Seed Germination—The Biochemical and Molecular Mechanisms

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The chemical energy synthesized during photosynthesis as carbohydrates, lipids and proteins accumulates in seeds and provides a food source for animals. Seeds are also important genetic delivery systems essential for sustainable agriculture and environmental control. Genetic information in elite cultivars of crop species accumulated during breeding programs is distributed in the form of seeds. Ensuring successful germination and seedling establishment is a significant first step in agricultural production. The molecular and biochemical mechanisms of seed germination are not fully understood. Our knowledge of the interactions between the embryo, endosperm and testa has been advanced through tomato seed research, a model system for seed germination research. Recent discoveries using Arabidopsis thaliana have provided additional information about the molecular and genetic mechanisms of seed dormancy and germination. Genes expressed during seed development determine the size, shape and chemical properties of mature seeds and affect seed dormancy. In imbibed seeds, genes associated with hormone biosynthesis and degradation play critical roles in radicle emergence. The physical, chemical and physiological changes in the embryo, endosperm and testa, as well as the interactions between these tissues all contribute to successful germination. Recent literature on seed science research needs to be compiled to provide a clear picture for seed germination. Hypotheses to explain global mechanisms of seed germination are examined in this review.

Key Words: seed, germination, dormancy, testa, embryo, endosperm, Arabidopsis.

Introduction

Seeds are excellent dispersal units which have emerged in the course of plant evolution. The chemical energy produced during photosynthesis accumulates as seed reserves in multiple forms including carbohydrates, lipids and proteins. Mobilization of these reserves following germination is essential for the embryo to complete seedling establishment and, also signals the start of a new life cycle, an important aspect of agricultural production. These seed storage reserves are used directly as a source of nutrition for animals including humans. Plant breeders have targeted and modified seed traits related to seed size and reserve accumulation, especially in cereals and beans (e.g. Rao et al. 1998, Khattak et al. 2003). However, selections focusing on seed germination capacity are not frequently performed in plant breeding.

Vegetable and flower cultivars are selected based on the quality of fresh products which is important in competitive markets. This sometimes conflicts with seed performance related to germination and storability. For example, in the case of sweet corn, efforts to develop a “sweeter” phenotype have reduced the capacity of kernels to accumulate starch. In general, since seeds which accumulate sucrose, instead of starch, have a lower water potential, more water is transported into seeds during development. During maturation drying, seeds lose water and eventually become shrunken (Dennis and Blakeley 2000). This often results in reduced vigor during corn seed (kernel) germination and seedling establishment in the fields (Welbaum et al. 2001). In general, larger spinach seeds produce more vigorous plants and directly affect the yield of this leafy vegetable. However, larger seeds tend to display a lower germination performance compared to smaller seeds, especially at high summer temperatures (H. Nonogaki, unpublished results). Botanically, spinach “seeds” are actually the fruit rather than the seed of the plant. The outermost layer of the “seed” is the pericarp which causes germination problems at high temperatures, most likely due to its limited oxygen permeability and mechanical resistance. It is unlikely that high temperatures reduce the oxygen permeability of the pericarp or increase its mechanical resistance. Probably, high temperatures decrease the growth potential of the embryo, and therefore, the embryo cannot overcome the existing barrier at high temperatures. Large spinach “seeds” have a thicker pericarp and show a slower and reduced germination rate at high temperatures compared to small seeds.

Thermodynamics of lettuce seeds is also problematic in lettuce production. Lettuce is often selected for different
The general mechanisms of seed germination related to the progress in seed germination research will be introduced. The molecular and genetic information obtained from these studies can be integrated into breeding programs over a long term. In this review, recent progress in seed germination research will be introduced. The general mechanisms of seed germination related to the seed structure will be described first, and then the importance of gene regulation in determining the seed morphology, dormancy and germination during seed development will be discussed. This will be followed by an examination of recent models depicting the biochemical and molecular mechanisms of sensu stricto seed germination.

**Seed development, structure and general mechanisms of germination**

To elucidate the mechanisms of seed germination, it is essential to analyze the structure of seeds common to many different species and also to recognize characteristics of seeds, such as the chemical and physical properties of the testa, which vary considerably among species. The properties of the testa will be analyzed in greater detail in the next section. The two major components of seed, the embryo and the endosperm and their interaction are presented here.

Seed is generally defined as a mature ovule. In angiosperm seeds, the embryo and the endosperm start to differentiate after double fertilization in the ovule. A pollen sperm nucleus penetrates into the ovule through one of the synergid cells and fuses with an egg cell nucleus forming a diploid embryo (Higashiyama et al. 2003). The embryo develops into globular, heart, torpedo and walking-stick stages and eventually exhibits a mature morphology (Bewley et al. 2000). The triploid endosperm, derived from the fusion between another pollen sperm nucleus and two central nuclei, also differentiates during embryogenesis as does the testa, which is derived from maternal integuments. Analyzing the genetic origin of seed tissues is important for interpreting and applying results from molecular and genetic studies. Investigations using Arabidopsis mutants indicated that the development of the testa and endosperm can occur independently of embryogenesis (Ohad et al. 1996, Chaudhury et al. 1997, Kiyosue et al. 1999). However, it is also possible that there are significant interactions and/or communication between these tissues during seed development.

In some species, the endosperm is not found in the mature seed. These seeds are referred to as non-endospermic seeds (Bewley and Black 1994). Even in these species, the endosperm tissue is formed during embryogenesis but is digested before development is completed. In endospermic seeds, the amount of endosperm present in the mature seed depends on when they are dispersed from the maternal plant. Fig. 1 shows five typical patterns of embryo/endosperm morphology found in mature seeds. In non-endospermic seeds or endospermic seeds with only remnants of endosperm tissues, the endosperm is completely or mostly digested during the developmental stages preceding dispersal. A mature seed of this type is occupied mainly by the embryo (Fig. 1, Type V). This group includes pea, bean, lettuce and Arabidopsis seeds. In endospermic seeds such as castor bean, pepper and tomato seeds, a substantial amount of endosperm tissue remains in the mature seed with linear (Type II) or curled embryos (Type III and IV). Mature seeds (“schizocarps” which are also fruits) of Umbelliferae species such as carrot, parsley and celery contain very small, rudimentary embryos (Type I). This type of seed is detached from the maternal plant at relatively early stages of development resulting in immature embryos in mature seeds. An immature embryo must grow inside the seed before germination. Therefore, two different programs—the developmental and the germinative programs—are sequentially observed in carrot seeds during imbibition (Homrichhausen et al. 2003).

Plant species that produce Type III, IV and V embryos disperse seeds at a relatively later stage than plants that produce Type I embryos. These embryos mature to a later developmental stage while still remaining attached to the maternal plant. In these species, the development of a delayed seed dispersal mechanism might have occurred in the course of evolution. Seed developmental programs, which are probably regulated by maternal signals, may be maintained in type III, IV and V seeds. This probably results in further maturation of the embryo remaining on the plant because of delayed seed detachment. (These mechanisms are hypothetical and have not been demonstrated experimentally.) In contrast, Type I embryos must develop during imbibition and they grow until they reach about two-thirds of the length of the seed prior to radicle emergence (Homrichhausen et al. 2003). Interestingly, when carrot seeds are imbibed in ABA, post-dispersal embryo growth is promoted in seeds. Although radicle emergence is inhibited by this hormone, excessive growth of the hypocotyls and cotyledons, which is not observed during normal germination, is promoted. The changes in embryo morphology in ABA-imibed seeds somewhat mimic the development of other endospermic seeds (Homrichhausen et al. 2003). These observations support the hypothesis mentioned above. Thus, the degree to which the developmental program of the embryos is completed seems to be determined by the timing of seed detachment from the maternal plant.

Although the endosperm thickness varies among species, in general, the occurrence of radicle protrusion in endospermic seeds is determined by a balance between the leaf phenotypes, but it is rare for lettuce cultivars to be selected for seed (or “achene” which is also a fruit) germination performance at high temperatures. If breeding programs could incorporate selection for seed germination tolerant of high temperatures, the performance of seeds in the fields for commercial lettuce production may change. Although this attempt has already been made by some seed companies, germination characteristics are seldom included in breeding programs.

It is important to consider seed performance for breeding purposes when new cultivars are developed. Significant progress in basic seed germination research was achieved during the last decade. The molecular and genetic information obtained from these studies can be integrated into breeding programs over a long term. In this review, recent progress in seed germination research will be introduced. The general mechanisms of seed germination related to the seed structure will be described first, and then the importance of gene regulation in determining the seed morphology, dormancy and germination during seed development will be discussed. This will be followed by an examination of recent models depicting the biochemical and molecular mechanisms of sensu stricto seed germination.
Grains have been extensively studied in relation to reserve mobilization (e.g., hormonal regulation of α-amylase secretion into starchy endosperm). However, since most of these events occur after radicle emergence, they are postgermination events. Information about the physiological mechanisms of “germination” *per se* is very limited in monocot seeds.

**Genes expressed during seed development affecting seed size, dormancy and germination**

During seed development, important genes that control the morphogenesis of seeds are expressed. Since extensive volumes of literature for embryogenesis-associated genes have already been published, these genes are not mentioned here. Instead, emphasis will be placed on other genes important in controlling the seed size, shape and dormancy/germination characteristics.

As mentioned above, seeds can exhibit a distinct performance in germination speed and high temperature sensitivity due to seed size differences. In natural ecosystems, the seed size also affects the survival of the plants. It appears that the concept “Larger seeds are always better” is also valid in some cases of seed survival. Interestingly, larger acorns of *Quercus ilex* show a decreased probability of survival compared to smaller acorns. Despite their superior performance in germination and seedling establishment, they are less likely to survive because they are more likely to be eaten by predators such as wild boars and wood mice (Gomez 2004). Thus, seed size determination is an important trait for plant survival. Natural selection pressures must have forced plants to develop strategies to control the seed size, some of which are probably mediated by gene expression. Therefore, it is likely that plants harbor some genes controlling the seed size. Of course, the seed size (or mass) is an important trait in agricultural species that is often modified through breeding programs. Identification of seed size determinants will exert a large impact on agriculture.

It is generally recognized that increased dosage from the maternal genome results in a smaller seed, while increased dosage from the paternal genome results in a larger seed. Interploidy reciprocal crosses between diploid and tetraploid *Arabidopsis* have shown that a double dosage of the maternal genomes inhibits endosperm development and produces a smaller embryo, while a double dosage of paternal genome promotes the growth of the endosperm and the embryo (Scott et al. 1998). This phenomenon is referred to as “parent-of-origin effect” and can be explained by genomic imprinting, which is caused by the conflict between the maternal and paternal genomes over resource allocation from the mother to the embryo (“parental conflict model”) (Haig and Westoby 1989, 1991, Moore and Haig 1991). Parent-of-origin effects can also be explained by other theories (see Dilkes and Comai 2004 for review). Mutations in genes associated with imprinting such as *FERTILIZATION INDEPENDENT ENDOSPERM (FIE)* (Ohad et al. 1996).
FERTILIZATION INDEPENDENT SEED (FIS) (Chaudhury et al. 1997) and MEDEA (MEA) (Grossniklaus et al. 1998, Kinoshita et al. 1999, Kiyosue et al. 1999) offer potential applications for agriculture. In these mutants, fruit and seed development is initiated without fertilization, although these mutations cause embryo abortion, indicating that sexually reproducing plants have a genetic potential for apomixis (Kiyosue et al. 1999). Apomixis allows breeders to reproduce desirable agronomic traits without crossing.

Information about other genes regulating the seed size is limited. Two separate research groups have reported that APETALLA2 (AP2) plays a key role in determining the seed mass/yield in Arabidopsis. Both groups observed that loss-of-function ap2 mutations caused an increase in seed weight (Jofuku et al. 2005, Ohito et al. 2005). AP2 or EREBP (ethylene responsive element binding protein) is a well-known transcription factor which is involved in the specification of flower organ identity (Okamura et al. 1993, Jofuku et al. 1994, Riechmann and Meyerowitz 1998). Although ap2 mutants exhibit abnormal flowers, the effect of mutation on seed mass is not due to reduced fertility but due to an increase in the cell number and size in the embryo (Jofuku et al. 2005, Ohito et al. 2005). By conducting reciprocal cross experiments, the authors also observed that AP2 controls the seed mass through the maternal sporophytic and endosperm genome. Maternal control of the seed size through integument and endosperm development has also been observed in mutations in HAiku (IKU) and TRANSPARENT TESTA GLABRA2 (TTG2) genes. In these cases, the control of cell elongation in the integuments and the endosperm by these genes was found to play a significant role in seed size determination (Garcia et al. 2005).

Although an increase in seed size is often compensated for by a decrease in total yield in seed production, in the ap2 mutation such a compensatory decrease in seed yield was not observed (Jofuku et al. 2005). The detailed mechanism underlying the control of the seed mass by the AP2 gene has not yet been elucidated. AP2 may control seed mass through its effects on the sugar metabolism (Ohito et al. 2005). The involvement of AP2 gene in suppressing the GA signaling pathway has also been suggested, based on genetic and physiological studies (Jofuku et al. 2005). GA is a well-known seed germination-promoting hormone and also plays significant roles in flowering (Jacobsen and Olzsewski 1993, Boss and Thomas 2002) and seed development (White and Rivin 2000, White et al. 2000, Swain and Singh 2005). Therefore, it is likely that AP2 controls the seed mass by modulating the hormonal balance in developing seeds.

Mutations in the brassinosteroid (BR) biosynthesis genes also affect the seed size in rice. Map-based cloning of the D11 gene in dwarf rice identified a novel cytochrome P450 (CYP724B1) which showed homology to the enzymes involved in BR synthesis. The d11 mutant produced smaller seeds which were shorter but with similar width compared to wild-type seeds. Seeds from plants complemented with the D11 gene showed a normal length, indicating that this gene actually controls seed size (Tanabe et al. 2005). Other BR mutants d2 and brdl also produced small seeds. In these mutant lines, both the length and width of the seeds were reduced (Hong et al. 2002, 2003).

AP2 and IKU change the size of the embryo and the endosperm which could affect the germination capacity indirectly. Many other genes directly affect seed dormancy and germination through the modulation of the testa properties, the best-known being those of the TRANSPARENT TESTA (tt) mutants, which exhibit a low seed dormancy (Debeaujon and Koornneef 2000, Debeaujon et al. 2000). Proanthocyanidins (or condensed tannins) are formed as colorless polymers through the flavonoid biosynthetic pathway and confer the brown color of Arabidopsis seeds after oxidation (Devic et al. 1999, Debeaujon et al. 2001). This pigmentation does not occur in the tt mutants. More than 20 loci cause tt mutations (Debeaujon et al. 2003). TT3, TT4, TT5, TT6 and TT7 encode enzymes in the flavonoid biosynthetic pathway, namely dihydroflavonol reductase (Shirley et al. 1992), chalcone synthase (Feinbaum and Ausubel 1988), chalcone isomerase (Shirley et al. 1992), flavonol synthase (Shirley et al. 1995) and flavonol 3′-hydroxylase, respectively (Koornneef et al. 1982b).

Other TT genes do not encode flavonoid synthetic enzymes but regulate the accumulation of flavonoids in developing seeds. TT12 encodes a protein similar to prokaryotic and eukaryotic secondary transporters with 12 transmembrane segments (the MATE [multidrug and toxic compound extrusion] family) and is possibly associated with the transport of flavonoids to vacuoles (Debeaujon et al. 2001). TT19 encodes a glutathione S-transferase (GST)-like protein (Kitamura et al. 2004). This protein is similar to the Petunia GST protein AN9 which is involved in flavonoid accumulation (Alfenito et al. 1998). Arabidopsis GST is also similar to the BZ2 protein in maize which is associated with the GST pump in vacuolar membranes (Marris et al. 1995). Identification of TT12 and TT19 suggests the importance of the vacuolar transport of flavonoids in testa pigmentation during seed development. The involvement of membrane transporters in testa pigmentation is also supported by the observation that mutations in a membrane protein AHA10 (auto-inhibited H+–ATPase) led to impaired vacuole development and induced TRANSPARENT TESTA phenotypes (Baxter et al. 2005).

Other TT genes encode transcription factors which are the upstream regulators of the flavonoid biosynthetic genes. BANYULS (BAN) encodes anthocyanidin reductase (Xie et al. 2003) which is involved in the flavonoid synthetic pathway. BAN expression is regulated by TT2 (R2R3 MYB protein), TT8 (basic helix-loop-helix [bHLH] protein) and TTG1 (WD-repeat protein [WDP]) (Nesi et al. 2000, 2001). Genetic analysis suggested that TT2, TT8 and TTG1 transcription factors are synergistically involved in BAN expression and control the seed testa pigmentation (Debeaujon et al. 2003). By combining molecular and genetic approaches, TT2, TT8 and TTG1 were found to physically interact, form a ternary complex and directly regulate the BAN promoter.
activity (Baudry et al. 2004). TT1 (WIP zinc finger protein, Sagasser et al. 2002) and TT16 (BSISTER MADS-box transcription factor, Nesi et al. 2002) are considered to act upstream of TT2 (Debeaujon et al. 2003, Baudry et al. 2004). TTG2 (WRKY transcription factor) acts downstream of TTG1 (Johnson et al. 2002). Characterization of testa mutants enabled to elucidate not only the biochemical pathways of flavonoid synthesis and its transport, but also the mechanisms that are important in the fine tuning of gene expression during seed development.

The testa mutant seeds display a low or a lack of dormancy, suggesting the biological significance of pigments in seed dormancy. The nature of this coat-imposed dormancy in Arabidopsis has not been elucidated. It is possible that the accumulation of pigments enhances the physical properties of the testa, providing mechanical resistance which prevents radicle protrusion (Debeaujon and Koornneef 2000). Alternatively, the chemical properties of the testa could also play an important role. Tannins of the testa may play antagonistic roles to GAs (Corcoran et al. 1972, Green and Corcoran 1975, Debeaujon et al. 2003). The role of the testa in seed germination and its interaction with other factors associated with germination will be examined later.

Chemical components other than the flavonoids also contribute to the properties of the testa and, as a consequence, to the regulation of seed germination. The polysaccharides deposited on the surface of the testa during seed development in Arabidopsis play a critical role in controlling water relations during seed imbibition. The polysaccharides form a mucilage around Arabidopsis seeds upon hydration. The myb61 mutant seeds exhibit a normal testa color but do not produce a mucilage. Comparison of the cell wall sugar composition between wild-type and myb61 mutant seeds indicated that the deficiency of mucilage in the mutant was mainly due to a reduction in the amount of rhamnose and galacturonic acid, suggesting that the mucilage consists mainly of rhamnogalacturonan-containing pectins (Penfield et al. 2001). Histochemical analysis of the developing testa in myb61 indicated that pectin secretion was impaired in epidermal cells of the developing testa in mutant seeds, which affected mucilage deposition in mature seeds. This led to a reduced germination capacity in the presence of an osmoticum such as polyethylene glycol (Penfield et al. 2001). These results indicate that the seed mucilage functions as a natural hydrogel (Zwieniecki et al. 2001) which facilitates the imbibition and retention of water around seeds under dry conditions (Penfield et al. 2001). Mutations in MUM4 (MUCILAGE-MODIFIED4) lead to a decrease in the amount of seed coat mucilage. This gene encodes a putative NDP-\(\gamma\)-rhamnose synthase, an enzyme required for rhamnogalacturonan synthesis and seems to be regulated by AP2 and TTG1. It regulates mucilage production through a pathway which is probably different from the MYB61 pathway (Western et al. 2004). These studies show the importance of “seed gel”, which can be found in many flower seeds such as pansy and chrysanthemum, in seed germination control.

The properties of the testa determined during seed development are physically present in mature seeds and affect dormancy and germination, which is the case in coat-imposed dormancy. The physiological status of seeds during development also affects the degree of dormancy, especially in the case of embryo dormancy where the embryo itself becomes quiescent. The DOF-AFFECTING GERMINATION1 (DAG1) zinc finger gene that is expressed during seed development is an important factor affecting seed dormancy and germination (Papi et al. 2000). Loss-of-function mutations in this gene lead to a reduction of seed dormancy and dag1 seeds are capable of germinating immediately after harvest. The after-ripened (or non-dormant) dag1 seeds germinate in the dark, while wild-type Arabidopsis seeds require light for germination. Since the germination of dag1 seeds is sensitive to far-red (FR) light, normal phytochrome sensing is present in this mutant. Other dormancy-lacking mutants exhibit defects in embryo development, like the lec1 (Meinke 1992, West et al. 1994), lec2 (Stone et al. 2001) and fus3 mutants (Keith et al. 1994) or in ABA sensitivity like the abi mutants (Koornneef et al. 1984, 1989, Finkelstein and Somerville 1990, Nambara et al. 1991, 1992, Giraudat et al. 1992, Finkelstein 1994). The dag1 mutant shows a normal embryo development and ABA sensitivity (Papi et al. 2000). Seeds produced in a DAG1/dag1 heterozygous maternal plant do not exhibit mutant phenotypes even though 25% of the seeds are expected to be homozygous dag1, suggesting the maternal control of mutant phenotypes. The testa of dag1 seeds is normal in terms of structure and physicochemical composition (Papi et al. 2002). DAG1 is not expressed in the embryo but is expressed in the vascular bundles of the funiculus which is connected to the placenta of the ovule (Papi et al. 2000). The authors assume that a germination controlling component is maintained in an inactive form by DAG1. It is transported from the maternal plant to the developing seeds through the vascular system and converted to an active form by Pfr (far-red light absorbing form phytochrome) during germination.

DAG2, another DoF zinc finger protein that is very similar to DAG1 plays the opposite role in seed germination; dag2 seeds are more dependent on light, GA and cold stratification for germination, suggesting that DAG2 is a positive regulator of seed germination (Gualberti et al. 2002). The function of DAG2 is also exerted maternally. Characterization of the dag1 dag2 double mutants indicated that DAG1 is epistatic over DAG2; the germination-suppressing effect of DAG1 outweighs the germination-promoting effect of DAG2. Since DAG1 and DAG2 contain an identical DNA binding domain and also show 77% amino acid identity outside the DoF domain, they probably act on the same maternal gene (Gualberti et al. 2002). The authors consider that DAG1 and DAG2 are activators and repressors of gene expression, respectively; and that they act on a maternal dormancy-promoting factor. In this hypothesis, DAG2 is assumed to bind to the same binding site as DAG1 but with less affinity than DAG1, or alternatively DAG2 could...
physically interact with DAG1 to inhibit its function as an activator (Gualberti et al. 2002). The hypothetical maternal target gene has not yet been identified. Thus, gene expression during seed development exerts a significant effect on dormancy and germination in mature seeds. The genes mentioned in this section (and the following section) are listed in Table 1.

Table 1. Genes associated with seed development and dormancy

<table>
<thead>
<tr>
<th>Genes</th>
<th>Species</th>
<th>Publications</th>
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<tr>
<td><strong>Seed Formation without Fertilization</strong></td>
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<td>FERTILIZATION INDEPENDENT ENDOSPERM (FIE)</td>
<td><em>Arabidopsis</em></td>
<td>Ohad et al. 1996</td>
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<td>FERTILIZATION INDEPENDENT SEED (FIS)</td>
<td><em>Arabidopsis</em></td>
<td>Chaudhury et al. 1997</td>
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<td>MEDEA (MEA)</td>
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<td><strong>Seed Size</strong></td>
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<td>APETALLA2 (AP2) or ETHYLENE RESPONSIVE ELEMENT</td>
<td><em>Arabidopsis</em></td>
<td>Jofuku et al. 2005</td>
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<td>BINDING PROTEIN (EREBP)</td>
<td><em>Arabidopsis</em></td>
<td>Otto et al. 2005</td>
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<td>HAIKU (IKU)</td>
<td><em>Arabidopsis</em></td>
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<td>TRANSPARENT TESTA GLABRA2 (TTG2)</td>
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<td>Dwarf11 (D11)</td>
<td><em>Rice</em></td>
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<td>Ebisu dwarf (D2)</td>
<td><em>Rice</em></td>
<td>Hong et al. 2003</td>
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<tr>
<td>BR-deficient dwarf 1 (BRD1, OsDWARF)</td>
<td><em>Rice</em></td>
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<td><strong>Seed Dormancy</strong></td>
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<td>TRANSPARENT TESTAs (TTs)</td>
<td><em>Arabidopsis</em></td>
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<td>Feinbaum and Ausubel 1988</td>
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<td>AUTO-INHIBITED H+-ATPase ISOFORM 10 (AHA10)</td>
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<td>BANYULS (BAN)</td>
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<td>MTB61</td>
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<td>SLEELY1 (SLY1)</td>
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<td>LEAFY COTYLEDONs (LEC)s</td>
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<td>Meinke 1992</td>
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<td>West et al. 1994</td>
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<td>FUSCA3 (FUS3)</td>
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<td>Keith et al. 1994</td>
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<td>ABA INSENSITIVES (ABIs)</td>
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<td>Koornneef et al. 1984, 1989</td>
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<td>GAI</td>
<td><em>Arabidopsis</em></td>
<td>Koornneef and van der Veen 1980</td>
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* This table is not a comprehensive list of the genes associated with seed development and dormancy, but contains only the genes mentioned in text.
status of seeds differs considerably at the germinative and postgerminative stages in terms of gene expression profiles and desiccation tolerance. Prior to radicle emergence, imbibed seeds can be dried and stored again. In contrast, the process is irreversible once the emerged root starts to elongate. Therefore, radicle emergence represents an extremely important developmental switch for seeds. Here, we focus on the mechanisms of sensu stricto germination which involves this critical decision.

Gene expression associated with the process leading to radicle emergence has not been fully elucidated. Compared to the genes expressed during seed development and the genes affecting dormancy and germination mentioned above, information about the genes associated with sensu stricto germination is limited, although some critical discoveries were made in the last decade. Arabidopsis seeds require light for germination, indicating the involvement of phytochrome in the induction of germination. Phytochrome was identified in lettuce seeds about 50 years ago by researchers in Beltsville, USA (Borthwick et al. 1952). The promotive effect of Pfr on seed germination had long been recognized. However, the events downstream of light perception by Pr (red light absorbing form of phytochrome) in seeds are largely unknown. Recently, PIL5, a basic helix-loop-helix (bHLH) protein has been identified as a mediator of phytochrome signaling in seeds (Oh et al. 2005). This protein is localized in the nucleus and preferentially binds to Pfr. PIL5 knockout seeds exhibit FR-resistant germination. Transgenic seeds overexpressing this gene are incapable of responding to red light, suggesting that this protein is a negative regulator of seed germination. This is the first phytochrome-interacting protein that was found to regulate seed germination.

One of the most significant discoveries in recent seed biology is the crosstalk between phytochrome and GA synthesis. GA has been known to promote seed germination in many plant species for over 50 years. Although potential crosstalk between phytochrome and GA was suggested (Peng and Harberd, 1997, Yang et al. 1997), the molecular mechanisms involved in phytochrome-GA crosstalk had not been elucidated. Yamaguchi et al. (1998) clearly demonstrated that gene expression of GA 3β-hydroxylase, an enzyme that catalyzes the final step of GA biosynthesis in Arabidopsis seeds, is controlled by phytochrome. Toyomasu et al. (1998) observed that 3β-hydroxylases, which are involved in the production of bioactive GA in lettuce seeds, are also regulated by phytochrome.

Moreover, the characterization of GA biosynthesis genes answered another key question concerning seed germination. Seed dormancy is broken by cold stratification, namely imbibition at low temperature—typically at 4°C. This cold treatment required for breaking seed dormancy can be replaced by GA application, and similarly GA requirement for breaking seed dormancy can be fulfilled by cold stratification in many species. Although cold stratification alone or cold stratification plus GA treatment is more efficient than simple GA application in breaking the dormancy of many vegetable and flower seeds, the function between exogenous GA and cold treatment frequently overlaps, suggesting the potential for crosstalk between GA and cold stimuli. Yamauchi et al. (2004) showed that GA3ox1, which encodes GA 3β-hydroxylase, is activated in a cold-inducible manner, suggesting that the promotion of seed germination by cold stratification is at least partly mediated by GA synthesis. These studies addressed the questions that seed biologists had been asking for many years.

What are the downstream events after GA synthesis in seeds? Does GA induce germination-promoting factors? Actually, rather than inducing a factor promoting germination, “repressing a repressor” of germination is more important in the switch from the dormant to germinative mode in Arabidopsis seeds. This is referred to as the de-repression of germination (Silverstone et al. 2001). The repressors are called DELLA proteins, and one of them, RGL2, plays a critical role in seed germination (Lee et al. 2002, Tyler et al. 2004). The seeds of the ga1 mutant which show a deficiency in GA biosynthesis, cannot germinate in the absence of exogenous GA (Koomen and van der Veen, 1980). However, this strong lack of germination phenotype of ga1 is rescued by the null mutation of RGL2; ga1 rgl2 double mutant seeds are capable of germinating without GA application (Lee et al. 2002). This suggests that the RGL2 DELLA protein, which is associated with the GA function, is a repressor of seed germination. Disappearance of the RGL2 protein 5 h after GA treatment in Arabidopsis seeds (Tyler et al. 2004) suggests that a quick turnover of this repressor induces germination. The loss-of-function mutant rgl2 is resistant to paclobutrazol (PAC), a GA synthesis inhibitor, while other DELLA mutants rgl1 and rgl3 do not confer PAC resistance (Tyler et al. 2004), supporting the specificity of RGL2 for seed germination regulation.

What is the mechanism of “de-repression”? How are the RGL2 proteins scavenged from seeds? An interesting mutant sleepy1 (sly1) (Steber et al. 1998) provides a clue to help understand this mechanism. The sly1 mutant was initially identified as a suppressor of the ABA-insensitive mutant ABI1-1. The plants are severely dwarfed, exhibit a dark green foliage, produce highly dormant seeds and strongly resemble other GA-deficient mutants. However, the sly1 phenotypes are not rescued by GA application, indicating that sly1 is a GA-insensitive mutant (Steber et al. 1998). SLY1, a potential downstream regulator of GA signal transduction in germination, is a SCF E3 ubiquitin ligase which is involved in protein degradation by the 26S-proteasome pathway (McGinnis et al. 2003). RGL2 proteins are most likely subjected to poly-ubiquitination by SCF E3 ligase and subsequently degraded by the 26S-proteasome pathway (see Ito et al. 2003 for review). RGA and GA1, other DELLA proteins that repress stem elongation “overaccumulate” in the sly mutant seedlings (McGinnis et al. 2003). RGL2 proteins do not disappear in the sly1 mutant seeds after GA treatment (Tyler et al. 2004). These data support the involvement of SCF^{SLY}.
in the degradation of RGL2 proteins in seed germination.

DELLA proteins are putative transcription factors that could be involved in gene regulation. The target of RGL2 is still unknown. Since GA is associated with cell elongation and GA biosynthesis genes are expressed exclusively in the embryonic axis (Yamaguchi et al. 2001), it is possible that RGL2 represses the genes associated with the embryo growth potential and prevents radicle elongation. However, whether the GA responses actually enhances the embryo growth potential during germination has not been unequivocally demonstrated (Bassel et al. 2004).

If the embryos of the gal-1, gal-3 and gal-11 mutants are excised from seeds, they can germinate at a 100% rate, indicating that the embryos display a growth potential in the absence of GA synthesis and that the impediment of the tissues covering the embryo prevents radicle emergence (Debeaujon and Koornneef 2000). As described above, the tt mutant seeds lack dormancy, indicating the restrictive role of the testa in germination. The gal tt4 and gal ttg1 mutant seeds germinate at a 100% rate without GA (Debeaujon and Koornneef 2000). These results suggest that GA is only required to overcome the restraint imposed by the testa. Probably, GA is necessary to generate the additional embryo growth potential required for the final step of germination. Testa mutants mimic ABA mutants; gal aba1 double mutant seeds are also capable of germinating without GA, as are gal tt4 and gal ttg1 (Koornneef et al. 1982a, Debeaujon and Koornneef 2000). However, the effect of ABA on seed germination is under embryonic genetic control (Kars sen et al. 1983, Debeaujon and Koornneef 2000). ABA may impose a restriction on the embryo and block an increase in the growth potential.

The molecular and genetic mechanisms of seed germination presented above, which have been revealed mainly through Arabidopsis seed research, have significantly contributed to the progress in the existing knowledge of germination which was obtained mainly from tomato, lettuce and other agricultural species. These findings need to be assembled to provide a clear picture of seed germination. Fig. 2 summarizes the concepts described above. In Hypothesis 1, the seed coat carries two distinct factors, the testa and the endosperm, restricting germination, although it remains to be determined whether the Arabidopsis endosperm provides a substantial mechanical resistance to the radicle. Another negative factor imposed by ABA is assumed to be associated with the embryo, which displays an initial growth potential sufficient to complete growth, if the testa and the endosperm are removed, but insufficient to complete germination in an intact seed. In this scheme, GA generates additional growth potential which triggers radicle emergence in wild-type seeds. The gal seeds do not germinate without GA application because this GA-dependent final increase in the growth potential is missing. However, the lack of germination phenotype of gal can be compensated by introducing the ABA deficiency mutation (gal aba1 double mutant). In this case, the restriction in the embryo is removed, which changes the balance of global forces in seed and triggers radicle emergence without requiring additional embryo growth potential. Likewise, the gal tt4 seeds can germinate without an increase in the GA-dependent growth potential, since another restriction associated with the testa is removed in this mutant.

Another hypothesis is that the additional increase in the embryo growth potential is not necessary, instead, the covering tissues are weakened (Fig. 2, Hypothesis 2). Since the testa is a dead tissue in the mature seeds of Arabidopsis and most (if not all) species, physiological changes are expected to occur in the living endosperm. Endosperm weakening is a well known phenomenon in the seeds of other plant species such as lettuce (Halmer et al. 1975), pepper (Watkins and Cantliffe 1983), tomato (Groot and Karssen 1987, Nonogaki et al. 1992, 1995, 1998a, 1998b, 2000), tobacco (Leubner-Metzger et al. 1996) and Datura (Sánchez and de Miguel 1997). Since the endosperm of Arabidopsis consists of only a single cell layer, it is less likely that endosperm weakening plays a decisive role in seed germination in Arabidopsis. However, the thin film-like endosperm of lettuce which consists of only two cell layers provides the mechanical resistance critical to seed germination (Halmer et al. 1975). It has also been suggested that the Arabidopsis endosperm acts, to some extent, as a barrier to germination (Liu et al. 2005a, 2005b). Probably, a combination of both hypotheses could fully explain the mechanisms of seed germination in Arabidopsis and many other species.

Despite recent major discoveries in seed biology, more information is necessary to revise and complete hypothetical models of seed germination mechanisms. The mechanisms of sensu stricto germination, which lead to the final increase in the embryo growth potential and radicle emergence, were reviewed in this article, with emphasis placed on GA synthesis and signal transduction. Yet, in terms of the transition from the developmental/dormant mode to the germinative mode, ABA plays another and/or potentially more important role than GA. As indicated in the studies on the gal aba1 double mutants, GA is not required for the induction of germination, when ABA is removed from seeds. The importance of the reduction in ABA synthesis (Grappin et al. 2000, Ali-Rachedi et al. 2004) and degradation of existing ABA (Kushiro et al. 2004) in the breaking of seed dormancy and induction of seed germination has been demonstrated. However, complete germination of the gal aba1 double mutants in the absence of GA still requires light (Debeaujon and Koornneef 2000), which implies the involvement of other factors controlled by light in addition to GA and ABA. The results obtained from the seeds of the Arabidopsis ethylene receptor mutant eru suggest that there is crosstalk between ethylene and GA, ABA, auxins and/or cytokinins during seed dormancy and germination (Chiwocha et al. 2005). Molecular characterization of such crosstalk should provide a clearer picture of seed dormancy and germination.
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Fig. 2. Hypotheses related to seed germination mechanisms. The occurrence of radicle emergence is determined by the balance between the mechanical resistance of the covering tissues such as the testa and the endosperm and the growth potential of the embryo. In Hypothesis 1, the increase in the growth potential of the embryo is associated with the effect of GA during germination. The two restrictive factors in the covering tissues—the mechanical resistance of the testa (brown arrow) and the endosperm (blue arrow) and another hypothetical restrictive factor imposed by ABA in the embryo (pink arrow) are balanced with, or more than, the initial growth potential of the embryo (three yellow arrows). Additional increase in the embryo growth potential (black arrow) which is induced by the action of GA synthesized in this tissue, changes the global balance of forces in seed to induce radicle protrusion. GA-deficient mutant ga1 seeds cannot germinate due to the lack of final embryo growth potential increase, which can be compensated by exogenous GA. In contrast, the ga1 aba1 double mutants are capable of germinating without GA application, since the absence of ABA alleviates its negative effect in the embryo and removes the GA requirement. In the testa pigmentation mutant tt4, the GA requirement is also removed because of the lack of restriction by the testa in this mutant. In Hypothesis 2, additional increase in the embryo growth potential is not required. Instead, the reduction in the mechanical resistance of the endosperm occurs, for example, by degradation of the cell walls of this tissue. The weakening of the endosperm is assumed to be inducible by GA inducible in this hypothesis. Lack of germination in the ga1 mutant can be explained by the lack of endosperm weakening. The seeds of the ga1 aba1 and ga1 tt4 double mutant seeds are still capable of germinating in the absence of GA, since another restrictive factor in the embryo and the testa, respectively, is missing in these mutants. These are not conflicting hypotheses; the increase in the embryo growth potential and the decrease in the mechanical resistance can occur simultaneously. Endo: endosperm, GP: growth potential.
Concluding remarks

This review provides a summary of the results derived from basic research on seed development and germination, and therefore the information presented here cannot be directly applied to breeding programs. However, some of the genes introduced are associated with important agronomic traits such as seed size and apomixis and have a potential for application. In the next step of research, the identification and characterization of the homologues of these genes in agronomic species will be taken up. This type of “translational biology” is necessary in the next step of research. Apparently, breeders have already been conducting selections for seed traits (de Leon et al. 2001, Miura et al. 2001, Abdelkhalik et al. 2005). More intensive selections for high and low temperature germination, drought tolerance, seedling vigor and dormancy phenotypes can be integrated into breeding programs. These fundamental screenings on seed performance will add values to new cultivars and increase their acceptance by growers.

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