24]. Jacobsen and Pressman [27] suggested that the embryo of celery (*Apium graveolens*) seeds does not secrete CWMPs but rather promote the activity of GA-inducible CWMPs in the endosperm. The depletion of the endosperm in this species generates a space where the embryo cells can expand and eventually penetrate the micropylar endosperm.

An overrepresentation analysis (ORA) of gene ontologies showed that transcription regulation is enriched in both the endosperm and the embryo transcriptomes of *Arabidopsis* seeds. The ORA analysis also showed that in the endosperm, the main biological processes are associated with cell wall metabolism, cell death, response to biotic stimulus, and defense and response to ABA. The main biological processes in the embryo include phosphate metabolic process, protein amino acid phosphorylation, hormone metabolic process (particularly auxin synthesis and transport), cell division and cell cycle, post-germination regulation of growth and organ development, and signaling [17].

3. Cell wall structure and composition

Plant cell walls are complex and highly dynamic structures composed of a variety of polysaccharides, proteins, and aliphatic or aromatic compounds [28, 29]. They are continually being modified throughout development and in response to environmental stimuli [30, 31]. Primary cell walls of flowering plants can be classified in two main groups depending on its general architecture and composition, as well as their biosynthetic processes [32, 33]. Type I cell walls are the most common, present in dicotyledonous and the non-commelinoid monocotyledonous plants (a more basal group of aroids, alismatids, and lilioids). Type II cell walls are found only in the commelinoid monocots that include the Poales (members of the families Poaceae, Bromeliaceae, and Cyperaceae) [32, 33].

3.1. Primary cell wall polysaccharides

Primary cell wall polysaccharides constitute the majority of the wall dry mass in land plants and can be grouped in three main classes: cellulose, hemicelluloses, and pectins [30]. Cellulose is a linear 1,4-β-D-glucan that assembles into partially crystalline microfibrils, each of which contains about 36 parallel polysaccharide chains [34]. Cellulose is synthesized *in muro* by the cellulose synthase complex (CSC), embedded within the plasma membrane and formed by 6 rosette subunits that contain 6 cellulose synthase proteins (CESA) [35]. Aside from cellulose, all other cell wall polysaccharides are synthesized and processed for wall targeting in the trans-Golgi system [5].

http://dx.doi.org/10.5772/64085

Hemicelluloses are polymers whose backbones consist of β-glucose, β-xylose, or β-mannose, with short side chains. In all vascular plants with type I walls, the most common hemicelluloses are xyloglucans (XyG), whereas type II cell walls contain less XyG, being the most abundant glucuronoarabinoxylans (GAX) and β1,3:β1,4 mixed glucans [33]. Hemicellulose chains adhere to cellulose microfibrils, in a rope-like manner, to restrain cell expansion [30, 34]. Also, in type I cell walls, hemicelluloses bind and cross-link with pectin and form the hydrated matrix [30].

The group generally known as pectins comprises over 30% of the cell wall total mass in dicots [31, 36]. Pectins are acidic heteropolymers that form a hydrated gel, in which cellulose and other molecules are embedded in the plant cell wall. Their main defining feature is 1,4-linked α-D-galacturonic acid residues (GalA). Pectins interact covalently and non-covalently with other pectin molecules or with hemicellulose xyloglucan or arabinogalactans [31]. Several studies support the hypothesis that the three major pectin classes, homoglacturonan (HG), rhamnogalacturonan I, and rhamnogalacturonan II, are covalently linked in the cell wall [29, 37], forming a hydrophilic macromolecular network. Pectin is deposited on the cell wall matrix in a highly methylesterified form [28]. The methyl group is removed by pectin methylesterases (PMEs) in muro, providing an anionically charged matrix and changing the mechanic properties of the cell wall. Increasing evidence shows that the regulation of the degree of methylesterification of the pectic matrix plays a fundamental role in plant growth, development, morphogenesis, cell-cell adhesion, cell expansion, seed hydration, and seed germination [5, 16, 36, 38].

Other polysaccharides present in primary cell walls of various species are the mannans, arabinoxylans, and arabinogalactans. Mannans are formed by mannosyl residues linked by β-1,4-glycosidic linkages. This mannosyl backbone can contain glucose residues (glucomannans) or be further substituted by single galactose residues with α-1,6-linkages (galactomannans). Arabinoxylan consists of a (1,4)-linked β-D-xylan backbone decorated with arabinose branches. Other residues, such as glucuronic acid and ferulic acid esters (FAE), are also attached in arabinoxylans that are particularly abundant in cereal grasses. Arabinogalactan and storage xyloglucans are used as reserves in cotyledons. The basic structure of storage xyloglucans differs from the primary wall xyloglucans in that it is not fucosylated [39].

3.2. Cell wall proteins

Primary cell walls are mainly constituted by polysaccharides; however, proteins account for about 10% of the total dry mass of the wall [40]. Proteins that contain a secretion signal peptide, which targets them to the secretory pathway and in most cases is excised to allow activation or proper protein function, are commonly referred as classical cell wall proteins [40–42]. In *Arabidopsis*, about 17% (~5000 genes) of the genome encodes for proteins targeted to the secretory pathway, and of this, about 1000–2000 genes could be cell wall proteins (CWPs). Cell wall proteins have several functions as structural, enzymatic, and defense and have been grouped in functional categories by different authors. The proteins with a structural function or those acting on polysaccharides are the two main functional categories [30, 42]. These

proteins are expressed in a tissue-specific and process-specific manner, contributing to the regulation of cell wall stabilization and rigidity [41].

3.2.1. Structural proteins

Structural proteins are usually classified by the predominant amino acids in their sequence, although some of them can belong to more than one category. The most common include the hydroxyproline-rich glycoproteins (HRGPs), the glycine-rich proteins (GRPs), the proline-rich proteins (PRPs), and the arabinogalactan proteins (AGPs). These proteins vary greatly in abundance within plant species, cell tissues, and environmental conditions [4]. Arabinogalactan proteins, that are widely distributed among plant families and comprise about 2–10% of the total protein in the wall, are highly glycosylated. Also, AGPs are rich in hydroxyproline, serine, alanine, threonine, and glycine, and resistant to proteolysis in their native state [41]. Extensins are a family of HRGPs particularly abundant in dicots that have been involved in modification of wall extensibility in elongating tissues [43].

3.2.2. Proteins acting on polysaccharides

Within the CWPs acting on polysaccharides, there are a broad variety of activities. For instance, the group of glycosyl hydrolases (GHs) include glycosidases (β-glucosidase, β-galactosidase, β-xylosidase, and α-xylosidase, and exo-polygalacturonases) and glycanases like β-mannanase, β-xylanase, (1→4)-β-glucanase "cellulase", endo-polygalacturonases, and xyloglucan endo-hydrolase (XEH). The combined activity of these kinds of enzymes is theoretically capable of hydrolyzing most of the glycosidic bonds in the cell wall polysaccharides but do not imply that all enzymes are active at the same time or tissue. The glycosyltransferases (GTs) activity involves the formation of a glycosyl-enzyme complex that is attacked by an acceptor substrate (another oligo/polysaccharide). This activity allows the integration of recently secreted polysaccharides into the matrix and the grafting of polysaccharides already present in the wall matrix [44]. This category includes the xyloglucan endotransglycosylase (XET). Both XEH and XET proteins are commonly grouped within the xyloglucan endotransglucosylase/hydrolase family (XTH) due to some of their members (like β-xylanase) that can have both GH and GT activities [30].

Polysaccharide lyases (PLs) promote cell separation by calcium-dependent de-polymerization of wall polygalacturonides. Plant pectate lyases are a group of enzymes that catalyze the cleavage of de-methylesterified pectin. PL activity has being described in cell wall degradation that occurs during fruit ripening [45], and Penfield et al. [7] report 34 pectate lyases that were downregulated in the endosperm of *Arabidopsis* imbibed seeds after treatment with ABA.

Carbohydrate esterases (CEs) include two enzyme families that have activity over pectins, the PMEs and pectin acetylesterases (PAEs), and the family of xylan acetylesterases. These enzymes cleave methyl or acetyl groups from the HG or Xyl backbone of polysaccharides [30].

PMEs catalyze the reaction by which methylesters are cleaved from a HG chain, producing a free carboxyl group and the release of a proton and methanol [46]. Plant PMEs are mainly alkaline isoforms bound to the wall matrix, while some isoforms are neutral and easily

http://dx.doi.org/10.5772/64085

solubilized or free apoplastic acidic isoforms. Alkaline isoforms seem to be the PMEs with most de-methylesterification activity, but the kinetics of PME activity is affected by the ionic composition of the matrix, thus influencing PME activity and mobility [47]. PME activity can lead to two different cell wall fates: the first one would be the formation of a rigid, stable structure by Ca^{2+} interaction with de-methylesterified GalA residues (>10) in the HG chains. The second fate of HG would be their degradation by polygalacturonases, where only small stretches or individual GalA residues are de-methylesterified, thus leading to a more relaxed matrix [28, 46]. Also, PME activity acidifies the cell wall; this acidification would allow expansin activity ("acid grow") [5]. PMEs are antagonistically regulated in the cell wall by proteinaceous PME inhibitors (PMEIs) meanwhile PGs by PG inhibitors (PGIPs) [28].

Expansins regulate cell wall loosening in a pH-dependent manner by disruption of the hydrogen bonds between xyloglucans and cellulose. Sequence analysis indicates that expansins contain an N-terminal domain slightly similar to the catalytic domain of the family-45 endoglucanases; however no catalytic activity has been reported.

In *Arabidopsis*, about 10% of the total CWPs described in cell wall proteomes from different tissues and plants correspond to gene families with domains of unknown function (DUF) [42]. Mewalal et al. [48] have pointed out the relevance of these DUF families on cell wall dynamics. In particular, the two plant-specific families DUF231 and DUF642 could be involved in pectin modification [49, 50].

3.3. Cell wall modification

The molecular modification of the wall network can result in the relaxation of wall stress or "wall loosening" by the controlled rearrangement of cellulose/matrix polymers, which involve sliding of a cross-link along a scaffold or the breakage of stress-bearing cross-links without substantial changes in wall dimensions. These rearrangements could include three processes: (a) the cleavage of the backbone of major matrix polymers, (b) the weakening of the non-covalent bonding between polysaccharides, and (c) the breakage of cross-links [5]. Following cell wall loosening, there are three main types of outcomes: cell expansion, cell separation, and wall stiffening. Cell wall enlargement occurs secondarily as a result of water uptake and the reduction of turgor pressure resulting from wall loosening [44].

Reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2), hydroxyl radical (·OH), and superoxide radical (O_2^-) have been proposed to play a major role in germination by participating in defense against pathogens, signaling, and promotion of cell wall loosening [2, 9]. ROS can negatively affect germination by reacting with almost all macromolecules stored in the seed, causing oxidative damage and cleavage of polysaccharide chains in the cell walls [5, 9]. The participation of ROS in cell wall loosening and promotion of germination might be indirect, through the ethylene signaling pathways that involve ROS production and downstream activation of CWMPs [9]. Cosgrove [5] suggests the revision of ROS participation in the process of wall loosening, since in most studies reporting ROS-mediated extensibility comprises only a small fraction (about 1% extensibility) and the assays with higher ROS concentrations provoke wall breakage.

3.4. Role and regulation of cell wall enzymes and proteins during germination

The study of plant cell wall structure and physiology has achieved a major progress from the input of "-omics" technologies in the past two decades. These -omics technologies are able to capture the complexity of biological processes, like seed germination and cell wall modification, with high sensitivity and spatial-temporal resolution. A tissue-specific transcriptome analysis in *Arabidopsis* showed that both endosperm and embryo share gene expression patterns and biological processes during seed germination. About 10,800 transcripts (~84% of total genes expressed) are present in both endosperm and embryo. Endosperm-specific genes comprise about 415 genes that were highly expressed [17]. Of this gene set, 154 are cell wall-related genes, with most of them being expressed at the onset of testa rupture. Transcript abundance of several CWMPs shows a transient peak with a 6–24 h interval in tomato [51], *Arabidopsis* [17], and *Lepidium* seeds [16]. Although a change in transcript levels does not necessarily correlate to changes in protein abundance or enzymatic activity, it has been demonstrated that during *rice* germination, most cell wall-related transcripts, as well as the resulting metabolites from cell wall modification, accumulate about 12–24 h after imbibition (HAI, although some metabolites can be detected by 3 HAI) [23].

Cell wall modification can occur at five different stages during seed germination: (a) during the cellular expansion process triggered by rehydration of tissues, (b) at the onset of testa rupture, (c) during endosperm weakening and rupture, (d) during cellular expansion related to radicle elongation, and (e) during wall degradation and mobilization of stored reserves in both living and nonliving storage tissues.

3.4.1. Rehydration-driven cellular expansion

Seed imbibition is given by the difference in water potential between the seed and the environment. Nonviable seeds swell faster than viable seeds, as viable seeds develop turgor pressure that restricts further water uptake [2]. However, rapid imbibition can still occur and lead to solute leakage and damage of membranes. Gradual rehydration of seed tissues has been detected in legumes like peas and beans, where hydration starts in the tissues near to the micropyle. As water diffuses in the outermost tissues, a waterfront is formed between imbibed tissues and those about to be imbibed. The testa plays a significant role in modulating imbibition kinetics and the waterfront formation [2]. The seeds of mutants with altered testa structure or altered deposition of protecting substances (like flavonoids, cutin, suberin, and lignin) have increased permeability and lower longevity than the wild type [52]. Testa structure usually consists of several layers of highly compressed dead cells where protective substances are deposited during seed development and maturation. Plant cell walls of living cells can also function as an interface that modulates water intake by changing wall porosity, thus allowing a gradual swelling of all tissues. This regulation could be achieved by rapid changes in wall extensibility as the ones generated by expansins. In support of this view, in whole unstratified *Arabidopsis* seeds, the upregulation of *EXPA1*, *EXPA2*, *EXPA3*, *EXPA8*, *EXPA9*, *EXPA15*, and *EXPA20* transcripts from 0 to 12 h has been reported. This induction was evident in seeds imbibed in the light and during moist cold stratification at 4°C in the dark [11]. Also, in whole-seed transcriptomes, the expression of *AtXTH5*, *AtXTH6*, and *AtXTH33* transcripts

within the first 6HAI and *AtXTH3* and *AtXTH*33 transcripts at 12HAI [24] was detected. The activity of XTHs from *chickpea* seeds has been detected in imbibed seeds from 1HAI until 24HAI, when the rate of radicle elongation slows down [53].

In many species from the Brassicaceae, Solanaceae, Linaceae, and Plantaginaceae, among others, the epidermis of the testa contains specialized cells that accumulate abundant pectins and heteroxylans, as well as some xyloglucans or arabinans during seed development. Upon imbibition these polysaccharides expand and burst out of the testa, generating a gel-like structure. This phenomenon, known as myxospermy, has been used as a model to study several hydrolases and PME activity [54, 55]. Although there is still uncertainty about the actual role of myxospermy, the proposed roles include regulating hydration, preventing desiccation, being an oxygen barrier, or allowing the seed to attach to the substrate and animals [54–56].

3.4.2. Testa rupture

In many plant species, testa rupture starts at the micropylar seed end. In tobacco (*Nicotiana tabacum*) seeds, the testa ruptures at the micropyle and follows predetermined breaking points due to the presence of channel-like structures underlying ridges in the testa [57]. In pea seeds the presence of xylogalacturonan in the inner walls of the testa was described, which coincides detached cells or with the junction sites between cells that are destined to detach, as described in other plant tissues [37]. In *Lepidium* seeds, HG composition shifts at the onset of testa rupture: while the seeds imbibe, non-esterified HG is ubiquitously distributed in all tissues, but by the time of testa rupture, this non-esterified HG is detected mostly on the endosperm and testa, meanwhile esterified HG is detected in the endosperm [38].

The seed testa is composed of several layers of nonliving cells, and thus the regulation and enzymes that facilitate cell separation in the testa must come from the living tissues underneath. At the onset of testa rupture (~25 HAI), it is possible to identify 90 cell wall-related genes from the 501 upregulated genes (~18%) in the micropylar endosperm and 58 from 282 genes (~20%) upregulated in the radicle. Also, about 8 (~8%) and 5 (~4%) genes were downregulated in both tissues, respectively [17]. In *Lepidium* seeds, tissue-specific transcript abundance patterns between the micropylar endosperm and the radicle accompany testa rupture, further supporting the view of this process as a decisive step in germination and in the regulation of cell wall-related genes [38]. In **Table 1**, these transcripts and its predicted biological/biochemical function are enlisted.

			Endosperm (HAI)				**RA**				**Endosperm (HAI)**				**RA**
Function	Gene ID	TS	0–12	16	25	31	25	Function	Gene ID	TS	0–12	16	25	31	25
EXPA10	AT1G26770	No	6–12		24		TR*	β-Gal	AT1G45130	EM	Not specified				
EXPA1	AT1G69530	No	3				TR*	β-Gal	AT5G08380	No	Not specified				
EXPA15	AT2G03090	No	12		24		TR*	β-Gal	AT1G77410	EN			24	31c	
EXPA6	AT2G28950	EM					TR	β-Gal	AT2G28470	No		16c	24		24

		Endosperm (HAI)				RA				Endosperm (HAI)				RA
EXPA4	AT2G39700	No		16			β-Gal	AT4G26140	No				31	
EXPA2	At5g05290	No	3		24		β-Glu	AT1G61820	EN	Not specified				
EXPA8	At2g40610	No	3–12		24		β-Glu	AT1G70710	No		16	TR*		
EXPA3	At2g37640	No	6–12		24		β-Glu	AT4G16260	EN	Not specified				
EXPA9	AT5G02260	No	12		24		β-Glu	AT1G26560	EM	Not specified				
EXPA20	AT4G38210	No	6–12		24		β-Glu	AT3G62750	No	3c				
EXPB1	At2g20750	No	12				β-Glu	AT2G44450	No			TR	31	
EXLA3	AT3G45960	No			TR		β-Glu	AT4G34480	No	12*				
EXLA1	AT3G45970	No		16	TR	TR	MAN5	AT4G28320	No	Not specified				
EXLA2	AT4G38400	No	6–12*		TR	TR	MAN6	AT5G01930	EN	Not specified				
EXT3	AT1G21310	EM	Not specified				MAN7	AT5G66460	No	6		24		
EXT10	AT5G06640	EM	Not specified				GH	AT5G49360	EN			TR		
EXTL	AT3G54590	EM	Not specified				GH	AT5G57560	No		16	TR		
EXTL	AT4G38770	EM	Not specified				GH	AT5G08370	No		16c	TR	31c	
EXTL	AT2G27380	RA	Not specified				GH	AT3G55430	No			TR	31	24*
XTH5	AT5G13870	No	6		24	24	GH	AT3G07320	No			24	31	24
XTH33	AT1G10550	No	12	16	TR	TR	GT	AT3G10320	EN				31c	
XTH	AT1G11545	No		16		TR	GH	AT3G13790	No		16		31	
XTH	AT1G32170	No			TR		GH	AT5G64570	No			24		TR
XTH17	AT1G65310	No			TR		GH-DUF3357	AT1G12240	No				31	TR
XTH	AT2G06850	No		16	TR	31 TR	GH	AT3G47010	EN			24		
XTH	AT2G36870	No				31	KOR2	AT1G65610	No				31	
XTH	AT3G23730	No			TR	TR	CESA5	AT5G09870	No			TR	31	24
XTH11	AT3G48580	No		16	24*	31	CSLC	AT4G07960	No			TR		TR
XTH	AT4G03210	No			TR	TR	AGP	AT3G11700	No			TR		TR
XTH	AT4G14130	No			TR	TR	AGP	AT5G44130	EN			TR		
XTH24	AT4G30270	EN		16	TR	TR	AGP	AT1G28290	No		16		31	
XTH18	AT4G30280	No		16	TR	TR	PRT	AT3G54400	No		16			24
XTH	AT4G30290	No		16		31	PRT	AT3G61820	No			TR*	31	24
XTH	AT4G37800	EM	Not specified				PRT	AT4G16563	No			TR		TR
XTH25	AT5G57550	No	6*		TR	TR	PL	AT3G24670	EM					24
XTR8	AT3G44990	EN		16c	24		PL	AT3G27400	No			TR		24
XTR6	AT4G25810	No			TR	TR	PL	AT4G13710	EN	Not specified				
PL	AT4G24780	No			TR*		PMEI	AT5G20740	No			TR		TR

http://dx.doi.org/10.5772/64085

		Endosperm (HAI)				RA			Endosperm (HAI)				RA
PL	AT5G48900	EM	Not specified				PMEI	AT5G46940	EN		TR		
PX	AT1G14540	EN		TR			PMEI	AT5G62340	No	16			TR
PX	AT1G14550	EN	Not specified				PMEI	AT5G64620	No			31c	
PX	AT1G30870	EM	Not specified				PG	AT3G59850	No	16	TR*	31	
PX	AT2G18980	No		TR	31		PG	AT3G61490	EM	Not specified			24
PX	AT2G43480	RA	Not specified				PG	AT4G23820	No	12	TR		24
PX	AT3G01190	EM	Not specified				Kinase	AT1G33590	No		TR	31	TR
PX	AT3G21770	EN			31c		Kinase	AT2G23770	No		TR		
PX	AT3G28200	No		TR	31		Kinase-DUF26	AT3G22060	MI	16		31	
PX	AT4G08770	EM	Not specified				Kinase	AT1G51940	No		TR		TR
PX	AT4G31760	EM	Not specified				LRR-p	AT4G26690	No		TR		TR
PX	AT5G05340	No			31		LRR-p	AT5G16590	No	16	TR	31	TR
PX	AT5G39580	No	3	TR			LRR-p	AT2G34930	No	16	TR	31	TR
PX	AT5G40150	EM	Not specified				DUF642	AT1G80240	EM	Not specified			
PX	AT5G64100	No	16	TR	31		DUF642	AT2G34510	EM	Not specified			
PX	AT5G64120	EN		TR	31		DUF642	AT2G41800	RA	Not specified			
PME	AT3G14310	No	6–12	24		24	DUF642	AT3G08030	No	3		31	
PME	AT1G04680	No			31	TR	DUF642	AT4G32460	No	16		31	
PME	AT1G57590	EM	Not specified			24	DUF642	AT5G11420	No	3		31	
PME	AT3G09410	EM	Not specified				DUF642	AT5G14150	EM	Not specified			
PME	AT3G10720	No	16	TR	31	TR	OX	AT1G62380	No	16c			
PME	AT3G62060	EM	Not specified			24	OX	AT1G76160	No		TR		
PME	AT4G19420	EN	Not specified				OX	AT2G46740	EN	16	TR	31	
PME	AT5G26670	RA	Not specified				OX	AT4G22010	No		TR		
PME	AT5G45280	No	3		31		OX	AT4G38420	No		TR		TR
PME	AT5G62330	EM	Not specified			24	OX	AT5G21105	No	16	TR	31	
PME2	AT1G02810	No	12	16	31		OX	AT5G44380	No		TR	31	
PME2	AT1G11580	No		16		24	PTRI	AT1G17860	No		TR	31	TR
PME2	AT2G26440	No		16	31	24	PTRI	AT2G38870	No			31	TR
PME2	AT3G47400	No			31	24	PTRI	AT4G22470	MI		TR	31	
PME2	AT3G49220	No		TR		TR	GT	AT1G64390	No	16	TR*	31	
PME2	AT4G02330	No	16	TR	31	TR	GT	AT2G02990	EN		TR		
PME2	AT4G33220	EM	Not specified				GT	AT2G14610	EN	Not specified			
PME2	AT5G64640	EM	12	24			GT	AT1G05170	EM				TR*

			Endosperm (HAI)			RA			Endosperm (HAI)		RA
PMEI	AT1G62770	RA	Not specified			GT	AT1G08280	No		31	
PMEI	AT2G47670	EM	3-12*	24*		PGIP	AT5G06860	No			TR
PMEI	AT4G00080	No		31	TR	PG	AT2G43860	MI	24	31	
PMEI	AT4G12390	EM	Not specified			PG	AT3G06770	No	TR		24

The endosperm expression profiles are subdivided by hours after imbibition (HAI) and testa rupture (TR, ~25HAI), whereas the radicle (Rad) expression only shows the moment of TR. *Abbreviations*: T-S, tissue-specific expression; EM, embryo; EN, endosperm; RA, radicle; MI, micropylar endosperm; EXPA, expansin; EXT, extensin; EXTL, extension-like protein; XTH, xyloglucan-transglycosylhydrolase; GH, glycosyl-hydrolase; β-Glu, β-glucosidase; β-Gal, β-galactosidase; AGP, arabinogalactan protein; CESA, cellulose synthase; PRT, protease; PL, pectate lyase; PX, peroxidase; CSLC, cellulose synthase-like; LRR-p, leucine-rich repeat protein; DUF, domain of unknown function; PME, pectin methylesterase; PME2, PME with an inhibitory domain; PMEI, PME inhibitor; PTRI, protease inhibitor; GT, transglycanase; PG, polygalacturonase; PGIP, PG inhibitor. The * means downregulation for that particular gene and time. The letter "c" beside a number means the expression was upregulated in the chalazal endosperm.

Table 1. Expression of some upregulated cell wall-modifying genes in *Arabidopsis* tissue-specific microarrays described by [17, 22] and expression profiles at http://bar.utoronto.ca/.

In *Arabidopsis* and *Lepidium* seeds, total PME activity increases gradually with imbibition time and peaks at the onset of testa rupture. ABA treatment does not affect testa rupture but endosperm rupture is delayed and PME activity fails to decrease following testa rupture [16, 38]. Two DUF642 genes, *BIIDXI* (*BDX*, *At4g32460*) and *At5g11420*, are expressed in the embryo and micropylar endosperm, respectively, during germination. In overexpression lines, testa and endosperm rupture of matrix-primed seeds occurred earlier compared to wild type. The germination performance of overexpression seeds was accompanied by an increase of total PME activity, compared to the wild type [50].

In non-endospermic seeds, testa rupture marks the end of germination. In this type of seeds, the testa rupture is accompanied by radicle elongation whose continued pressure in the inner face of the testa promotes cell separation [53].

3.4.3. Endosperm weakening for radicle protrusion

The endosperm functions as a barrier to control radicle protrusion as it can impose primary dormancy in many species like *Arabidopsis*, *Lepidium*, and yellow cedar, among others [2]. Endosperm structure of mature seeds varies greatly within species, where it can comprise one layer of cells as in *Arabidopsis*, *Lepidium*, and cucumber or to several layers as in tomato or tobacco [58, 59]. Structural studies in hard-seeded species like fenugreek (*Trigonella foenum-graecum*) and coffee (*Coffea arabica*) have demonstrated that near the micropyle a zone of thin-walled cells that can be a low-resistance area for radicle protrusion exists. Endosperm cell wall composition varies considerably among species: in the closely related species, *Arabidopsis* and *Lepidium* are rich in cellulose, non-esterified HG, arabinans, and XG. However, these polysaccharides are not uniformly distributed in the endosperm: in *Lepidium*, an epitope for arabinans (LM13) was localized in the inner and outer walls of the cells, but absent from the traverse walls. The endosperm of tomato seed contains mannans that have been shown to contribute

http://dx.doi.org/10.5772/64085

to the control of radicle protrusion and general endosperm hardness rather than a storage function [39].

The hydrolytic activity of β-glucanases or endo-β-mannanases can contribute to endosperm cell wall weakening in Brassicaceae and Solanaceae species, which have cell walls rich in mannans [59]. In *Arabidopsis* seeds the activity of *AtMAN5*, *AtMAN6*, and *AtMAN7* and regulation of their activity by the basic leucine-zipper 44 transcription factor, *AtbZIP44*, whose knockout mutants have delayed germination have been described [60]. In hard-seeded species, there was a negligible activity in the radicle and micropylar endosperm of endo-β-mannanases that could not be associated with endosperm weakening to allow radicle protrusion [61]. However it is still unexplored the activity of other CWMPs that could contribute to wall rearrangements during germination in these species. The upregulation of wall-related genes in the endosperm has been reported; this includes α-expansins (*AtEXPA2*, *AtEXPA8*, and *AtEXPA9*), β-expansin (*AtEXPB1*), expansin-like protein (*AtEXPL1*), cellulose synthase-like proteins (*CSLA2* and *CSLC4*), xyloglucan endotransglycosylases (*AtXTH11*, *AtXTH17*, *AtXTH18*, *AtXTH33*, *AtXTH31*, *AtXTH23*, and *AtXTH24*), and mannanase (*AtMAN7*) [62].

Several reports indicate that endosperm weakening and rupture are inhibited or delayed by ABA, in a dose-dependent manner, in some species of the Brassicaceae family [8, 16] and tobacco [63]. Microarray analysis of *Arabidopsis* seeds treated with exogenous ABA at the onset of radicle protrusion has shown a downregulation of several wall-related transcripts in the endosperm, including PMEs, AGPs, and PLs [7]. In tobacco, ABA delays the accumulation and activity of β-1,3-glucanase in the micropyle before radicle protrusion [63]. PMEs contribute to seed germination in several species by modulating the degree of methylesterification of pectins in the endosperm [16, 38, 64, 65]. In yellow cedar a loss of the internal structure of the megagametophyte surrounding the radicle during germination was described. The resulting decrease in the mechanical strength of the megagametophyte would allow radicle protrusion. There is a positive correlation between dormancy alleviation and PME activity, as well as with germination performance and PME activity in both the megagametophyte and the embryo. PME activity has also been demonstrated to positively correlate with germination performance in *Arabidopsis* [64]. The *LeXET4* gene transcripts, which are restricted to the endosperm cap, are detected in tomato seeds within 12 h of imbibition and reach a maximum at 24 h [66]; they decline after radicle emergence despite a continued degradation of the lateral endosperm cell walls. PG *At2g43860* is expressed within the endosperm cells of the seed adjacent to the site of the emerging radicle [67]. In germinating tomato seeds, several reports indicate the expression of expansins [51], PGs (*LeXPG1*) [68], and XTHs (*LeXET4*), which accumulate in the endosperm region adjacent to the expanding radicle (~40HAI). Transcripts are detected within 12HAI and generally peak by 24HAI, consistent with the endosperm weakening.

3.4.4. Embryonic axis elongation

Cell elongation, rather than cellular division, is the main process that drives embryo growth [14]. Cell division occurs after radicle protrusion and contributes to the rapid growth of the embryonic axis by generating new elongating cells [2]. Cell elongation that drives radicle

protrusion occurs at the transition zone, which comprises the cells between the last proximal root hair cell in the radicle and the lower basal cells of the hypocotyl [69]. In *Arabidopsis* Col seeds that have been previously stratified, the radicle protrusion can initiate as early as 32 HAI. By this time, and immediately prior to the radicle emergence through the endosperm, the cells in the transition zone had incremented their size by 44% while the cells in the radicle 10% and in the hypocotyl 30%. By 40HAI, the radicle has already protruded and the elongated cells in the seedling have increased their size by 15% in the radicle, 52% in the hypocotyl, and 108% in the transition zone. Elongation is often accompanied by an increase in DNA content without subsequent mitosis (endoreduplication) [69].

In the micropylar endosperm of tomato seeds, an important mobilization of protein bodies occurs, but it seems that there is no cell degradation as the radicle protrudes. Instead, a process similar to cell separation to allow radical protrusion was suggested [70]. A similar process was observed in celery seeds, where the radicle tip also seems to penetrate the micropylar endosperm by separating the endosperm cells, but, since the embryo needs to grow before germination is completed, cell degradation for storage mobilization occurs in the endosperm adjacent to the embryo [27]. The expression of *LeEXP8* and *LePG1* in the embryo elongation zone of tomato seeds has been reported at the onset of radicle protrusion [51, 68].

3.4.5. Cell wall participation in the mobilization of stored reserves

Major reserve mobilization occurs once germination has concluded, and these reserves are utilized to feed the growing seedling rather than to fuel radicle protrusion. However, in cereal grains, the preparation for starch and oligosaccharide mobilization occurs within the first hours of germination [15]. In cereals, the endosperm is a nonliving storage tissue, and the endosperm cell walls protect its contents from enzymatic attack. Accordingly, the degradation of cell walls is a limiting step in storage reserve mobilization that is induced by the GA produced by the embryo (at the scutellum) and secreted to the aleurone layer [2].

In most endospermic seeds this tissue is still living. Mannans in the endosperm cell walls of date palm (*Phoenix dactylifera*) and coffee are mobilized to support embryo development. It has been proposed that the mobilization of storage xyloglucans can be coupled to the growth rate of the seedling by transglycosylation. In legumes, endosperm galactomannans seems to function as reserves; they can constitute up to 30% of total seed dry weight. In fenugreek and *Schizolobium parahyba*, the cell walls of the endosperm are thickened with galactomannan and in some cases the cytoplasm is nonexistent. In *Tamarindus indica* and *Hymenaea courbaril*, reserve xyloglucans are stored between two primary walls and are degraded without hydrolyzing both walls [39]. During germination of celery seeds, the surrounding endosperm degrades leaving a small amount of un-degraded polymers of the cell wall, except for the micropylar endosperm, in which only some protein bodies are mobilized and the rest of the cells persist until the radicle pushes through; once radicle protrusion has started, these micropylar cells are degraded [27].

http://dx.doi.org/10.5772/64085

4. Concluding remarks

In-depth temporal screening of cell wall-related transcripts and proteins has provided an important overview of the possible actors involved in the five stages during germination where wall modification is involved, as described above. In *rice*, a valuable integrative effort using -omics approaches has been done to understand seed germination [23]. This analysis suggested that the changes in transcript levels during early germination (3–12 HAI) drive the subsequent changes in the metabolome (12–24 HAI) of germinating seeds, supporting that most of the changes observed at the transcriptional level are related to the cellular processes involved in germination. Other authors have associated transcript abundance with specific seed compartments and some enzymatic activity assays, demonstrating the relevance of understanding tissue-specific expression profiles [17, 38]. Much of the information available related to CWMPs still needs to be validated through enzymatic activity or in vivo interaction assays. Only about 121 (~12%) of the total cell wall-related genes are experimentally validated [48]. Also, many cell wall-related proteins belong to families of unknown function. The -omics approach can be useful to propose hypothesis of wall-modification complexes, whose activity could be regulated at several levels, and coordinated by unknown function proteins that could act as scaffolding proteins and direct this complex activity to specific polysaccharides. Cosgrove [44] proposed that CWMPs could be functionally classified into primary or secondary modifiers, but this idea has not being reflected in other studies. Following Cosgrove, the analysis of cell wall modification considering an alternating activity of primary or secondary modifiers could facilitate the understanding of the dynamics of cell wall modification during seed germination. For instance, expansins could be primary modifiers as they affect cell wall loosening and extensibility, but they do not remove or transfer polysaccharides into the wall during imbibition; other primary modifiers could be PMEs, as their activity precedes PGs and promotes cell expansion or cell separation, or the resulting exposed GAL residues can be cross-linked with Ca^{2+} and promote wall stiffening. Secondary modifiers would include GHs and GTs that would act on exposed residues either promoting cell expansion, separation, or stiffening. In assays to study mucilage properties, sequential treatment with different hydrolases allows solubilization of other components, which are masked to the activity of other enzymes [55, 71]. The alternate perspective of primary and secondary modifiers could help in identifying potential interactions in silico and tested in vivo.

Spatial transcriptomic analyses that include the different seed compartments and the analysis of cell wall composition changes using specific antibodies for in situ localization of the different polysaccharide epitopes in seed tissues provide valuable information. Although *Arabidopsis* is the best-known plant model, several authors demonstrate that comparative analysis allows higher resolution of tissue-specific cell wall microdomains that are not achievable in *Arabidopsis* [8, 10, 60]. As an example, Lee et al. [59] describe the presence of LM13 epitopes in the inner and outer cell walls, but absent in the transverse cell walls of the endosperm in *Lepidium* seeds; in tobacco, which has a thicker endosperm than *Arabidopsis* or *Lepidium*, XGs were abundant in the embryo, and at the micropyle (rich in heteromannans), these polysaccharides were only present in the middle lamella and intercellular regions. Thus, the analysis of cell wall-modification processes would benefit from the multispecies compar-

ison of in situ localization of polysaccharide epitopes in seed tissues. The characterization of wall microdomains could be combined with the valuable information generated by -omics technologies, to propose new hypothesis of regulation and coordinated activity of CWMPs. Ultimately, the activity of these CWMPs must be confirmed by in situ localization, in vivo protein interactions, and enzymatic activity. By combining the resources available for model species with the selection of other plant systems with bigger-easy-to-handle seeds, it could be possible to achieve a comprehensive view of seed-compartment functions and regulation during germination. The endosperm role during germination is fundamental in endospermic seeds; however, in non-endospermic seeds, this role must befall on either the embryo or the testa. Since the testa is a nonliving tissue, the radicle most certainly assumes part of this regulatory role, but a comparative analysis is needed to ascertain this supposition and to determine if some of the endosperm functions are developed by the testa while still in the maturation program. The occurrence of endospermic and non-endospermic seeds within the same taxa is relatively common in legumes such as *soybean*, which could offer a model for analyzing transcriptomic differences within embryo compartments comparable to the differences described between the endosperm and the radicle.

ROS participation in germination is supported by several reports and transcriptomic profiles of germinating seeds [9, 14, 17]. However, the actual role of ROS and ROS-related enzymes in promoting cell wall loosening needs to be further analyzed, since the physiological concentrations of ROS during germination do not seem to be sufficient to induce wall extension, and attempts of increasing ROS concentration lead to wall breakage [5]. Müller et al. [72] describe abnormal rupture of the micropylar endosperm of *Lepidium* seeds treated with H_2O_2, while the treatment with myrigalone A [73], which inhibits the hormone-mediated accumulation of ROS during germination, also induces abnormal endosperm breakage. These observations further support the notion of ROS as a signaling agent that induces downstream activation of CWMPs than inducing wall loosening on its own.

Acknowledgements

This work was supported by Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT) IN207915 and SEP-CONACyT grant 155074. Ximena Gómez-Maqueo acknowledges the scholarship by the Consejo Nacional de Ciencia y Tecnología (CONACyT).

Author details

Ximena Gómez-Maqueo and Alicia Gamboa-deBuen*

*Address all correspondence to: agamboa@ecologia.unam.mx

Ecological Physiology Lab., Ecology Institute, National Autonomous University of Mexico, UNAM, Mexico City, Mexico

References

[1] Baskin CC, Baskin JM. Seeds: ecology, biogeography, and evolution of dormancy and germination. San Diego, Elsevier; 1998. 666 p. ISBN-13: 978-0120802609

[2] Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H. Seeds, physiology of development, germination and dormancy. 3rd ed. New York, Springer; 2013. 392p. DOI 10.1007/978-1-4614-4693-4.

[3] Fenner M, Thompson K. The ecology of seeds. Cambridge, Cambridge University Press; 2005. 250p.

[4] Taiz L, Zeiger E. Plant physiology. 5th Ed. Sunderland: Sinauer Assoc; 2010. 782p. ISBN-13: 978-0878938667

[5] Cosgrove DJ. Growth of the plant cell wall. Nature Reviews Molecular Cell Biology. 2005;6:850-861. DOI: 10.1038/nrm1746

[6] Donohue K, Dorn L, Griffith C, Kim E, Aguilera A, Polisetty CR, Schmitt J. The evolutionary ecology of seed germination of *Arabidopsis thaliana*: variable natural selection on germination timing. Evolution. 2005;59:758-770. DOI: 10.1111/j.0014-3820.2005.tb01751.x

[7] Penfield S, Li Y, Gilday AD, Graham S, Graham IA. Arabidopsis ABA INSENSITIVE4 regulates lipid mobilization in the embryo and reveals repression of seed germination by the endosperm. The Plant Cell. 2006;18:1887-1899. DOI: http://dx.doi.org/10.1105/tpc.106.041277

[8] Müller K, Tintelnot S, Leubner-Metzger G. Endosperm-limited Brassicaceae seed germination: abscisic acid inhibits embryo-induced endosperm weakening of *Lepidium sativum* (cress) and endosperm rupture of cress and *Arabidopsis thaliana*. Plant and Cell Physiology. 2006;47:864-877. DOI: 10.1093/pcp/pcj059

[9] Linkies A, Leubner-Metzger G. Beyond gibberellins and abscisic acid: how ethylene and jasmonates control seed germination. Plant Cell Reports. 2012;31:253-270. DOI: 10.1007/s00299-011-1180-1

[10] Linkies A, Müller K, Morris K, Turečková V, Wenk M, Cadman CS, Corbineau F, Strnad M, Lynn JR, Finch-Savage WE, Leubner-Metzger G. Ethylene interacts with abscisic acid to regulate endosperm rupture during germination: a comparative approach using *Lepidium sativum* and *Arabidopsis thaliana*. The Plant Cell. 2009;21:3803-3822. DOI: http://dx.doi.org/10.1105/tpc.109.070201

[11] Kanai M, Nishimura M, Hayashi M. A peroxisomal ABC transporter promotes seed germination by inducing pectin degradation under the control of ABI5. The Plant Journal. 2010;62:936-947. DOI: 10.1111/j.1365-313X.2010.04205.x

[12] Bewley JD. Seed germination and dormancy. The Plant Cell. 1997;9(7):1055. Plant Cell. 1997;9:1055–1066. DOI: 10.1105/tpc.9.7.1055

[13] Nonogaki H, Bassel GW, Bewley JD. Germination—still a mystery. Plant Science. 2010;179:574-581. DOI: 10.1016/j.plantsci.2010.02.010

[14] Weitbrecht K, Müller K, Leubner-Metzger G. First off the mark: early seed germination. Journal of Experimental Botany. 2011;62:3289-3309. DOI: 10.1093/jxb/err030

[15] Rosental L, Nonogaki H, Fait A. Activation and regulation of primary metabolism during seed germination. Seed Science Research. 2014;24:1-5. DOI: http://dx.doi.org/10.1017/S0960258513000391

[16] Müller K, Levesque-Tremblay G, Bartels S, Weitbrecht K, Wormit A, Usadel B, Haughn G, Kermode AR. Demethylesterification of cell wall pectins in Arabidopsis plays a role in seed germination. Plant Physiology. 2013;161:305-316. DOI: http://dx.doi.org/10.1104/pp.112.205724

[17] Dekkers BJ, Pearce S, van Bolderen-Veldkamp RP, Marshall A, Widera P, Gilbert J, Drost HG, Bassel GW, Müller K, King JR, Wood AT. Transcriptional dynamics of two seed compartments with opposing roles in Arabidopsis seed germination. Plant Physiology. 2013;163:205-215. DOI: http://dx.doi.org/10.1104/pp.113.223511

[18] Yan D, Duermeyer L, Leoveanu C, Nambara E. The functions of the endosperm during seed germination. Plant Cell and Physiology. 2014;55:1521-1533. DOI: 10.1093/pcp/pcu089

[19] Narsai R, Law SR, Carrie C, Xu L, Whelan J. In-depth temporal transcriptome profiling reveals a crucial developmental switch with roles for RNA processing and organelle metabolism that are essential for germination in Arabidopsis. Plant Physiology. 2011;157:1342-1362. DOI: http://dx.doi.org/10.1104/pp.111.183129

[20] Jia H, Suzuki M, McCarty DR. Regulation of the seed to seedling developmental phase transition by the LAFL and VAL transcription factor networks. Wiley Interdisciplinary Reviews: Developmental Biology. 2014;3:135-145. DOI: 10.1002/wdev.126

[21] Kermode AR, Finch-Savage BE. Desiccation sensitivity in orthodox and recalcitrant seeds in relation to development. In: Black M, Pritchard HW, editors. Desiccation and survival in plants: drying without dying. New York: Cabi; 2002; p:149-184

[22] Nakabayashi K, Okamoto M, Koshiba T, Kamiya Y, Nambara E. Genome-wide profiling of stored mRNA in *Arabidopsis thaliana* seed germination: epigenetic and genetic regulation of transcription in seed. The Plant Journal. 2005;41:697-709. DOI: 10.1111/j.1365-313X.2005.02337.x

[23] Howell KA, Narsai R, Carroll A, Ivanova A, Lohse M, Usadel B, Millar AH, Whelan J. Mapping metabolic and transcript temporal switches during germination in rice highlights specific transcription factors and the role of RNA instability in the germi-

nation process. Plant Physiology. 2009;149:961-980. DOI: http://dx.doi.org/10.1104/pp.108.129874

[24] Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y, Yamaguchi S. Gibberellin biosynthesis and response during Arabidopsis seed germination. The Plant Cell. 2003;15:1591-1604. DOI: http://dx.doi.org/10.1105/tpc.011650

[25] Silverstone AL, Chang CW, Krol E, Sun TP. Developmental regulation of the gibberellin biosynthetic gene GA1 in *Arabidopsis thaliana*. The Plant Journal. 1997;12:9-19. DOI: 10.1046/j.1365-313X.1997.12010009.x

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

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

[28] Pelloux J, Rusterucci C, Mellerowicz EJ. New insights into pectin methylesterase structure and function. Trends in Plant Science. 2007;12:267-277. DOI: 10.1016/j.tplants.2007.04.001

[29] Caffall KH, Mohnen D. The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. Carbohydrate Research. 2009;344:1879-1900. DOI: 10.1016/j.carres.2009.05.021

[30] Franková L, Fry SC. Biochemistry and physiological roles of enzymes that 'cut and paste' plant cell-wall polysaccharides. Journal of Experimental Botany. 2013;64:3519–3550. DOI: 10.1093/jxb/ert201

[31] Levesque-Tremblay G Pelloux J, Braybrook SA. Tuning of pectin methylesterification: consequences for cell wall biomechanics and development. Planta. 2015; 242(4):791–811. DOI 10.1007/s00425-015-2358-5

[32] Carpita NC. Structure and biogenesis of the cell walls of grasses. Annual Review of Plant Biology. 1996;47:445-476. DOI: 10.1146/annurev.arplant.47.1.445

[33] Yokoyama R, Nishitani K. Genomic basis for cell-wall diversity in plants. A comparative approach to gene families in rice and Arabidopsis. Plant and Cell Physiology. 2004;45:1111-1121. DOI: 10.1093/pcp/pch151

[34] Reiter W. Biosynthesis and properties of the plant cell wall. Current Opinion in Plant Biology. 2002;5:536-542. DOI: 10.1016/S1369-5266(02)00306-0

[35] Bashline L, Li S, Gu Y. The trafficking of the cellulose synthase complex in higher plants. Annals of Botany. 2014;114:1059-1067. DOI: 10.1093/aob/mcu040

[36] Mohnen, D. Pectin structure and biosynthesis. Current Opinion in Plant biology. 2008;11:266-277. DOI: 10.1016/j.pbi.2008.03.006

[37] Willats WG, McCartney L, Steele-King CG, Marcus SE, Mort A, Huisman M, van Alebeek GJ, Schols HA, Voragen AG, Le Goff A, Bonnin E. A xylogalacturonan epitope

is specifically associated with plant cell detachment. Planta. 2004;218:673-681. DOI: 10.1007/s00425-003-1147-8

[38] Scheler C, Weitbrecht K, Pearce SP, Hampstead A, Büttner-Mainik A, Lee KJ, Voegele A, Oracz K, Dekkers BJ, Wang X, Wood AT. Promotion of testa rupture during garden cress germination involves seed compartment-specific expression and activity of pectin methylesterases. Plant Physiology. 2015;167:200-215. DOI: 10.1104/pp.114.247429

[39] Buckeridge MS. Seed cell wall storage polysaccharides: models to understand cell wall biosynthesis and degradation. Plant Physiology. 2010;154:1017-1023. DOI: 10.1104/pp.110.158642

[40] Watson BS, Lei Z, Dixon RA, Sumner LW. Proteomics of *Medicago sativa* cell walls. Phytochemistry. 2004;65:1709-1720. DOI: 10.1016/j.phytochem.2004.04.026

[41] Showalter AM. Structure and function of plant cell wall proteins. The Plant Cell. 1993;5:9-23. DOI: 10.1105/tpc.5.1.9

[42] Jamet E, Canut H, Boudart G, Pont-Lezica RF. Cell wall proteins: a new insight through proteomics. Trends in Plant Science. 2006;11:33-39. DOI: 10.1016/j.tplants.2005.11.006

[43] Dubreucq B, Berger N, Vincent E, Boisson M, Pelletier G, Caboche M, Lepiniec L. The Arabidopsis AtEPR1 extensin-like gene is specifically expressed in endosperm during seed germination. The Plant Journal. 2000;23:643-652. DOI: 10.1046/j.1365-313x.2000.00829.x

[44] Cosgrove DJ. Enzymes and other agents that enhance cell wall extensibility. Annual Review of Plant Biology. 1999;50:391-417. DOI: 10.1146/annurev.arplant.50.1.391

[45] Marín-Rodríguez MC, Orchard J, Seymour GB. Pectate lyases, cell wall degradation and fruit softening. Journal of Experimental Botany. 2002;53:2115-2109. DOI: 10.1093/jxb/erf089

[46] Wolf S, Mouille G. Pelloux J. Homogalacturonan methyl-esterification and plant development. Molecular Plant. 2009;2:851-860. DOI: 10.1093/mp/ssp066

[47] Bordenave M, Goldberg R. Immobilized and free apoplastic pectinmethylesterases in mung bean hypocotyl. Plant Physiology. 1994;106:1151-1160. DOI: http:/dx.doi.org/10.1104/pp.106.3.1151

[48] Mewalal R, Mizrachi E, Mansfield SD, Myburg AA. Cell wall-related proteins of unknown function: missing links in plant cell wall development. Plant and Cell Physiology. 2014;55:1031-1043. DOI: 10.1093/pcp/pcu050

[49] Manabe Y, Nafisi M, Verhertbruggen Y, Orfila C, Gille S, Rautengarten C, Knox JP. Loss-of-function mutation of REDUCED WALL ACETYLATION2 in Arabidopsis leads to reduced cell wall acetylation and increased resistance to *Botrytis cinerea*. Plant Physiology. 2011;155:1068-1078. DOI: http://dx.doi.org/10.1104/pp.110.168989

[50] Zúñiga-Sánchez E, Soriano D, Martínez-Barajas E, Orozco-Segovia A, Gamboa-deBuen A. *BIIDXI*, the *At4g32460* DUF642 gene, is involved in pectin methyl esterase regula-

tion during *Arabidopsis thaliana* seed germination and plant development. BMC Plant Biology. 2014;14:338. DOI: 10.1186/s12870-014-0338-8

[51] Chen F, Bradford KJ. Expression of an expansin is associated with endosperm weakening during tomato seed germination. Plant Physiology. 2000;124:1265-1274. DOI: http://dx.doi.org/10.1104/pp.124.3.1265

[52] Sano N, Rajjou L, North HM, Debeaujon I, Marion-Poll A, Seo M. Staying alive: molecular aspects of seed longevity. Plant and Cell Physiology. 2015 Dec 3:pcv186. DOI: 10.1093/pcp/pcv186.

[53] Hernández-Nistal J, Labrador E, Martín I, Jiménez T, Dopico B. Transcriptional profiling of cell wall protein genes in chickpea embryonic axes during germination and growth. Plant Physiology and Biochemistry. 2006;44:684-692. DOI: 10.1016/j.plaphy.2006.10.017

[54] Saez-Aguayo S, Ralet MC, Berger A, Botran L, Ropartz D, Marion-Poll A, North HM. PECTIN METHYLESTERASE INHIBITOR6 promotes Arabidopsis mucilage release by limiting methylesterification of homogalacturonan in seed coat epidermal cells. The Plant Cell. 2013;25:308-323. DOI: http://dx.doi.org/10.1105/tpc.112.106575

[55] Huang D, Wang C, Yuan J, Cao J, Lan H. Differentiation of the seed coat and composition of the mucilage of *Lepidium perfoliatum* L.: a desert annual with typical myxospermy. Acta Biochimica et Biophysica Sinica. 2015;47:775-787. DOI: 10.1093/abbs/gmv078

[56] Kreitschitz A, Vallès J. Achene morphology and slime structure in some taxa of Artemisia L. and Neopallasia L.(Asteraceae). Flora-Morphology, Distribution, Functional Ecology of Plants. 2007;202:570-580. DOI: 10.1016/j.flora.2006.12.003

[57] Leubner-Metzger G. Functions and regulation of β-1,3-glucanases during seed germination, dormancy release and after-ripening. Seed Science Research. 2003;13:17-34. DOI: http://dx.doi.org/10.1079/SSR2002121

[58] Salanenka YA, Goffinet MC, Taylor AG. Structure and histochemistry of the micropylar and chalazal regions of the perisperm–endosperm envelope of cucumber seeds associated with solute permeability and germination. Journal of the American Society for Horticultural Science. 2009;134:479-487.

[59] Lee KJ, Dekkers BJ, Steinbrecher T, Walsh CT, Bacic A, Bentsink L, Leubner-Metzger G, Knox JP. Distinct cell wall architectures in seed endosperms in representatives of the Brassicaceae and Solanaceae. Plant Physiology. 2012;160:1551-1566. DOI: http://dx.doi.org/10.1104/pp.112.203661

[60] Iglesias-Fernández R, Rodríguez-Gacio MC, Barrero-Sicilia C, Carbonero P, Matilla A. Three endo-β-mannanase genes expressed in the micropylar endosperm and in the radicle influence germination of *Arabidopsis thaliana* seeds. Planta. 2011;233:25-36. DOI: 10.1007/S00425-010-1257-z

[61] Gong X, Bassel GW, Wang A, Greenwood JS, Bewley JD. The emergence of embryos from hard seeds is related to the structure of the cell walls of the micropylar endosperm, and not to endo-β-mannanase activity. Annals of Botany. 2005;96:1165-1173. DOI: 10.1093/aob/mci269

[62] Endo A, Tatematsu K, Hanada K, Duermeyer L, Okamoto M, Yonekura-Sakakibara K, Saito K, Toyoda T, Kawakami N, Kamiya Y, Seki M. Tissue-specific transcriptome analysis reveals cell wall metabolism, flavonol biosynthesis and defense responses are activated in the endosperm of germinating *Arabidopsis thaliana* seeds. Plant and Cell Physiology. 2012;53:16-27. DOI: 10.1093/pcp/pcr171

[63] Leubner-Metzger G, Frundt C, Vogeli-Lange R, Meins Jr F. Class I [β]-1, 3-Glucanases in the endosperm of tobacco during germination. Plant Physiology. 1995;109:751-759. DOI: http://dx.doi.org/10.1104/pp.109.3.751

[64] Ren C, Kermode AR. An increase in pectin methyl esterase activity accompanies dormancy breakage and germination of yellow cedar seeds. Plant Physiology. 2000;124:231-242. DOI: http://dx.doi.org/10.1104/pp.124.1.231

[65] Martínez-Andújar C, Pluskota WE, Bassel GW, Asahina M, Pupel P, Nguyen TT, Takeda-Kamiya N, Toubiana D, Bai B, Górecki RJ, Fait A. Mechanisms of hormonal regulation of endosperm cap-specific gene expression in tomato seeds. The Plant Journal. 2012;71:575-86. DOI: 10.1111/j.1365-313X.2012.05010.x

[66] Chen F, Nonogaki H, Bradford KJ. A gibberellin-regulated xyloglucan endotransglycosylase gene is expressed in the endosperm cap during tomato seed germination. Journal of Experimental Botany. 2000;53:215-223. DOI: 10.1093/jexbot/53.367.215

[67] González-Carranza ZH, Elliott KA, Roberts JA. Expression of polygalacturonases and evidence to support their role during cell separation processes in *Arabidopsis thaliana*. Journal of Experimental Botany. 2007;58:3719-3730. DOI: 10.1093/jxb/erm222

[68] Sitrit Y, Hadfield KA, Bennett AB, Bradford KJ, Downie AB. Expression of a polygalacturonase associated with tomato seed germination. Plant Physiology. 1999;121:419-428. DOI: http://dx.doi.org/10.1104/pp.121.2.419

[69] Sliwinska E, Bassel GW, Bewley JD. Germination of *Arabidopsis thaliana* seeds is not completed as a result of elongation of the radicle but of the adjacent transition zone and lower hypocotyl. Journal of Experimental Botany. 2009;60:3587-3594. DOI: 10.1093/jxb/erp203

[70] Karssen CM, Haigh A, Van der Toorn P, Weges R. Physiological mechanisms involved in seed priming. In: Taylorson, R.B. Recent advances in the development and germination of seeds. 1989 (pp. 269-280). New York, Springer US.

[71] North HM, Berger A, Saez-Aguayo S, Ralet MC. Understanding polysaccharide production and properties using seed coat mutants: future perspectives for the

exploitation of natural variants. Annals of Botany. 2014;114:1251-1263. DOI: 10.1093/aob/mcu011

[72] Müller K, Linkies A, Vreeburg RA, Fry SC, Krieger-Liszkay A, Leubner-Metzger G. In vivo cell wall loosening by hydroxyl radicals during cress seed germination and elongation growth. Plant Physiology. 2009;150:1855-65. DOI: http://dx.doi.org/10.1104/pp.109.139204

[73] Oracz K, Voegele A, Tarkowská D, Jacquemoud D, Turečková V, Urbanová T, Strnad M, Sliwinska E, Leubner-Metzger G. Myrigalone A inhibits *Lepidium sativum* seed germination by interference with gibberellin metabolism and apoplastic superoxide production required for embryo extension growth and endosperm rupture. Plant and Cell Physiology. 2012;53:81-95. DOI: 10.1093/pcp/pcr124

IntechOpen