Seed dormancy and germination

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Seed dormancy and germination are complex adaptive traits of higher plants that are influenced by a large number of genes and environmental factors. Studies of genetics and physiology have shown the important roles of the plant hormones abscisic acid and gibberellin in the regulation of dormancy and germination. More recently, the use of quantitative genetics and mutant approaches has allowed the further genetic dissection of these traits and the identification of previously unknown components. Molecular techniques, and especially expression studies and transcriptome and proteome analyses, are novel tools for the analysis of seed dormancy and germination. These tools preferentially use *Arabidopsis thaliana* because of the molecular genetic resources available for this species. However, Solanaceae and cereals also provide important models for dormancy research.

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Abbreviations

ABA	abscisic acid
abi	*ABA insensitive*
BR	brassinosteroid
ctr1	*constitutive triple response1*
cts	*comatose*
Dof	DNA-binding with one finger
ein2	*ethylene insensitive2*
fus3	*fusca3*
GA	gibberellin
βGlu I	β–1,3-glucanase
lec	*leafy cotyledon*
QTL	quantitative trait loci
sly1	*sleepy1*

Introduction

The seed is the structure in which a usually fully developed plant embryo is dispersed, and which enables the embryo to survive the period between seed maturation and seedling establishment, thereby ensuring the initiation of the next generation. The dry dormant seed is well equipped to survive extended periods of unfavorable conditions. Seed dormancy is defined as the failure of an intact viable seed to complete germination under favorable conditions [1] and is controlled by several environmental factors, such as light, temperature and the duration of seed storage (after ripening). Dormancy and germination are determined by the co-action of the growth potential of the embryo and the restraints imposed by the tissues surrounding it. Bewley [1] concluded in his recent review that little is known about the mechanism of dormancy and germination.

This review focuses on recent progress made in seed dormancy/germination research, especially through the use of molecular genetics. *Arabidopsis* is developing as a favored model in this field because of its excellent suitability for genetic and molecular studies, and because its germination responses are similar to those of many species used in physiological seed research. However, some cereal species and Solanaceae species such as tobacco and tomato, in which the embryo is embedded in a rigid endosperm [2], are also suitable models and have contributed significantly to the progress made in understanding germination biology.

The genetic analysis of 'natural' differences in seed dormancy and germination characteristics

Genetic variation for seed dormancy within species is present both among accessions of wild plants and among varieties of cultivated plants. The substantial influence of environmental effects on the expression of germination characteristics and the involvement of many genes make dormancy a typical quantitative trait. Such traits are becoming more amenable to genetic analysis because the position of individual quantitative trait loci (QTL) and the relative contribution of these loci can now be determined. QTL analysis for seed dormancy requires permanent or immortal mapping populations, such as recombinant inbred lines (RILs), because these allow the testing of a large number of genetically identical seeds (i.e. seeds from the same RIL) in different environmental conditions. QTL analysis of seed dormancy has been reported for *Arabidopsis thaliana* [3], barley [4], rice [5] and wheat [6]. It appears that QTL identified for wheat co-locate with barley QTL but not with rice QTL [6]. Wild species often show stronger dormancy than cultivated genotypes, making crosses between wild and cultivated genotypes useful for QTL analysis [7,8]. QTL analysis can be followed by the study of individual genes (or chromosome regions) containing specific dormancy QTL and by fine mapping. Such studies have been initiated in barley [9,10] and *Arabidopsis* (L Bentsink, unpublished data). It is expected that the study of such QTL will allow the molecular identification of genes that affect dormancy in these species by map-based cloning. However, the cloning of such dormancy QTL has not yet been reported.

Mutants in dormancy and germination research

Studies of gibberellin (GA)-deficient, abscisic acid (ABA)-deficient, and signaling mutants in *Arabidopsis* and tomato have identified the crucial role of ABA in seed dormancy, as well as the requirement for GA for germination [11,12]. The isolation of a *Tos17*-transposon-induced *viviparous* (non-dormant) mutant in rice, which was shown to be defective in a zeaxanthin epoxidase gene (encoding one of the enzymes of the ABA-synthetic pathway) [13], showed that ABA is also important in dormancy control in cereals.

Manipulation of seed ABA content by genetic modification of tobacco has shown that overexpression of zeaxanthin epoxidase results in increased dormancy, whereas 'knocking out' the gene encoding this enzyme by antisense techniques yields phenotypes that are less dormant [14]. The observation that inhibitors of ABA biosynthesis, such as norfluorazon, promote germination [12] indicates that the maintenance of dormancy in imbibed seeds is an active process involving *de novo* ABA synthesis, as has also been found for *Nicotiana plumbaginifolia* [15•]. These findings complement those of earlier studies that emphasized the role of ABA during seed development.

In addition to the well-known *ABA insensitive* (*abi*) and *enhanced response to ABA* (*era*) mutants, which all have a seed germination phenotype, it was recently found that the *ethylene insensitive2* (*ein2*) and *ethylene response* (*etr*) mutants of *Arabidopsis* are also hypersensitive to ABA [16•,17]. This finding is consistent with the fact that *ein2* mutants were isolated as suppressors of the *abi1* mutant. The *constitutive triple response1* (*ctr1*) mutant, which is characterized by a constitutive ethylene response, was among mutants selected as enhancers of the ABA-insensitive mutant *abi1-1*. The *ctr1* monogenic mutants are also slightly ABA resistant [16•]. These findings, in combination with the non-dormant phenotype of the *ein2 abi3-4* double mutant, indicate that ethylene may suppress seed dormancy by inhibiting ABA action [16•]. In addition, the presence of cross-talk between sugar signaling and ethylene signaling is suggested by the sugar-insensitive phenotype of *ctr1* [18] and the sugar-hypersensitive phenotype of *etr* [19]. Apparently, ABA, ethylene and sugar signaling strongly interact during the regulation of germination and early seedling growth. This interaction is further supported by the observation that many sugar-signaling mutants turn out to be ABA-biosynthesis mutants or alleles of *abi4* and *abi5*, which represent a subclass of the ABA-insensitive mutants [20•].

Detailed analysis of the seed-maturation mutants *leafy cotyledon* (*lec*), *fusca3* (*fus3*) and *abi3* has shown that they differ in the time at which they can undergo premature germination. The *LEC1* and *FUS3* loci probably regulate developmental arrest, as mutations in these genes cause a continuation of growth in immature embryos. Control of dormancy by ABA (via *ABA* and *ABI*) might represent a different mechanism to prevent germination, which occurs later and is additive to the developmental arrest controlled by *LEC1* and *FUS3* [21].

Mutants have also been useful in establishing the role of brassinosteroids (BRs) in seed germination. The *Arabidopsis* BR mutants *de-etiolated2* (*det2*) and *brassinosteroid insensitive1* (*bri1*) show reduced germination but eventually germinate without BR, indicating that, in contrast to GAs, BRs are not absolutely required for germination [22].

Recently, *sleepy1* (*sly1*), an *Arabidopsis* mutant that has a severe germination defect, was selected in a screen for suppressors of the ABA-insensitive *abi1-1* mutant. This mutant strongly resembles the GA auxotrophs. However, the lack of germination of *sly1* cannot be rescued by GA, therefore, *SLY1* has been postulated to be a key factor in GA reception [23]. Another mutant with a marked reduction in germination potential is *comatose* (*cts*). Although the morphology of *cts* plants is not altered, mature *cts* seeds do not respond to gibberellin. It has therefore been suggested that *CTS* promotes increased germination potential, represses embryo dormancy and might be involved in seed-specific GA signaling [24].

In addition to these mutants affecting the embryo proper, mutants have been selected that control dormancy through the seed coat or other maternal factors. A number of seed-coat or testa mutants [25•] have a maternally inherited reduced seed dormancy. This indicates the importance of the testa structure as a constraint to radicle emergence. In *Arabidopsis*, dormancy is apparently imposed by the seed coat because removal of the testa allows the germination of both GA-deficient mutants [12] and accessions that have a very strong dormancy (L Bentsink, unpublished data). Evidently, lack of germination may also be due to a reduced growth potential of the embryo. A knockout mutant of the *Dof AFFECTING GERMINATION1* (*DAG1*) gene, which encodes a Dof (DNA-binding with one finger) transcription factor, caused reduced dormancy [26•]. In contrast to those of other reduced dormancy mutants [27,28], this phenotypic effect is determined by the maternal genotype. This maternal inheritance is consistent with the expression pattern of the *DAG1* gene in the vascular tissue that enters the developing seeds, which is genetically derived from the mother plant [26•].

Genes with an expression pattern correlated to dormancy and germination

In addition to the identification and subsequent cloning of genes through the use of mutants, genes controlling seed dormancy and germination can also be identified on the basis of their expression pattern. This may involve an unbiased search of genes with germination-specific expression or may focus on genes with assumed functions that are related to seed germination.

Examples of genes identified by the latter strategy include a 3 β hydroxylase gene controlling gibberellin biosynthesis in a light-induced and seed-specific way [29], and the gene encoding a dormancy-specific $NADP^+$ phosphatase, which has a higher activity in dormant seeds than in non-dormant seeds of *Avena sativa* [30].

During seed maturation the expression of many genes is altered and specific classes of mRNAs such as those of the *LATE-EMBRYOGENESIS-ABUNDANT* (*LEA*) genes appear. However, none of these genes has a proven specific function in seed dormancy. Although it appears that seed maturation and post-germination growth have a distinct gene-expression profile, some genes that are highly

expressed after germination are also expressed during the later stages of seed development (reviewed in [31]), suggesting that some aspects of post-germination growth are initiated during maturation. The onset of early germination is also obvious in some of the *Arabidopsis* maturation mutants: *lec*, *fus3* and *abi3*. To study genes that are activated during late embryo development and germination, mRNAs from immature siliques of the *abi3 fus3* double mutant were compared with those from wildtype siliques using a differential display [32]. The genes that were identified as being active during late embryo development and germination encode a variety of metabolic enzymes, regulatory proteins and a number of ribosomal proteins. Cellular processes involved in growth, the activation of protection mechanisms (such as those involved in protection against oxidative stress), and storage-compound metabolism are expected to be related to germination.

Germination in tomato and tobacco is controlled by interactions between the embryonic radicle tip and the enclosing endosperm cap. Weakening of the endosperm cap, by enzymatic hydrolysis, is required to allow radicle protrusion. Enzymes involved in this process are expansin [33] and endo-β-mannanase [34], which are specifically expressed in the endosperm cap of tomato. A close correlation between class I β–1,3-glucanase (βGlu I) induction and endosperm rupture in response to plant hormones and environmental factors in tobacco suggested that βGlu I may also promote radicle protrusion [35]. The involvement of this enzyme in germination was supported by the observation that transgenic plants with a sense construct of the gene encoding βGlu I under control of an ABA inducible promoter had both increased βGlu I activity and increased endosperm rupture [36•]. In tomato, βGlu I was also expressed specifically in the endosperm cap [37]. However, a correlation between the expression of this gene and endosperm weakening could not be shown as the activity of βGlu I was inhibited by applied ABA, which did not inhibit endosperm weakening [37]. In addition to this, Toorop *et al.* [38] demonstrated that endosperm cap weakening in tomato is a biphasic process and that inhibition of germination by ABA occurs exclusively at the second step in this process.

Gene or promoter trapping with a reporter gene, such as β-glucuronidase (GUS), may identify genes with a specific expression. Dubreucq *et al.* [39•] isolated gene traps that are expressed during seed germination, among which they identified an insertion close to *AtEPR1*. This gene encodes an extensin-like protein, is specifically expressed in the endosperm during seed germination and is under control of GAs [39•].

The use of genomics and proteomics in seed research

Microarrays containing 2600 genes expressed in developing *Arabidopsis* seeds were described by Girke *et al.* [40•]. These microarrays revealed many genes of unknown function that are highly expressed in seeds. The analysis of protein patterns by 2D gel electrophoresis and the subsequent identification of a number of those proteins, showed that among the 1300 seed proteins detected, 74 changed in abundance during the imbibition phase or during the radicle protrusion of *Arabidopsis*. Many of these proteins had previously been described as being involved in germination (e.g. in the mobilization of food reserves). In addition, proteins not previously associated with these processes were identified [41•].

Conclusions and perspectives

Dormancy and germination are complex traits that are controlled by a large number of genes, which are affected by both developmental and environmental factors. Seed dormancy and germination depend on seed structures, especially those surrounding the embryo, and on factors affecting the growth potential of the embryo. The latter may include compounds that are imported from the mother plant and also factors that are produced by the embryo itself, including several plant hormones. Genetic analysis has identified the crucial role of ABA in seed dormancy, as well as the requirement for GAs for germination. QTL and mutant analyses are identifying additional genes. Whether these genes with unknown functions are downstream targets of ABA and GA, or whether they affect seed dormancy/germination in an independent way is currently not known. The molecular identification of all these genes will be important, as will the identification of more target genes. Using whole transcriptome and proteome approaches will be the most efficient way to identify target genes.

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