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# The Functions of the Endosperm During Seed Germination

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**In angiosperms, a double fertilization event initiates the development of two distinct structures, the embryo and endosperm. The endosperm plays an important role in supporting embryonic growth by supplying nutrients, protecting the embryo and controlling embryo growth by acting as a mechanical barrier during seed development and germination. Its structure and function in the mature dry seed is divergent and specialized among different plant species. A subset of endospermic tissues are composed of living cells even after seed maturation, and play an active role in the regulation of seed germination. Transcriptome analysis has provided new insights into the regulatory functions of the endosperm during seed germination. It is well known that the embryo secretes signals to the endosperm to induce the degradation of the seed reserve and to promote endosperm weakening during germination. Recent advances in seed biology have shown that the endosperm is capable of sensing environmental signals, and can produce and secrete signals to regulate the growth of the embryo. Thus, germination is a systemic response that involves bidirectional interactions between the embryo and endosperm.**

**Keywords:** Abscisic acid • Embryo • Endosperm • Germination • Gibberellin • Seed.

**Abbreviations:** ABI3, ABSCISIC ACID-INSENSITIVE3; ABRE, ABA-responsive element; bZIP, basic leucine zipper; CWRE, cell wall remodeling enzyme; DOF, DNA with one finger; δVPE, delta-vacuolar processing enzyme; FUS3, FUSCA3; FR, far-red; GARE, gibberellin-responsive element; LEC2, LEAFY COTYLEDON2; MyA, myrigalone A; MAN, endo-β-mannanase; ME, micropylar endosperm; O2, Opaque-2; OA, okadaic acid; PAC, paclobutrazol; PBF, prolamin-binding-factor; PCD, programmed cell death; PME, pectin methylesterase; R, red; SCBA, seed coat bedding assay; TAG, triacylglycerol; TF, transcription factor; VP1, VIVIPAROUS1; WT, wild type; XTH/XTR, xyloglucan endotransglycosylase hydrolase/XET-related; XYL3, β-D-XYLOSIDASE3.

## Introduction

Seeds are able to sense and respond to environmental factors such as light (Oh et al. 2007), temperature (Yamauchi et al. 2004, Toh et al. 2012), nutrients (Matakiadis et al. 2009) and water (Preston et al. 2009, Bai et al. 2012), in order to control the precise timing of germination. Once the embryonic growth potential exceeds the mechanical constraint of the surrounding tissues, including the endosperm, germination is then complete (Finch-Savage and Leubner-Metzger 2006). In a study by Finch-Savage et al. (2007), it was reported that different germination stimuli (nitrate, after-ripening, light and temperature) trigger similar changes in the transcriptome. This suggests that these environmental factors regulate common downstream events, probably targeting and acting on plant hormone metabolism and signaling. In support of this view, it has been shown that environmental signals can regulate hormone metabolism in the seed, as well as seed responsiveness to hormones (Seo et al. 2009, Nambara et al. 2010, Kim et al. 2013).

Seed germination is regulated in many plant species by two antagonistic plant hormones, ABA and gibberellins. ABA is involved in the induction and maintenance of seed dormancy and inhibits seed germination (Nambara et al. 2010). During seed development, the production of maternal and embryonic ABA is important for preventing precocious germination (Karssen et al. 1983, Kanno et al. 2010). Gibberellin promotes seed germination (Yamaguchi 2008), and both gibberellin metabolism and signaling are influenced by developmental and environmental factors (Ogawa et al. 2003, Rodríguez et al. 2012). Other hormones and regulators (i.e. reactive oxygen species and small regulatory RNAs) contribute towards the fine-tuning of seed dormancy and germination (Nonogaki, 2010, Fernández-Arbaizar et al. 2012, Leymarie et al. 2012, Toh et al. 2012).

In addition to endogenous signals, seed germination is regulated by external signals such as allelochemicals, which are secreted signals that influence the growth and development of surrounding organisms. Plant-derived phytotoxins can be secreted and act as allelochemicals. For example, sweet gale fruit leachate contains myrigalone A (MyA), a C-methylated dihydrochalcone. By utilizing cress (*Lepidium sativum*) seeds as a target, Oracz et al. (2012) demonstrated that MyA modulates endosperm weakening of surrounding cress seeds by regulating gibberellin biosynthesis.

Increased knowledge of endosperm function has made it an attractive target for breeding and biotechnology. In particular, since the endosperm in cereals acts as the primary storage organ for the seed, it can be modified or manipulated to accumulate various metabolites or proteins (Paine et al. 2005, Li et al. 2013, Wang et al. 2013, Tuncel et al. 2014). In addition,

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the endosperm expresses unique genes that have specialized functions in stress tolerance. Such endospermic proteins have been used to acquire stress tolerance when expressed in a heterologous organism (Amara et al. 2012).

In cereals, the role of the endosperm in seed germination has been well documented. The scutellum of the embryo synthesizes and secretes gibberellin to the aleurone layer of the endosperm. Gibberellin induces the synthesis of α-amylase in the aleurone layer, which secretes such hydrolysis enzymes to the starchy endosperm. The embryo utilizes sugars released by starch degradation for its growth. The embryo-less half-seed (the dissected aleurone layer and starchy endosperm from the embryo) has traditionally been used for examining the function of the endosperm in cereal physiology and biochemistry. More recently, dissection of the endosperm is now being utilized for the study of the endosperm in other species (Muller et al. 2006, Penfield et al. 2006, Lee et al. 2010). A major focus in seed biology at the moment is the elucidation of the function of individual cell types or tissues, and the mechanism by which these interact to coordinate a systemic response. In this regard, identifying the sites (i.e. cell types, tissues or organs) of environmental sensing, signal synthesis and perception is crucial for understanding the molecular mechanisms of seed germination. Current advances in crop functional genomics have produced novel tools, concepts and methodologies, and allowed for the comparison of conserved and divergent functions of each organ. This has promoted the accelerated translation of seed biology knowledge into agricultural and biotechnological uses (Martinez-Andujar et al. 2012, Nambara and Nonogaki 2012). This review article summarizes the current progress in our understanding of the endosperm, and its function during seed germination.

## Endosperm Development and its Physiological Roles

### Endosperm development and its role in seed development

Cells of the endosperm are triploid (3*n*) and arise from the fusion of the polar nuclei (2*n*) with the sperm nucleus (*n*) through a double fertilization event. The development of the endosperm is divergent among plant species. Nonetheless, the comparison of endosperm development between cereals and Arabidopsis (*Arabidopsis thaliana*) displays some similar features (Olsen 2004). The development of the endosperm in these plants can be divided into several phases, and includes the formation of the nuclear endosperm (or coenocyte-type endosperm), cellularization, differentiation, maturation and cell death. The development of the nuclear endosperm involves repetitive nuclear divisions without separation of nuclei by the cell wall. This is followed by cellularization which separates sister nuclei by the formation of a periclinal cell wall. There are four main cell types in the cereal endosperm: the starchy endosperm, the aleurone layer, transfer cells and the region surrounding the embryo. The starchy endosperm is the major tissue for seed reserve accumulation in cereal grains, which serves as a nutrient source for seed germination and seedling establishment. In contrast to cereals, in dicot seeds the endosperm is cellularized and consumed for use as an energy source for embryo growth during seed development (Lopes and Larkins 1993). Arabidopsis mutants defective in endosperm cellularization produce small mature dry seeds due to the lack of nutrients required for embryonic growth (Sørensen et al. 2002). The endosperm adjacent to the radicle, the part that protrudes during seed germination, is referred to as the micropylar endosperm (ME). The ME expresses a set of endosperm-specific genes, including those related to cell wall loosening (Dekkers et al. 2013). ME-specific gene expression has been visualized by several techniques such as tissue print (Nonogaki et al. 2000), in situ hybridization (Iglesias-Fernandez et al. 2013) and enhancer trap in Arabidopsis (Liu et al. 2005).

### Structural diversity of the endosperm in mature seeds

The structure of the endosperm in the mature seed varies considerably between different species. The tomato (*Solanum lycopersicum*) contains a hard and thick endosperm cell layer in the mature seed, which undergoes extensive weakening during germination (**Fig. 1A**; Nonogaki et al. 2000). In contrast, exalbuminous seeds such as soybean (*Glycine max*) and pea (*Pisum sativum*) contain very little or no endosperm in the mature seed, as it is fully consumed during seed development (**Fig. 1B**; Lackey 2010). In Arabidopsis, the endosperm is confined to a peripheral aleurone-like cell layer in the mature seed (**Fig. 1C**; K.J.D. Lee et al. 2012). It acts as a mechanical barrier to inhibit embryonic growth, and as a nutrient reserve for seed germination and early seedling establishment. The seed coat undergoes programmed cell death (PCD) during seed development, and as a consequence is no longer alive in the mature seed. In contrast, cereal seeds have more complex structures with a large starchy endosperm and living aleurone layers. In most cereal grains the aleurone is a single cell layer (**Fig. 1D**). The exception is the barley (*Hordeum vulgare*) grain, which contains three cell layers.

### A role for the endosperm: supplier of nutrients

In cereals, starch and proteins are catabolized upon the initiation of germination by hydrolytic enzymes secreted from the aleurone layer. The hydrolyzed starch and proteins act as an energy source, providing carbon and nitrogen for seed germination, and subsequent seedling establishment. The one cell layer endosperm in Arabidopsis accumulates lipids in the form of triacylglycerols (TAGs). TAGs are catabolized into sucrose through gluconeogenesis during and after germination (Penfield et al. 2004). Strikingly, lipids found within the Arabidopsis endosperm differ from those found within the embryo not only in composition, but also in how their catabolism is regulated. Tissue-specific proteomes have been reported for the ME of germinating cress seeds (Muller et al. 2010). Germinating cress endosperm accumulates proteins involved in energy production, protein folding, protein stability

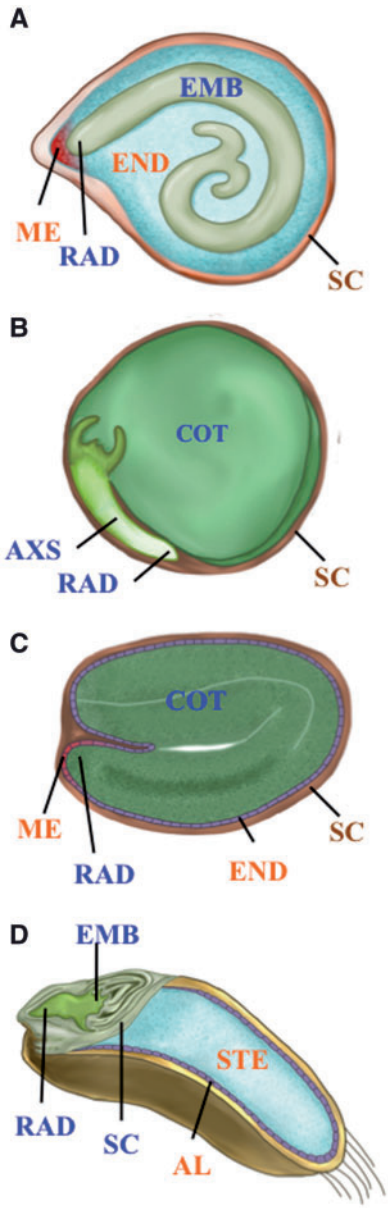


**Fig. 1** Structures of the embryo and endosperm. The endospermic and embryonic tissues are labeled with red and blue, respectively. END, endosperm; EMB, embryo; ME, micropylar endosperm; RAD, radicle; SC, seed coat; COT, cotyledons; AXS, embryonic axis; STE, starchy endosperm; AL, aleurone layer; SC, scutellum. (A) Tomato, (B) pea, (C) Arabidopsis, (D) wheat. The region of ME is colored with red (A and C).

and defense. Their abundance is associated temporally, hormonally and spatially with ME weakening and rupture. This work supports the view that the ME of cress has a regulatory function for seed germination through the modification of cell walls, and does not act exclusively as a source of nutrition (Muller et al. 2010).

## Cell wall loosening at the ME

Modification of the cell wall at the ME is a common strategy to regulate seed germination in dicot seeds that contain living endosperm cells in the mature seeds. The cell wall forms a complex three-dimensional network composed of cellulose, hemicellulose and pectin. Cellulose is a linear polymer of β(1→4)-linked glucose units, while hemicellulose and pectin are heteropolysaccharides composed of different sugar monomors. Hemicellulose cross-links cellulose microfibrils, and pectin is embedded in the cell wall matrix. The endosperm cell wall often contains mannan-rich hemicellulose as a major component in some species such as tomato and tobacco (*Nicotiana tabacum*) (Rodriguez-Gacio et al. 2012), although the detailed composition and components of the cell wall in the endosperm vary among plant species. Cell wall remodeling enzymes (CWREs) are important for synthesizing, loosening and reinforcing the cell walls. The endosperm cell wall is dynamic, as its structure changes in response to environmental, hormonal and developmental signals, all of which can affect the outcome of germination (Leubner-Metzger 2003, Rodriguez-Gacio et al. 2012). The endosperm weakening at the ME is inhibited by ABA, while gibberellin stimulates it (Leubner-Metzger 2003, Muller et al. 2006) by modulating expression of genes encoding CWREs. Both the composition and abundance of CWREs alter the tensile properties of the endosperm to influence the rate of seed germination.

The endosperm cell walls have diverse structure and compositions of hemicellulose and pectin monomers. The architecture of the cell wall for the tobacco endosperm is asymmetrical (K.J.D. Lee et al. 2012), with abundant mannans localized to the ME. In contrast to tobacco, Arabidopsis and cress display a largely uniform architectural arrangement of the endosperm (K.J.D. Lee et al. 2012). The endosperm of Arabidopsis and cress, while composed of cellulose and xyloglucan like tobacco, additionally contains evenly distributed unesterified galacturonan and arabinan. The endosperm of tobacco also accumulates callose, a polymer of β(1→3)-linked glucoses, which is not commonly observed in the cell wall of the endosperm (Leubner-Metzger 2003). Upon imbibition after testa rupture in tobacco, mannans are degraded in the ME to promote endosperm weakening (Leubner-Metzger 2003). Two types of CWREs, endo-β-mannanases (MANs) in tomato (Groot and Karssen 1987, Groot et al. 1988, Nonogaki 2000), and class I β-1,3-glucanases in both tomato and tobacco (Leubner-Metzger 2003), were extensively analyzed for their localized expression to the ME after testa rupture. Both CWREs were thus predicted to play a role in endosperm weakening.

MANs contribute to the regulation of seed germination in Arabidopsis. Four out of seven Arabidopsis MANs are expressed in both the ME and embryo before radicle protrusion (Iglesias-Fernandez et al. 2011). Loss-of-function mutants of *AtMAN5*, *AtMAN6* or *AtMAN7* show delayed germination when compared with wild-type (WT) seeds, suggesting that these MANs are involved in both the endosperm weakening and promotion of embryonic growth during seed germination. β-D-XYLOSIDASE3 (XYL3) plays a role in the hydrolysis of arabinan in Arabidopsis seeds (Minic et al. 2006). *XYL3* is expressed in the endosperm during the early stage in seed development, but is not expressed during the late stage in seed development. *xyl3* mutants exhibit delayed germination and small seed size. It is currently unknown whether delayed germination is due to

the defect in endosperm development or to reduced embryonic growth potential.

In addition to their role in endosperm cell wall weakening, other CWREs such as Arabidopsis xyloglucan endotransglycosylase hydrolase 31/XET-related 8 (XTH31/XTR8) and pectin methylesterases (PMEs) have been implicated in contributing to the reinforcement of the endosperm cell wall during germination. XTH/XTR catalyze the cleavage and reformation of xyloglucan polymers (Rose et al. 2002). *XTH31/XTR8* was highly up-regulated in the endosperm during germination, and the *xth31/xtr8* mutants germinated faster than WT seeds (Endo et al. 2012). Galacturonans are demethylesterified by PMEs, which are highly expressed in the Arabidopsis endosperm during germination (Muller et al. 2013). When PME expression is inhibited, galacturonans remain highly methylesterified in the endosperm cell wall and, as a result, seeds are much larger and germination speed is enhanced. The overall breakdown of the endosperm in seed germination is a complex process that strikes a balance between cell wall strengthening and weakening.

## Programmed cell death

PCD plays an important role in the developmental program of the plant (van Doorn et al. 2011). It is well documented that PCD occurs during seed germination in cereal aleurone cells in order to promote the mobilization of stored nutrients required to support germination and early seedling growth (Young and Gallie 2000). The starchy endosperm of cereals is subjected to PCD during seed development, while the outer aleurone layer remains viable in the mature seed (Young and Gallie 2000). Gibberellin induces PCD in cereal aleurone layers and ABA functions as an antagonist of this process (Kuo et al. 1996, Bethke et al. 1999, Bethke et al. 2002). During PCD, the cells of the aleurone layer synthesize hydrolytic enzymes to break down the nuclear DNA of the cell (Bethke et al. 1999). In addition to hormones, this response is mediated by environmental stimuli, such as hypoxia (Kuo et al. 1996). By analyzing the effect of okadaic acid (OA), Kuo et al. (1996) demonstrated that wheat (*Triticum aestivum*) aleurone cells integrate multiple signal transduction pathways. OA is a protein phosphatase inhibitor that impedes gibberellin-induced PCD, but has no effect on the response to hypoxia. This suggests a possible role for protein phosphatases in gibberellin-induced PCD. PCD also occurs in the aleurone layers of other plant species such as Arabidopsis (Bethke et al. 2007).

PCD occurs via vacuolation, a conserved process by which multiple vacuoles fuse to form a single large lytic vacuole (Bethke et al. 1999, Bethke et al. 2007,Bolte et al. 2011). This compromises the integrity of the plasma membrane, causing the collapse and subsequent death of the cell (Bethke et al. 1999). The degradation of DNA begins during this highly vacuolated state (Bethke et al. 1999), accompanied by the degradation of proteins through the action of cysteine proteases released from small organelles called ricinosomes (Schmid et al. 1998, Schmid et al. 1999). In Arabidopsis, delta-vacuolar processing enzyme (δVPE) is a cysteine protease that has been found to play a role in vacuolation during germination (Endo et al. 2012). Despite delayed vacuolation, Arabidopsis δvpe mutants have a normal germination phenotype. This suggests that vacuolation does not trigger, but rather is a consequence of, germination.

## Gene Expression and Transcriptomes in the Endosperm

Gene expression in the endosperm has been extensively examined in Arabidopsis. Penfield et al. (2006) reported the microarray expression analysis on dissected endosperms from non-dormant Arabidopsis seeds that were imbibed for 24 h after 3 d of stratification. Though a number of embryo- and endosperm-specific genes were found, it was largely determined that the transcriptomes of the embryo and endosperm were quite similar. Thus, the two tissues express similar genetic programs that are intrinsic to the seed. When seeds were treated with ABA and paclobutrazol (PAC), a comparison between the two transcriptomes revealed that both the embryo and endosperm expressed ABA- and gibberellin-regulated genes. Additionally, it was shown that the endosperm of non-dormant seeds was less sensitive to ABA treatment, compared with the embryo (Penfield et al. 2006). Interestingly, both the embryo and endosperm display feedback regulation of gibberellin biosynthetic genes in PAC-treated seeds.

Using the dormant accession C24 to examine the function of the Arabidopsis endosperm, Bethke et al. (2007) demonstrated that the endosperm of imbibed dormant seeds respond to nitric oxide (NO) and gibberellin. It was also found that the endosperm of imbibed dormant seeds was highly sensitive to ABA (Bethke et al. 2007). Endo et al. (2012) performed a tilling array-based transcriptome analysis of the embryo and endosperm of 6 and 24 h imbibed, non-dormant Arabidopsis seeds. This study showed that similar Gene Ontology (GO) categories (i.e. cellular transport, cell wall modification, defense and metabolism) are over-represented in the endosperm transcriptomes at both 6 and 24 h. In addition, the number of expressed endosperm-specific genes increased in 24 h imbibed seeds, indicating that the endosperm acquires specific functions during the progression of seed germination. Dekkers et al. (2013) have generated a web-accessible visualization tool showing detailed spatial and temporal transcriptome analyses of the germinating seed (vseed.nottingham.ac.uk). This high-resolution transcriptome analysis demonstrated that transcriptome patterns of imbibed Arabidopsis seeds are divided into two phases: before and after testa rupture. This work also indicates that mechano-induced signaling genes are associated with testa rupture, and that the transcriptomes of the endosperm and radicle display distinct fates with respect to senescence and growth post-testa rupture (Dekkers et al. 2013). The finding that the cell wall remodeling and hormone-responsive genes showed different temporal and spatial expression patterns suggested that these genes might be under the regulation of multiple different factors. This might establish a complex

regulatory network that responds to multiple signals, depending on the environmental cue and/or hormonal signal.

*Lepidium sativum*, a Brassicaceae species that is a close relative of Arabidopsis, produces large seeds, making it suitable for cross-species microarrays (Linkies et al. 2009, Morris et al. 2011). Such analyses have shown that the endosperm is an important target of gibberellin, ABA and ethylene, and regulates seed dormancy and germination (Linkies et al. 2009, Morris et al. 2011).

## Transcriptional Regulators in the Endosperm

There are two classes of endosperm regulators during germination. The first class includes those that are expressed during both seed development and germination, while the second includes those that are expressed only during germination. Seed maturation regulators possibly function in the endosperm to control germination, either directly or indirectly. Since there are functional links between seed development and germination, we describe transcriptional regulators of endosperm function(s) that are expressed either in seed development, germination or both.

Transcriptional regulation in the endosperm is determined by the *cis*-acting elements found within the promoters of endosperm-expressed genes, as well as the expression of their corresponding transcription factors (TFs) (**Table 1**). As microarray analyses demonstrate, transcriptomes of the embryo and endosperm in germinating seeds are largely similar to one another. Consistent with this observation, many master regulators for seed maturation and germination function in both the embryo and endosperm.

To date, the most well studied *cis*-acting elements for endosperm-specific gene expression in cereals is the bipartite endosperm box (the E box). The E box contains the GCN4-like motif (GLM; 5′-TGASTCA-3′) targeted by basic leucine zipper (bZIP) TFs and the prolamin box (PB; 5′-TGHAAAG-3′) that is bound by the DNA with one finger (DOF) TFs. bZIP and DOF TFs activate endosperm-specific transcription during grain development. The gibberellin-responsive element (GARE), 5′-AACA/TA-3′, is required for gibberellin-induced transcription in the aleurone layer during germination in cereals.

Maize *opaque-2* (*o2*) mutants have a soft and chalky endosperm. *O2* encodes a bZIP TF that binds to the GCN4-like motif, a part of the E box (Schmidt et al. 1990, Lohmer et al. 1991). The *o2* mutation causes a reduction in the synthesis of endosperm-specific seed storage proteins, such as 22 kDa α-zein, and an increase in non-zein proteins. This leads to an increase in total lysine and tryptophan content. Since *o2* lines have high nutritional value, many research groups have attempted to select for *o2* lines containing high lysine content. Knock-down of rice *RISBZ1*, an ortholog of maize *O2*, causes an increase in free lysine content in the rice grain (Kawakatsu and Takaiwa 2010). In Arabidopsis, AtbZIP10 and AtbZIP25, two bZIP TFs orthologous to O2, are expressed in both the embryo and endosperm during seed development (Lara et al. 2003). AtbZIP10 and AtbZIP25 bind to the promoters of seed storage protein genes, and activate transcription in a synergistic manner with other TFs such as ABI3 and bZIP53 (Lara et al. 2003, Alonso et al. 2009).

DOF TFs are characterized by a zinc finger domain that binds to the prolamin box located in the promoters of cereal endosperm proteins. Evolved from a single gene in *Chlamydomonas reinhardtii*, the DOF TF family has expanded into different taxonomic groups with multiple members in vascular plants, and plays important roles in multiple biological processes (Moreno-Risueno et al. 2007b). DOF TFs are well known in cereal seeds for their regulatory roles during seed maturation and germination. Prolamin-binding factor (PBF) from maize and its orthologs, HvDof24/BPBF from barley and WPBF from wheat, activate the transcription of endosperm-specific genes during seed maturation, and contribute to the accumulation of storage reserves in the mature seeds (Mena et al. 1998). In addition, HvDof24/BPBF also acts as a repressor of a gibberellin-responsive hydrolase gene (*cathepsin-B-like cysteine protease Al21*) in aleurone cells upon germination (Mena et al. 2002). HvDOF19 and HvDOF17 mediate the ABA-dependent repression of AI21 in germinating barley aleurone cells (Moreno-Risueno et al. 2007a). The regulatory roles of DOF TFs in seed germination were also reported in Arabidopsis, though a role for the two DOFs, DAG1 and DAG2, in the endosperm has not been reported (Gualberti et al. 2002).

The gibberellin-inducible R2R3MYB TF known as GAMYB was initially identified in the cereal aleurone (Gubler et al. 1995, Gubler et al. 1997). GAMYB binds to the GARE to trans-activate a number of hydrolase genes required for starch mobilization during seed germination (Gubler et al. 1999). Additionally, GAMYB has been shown to be involved in gibberellin-mediated PCD in the aleurone cells of both cereal and Arabidopsis (Guo and Ho 2008, Alonso-Peral et al. 2010). The GAMYB/GAMYB-like genes are conserved across plant genomes (Woodger et al. 2003). In Arabidopsis, three GAMYB-like proteins, MYB101, MYB33 and MYB65, have similar functions to that of GAMYB in cereals (Alonso-Peral et al. 2010). Both rice *GAMYB* and Arabidopsis *MYB101/33/65* are expressed in the aleurone layer. The rice *gamyb* mutant and Arabidopsis *myb33myb65-myb101* triple mutant show defects in gibberellin-induced responses in the aleurone layer of the endosperm (Kaneko et al. 2004, Alonso-Peral et al. 2010). Microarray analysis of gibberellin-treated and untreated embryo-less half-seeds of rice has shown that the *gamyb* mutation leads to a failure of gibberellin-induced transcription in rice aleurone layers during germination (Tsuji et al. 2006). In addition, OsGAMYB induces a different set of gibberellin-responsive genes in the aleurone layer from that of the anthers, indicating that other TFs may be required for the endosperm-specific gibberellin response (Tsuji et al. 2006). It is now known that *miR159* determines the endosperm-specific expression patterns of these genes, since it has been demonstrated that loss of *miR159* function shows ectopic expression of GAMYB in rice and Arabidopsis (Tsuji et al. 2006, Alonso-Peral et al. 2010).

GAMYB is an important regulator for the function of the endosperm during seed development and germination. It interacts with multiple DOF TFs including HvDof24/BPBF, HvDOF23/SAD (Diaz et al. 2005), HvDOF19 and HvDOF17

**Table 1** Transcription factors which function in the endosperm

| Gene | Species | Binding site | Target gene | Expression | Phenotypes | Reference |
|---|---|---|---|---|---|---|
| bZIP transcription factors | | | | | | |
| Opaque2 (O2) | Maize | GCN4 like motif (TGASTCA) | a-zein, 32 kDa albumin(b-32) | Endosperm-specific | Soft and chalky endosperm with high lysine and tryptophan | Lohmer et al. (1991); Schmidt et al. (1990) |
| BLZ1 | Barley | | Itr1 | Endosperm, roots and leaves | NA | Vicente-Carbajosa et al. (1998) |
| BLZ2 | Barley | | Hor-2 | Endosperm-specific | NA | Onate et al. (1999) |
| SPA | Wheat | | LMWG-1D1 | Seed-specific | NA | Albani et al. (1997) |
| RISBZ1 | Rice | | OsLKR/SDH | Endosperm-specific | Higher lysine content | Kawakatsu and Takaiwa (2010); Kawakatsu et al. (2009) |
| TRAB1 | Rice | ABRE (ACGT box) | Osem | Embryo, roots and leaves | NA | Hobo et al. (1999) |
| HvABI5 | Barley | | HVA1, HVA22 | Aleurone layer[a] | HvABI5 RNAi inhibits the ABA activation of ABRC–GUS | Casaretto and Ho (2003) |
| AtABI5 | Arabidopsis | | AtEm6, AtEm1 | Embryo and micropylar endosperm | Reduced sensitivity to ABA inhibition of germination | Carles et al. (2002); Kim et al. (2002); Penfield et al. (2006) |
| AtbZIP44 | Arabidopsis | G-box (CACGTG) | AtMAN7 | Embryo and micropylar endosperm | Delayed germination | Iglesias-Fernandez et al. (2013) |
| DOF transcription factors | | | | | | |
| PBF | Maize | Prolamin box (TGHAAAG) | γ-zein | Endosperm specific | NA | Marzabal et al. (2008) |
| WPBF | Wheat | | α-gliadin | Root, cotyledon, leaf, stem, flower, seeds | NA | Dong et al. (2007) |
| HvDOF24/ BPBF | Barley | | Hor-2, Al21, Amy2/ 32b | Endosperm specific | NA | Mena et al. (1998); Mena et al. (2002) |
| HvDOF23/ SAD | Barley | | Itr1, Hor-2 and Al21 | Starchy endosperm, aleurone cells, nucellar projection, vascular tissues and the immature embryo | NA | Diaz et al. (2005); Isabel-LaMoneda et al. (2003) |
| HvDOF19 | Barley | | Al21 | Aleurone layer, embryo | NA | Moreno-Risueno et al. (2007a) |
| GAMYB transcription factors | | | | | | |
| HvGAMYB | Barley | GARE (T/C)AAC(A/T)AC | Hor-2 and Itr1 | Aleurone layer, starchy endosperm, nucellar projection, vascular tissue and immature embryo | Transient expression of HvGAMYB RNAi blocks gibberellin-induced vacuolation in aleurone cells | Diaz et al. (2002); Guo and Ho (2008) |

(continued)

**Table 1** Continued

| Gene | Species | Binding site | Target gene | Expression | Phenotypes | Reference |
|---|---|---|---|---|---|---|
| OsGAMYB | Rice | GARE (TAACAAA) | RAmy1A | Aleurone cells and anthers | Defects in gibberellin-induced gene expression in the endosperm. Incomplete heading, sterile panicle | Gubler et al. (1995); Tsuji et al. (2006) |
| AtMYB101, AtMYB33, AtMYB65 | Arabidopsis | NA | NA | Endosperm, embryo, anthers. MYB101 is endosperm-specific | Defects in gibberellin-induced vacuolation in germinating endosperm | Alonso-Peral et al. (2010); Penfield et al. (2006) |
| DELLA proteins | | | | | | |
| SLN1 | Barley | NA | NA | NA | Constitutive expression of α-amylase in aleurone layer, slender plants | Gubler et al. (2002) |
| SLR1 | Rice | NA | NA | NA | Constitutive expression of α-amylase in aleurone layer, slender plants | Ikeda et al. (2001) |
| RGL2 | Arabidopsis | NA | NA | NA | Inability to secrete ABA from the endosperm | Lee et al. (2010) |
| B3-domain transcription factors | | | | | | |
| Viviparous1 (VP1) | Maize | RY/Sph motif (CATGCA) | C1, a regulator for anthocyanin biosynthesis | Embryo and aleurone layer | ABA-insensitive seed. Reduced accumulation of anthocyanins in kernels. Vivipary | Cao et al. (2007); McCarty et al. (1989); Suzuki et al. (1997) |
| OsVP1 | Rice | | Osem | Embryo and aleurone layer | NA | Hattori et al. (1995); Miyoshi et al. (2002) |
| AtABI3 | Arabidopsis | | SOMNUS (SOM), a set of 98 genes | Embryo and endosperm | ABA-insensitive seed. Severe defects in seed maturation. Desiccation-intolerant seeds | Monke et al. (2012); Park et al. (2011); Penfield et al. (2006) |
| HvFUS3 | Barley | | Hor-2 and Itr1 | Embryo, endosperm and aleurone cells | HvFUS3 complements Arabidopsis *fus*3 mutant | Moreno-Risueno et al. (2008) |
| bHLH transcription factors | | | | | | |
| AtPIL5 | Arabidopsis | G-box (CACGTG) | SOMNUS (SOM), GAI, RGA | Both embryo and endosperm in germinating seeds[b], seedling | PhyB-independent germination. Dissected endosperm secretes ABA in a light-dependent manner. | Lee et al. (2012); Oh et al. (2007); Park et al. (2011); Zhang et al. (2013) |
| WRKY transcription factors | | | | | | |
| HvWRKY38 | Barley | W-box (TTGACY) | Amy32b | NA | NA | Mare et al. (2004) |

NA, information not available.
[a] The cDNA library was made from the aleurone layer.
[b] Expression data are obtained from tilling array data on the embryo and endosperm of 24 h imbibed seeds (Endo et al. 2012).



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(Moreno-Risueno et al. 2007a). Other TFs acting together with GAMYB regulate transcription of the α-*amylase* (*Amy32b*) gene in barley (Zou et al. 2008). Two GAMYB-interacting repressors from barley, HvWRKY38 and HvDof24/BPBF, suppress the physical interaction of HvDOF23/SAD and GAMYB upon induction of *Amy32b* in the absence of gibberellin. A detailed analysis of the interactions between different TFs on the barley *Al21* promoter proposed that in the early germination phase when the ABA:gibberellin ratio is high, the transcription of *Al21* is repressed by ABA through HvDOF19. HvDOF17 and HvDof24/BPBF act to repress *Al21* expression independently of ABA. In late germination when the ABA:gibberellin ratio decreases, repression of *Al21* transcription can be overcome, as the expression of the positive effectors GAMYB and HvDOF23/SAD increases (Moreno-Risueno et al. 2007a).

The maize VIVIPAROUS1 (VP1) B3-domain TF is an important regulator of seed ABA responses (Suzuki et al. 1997). VP1 binds to the Sph seed-specific enhancer and activates ABA-induced transcription via interaction with the ABA-responsive element (ABRE)-binding bZIP TF TRAB1 in rice (Hobo et al. 1999), and HvABI5 in barley (Casaretto and Ho 2003). OsVP1 is expressed in both the embryo and endosperm, and is abundantly expressed in the aleurone layer, suggesting that VP1 plays a prominent role in this tissue (Miyoshi et al. 2002). Penfield et al. (2006) reported that Arabidopsis *ABSCISIC ACID-INSENSITIVE3* (*ABI3*) and *ABI5*, encoding an ortholog of VP1 and an ABRE-binding bZIP TF, respectively, are expressed in both the embryo and endosperm during germination. In contrast, *ABI4*, which encodes an AP2-domain TF, is expressed specifically in the embryo. Consistent with this expression pattern, the endosperm of *abi4* mutants is ABA sensitive, while the embryo is ABA insensitive (Penfield et al. 2006).

In Arabidopsis, the B3 domain TFs ABI3, FUSCA3 (FUS3) and LEAFY COTYLEDON2 (LEC2) are master regulators of seed maturation and germination (Kagaya et al. 2005, Santos Mendoza 2005, Verdier and Thompson 2008). These mutants show severe defects in seed maturation and produce seeds that are desiccation intolerant. HvFUS3, a barley ortholog of FUSCA3, is most abundantly expressed in the aleurone layer during seed development (Moreno-Risueno et al. 2008), which is different from ubiquitous expression of Arabidopsis B3 TFs in both the embryo and endosperm.

Iglesias-Fernandez et al. (2013) analyzed the transcriptional regulation of *AtMAN7* in the endosperm during germination. A comparative analysis of Brassicaceae *AtMAN7* promoters identified several conserved regions. AtbZIP44, a bZIP TF, was successfully identified based on its ability to bind a conserved region in the *AtMAN7* promoter, and to activate its transcription in the endosperm during germination (Iglesias-Fernandez et al. 2013). *atbzip44* mutants showed delayed germination, while its overexpression showed the opposite germination phenotype. *AtbZIP44* is expressed in both the embryo and endosperm, indicating that other regulators/signals may determine or regulate the endosperm-specific expression of *AtMAN7*. This illustrates the advantage of using multiple plant genome sequences of closely related species for the purpose of identifying conserved *cis*-elements and their corresponding TFs.

## The Endosperm: A Mediator of Communication Between the Embryo and its Environment

### Environmental sensing and responses

The endosperm is the outermost living layer in the seeds, and its function is influenced by both the embryo and the surrounding environment. The endosperm responds to environmental factors, inducing the expression of Arabidopsis hormone metabolism genes in response to imbibition (Okamoto et al. 2006), as well as low temperature (Yamauchi et al. 2004). Cold temperatures activate *AtGA3ox1* expression, the rate-limiting enzyme for gibberellin biosynthesis during germination, at both the embryonic axis and endosperm. In contrast, red (R) light induces *AtGA3ox1* expression at the embryonic axis, but not at the endosperm (Yamauchi et al. 2004). Despite the availability of extensive gene expression data, it remains to be determined if endosperm cells are able to sense various environmental changes in a cell-autonomous fashion.

The function of the endosperm often requires its attachment to the embryo during germination. The tomato endosperm is weakened prior to radicle protrusion, which is induced by embryo-derived diffusible signal(s) (Groot and Karssen 1987). The endosperm weakening of cress is also promoted by the attachment of the embryo (Muller et al. 2006). In addition, genes encoding cell wall weakening enzyme are expressed at the ME (Nonogaki et al. 2000, Linkies et al. 2009, Dekkers et al. 2013). The progression of vacuolation of the Arabidopsis endosperm is dependent on its attachment to the embryo (Debeaujon and Koornneef 2000, Bethke et al. 2007). Genes related to endosperm weakening and vacuolation are gibberellin inducible (Groot et al. 1988, Toorop et al. 2000); thus gibberellin may act as a diffusible signal from the embryo. Indeed, application of gibberellin induces β-mannanase activity in the detached endosperm of the tomato (Groot et al. 1988).

The endosperm cells are capable of sensing ABA and gibberellin. In contrast, limited information is available regarding the ability of endosperm cells to sense environmental signals. For the investigation of the function of the endosperm, Lee et al. (2010) designed a novel assay system called the seed coat bedding assay (SCBA). Arabidopsis seeds were dissected for the purpose of removing the embryo from the endosperm and testa (endosperm/testa). The embryos were then placed and grown on a bed of endosperm/testa, to examine embryonic growth. Various combinations of embryos and endosperm/testa from different genetic and physiological backgrounds can be used to test the interaction between the embryo and endosperm by this assay. The authors treated dissected embryos and endosperm/testa with R light, and far-red (FR) light pulses. The SCBA results showed that embryonic growth is dependent on the endosperm, indicating that Arabidopsis endosperms are capable of sensing R and FR light to activate downstream events (K.P. Lee et al. 2012). It should be noted that the sites of light sensing that act to induce germination may differ between plant species. Physiological experiments

using lettuce seeds suggest that the embryonic axis is a possible site for sensing R light, as well as gibberellin-mediated embryonic growth (Takeba 1980).

The endosperm and embryo display differential responses to high temperature. Thermoinhibition inhibits seed germination under supraoptimal temperatures. Arabidopsis seeds are unable to germinate when imbibed at high temperatures (Tamura et al. 2006), while non-germinated seeds imbibed at high temperatures induce the expression of *NCED2*, *NCED5* and *NCED9*, genes that encode the rate-limiting enzyme of ABA biosynthesis. High temperatures induce *NCED9* expression effectively in the embryo, and *NCED5* expression is induced by high temperatures more effectively in the endosperm than in the embryo (Toh et al. 2008). High temperature-induced *NCED9* expression is a rapid response, detected by 6 h post-imbibition. In contrast, high temperature-induced *NCED5* expression is a late response, as it can only be detected 24 h post-imbibition. Based on the difference in the kinetics of *NCED5* and *NCED9* expression, it is possible that the embryo detects the high temperature, and, once detected, a signal is sent to the endosperm. The expression pattern of the *NCED* genes suggests that the induction of the ABA biosynthetic gene in seeds imbibed at supraoptimal temperatures is a common response in both the embryo and endosperm, despite their differential regulation.

## Signal synthesis and secretion by the endosperm

A signal molecule synthesized in the seed is able to regulate the cellular activity of its own cells, the surrounding cells, the distal cells of the same seed or those of neighboring seeds. Studies examining the function of the endosperm demonstrate that the endosperm secretes signals to control the growth of the embryo. Lee et al. (2010) reported that the dissected endosperm is capable of synthesizing and releasing ABA to regulate embryonic growth. The SCBA showed that ABA released from the dissected endosperms is capable of blocking the growth of the coat-less embryos. The SCBA also showed that the endosperm is capable of synthesizing ABA in response to light signals (K.P. Lee et al. 2012). These findings demonstrate that the cells of the endosperm are able to sense the environmental changes and synthesize signals for the purpose of regulating embryonic growth. Thus, communication and interaction(s) between the embryo and endosperm is bidirectional.

## Conclusion

The structures of the endosperm are divergent and functionally specialized in each plant species. While the structure of the endosperm differs and takes on diverse functions, the main role remains that of a primary nutrient source, and/or as a regulator of embryonic growth during seed development and germination. Current functional genomic research in Arabidopsis and cereal crops indicates interesting features in the function of the endosperm during seed germination.

One interesting feature is that a similar set of seed regulators has been utilized among different plant species to establish the common and distinct regulatory networks in these seeds. Despite the similarity of these conserved members of seed regulators, their spatial and temporal expression patterns are varied among different plant species. This may contribute to the acquisition of specialized functions for the embryo and endosperm in each plant species.

Another interesting finding is that the endosperm is capable of sensing light signals and interacting with the embryo through bidirectional communication. This indicates that the endosperm is not merely a nutrient source or a mechanical barrier of germination, to be controlled by embryonic signals, but also directs embryonic growth by actively secreting signals. Examining the function of individual seed cell types, including the endosperm, will provide an excellent model for understanding the mechanism of cell–cell communication. It will also provide insight into how cell–cell communication directs or coordinates the systemic responses of the seed. Continued study into the role of the endosperm will facilitate the application of seed biology knowledge to the development of robust and sustainable agricultural practices.

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The authors have no conflicts of interest to declare.

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