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Seed Dormancy and Germination

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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 unfavorable conditions. To optimize 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 dormancy, as is the case for many other plant species, which is controlled by environmental factors such as light, temperature and time of dry storage as well as by genetic factors (Koornneef and Karssen, 1994). 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 factors and genes controlling seed dormancy and germination in Arabidopsis.

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). During normal seed development, embryo arrest and dormancy are reversed upon germination, which occurs when proper environmental conditions are provided and the dry seed imbibes water.

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 failure of an intact, viable seed to complete germination under favorable conditions (Bewley, 1997). Since in Arabidopsis removal of the seed coat allows germination of non-germinating and strongly dormant genotypes, dormancy in Arabidopsis should be described as coat-enhanced

dormancy (Bewley, 1997). However, in addition to the structures 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.

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 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 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). The visible sign that germination is complete is usually the penetration of the structures surrounding the embryo by the radicle. 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. 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 male parent. Although reciprocal differences might also be attributed to cytoplasmic inheritance of the trait, genetic segregation in the subsequent generations allows the distinction between cytoplasmic and maternal inheritance (Léon-Kloosterziel et al., 1994).

Seed dormancy in Arabidopsis can be overcome by, or seed germination is activated by, the common germination promoting factors such as after-ripening, light, cold treatment (also called stratification) and chemicals such as gibberellins and KNO_3 (Derkx and Karssen, 1993b). None of these exogenous 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.

Non-dormant seeds that are exposed for some time to unfavorable 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 1993a).

The challenge in dormancy and germination research is to identify the nature of the crucial regulator(s) that prevent(s) the onset of germination (dormancy) and that trigger(s) the germination process and their mutual interaction.

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. That even small differences in dormancy are also amenable to genetic analysis in Arabidopsis was shown by Quantitative Trait Loci (QTL) analysis of the differences in seed germination between two commonly used Arabidopsis accessions L*er* and Col (van der Schaar et al., 1997). Germination tests were performed with seeds grown from a set of 98 recombinant inbred lines (RILs), in three different maternal environments, and each seed batch was tested in three different germination environments: in light, in darkness and in the presence of the gibberellin biosynthesis and germination inhibitor, paclobutrazol. For nine out of the fourteen loci detected, no significant interaction between the locus and environmental factors could be detected. However, three distinct loci controlling germination behavior in the presence of paclobutrazol had a much lower or no effect when germination was tested in water. Two other loci affecting germination in darkness and/or light had practically no effect on germination in paclobutrazol. The effects of the individual loci were small in all cases, which makes a thorough molecular analysis very difficult. However, the presence of QTL in specific regions, which may represent quantitative effects of specific alleles, can be studied when genes affecting seed dormancy are later identified in those regions. QTL analysis performed on RILs between accessions with a larger difference in dormancy; L*er* (low level of dormancy) and Cvi (strong dormancy), revealed individual QTL with much stronger effects. Seven chromosomal regions affecting this trait have been identified and the effects of the individual loci could be confirmed by analyzing Near Isogenic Lines (NILs) (L. Bentsink, unpublished results). These NILs are being used to clone the QTL and also for further mutagenesis experiments that may lead to mutations in the respective QTL or in genes acting elsewhere in the dormancy/germination pathways.

It is expected that the analysis of more accessions, of which many are more dormant than the commonly used laboratory accessions (Ratcliffe, 1976; Koornneef et al., 2000), will reveal more loci affecting seed dormancy and germination.

Mutants affected in seed dormancy/germination

There are two groups of seed dormancy/germination mutants that can be recognized. In one group seed dormancy is reduced (germination is promoted) and in the other seed dormancy is increased (germination is inhibited).

Mutants that reduce and/or prevent seed dormancy or promote 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*) play prominent roles in controlling mid- and late seed development (Meinke et al., 1994). Mutations at any of these loci affect multiple processes, including accumulation of storage proteins, as well as acquisition of dormancy and desiccation tolerance. It appears that these four genes have partially overlapping functions in the overall control of seed maturation (Parcy et al., 1997). It has been suggested that the non-dormant phenotype of *lec1, fus3* and *abi3* mutants is due to defective seed maturation and that mutant seeds germinate because this is the default state (Nambara et al., 2000). However, detailed analysis of *lec1, fus3* and *abi3* mutants showed that they differ in the time at which premature germination can occur. *LEC1 (*probably *LEC2* as well) and *FUS3* loci regulate developmental arrest, as mutations in these genes cause a continuation of growth in

immature embryos. Abscisic acid (ABA) controlled dormancy (via *ABA* and *ABI*) occurs later and is additive to the developmental arrest controlled by *LEC1* and *FUS3* (Raz et al., 2001). The dependency of germination on GA is maintained in *fus3* mutants but not in *lec1*, which suggests that these mutants affect the germination potential of seeds in different ways.

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., 1996a). 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). Since in wild type ABA levels decrease at the end of seed maturation it was proposed that after the onset of dormancy endogenous ABA is not required for its maintenance (Karssen et al., 1983). However, the observation that inhibitors of ABA biosynthesis, such as norfluorazon, promote germination (Debeaujon and Koornneef, 2000) indicates that the maintenance of dormancy in imbibed seeds is an active process involving de novo ABA synthesis as was found also for *Nicotiana plumbaginifolia* (Grappin et al., 2000). Because ABA has such a major effect on seed dormancy 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 *abi1* to *abi5* are able to germinate in the presence of ABA concentrations that are inhibitory to the wild type. In contrast, germination of the *era1* (enhanced response to ABA) to *era3* mutant seed is prevented by low concentrations of ABA that ordinarily permit germination of the wild-type seed (Cutler et al., 1996). As judged from their impact on seed dormancy, these two sets of mutations also alter the regulation of seed germination by endogenous ABA. Like ABA-deficient mutants, the ABA-insensitive mutants *abi1* to *abi3* display marked reductions in seed dormancy. Conversely, the ABA-supersensitive mutant *era1* confers enhanced seed dormancy (Cutler et al., 1996). 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) in strong alleles. Surprisingly no dormancy or other seed maturation phenotype was observed in *abi4* and *abi5* mutants (Finkelstein, 1994), 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).

Genetic screens based on ABA-regulated reporter genes in vegetative tissues revealed two additional ABA related mutants. The *ade1* (ABA-deregulated gene expression) and *hos5* (high expression of osmotically responsive genes) mutations enhance gene expression in response to ABA, (Foster and Chua, 1999; Xiong et al., 1999) but have little effect on seed germination. This is also observed for the *aao3* mutant, which affects a step in ABA biosynthesis, different from those in the *aba1 – aba3* mutants (Seo et al., 2000), probably because other redundant genes with seed-specific expression compensate for the function of the mutated genes.

It has been found that sugars such as sucrose and various hexoses inhibit seed germination independently of their osmotic effects (Pego et al., 2000). Mutants that were insensitive to the inhibiting effect of glucose and sucrose were isolated by several groups and appeared to be defective in ABA biosynthesis or are among the ABA insensitive mutants. The *sugar insensitive, sis4* and *sis5* mutants are allelic to respectively *aba2* and *abi4* (Laby et al., 2000) *and sucrose uncoupled* (*sun6*) is also an *abi4* allele (Huijser et al., 2000). These results indicate that germination is controlled in an ABA and sugar dependent way. In respect to this, Garciarrubio et al (1997) published that the addition of sugars and amino acids allowed seeds to germinate in otherwise inhibitory concentrations of ABA and suggested that ABA inhibits the mobilization of food reserves. However, it cannot be excluded that these sugar effects are mediated by sugar signaling effects (Smeekens, 1998; Gibson and Graham, 1999).

A class of mutants that was directly selected on the basis of reduced dormancy are the *rdo1-rdo4* mutants (Léon-Kloosterziel et al., 1996b; A.J.M. Peeters unpublished results). 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 mutants differ from

each other in their pleiotropic and epistatic effects, indicating that not one single pathway is involved. Recent data suggest that *RDO2*, *RDO3* and probably *RDO1* affect the GA requirement, although in a less severe way than ABA, probably due to redundancy of the function in the dormancy control of the various genes. However, *RDO4* must act on a target different from GA requirement (A.J.M. Peeters unpublished results).

The *dag1-1* mutant 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. Recently, a related gene, named *DAG2,* with a similar expression pattern as *DAG1* was isolated. However, the germination phenotype of *dag2* mutant seeds is opposite to that of *dag1* seeds (Vittorioso et al., 2001) as it shows increased dormancy. Additional mutants with a reduced dormancy phenotype at other loci, including mutants with no obvious pleiotropic effect have been isolated (unpublished results) indicating the complexity of the genetic regulation of seed dormancy.

Mutants with an altered seed coat or testa also show reduced seed dormancy (Debeaujon et al., 2000). 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 (Mohamed-Yasseen et al., 1994; Weber and Wobus, 1999). 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 morphological differentiation of the outer integument, which excretes mucilage upon imbibition (Willats et al., 2001) has been reported by Western et al. (2000) and Windsor et al. (2000).

The seed coat 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 have reduced brown pigments, 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.

The second group is represented by mutants affected in testa structure. The aberrant testa shape (*ats*) mutant that controls the differentiation between inner and outer integuments has only one integument with 3 cell layers instead of 2 with 2 + 3 cell layers (Léon-Kloosterziel et al., 1994). The fact that this mutant has no adult phenotype suggests that its function is restricted to this phase of development.

Mutants that increase seed dormancy or reduce the germination potential of seeds

Many mutants that increase seed dormancy or reduce the germination capacity have mutations in the biosynthesis or signaling pathways of plant hormones that stimulate seed germination such as gibberellins, brassinosteroids and ethylene.

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). 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. 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. This light effect was supported by Yamaguchi et al., (1998), who showed that one of two 3-b hydroxylase enzymes, encoded by the *GA4H* gene is induced in germinating seeds by phytochrome. Cold treatments do not stimulate GA biosynthesis, but rather increase their sensitivity to GAs (Debeaujon and Koornneef, 2000). Two different mechanisms of action have been proposed to explain the role of endogenous GAs in the control of germination. The first one is the induction of genes encoding enzymes that reduce the mechanical resistance to radicle protrusion. This has not been proven for Arabidopsis. The second mechanism consists of a direct effect on the growth

potential of the embryo (Karssen and Laçka, 1986). The latter is assumed to be restricted by the plant hormone ABA, which is produced in the embryo. GA is required to overcome the ABA-induced dormant state. Severe mutations in genes acting early in GA biosynthesis (*GA1*, *GA2* and *GA3*) display a number of GA-rescuable phenotypes. These include failure to germinate, growth of the plant as a dark green dwarf, underdeveloped petals and stamen accompanied by reduced fertility, delayed flowering in short days, reduced apical dominance, and delayed senescence (Koornneef and van der Veen, 1980). A screen aimed specifically at isolating T-DNA tagged mutants that do not germinate was reported by Dubreucq et al., (1996). This screen yielded two mutants in the first 3500 screened T-DNA lines, among which was one *ga1* mutant.

A single dominant mutant with decreased GA signal transduction has been described, *gai1* (GA-insensitive) (Koornneef et al., 1985). *Gai1* has a reduced sensitivity to GA but does not exhibit strongly reduced germination. However, loss of function alleles of this locus require slightly less GA for growth than wild type (Peng et al., 1997). This GA hypersensitive phenotype resembles the *rga* (repressor of *ga1-3*) mutant. The *RGA* gene shares 82% sequence identity with *GAI.* The *GAI* and *RGA* genes belong to the GRAS family and have overlapping but not redundant functions in transcriptional regulation (Sun, 2000) acting as repressors of GA action.

The *sleepy1 (sly1)* mutant was selected in a screen for suppressors of the ABA insensitive mutant *abi1-1*. This mutant strongly resembles the GA auxotrophs described above. However, the lack of germination of *sly1* cannot be rescued by GA and therefore *sly1* is the first GA-insensitive mutant that reflects the full spectrum of GA-associated phenotypes. Therefore, *SLY1* has postulated to be a key factor in GA reception (Steber et al., 1998).

Mutations in the *COMATOSE* (*CTS*) gene also results in a marked reduction in germination potential. Whilst the morphology of *comatose* embryos is not altered, physiological analysis reveals that mature *cts* seeds do not respond to gibberellin. Prolonged chilling of imbibed seeds only partially restores germination potential. It is suggested that *CTS* promotes increased germination potential, represses embryo dormancy and might be involved in GA signaling specific for seeds (Russel et al., 2000).

Brassinosteroids (BRs), a group of over 40 naturally occurring plant steroid hormones found in a wide variety of plant species (Clouse and Sasse, 1998; Schumacher and Chory, 2000) are also involved in the control of germination in Arabidopsis. It is suggested that the BR signal is required to reverse ABA-induced dormancy and to stimulate germination (Steber and McCourt, 2001). BRs could overcome the lack of germination of the *sleepy1* mutant, probably by bypassing its GA requirement. Two BR mutants *DET2* and *BRI1* show reduced germination but eventually germinate without BR, indicating that in contrast to GAs BRs are not absolutely required for germination (Steber, 2001).

Mutants in ethylene signaling 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 mimics the action of gibberellins as applied ethylene allows germination of seeds of the GA-deficient mutant (which normally only germinate in the presence of GA_{4+7}) (Karssen et al., 1989). 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). Ethylene mutants show phenotypes that resemble ABA and sugar signaling mutants. 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 characterized 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) suggested that, in addition to signaling, the effect of *EIN2* might also involve effects on ABA biosynthesis. The presence of cross talk between sugar signaling 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 signaling strongly interact at the level of germination and early seedling growth.

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 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 McCollough 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 behavior of mature seeds. Recently

Munir et al. (2001) showed that photoperiod conditions during seed formation may also influence seed germination. However, this effect was strongly genotype dependent.

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 such as *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 presence of genetic variation for storability is clear in desiccation intolerant genotypes such as *abi3, lec1* and *fus3* mutants (Meinke et al., 1994; Weber and Wobus, 1999) and effects on testa mutants are reported as well (Debeaujon et al., 2000). QTL analysis in the Cvi/Ler RIL lines (Bentsink et al., 2000) 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. Another mutant that affects specifically seed storage compounds, but is without a reported effect on seed germination is *wrinkled1* (*wri1*; Focks and Benning, 1998), which has an 80% reduction in seed oil content. It is suggested that *wri1* is not directly affected in fatty acid triacylglycerol biosynthesis, but controls the conversion of glucose into fatty acid precursors. It can be expected that these mutants have problems in seedling establishment as well.

The quantity of storage material is related to the size of seeds and also for this character considerable variation exist among Arabidopsis accessions (Krannitz et al., 1991), where the larger seeds also produce larger seedlings. This natural variation for seed size was genetically analyzed in the L*er* x Cvi RIL population by Alonso-Blanco et al. (1999) who found that for this trait very large reciprocal differences exist that indicate the presence of a maternal inherited factor in combination with specific paternal effects that might be related to imprinting phenomena. The number of loci that are segregating for seed size in this population is 11, some of which seem to control the number of ovules formed per unit silique length as a pleiotropic effect.

For the mobilization of food reserves during germination and early seedling growth, the activity of lipid and

carbohydrate degrading enzymes such as malate synthase (MLS) and iso-citrate lyase (ICL) is required. These enzymes, which are unique to the glyoxylate cycle, are expressed specifically upon imbibition of oilseeds such as Arabidopsis. Knock-out mutants in these genes (*icl* and *mls*) confirmed that in these mutants the defect is not in germination per sé, but specifically in seedling establishment. The *icl* and *mls* mutant seeds germinate and become photoautotropic when grown on agar plates or on soil in the greenhouse. Therefore, the glyoxylate cycle can be described as being non-essential in Arabidopsis, although it does play an important physiological role because the survival rate of *icl* mutant seedlings declines when they are grown in sub-optimal light conditions (Eastmond and Graham, 2001). The crucial role of GA in mobilization of starch degrading enzymes in the cereal aleuron layer has not been shown in Arabidopsis, where the aleuron layer comprises only one cell layer at maturity. However, the granule structures in this cell layer disappear upon imbibition (Debeaujon and Koornneef, 2000), which also occurs when germination takes place in a GA deficient mutant in which the testa has been removed.

GENES WITH AN EXPRESSION PATTERN CORRELATED WITH DORMANCY AND GERMINATION

In addition to searching for mutants and the subsequent cloning of genes, genes controlling seed dormancy and germination can also be identified on the basis of their expression pattern. However, even abolishment of gene function may not always result in an altered phenotype due to redundancy of other genes.

Various differential screening procedures using subtractive probes have proven successful in identifying tissue-specific genes. These techniques, however, are limited in application in seeds due to their requirement of large amounts of poly(A)$^+$ RNA, and also due to the relatively high expression of seed-storage protein genes which tend to mask less abundant messengers.

Many experiments have shown that during seed maturation the expression of many genes is altered and specific classes of mRNAs such as LEA appear. However, for none of these genes has a specific function in seed dormancy/germination been proven. Although it appears that seed maturation and post-germinative growth have a distinct gene expression profile, some genes that are highly expressed after germination are also expressed during the later stages of seed development (reviewed by Harada, 1997), suggesting that some aspects of post-germinative growth are initiated during maturation. The onset of early germination is also obvious in some of the maturation mutants.

To study genes which are activated during late embryo development and germination a differential display has been performed on immature siliques of the *abi3 fus3* double mutant in comparison with wild-type (Nambara et al., 2000). In addition the same authors identified 16 clones that were activated during wild-type germination of which 11 are de-repressed in the *abi3 fus3* double mutant that apparently have an activated germination process. The genes that were identified represent a variety of metabolic enzymes, regulatory proteins and a number of ribosomal proteins. Cellular processes aiming at growth and activation of protection mechanisms, such as those involved in protection against oxidative stress, and storage compound metabolism are expected to be related to germination. Such proteins were also found in a proteomic analysis of seed germination (Gallardo et al., 2001).

mRNA that are differentially accumulated in dormant versus non-dormant seed have been identified in several species (Dyer, 1993; Johnson et al., 1995). However, here again the essential role of such genes has not been established, and even the expression level of only a surprising low number of genes has been studied into some detail. The peroxiredoxin gene was an orthologue of a barley gene associated with dormancy, but since expression in freshly harvested (dormant) seeds is much higher than in after ripened seeds, it is also in Arabidopsis associated with dormancy (Haslekas et al., 1998).To prove that genes identified by these criteria have essential functions in seed germination, knock out mutants of such genes need to be studied.

Gene or promoter trapping with reporter genes such as GUS may identify genes with a specific expression. Dubreucq et al., (2000) isolated gene traps with an expression during seed germination and identified an insertion close to the *AtEPR1*. This gene encodes an extensin-like protein and is specifically expressed in the endosperm during seed germination, under the control of GAs (Dubreucq et al., 2000). A novel medium-chain acyl-CoA oxidase, which is transcriptionally induced during seed germination has been identified by promoter trapping (Eastmond et al., 2000).

Several novel high throughput techniques for a large scale monitoring of gene expression, such as serial analysis of gene expression (SAGE) and gel-based RNA fingerprinting techniques (cDNA-AFLP) have been added to the available techniques of gene expression analysis. Recent progress in proteome and whole transcriptome analysis using microarrays and DNA chips may provide a

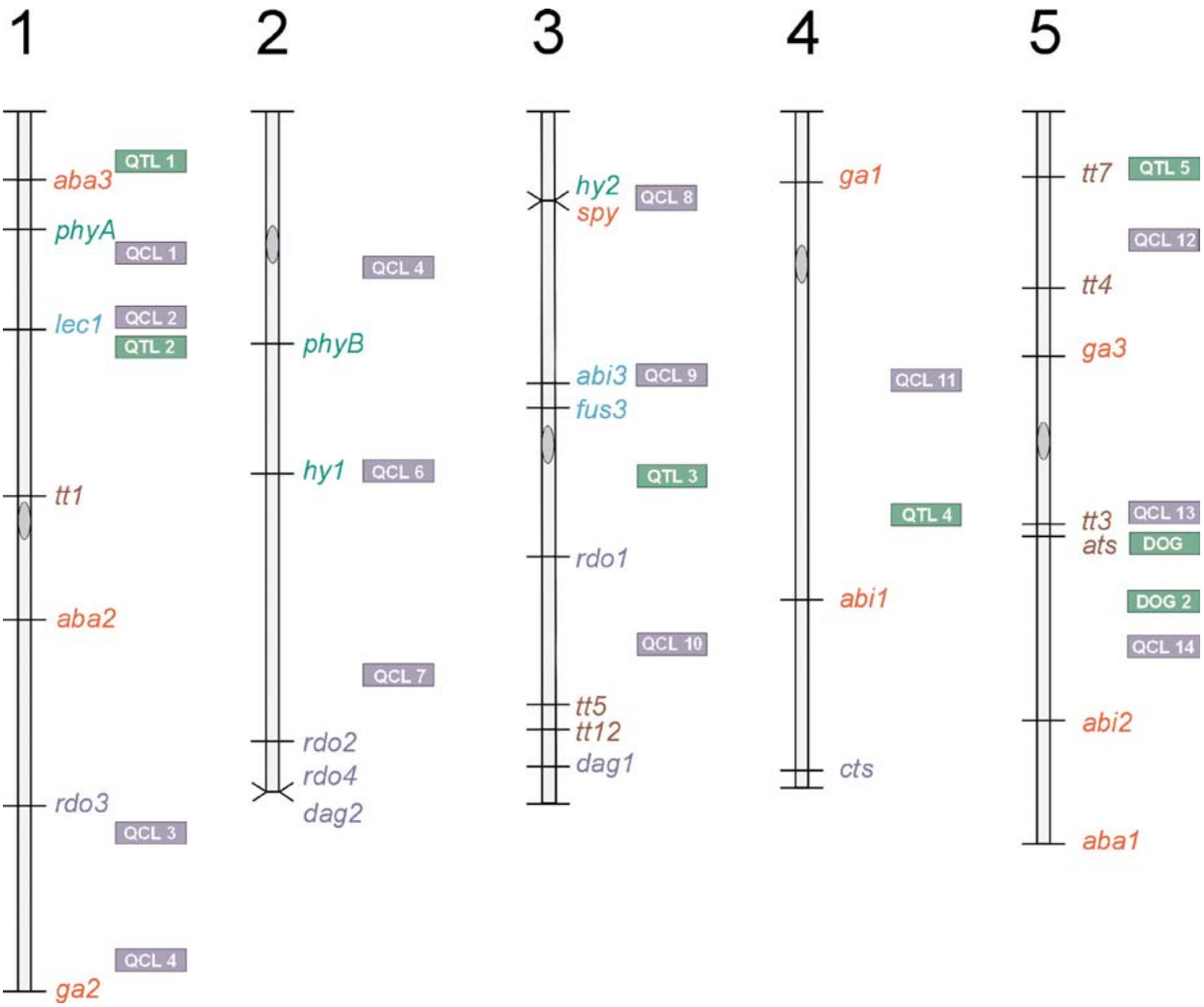

Figure 1. Arabidopsis genetic map showing the mutant loci and polymorphic QTL identified affecting germination/dormancy. Mutants are divided in groups, every group is represented by a different colour. Germination/dormancy affected by hormones (red), testa mutants (brown), mutants affected in light response (green), seed maturation mutants (blue) and miscellaneous dormancy mutants (purple). Purple blocks represent QTL mapped in the Ler/Col RILs (van der Schaar et al., 1997) and the green blocks QTL mapped in the Ler/Cvi RILs (C. Alonso-Blanco and L. Bentsink, unpublished results).

complete picture of genes involved in the seed germination process.

Microarrays containing 2600 seed-expressed genes for developing Arabidopsis seeds were described by Girke et al., (2000) and revealed many genes of unknown function that are highly expressed in seeds.

CONCLUSIONS AND PERSPECTIVES

Dormancy is a very complex trait under the control of a large number of genes. Mutants and the molecular/biochemical function of the respective genes, as so far known, are listed in appendix 1. Seed dormancy and germination deal with structural factors, especially in the structures surrounding the embryo and factors affecting the growth potential of the embryo. The latter may include compounds that are imported from the mother plant and also factors that are produced by the embryo itself, among which are several plant hormones. The genetic analysis of

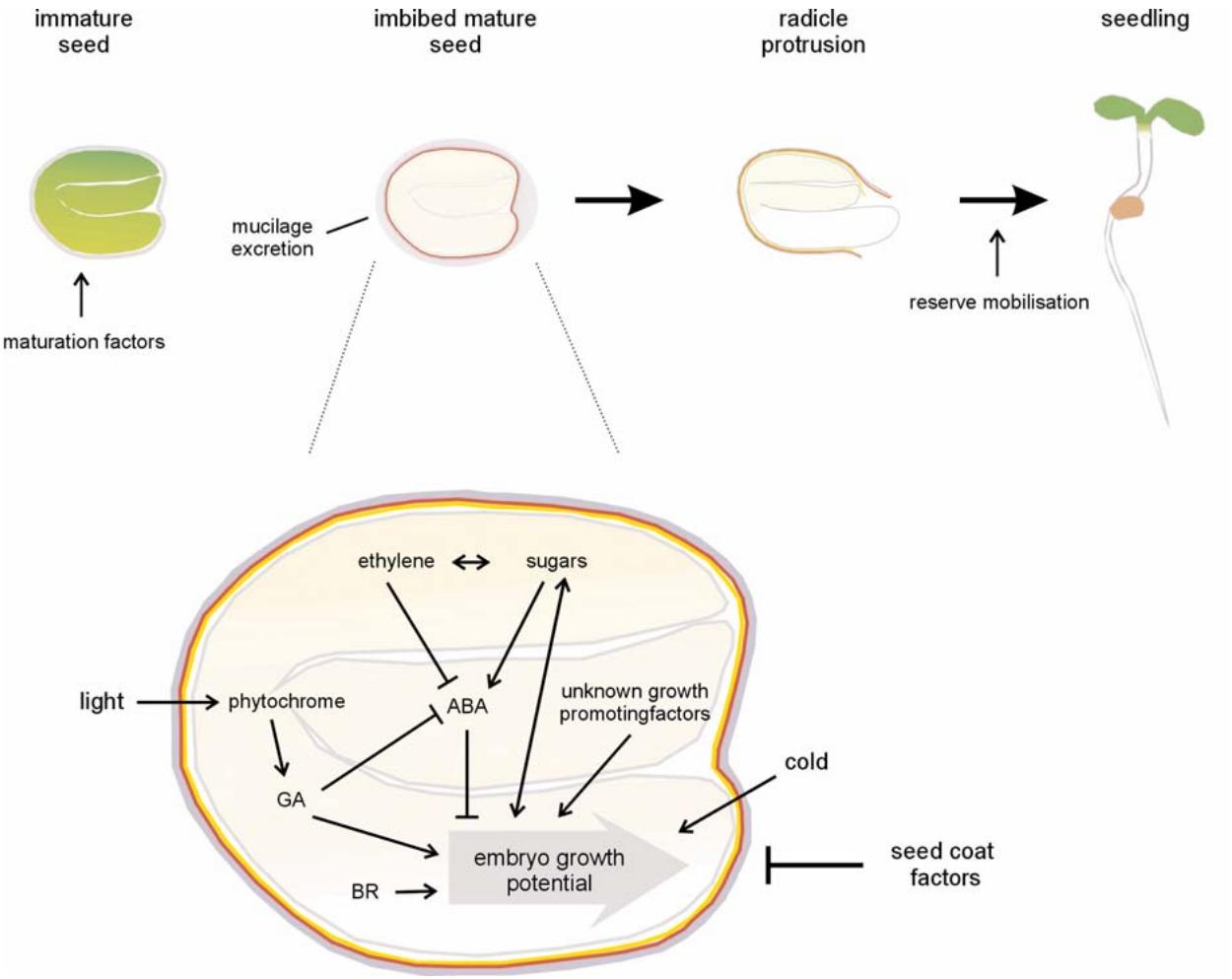

Figure 2. General scheme of the factors controlling germination of Arabidopsis.

Arabidopsis identified the crucial role of ABA in seed dormancy, as well as the requirement of GAs for germination. The latter requirement only exists when ABA was and or is present and when the envelopes surrounding the seed can prevent germination. For GAs the requirement of de novo GA synthesis upon imbibition is convincingly shown and light acting through the phytochrome system controls this step through induction of a seed specific GA 3b hydroxylase. The importance of the mechanical barrier of the maternally inherited testa was shown by the reduced dormancy of a large collection of testa mutants in Arabidopsis. However, if the single layer of endosperm tissue (the aleurone layer) provides a barrier for germination in Arabidopsis, as it does in some species such as tomato and *Datura ferox* (Bewley, 1997), which have a thicker endosperm layer is not known. The nature of other germination inhibiting or promoting factors, of which the existence is shown by mutant and natural variants in the respective genes is not known. With the exception of two DOF transcription factors none of these genes has been cloned. The complexity of the genetic control of seed dormancy and germination is shown by the large number of loci identified already (Figure 1) and which probably reflect only the tip of an iceberg. In what way the genes with unknown functions are downstream targets of ABA and GA or if they affect seed dormancy/germination

in an independent way is currently not known. An important aspect of seed biology is also the storability and stress tolerance of seeds and factors influencing seedling establishment. For both processes genetics only very recently started to be used for their analysis. A general scheme of the factors controlling germination is shown in Figure 2.

The molecular identification of all these genes will be important as well as the identification of more target genes. The latter can and will be done most efficiently by using whole transcriptome and proteome approaches.

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Appendix

Table of mutants which are related to germination/dormancy. Germination phenotype of the mutants compared to wild type is indicated, better germination (+) and reduced or no germination (-). Proteins are divided in the following classes; transcription factor (TF), regulatory protein (RP), enzyme (E), photoreceptor (P) and transporter protein(T).

Seed maturation mutants

Mutant	Gene	Germination phenotype	Encoded protein	Protein class	Reference	Accession number
abi3	*ABI3*	+	B3 domain protein with B1 and B2 domain	TF	Giraudat et al., 1992	AJ002473
fus3	*FUS3*	+	B3 domain protein with B2 domain	TF	Luerssen et al., 1998	AF016264
lec1	*LEC1*	+	HAP3 subunit of CCAAT box binding protein	TF	Lotan et al., 1998	AF036684
lec2	*LEC2*	+	B3 domain transcription factor	TF	Stone et al., 2001	AF400124

Abscisic acid biosynthesis and signaling mutants

Mutant	Gene	Germination phenotype	Encoded protein	Protein class	Reference	Accession number
abi1	*ABI1*	+	serine/threonine phosphatase 2C	RP	Leung et al., 1994; Meyer et al., 1994	X78886
abi2	*ABI2*	+	serine/threonine phosphatase 2C	RP	Leung et al., 1997	Y11840
abi4	*ABI4*	+*	APETELA2 domain protein	TF	Finkelstein et al., 1994; Finkelstein et al., 1998	AF040959
abi5	*ABI5*	+*	basic leucine zipper transcription factor	TF	Finkelstein et al., 1994; Finkelstein and Lynch, 2000	AC006921.5
aba1	*ABA1*	+	zeaxanthin epoxidase	E	Rock and Zeevaart, 1991; Meyer et al., 1994	AF283761
aba2	*ABA2*	+	xanthoxin oxidase	E	Schwartz et al., 1997; Léon-Kloosterziel et al., 1996a	
aba3	*ABA3*	+	molybdenum cofactor sulfurase	E	Schwartz et al., 1997; Xiong et al., 2001	AF325457
era1	*ERA1*	-	farnesyl transferase	RP	Cutler et al., 1996	AF214106

* germination on ABA concentrations that inhibit wild type germination

Gibberellin biosynthesis and signaling mutants

Mutant	Gene	Germination phenotype	Encoding protein	Protein class	Reference	Accession number
ga1	*GA1*	-	copalyl diphosphate synthase (CPS)	E	Koornneef and van der Veen, 1980; Sun et al., 1992	U11034
ga2	*GA2*	-	ent-kaurene synthase (EKS)	E	Koornneef and van der Veen, 1980; Yamaguchi et al., 1998	AF034774
ga3	*GA3*	-	ent-kaurene oxidase	E	Koornneef and van der Veen, 1980; Helliwell et al., 1998	AF047719
spy	*SPY*	+	threonine-O-linked N-acetylglucosine transferase	RP	Jacobsen et al., 1996	U62135
sly1		-	unknown		Steber et al., 1998	

Ethylene and Brassinosteroid mutants

Mutant	Gene	Germination phenotype	Encoding protein	Protein class	Reference	Accession number
crt1	*CTR1*	+	raf family of protein kinases	RP	Kieber et al,1993; Beaudoin et al., 2000	L08790
ein2	*EIN2*	-	bifunctional transducer	RP	Alonso et al., 1999; Beaudoin et al., 2000	AF141202
etr1	*ETR1*	-	ethylene receptor with histidine kinase activity	RP	Bleecker et al., 1988; Chang et al., 1993	L24119
det2	*DET2*	-	steroid 5 -reductase	E	Li et al.,1996; Steber and McCourt, 2001	U53860
bri1	*BRI1*	-	transmembrane receptor kinase	RP	Li and Chory, 1997; Steber and McCourt, 2001	AF017056

Seed coat mutants

Mutant	Gene	Germination phenotype	Encoding protein	Protein class	Reference	Accession number
ats		+	unknown		Léon-Kloosterziel et al., 1994	
tt1		+	unknown		Debeaujon et al., 2000	
tt2	*TT2*	+	R2R3 MYB domain protein	TF	Nesi et al., 2001	AJ299452
tt3	*DFR*	+	dihydroflavonol-4-reductase	E	Shirley et al., 1992	AB033294
tt4	*CHS*	+	chalcone synthase	E	Feinbaum and Ausubel, 1988	AF112086
tt5	*CHI*	+	chalcone isomerase	E	Shirley et al., 1992	M86358
tt6	*F3H*	+	flavonol 3-hydroxylase	E	Wisman et al., 1998; Pelletier and Shirley, 1996	U33932
tt7	*F3'H*	+	flavonol 3'-hydroxylase	E	Schoenbohm et al., 2000	AF155171
tt8	*TT8*	+	basic helix-loop-helix domain protein	TF	Nesi et al., 2000	AJ277509
tt9		+	unknown		Debeaujon et al., 2000	
tt10		+	unknown		Debeaujon et al., 2000	
tt11		+	unknown		Debeaujon et al., 2000	
tt12	*TT12*	+	MATE family protein	T	Debeaujon et al., 2001	AJ294464
tt13		+	unknown		Debeaujon et al., 2000	
tt14		+	unknown		Debeaujon et al., 2000	
tt15		+	unknown		Focks et al., 1999	
ttg1		+	WD40-repeat protein	RP	Walker et al., 1999	AJ133743
ban	*LAR*	+	leucoanthocyanidin reductase	E	Devic et al., 1999	AF092912

Miscellaneous

Mutant	Gene	Germination phenotype	Encoding protein	Protein class	Reference	Accession number
dag1	*DAG1*	+	DOF transcription factor	TF	Papi et al., 2000	AJ224122
dag2	*DAG2*	-	DOF transcription factor	TF	Vittorioso et al., 2001	AJ237810
rdo1		+	unknown		Léon-Kloosterziel et al., 1996a	
rdo2		+	unknown		Léon-Kloosterziel et al., 1996a	
rdo3		+	unknown		A.J.M. Peeters unpublished results	
rdo4		+	unknown		A.J.M. Peeters unpublished results	
cts		-	unknown		Russel et al., 2000	
phyA	*PHYA*		phytochrome A apoprotein	P	Sharrock and Quail, 1989; Shinomura et al., 1996	X17341
phyB	*PHYB*	-	phytochrome B apoprotein	P	Sharrock and Quail, 1989; Shinomura et al., 1996	X17342
hy1	*HY1*	-	ferredoxin-dependent heme oxygenase	E	Cone and Kendrick, 1985; Muramoto et al., 1999	AB021858
hy2	*HY2*	-	phytochromobilin synthase	E	Cone and Kendrick, 1985; Kohchi et al., 2001	AB045112
hot1	*HPS101*	-**	heat shock 101/ClpB protein	RP	Hong and Vierling, 2001	AF218796
icl	*ICL*	-***	iso-citrate lyase	E	Eastmond et al., 2000; Eastmond and Graham, 2001	AB025634
mls	*MLS*	-***	malate synthase	E	Eastmond and Graham, 2001	At5g03860

** reduced germination after high temperature treatment
*** reduced seedling establisment