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Seed Dormancy and Germination

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Seed dormancy allows seeds to overcome periods that are unfavourable for seedling established and is therefore important for plant ecology and agriculture. Several processes are known to be involved in the induction of dormancy and in the switch from the dormant to the germinating state. The role of plant hormones, the different tissues and genes involved, including newly identified genes in dormancy and germination are described in this chapter, as well as the use transcriptome, proteome and metabolome analyses to study these mechanistically not well understood processes.

INTRODUCTION

The seed is an important stage in the higher plant life cycle with respect to its survival as a species. It is the dispersal unit of the plant, which is able to survive the period between seed maturation and the establishment of the next generation as a seedling after it has germinated. For this survival, the seed, mainly in a dry state, is well equipped to sustain extended periods of unfavourable conditions. To optimise germination over time, the seed enters a dormant state. Dormancy prevents pre-harvest germination as well. Numerous studies have been performed to better understand how germination is controlled by various environmental factors and applied chemicals. However, still very little is known about the process by which the embryo emerges from the seed to complete germination and how embryo emergence is blocked in dormant seeds (Bewley, 1997).

Arabidopsis possesses seed dormancy, as is the case for many other plant species. This property is controlled by environmental factors such as light, temperature and time of seed dry storage as well as by genetic factors. The use of genetics and molecular genetics in Arabidopsis is starting to shed light on some aspects of the mechanism of dormancy and germination by the identification of mutants and genes that control these processes. This review provides an overview of current knowledge of seed dormancy and germination in Arabidopsis based mainly on the contribution that molecular genetics made to the study of this process including a table with genes that are related to germination/dormancy. Several recent reviews (Finch Savage and Leubner-Metzger 2006, Finkelstein et al., 2008, Holdsworth et al., 2008a) describe more details of the molecular mechanism that were derived from the mainly molecular genetic studies combined with physiological experiments.

SEED DEVELOPMENT

Seed development comprises two major phases: embryo development and seed maturation. Embryogenesis, which is a morphogenesis phase, starts with the formation of a single-cell zygote and ends in the heart stage when all embryo structures have been formed (Mayer et al., 1991). It is followed by a growth phase during which the embryo fills the seed sac (Goldberg et al., 1994). At the end of the embryo growth phase, cell division in the embryo arrests (Raz et al., 2001). Hereafter, the seed, containing a full sized embryo, undergoes maturation during which food reserves accumulate and dormancy and desiccation tolerance develops (Goldberg et al., 1994). Several detailed studies have been published on the developmental (Baud et al. 2002), metabolic (Fait et al., 2006), proteomic (Fu et al., 2005; Gallardo et al., 2001, 2002; Rajjou et al., 2004 and Chibani et al., 2006 and gene expression (Girke et al., 2000; Nakabayashi et al., 2005; Finch-Savage et al., 2007) changes during seed development, in dry seeds and subsequent germination.

Seed development has been extensively studied using mutants defective in various aspects of the process. Mutants affected in the morphogenesis phase result in lethality or have a seedling phenotype (Mayer et al., 1991; Meinke, 1995). In seed germination mutants, properties of germination and dormancy are affected which sometimes are accompanied by pleiotropic effects that are specific for maturation, such as desiccation intolerance (Goldberg et al., 1994; Koornneef and Karssen, 1994).

SEED DORMANCY AND GERMINATION

Seed dormancy has been defined as the incapacity of a viable seed to germinate under favorable conditions (Bewley, 1997;

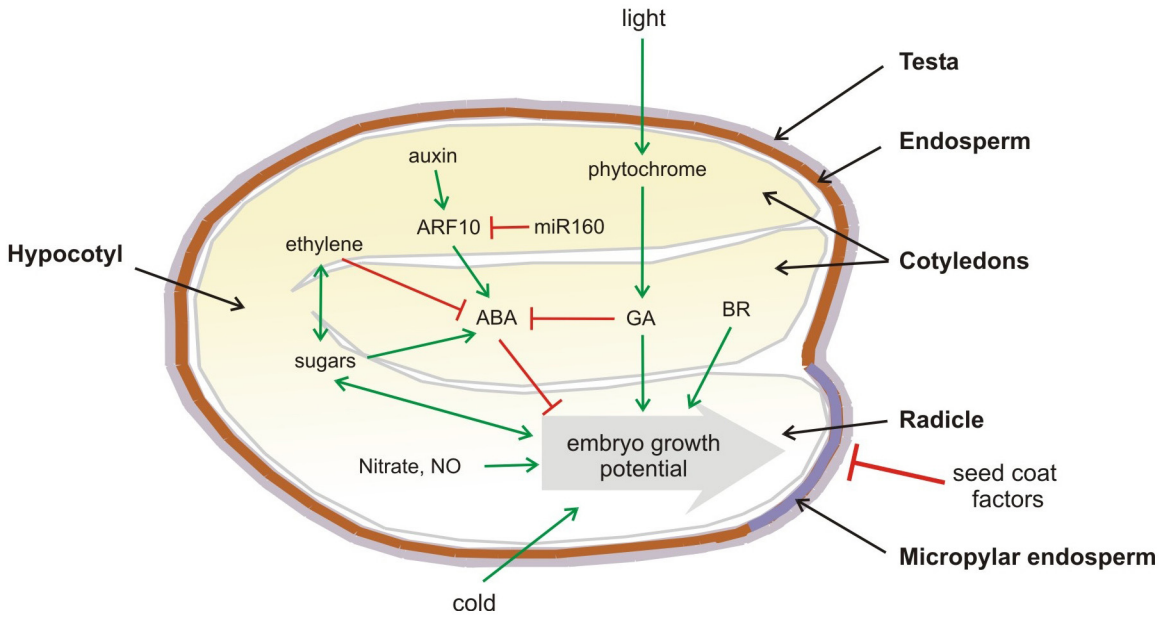

Figure 1. Schematic presentation of processes controlling seed dormancy and germination in an Arabidopsis seed. The Arabidopsis seed is characterized by the embryo with two cotyledons and a single cell layer endosperm. Germination promoting (green arrows) and inhibiting factors (red arrows) are indicated.

Finch-Savage and Leubner–Metzger, 2006). Dormancy in Arabidopsis should be described as physiologically non-deep, meaning that embryos released from surrounding structures grow normally, and that dormancy is lost through moist chilling (stratification) or after-ripening (Baskin and Baskin, 2004). However, in addition to the testa and endosperm layer surrounding the embryo, the growth potential of the embryo is also important to overcome the constraint of these structures and thereby affects the dormancy state of a seed (Kucera et al., 2006).

Since dormancy is regulated at different developmental phases, in interaction with environmental factors, it is difficult to detect when the genetic and physiological differences are established. This difficulty arises because all dormancy assays are based on seed germination, which is the result of the balance between the degree of dormancy and the capacity of the embryo to overcome dormancy. Mechanistically one can distinguish factors that influence dormancy and germination on the basis of their effect on germination, being either inhibiting or promoting. Mutants that germinate better or faster can represent genes that promote dormancy or those that repress germination. A further distinction can be made by defining the timing and site of action of these factors (during maturation or during imbibition of the seeds, in the embryo, the endosperm or in the testa). The interaction between these factors and the large effect of the environment, both during seed development and during imbibition, make seed dormancy a very complex trait.

By definition, germination incorporates those events that commence with the uptake of water by the quiescent dry seed and terminates with the elongation of the embryonic axis (Bewley and Black, 1994). Water uptake by a seed is triphasic; phase I rapid initial uptake; phase II plateau phase and in phase III further increase of water uptake, however, only when germination occurs (Schopfer and Plachy, 1984; Bewley, 1997; Manz et al., 2005). The first signs of germination are the resumption of essential processes, including transcription, translation and DNA repair followed by cell-elongation and eventually at the time of radicle protrusion, resumption of cell division (Barroco et al., 2005; Masubelele et al., 2005). Physically germination is a two-stage process, where testa rupture is followed by endosperm rupture. Following rupture of the micropylar endosperm by the emerging radicle, germination is complete (Fig. 2; Karssen, 1976; Hepher and Roberts 1985; Leubner-Metzger et al., 1995; Krock et al., 2002; Petruzzelli et al., 2003; Leubner-Metzger, 2003; Liu et al., 2005). Germination assays in Arabidopsis are often performed in light on seeds freshly harvested or stored for a limited time (Léon-Kloosterziel et al., 1996a). Other parameters are the germination rate after different periods of cold treatment (Cutler et al., 1996) and germination in darkness (Meng et al., 2008; Kim et al., 2008). In addition to testing mature seeds, germination of immature seeds, either excised from the silique or within fruits detached from the plant, can be used to investigate genetic variation during the early stages of seed development (Raz et al., 2001).

Since tissues from both maternal (testa) and zygotic origin (embryo and endosperm) contribute to seed germination, genetic analyses of seed dormancy have to take into account these different tissue origins. Maternal effects, in contrast to zygotic factors are maternally inherited and might be due to the genetic make up of the testa surrounding the embryo, but can also be due to genetic differences related to factors that are transported into the seed from the mother plant. Maternal inheritance can be deduced from the germination of seeds obtained after reciprocal crosses, where the parental genotypes are used both as female and as

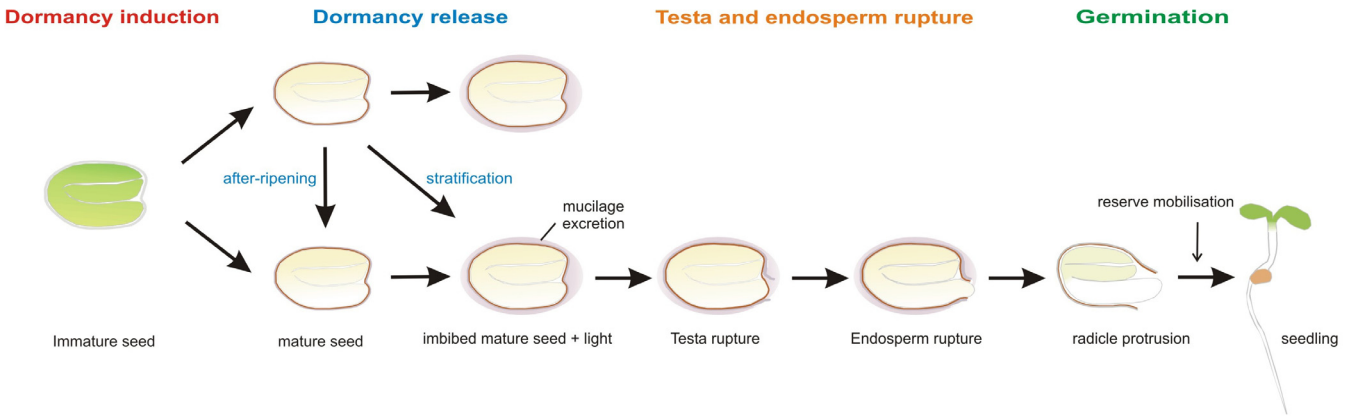

Figure 2. Arabidopsis seed germination. A mature Arabidopsis seed can be either dormant or non-dormant. A dormant seed will not germinate when it will be exposed to the right environmental conditions (light and water). Seed dormancy can be broken by dry storage or by a cold imbibition (stratification). Seed germination in Arabidopsis is two-step: testa rupture followed by endosperm rupture.

male parent. The endosperm is the product of fertilization. However the genomic contribution of the female parent is twice that of the male parent in this triploid tissue, which in some cases may lead to differences in reciprocal crosses when dose effects are involved. The role of this single endosperm layer in dormancy and the need to weaken this cell layer for germination has recently been shown (Müller et al., 2006; Bethke et al., 2007a; for review Holdsworth et al., 2008a).

Seed dormancy in Arabidopsis can be overcome by germination promoting factors such as after-ripening, light, cold treatment (also called stratification). Furthermore applied chemicals such as gibberellins and KNO_3 (Derkx and Karssen, 1993a; Alboresi et al. 2005; Ali-Rachedi et al. 2004), NO (Bethke et al., 2007b) also have germination promoting effects (Fig. 1). None of these environmental factors are an absolute requirement for germination because the need for one factor depends on the other factors, as shown for the interaction between light and temperature by Cone and Spruit (1983). This requirement for exogenous factors depends very much on the genotype. Dormancy, released by dry after-ripening, or by exposure to cold or nitrate followed by light exposure has been studied in the accession of the Cape Verde Islands (Cvi). It appeared that the sensitivity of seeds to cold, nitrate and light was dependent upon the length of time that seeds had been dry after-ripened. The seeds became first sensitive to nitrate, then to cold and finally to light (Finch-Savage et al., 2007). Furthermore it has been shown that the rate of increase of sensitivity to environmental signals was not fixed, seeds produced in different years had a different response. This is consistent with the fact that the depth of dormancy is not only determined genetically, but also by the ambient environment during seed formation (Donohue, 2005).

Non-dormant seeds that are exposed for some time to unfavourable germination conditions (imbibed seeds kept at relatively high temperature in darkness for example) may enter a state of dormancy again, which is called secondary dormancy (Cone and Spruit, 1983; Derkx and Karssen 1993b; Hilhorst, 2007).

A challenge in dormancy and germination research is to identify the nature of the crucial regulator(s) that prevent(s) the onset of germination (dormancy), that trigger(s) the germination process and their mutual interaction. Furthermore, it is important to know how the environmental factors such as light and cold affect the endogenous factors that control germination.

GENETIC VARIATION FOR GERMINATION CHARACTERS

Genetic variation can be induced by mutagenesis, but is also present among the many natural accessions (ecotypes) of Arabidopsis. Genetic variation for germination can be detected when genotypes are compared in identical environments. This implies that not only the conditions of the germination test must be identical, but also growth conditions during seed development and storage conditions, including the time that the seeds are stored, must be the same. Furthermore, the test conditions must be discriminative between genotypes.

Germination tests can be used efficiently for mutant screens because of the large numbers that can be assayed. However, variability of the germination trait may lead to genetic misclassification of individual seeds and therefore to false-positives in mutant screens. The Arabidopsis genotypes Landsberg *erecta* (L*er*) and Columbia (Col), which are mostly used in Arabidopsis research, show only a low level of dormancy. This dormancy disappears after approximately one month of after-ripening (van der Schaar et al., 1997). Because of this relatively low level of dormancy it is impossible to saturate mutations in dormancy genes. However this problem can be overcome by the use of more dormant accessions (Koornneef et al., 2000).

The genetic variants described in this review have in common that the seeds are viable, as shown by their ability to germinate after special treatments, such as disruption of the seed coat or the application of specific chemicals. Mutants that do not germinate because they are lethal, including early ovule mutants (Schneitz, 1999) and many of the so-called embryo lethals (Meinke, 1995) are not described in this review because they mainly have developmental defects that do not control specifically dormancy and germination.

The analysis of natural variation

Arabidopsis is an annual plant for which large differences in dormancy can be found between accessions collected from nature (Ratcliffe, 1976). Kugler (1951) performed the first genetic study of differences in germination between Arabidopsis accessions. Differences in dormancy are amenable to genetic analysis in Arabidopsis as was shown by Quantitative Trait Loci (QTL) analysis for seed germination/dormancy characteristics in several recombinant inbred line (RIL) populations (van der Schaar et al., 1997; Alonso-Blanco et al., 2003 and Clerkx et al., 2004). These RIL populations have been made by crossing accessions that have different levels of seed dormancy to the standard laboratory accession L*er*. In the analysis of van der Schaar et al. (1997), the L*er*/Col RILs were grown in three independent greenhouse experiments and tested in different germination conditions. Despite little difference in dormancy between the parental lines, altogether 14 QTL could be identified, of which most had only small effects. Many of these were only detected in specific germination conditions or in seeds from one or two harvest dates, indicating the complexity of the genetic control of dormancy differences.

QTL analyses for seed dormancy that led to the identification of loci with an effect on seed dormancy that could be confirmed using Near Isogenic Lines (NILs) were described for the L*er*/Cvi RIL population (Alonso-Blanco et al., 2003). This QTL study identified loci affecting the after-ripening requirement, measured as the number of *days of seed dry storage required to reach 50%* germination (DSDS50). Thus, seven QTL were identified and named *Delay Of Germination* (*DOG*) 1–7. To confirm and characterize these loci NILs carrying Cvi introgression fragments in a L*er* genetic background were constructed. The analysis of these lines for germination confirmed four QTL (*DOG1*, *DOG2*, *DOG3*, and *DOG6*). The same type of analyses in the L*er*/Shahdara (Sha) RIL population revealed four QTL. Three of them, *DOG1*, *DOG3* and *DOG6* were at positions, identified before (Alonso-Blanco et al., 2003) and most likely representing the same loci. In addition a QTL on chromosome 2 was identified (Clerkx et al., 2004).

The first of these seed dormancy QTL, *DOG1* a major QTL in the L*er*/Cvi population has been cloned (Bentsink et al. 2006). *DOG1* is expressed during seed development and the transcript remains in the mature dry and after-ripened seed but upon imbibition the transcript disappears. *DOG1* is a member of a small gene family of unknown molecular function and has not been related with seed dormancy before, indicating that natural variation can be valuable to identify novel seed dormancy genes (Bentsink et al., 2006).

In total seven RIL populations derived form crosses with L*er* have presently been analysed, leading to the identification of eleven *DOG* QTL (Bentsink et al., unpublished). This screen for after-ripening requirement is reaching saturation as in newly analysed populations the same QTL are being identified also in a cross were L*er* has not been involved (X. Huang, M. Koornneef and K. Donohue unpublished data).

Analyses for seed germination in response to cold and dark showed the feasibility of fine mapping and cloning of QTL responsible for cold-tolerant, dark germination (Meng et al., 2008). The authors identified three major loci responsible for the variability of this trait in the Bay-0/Sha RIL population. One of these QTL, *CDG-1* (*Cold-tolerant Dark Germination*) was localized on the same genomic region as the *delay of germination* loci *DOG2* and *DOG3* (Alonso-Blanco et al., 2003) and QTL that affect controlled deterioration and germination speed (Clerkx et al., 2004).

Another seed trait for which natural variation has been described is for mucilage release during imbibition. The Sha accession from central Asia carries a mutation in the *MUM2* gene (Macquet et al., 2007) resulting in the absence of mucilage.

Genes and factors affected in seed dormancy/germination

Genes and or factors that induce seed dormancy or inhibit germination

The first important stage for dormancy induction is probably the end of the morphogenetic program, when all tissues present in a mature embryo have been formed and the embryo enters a phase of growth arrest. *ABA-INSENSITIVE3* (*ABI3*), *FUSCA3* (*FUS3*) and *LEAFY COTYLEDON* (*LEC1* and *LEC2*) are four key regulators that play prominent roles in controlling mid- and late seed development (Meinke et al., 1994; Raz et al., 2001). *ABI3*, *FUS3* and *LEC2* encode related plant-specific transcription factors containing the conserved B3 DNA binding domain (Giraudat et al., 1992; Luerssen et al., 1998; Stone et al., 2001), whereas *LEC1* encodes a HAP3 subunit of the CCAAT binding transcription factor (CBF, also known as NF-Y (Lotan et al., 1998)). All four *abi3*, *lec1*, *lec2* and *fus3* mutants are severely affected in seed maturation and share some common phenotypes, such as decreased dormancy at maturation (Raz et al., 2001) and reduced expression of seed storage proteins (Gutierrez et al., 2007). However, they also show specific phenotypes, such as the absence of chlorophyll degradation in the dry seed (*abi3*), a reduced sensitivity to ABA (*abi3* and, to a lesser extent, *lec1*), the accumulation of anthocyanins (*fus3*, *lec1*, and, to a lesser extent, *lec2*), an intolerance to desiccation (*abi3*, *fus3*, and *lec1*), or defects in cotyledon identity (*lec1*, *fus3*, and *lec2*) (Bäumlein et al., 1994; Keith et al., 1994; Meinke et al., 1994; Parcy et al., 1994; Parcy & Giraudat, 1997; Luerssen et al., 1998; Vicient et al., 2000; Raz et al., 2001; Stone et al., 2001; Kroj et al., 2003). It was recently shown that several of the *fus3* phenotypes are due to pleiotropic effects caused by truncated gene products of the mutant alleles. The direct effects of *FUS3* are probably restricted to embryo-derived dormancy and determination of cotyledon epidermis cell identity (Tiedemann et al., 2008).

The *LEC1* gene is required for normal development during early and late phases of embryogenesis and is sufficient to induce embryonic development in vegetative cells (Lotan et al., 1998). Loss of function of *LEC1* leads to germination of excised embryos at a similar stage (between 8-10 days after pollination) as *lec2* and *fus3* mutants, but earlier during embryo development than found for *abi3* mutants (Raz et al., 2001). Ten HAP3 (AHAP3) subunits have been identified in Arabidopsis, which can be divided into two classes based on sequence identity in their central, conserved B domain (Kwong et al., 2003). *LEC1* and the closely related subunit, *LEC1-LIKE* (*L1L*), constitute *LEC1*-type AHAP3 subunits, whereas the remaining eight are designated non-*LEC1*-type. Similar to *LEC1*, *L1L* is expressed primarily during seed development. However, suppression of *L1L* gene expression induced defects in embryo development that differed from those of *lec1* mutants, suggesting that *LEC1* and *L1L* play different roles in embryogenesis (Kwong et al., 2003).

LEC2 directly controls a transcriptional program involved in the maturation phase of seed development. Induction of *LEC2* activity in seedlings causes rapid accumulation of RNAs normally present primarily during the maturation phase, including seed storage and lipid-body proteins. Promoters of genes encoding these maturation RNAs all possess RY motifs (cis-elements bound by B3 domain transcription factors) (Braybrook et al., 2006). This provides strong evidence that these genes represent transcriptional targets of *LEC2*. One of these genes is *DOG1*, the first seed dormancy gene accounting for variation occurring in natural populations that has been identified at the molecular level (Bentsink et al., 2006).

It has been shown that *ABI3*, *FUS3*, *LEC1* and *LEC2* interact as a network to control various aspects of seed maturation. *LEC1* was shown to regulate the expression of both *ABI3* and *FUS3* (Kagaya et al., 2005), *FUS3* and *LEC2* have been shown to act in a partially redundant manner to control gene expression of seed specific proteins, and *LEC2* was shown to locally regulate *FUS3* expression in regions of the cotyledons (Kroj et al., 2003). The indication of redundant regulation within this group of genes was recently shown (To et al., 2006). By analyzing *ABI3* and *FUS3* expression in various single, double, and triple maturation mutants, multiple regulatory links among all four genes were identified. It was found that one of the major roles of *LEC2* was to up-regulate *FUS3* and *ABI3*. The *lec2* mutation leads to a dramatic decrease in *ABI3* and *FUS3* expression, and most *lec2* phenotypes can be rescued by *ABI3* or *FUS3* constitutive expression. In addition, *ABI3* and *FUS3* were shown to positively regulate themselves and each other, thereby forming feedback loops essential for their sustained and uniform expression in the embryo. Finally, *LEC1* also positively regulates *ABI3* and *FUS3* in the cotyledons (To et al., 2006). Although multiple regulatory links were identified amongst these four genes, molecular mechanisms underlying this network, and the downstream targets of the network associated with dormancy induction still require further investigation.

Apart from mutants that influence general seed maturation, other mutants more specifically influence seed dormancy, i.e. mutants, which are altered in ABA biosynthesis or its mode of action. ABA regulates various aspects of plant growth and development, including seed dormancy. The absence of ABA-induced dormancy allows seeds to germinate without gibberellins. Therefore, the selection of mutants that germinate in the presence of GA biosynthesis inhibitors, such as paclobutrazol and tetcyclacis, is an effective way to isolate ABA biosynthesis mutants (Léon-Kloosterziel et al., 1996b). Reciprocal crosses between wild type and the ABA deficient *aba1* mutants showed that dormancy is controlled by the ABA genotype of the embryo and not by that of the mother plant. The latter is responsible for the relatively high ABA levels found in seeds halfway through seed development (Karssen et al., 1983). At this phase ABA may prevent precocious germination as shown by the maternal ABA effects in the extreme *aba abi3-1* double mutants (Koornneef et al., 1989). Key genes for ABA biosynthesis during seed development are *NCED6* and *NCED9,* both members of the 9-cis-epoxycarotenoid dioxygenese family (Lefebvre et al., 2006). In contrast to what was proposed by Karssen et al (1983) it is now clearly shown that endogenous ABA is required for the maintenance of seed dormancy. In wild type ABA levels decrease at the end of seed maturation and during imbibition due to the activity of ABA catabolism genes belonging to the P450 CYP707A family (Okamoto et al., 2006), indicating that ABA levels can be modified at different phases of seed development and germination with significant effects on germination. Furthermore, the observation that inhibitors of ABA biosynthesis, such as norfluorazon, promote germination (Debeaujon and Koornneef, 2000) indicated that the maintenance of dormancy in imbibed seeds is an active process involving de novo ABA synthesis as was also shown for dormant seed batches of the accession Cvi (Ali-Rachedi et al., 2004).

ABA has a major effect on seed dormancy and therefore it can be expected that defective ABA signalling also leads to changes in germination characteristics. Substantial progress has been made in the characterization of such ABA signal transduction pathways (Bonetta and McCourt, 1998; Leung and Giraudat, 1998). Genetic screens to identify ABA signalling mutants were based primarily on the inhibition of seed germination by applied ABA. The ABA-insensitive (*abi*) mutants (Koornneef et al., 1984) and several others described thereafter (Rock, 2000 and Holdsworth et al., 2008a) are able to germinate and grow in the presence of ABA concentrations that are inhibitory to the wild type. It was expected that such screens would yield ABA receptor and signal transduction mutants. However most of ABA receptor genes were identified using reverse genetics in which screening for germination characteristics were performed that often showed no or small effects on ABA sensitivity for germination and dormancy it self was often not tested (reviewed in Holdsworth et al., 2008a). Forward screens for mutants in which seed germination is prevented by low concentrations of ABA that ordinarily permit germination of the wild-type seed were first described by Cutler et al. (1996) resulting in the *era1* (enhanced response to ABA) to *era3* mutants. Subsequently using similar screens identified many additional loci that are involved in removal of sensitivity to ABA function, that when mutated lead to ABA hypersensitivity of imbibed after-ripened or moist-chilled seeds (Hugouvieux et al., 2001; Xiong et al., 2001; Nishimura et al., 2004; Katagiri et al., 2005; Zhang et al., 2005; Pandey et al., 2006; Saez et al., 2006; Yoine et al., 2006; Nishimura et al., 2007). These loci encode diverse functions, including those associated with RNA translation and metabolism, protein degradation pathways and phosphatase components of signalling pathways and transcription factors (Holdsworth et al., 2008a). As judged from their effects on seed dormancy, these two sets of mutations also alter the regulation of seed germination by endogenous ABA. The *abi3*, *abi4* and *abi5* mutants exhibit reduced expression of various seed maturation genes but only *abi3* mutants are non-dormant, which coincides with desiccation intolerance (Nambara et al., 1992, Ooms et al., 1993, Bies et al., 1999, Finkelstein et al., 2008) in strong alleles. Surprisingly no dormancy or other seed maturation phenotype was observed in *abi4* and *abi5* mutants (Finkelstein, 1994; Finkelstein et al., 2008), except reduction of some seed maturation specific mRNAs (Finkelstein and Lynch, 2000; Söderman, et al., 2000). This may indicate that other genes are redundant in function to these seed specific transcription factors, which are members of the APETALA2 domain (*ABI4*, Finkelstein et al., 1998; Söderman, et al., 2000) and basic leucine zipper factor family (*ABI5*, Finkelstein and Lynch, 2000; Lopez-Molina et al., 2001).

According to a recent report, ABA levels might be positively regulated by DELLA protein through upregulation of *XERICO* expression (Zentella et al., 2007). *XERICO* over-expression, which

encodes an E3 ubiquitin *ligase*, leads to both an elevated level of ABA and increased drought tolerance, although the mechanism is still unknown (Ko et al., 2006).

A class of mutants that was directly selected on the basis of reduced dormancy are the *rdo1-rdo4* mutants (Léon-Kloosterziel et al., 1996a; Peeters et al., 2002). The fact that all four mutants show some mild pleiotropic effects in adult plants indicates that the genes are not specific for dormancy/germination but affect other processes as well. The *RDO4* (renamed as *HUB1*) was shown to encode a C3HC4 Ring finger protein involved in the monoubiquitination of histone H2B, revealing a role for chromatin modelling in seed dormancy (Liu et al., 2007). The *dag1-1* mutant also displays reduced dormancy, but in contrast to the *rdo* mutants the effect is determined by the maternal genotype. This is in agreement with the expression pattern of the *DAG1* gene in the vascular tissue of the developing seed. *DAG1*, which encodes a DOF transcription factor, may influence the import of compounds from the mother plant into the seed (Papi et al., 2000). It is the first gene identified as being specifically involved in maternal control of seed germination. However, the germination phenotype of *dag2*, mutant in the related *DAG2* gene, with a similar expression pattern as *DAG1* is opposite to that of *dag1* seeds (Gualaberti et al., 2002) showing increased dormancy. Additional mutants with a reduced dormancy phenotype at other loci, including mutants with no obvious pleiotropic effect have been isolated (Bentsink et al., 2006 and M. Schwab and W. Soppe unpublished results), indicating the complexity of the genetic regulation of seed dormancy. Instead of selecting for mutants that germinate when the wild type is still dormant, Salaita et al. (2005) used germination speed at 10°C as a selection criterion when screening activation tagged lines of the Col accession. Except for two *tt* mutants (see below) none of these cold temperature germination (*ctg*) has been characterized molecularly.

Another group of mutants that shows reduced seed dormancy are mutants with an altered seed coat or testa (Debeaujon et al., 2000; reviewed in Debeaujon et al., 2007; Lepiniec et al., 2006). The seed coat is a multifunctional organ that plays an important role in embryo nutrition during seed development and in protection against detrimental agents from the environment afterwards (Debeaujon et al., 2007). The seed coat is formed from two integuments of epidermal origin that surround the mature ovule. The development of the seed coat from the ovule has been described by Beeckman et al. (2000).

The seed coat together with the endosperm layer exerts a germination-restrictive action, either by being impermeable to water and /or oxygen, by producing germination inhibiting compounds or by its mechanical resistance to radicle protrusion. In Arabidopsis, phenolic compounds and their derivatives present in the inner layer of the testa, called endothelium, affect seed coat properties that influence germination as can be concluded from the reduced dormancy phenotype of many testa mutants.

Seed coat mutants consist of two major groups. One group, affected in flavonoid pigmentation is represented by the *transparent testa* (*tt*) and *transparent testa glabra* (*ttg*) mutants. Mutants identified are *tt1* to *tt15*, *ttg1* and *ttg2* and *banyuls* (*ban*). The color of the *tt* mutants ranges from yellow to pale brown (Debeaujon et al., 2000). *Ban* mutants accumulate pink flavonoid pigments in the endothelium of immature seeds, but do not contain proanthocyanins due to a mutation in the anthocyanin reductase (*ANR*) gene (Xie et al., 2003) resulting in grayish-green, spotted mature seeds (Albert et al, 1997; Devic et al, 1999). The *ttg1* mutant lacks mucilage and trichomes and is affected in the morphology of the outer layer of the seed coat as well as in pigment production. Many of the seed coat mutants have now been cloned, for more details see supplemental Table 1 online and Debeaujon et al. (2007).

The second group is represented by mutants affected in testa structure. The aberrant testa shape (*ats*) mutant, mutated in the *KANADI 4* gene (McAbee et al., 2006) produces a single integument instead of the two integuments seen in wild-type ovules and shows less dormancy.

Genes and or factors that decrease seed dormancy or promote the germination potential of seeds

The germination of Arabidopsis seeds is under phytochrome-mediated photocontrol. It is therefore to be expected that phytochrome deficient mutants are affected in seed germination. The complexity of the phytochrome system comes from the presence of distinct types of phytochromes, for which five genes in the Arabidopsis genome encode different, but related, apoproteins (Sharrock and Quail, 1989). In addition different modes of action of phytochrome, described as very-low-fluence response (VLFR), low-fluence response (LFR) and high-irradiance response (HIR), which have their own fluence dependency, can affect germination (reviewed by Casal and Sánchez, 1998). Mutants lacking phytochrome B (phyB) show a reduced sensitivity to red light, indicating that phyB has a primary role in seed germination. PhyA can only induce germination after a prolonged imbibition of seeds (Shinomura et al., 1994). Detailed action spectra for seed germination performed in wild type, *phyA* and *phyB* mutants revealed a typical red/ far-red (R/FR) -reversible LFR mediated by phyB, whereas the germination response mediated by phyA turned out to be a VLFR with a 10^4 -fold higher sensitivity to light (Shinomura et al, 1996). The observation that also *phyA phyB* double mutants show some light- dependent germination indicates the involvement of another R/FR-reversible photoreceptor system (Yang et al., 1995; Poppe and Schäfer, 1997) probably mediated by phyC, D, and/or E.

Although the main role of phytochrome is in light-induced stimulation of seed germination, a role in the onset of dormancy or the setting of the light requirement is suggested by the experiments of McCullough and Shropshire (1970) and Hayes and Klein (1974). These authors showed that the R/FR ratio experienced by the mother plant and therefore during seed maturation, affects the subsequent germination behaviour of mature seeds. Munir et al. (2001) showed that photoperiod conditions during seed formation may also influence seed germination. However, this effect was strongly genotype dependent. In addition, it appears that phytochrome mediated pathways are required to break cold-induced dormancy (Donohue et al., 2007). Cool temperatures during seed maturation induced seed dormancy which could not be overcome in the *hy2-1* (deficient in phytochrome chromophore, common to all five phytochromes) mutant.

The plant hormone gibberellin plays an important role in promoting seed germination. GA-deficient mutants are unable to germinate without exogenous GAs (Koornneef and van der Veen, 1980; Mitchum et al., 2006). De novo biosynthesis of GAs is required during imbibition, as was concluded from the observation

that inhibitors of GA biosynthesis, such as paclobutrazol and tetcyclacis prevent germination (Karssen et al., 1989) unless ABA is absent. As expected also GA signalling mutants such as the sleepy1 (*sly1*) mutant, which was selected in a screen for suppressors of the ABA insensitive mutant *abi1-1* (Steber et al., 1998) and Della protein encoding genes such as *RGL2* (Lee et al., 2002; Bassel et al., 2004) have germination defective phenotypes. For the GA receptors mutants (*gid1a*, *gid1b*, *gid1c*) triple mutants had to be constructed to see this phenotype due to redundancy of the function of these genes (Griffith et al., 2006, Iuchi et al., 2007, reviewed by Hirano et al., 2008). GAs can promote germination by their ability to overcome germination constraints that exist in seeds requiring after-ripening, light and cold. This led to the suggestion that such environmental factors may induce GA biosynthesis during the early phases of germination. At present the changes of GA content and the expression of GA biosynthesis and catabolism genes during dormancy release and germination is well documented (Yamauchi et al., 2007).

The phytochrome (light) effect was supported by Yamaguchi et al. (1998), who showed that one of two 3-β hydroxylase enzymes, encoded by the *GA4H* gene is induced in germinating seeds by phytochrome. The mechanism of the GA signalling proteins and the effect of light is now well established. Crucial in this are the DELLA proteins which repress GA action, RGL2 (repressor of GA1-3 like 2) is the major DELLA regulating seed germination. These DELLA proteins are degraded by the 26S proteosome therefore GA charged GID1 DELLA proteins interact with the F box protein SLY1 needed for DELLA ubiquitination (Steber, 2007, Finkelstein et al., 2008). An important signal transduction component of light induced germination is the bHLH transcription factor *PIF1* (*Phytochrome Interacting Factor 1*) also named *PIL5* (*Phytochrome-Interacting factor 3-Like 5*) (Oh et al., 2004, 2006, Castillon et al., 2007), which can bind to Pfr that thereafter results in proteosome degradation (Oh et al., 2006). This increases GA levels because GA biosynthesis genes such as GA3ox1 and GA3ox2 are repressed by *PIF1*/*PIL5*, whereas the GA catabolic GA2ox gene and genes encoding DELLA proteins are activated (Oh et al., 2007). These higher GA levels further lead to inactivation of the DELLA proteins as described above. *Somnus* (*SOM*) which encodes a nucleus-localized CCCH-type zinc finger protein is another gene acting downstream of *PIL5* (Kim et al., 2008). The *som* mutant germinates in darkness, independently of various light regimes. Kim et al. (2008) showed that *PIL5* activates the expression of *SOM* by binding directly to the *SOM* promoter. It is suggested that PIL5 regulates ABA and GA metabolic genes partly through SOM.

Cold treatments were also found to stimulate GA biosynthesis (Yamauchi et al., 2004) by inducing the GA3ox1 and GA3ox2 genes. This cold effect is mediated by a light stable bHLH transcription factor SPATULA (SPT), which suppresses the expression of these genes (Penfield et al., 2005). In addition cold may increase the sensitivity of seeds to GAs because it also has an effect in GA deficient mutants (Debeaujon and Koornneef, 2000).The fact that often stratification is more effective than GA treatment (Alonso-Blanco et al., 2003) suggests that also other factors, promoting germination, are affected by stratification.

Brassinosteroids (BRs), are naturally occurring plant steroid hormones found in a wide variety of plant species (Clouse and Sasse, 1998) are also involved in the control of germination in Arabidopsis. The BR signal leads to reduced sensitive to ABA and thereby stimulates germination, although the normal germination of BR deficient and BR signalling mutants indicates that there is no absolute BR requirement for germination (Steber and McCourt, 2001). BRs could overcome the lack of germination of the *sleepy1* mutant, probably by bypassing its GA requirement through a GA independent mechanism (Steber and McCourt, 2001; Finkelstein et al., 2008).

Mutants in ethylene signalling are also affected in their germination response. Ethylene is produced in trace amounts by almost all higher plants and is involved in the control of growth and developmental processes that range from germination to senescence. Often seeds that respond to ethylene are light sensitive for germination (Kepczynski and Kepczynska, 1997). Ethylene insensitive mutants such as *etr* and *ein2* germinate less well or after a longer period of after-ripening than wild type (Bleecker et al., 1988; Beaudoin et al., 2000). The *ein2* and *etr* mutants are hypersensitive to ABA (Beaudoin et al., 2000; Ghassemian et al., 2000), which agrees with the observation that *ein2* mutants were isolated as *abi1-1* suppressors. The *ctr1* mutant, which is characterised by a constitutive ethylene response, appeared among mutants selected as enhancers of the ABA insensitive mutant *abi1-1* and *ctr1* monogenic mutants are also slightly ABA resistant (Beaudoin et al., 2000). These observations, in combination with the non-dormant phenotype of the *ein2 abi3-4* double mutant indicated that ethylene negatively regulates seed dormancy by inhibiting ABA action (Beaudoin et al., 2000). However, Ghassemian et al., (2000) and Chiwocha et al. (2005) showed that, in addition to signalling, the slightly more dormant ethylene insensitive mutants, such as *ein2* and *etr1-2* have higher ABA levels. The higher levels of germination promoting hormones indicate compensation effects. The presence of cross talk between sugar signalling and ethylene was suggested by the sugar insensitive phenotype of *ctr1* (Gibson et al., 2001) and the sugar hypersensitive phenotype of *etr* (Zhou et al., 1998). Apparently ABA, ethylene and sugar signalling strongly interact at the level of germination and early seedling growth.

Auxins are known to play important roles in embryogenesis. However, its role in the regulation of germination and seedling establishment remained obscure (Kucera et al., 2006). Auxin alone was not generally considered to be important in the control of seed germination but cross-talk between auxin, ABA, GA and ethylene was suggested to both affect germination and seedling establishment (Fu and Harberd, 2003; Ogawa et al., 2003; Chiwocha et al., 2005, Carrera et al., 2008; Liu, PP et al., 2007 reviewed by Holdsworth et al., 2008a). Analysis of the expression of the DR5:GUS auxin reporter indicated that auxin accumulates during embryogenesis and is present in the seed following imbibition. Expression was observed at the radicle tip prior to germination in one study (Liu, PP et al., 2007) and throughout the embryo at the end of embryogenesis in another study (Ni et al., 2001). Analysis of transcriptome expression showed that RNA encoding auxin transporters AUX1, PIN2, and PIN7 were highly up-regulated in response to treatment of *ga1* mutant seeds with GA (Ogawa et al., 2003), and that both efflux and influx transporters are up-regulated in after-ripened compared to dormant seeds (Carrera et al., 2008). This may indicate a role for these transporters in germination per-se, or with the establishment of the root apex and gravitropism following radicle emergence. Clearer genetic evidence of a role for auxin in germination has been obtained from an analysis

of the regulation of *Auxin Response Factor10* (*ARF10*) by microRNA (miRNA) miR160 (Liu, PP et al., 2007). miRNAs have been shown to down-regulate target genes at the post-transcriptional level, and play crucial roles in a broad range of developmental processes (Dugas & Bartel, 2004). It was shown that transgenic seeds expressing an miR160-resistant form of *ARF10* (*mARF10*) were hypersensitive to germination inhibition by exogenous ABA, whereas ectopic expression of miR160 resulted in reduced sensitivity to ABA (Liu, PP et al., 2007).

These results indicate a role of auxin in germination associated pathways, and suggest that interactions between auxin and ABA signalling pathways may contribute to the germination potential of seeds. An analysis of the function of key components of auxin signalling in relation to after-ripening, germination potential and vigour may reveal novel roles for auxin in these processes.

Compounds that have been identified as being important stimulants of germination, mainly using farmacological tools are several nitrogen-containing compounds, including nitric oxide (NO) gas (Bethke et al., 2006), nitrite (NO_2^-) and nitrate (NO_3^-) (Alboresi et al., 2005; Bethke et al., 2007a for review). It is suggested (Bethke et al., 2007a) that all N compounds affect germination via conversion into NO. Enzymatic NO production occurs mainly via nitrate reductase as by product of lipid catabolism or nitric oxide synthase (Crawford and Guo, 2005). Non-enzymatic conversion of nitrite to NO, has also been demonstrated and was suggested to have special significance for seeds (Bethke et al., 2007a). The observation by Alboresi et al. (2005) that the high nitrate levels that accumulate in nitrate reductase deficient mutants lead to reduced dormancy implies that nitrate reductase is not essential either because NO is generated by this non-enzymatic pathway or nitrate acts on its own.

An effect of NO is that it might function as an antioxidant (Lamatinna et al., 2003). However, it is also reported that NO inhibits catalase leading to higher H_2O_2 and reactive oxygen species (ROS), which has the opposite effect as antioxidants (Bethke et al., 2007a). ROS are a by product of β–oxidation of stored seed fatty acids. Thereby ROS are increased, which it self may alleviate dormancy (Bailly et al., 2004). However, other and additional mechanisms have also been suggested and include interaction with ABA catabolism enzymes and light and GA signalling (Finkelstein et al., 2008, Bethke et al., 2007b)

THE GENETIC CONTROL OF SEED STORAGE COMPOUNDS, SEED DETERIORATION AND EARLY SEEDLING DEVELOPMENT

Seeds can survive for a long time without germinating, either when stored in dry conditions or when buried in the soil. These seeds form the seed bank waiting until environmental conditions become favorable for germination. Despite the strong desiccation tolerance of many seeds, storage also under dry conditions ultimately leads to a loss of viability. It has been suggested that compounds such as certain sugars and proteins, such as LEA (Late embryogenesis abundant) (Skriver and Mundy, 1990) and Heat shock proteins (Hsps) (Wehmeyer and Vierling, 2000; Hong and Vierling, 2001), that accumulate during the later stages of seed development, have a desiccation protective role. However, mutants like *abi5*, which show a strong reduction in some LEA proteins (Finkelstein and Lynch, 2000) do not have obvious defects in seed storability. A knock-out mutant of Hsp101 shows a loss of thermotolerance during seed germination (Hong and Vierling, 2001), but under optimal conditions germination seems unaffected, suggesting that at least Hsp101 does not have a general function in the survival of desiccated seeds. The expression of these heath shock proteins are developmentally controlled by the seed specific heat stress transcription factor HsfA9, which is regulated by the ABI3 protein (Kotak et al., 2007).

The presence of genetic variation for storability is clear in desiccation intolerant genotypes such as *abi3*, *lec1*, *fus3* and *dog1* mutants (Meinke et al., 1994; Weber and Wobus, 1999; Bentsink et al., 2006) and effects on testa mutants are reported as well (Debeaujon et al., 2000). QTL analysis in the L*er*/Cvi and L*er*/Sha RIL lines (Bentsink et al., 2000; Clerkx et al., 2004) also revealed genetic variation for this trait, which was however not related to the levels of raffinose series oligosaccharides segregating in the same population.

For a seed it is important that after surviving a period in which it could not germinate, it can grow into a vigorous seedling that can compete with other seedlings. Factors that are important for seedling establishment probably mainly have to do with the availability and mobilization of storage materials. The accumulation of storage proteins and lipids is defective in seed maturation mutants such as *lec1*, *lec2* and *fus3*, which although they germinate on filter paper they cannot be transferred efficiently to soil from this substrate, in contrast to other seeds, and require establishment as seedling on sugar supplemented media before they can be transplanted to soil. The *wrinkled1* (*wri1*; Focks and Benning, 1998) mutant affects specifically seed storage compounds with a strong reduction in seed oil content. In addition it shows reduced germination potential and reduced seedling establishment and responds different than wild type to sugar and ABA signals (Cernac et al., 2006). *WRI1* encodes an AP2/EREB family transcription factor (Cernac and Benning, 2004), which itself is regulated by LEC2 (Baud et al., 2007). WRI1 controls carbon metabolism in developing seeds and is a prerequisite for oil accumulation in seeds.

The mobilization of food reserves during germination and early seedling growth require the activity of lipid and carbohydrate degrading enzymes. Storage lipid breakdown is a complex process involving a number of steps for which many of the genes have been identified in recent years and their effect of mutations in these genes have been studied in mutants and double mutants (reviewed in Penfield et al., 2007; Graham et al., 2008). Fatty acids are stored in oil bodies as triacylglycerol (TAG), which are hydrolysed by lipases. The released fatty acids are passed to glyoxysomes (a peroxisome in which the glyoxylate cycle occurs) via an ABC transporter encoded by the CTS (comatose) protein (Russell et al., 2000; Footitt et al., 2002). In here β–oxidation takes place converting fatty acids, activated to acyl-CoA esters to acetyl Co-A, which is subsequently converted to four-carbon sugars. These sugars are then transported to the mitochondria from where they are converted to malate and transported to the cytosol for gluconeogenesis or used for respiration. Gene expression profiling revealed that flavonoids were absent from *cts* and *kat2*-1 mutant seeds, but accumulated in the presence of sucrose. This indicates an essential role for β–oxidation in inducing flavonoid biosynthetic genes. The same analyses revealed that CTS functions very late

in phase II of germination and that its function is required for the expression of specific gene sets related to an important biochemical pathway associated with seedling establishment and survival (Carrera et al., 2007).

Proteins that play a role in β–oxidation are LACS (Acyl-CoA synthetase), ACX (Acyl-CoA oxidase), MFP2 (Multifunctional protein 2) and KAT2 (3-keto-acyl-CoA thiolase). The glyoxylate cycle involve citrate synthase (CSY), isocitrate lyase (ICL), malate synthase (MLS) and phosphophoenolpyruvate carboxykinase (PCK1) (reviewed by Penfield et al., 2007; Graham et al., 2008).

As expected from mutants that are deficient in storage lipids, many mutants in these genes described above (or double mutants when duplicated genes control the step) have defects in hypocotyl elongation in darkness and seedling establishment that can be rescued by sugars (Eastmond et al., 2000; Cornah et al., 2004). Therefore, the glyoxylate cycle can be described as being non-essential in Arabidopsis germination, although it does play an important physiological role in seedling establishment. However a subset of the lipid mobilisation single mutants (*cts*, *kat2*) and double (in case of *acx* and *csy*) show strongly reduced germination, which explains why *cts* mutants were isolated in a seed germination screen (Russell et al., 2000). All these mutants have in common that they act in the glyoxysome and are involved in transport into (CTS) or, export from (CSY, Pracharoenwattana et al., 2005, Graham, 2007 for review) or β-oxidation in this organelle. This suggests that a product(s) of β-oxidation apparently has a germination promoting function. The nature of this signal, which is not the absence of lipid mobilisation as such, is not known. Penfield et al. (2007) mention a number of possibilities, such as involvement of jasmonate biosynthesis or redox control. However, the direct involvement of these factors is unlikely because other mutants in these factors do not show a germination phenotype (Penfield et al., 2007).

An example of another gene controlling metabolism in developing and germinating seeds is plastidic pyruvate kinase (*PKP1*) controlling the last step of glycolysis. *Pkp1* mutants which are reduced in oil accumulation, contain less chlorophyll and tocopherol and consequently a seedling establishment defect (Andre et al., 2007; Baud et al., 2007).

Sugars arrest early seedling establishment. It has been shown that this process is closely associated with plant hormone biosynthesis and signalling, in particular with that of ABA (for review see Finkelstein and Gibson, 2001; Gazzarini and McCourt, 2001; León and Sheen, 2003; Rook et al., 2006; Dekkers and Smeekens, 2007; Rognoni et al., 2007). Mutants that were insensitive to the inhibiting effect of glucose and sucrose on early seedling development were isolated by several groups and identified ABA deficient mutants (i.e. *aba2*/*isi4*/*gin1*/*sis4* and *aba3*/*gin5*) and *ABA insensitive4* (*abi4*/*sun6*/*isi3*/*gin6*/*sis5*) as sugar insensitive (Arenas-Huertero et al., 2000; Huijser et al., 2000; Laby et al., 2000; Rook et al., 2001). The fact that *ABI4* encodes an AP2 domain containing transcription factor that binds a CE1-like element present in many ABA and sugar regulated promoters (Finkelstein et al., 1998; Niu et al., 2002; Acevedo-Hernandez et al., 2005) linked sugar regulation to ABA signalling. However there are many more examples of processes and genes that are regulated by ABA and sugar. Recently Dekkers et al. (2008) showed that *ABI3* is a key regulator of glucose insensitive (*GIN*) signalling which is in agreement with previous reports showing that *ABI3* overexpression (either *AtABI3* or *CnABI3*) in Arabidopsis results in a glucose oversensitive phenotype (Finkelstein et al., 2002; Zeng and Kermode, 2004). The same authors showed that ABA and glucose signalling overlap to a larger extend than originally thought (Dekkers et al., 2008). Recently it has been identified that *DOG1* is involved as well in the ABA mediated sugar signalling pathway in seedlings. This role is specific for the *GSQ5*/*DOG1* Cvi allele as glucose addition induced the expression of the *GSQ5*/*DOG1* Cvi allele whereas the L*er* and Col allele did not respond (Teng et al., 2008).

"OMIC" ANALYSES RELATED TO DORMANCY AND GERMINATION

Several recent studies have analysed the expression of the genome in Arabidopsis seeds at both the transcriptome and proteome levels (reviewed by Holdsworth et al., 2008b). These studies have identified a major role for translation in germination and dormancy release. Rajjou et al. (2004) reported that transcription is not the restricting step in the completion of germination following imbibition of non-dormant seeds. Imbibition of seeds in the presence of an excess of -amanitin (a specific inhibitor of DNA-dependent RNA polymerase II) failed to inhibit germination completion, whereas the translational inhibitor cyclohexamide totally inhibited germination. This indicates the importance of the in the dry seeds stored mRNA that are used for translation in imbibed seeds (Rajjou et al., 2004)

Several recent studies have shown that important transcript and protein changes are happening in the 'dry' seeds during storage and that these changes might be targeted to release of dormancy as seeds after-ripen. These studies indicate that both the accumulation of specific proteins (Chibani et al., 2006) and gene transcripts (Bove et al., 2005, Leubner-Metzger, 2005) can occur. The "dry" state, as very clearly described by Holdsworth et al. (2008b), are mature seeds containing 5-10% water depending on the species. This residual water is not uniformly distributed in the seed tissues indicating that some areas (hydrated pockets within cells) may contain enough water to support gene expression (Leubner-Metzger, 2005).

The transcriptome

Developmental phases during late embryonesis and subsequent germination are characterized by spatial and temporal patterns of gene expression (Nambara et al., 2000; Parcy et al., 1997). Several studies have been performed in order to understand processes that occur in the "dry" seed, for example during after-ripening. Carrera et al. (2008) performed a study in order to understand the relationship between ABA and after-ripening. It has been shown that mutants defective in seed ABA synthesis (*aba1-1*) or perception (*abi1-1*) which do not show dormancy, exhibit changes in global gene expression resulting from dry after-ripening that were comparable with changes occurring in wild type seeds. Thus, the action of ABA has been separated from after-ripening and dry storage regulated gene expression. The authors also use their seed-specific gene ontology (GO) TAGGIT, which have been described in an earlier paper (Carrera et al., 2007), which facilitates the identification and visualisation of the germination signature.

Stored mRNA species in dry seeds are thought to be required for late embryogenesis and seed germination. To differentiate these classes Nakabayaski et al. (2005) analyzed expression profiles obtained from seeds at 6, 12 and 24 hours after imbibition. It has been indicated that embryonic genes are largely down-regulated within 6 hours of imbibition, and that induction of metabolic genes involved in germination started around 12 hours after imbibition. The same study revealed that upstream regions of genes that are highly expressed in dry seeds over-represent ABREs (ABA-responsive elements). The ABRE requires a second element to form a functional ABA response complex. The coupling element (CE) is such a motif and elicits a response to ABA when located adjacent to an ABRE (ABRC; Shen and Ho., 1995). It was indeed shown, that genes that contain both ABRE and CE are expressed at higher levels in seeds. In addition to this also the RY/Sph motif (known to regulate seed specific expression; Bäumlein et al., 1992; Hattori et al., 1992) showed enrichment in seeds when associated with ABRE (Nakabayashi et al., 2005).

Penfield et al. (2006) established the relative roles of embryo and endosperm in the control of seed germination and seedling establishment. They showed that the endosperm responds to both ABA and gibberellins, but that ABA in particular regulates nuclear but not plastid-encoded photosynthetic gene expression in the embryo. They also showed that *ABI4* expression is confined to the embryo, accounting for the major differences in embryo response to ABA, and defining a role for ABI4 as a repressor of lipid breakdown. Furthermore, *ABI5* expression in the endosperm defines a second region of altered ABA signalling in the micropylar endosperm cap. Finally, embryo and endosperm ABA signalling mutants demonstrate the spatial specificity of ABA action in seed germination. It was concluded that the single cell endosperm plays an active role in the regulation of seed germination in Arabidopsis (Penfield et al., 2006).

Other studies have produced microarray data related to germination under different conditions. Experiments using GA-deficient seeds (Ogawa et al., 2003) have described temporal transcript abundance changes in GA-deficient mutants seeds stimulated to germinate by GA treatment or in nongerminating water-imbibed controls. Dormancy cycling was studied in the Arabidopsis accession Cvi in a range of dormant and after-ripening states. The data support an ABA-GA hormone balance mechanism controlling cycling through dormant states that depends of synthetic and catabolic pathways of both hormones (Cadman et al., 2006). The same authors studied the effect of after-ripening, low temperature, nitrate and light on gene expression during dormancy release (Finch-Savage et al., 2007). Here they show that dormancy and after-ripening gene sets respond in a quantitative way to specific environmental signals. Recently, Bassel et al. (2008) have performed a chemical genetic approach in order to identify small-molecules that inhibit germination. The authors identified three mechanistically distinct inhibitors which they used subsequently to isolate a transcriptional signature that defines germinating seeds. The data was compared to data published by Carrera et al. (2007).

To help to visualize the data mentioned above a seed mRNA expression browser was designed using the electronic Fluorescent Pictograph platform (eFP). This eFP is included as part of the Bio-Array (BAR) online bioinformatics tools package (www.bar.utoronto.ca). The seed eFP browser which is developed and described by Bassel et al. (2008) visualizes experimental gene expression data painted onto pictographic representations of the tissues and conditions from which the RNA samples were obtained. The TAGGIT GO (Carrera et al., 2007) has also been integrated into the eFP browser.

The proteome

Several proteome analyses have been performed to analyse protein abundance in Arabidopsis seeds in relation to dormancy and germination using two-dimensional gels. Gallardo et al. (2001, 2002a) described protein abundance related to germination and seed priming (pre-germination treatment which overcomes seed dormancy) and the role of gibberellins in seed germination. In addition the importance of methionine biosynthesis for Arabidopsis seed germination and seedling growth has been shown (Gallardo et al., 2002b). Results indicated that methionine synthase and S-adenosylmethionine synthetase are fundamental components controlling metabolism in the transition from a quiescent to a highly active state during seed germination.

Recently a genome-scale proteomics analyses was published were proteome dynamics are described for different plant organs using mass spectrometry (Baerenfaller et al., 2008). This research includes data on after-ripened “dry” seeds, in these seeds 13,901 distinct peptides were detected that represent 3,789 proteins. In total 133 seed specific biomarkers have been identified. These biomarkers were enriched for specific functional classes that are: embryonic development ending in seed dormancy (GO:0009793), sequestering of lipids (GO:0019915), lipid transport (GO:0006869), seed dormancy (GO:0010162) and response to water (GO:0009415). Although this paper only describes a protein survey in “dry” after-ripened seeds, the seed specific peptide map shows that proteomics can be used as a routine scoring method comparing different dormancy and germination states.

The metabolome

Fait et al. (2006) have analysed metabolic profiles using gas chromatography-mass spectrometry (GS-MS) on seeds throughout development and germination. The authors conclude that seed maturation was associated with a significant reduction of most sugars, organic acids and amino acids, suggesting their incorporation into storage reserves. Furthermore, it appeared that the transition from reserve accumulation to seed desiccation was associated with a major metabolic switch, resulting in the accumulation of distinct sugars, organic acids, nitrogen-rich amino acids, and shikimate-derived metabolites. In contrast to this, seed stratification was associated with a decrease in the content of several of the metabolic intermediates accumulated during seed desiccation, implying that these intermediates might support the metabolic reorganization needed for seed germination. Concomitantly, the levels of other metabolites significantly increased during stratification and were boosted further during germination sensu stricto, implying their importance for germination and seedling establishment. The metabolic switches during seed maturation and germination were also associated with distinct patterns of expression of genes encoding metabolism-associated gene products, as

determined by semi-quantitative RT-PCR and analysis of publicly available microarray data. In conclusion, this study provides a comprehensive picture of the coordinated changes in primary metabolism that underlie seed development and germination in Arabidopsis. They furthermore imply that the metabolic preparation for germination and efficient seedling establishment initiates already during seed desiccation and continues by additional distinct metabolic switches during stratification and early germination (Fait et al., 2006).

CONCLUSIONS

Dormancy and germination are very complex traits under the control of a large number of genes. The number of mutants and genes that are involved has increased rapidly in recent years. These studies confirmed the crucial role of the plant hormones GA and ABA. However it also showed the involvement and complex interaction with other plant hormones such as ethylene, brassinosteriods and auxin. Furthermore novel players were detected such as the *HUB* genes affecting histone modifications and genes such as *DOG1* for which loss of function mutants show their importance but for which the biochemical function is unknown. The use of transcriptomic and proteomic analysis refined the variation and similarities of the pathways affected by dormancy and germination and provides a basis for studying the role of individual genes in these pathways. In future studies apart from distinguishing dormancy and germination promotion also the temporal and spatial components will be taken into account, as well as the specific roles and sensing mechanisms of environmental factors both during seed development, seed storage and seed germination. This together should allow an in depth insight in this essential and intriguing biological system.

References

Albert, S., Delseny, M., and Devic, M. (1997) BANYULS, a novel negative regulator of flavonoid biosynthesis in the Arabidopsis seed coat. Plant J. **11:** 289-299.

Alboresi, A., Gestin, C., Leydecker, M.T., Bedu, M., Meyer, C., and Truong, H.N. (2005) Nitrate, a signal relieving seed dormancy in Arabidopsis. Plant Cell Environ. **28:** 500-512.

Ali-Rachedi, S., Bouinot, D., Wagner, M.H., Bonnet, M., Sotta, B., Grappin, P., and Jullien, M. (2004) Changes in endogenous abscisic acid levels during dormancy release and maintenance of mature seeds: studies with the Cape Verde Islands ecotype, the dormant model of *Arabidopsis thaliana*. Planta **219:** 479-488.

Alonso-Blanco, C., Bentsink, L., Hanhart, C.J., Blankestijn-De Vries, H., and Koornneef, M. (2003) Analysis of natural allelic variation at seed dormancy loci of *Arabidopsis thaliana*. Genetics **164:** 711-729.

Alonso, J., Hirayama, T., Roman, G., Nourizadeh, S., and Ecker, J.R. (1999) EIN2, a bifunctional transducer of ethylene and stress responses in Arabidopsis. Science **284:** 2148-2155.

Andre, C., Froehlich, J.E., Moll, M.R., and Benning, C. (2007) A heteromeric plastidic pyruvate kinase complex involved in seed oil biosynthesis in Arabidopsis. Plant Cell **19:** 2006-2022.

Arenas-Huertero, F., Arroyo, A., Zhou, L., Sheen, J., and Leon, P. (2000) Analysis of Arabidopsis glucose insensitive mutants, gin5 and gin6, reveals a central role of the plant hormone ABA in the regulation of plant vegative development by sugar. Genes Dev. **14:** 2085-2096.

Baerenfaller, K., Grossmann, J., Grobei, M.A., Hull, R., Hirsch-Hoffmann, M., Yalovsky, S., Zimmermann, P., Grossniklaus, U., Gruissem, W., and Baginsky, S. (2008) Genome-scale proteomics reveals *Arabidopsis thaliana* gene models and proteome dynamics. Science **320:** 938-941.

Bailly, C. (2004) Active oxygen species and antioxidants in seed biology. Seed Sci. Res. **14:** 93-107.

Barroco, R.M., Van, P.K., Bergervoet, J.H., De, V.L., Groot, S.P., Inze, D., and Engler, G. (2005) The role of the cell cycle machinery in resumption of postembryonic development. Plant Physiol. **137:** 127-140.

Baskin, J.M. and Baskin, C.C. (2004) A classification system for seed dormancy. Seed Sci. Res. **14:** 1-16.

Bassel, G.W., Zielinska, E., Mullen, R.T., and Bewley, J.D. (2004) Down-regulation of DELLA genes is not essential for germination of tomato, soybean, and Arabidopsis seeds. Plant Physiol. **136:** 2782-2789.

Bassel, G.W., Fung, P., Freeman Chow T-f., Foong, J.A., Provart, N.J. and Cutler, S.R. (2008) Elucidating the germination transcriptional program using small molecules. Plant Physiol. **147:** 143-155.

Baud, S., Boutin, J.P., Miquel, M., Lepiniec, L., and Rochat, C. (2002) An intergrated overview of seed development in *Arabidopsis thaliana* ecotype Ws. Plant Physiol. Biochem. **40:** 151-160.

Baud, S., Mendoza, M.S., To, A., Harscoet, E., Lepiniec, L., and Dubreucq, B. (2007) WRINKLED1 specifies the regulatory action of LEAFY COTYLEDON2 towards fatty acid metabolism during seed maturation in Arabidopsis. Plant J. **50:** 825-838.

Baumlein, H., Nagy, I., Villarroel, R., Inze, D., and Wobus, U. (1992) Cis-analysis of a seed protein gene promoter: the conservative RY repeat CATGCATG within the legumin box is essential for tissue-specific expression of a legumin gene. Plant J. **2:** 233-239.

Bäumlein, H., Miséra, S., Luersen, H., Kölle, K., Horstmann, C., Wobus, U., and Müller, A.J. (1994) The FUS3 gene of *Arabidopsis thaliana* is a regulator of gene expression during late embryogenesis. Plant J. **6:** 379-387.

Beaudoin, N., Serizet, C., Gosti, F., and Giraudat, J. (2000) Interactions between abscisic acid and ethylene signaling cascades. Plant Cell **12:** 1103-1115.

Beeckman, T., De Rycke, R., Viane, R., and Inze, D. (2000) Histological study of seed coat development in *Arabidopsis thaliana*. J. Plant Res. **113:** 139-148.

Bennett, M.J., Marchant, A., Green, H.G., May, S.T., Ward, S.P., Millner, P.A., Walker, A.R., Schulz, B., and Feldmann, K.A. (1996) Arabidopsis AUX1 gene: a permease-like regulator of root gravitropism. Science **273:** 948-950.

Bentsink, L., Alonso-Blanco, C., Vreugdenhil, D., Tesnier, K.J.Y., Groot, S.P.C., and Koornneef, M. (2000) Genetic analysis of seed-soluble oligosaccharides in relation to seed storability of Arabidopsis. Plant Physiol. **124:** 1595-1604.

Bentsink, L., Jowett, J., Hanhart, C.J., and Koornneef, M. (2006) Cloning of DOG1, a quantitative trait locus controlling seed dormancy in Arabidopsis. Proc. Natl. Acad. Sci. USA **103:** 17042-17047.

Bethke, P.C., Libourel, I.G., and Jones, R.L. (2006) Nitric oxide reduces seed dormancy in Arabidopsis. J.Exp.Bot. **57**: 517-526.

Bethke, P.C., Libourel, I.G., and Jones, R.L. (2007a) Nitric oxide in seed dormancy and germination. In Seed development, dormancy and germination, K.Bradford and H.Nonogaki, eds (Oxford, UK: Blackwell Publishing), pp. 153-171.

Bethke, P.C., Libourel,I.G., Aoyama,N., Chung,Y.Y., Still,D.W., and Jones, R.L. (2007b) The Arabidopsis aleurone layer responds to nitric oxide, gibberellin, and abscisic acid and is sufficient and necessary for seed dormancy. Plant Physiol **143:** 1173-1188.

Bewley, J.D. and Black, M. (1994) Seeds; Physiology of development and germination. New York: Plenum Press.

Bewley, J.D. (1997) Seed germination and dormancy. Plant Cell **9:** 1055-1066.

Bies, N., Silva Conceicao, A., Giraudat, J., Koornneef, M., Leon-Kloosterziel, K.M., Valon, C., and Delseny, M. (1999) Importance of the B2 domain of the Arabidopsis ABI3 protein for EM and 2S albumin gene regulation. Plant Mol. Biol. **40:** 1045-1054.

Bleecker, A.B., Estelle, M.A., Somerville, C., and Kende, H. (1988) Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. Science **241:** 1086-1089.

Bonetta, D. and McCourt, P. (1998) Genetic analysis of ABA signal transduction pathways. Trends Plant Sci. **3:** 231-235.

Bove, J., Lucas, P., Godin, B., Oge, L., Jullien, M., and Grappin,P. (2005) Gene expression analysis by cDNA-AFLP highlights a set of new signaling networks and translational control during seed dormancy breaking in *Nicotiana plumbaginifolia*. Plant Mol. Biol. **57:** 593-612.

Braybrook, S.A., Stone, S.L., Park, S., Bui, A.Q., Le, B.H., Fischer, R.L., Goldberg, R.B., and Harada, J.J. (2006) Genes directly regulated by LEAFY COTYLEDON2 provide insight into the control of embryo maturation and somatic embryogenesis. Proc. Natl. Acad. Sci. USA **103:** 3468-3473.

Cadman, C.S., Toorop, P.E., Hilhorst, H.W., and Finch-Savage, W.E. (2006) Gene expression profiles of Arabidopsis Cvi seeds during dormancy cycling indicate a common underlying dormancy control mechanism. Plant J. **46:** 805-822.

Carrera, E., Holman, T., Medhurst, A., Peer, W., Schmuths, H., Footitt, S., Theodoulou, F.L., and Holdsworth, M.J. (2007) Gene expression profiling reveals defined functions of the ATP-binding cassette transporter COMATOSE late in phase II of germination. Plant Physiol. **143:** 1669-1679.

Carrera, E., Holman, T., Medhurst, A., Dietrich, D., Footitt, S., Theodoulou, F.L., and Holdsworth, M.J. (2008) Seed after-ripening is a discrete developmental pathway associated with specific gene networks in Arabidopsis. Plant J. **53:** 214-224.

Casal, J.J. and Sánchez, R.A. (1998) Phytochromes and seed germination. Seed Sci. Res. **8:** 317-329.

Castillon, A., Shen, H., and Huq, E. (2007) Phytochrome interacting factors: central players in phytochrome-mediated light signaling networks. Trends Plant Sci. **12:** 514-521.

Cernac, A. and Benning, C. (2004) WRINKLED1 encodes an AP2/EREB domain protein involved in the control of storage compound biosynthesis in Arabidopsis. Plant J. **40:** 575-585.

Cernac, A., Andre, C., Hoffmann-Benning, S., and Benning, C. (2006) WRI1 is required for seed germination and seedling establishment. Plant Physiol. **141:** 745-757.

Chang, C., Kwok, S.F., Bleecker, A.B., and Meyerowitz, E.M. (1993) Arabidopsis ethylene-response gene ETR1: similarity of product to two-component regulators. Science **262:** 539-544.

Chibani, K., Ali-Rachedi, S., Job, C., Job, D., Jullien, M., and Grappin, P. (2006) Proteomic analysis of seed dormancy in Arabidopsis. Plant Physiol. **142:** 1493-1510.

Chiwocha, S.D.S., Cutler, A.J., Abrams, S.R., Ambrose, S.J., Yang, J., Ross, A.R.S., and Kermode, A.R. (2005) The etr1-2 mutation in *Arabidopsis thaliana* affects the abscisic acid, auxin, cytokinin and gibberellin metabolic pathways during maintenance of seed dormancy, moist-chilling and germination. Plant J. **42:** 35-48.

Clerkx, E.J.M., El Lithy, M.E., Vierling, E., Ruys, G.J., Blankestijin-De Vries, H., Groot, S.P.C., Vreugdenhil, D., and Koornneef, M. (2004) Analysis of natural allelic variation of Arabidopsis seed germination and seed longevity traits between the accessions Landsberg erecta and Shakdara, using a new recombinant inbred line population. Plant Physiol. **135:** 432-443.

Clouse, S. and Sasse, J.M. (1998) Brassinosteroids: Essential regulators of plant growth and development. Annu. Rev. Plant. Physiol. Plant Mol. Biol. **49:** 427-451.

Cone, J.W. and Spruit, C.J.P. (1983) Imbibition conditions and seed dormancy of *Arabidopsis thaliana*. Physiol. Plant **59:** 416-420.

Cone, J.W. and Kendrick, R.E. (1985) Fluence-response curves and action spectra for promotion and inhibition of seed germination in wildtype and long-hypocotyl mutants of *Arabidopsis thaliana* L. Planta *163:* 43-54.

Cornah, J.E., Germain, V., Ward, J.L., Beale, M.H., and Smith, S.M. (2004) Lipid utilization, gluconeogenesis, and seedling growth in Arabidopsis mutants lacking the glyoxylate cycle enzyme malate synthase. J. Biol. Chem. **279:** 42916-42923.

Crawford, N.M. and Guo, F.Q. (2005) New insights into nitric oxide metabolism and regulatory functions. Trends Plant Sci. **10:** 195-200.

Cutler, S., Ghassemian, M., Bonetta, D., Cooney, S., and McCourt, P. (1996) A protein farnesyl transferase involved in ABA signal transduction in Arabidopsis. Science **273:** 1239-1241.

Dall'Osto, L., Cazzaniga, S., North, H., Marion-Poll, A., and Bassi, R. (2007) The Arabidopsis aba4-1 mutant reveals a specific function for neoxanthin in protection against photo-oxidative stress. Plant Cell **19:** 1048-1064.

Debeaujon, I., Leon-Kloosterziel, K.M., and Koornneef, M. (2000) Influence of the testa on seed dormancy, germination and longevity in *Arabidopsis thaliana*. Plant Physiol. **122:** 403-414.

Debeaujon, I. and Koornneef, M. (2000) Gibberellin requirement for *Arabidopsis thaliana* seed germination is determined both by testa characteristics and embryonic ABA. Plant Physiol. **122:** 415-424.

Debeaujon, I., Peeters, A.J.M., Leon-Kloosterziel, K.M., and Koornneef, M. (2001) The TRANSPARENT TESTA 12 gene of Arabidopsis encodes a multidrug secondary transporter-like protein required for flavonoid sequestration in vacuoles of the seed coat endothelium. Plant Cell **13:** 853-871.

Debeaujon, I., Lepiniec, L., Pourcel, L., and Routaboul, J.-M. (2007) Seed coat development and dormancy. In Seed development, dormancy and germination, K. Bradford and H. Nonogaki, eds (Oxford, UK: Blackwell Publishing), pp. 25-43.

Dekkers, B.J. and Smeekens, S. (2007) Sugar and abscisic acid regulation of germination and transition to seedling growth. In Seed development, dormancy and germination, K.Bradford and H.Nonogaki, eds (Oxford, UK: Blackwell Publishing), pp. 305-322.

Dekkers, B.J., Schuurmans, J.A., and Smeekens, S.C. (2008) Interaction between sugar and abscisic acid signalling during early seedling development in Arabidopsis. Plant Mol. Biol. **67:** 151-167.

Derkx, M.P.M. and Karssen, C.M. (1993a) Variability in light-gibberellin- and nitrate requirement of *Arabidopsis thaliana* seeds due to harvest time and conditions of dry storage. J. Plant Physiol. **141:** 574-582.

Derkx, M.P.M. and Karssen, C.M. (1993b) Changing sensitivity to light and nitrate but not to gibberellins regulates seasonal dormancy patterns in *Sisymbrium officinale* seeds. Plant Cell Environ. **16:** 469-479.

Devic, M., Guilleminot, J., Debeaujon, I., Bechtold, N., Bensaude, E., Koornneef, M., Pelletier, G., and Delseny, M. (1999) The BANYULS gene encodes a DFR-like protein and is a marker of early seed coat development. Plant J. **19:** 387-398.

Donohue, K., Dorn, L., Griffith, C., Kim, E., Aguilera, A., Polisetty, C.R., and Schmitt, J. (2005) Environmental and genetic influences on the germination of *Arabidopsis thaliana* in the field. Evolution **59:** 740-757.

Donohue, K., Heschel, M.S., Chiang, G.C., Butler, C.M., and Barua, D. (2007) Phytochrome mediates germination responses to multiple seasonal cues. Plant Cell Environ. **30:** 202-212.

Dugas, D.V. and Bartel, B. (2004) MicroRNA regulation of gene expression in plants. Curr. Opin. Plant Biol. **7:** 512-520.

Dugas, D.V. and Bartel, B. (2008) Sucrose induction of Arabidopsis miR398 represses two Cu/Zn superoxide dismutases. Plant Mol.Biol. **67:** 403-417.

Eastmond, P.J., Germain, V., Langa, P.R., Bryce, J.H., Smith, S.M., and Graham, I.A. (2000) Postgerminative growth and lipid catabolism in oilseeds lacking the glyoxylate cycle. Proc. Natl. Acad. Sci. USA **97:** 5669-5674.

Eastmond, P.J. and Graham, I.A. (2001) Re-examining the role of the glyoxylate cycle in oilseeds. Trends Plant Sci. **6:** 72-77.

Eastmond, P.J. (2006) SUGAR-DEPENDENT1 encodes a patatin domain triacylglycerol lipase that initiates storage oil breakdown in germinating Arabidopsis seeds. Plant Cell **18:** 665-675.

Fait, A., Angelovici, R., Less, H., Ohad, I., Urbanczyk-Wochniak, E., Fernie, A.R., and Galili, G. (2006) Arabidopsis seed development and germination is associated with temporally distinct metabolic switches. Plant Physiol. **142:** 839-854.

Feinbaum, R.L. and Ausubel, F.M. (1988) Transcriptional regulation of the Arabidopsis chalcone synthase gene. Mol. Cell. Biol. **8:** 1985-1992.

Finch-Savage, W.E. and Leubner-Metzger, G. (2006) Seed dormancy and the control of germination. New Phytol. **171:** 501-523.

Finch-Savage, W.E., Cadman, C.S., Toorop, P.E., Lynn, J.R., and Hilhorst, H.W. (2007) Seed dormancy release in Arabidopsis Cvi by dry after-ripening, low temperature, nitrate and light shows common quantitative patterns of gene expression directed by environmentally specific sensing. Plant J. **51:** 60-78.

Finkelstein, R., Wang, M.L., Lynch, T.J., Rao, S., and Goodman, H.M. (1998) The Arabidopsis Abscisic Acid response locus ABI4 encodes an APETALA2 domain protein. Plant Cell **10:** 1043-1054.

Finkelstein, R., Reeves, W., Ariizumi, T., and Steber, C. (2008) Molecular aspects of seed dormancy. Annu. Rev. Plant Biol. **59:** 387-415.

Finkelstein, R.R. (1994) Mutations at two new Arabidopsis ABA response loci are similar to the abi3 mutations. Plant J. **5:** 765-771.

Finkelstein, R.R. and Lynch, T.J. (2000) The Arabidopsis abscisic acid response gene ABI5 encodes a basic leucine zipper transcription factor. Plant Cell **12:** 599-609.

Finkelstein, R.R. and Gibson, S.I. (2001) ABA and sugar interactions regulating development: cross-talk or voices in a crowd? Curr. Opin. Plant Biol. **5:** 26-32.

Finkelstein, R.R., Gampala, S.S., and Rock, C.D. (2002) Abscisic acid signaling in seeds and seedlings. Plant Cell **14 Suppl:** S15-S45.

Focks, N. and Benning, C. (1998) Wrinkled1: A novel, low-seed-oil mutant of Arabidopsis with a deficiency in the seed-specific regulation of carbohydrate metabolism. Plant Physiol. **118:** 91-101.

Focks, N., Sagasser, M., Weisshaar, B., and Benning, C. (1999) Characterization of tt15, a novel transparent testa mutant of *Arabidopsis thaliana* (L.) Heynh. Planta **208:** 352-357.

Footitt, S., Slocombe, S.P., Larner, V., Kurup, S., Wu, Y., Larson, T., Graham, I., Baker, A., and Holdsworth, M. (2002) Control of germination and lipid mobilization by COMATOSE, the Arabidopsis homologue of human ALDP. EMBO J. **21:** 2912-2922.

Fu, Q., Wang, B.C., Jin, X., Li, H.B., Han, P., Wei, K.H., Zhang, X.M., and Zhu, Y.X. (2005) Proteomic analysis and extensive protein identification from dry, germinating Arabidopsis seeds and young seedlings. J. Biochem. Mol. Biol. **38:** 650-660.

Fu, X.D. and Harberd, N.P. (2003) Auxin promotes Arabidopsis root growth by modulating gibberellin response. Nature **421:** 740-743.

Gallardo, K., Job, C., Groot, S.P.C., Puype, M., Demol, H., Vandekerckhove, J., and Job, D. (2001) Proteomic analysis of Arabidopsis seed germination and priming. Plant Physiol. **126:** 835-848.

Gallardo, K., Job, C., Groot, S.P.C., Puype, M., Demol, H., Vandekerckhove, J., and Job, D. (2002) Importance of methionine biosynthesis for Arabidopsis seed germination and seedling growth. Physiol. Plant. **116:** 238-247.

Gallardo, K., Job, C., Groot, S.P.C., Puype, M., Demol, H., Vandekerckhove, J., and Job, D. (2002) Proteomics of Arabidopsis seed germination. A comparative study of wild-type and gibberellin-deficient seeds. Plant Physiol. **129:** 823-837.

Gazzarrini, S. and McCourt, P. (2001) Genetic interactions between ABA, ethylene and sugar signaling pathways. Curr. Opin. Plant Biol. **4:** 387-391.

Germain, V., Rylott, E.L., Larson, T.R., Sherson, S.M., Bechtold, N., Carde, J.P., Bryce, J.H., Graham, I.A., and Smith, S.M. (2001) Requirement for 3-ketoacyl-CoA thiolase-2 in peroxisome development, fatty acid beta-oxidation and breakdown of triacylglycerol in lipid bodies of Arabidopsis seedlings. Plant J. **28:** 1-12.

Ghassemian, M., Nambara, E., Cutler, S., Kawaide, H., Kamiya, Y., and McCourt, P. (2000) Regulation of abscisic acid signaling by the ethylene response pathway in Arabidopsis. Plant Cell **12:** 1117-1126.

Gibson, S.I., Laby, R.J., and Kim, D. (2001) The sugar-insensitive1 (sis1) mutant of Arabidopsis in allelic to ctr1. Biochem. Biophys. Res. Comm. **280:** 196-203.

Giraudat, J., Hauge, B.M., Valon, C., Smalle, J., Parcy, F., and Goodman, H.M. (1992) Isolation of the Arabidopsis ABI3 gene by positional cloning. Plant Cell **4:** 1251-1261.

Girke, T., Todd, J., Ruuska, S., White, J., Benning, C., and Ohlrogge, J. (2000) Microarray analysis of developing Arabidopsis seeds. Plant Physiol. **124:** 1570-1581.

Goldberg, R.B., Paiva, G., and Yadegari, R. (1994) Plant embryogenesis: zygote to seed. Science **266:** 605-614.

Graham, I.A. (2008) Seed storage oil mobilization. Annu.Rev.Plant Biol. **59:** 115-142.

Griffiths, J., Murase, K., Rieu, I., Zentella, R., Zhang, Z.L., Powers, S.J., Gong, F., Phillips, A.L., Hedden, P., Sun, T.P., and Thomas, S.G. (2006) Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. Plant Cell **18:** 3399-3414.

Gualberti, G., Papi, M., Bellucci, L., Ricci, I., Bouchez, D., Camilleri, C., Costantino, P., and Vittorioso, P. (2002) Mutations in the Dof zinc finger genes *DAG2* and *DAG1* influence with opposite effects germination of Arabidopsis seeds. Plant Cell **14:** 1253-1263.

Guilfoyle, T.J. and Hagen, G. (2007) Auxin response factors. Curr. Opin. Plant Biol. **10:** 453-460.

Gutierrez, L., Van Wuytswinkel, O., Castelain, M., and Bellini, C. (2007) Combined networks regulating seed maturation. Trends Plant Sci **12:** 294-300.

Hattori, T., Vasil, V., Rosenkrans, L., Hannah, L.C., McCarty, D.R., and Vasil, I.K. (1992) The Viviparous-1 gene and abscisic acid activate the C1 regulatory gene for anthocyanin biosynthesis during seed maturation in maize. Genes Dev. **6:** 609-618.

Hayashi, M., Toriyama, K., Kondo, M., and Nishimura, M. (1998) 2,4-dichlorophenoxybutyric acid-resistant mutants of Arabidopsis have defects in glyoxysomal fatty acid beta-oxidation. Plant Cell **10:** 183-195.

Hayes, R.G. and Klein, W.H. (1974) Spectral quality influence of light during development of *Arabidopsis thaliana* plants in regulating seed germination. Plant Cell Physiol. **15:** 643-653.

Helliwell, C.A., Sheldon, C.C., Olive, M.R., Walker, A.R., Zeevaart, J.A.D., Peacock, W.J., and Dennis, E.S. (1998) Cloning of the Arabidopsis entkaurene oxidase gene GA3. Proc. Natl. Acad. Sci. USA **95:** 9019-9024.

Hepher, A. and Roberts, J.A. (1985) The control of seed germination in *Trollius ledebouri*: the breaking of dormancy. Planta **166:** 314-320.

Hilhorst, H.W. (2007) Definitions and hypotheses of seed dormancy. In Seed development, dormancy and germination, K.Bradford and H.Nonogaki, eds (Oxford, UK: Blackwell Publishing), pp. 50-67.

Hirano, K., Ueguchi-Tanaka, M., and Matsuoka, M. (2008) GID1-mediated gibberellin signaling in plants. Trends Plant Sci. **13:** 192-199.

Holdsworth, M.J., Bentsink, L., and Soppe, W.J. (2008a) Molecular networks regulating Arabidopsis seed maturation, after-ripening, dormancy and germination. New Phytol. **179:** 33-54.

Holdsworth, M.J., Finch-Savage, W.E., Grappin, P., and Job, D. (2008b) Post-genomics dissection of seed dormancy and germination. Trends Plant Sci. **13:** 7-13.

Hong, S.H. and Vierling, E. (2001) Hsp101 is necessary for heat tolerance but dispensable for development and germination in the absence of stress. Plant J. **26:** 1-12.

Hugouvieux, V., Kwak, J.M., and Schroeder, J.I. (2001) An mRNA cap binding protein, ABH1, modulates early abscisic acid signal transduction in Arabidopsis. Cell **106:** 477-487.

Huijser, C., Kortstee, A.J., Pego, J.V., Weisbeek, P., Wisman, E., and Smeekens, S. (2000) The Arabidopsis SUCROSE UNCOUPLED-6 gene is identical to abscisic acid insensitive-4: involvement of abscisic acid in sugar responses. Plant J. **23:** 577-585.

Iuchi, S., Suzuki, H., Kim, Y.C., Iuchi, A., Kuromori, T., Ueguchi-Tanaka, M., Asami, T., Yamaguchi, I., Matsuoka, M., Kobayashi, M., and Nakajima, M. (2007) Multiple loss-of-function of Arabidopsis gibberellin receptor AtGID1s completely shuts down a gibberellin signal. Plant J. **50:** 958-966.

Jacobsen, S.E., Binkowski, K.A., and Olszewski, N.E. (1996) SPINDLY, a tetratricopeptide repeat protein involved in gibberellin signal transduction in Arabidopsis. Proc. Natl. Acad. Sci. USA **93:** 9292-9296.

Johnson, C.S., Kolevski, B., and Smyth, D.R. (2002) TRANSPARENT TESTA GLABRA2, a trichome and seed coat development gene of Arabidopsis, encodes a Wrky transcription factor. Plant Cell **14:** 1359-1375.

Kagaya, Y., Toyoshima, R., Okuda, R., Usui, H., Yamamoto, A., and Hattori, T. (2005) LEAFY COTYLEDON1 controls seed storage protein genes through its regulation of FUSCA3 and ABSCISIC ACID INSENSITIVE3. Plant Cell Physiol. **46:** 399-406.

Karssen, C.M. (1976) Uptake and effect of abscisic acid during induction and progress of radicle growth in seeds of *Chenopodium album*. Physiol. Plant. **36:** 259-263.

Karssen, C.M., Brinkhorst-van der Swan, D.L.C., Breekland, A.E., and Koornneef, M. (1983) Induction of dormancy during seed development by endogenous abscisic acid: studies on abscisic acid deficient genotypes of *Arabidopsis thaliana* (L.) Heynh. Planta **157:** 158-165.

Karssen, C.M., Zagórski, S., Kepczynski, J., and Groot, S.P.C. (1989) Key role for endogenous gibberellins in the control of seed germination. Ann. Bot. **63:** 71-80.

Katagiri, T., Ishiyama, K., Kato, T., Tabata, S., Kobayashi, M., and Shinozaki, K. (2005) An important role of phosphatidic acid in ABA signaling during germination in *Arabidopsis thaliana*. Plant J. **43:** 107-117.

Keith, K., Kraml, M., Dengler, N.G., and McCourt, P. (1994) Fusca3: A heterochronic mutation affecttng late embryo development in Arabidopsis. Plant Cell **6:** 589-600.

Kepczynski, J. and Kepczynska, E. (1997) Ethylene in seed dormancy and germination. Physiol. Plant. **101:** 720-726.

Kieber, J.J., Rothenberg, M., Roman, G., Feldmann, K.A., and Ecker, J.R. (1993) CTR1, a negative regulator of the ethylene response pathway in Arabidopsis, encodes a member of the raf family of protein kinases. Cell **72:** 427-441.

Kim, D.H., Yamaguchi, S., Lim, S., Oh, E., Park, J., Hanada, A., Kamiya, Y., and Choi, G. (2008) SOMNUS, a CCCH-type zinc finger protein in Arabidopsis, negatively regulates light-dependent seed germination downstream of PIL5. Plant Cell. **20:** 1260-1277.

Kitamura, S., Shikazono, N., and Tanaka, A. (2004) Subcellular localization of TT19, which is involved in flavonoid accumulation in Arabidopsis. Plant Cell Physiol. **45:** S180.

Ko, J.H., Yang, S.H., and Han, K.H. (2006) Upregulation of an Arabidopsis RING-H2 gene, XERICO, confers drought tolerance through increased abscisic acid biosynthesis. Plant J. **47:** 343-355.

Kohchi, T., Mukougawa, K., Frankenberg, N., Masuda, M., Yokota, A., and Lagarias, J.C. (2001) The Arabidopsis HY2 gene encodes phytochromobilin synthase, a ferredoxin-dependent biliverdin reductase. Plant Cell **13:** 425-436.

Koornneef, M. and Van der Veen, J.H. (1980) Induction and analysis of gibberellin-sensitive mutants in *Arabidopsis thaliana* (L.) Heynh. Theor. Appl. Genet. **58:** 257-263.

Koornneef, M., Reuling, G., and Karssen, C.M. (1984) The isolation and characterization of abscisic acid-insensitive mutants of *Arabidopsis thaliana*. Physiol. Plant. **61:** 377-383.

Koornneef, M., Hanhart, C.J., Hilhorst, H.W.M., and Karssen, C.M. (1989) In vivo inhibition of seed development and reserve protein accumulation in recombinants of abscisic acid biosynthesis and responsiveness mutants in *Arabidopsis thaliana*. Plant Physiol. **90:** 463-469.

Koornneef, M. and Karssen, C.M. (1994) Seed dormancy and germination. In Arabidopsis, E.M.Meyerowitz and C.R.Somerville, eds (Cold Spring Harbor: Cold Spring Harbor Laboratory Press), pp. 313-334.

Koornneef, M., Alonso-Blanco, C., Bentsink, L., Blankenstijn-de Vries, H., Debeaujon, I., Hanhart, C.J., Leon-Kloosterziel, K.M., Peeters, T., and Raz, V. (2000) The genetics of seed dormancy in *Arabidopsis thaliana*. In Dormancy in plant, J.D.Viémont and J.Crabbé eds (CAB International, Wallingford UK), pp. 365-373.

Kotak, S., Vierling, E., Baumlein, H., and von Koskull-Doring, P. (2007) A novel transcriptional cascade regulating expression of heat stress proteins during seed development of Arabidopsis. Plant Cell **19:** 182-195.

Krock, B., Schmidt, S., Hertweck, C., and Baldwin, I.T. (2002) Vegetation-derived abscisic acid and four terpenes enforce dormancy in seeds of the post-fire annual, *Nicotiana attenuata*. Seed Sci. Res. **12:** 239-252.

Kroj, T., Savino, G., Valon, C., Giraudat, J., and Parcy, F. (2003) Regulation of storage protein gene expression in Arabidopsis. Development **130:** 6065-6073.

Kucera, B., Cohn, M.A., and Leubner-Metzger, G. (2006) Plant hormone interactions during seed dormancy release and germination. Seed Sci. Res. **15:** 281-307.

Kugler, I. (1951) Untersuchungen über das Keimverhalten einiger Rassen von *Arabidopsis thaliana* (L.) Heynh. Ein Beitrag zum Problem der Lichtkeimung. Beitr.Biol.Pflanzen **28:** 211-243.

Kushiro, T., Okamoto, M., Nakabayashi, K., Yamagishi, K., Kitamura, S., Asami, T., Hirai, N., Koshiba, T., Kamiya, Y., and Nambara, E. (2004) The Arabidopsis cytochrome P450 CYP707A encodes ABA 8'-hydroxylases: key enzymes in ABA catabolism. EMBO J. **23:** 1647-1656.

Kwong, R.W., Bui, A.Q., Lee, H., Kwong, L.W., Fischer, R.L., Goldberg, R.B., and Harada, J.J. (2003) LEAFY COTYLEDON1-LIKE defines a class of regulators essential for embryo development. Plant Cell **15:** 5-18.

Laby, R.J., Kincaid, S., Kim, D., and Gibson, S.I. (2000) The Arabidopsis sugar-insensitive mutants sis4 and sis5 are defective in abscisic acid synthesis and response. Plant J. **23:** 587-596.

Lamattina, L., Garcia-Mata, C., Graziano, M., and Pagnussat, G. (2003) Nitric oxide: the versatility of an extensive signal molecule. Annu. Rev. Plant Biol. **54:** 109-136.

Lee, S., Cheng, H., King, K.E., Wang, W., He, Y., Hussain, A., Lo, J., Harberd, N.P., and Peng, J. (2002) Gibberellin regulates Arabidopsis seed germination via RGL2, a GAI/RGA-like gene whose expression is up-regulated following imbibition. Genes Dev. **16:** 646-658.

Lefebvre, V., North, H., Frey, A., Sotta, B., Seo, M., Okamoto, M., Nambara, E., and Marion-Poll, A. (2006) Functional analysis of Arabidopsis NCED6 and NCED9 genes indicates that ABA synthesized in the endosperm is involved in the induction of seed dormancy. Plant J. **45:** 309-319.

Leon, P. and Sheen, J. (2003) Sugar and hormone connections. Trends Plant Sci. **8:** 110-116.

Lepiniec, L., Debeaujon, I., Routaboul, J.M., Baudry, A., Pourcel, L., Nesi, N., and Caboche, M. (2006) Genetics and biochemistry of seed flavonoids. Annu.Rev.Plant Biol. **57:** 405-430.

Leubner-Metzger, G., Fründt, C., Vögeli-Lange, R., and Meins, F. (1995) Class I b-1,3-glucanases in the endosperm of tobacco during germination. Plant Physiol. **109:** 751-759.

Leubner-Metzger, G. (2003) Functions and regulation of beta-1,3-glucanases during seed germination, dormancy release and after-ripening. Seed Sci. Res. **13:** 17-34.

Leubner-Metzger, G. (2005) beta-1,3-Glucanase gene expression in low-hydrated seeds as a mechanism for dormancy release during tobacco after-ripening. Plant J. **41:** 133-145.

Leung, J., Bouvier-Durand, M., Morris, P.C., Guerrier, D., Chefdor, F., and Giraudat, J. (1994) Arabidopsis ABA-response gene ABI1: features of a calcium-modulated protein phosphatase. Science **264:** 14481-1452.

Leung, J., Merlot, S., and Giraudat, J. (1997) The Arabidopsis ABI2 gene is a homologue of ABI1 and implicates redundant protein serine/threonine phosphatases 2C in abscisic acid signal transduction. Plant Cell **9:** 759-771.

Leung, J. and Giraudat, J. (1998) Cloning genes of *Arabidopsis thaliana* by chromosome walking. Methods Mol. Biol. **82:** 277-303.

Léon-Kloosterziel, K.M., Keijzer, C.J., and Koornneef, M. (1994) A seed shape mutant of Arabidopsis that is affected in integument development. Plant Cell **6:** 385-392.

Léon-Kloosterziel, K.M., van de Bunt, G.A., Zeevaart, J.A.D., and Koornneef, M. (1996a) Arabidopsis mutants with a reduced seed dormancy. Plant Physiol. **110:** 233-240.

Léon-Kloosterziel, K.M., Gil, M.A., Ruijs, G.J., Jacobsen, S.E., Olszewski, N.E., Schwartz, S.H., Zeevaart, J.A.D., and Koornneef, M. (1996b) Isolation and characterization of abscisic acid-deficient Arabidopsis mutants at two new loci. Plant J. **10:** 655-661.

Li, J., Nagpal, P., Vitart, V., McMorris, T.C., and Chory, J. (1996) A role for brassinosteroids in light-dependent development of Arabidopsis. Science **272:** 398-401.

Li, J. and Chory, J. (1997) A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. Cell **90:** 825-827.

Liu, P.P., Koizuka, N., Homrichhausen,T .M., Hewitt, J.R., Martin, R.C., and Nonogaki, H. (2005) Large-scale screening of Arabidopsis enhancer-trap lines for seed germination-associated genes. Plant J. **41:** 936-944.

Liu, P.P., Montgomery, T.A., Fahlgren, N., Kasschau, K.D., Nonogaki, H., and Carrington, J.C. (2007) Repression of AUXIN RESPONSE FACTOR10 by microRNA160 is critical for seed germination and post-germination stages. Plant J. **52:** 133-146.

Liu, Y., Koornneef, M., and Soppe, W.J. (2007) The absence of histone H2B monoubiquitination in the Arabidopsis hub1 (rdo4) mutant reveals a role for chromatin remodeling in seed dormancy. Plant Cell **19:** 433-444.

Lopez-Molina, L., Mongrand, S., and Chua, N.H. (2001) A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in Arabidopsis. Proc. Natl. Acad. Sci. USA **98:** 4782-4787.

Lotan, T., Ohto, M., Yee, K.M., West, M.A., Lo, R., Kwong, R.W., Yamagishi, K., Fischer, R.L., Goldberg, R.B., and Harada, J.J. (1998) Arabidopsis LEAFY COTYLEDON1 is sufficient to induce embryo development in vegetative cells. Cell **93:** 1195-1205.

Luerssen, H., Kirik, V., Herrmann, P., and Misera, S. (1998) FUSCA3 encodes a protein with a conserved VP1/AB13-like B3 domain which is of functional importance for the regulation of seed maturation in *Arabidopsis thaliana*. Plant J. **15:** 755-764.

Macquet, A., Ralet, M.C., Loudet, O., Kronenberger, J., Mouille, G., Marion-Poll, A., and North, H.M. (2007) A naturally occurring mutation in an Arabidopsis accession affects a beta-D-galactosidase that increases the hydrophilic potential of rhamnogalacturonan I in seed mucilage. Plant Cell **19:** 3990-4006.

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

Masubelele, N.H., Dewitte, W., Menges, M., Maughan, S., Collins, C., Huntley, R., Nieuwland, J., Scofield, S., and Murray, J.A.H. (2005) D-type cyclins activate division in the root apex to promote seed germination in Arabidopsis. Proc. Natl. Acad. Sci. USA **102:** 15694-15699.

Mayer, U., Ruiz, R.A.T., Berleth, T., Misera, S., and Jürgens, G. (1991) Mutations affecting body organization in the Arabidopsis embryo. Nature **353:** 402-407.

Mazzella, M.A., Arana, M.V., Staneloni, R.J., Perelman, S., Rodriguez Batiller, M.J., Muschietti, J., Cerdan, P.D., Chen, K., Sanchez, R.A., Zhu, T., Chory, J., and Casal, J.J. (2005) Phytochrome control of the Arabidopsis transcriptome anticipates seedling exposure to light. Plant Cell **17:** 2507-2516.

Mcabee, J.M., Hill, T.A., Skinner, D.J., Izhaki, A., Hauser, B.A., Meister, R.J., Reddy, G.V., Meyerowitz, E.M., Bowman, J.L., and Gasser, C.S. (2006) ABERRANT TESTA SHAPE encodes a Kanadi family member, linking polarity determination to separation and growth of Arabidopsis ovule integuments. Plant J. **46:** 522-531.

McCullough, J.M. and Shropshire, W.J. (1970) Physiological predetermination of germination responses in *Arabidopsis thaliana* (L.) Heynh. Plant Cell Physiol. **11:** 139-148.

McGinnis, K.M., Thomas, S.G., Soule, J.D., Strader,L.C., Zale, J.M., Sun, T.P., and Steber, C.M. (2003) The Arabidopsis SLEEPY1 gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. Plant Cell **15:** 1120-1130.

Meinke, D.W. (1994) Seed development in *Arabidopsis thaliana*. In Arabidopsis, E.M.Meyerowitz and C.R.Somerville, eds (Cold Spring Harbor: Cold Spring Harbor Laboratory Press), pp. 253-298.

Meinke, D.W. (1995) Molecular genetics of plant embryogenesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 46: 369-394.

Meng, P.-H., Macquet, A., Loudet, O., Marion-Poll, A., and North, H. (2008) Analysis of natural allelic variation controlling *Arabidopsis thaliana* seed germinability in response to cold and dark: Indentification of three major quantitative trait loci. Mol. Plant **1:** 145-154.

Meyer, K., Leube, M.P., and Grill, E. (1994) A protein phosphatase 2C involved in ABA signal transduction in *Arabidopsis thaliana*. Science **264:** 1452-1455.

Mitchum, M.G., Yamaguchi, S., Hanada, A., Kuwahara, A., Yoshioka, Y., Kato, T., Tabata, S., Kamiya, Y., and Sun, T.P. (2006) Distinct and overlapping roles of two gibberellin 3-oxidases in Arabidopsis development. Plant J. **45:** 804-818.

Muller, A., Guan, C., Galweiler, L., Tanzler, P., Huijser, P., Marchant, A., Parry, G., Bennett, M., Wisman, E., and Palme, K. (1998) AtPIN2 defines a locus of Arabidopsis for root gravitropism control. EMBO J. **17:** 6903-6911.

Muller, K., Tintelnot, S., and Leubner-Metzger, G. (2006) Endosperm-limited Brassicaceae seed germination: abscisic acid inhibits embryo-induced endosperm weakening of *Lepidium sativum* (cress) and

endosperm rupture of cress and *Arabidopsis thaliana*. Plant Cell Physiol. **47:** 864-877.

Munir, J., Dorn, L.A., Donohue, K., and Schmitt, J. (2001) The effect of maternal photoperiod on seasonal dormancy in *Arabidopsis thaliana* (Brassicaceae). Amer. Jour. Bot. **88:** 1240-1249.

Muramoto, T., Kohchi, T., Yokota, A., Hwang, I., and Goodman, H.M. (1999) The Arabidopsis photomorphogenic mutant hy1 is deficient in phtyochrome chromophore biosynthesis as a result of a result of a mutation in a plastid heme oxygenase. Plant Cell **11:** 335-347.

Nakabayashi, K., Okamoto, M., Koshiba, T., Kamiya, Y., and Nambara, E. (2005) Genome-wide profiling of stored mRNA in *Arabidopsis thaliana* seed germination: epigenetic and genetic regulation of transcription in seed. Plant J. **41:** 697-709.

Nambara, E., Naito, S., and McCourt, P. (1992) A mutant of Arabidopsis which is defective in seed development and storage protein accumulation is a new abi3 allele. Plant J. **2:** 435-441.

Nambara, E., Hayama, R., Tsuchiya, Y., Nishimura, M., Kawaide, H., Kamiya, Y., and Naito, S. (2000) The role of ABI3 and FUS3 loci in *Arabidopsis thaliana* on phase transition from late embryo development to germination. Devel. Biol. **220:** 412-423.

Nesi, N., Debeaujon, I., Jond, C., Pelletier,G., Caboche, M., and Lepiniec, L. (2000) The TT8 gene encodes a basic helix-loop-helix domain protein required for expression of DFR and BAN genes in Arabidopsis siliques. Plant Cell **12:** 1863-1878.

Nesi, N., Jond, C., Debeaujon, I., Caboche, M., and Lepiniec, L. (2001) The Arabidopsis TT2 gene encodes an R2R3 MYB domain protein that acts as a key determinant for accumulation in developing seed. Plant Cell **13:** 2099-2114.

Nesi, N., Debeaujon, I., Jond, C., Stewart, A.J., Jenkins, G.I., Caboche, M., and Lepiniec, L. (2002) The TRANSPARENT TESTA16 locus encodes the ARABIDOPSIS B SISTER MADS domain protein and is required for proper development and pigmentation of the seed coat. Plant Cell **14:** 2463-2479.

Ni, D.A., Wang, L.J., Ding, C.H., and Xu, Z.H. (2001) Auxin distribution and transport during embryogenesis and seed germination of Arabidopsis. Cell Res. **11:** 273-278.

Nishimura, N., Yoshida, T., Murayama, M., Asami, T., Shinozaki, K., and Hirayama, T. (2004) Isolation and characterization of novel mutants affecting the abscisic acid sensitivity of Arabidopsis germination and seedling growth. Plant Cell Physiol. **45:** 1485-1499.

Nishimura, N., Yoshida, T., Kitahata, N., Asami, T., Shinozaki, K., and Hirayama, T. (2007) ABA-Hypersensitive Germination1 encodes a protein phosphatase 2C, an essential component of abscisic acid signaling in Arabidopsis seed. Plant J. **50:** 935-949.

Niu, X.P., Helentjaris, T., and Bate, N.J. (2002) Maize AB14 binds coupling element1 in abscisic acid and sugar response genes. Plant Cell **14:** 2565-2575.

North, H.M., De Almeida, A., Boutin, J.P., Frey, A., To, A., Botran, L., Sotta, B., and Marion-Poll, A. (2007) The Arabidopsis ABA-deficient mutant *aba4* demonstrates that the major route for stress-induced ABA accumulation is via neoxanthin isomers. Plant J. **50:** 810-824.

Ogawa, M., Hanada, A., Yamauchi, Y., Kuwahara, A., Kamiya, Y., and Yamaguchi, S. (2003) Gibberellin biosynthesis and response during Arabidopsis seed germination. Plant Cell **15:** 1591-1604.

Oh, E., Kim, J., Park, E., Kim, J.I., Kang, C., and Choi, G. (2004) PIL5, a phytochrome-interacting basic helix-loop-helix protein, is a key negative regulator of seed germination in *Arabidopsis thaliana*. Plant Cell **16:** 3045-3058.

Oh, E., Yamaguchi, S., Kamiya, Y., Bae, G., Chung, W.I., and Choi, G. (2006) Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in Arabidopsis. Plant J. **47:** 124-139.

Oh, E., Yamaguchi, S., Hu, J., Yusuke, J., Jung, B., Paik, I., Lee, H.S., Sun, T.P., Kamiya, Y., and Choi, G. (2007) PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the GAI and RGA promoters in Arabidopsis seeds. Plant Cell **19:** 1192-1208.

Okamoto, M., Kuwahara, A., Seo, M., Kushiro, T., Asami, T., Hirai, N., Kamiya, Y., Koshiba, T., and Nambara, E. (2006) CYP707A1 and CYP707A2, which encode abscisic acid 8'-hydroxylases, are indispensable for proper control of seed dormancy and germination in Arabidopsis. Plant Physiol. **141:** 97-107.

Ooms, J.J.J., Léon-Kloosterziel, K.M., Bartels, D., Koornneef, M., and Karssen, C.M. (1993) Acquisition of desiccation tolerance and longevity in seeds of *Arabidopsis thaliana* - A comparative study using abscisic acid- insensitive abi3 Mutants. Plant Physiol. **102:** 1185-1191.

Pandey, S., Chen, J.G., Jones, A.M., and Assmann, S.M. (2006) G-protein complex mutants are hypersensitive to abscisic acid regulation of germination and postgermination development. Plant Physiol. **141:** 243-256.

Papi, M., Sabatini, S., Bouchez, D., Camilleri, C., Costantino, P., and Vittorioso, P. (2000) Identification and disruption of an Arabidopsis zinc finger gene controlling seed germination. Genes Dev. **14:** 28-33.

Paponov, I.A., Teale, W.D., Trebar, M., Blilou, K., and Palme, K. (2005) The PIN auxin efflux facilitators: evolutionary and functional perspectives. Trends Pl. Sci. **10:** 170-177.

Parcy, F., Valon, C., Raynal, M., Gaubier-Comella, P., Delseny, M., and Giraudat, J. (1994) Regulation of gene expression programs during Arabidopsis seed development: roles of the ABI3 locus and of endogenous abscisic acid. Plant Cell **6:** 1567-1582.

Parcy, F. and Giraudat, J. (1997) Interactions between the ABI1 and the ectopically expressed ABI3 genes in controlling abscisic acid responses in Arabidopsis vegetative tissues. Plant J. **11:** 693-702.

Peeters, A.J., Blankestijn-De Vries, H., Hanhart, C.J., Leon-Kloosterziel, K.M., Zeevaart, J.A., and Koornneef, M. (2002) Characterization of mutants with reduced seed dormancy at two novel rdo loci and a further characterization of rdo1 and rdo2 in Arabidopsis. Physiol. Plant **115:** 604-612.

Pelletier, M.K. and Shirley, B.W. (1996) Analysis of flavanone 3-hydroxylase in Arabidopsis seedlings. Coordinate regulation with chalcone synthase and chalcone isomerase. Plant Physiol. **111:** 339-345.

Penfield, S., Rylott, E.L., Gilday, A.D., Graham, S., Larson, T.R., and Graham, I.A. (2004) Reserve mobilization in the Arabidopsis endosperm fuels hypocotyl elongation in the dark, is independent of abscisic acid, and requires PHOSPHOENOLPYRUVATE CARBOXYKINASE1. Plant Cell **16:** 2705-2718.

Penfield, S., Josse, E.M., Kannangara, R., Gilday, A.D., Halliday, K.J., and Graham, I.A. (2005) Cold and light control seed germination through the bHLH transcription factor SPATULA. Curr.Biol. **15:** 1998-2006.

Penfield, S., Li, Y., Gilday, A.D., Graham, S., and Graham, I.A. (2006) Arabidopsis ABA INSENSITIVE4 regulates lipid mobilization in the embryo and reveals repression of seed germination by the endosperm. Plant Cell **18:** 1887-1899.

Penfield, S., Pinfield-Wells, H., and Graham, I. (2007) Lipid metabolism in seed dormancy. K.Bradford and H.Nonogaki, eds (Oxford, UK: Blackwell Publishing), pp. 131-152

Petruzzelli, L., Muller, K., Hermann, K., and Leubner-Metzger, G. (2003) Distinct expression patterns of beta-1,3-glucanases and chitinases during the germination of Solanaceous seeds. Seed Sci. Res. **13:** 139-153.

Poppe, C. and Schäfer, E. (1997) Seed germination of *Arabidopsis thaliana* phyA/phyB double mutants is under phytochrome control. Plant Physiol. **114:** 1487-1492.

Pourcel, L., Routaboul, J.M., Kerhoas, L., Caboche, M., Lepiniec, L., and Debeaujon, I. (2005) TRANSPARENT TESTA10 encodes a laccase-like enzyme involved in oxidative polymerization of flavonoids in Arabidopsis seed coat. Plant Cell **17:** 2966-2980.

Pracharoenwattana, I., Cornah, J.E., and Smith, S.M. (2005) Arabidopsis peroxisomal citrate synthase is required for fatty acid respiration and seed germination. Plant Cell **17:** 2037-2048.

Rajjou, L., Gallardo, K., Debeaujon, I., Vandekerckhove, J., Job, C., and Job, D. (2004) The effect of alpha-amanitin on the Arabidopsis seed proteome highlights the distinct roles of stored and neosynthesized mRNAs during germination. Plant Physiol. **134:** 1598-1613.

Ratcliffe, D. (1976) Germination characteristics and their inter- and intrapopulation variability in Arabidopsis. Arab. Inf. Serv. **13:** 34-45.

Raz, V., Bergervoet, J.H., and Koornneef, M. (2001) Sequential steps for developmental arrest in Arabidopsis seeds. Development **128:** 243-252.

Rock, C.D. and Zeevaart, J.A.D. (1991) The aba mutant of *Arabidopsis thaliana* is impaired in epoxy-carotenoid biosynthesis. Proc. Natl. Acad. Sci. USA **88:** 7496-7499.

Rock, C.D. (2000) Pathways to abscisic acid-regulated gene expression. New Phytol. **148:** 357-396.

Rognoni, S., Teng, S., Arru, L., Smeekens, S.C.M., and Perata, P. (2007) Sugar effects on early seedling development in Arabidopsis. Plant Growth Reg. **52:** 217-228.

Rook, F., Corke, F., Card, R., Munz, G., Smith, C., and Bevan, M.W. (2001) Impaired sucrose-induction mutants reveal the modulation of sugar-induced starch biosynthetic gene expression by abscisic acid signaling. Plant J. **26:** 421-433.

Rook, F., Hadingham, S.A., Li, Y., and Bevan, M.W. (2006) Sugar and ABA response pathways and the control of gene expression. Plant Cell Envir. **29:** 426-434.

Russell, L., Larner, V., Kurup, S., Bougourd, S., and Holdsworth, M.J. (2000) The Arabidopsis COMATOSE locus regulates germination potential. Development **127:** 3759-3767.

Rylott, E.L., Rogers, C.A., Gilday, A.D., Edgell, T., Larson, T.R., and Graham, I.A. (2003) Arabidopsis mutants in short- and medium-chain acyl-CoA oxidase activities accumulate acyl-CoAs and reveal that fatty acid beta-oxidation is essential for embryo development. J. Biol. Chem. **278:** 21370-21377.

Rylott, E.L., Eastmond, P.J., Gilday, A.D., Slocombe, S.P., Larson, T.R., Baker, A., and Graham, I.A. (2006) The *Arabidopsis thaliana* MULTIFUNCTIONAL PROTEIN gene (MFP2) of peroxisomal beta-oxidation is essential for seedling establishment. Plant J. **45:** 930-941.

Saez, A., Robert, N., Maktabi, M.H., Schroeder, J.I., Serrano, R., and Rodriguez, P.L. (2006) Enhancement of abscisic acid sensitivity and reduction of water consumption in Arabidopsis by combined inactivation of the protein phosphatases type 2C ABI1 and HAB1. Plant Physiol. **141:** 1389-1399.

Sagasser, M., Lu, G.H., Hahlbrock, K., and Weisshaar, B. (2002) *A. thaliana* TRANSPARENT TESTA 1 is involved in seed coat development and defines the WIP subfamily of plant zinc finger proteins. Genes Devel. **16:** 138-149.

Salaita, L., Kar, R.K., Majee, M., and Downie, A.B. (2005) Identification and characterization of mutants capable of rapid seed germination at 10 degrees C from activation-tagged lines of *Arabidopsis thaliana*. J. Exp. Bot. **56:** 2059-2069.

Schneitz, K. (1999) The molecular and genetic control of ovule development. Curr. Opin. Plant Biol. **2:** 13-17.

Schoenbohm, C., Martens, S., Eder, C., Forkmann, G., and Weisshaar, B. (2000) Identification of the *Arabidopsis thaliana* flavonoid 3'-hydroxylase gene and functional expression of the encoded P450 enzyme. Biol. Chem. **381:** 749-753.

Schopfer, P. and Plachy, C. (1984) Control of seed germination by abscisic acid. II. Effect on embryo water uptake in *Brassica napus* L. Plant Physiol. **76:** 155-160.

Schwartz, S.H., Léon-Kloosterziel, K.M., Koornneef, M., and Zeevaart, J.A.D. (1997) Biochemical characterization of the aba2 and aba3 mutants in *Arabidopsis thaliana*. Plant Physiol. **114:** 161-166.

Sharrock, R.A. and Quail, P.H. (1989) Novel phytochrome sequences in *Arabidopsis thaliana*: structure, evolution, and differential expression of a plant regulatory photoreceptor family. Genes Devel. **3:** 1745-1757.

Shen, Q. and Ho, T.H. (1995) Functional dissection of an abscisic acid (ABA)-inducible gene reveals two independent ABA-responsive complexes each containing a G-box and a novel cis-acting element. Plant Cell **7:** 295-307.

Shikazono, N., Yokota, Y., Kitamura, S., Suzuki, C., Watanabe, H., Tano, S., and Tanaka, A. (2003) Mutation rate and novel tt mutants of *Arabidopsis thaliana* induced by carbon. Genetics **163:** 1449-1455.

Shinomura, T., Nagatani, A., Chory, J., and Furuya, M. (1994) The induction of seed germination in *Arabidopsis thaliana* is regulated principally by phytochrome B and secondarily by phytochrome A. Plant Physiol. **104:** 363-371.

Shinomura, T., Nagatani, A., Hanzawa, H., Kubota, M., Watanabe, M., and Furuya, M. (1996) Action spectra for phytochrome A- and B specific photoinduction of seed germination in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **93:** 8129-8133.

Shirley, B.W., Henley, S., and Goodman, H.M. (1992) Effects of ionizing radiation on a plant genome: Analysis of two Arabidopsis transparent testa mutations. Plant Cell **4:** 333-347.

Skriver, K. and Mundy, J. (1990) Gene expression in response to abscisic acid and osmotic stress. Plant Cell **2:** 503-512.

Soderman, E.M., Brocard, I.M., Lynch, T.J., and Finkelstein, R.R. (2000) Regulation and function of the Arabidopsis ABA-insensitive gene in seed abscisic acid response signaling networks. Plant Physiol. **124:** 1752-1765.

Steber, C. (2007) De-repression of seed germination by GA signaling. In Seed development, dormancy and germination, K.Bradford and H.Nonogaki, eds (Oxford, UK: Blackwell Publishing), pp. 248-260.

Steber, C.M., Cooney, S., and McCourt, P. (1998) Isolation of the GA-response mutant sly1 as a suppressor of ABI1-1 in *Arabidopsis thaliana*. Genetics. **149:** 509-521.

Steber, C.M. and McCourt, P. (2001) A role for brassinosteriods in germination in Arabidopsis. Plant Physiol. **125:** 763-769.

Stone, S.L., Kwong, L.W., Yee, K.M., Pelletier, J., Lepiniec, L., Fischer, R.L., Goldberg, R.B., and Harada, J.J. (2001) Leafy cotyledon2 encodes a B3 domain transcript factor that induces embryo development. Proc. Natl. Acad. Sci. USA **98:** 11806-11811.

Sun, T., Goodman, H.M., and Ausubel, F.M. (1992) Cloning the Arabidopsis GA1 locus by genomic subtraction. Plant Cell **4:** 119-128.

Teng, S., Rognoni, S., Bentsink, L., and Smeekens, S. (2008) The Arabidopsis GSQ5/DOG1 Cvi allele is induced by the ABA-mediated sugar signaling pathway and enhances sugar sensitivity by stimulating ABI4 expression. Plant J. **55:** 372-381.

Tiedemann, J., Rutten, T., Monke, G., Vorwieger, A., Rolletschek, H., Meissner, D., Milkowski, C., Petereck, S., Mock, H.P., Zank, T., and Baumlein, H. (2008) Dissection of a complex seed phenotype: novel insights of FUSCA3 regulated developmental processes. Dev. Biol. **317:** 1-12.

To, A., Valon, C., Savino, G., Guilleminot, J., Devic, M., Giraudat, J., and Parcy, F. (2006) A network of local and redundant gene regulation governs Arabidopsis seed maturation. Plant Cell **18:** 1642-1651.

Ueguchi-Tanaka, M., Ashikari, M., Nakajima, M., Itoh, H., Katoh, E., Kobayashi, M., Chow, T.Y., Hsing, Y.I.C., Kitano, H., Yamaguchi, I., and

Matsuoka, M. (2005) Gibberellin insensitive dwarf1 encodes a soluble receptor for gibberellin. Nature **437:** 693-698.

Van der Schaar, W., Alonso-Blanco, C., Léon-Kloosterziel, K.M., Jansen, R.C., Van Ooijen, J.W., and Koornneef, M. (1997) QTL analysis of seed dormancy in Arabidopsis using recombinant inbred lines and MQM mapping. Heredity **79:** 190-200.

Vicient, C.M., Bies-Etheve, N., and Delseny, M. (2000) Changes in gene expression in the leafy cotyledon1 (lec1) and fusca3 (fus3) mutants of *Arabidopsis thaliana* L. J. Exp. Bot. **51:** 995-1003.

Walker, A.R., Davidson, P.A., Bolognesi-Winfield, A.C., James, C.M., Srinivasan, N., Blundell, T.L., Esch, J.J., Marks, M.D., and Gray, J.C. (1999) The TRANSPARENT TESTA GLABRA 1 locus, which regulates trichome differentiation and anthocyanin biosynthesis in Arabidopsis, encodes a WD40 repeat protein. Plant Cell **11:** 1337-1349.

Weber, H. and Wobus, U. (1999) Sugars as signal molecules in plant seed development. Biol.Chem. **380:** 937-944.

Wehmeyer, N. and Vierling, E. (2000) The expression of small heat shock proteins in seeds responds to discrete developmental signals and suggests a general protective role in desiccation tolerance. Plant Physiol. **122:** 1099-1108.

Wisman, E., Hartmann, U., Sagasser, M., Baumann, E., Palme, K., Hahlbrock, K., Saedler, H., and Weisshaar, B. (1998) Knock-out mutants from an En-1 mutagenized *Arabidopsis thaliana* population generate phenylpropanoid biosynthesis phenotypes. Proc. Natl. Acad. Sci. USA **95:** 12432-12437.

Xie, D.Y., Sharma, S.B., Paiva, N.L., Ferreira, D., and Dixon, R.A. (2003) Role of anthocyanidin reductase, encoded by BANYULS in plant flavonoid biosynthesis. Science **299:** 396-399.

Xiong, L., Ishitani, M., Lee, H., and Zhu, J.K. (2001) The Arabidopsis LOS5/ABA3 locus encodes a molybdenum cofactor sulferase and modulates cold stress- and osmotic stress-responsive gene expression. Plant Cell **13:** 2063-2083.

Yamaguchi, S., Smith, M.W., Brown, R.G.S., Kamiya, Y., and Sun, T. (1998) Phytochrome regulation and differential expression of gibberellin 3β-hydroxylase genes in germinating Arabidopsis seeds. Plant Cell **10:** 2115-2126.

Yamaguchi, S., Kamiya, Y., and Sun, T.P. (2001) Distinct cell-specific expression patterns of early and late gibberellin biosynthetic genes during Arabidopsis seed germination. Plant J. **28:** 443-453.

Yamauchi, Y., Ogawa, M., Kuwahara, A., Hanada, A., Kamiya, Y., and Yamaguchi, S. (2004) Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of *Arabidopsis thaliana* seeds. Plant Cell **16:** 367-378.

Yamauchi, Y., Takeda-Kamiya, N., Hanada, A., Ogawa, M., Kuwahara, A., Seo, M., Kamiya, Y., and Yamaguchi, S. (2007) Contribution of gibberellin deactivation by AtGA2ox2 to the suppression of germination of dark-imbibed *Arabidopsis thaliana* seeds. Plant Cell Physiol. **48:** 555-561.

Yang, Y.Y., Nagatani, A., Zhao, Y.J., Kang, B.J., Kendrick, R.E., and Kamiya, Y. (1995) Effects of gibberellins on seed germination of phytochrome- deficient mutants of *Arabidopsis thaliana*. Plant Cell Physiol. **36:** 1205-1211.

Yoine, M., Ohto, M.A., Onai, K., Mita, S., and Nakamura, K. (2006) The lba1 mutation of UPF1 RNA helicase involved in nonsense-mediated mRNA decay causes pleiotropic phenotypic changes and altered sugar signaling in Arabidopsis. Plant J. **47:** 49-62.

Zeng, Y. and Kermode, A.R. (2004) A gymnosperm ABI3 gene functions in a severe abscisic acid-insensitive mutant of Arabidopsis (abi3-6) to restore the wild-type phenotype and demonstrates a strong synergistic effect with sugar in the inhibition of post-germinative growth. Plant Mol. Biol. **56:** 731-746.

Zentella, R., Zhang, Z.L., Park, M., Thomas, S.G., Endo, A., Murase, K., Fleet, C.M., Jikumaru, Y., Nambara, E., Kamiya, Y., and Sun, T.P. (2007) Global analysis of della direct targets in early gibberellin signaling in Arabidopsis. Plant Cell **19:** 3037-3057.

Zhang, F., Chen, G., Huang, Q., Orion, O., Krugman, T., Fahima, T., Korol, A.B., Nevo, E., and Gutterman, Y. (2005) Genetic basis of barley caryopsis dormancy and seedling desiccation tolerance at the germination stage. Theor. Appl. Genet. **110:** 445-453.

Zhou, L., Jang, J.C., Jones, T.L., and Sheen, J. (1998) Glucose and ethylene signal transduction crosstalk revealed by an Arabidopsis glucose-insensitive mutant. Proc. Natl. Acad. Sci. USA **95:** 10294-10299.