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Corresponding Author	Family Name	**Hilhorst**
	Particle	
	Given Name	**Henk W. M.**
	Suffix	
	Division	Lab of Plant Physiology
	Organization	Wageningen University
	Address	Droevendaalsesteeg 1, 6708, Wageningen, The Netherlands
	Email	henk.hilhorst@wur.nl
Author	Family Name	**Finch-Savage**
	Particle	
	Given Name	**William E.**
	Suffix	
	Division	Warwick HRI
	Organization	Warwick University
	Address	Wellesbourne, CV35 9EF, Warwick, UK
	Email	bill.finch-savage@warwick.ac.uk
Author	Family Name	**Buitink**
	Particle	
	Given Name	**Julia**
	Suffix	
	Division	UMR 1191 Molecular Seed Physiology
	Organization	INRA
	Address	16 Bd Lavoisier, 49045, Angers, France
	Email	julia.buitink@angers.inra.fr
Author	Family Name	**Bolingue**
	Particle	
	Given Name	**William**
	Suffix	
	Division	UMR 1191 Molecular Seed Physiology
	Organization	INRA
	Address	16 Bd Lavoisier, 49045, Angers, France
	Email	william.bolingue@angers.inra.fr
Author	Family Name	**Leubner-Metzger**
	Particle	
	Given Name	**Gerhard**
	Suffix	

Division	
Organization	Institut fuer Biologie II, Botanik/Pflanzenphysiologie, Albert-Ludwigs-Universitaet Freiburg
Address	Schaenzlestr. 1, D-79104, Freiburg i. Br., Germany
Email	gerhard.leubner@biologie.uni-freiburg.de

Abstract

Seed dormancy has been studied intensely over the past decades and, at present, knowledge of this plant trait is at the forefront of plant biology. The main model species is *Arabidopsis thaliana*, an annual weed, possessing nondeep physiological dormancy. This overview presents the state-of-the-art of seed dormancy research, focusing mainly on physiological and molecular-genetic aspects in this species. It has become clear that, like in many other organisms, the dormancy and stress responses are tightly associated in seeds. The plant hormones, abscisic acid and gibberellins, play a pivotal role in the acquisition of developmental arrest or repression of metabolic inactivity, respectively. Some attention is given to the overlapping dormancy and stress responses, commonly studied in many other organisms but only marginally in seeds.

Chapter 4
Dormancy in Plant Seeds

Henk W.M. Hilhorst, William E. Finch-Savage, Julia Buitink, William Bolingue, and Gerhard Leubner-Metzger

Abstract Seed dormancy has been studied intensely over the past decades and, at present, knowledge of this plant trait is at the forefront of plant biology. The main model species is *Arabidopsis thaliana*, an annual weed, possessing nondeep physiological dormancy. This overview presents the state-of-the-art of seed dormancy research, focusing mainly on physiological and molecular-genetic aspects in this species. It has become clear that, like in many other organisms, the dormancy and stress responses are tightly associated in seeds. The plant hormones, abscisic acid and gibberellins, play a pivotal role in the acquisition of developmental arrest or repression of metabolic inactivity, respectively. Some attention is given to the overlapping dormancy and stress responses, commonly studied in many other organisms but only marginally in seeds.

H.W.M. Hilhorst (✉)
Lab of Plant Physiology, Wageningen University, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands
e-mail: henk.hilhorst@wur.nl

W.E. Finch-Savage
Warwick HRI, Warwick University, Wellesbourne, Warwick CV35 9EF, UK
e-mail: bill.finch-savage@warwick.ac.uk

J. Buitink and W. Bolingue
UMR 1191 Molecular Seed Physiology, INRA, 16 Bd Lavoisier, 49045, Angers, France
e-mails: julia.buitink@angers.inra.fr; william.bolingue@angers.inra.fr

G. Leubner-Metzger
Institut fuer Biologie II, Botanik/Pflanzenphysiologie, Albert-Ludwigs-Universitaet Freiburg, Schaenzlestr. 1, D-79104, Freiburg i. Br., Germany
e-mail: gerhard.leubner@biologie.uni-freiburg.de

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4.1 Introduction

Seeds are the principal propagules of the majority of higher plants. They ensure dispersal of the species in space and time and, by their adaptation potential, make an important contribution to the introduction and survival of species. Seed forms and shapes are highly varied, in line with specific environmental requirements for dispersal and establishment. Most seeds consist of an embryo, surrounded by one or more covering layers. The covering layers usually consist of a living endosperm of one to several cell layers and a testa, which is mostly dead tissue (Fig. 4.1a, b). Most seeds can withstand desiccation to water contents as low as 2–3% and this gives seeds the ability to survive for long periods under adverse conditions.

Seed germination and dormancy represent key ecological and agronomical traits that determine plant establishment in natural or agricultural ecosystems. Seeds are mostly shed from the mother plant in a dry state in which the seed tissues (embryo, covering layers) are preserved at low water content. Seed germination commences with the uptake of water by the dry seed, followed by embryo expansion growth. Germination is completed when the radicle has protruded through the surrounding covering layers. Seed germination depends on the interaction of the seed with the environment, and occurs under favourable conditions with the key environmental factors: water availability, appropriate temperature and in some cases light.

Germination timing is a plant trait with the highest selection pressure by the environment and has, during seed evolution, led to a connected second key trait: seed dormancy. This can be defined as the (temporary) incapacity of a viable imbibed seed to germinate under favourable conditions. Primary dormancy (PD)

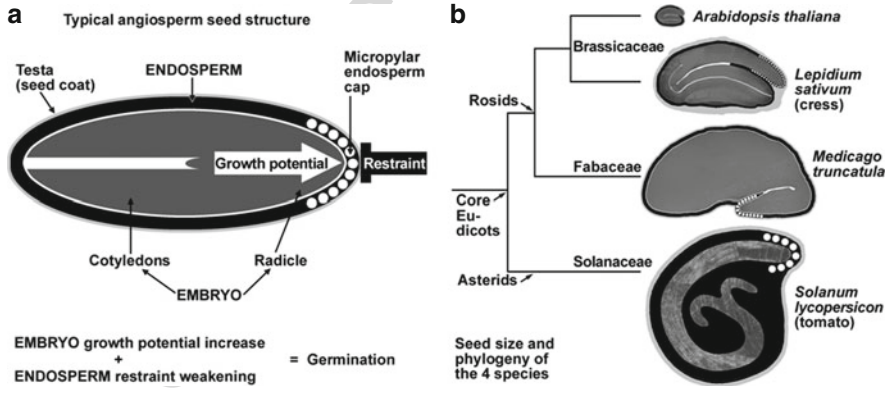

Fig. 4.1 Seed structures, sizes and phylogenetic relationships of model and crop species. (**a**) Generalised structure of an angiosperm (eudicot) seed with EMBRYO and ENDOSPERM as the two important seed components. The direction of embryo growth (*arrow* 'Growth potential') that results in germination (rupture of the endosperm) and repressive function of endosperm (*block* 'restrain') are shown. (**b**) Phylogenetic relationship and seed size comparison for *Arabidopsis*, *Lepidium*, *Medicago*, and tomato. The four species represent important model and crop plants. Figure reproduced with permission from The Seed Biology Place (http://www.seedbiology.de)

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refers to the type of dormancy that occurs prior to dispersal as part of the seed developmental program, whereas secondary dormancy (SD) refers to the acquisition of dormancy in a mature seed after imbibition as a result of the lack of proper conditions for germination (Amen 1968).

Dormancy may be located in the embryo or imposed by the tissues that surround the embryo. In a number of species, both the embryo and the tissues enclosing it impose dormancy. The completion of germination (radicle protrusion) is the net result of the opposing forces: the "thrust" of the embryo and the restraints by the surrounding tissues. In the case of embryo dormancy, the properties of the embryo are of principal importance. In coat-imposed dormancy, the properties of the covering tissues are the determinants, including mechanical, chemical and permeability features, all of which may interfere with the successful completion of germination. For example, many seeds possess a seed coat that poses a mechanical restraint to embryonic growth and that may also contain chemical inhibitors, such as phenolic compounds, that prevent embryo growth (mechanical and chemical dormancy). Endosperm tissue may restrict embryo growth until the thick endosperm cell walls are degraded by hydrolytic enzymes that can be induced by factors (e.g. plant hormones) derived from the embryo (physiological/mechanical dormancy). Both embryo and coat-imposed dormancy are common and there does not seem to be a preference for a specific category or type of dormancy among plant families or genera (Baskin and Baskin 1998). Among the several different types and classes of dormancy, the study of physiological dormancy has received most attention. This class of dormancy is caused by metabolic blocks in the seed and is essentially reversible. This enables the seed (in the soil) to go through several successive cycles of dormancy break and induction until the conditions for germination and seedling establishment are optimal (Hilhorst 2007). Here, we will give an update on the progress in dormancy research of physiological dormancy and mainly in *Arabidopsis thaliana*. For a full account of the other dormancy types, the reader is referred to several excellent reviews (Baskin and Baskin 1998, 2004).

4.1.1 Embryo–Endosperm Interaction as a Mechanistic Model for Germination

The mature seeds of most angiosperm species are endospermic, that is have retained a more or less abundant endosperm layer (Finch-Savage and Leubner-Metzger 2006; Holdsworth et al. 2008a). In typical seeds, the embryo is surrounded by two covering layers ('coats', Fig. 4.1): the endosperm (living cells in most species) and the testa (seed coat, dead cells). On the mechanistic level, successful seed germination/breaking of dormancy depends simply on the net sum between two opposing forces:

- The embryo growth potential (mainly associated with the radicle) must increase to allow radicle extension growth and protrusion of the covering layers (EMBRYO = promotive).

- The restraint of the covering layers (testa, endosperm) must be weakened and weakening of the micropylar endosperm cap covering the radicle is of utmost importance (endosperm cap weakening, ENDOSPERM = repressive).

Radicle extension growth, 'coat' dormancy release and endosperm cap weakening are the key processes of seed germination and dormancy break in most species and share known molecular mechanisms of which several are evolutionary conserved.

4.2 Seed Dormancy Research: An Update

4.2.1 Global Analysis

There have been several recent reviews reporting advances in our understanding of dormancy and the control of germination in seeds resulting from large-scale gene expression profiling at both RNA and protein levels (Finch-Savage and Leubner-Metzger 2006; Bradford and Nnogaki 2007; Holdsworth et al. 2008a, b; Catusse et al. 2008a, b; Finkelstein et al. 2008). It is clear from work on both transcriptome (Ogawa et al. 2003; Nakabayashi et al. 2005; Cao et al. 2006; Cadman et al. 2006; Finch-Savage et al. 2007; Carrera et al. 2007, 2008) and proteome (Gallardo et al. 2001; Rajjou et al. 2004; Job et al. 2005; Chibani et al. 2006; Oracz et al. 2007) that there are extensive changes in genome expression involved in the control of cycling through different levels of dormancy and the final transition to the completion of germination. Holdsworth et al. (2008b) conclude from this work that RNA translation and post-translation are the major levels of control for germination completion and that transcriptome changes reflect more the alteration in dormancy status, enhancement of germination potential and effects on post-germination functions related to seedling growth. However, Nakabayashi et al. (2005) have shown that more than half (>12,000) of all genes in *Arabidopsis* have transcripts present in dry mature seeds. Holdsworth et al. (2008b), therefore, also suggest that changes in the transcriptome following seed imbibition indicate a dynamic relationship between these RNAs 'stored' from late seed development and synthesis of new RNAs related to post-imbibition germinating or dormant seed states. A further dynamic is now also thought to exist through changes in the 'dry state', apparently resulting from transcription and protein metabolism, which are manifested as altered dormancy status upon imbibition. These various levels of control are temporally coordinated from seed maturation through dormancy to germination and this provides the flexibility that is required for seeds to respond to the variable environment that surrounds them (Finch-Savage and Leubner-Metzger 2006; Fig. 4.2). In this way, seeds continually change their dormancy status to optimise the timing of germination completion in tune with seasonal cycles to maximise subsequent plant survival and reproduction.

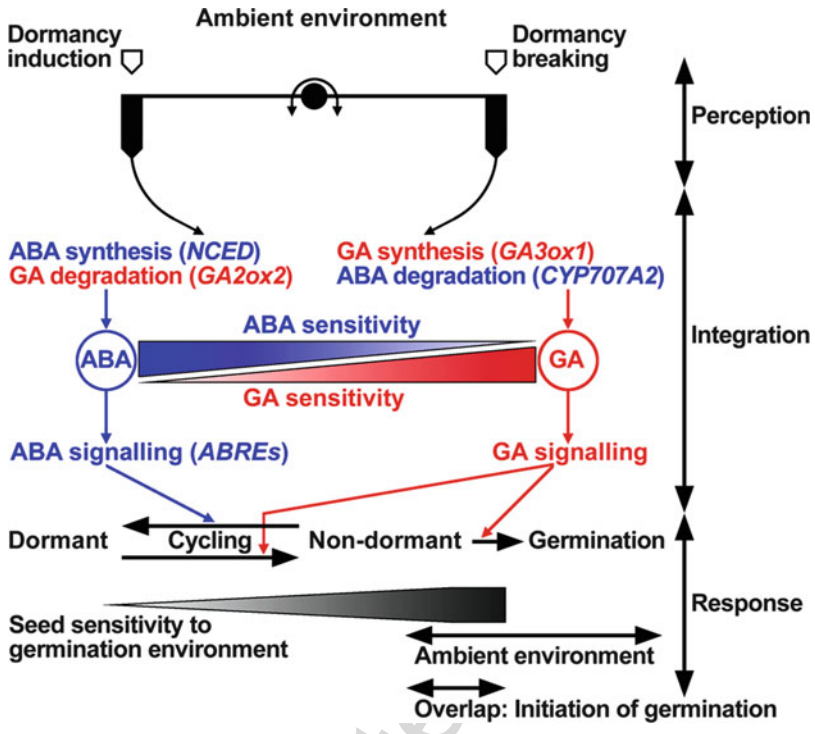

Fig. 4.2 Model for the regulation of dormancy and germination by ABA and GA in response to the environment. According to this model ambient environmental factors (e.g. temperature) affect the ABA/GA balance and the sensitivity to these hormones. ABA synthesis and signalling (GA catabolism) dominates the dormant state, whereas GA synthesis and signalling (ABA catabolism) dominates the transition to germination. The complex interplay between hormone synthesis, degradation and sensitivities in response to ambient environmental conditions can result in dormancy cycling. Change in the depth of dormancy alters the requirements for germination (sensitivity to the germination environment); when these overlap with changing ambient conditions, germination will proceed to completion. Model based on work with *A. thaliana* ecotype Cvi, modified from Cadman et al. (2006). Key target genes are in parenthesis. Figure reproduced from Finch-Savage and Leubner-Metzger (2006) with permission from Elsevier Ltd

4.2.1.1 Similarities and Differences between Physiological States

The analysis of global expression patterns has provided a new opportunity to address some old questions such as: whether seeds imbibed in the dormant state are fundamentally different from those in a non-dormant state; and whether different dormant states such as primary dormancy (PD) and secondary dormancy (SD) are similar. It is clear that specific sets of transcripts have higher abundance in seeds that will complete germination, when compared to seeds that will remain dormant and vice versa (Cadman et al. 2006; Carrera et al. 2007, 2008). From this, characteristic sets of gene transcripts have been assigned to dormant states (D-set) and fully after-ripened states (AR-set). However, principle component analysis has

shown that primary dormant seeds that have had relatively short periods of imbibition (24 and 48 h) group separately from seeds that have been imbibed for longer and have entered a 'maintained' primary or secondary dormant state (Cadman et al. 2006). Thus, on the basis of transcript abundance there is little difference between these maintained states during dormancy cycling. However, it is likely that newly imbibed primary dormant seeds are dominated by transcripts remaining from seed development (stored) and thus differ from maintained states. Most published work on dormancy has centred on the former and this may have produced some misconceptions about the dormant state.

In complementary work, transcriptomes were compared from *Arabidopsis* seeds of the deeply dormant Cvi accession, exposed to different dormancy releasing factors (after-ripening, cold, nitrate, light; Finch-Savage et al. 2007). To complete germination, these seeds require more than one of these factors and thus exposure to only one factor or an incorrect combination of factors will result in different depths of dormancy. Principal component analyses of the expression patterns observed grouped physiological states in a way that related to this depth of seed dormancy, rather than the type of environmental exposure (Finch-Savage et al. 2007). This suggests similarity in the response to different environments. Furthermore, opposite changes in transcript abundance of genes in D- and AR-sets were also related to the depth of dormancy and common to different environments. Thus, transcription of these gene sets responds in a quantitative way to specific environmental signals when they are presented to the seeds in the order appropriate to relieve dormancy and facilitate the completion of germination in seasonal conditions that are suitable to sustain subsequent growth.

In addition to these common quantitative changes, environment-specific gene expression patterns during dormancy relief were also found. For example, higher transcript abundance for genes linked to the process of nitrate accumulation and reduction was associated with dormancy relief. Further patterns were consistent with a role for the balance of the plant hormones abscisic acid (ABA) and gibberellins (GAs) in integrating dormancy-relieving environmental signals, which is discussed further below.

4.2.1.2 Genes Associated with Different States

Work at the level of gene expression has confirmed that dormancy is an active state, with complex regulatory networks continuously integrating environmental signals and responding to them by positive maintenance of dormancy through de novo ABA synthesis and/or negative regulation of germination (Fig. 4.1; Cadman et al. 2006; Carrera et al. 2007, 2008; Finch-Savage et al. 2007). Cadman et al. (2006) show that changes in dormancy status are consistent with differential expression of large numbers of transcription factors present in the D- and AR-sets, along with genes encoding histones, which are suggestive of a complete switch in gene expression, resulting from a change in chromatin structure. Genes in the D-set are associated with embryo maturation including storage proteins, heat shock proteins

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and dehydrins etc. It was also found that ABA-, stress- and dormancy responses significantly overlap at the transcriptome level (Cadman et al. 2006; Finch-Savage et al. 2007). Many of the genes more highly expressed in the dormant states appeared to be related to stress. This may be linked to the synthesis of ABA, which appears essential to the maintenance of dormancy. Under prolonged conditions that are non-permissive to germination, there is an increase of ABA content (Ali-Rachedi et al. 2004). These conditions will, therefore, always lead to co-expression of dormant- and stress-related genes that are controlled by ABA. There is an evolutionary advantage in this co-expression, since dormancy is a mechanism to survive prolonged periods of environmental stress that are unfavourable for growth. In contrast, many genes of the AR-set are associated with the establishment of translation machinery, the potential for cell-wall remodelling and reserve mobilisation in advance of germination completion. The genes represented in this set appear also to, at least partly, anticipate the next likely stage of development, that is radicle extension and subsequent seedling growth.

Results from Rajjou et al. (2004) have shown that chemical inhibition of transcription in non-dormant *Arabidopsis* seeds did not affect the eventual completion of germination, but inhibited further growth of the seedling after radicle protrusion. In contrast, translation inhibitors effectively blocked the completion of germination. This suggests that the transcripts for the completion of germination of non-dormant seeds are pre-formed during their development (stored transcripts), and then translated to enable progress of germination all the way to completion. Thus, transcription is not essential for the completion of germination in these previously unimbibed non-dormant seeds. However, the work of Cadman et al. (2006) shows that dormancy is characterised by an absence of transcripts related to establishing translational machinery, whereas dormancy release is accompanied by transcription of genes associated with the completion of germination (AR-set) including those encoding for proteins involved in translation machinery. They, therefore, hypothesise that an important molecular event in release from the maintained dormant state, when stored transcripts may no longer be available, is establishing the capacity for the translational control of germination completion.

4.2.1.3 The Hormone Balance and Regulation of Dormancy

A dynamic balance of hormone synthesis and catabolism operates that establishes a controlling balance of ABA–GA ratio (Ali-Rachedi et al. 2004; Cadman et al. 2006). This intrinsic balance directs signalling pathways that regulate dormancy level by altering the seeds sensitivity to the ambient germination environment (Fig. 4.2). While the release of primary dormancy in Cvi seeds occurs effectively by after-ripening, stratification or inhibition of ABA biosynthesis, the addition of GA appears less effective and can cause a transient increase in ABA levels (Ali-Rachedi et al. 2004; Finch-Savage et al. 2007). This suggests that in dormant seeds a feedback mechanism exists that maintains a high ABA–GA ratio. However, dormancy release also involves a net shift to increased GA biosynthesis and ABA

degradation resulting in a low ABA–GA ratio (Ali-Rachedi et al. 2004; Cadman et al. 2006).

D-set genes had an over-representation of ABA-responsive elements (ABRE) in their promoters, and of genes for transcription factors that bind to the ABRE (Cadman et al. 2006). Such an over-representation of ABRE-containing genes is also evident in stored mRNAs of dry *A. thaliana* seeds (Nakabayashi et al. 2005). ABRE-binding transcription factors appear to be master regulators that mediate ABA responses in seeds including the regulation of dormancy. On the other hand, during imbibition of non-dormant seeds, there are many GA-responsive genes induced, but GA also causes down-regulation of many ABRE-containing genes (Yamaguchi and Kamiya 2002; Ogawa et al. 2003; Yamauchi et al. 2004).

4.2.1.4 Exposure to Dormancy Releasing Environmental Factors

The timing, extent and pattern of seed germination and subsequent seedling emergence within a seed population are determined by a complex interaction of ambient weather conditions, soil, and seed characteristics (Finch-Savage and Leubner-Metzger 2006). The key weather/soil factors for germination and dormancy are:

- Water availability
- Temperature
- Light
- Abiotic stresses

In crops, the rate and extent of seed germination is key to successful seedling establishment, which in turn is the cornerstone of sustainable and profitable crop production. Transitions from the primary dormant to the non-dormant state and from the non-dormant state to germination or the secondary dormant state depend on the ambient environment, which determines both rate and extent of the response. This interactive process can be very complex, but population-based threshold models provide a universal approach to quantifying the array of ecophysiological responses exhibited by seeds (Finch-Savage and Leubner-Metzger 2006). The models use biological time in which the process of germination progresses to completion at different rates according to the ambient conditions. The quantitative effects of temperature (thermal time), water availability (hydrotime), and the combination of both (hydrothermal time), as well as seed after-ripening, dormancy or any abiotic stress can be described by these models. They can be used to simulate and predict the impact of environment on seed germination in field soils.

In fully AR seeds that require only light to germinate and those exposed to light, the transcript expression of AtGA3ox2 increases dramatically (Yamaguchi et al. 1998; Cadman et al. 2006; Finch-Savage et al. 2007) presumably facilitating the final step of the biosynthesis of biologically active GA. Cold release of dormancy is also mediated, at least in part, by promoting GA biosynthesis via enhanced expression of AtGA3ox (Yamaguchi and Kamiya 2002; Oh et al. 2004; Yamauchi et al. 2004; Liu et al. 2005a, b; Penfield et al. 2005) and by promoting ABA catabolism AU2

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via activity of the flowering gene *FLC (FLOWERING LOCUS C*; Chiang et al. 2009).

Dormancy can also be released by AR at rates that are determined by moisture and oil content, seed-covering structures and temperature (e.g. Manz et al. 2005). Bove et al. (2005) provide evidence that Nicotiana seed AR generates a developmental switch at the transcript level that is evident upon imbibition and this is supported by work with *Arabidopsis* Cvi (Cadman et al. 2006). In part, this may result from gene expression in air-dry seeds during after-ripening (Bove et al. 2005; Leubner-Metzger 2005). Carrera et al. (2008) studied changes in gene expression in imbibed non-dormant mutants (aba1 and abi1) and compared them to wild-type seeds with and without AR. This indicated that AR acts as a developmental pathway that can be separated from dormancy of the imbibed seed. This work also showed that exogenous application of ABA did not re-impose the gene expression of seeds that had not been after-ripened, and that seeds of the non-dormant mutants demonstrated changes in genome expression during dry storage that were characteristic of AR. This provided a clear demonstration that ABA is not a major regulator of AR in dry seed (Holdsworth et al. 2008a).

It is also clear that exogenous application of ABA to seeds does not result in seed phenotypes that mimic dormancy at the proteome (Chibani et al. 2006) or transcriptome levels (Carrera et al. 2008). Despite this, in the studies so far carried out where samples are comparable there is little correlation between observations at the transcriptome and proteome levels, for example following imbibition of AR seeds (Cadman et al. 2006 and Chibani et al. 2006 respectively). To date the reason for this is open to speculation and more work is required. However, a proteome study of dormancy relief by AR in sunflower by Oracz et al. (2007) has highlighted the potential importance of reactive oxygen species (ROS) in this process. From this work, they raise the hypothesis that dormancy release involves a change in proteome oxidation, resulting from the accumulation of ROS during AR. ROS accumulation, therefore, appears to be a key signal governing cell activity during AR (Oracz et al. 2007). They suggest that this mechanism may also have relevance for dormancy breaking in the imbibed state.

4.2.1.5 Different Seed Tissues and Sensitivities

Global expression analyses are consistent with the induction and maintenance of the dormant state being characterised by increased ABA biosynthesis and GA degradation and the reverse during dormancy release. In seeds of different species these changes in the two hormones may occur at the same time or at different times and at different sites within the seed. However, the emerging picture is incomplete without considering the influence of the seed coat and the antagonism of different tissues (embryo, endosperm) within the seed and hormone sensitivities. The sensitivities for GA and ABA, their perception by receptors, their interconnected signalling chains, and their developmental regulation are of utmost importance for germination and dormancy (Kucera et al. 2005). Thus, dormancy loss in many

seeds is also characterised by a decrease in ABA sensitivity and an increase in GA sensitivity (e.g. Le Page-Degivry et al. 1996; Corbineau et al. 2002; Koornneef et al. 2002; Leubner-Metzger 2002; Ali-Rachedi et al. 2004; Chiwocha et al. 2005).

In endospermic seeds, the endosperm acts as a mechanical barrier to germination and is, therefore, intimately involved in dormancy mechanisms (Kucera et al. 2005; Finch-Savage and Leubner-Metzger 2006). Again the emergence of the radicle through the endosperm is regulated via the ratio of ABA–GA, which controls weakening of the micropylar endosperm in many species (reviewed by Finch-Savage and Leubner-Metzger 2006). Recent evidence suggests the endosperm may be the primary determinant of seed dormancy in *Arabidopsis* (Bethke et al. 2007). It is anticipated that future genome wide expression studies will have emphasis on the separate analysis of seed tissues (Holdsworth et al. 2008b). Indeed, differential global gene expression patterns have already been demonstrated between different seed tissues of *Arabidopsis* at the level of the transcriptome (Penfield et al. 2006), and of sugar beet at the proteome level (Catusse et al. 2008a, b).

4.2.2 Specific Analyses: Key Genes and Processes Related to the Hormonal Regulation of Dormancy, After-Ripening and Germination

The previous sections provide a global overview and introduced the general concept of the antagonistic hormonal interactions like GA–ABA and the importance of the seed tissues for dormancy, after-ripening and germination. The following parts present specific examples for key genes and processes in seeds that are exemplary. They are of course not exclusive, and other important case studies are summarised in several recent reviews (Kucera et al. 2005; Finch-Savage and Leubner-Metzger 2006; Bentsink and Koornneef 2008; Finkelstein et al. 2008; Holdsworth et al. 2008a).

4.2.2.1 ABA: A Positive Regulator of Dormancy Induction and Maintenance, and a Negative Regulator of Germination

In many plant species, endogenous ABA is involved in the induction and perhaps in the maintenance of the dormant state (reviews: Hilhorst 1995; Kucera et al. 2005; Holdsworth et al. 2008a). Mutants with reduced seed ABA biosynthesis exhibit reduced dormancy. Over-expression of genes for ABA biosynthesis can increase seed ABA content and enhance seed dormancy or delay germination (e.g. Grappin et al. 2000; Nambara and Marion-Poll 2003). Enhanced dormancy is also evident in *Arabidopsis cyp707a2* mutants with increased ABA content due to a block of seed ABA catabolism (ABA 8′ hydroxylase, Kushiro et al. 2004; Müller et al. 2006).

Several of the *Arabidopsis* ABA-insensitive (*abi*) response mutants, *abi1* to *abi5* and *abi8*, exhibit, like the ABA-deficient mutants, a marked reduction in seed dormancy (Kucera et al. 2005; Finkelstein et al. 2008; Holdsworth et al. 2008a). The seed responses of strong alleles of the *Arabidopsis ABI3* gene are severe compared to the *abi1*, *abi2* and the ABA-deficient mutant alleles. ABI3 may play a major role in seed and bud dormancy (Rohde et al. 2000; Bassel et al. 2006). The ABA-insensitive *viviparous1* (*vp1*) mutant of maize is characterised by severe seed responses, including reduced sensitivity of germination to exogenous ABA and vivipary. The *Arabidopsis ABI3* and the maize *VP1* are orthologous genes that encode transcription factors of the B3 domain class that are essential for ABA action. VP1/ABI3-like proteins are multifunctional transcription factors that integrate ABA and other regulatory signals of seed maturation and developmental arrest. Post-translational targeting of ABI3 for protein degradation and perhaps also farnesylation of ABI3 are mechanisms to regulate ABI3-mediated ABA signalling (Finkelstein et al. 2008). The interaction of ABI3 with other factors in the network that establishes seed dormancy during seed maturation is summarised by Holdsworth et al. (2008a).

ABA is not only a positive regulator of dormancy induction; it also inhibits seed germination and has been proposed to be a positive regulator of dormancy maintenance. ABA inhibits embryo growth potential and endosperm cap weakening during coffee seed germination (da Silva et al. 2004). A transient rise in ABA content in the embryo was evident early during imbibition. ABA treatment inhibits and fluridone treatment accelerates radicle protrusion of coffee seeds. Vegetation-derived ABA is also of ecological importance in the regulation of seed dormancy and germination. ABA leached from plant litter plays an important role in the germination control of the post-fire annual Nicotiana attenuata (Krock et al. 2002; Schwachtje and Baldwin 2004).

Rupture of the testa and the endosperm are distinct and temporally separate events during the germination of many species; such two-step germination with testa rupture subsequently followed by endosperm rupture, is known for Nicotiana spp. (Solanaceae, e.g. Leubner-Metzger 2003), *Lepidium sativum* (cress) and *A. thaliana* (Liu et al. 2005a, b; Müller et al. 2006; Piskurewicz et al. 2008). Addition of ABA to the medium during imbibition resembles the effects of maternal ABA during seed development and residual ABA in mature seeds. In after-ripened seeds, this does not appreciably affect the kinetics of testa rupture, but it delays endosperm rupture and results in the formation of a novel structure, consisting of the enlarged radicle with a sheath of greatly elongated endosperm tissue (Leubner-Metzger and Meins 2000; Leubner-Metzger 2003).

4.2.2.2 Gibberellins Release Coat Dormancy, Promote Germination and Counteract ABA Effects

According to the revised hormone-balance hypothesis for seed dormancy proposed by Karssen and Laçka (1986), ABA and GA act at different times and sites during

the 'seed life'. ABA induces dormancy during maturation, and GAs play a key role in dormancy release and in the promotion of germination. GA biosynthesis in developing seeds of many species leads to the accumulation and storage of either bioinactive GA precursors or bioactive GA (Yamaguchi and Kamiya 2002; Kucera et al. 2005). GA biosynthesis in developing seeds appears not to be involved in the establishment of primary dormancy per se, but in other aspects of seed development, including fertilisation, embryo growth, assimilate uptake, fruit growth, and the prevention of seed abortion.

The temporal and spatial expression pattern of GA biosynthesis genes has been investigated during *Arabidopsis* seed germination (Yamaguchi et al. 2001; Ogawa et al. 2003; Yamauchi et al. 2004). Bioactive GAs accumulate just prior to radicle protrusion and appear to occur in two separate locations within the embryo: (1) the early biosynthetic pathway, including the geranylgeranyl diphosphate cyclisation reaction catalysed by ent-copalyl diphosphate synthetase (CPS), in the provascular tissue where *AtCPS1* gene promoter activity is localised, and (2) the late biosynthetic pathway, including the formation of bioactive GA by GA 3-oxidase, in the cortex and endodermis of the root where *AtGA3ox1* and *AtGA3ox2* transcripts accumulate and *AtGA3ox2* gene promoter activity is localised. This implies that intercellular transport of an intermediate of the GA biosynthetic pathway (probably ent-kaurene) is required to produce bioactive GA. Two functions for GA during seed germination have been proposed (reviews: Hilhorst 1995; Bewley 1997a, b; Kucera et al. 2005; Finch-Savage and Leubner-Metzger 2006). First, GA increases the growth potential of the embryo. Second, GA is necessary to overcome the mechanical restraint conferred by the seed-covering layers by weakening of the tissues surrounding the radicle. The localisation of seed GA biosynthesis in the *Arabidopsis* radicle (Yamaguchi et al. 2001) is consistent with the hypothesis that embryonic GA is released and triggers the weakening of seed-covering layers. This is further supported by the finding that at least some GA responsive genes are expressed in non-GA-producing seed tissues (Ogawa et al. 2003). Environmental cues like light and temperature can alter the tissue-specific localisation of GA biosynthesis (Yamauchi et al. 2004). The temporal and spatial pattern of GA biosynthesis and sensitivity are both important for the GA-mediated seed responses. Seed germination of GA-deficient biosynthesis mutants of *Arabidopsis* (e.g. ga1) and tomato (e.g. gib-1) absolutely depends on the addition of GA to the medium during imbibition (Hilhorst 1995; Kucera et al. 2005). The mechanisms imposing a GA requirement to promote the germination of dormant and non-dormant *Arabidopsis* seeds have been analysed using the GA-deficient mutant ga1 and the ABA-deficient mutant aba1, and is described in Sect. 2.2.5.

Among the GA-response mutants of *Arabidopsis*, some of the GA-insensitive DELLA repressor mutants, including *gai* (GA-insensitive), *rga* (repressor-of-ga1-3), *rgl1* (*rga-like1*), *rgl2* and *rgl3*, have been investigated in detail (e.g. Richards et al. 2001; Kucera et al. 2005; Achard et al. 2008; Piskurewicz et al. 2008). GA signalling causes proteasome-mediated degradation of these repressor proteins which is the mechanism by which many GA responses are mediated. The gain-of-function mutants in these DELLA repressor mutants are characterised

by dominant GA-insensitive repression of GA responses leading to a dwarf phenotype, increased GA content and complex seed effects that are consistent with a severely decreased GA-sensitivity of dormancy release and germination. It has been proposed that *RGL1* plays a greater role in seed germination than do *GAI* and *RGA* (Wen and Chang 2002), but *RGL2* has been proposed to be the most important regulator of *Arabidopsis* seed germination in response to GA (Lee et al. 2002; Tyler et al. 2004; Cao et al. 2005). However, two detailed studies demonstrate that the involvement of DELLA repressor degradation in seed germination is complex: A careful time-course analysis of *Arabidopsis* seed germination showed that the *RGL2* mRNA decline occurred after radicle emergence, that is after germination had been completed (Bassel et al. 2004). The work of Piskurewicz et al. (2008) shows by a combination of time course analyses of testa rupture and endosperm rupture, transcript and protein analyses, that RGL2 inhibits *Arabidopsis* seed germination by stimulating ABA synthesis and ABI5 activity. These results support the notion that ABI5 acts as the final common repressor of germination in response to changes in ABA and GA levels.

4.2.2.3 Identification of Dormancy-Specific Genes and Other Key Genes that Control Germination Timing

While a major role for ABA in the establishment and maintenance of seed dormancy is evident, hardly anything is known about its downstream targets and the molecular mechanisms of the induction of dormancy and the release by temperature and after-ripening. Due to the overall importance of ABA in plant development, the ABA-related mutants exhibit pleiotropic phenotypes and are, therefore, not seed- or dormancy-specific. ABA-independent pathways and genes specific for seed dormancy are evident from the *Arabidopsis rdo* (reduced dormancy) and *dog* (delay of germination) mutants (Bentsink and Koornneef 2008; Holdsworth et al. 2008a). Besides a mild pleiotropic phenotype, the *rdo* mutants are ABA-independent, have a strong effect on dormancy, and *rdo2* and *rdo4* mutant seeds are thermoinhibition resistant (Peeters et al. 2002; Tamura et al. 2006). The *RDO4* (*REDUCED DORMANCY4*) = *HUB1* (*HISTONE MONOUBIQUITINATION1*) gene encodes a RING finger protein necessary for monoubiquitination of histone H2B (Liu et al. 2007). The importance of the peroxisome has been highlighted by the observation that the ABC transporter COMATOSE (CTS) controls germination (Carrera et al. 2007; Holdsworth et al. 2008a).

A very promising and successful approach to find specific genes involved in *Arabidopsis* seed dormancy is based on natural genetic variation, as it exists between the ecotype Ler (low dormancy) and the deeply dormant ecotype Cvi (Alonso-Blanco et al. 2003; Koornneef et al. 2004; Bentsink et al. 2006). The substantial influence of environmental effects on the expression of germination characteristics and the involvement of many genes make dormancy a typical quantitative trait. Such traits are becoming more amenable to genetic analysis, because the position of individual quantitative trait loci (QTL) and the relative

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contribution of these loci can now be determined. QTL analysis for seed dormancy requires permanent mapping populations, such as recombinant inbred lines (RILs), because these allow the testing of a large number of genetically identical seeds, that is seeds from the same RIL, in different environmental conditions. Seven dormancy QTLs, *DOG1* to *DOG7*, have been identified by Alonso-Blanco et al. (2003) and several more by Laserna et al. (2008). Cvi alleles at six loci (*DOG1, DOG3–DOG7*) increased dormancy, while Cvi alleles at *DOG2* decreased dormancy, compared to Ler alleles. The cloning of such a dormancy QTL has yet been published only for the case of *DOG1* (Bentsink et al. 2006). *A. thaliana DOG1*, for which the Cvi allele increases the level of seed dormancy, explains 12% of the variance observed in seed dormancy. The *dog1* mutant lacks dormancy, but it does not show any obvious pleiotropic effects and is, therefore, a dormancy-specific mutant. The positional cloning of this major seed dormancy QTL *DOG1* has been reported by Bentsink et al. (2006). With the isolation of *DOG1*, the first seed dormancy gene accounting for genetic variation in natural populations has been identified at the molecular level. The *DOG1* gene encodes a novel protein of unknown mode of action, but it is absolutely required for *Arabidopsis* seed dormancy. *DOG1* transcripts are expressed during seed development, are present in dry fresh (dormant; higher *DOG1* mRNA content) and dry after-ripened (non-dormant; lower *DOG1* mRNA content) seeds, and disappear upon imbibition of fresh and after-ripened seeds. A recent transcriptome analysis with *Arabidopsis* Cvi seeds demonstrated that *DOG1* transcript expression is regulated in a complex manner during dormancy induction and release (Finch-Savage et al. 2007). *DOG1* is not specifically involved in ABA signal transduction; the *dog1* mutant has a normal sensitivity to applied ABA. DOG1 function is, however, clearly related to ABA, it might affect dry seed ABA levels (Bentsink et al. 2006). The *DOG1* Cvi allele is induced by the ABA-mediated sugar signalling pathway, and enhances sugar sensitivity by stimulating *ABI4* expression (Teng et al. 2008).

4.2.2.4 Control of Germination by the Seed Coat: Testa Mutant Studies

Embryo and coat (testa and/or endosperm) dormancy are the components of physiological dormancy, their sum and interaction determine the degree of 'whole-seed' dormancy (Kucera et al. 2005; Finch-Savage and Leubner-Metzger 2006; Bentsink and Koornneef 2008; Holdsworth et al. 2008a). Embryo dormancy is characterised by an intrinsic block within the embryo itself that inhibits extension growth, and therefore excised embryos do not grow. Coat dormancy is characterised by a block to germination that is conferred to the seed by the covering layers ('coats'). 'Coat' is used in a loose sense and can be any embryo-covering structure, for example testa and/or endosperm. Based on this definition, the physiological seed dormancy of *A. thaliana* is due to coat dormancy: testa (Debeaujon and Koornneef 2000) and endosperm (Bethke et al. 2007) confer a (mechanical, chemical, etc.) resistance, which in the dormant state prevents embryo growth. In physiologically dormant seeds the embryo-covering layers can confer mechanical

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constraint (coat dormancy) that must be overcome by the growth potential of the embryo (Finch-Savage and Leubner-Metzger 2006; Bentsink and Koornneef 2008). For dead seed covering layers, for example the testa, pre-determined breaking points may facilitate tissue rips prior to germination. Enzymes that facilitate testa rupture might be released by the endosperm and/or the radicle. The testa is a maternal tissue and the reduced seed dormancy phenotype is inherited maternally. A series of *A. thaliana* testa mutants show reduced dormancy that is caused by alterations of the testa characteristics (Debeaujon and Koornneef 2000; Koornneef et al. 2002; Rajjou et al. 2004) and highlight the importance of the testa structure as a constraint to radicle emergence. The GA requirement for *A. thaliana* seed germination is determined by testa characteristics, embryonic growth potential and by embryonic ABA.

4.2.2.5 Control of Germination by the Endosperm: Endosperm Dormancy and Endosperm Weakening

Endosperm dormancy requires that the restraint of the embryo-covering layers must be overcome by the growth potential of the embryo (Finch-Savage and Leubner-Metzger 2006; Holdsworth et al. 2008a). Since the endosperm in many species is a living tissue, seed-covering weakening occurs prior to germination and the tissue itself can produce enzymes for this process. The work of Bethke et al. (2007) demonstrates the importance of the endosperm for *Arabidopsis* seed dormancy: when the testas of dormant seeds were removed, the endosperm prevented the germination upon imbibition. Treatments, known to release *Arabidopsis* seed dormancy, induced endosperm rupture and radicle emergence of these 'testa-less' seeds. Excised *Arabidopsis* embryos, even from seeds of the deeply dormant accessions Cvi and C24 (Bethke et al. 2007) or from GA-deficient or-insensitive mutants (e.g. Iuchi et al. 2007), have coat-dormancy; their excised embryos grow and exhibit at least the initial extension growth required for germination. Thus, based on current knowledge, the testa and the endosperm are both major determinants conferring coat dormancy to *Arabidopsis* seeds; the excised embryos grow, but may exhibit reduced growth potential. The contributions of the different tissues to the degree of the 'whole-seed' dormancy are a matter of controversial debate. The small size of *Arabidopsis* seeds is a disadvantage for directly quantifying these tissue-specific processes in order to calculate the degree of the 'whole-seed' dormancy. It is not precisely known if 'dormancy genes' affect only the embryo, only the endosperm, only the testa, or any combination of the three seed components.

The endosperm acts as a mechanical barrier to the germination of seeds in several angiosperm clades (Finch-Savage and Leubner-Metzger 2006). A decline in this mechanical resistance of the micropylar endosperm (the endosperm layer covering the radicle tip) appears to be a prerequisite for radicle protrusion during seed germination. This endosperm weakening can be promoted by GA and, at least in part, inhibited by ABA. Solanaceae species like tomato, tobacco, pepper and Datura have become model species for endosperm weakening.

Direct biomechanical measurement of endosperm weakening by puncture-force experiments with coffee and tomato seeds, have shown that endosperm weakening is biphasic with regard to the ABA inhibition (Finch-Savage and Leubner-Metzger 2006). The first phase is ABA-insensitive and this is followed by the second phase that is inhibited by ABA (Toorop et al. 2000; da Silva et al. 2004). In coffee seeds ABA controls germination by inhibiting both the embryo growth potential and the second step of endosperm weakening (da Silva et al. 2004). Coffee (Rubiaceae) and tomato (Solanaceae) belong to the Asterid clade of angiosperms. Endosperm weakening appears to be a widespread phenomenon and has also been demonstrated for the Rosid clade of angiosperms: in Brassicaceae seeds the endosperm is also a constraint to germination (Müller et al. 2006). In this work, seeds of both *A. thaliana* and its' much larger-seeded relative *L. sativum* (garden cress) were studied. Both species belong to the subclade I of the Brassicaceae and are highly similar in seed structure and physiology. Testa rupture and endosperm rupture are separate events and only the latter is inhibited by ABA in after-ripened seeds of both species. Direct biomechanical measurement of the puncture force required to rupture the endosperm showed that the *L. sativum* micropylar endosperm weakened prior to radicle emergence (Müller et al. 2006). ABA delayed the onset and inhibited the rate of endosperm weakening in a dose-dependent manner. An early embryo signal which was required to induce endosperm weakening could be replaced by GA, and that weakening was found to be regulated by the GA–ABA ratio. These results suggest that the control of radicle protrusion in *L. sativum* and probably also *A. thaliana* seeds is mediated, at least in part, by endosperm weakening. In contrast to coffee and tomato, a 'one-phase' ABA-inhibited endosperm weakening is evident in *Lepidium* seeds (Müller et al. 2006). Based on the 'comparative seed biology' approach with *Lepidium* and *Arabidopsis*, one can speculate that during evolution the endospermic Brassicaceae seeds have retained ABA-inhibitable and evolutionary conserved molecular mechanism(s) found in both clades, whereas the ABA-insensitive phase of endosperm weakening was lost.

Ikuma and Thimann (1963) in their 'hatching hypothesis' of seed biology suggested that '... the final step in the germination control process is the production of an enzyme whose action enables the tip of the radicle to penetrate through the coat'. In searching for this 'hatching enzyme', evidence has been uncovered for the contribution of various cell-wall modifying proteins, including endo-β-1,4-mannanases and endo-β-1,3-glucanases (summarised in: Hilhorst 1995; Bewley 1997a; Leubner-Metzger 2003; Kucera et al. 2005; Finch-Savage and Leubner-Metzger 2006; Holdsworth et al. 2008a). Taken together, the current findings support the view that germination control by the seed-covering layers is achieved through the combined or successive action of several cell-wall modifying proteins. One intriguing issue arising from these studies is that there seem to be evolutionary conserved molecular mechanisms as well as species-specific adaptations for endosperm weakening and/or coat dormancy release. Analysis of endosperm-specific transcriptome data sets of germinated *Arabidopsis* seeds, provide information about the expression of genes for cell-wall modifying proteins (Penfield et al. 2006; Holdsworth et al. 2008a). In addition to typical cell-wall polysaccharide hydrolases,

ROS seem to be involved in seed dormancy release and germination and may contribute to endosperm weakening and embryo growth (Bailly 2004; Oracz et al. 2007).

4.3 Dormancy and Harsh Environments

4.3.1 Seed Dormancy and Tolerance in the Dry State

At the final stages of seed maturation, the induction of dormancy and subsequent desiccation on the mother plant results in dry seeds that are extremely tolerant to many types of stress. For instance, dry seeds can survive exposure to extremely high (120°C) or low (liquid nitrogen) temperatures or vacuum (Leprince and Vertucci 1995). The lifespan of seeds in the dry state can be extremely long, ranging from decades to centuries and even millennia. The most remarkable discovery was that on ancient seeds of Sacred Lotus from China; radiocarbon dating showed an age of these seeds of 1,288 ± 271 years while still being capable to germinate (Shen-Miller et al. 1995). The main reason for long-term protection is that the removal of water results in glass formation of the cytoplasm. Glasses are semi-equilibrium solid liquids with an extremely high viscosity (see Buitink and Leprince 2004, for review). Low temperatures and low water contents drive the viscosity to such high values that the cytoplasm will form a glassy state. The high viscosity is thought to be responsible for the decreased ageing rates observed at these low water contents and temperatures. Indeed, cellular viscosity and molecular mobility measurements in the cytoplasm correlate with seed longevity over a wide range of temperatures and water contents (Buitink et al. 2000). Thus, this intracellular glass formation, together with direct interaction between molecules that are imbedded in the glassy matrix through hydrogen bonding will maintain structural integrity lead to optimal preservation of the dormant seeds in the dry state (reviewed in Buitink and Leprince 2004).

Although glass formation in seeds drastically decreases molecular mobility, the molecules in a glass are not completely restricted in their movement, and this can have known repercussions on the survival in the dry state and probably as well on the dormancy status. In time, diffusion will be possible in the dry state, albeit at a rate considerably slower than that in hydrated cytoplasm. Using theoretical considerations coupled to measurements of relaxation times, Walters (2004) demonstrated that mobility is not restricted until at least 70°C below the glass transition temperature. This explains why seeds still age, because deteriorative processes such as lipid oxidation can take place, though at a very slow rate. This could also explain the natural after-ripening process that occurs in 'dry' seeds after harvest, during which seeds escape dormancy (reviewed in Holdsworth et al. 2008a). The processes taking place in dry seeds, that is with a water content below 0.10 g H_20 g/g DW (corrected for lipid content), can not involve true metabolism (i.e. ATP production

via electron transport chains) because it has been shown to be arrested at water contents below 0.2 g/g. However, the release of dormancy could well be determined by the diffusion rate of certain molecules released from or diffusing within the glassy cytoplasm. Interestingly, both the rate of after-ripening and aging increase with increasing water content and temperature, as does the molecular mobility of the cytoplasm (J. Buitink, unpublished data).

Another interesting question that remains to be answered is whether dry seeds in a dormant state are more tolerant to stress than non-dormant seeds. Although this has been suggested, we found no experimental evidence in the literature. Seed longevity and seed dormancy seem to be controlled by different genetic factors in rice as well as in *Arabidopsis* seeds (Miura et al. 2002; Clerkx et al. 2004) as suggested by the different chromosomal location of QTL for these traits. Mutant seeds of abi3, a master-regulator of seed maturation, are affected both in dormancy and longevity (Ooms et al. 1993), but this regulation could involve independent signalling pathways. The only mutation that directly affects both dormancy and longevity is related to the seed testa of *Arabidopsis* (Debeaujon et al. 2000). These testa mutants were shown to take up tetrazolium much more readily than the wild types. This was related to defects in the pigmentation of the endothelium and its neighbouring crushed parenchymatic layers. The degree of seed deterioration was not strictly correlated with dormancy characteristics. Where the increased permeability of the seed coat may result in reduced dormancy, it is most likely the absence of the flavonoids that affect longevity, play a protective role against solute leakage, imbibition damage, and oxidative stress.

4.3.2 Stress Tolerance of Dormant Seeds in the Hydrated State

Although seeds that remain dry are very tolerant, environmental conditions fluctuate in nature, and seeds in soil banks are submitted to hydration and dehydration cycles. Regardless of their dormancy status, seeds can undergo several cycles of hydration and dehydration and, prior to radicle emergence, seeds remain desiccation-tolerant, unless this cycle is repeated too often (Sliwinska and Jendrzejczak 2002). Interestingly, seed mitochondria of desiccation-tolerant, non-germinated pea seeds have a remarkable temperature tolerance in response to both cold and heat stress, when compared to mitochondria isolated from etiolated epicotyls, and contain large amounts of a small heat shock protein, HSP22, and a late embryogenesis abundant (LEA) protein, LEAm (Stupnikova et al. 2006). It has been even shown that re-drying hydrated, even germinated seeds can re-induce desiccation tolerance (Buitink et al. 2003; Faria et al. 2005; Buitink et al. 2006). A transcriptomic profiling of this re-induction of desiccation tolerance in *Medicago truncatula* demonstrated that a large number of genes was re-induced when hydrated radicles were submitted to a partial drying by an osmotic solution. Many of these genes are related to protection against a wide range of stresses. For instance, a number of genes encode regulatory genes that are typically expressed during abiotic/drought

stresses as well as maturation. Furthermore, highly induced expression is found for genes encoding LEA proteins, detoxification enzymes and heat-shock proteins (Buitink et al. 2006). During this partial dehydration, a massive repression of genes occurred belonging to numerous classes, including cell cycle, biogenesis, primary and energy metabolism, suggesting that the re-establishment of DT in the germinated radicles goes together with an active regulation to prepare for the return to the quiescent state imposed by the incipient lack of water. Although in *M. truncatula*, the re-induction of desiccation tolerance does not re-induce dormancy in the seeds, it has been reported that rehydration–dehydration cycles can also re-induce dormancy. Batlla and Benech-Arnold (2006) demonstrated that seeds in weed seed banks under field conditions that were subjected to fluctuating soil water content regime generally showed an increase in their dormancy level after periods of storage under dry soil conditions, and a decrease in their dormancy level after periods of storage under moist soil conditions.

Dormant seeds that remain hydrated need also to be protected against sudden unfavourable environmental conditions. Indeed, protective mechanisms seem to be activated in imbibed seeds. For example, the seed coats of dormant barrel medic seeds remain devoid of any contaminating fungi and bacteria for months, whereas isolated seed coats are readily infected (personal observation W. Bolingue). Also, expression studies in dormant *Arabidopsis* seeds demonstrate that genes related to defence and protection are highly expressed (Cadman et al. 2006). We re-analysed the transcriptome data of imbibed dormant *Arabidopsis* seeds (D-dataset) from Cadman et al. (2006) to screen for genes encoding putative protective molecules. Several genes (6) encode LEA proteins, out of which two belong to group 1 (PF00477) and two to group 5 (seed maturation protein, PF04927) (see Chapter xx). AU3 In addition, nine genes encode small heat shock proteins, HSP70 and chaperone proteins dnaJ. Furthermore, 14 genes involved in detoxification are highly expressed, such as metallothionein, aldo-reductase, glutathione reductase-S-transferase and peroxiredoxin. Interestingly, a similar set of genes are also highly expressed in relation to desiccation tolerance, indicating partially similar regulatory mechanisms underlying both dormancy and desiccation tolerance. Genes related to biotic stress are equally expressed (8), such as defensins or CC-NBS-LRR class disease resistance proteins. In barrel medic, a number of genes are up-regulated during imbibition in dormant seeds that are related to secondary metabolism and defence responses, whereas their expression remains low in imbibed seeds that are non-dormant and will readily germinate (W. Bolingue and J. Buitink, unpublished data). Apparently, regulation of gene expression related to protection and defence is constitutively activated in dormant seeds.

In conclusion, in order to survive long time in seed banks, dormant seeds need to be resistant in the dry as well as hydrated state against biotic and abiotic factors that they are likely to encounter. A number of these mechanisms are likely to overlap with those acquired during maturation, with the acquisition of desiccation tolerance and longevity. Armed with these mechanisms, seeds can overcome those times under which conditions are unfavourable for seedling establishment, and will as such assure the propagation of future generations.

4.4 Future Prospects

In the last decade, enormous progress has been made in the understanding of the mechanisms and regulation of seed dormancy and germination, with a strong focus on model systems, such as *A. thaliana* and *M. truncatula*. It has become clear that environmental cues modulate the levels and balance of the plant hormones ABA and GA in a complex way and thus determine the occurrence of dormancy and germination. Annual dormancy cycling is mostly driven by changes in seasonal temperatures, whereas the breaking of dormancy is influenced by environmental cues such as light, nitrate and temperature/time. The regulation of seed dormancy (cycling) and germination in *A. thaliana* at the molecular level is complex and involves at least several hundreds of genes (Finch-Savage et al. 2007). It is, therefore, likely that transcriptional networks and their associated transcription factors are operational in the control of dormancy and germination. In order to identify clusters within the network, co-expression analysis may be performed on the different gene sets associated with different dormant states. In addition, to identify potential transcription factors, sequence motifs in both the promoter and non-coding regions of co-expressed genes may be identified. In addition to this (transcriptional) network analysis, a system's biology approach of seed germination and dormancy appears timely. Such an approach would incorporate all levels of complexity, from molecules to cells, to tissues to the whole seed and to the environment. It would also include responses to biotic stresses that occur in parallel to the dormancy/germination response, indicating a tight association between stress and dormancy, as in many other organisms.

References

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Achard P, Renou JP, Berthome R, Harberd NP, Genschik P (2008) Plant DELLAs restrain growth and promote survival of adversity by reducing the levels of reactive oxygen species. Curr Biol 18:656–660

Ali-Rachedi S, Bouinot D, Wagner MH, Bonnet M, Sotta B, Grappin P, 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 CJ, Blankestijn de Vries H, Koornneef M (2003) Analysis of natural allelic variation at seed dormancy loci of *Arabidopsis thaliana*. Genetics 164:711–729

Amen RD (1968) A model of seed dormancy. Bot Rev 34:1–31

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

Baskin CC, Baskin JM (1998) Seeds; ecology, biogeography and evolution of dormancy and germination. Academic, San Diego

Baskin JM, Baskin CC (2004) A classification system for seed dormancy. Seed Sci Res 14:1–16

Bassel GW, Zielinska E, Mullen RT, Bewley JD (2004) Down-regulation of DELLA genes is not essential for germination of tomato, soybean, and Arabidopsis seeds. Plant Physiol 136:2782–2789

Bassel GW, Mullen RT, Bewley JD (2006) ABI3 expression ceases following, but not during, germination of tomato and Arabidopsis seeds. J Exp Bot 57:1291–1297

Batlla D, Benech-Arnold RL (2006) The role of fluctuations in soil water content on the regulation of dormancy changes in buried seeds of Polygonum aviculare L. Seed Sci Res 16:47–59

Bentsink L, Koornneef M (2008) Seed dormancy and germination. In: Somerville CR, Meyerowitz EM (eds) The Arabidopsis book. American Society of Plant Biologists, Rockville, MD. doi:10.1199/tab.0119

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

Bethke PC, Libourel IGL, Aoyama N, Chung Y-Y, Still DW, Jones RL (2007) 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 JD (1997a) Breaking down the walls – a role for endo-β-mannanase in release from seed dormancy? Trends Plant Sci 2:464–469

Bewley JD (1997b) Seed germination and dormancy. Plant Cell 9:1055–1066

Bove J, Lucas P, Godin B, Ogé L, Jullien M, 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 57:93–612

Bradford KB, Nnogaki H (2007) Seed development, dormancy and germination. Annual plant reviews. Wiley-Blackwell, Oxford

Buitink J, Leprince O (2004) Glass formation in plant anhydrobiotes: survival in the dry state. Cryobiology 48:215–228

Buitink J, Leprince O, Hemminga MA, Hoekstra FA (2000) Molecular mobility in the cytoplasm: a new approach to describe and predict lifespan of dry germplasm. Proc Natl Acad Sci USA 97:2385–2390

Buitink J, Ly Vu B, Satour P, Leprince O (2003) A physiological model to study the re-establishment of desiccation tolerance in germinated radicles of *Medicago truncatula* Gaertn. seeds. Seed Sci Res 13:273–286

Buitink J, Leger JL, Guisle I, Ly-Vu B, Wuillème S, Lamirault G, Le Bars A, Le Meur N, Becker A, Küster K, Leprince O (2006) Transcriptome profiling uncovers metabolic and regulatory processes occurring during the transition from desiccation sensitive to -tolerant stages in Medicago truncatula seeds. Plant J 47:735–750

Cadman CSC, Toorop PE, Hilhorst HWM, Finch-Savage WE (2006) Gene expression profiles of Arabidopsis Cvi seed during cycling through dormant and non-dormant states indicate a common underlying dormancy control mechanism. Plant J 46:805–822

Cao D, Hussain A, Cheng H, Peng J (2005) Loss of function of four DELLA genes leads to light- and gibberellin-independent seed germination in Arabidopsis. Planta 223:105–113

Cao DN, Cheng H, Wu W, Soo HM, Peng JR (2006) Gibberellin mobilizes distinct DELLA-dependent transcriptomes to regulate seed germination and floral development. Plant Physiol 142:509–525

Carrera E, Holman T, Medhurst A, Peer W, Schmuths H, Footitt S, Theodoulou FL, Holdsworth MJ (2007) Gene expression profiling reveals defined functions of the ABC transporter COMATOSE late in phase II of germination. Plant Physiol 143:1669–1679

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

Catusse J, Job C, Job D (2008a) Transcriptome- and proteome-wide analyses of seed germination. C R Biol 331:815–822

Catusse J, Strub J-M, Job C, Van Dorsselaer A, Job D (2008b) Proteome-wide characterization of sugarbeet seed vigour and its tissue specific expression. Proc Natl Acad Sci USA 105:10262–10267

Chiang GCK, Barua D, Kramer EM, Amasino RM, Donohue K (2009) Major flowering time gene, *FLOWERING LOCUS C*, regulates seed germination in *Arabidopsis thaliana*. Proc Natl Acad Sci USA 106:11661–11666

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

Chiwocha SDS, Cutler AJ, Abrams SR, Ambrose SJ, Yang J, Ross ARS, Kermode AR (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 EJM, El-Lithy ME, Vierling E, Ruys GJ, Blankestijn-De Vries H, Groot SP, Vreugdenhil D, 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

Cohn MA (1996) Operational and philosophical decisions in seed dormancy research. Seed Sci Res 6:147–153

Corbineau F, Bianco J, Garello G, Come D (2002) Breakage of Pseudotsuga menziesii seed dormancy by cold treatment as related to changes in seed ABA sensitivity and ABA levels. Physiol Plant 114:313–319

da Silva EAA, Toorop PE, van Aelst AC, Hilhorst HWM (2004) Abscisic acid controls embryo growth potential and endosperm cap weakening during coffee (Coffea arabica cv. Rubi) seed germination. Planta 220:251–261

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

Debeaujon I, Léon-Kloosterziel KM, Koornneef M (2000) Influence of the testa on seed dormancy, germination, and longevity in Arabidopsis. Plant Physiol 122:403–413

Faria JMR, Buitink J, van Lammeren AAM, Hilhorst HWM (2005) Changes in DNA and microtubules during loss and re-establishment of desiccation tolerance in germinating Medicago truncatula seeds. J Exp Bot 56:2119–2130

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

Finch-Savage WE, Cadman CSC, Toorop PE, Lynn JR, Hilhorst HWM (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, Reeves W, Ariizumi T, Steber C (2008) Molecular aspects of seed dormancy. Annu Rev Plant Biol 59:387–415

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

Grappin P, Bouinot D, Sotta B, Miginiac E, Jullien M (2000) Control of seed dormancy in Nicotiana plumbaginifolia: post-imbibition abscisic acid synthesis imposes dormancy maintenance. Planta 210:279–285

Hilhorst HWM (1995) A critical update on seed dormancy. I. Primary dormancy. Seed Sci Res 5:61–73

Hilhorst HWM (2007) Definition and hypotheses of seed dormancy. In: Bradford KJ, Nonogaki H (eds) Seed development, dormancy and germination. Annual plant reviews, vol 27. Blackwell, Sheffield, UK, pp 50–71

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

Holdsworth MJ, Finch-Savage WE, Grappin P, Job D (2008b) Post-genomics dissection of seed dormancy and germination. Trends Plant Sci 13:7–13

Ikuma H, Thimann KV (1963) The role of the seed-coats in germination of photosensitive lettuce seeds. Plant Cell Physiol 4:169–185

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

Job C, Rajjou L, Lovigny Y, Belghazi M, Job D (2005) Patterns of protein oxidation in Arabidopsis seeds and during germination. Plant Physiol 138:790–802

Karssen CM, Laçka E (1986) A revision of the hormone balance theory of seed dormancy: studies on gibberellin and/or abscisic acid-deficient mutants of Arabidopsis thaliana. In: Bopp M (ed) Plant growth substances 1985. Springer, Berlin, pp 315–323

Koornneef M, Bentsink L, Hilhorst H (2002) Seed dormancy and germination. Curr Opin Plant Biol 5:33–36

Koornneef M, Alonso-Blanco C, Vreugdenhil D (2004) Naturally occurring genetic variation in Arabidopsis thaliana. Ann Rev Plant Biol 55:141–172

Krock B, Schmidt S, Hertweck C, Baldwin IT (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

Kucera B, Cohn MA, Leubner-Metzger G (2005) Plant hormone interactions during seed dormancy release and germination. Seed Sci Res 15:281–307

Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, Asami T, Hirai N, Koshiba T, Kamiya Y, Nambara E (2004) The Arabidopsis cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. EMBO J 23:1647–1656

Laserna MP, Sánchez RA, Botto JF (2008) Light-related loci controlling seed germination in L*er* x Cvi and Bay-0 x Sha recombinant inbred-line populations of *Arabidopsis thaliana*. Ann Bot 102:631–642

Le Page-Degivry MT, Bianco J, Barthe P, Garello G (1996) Change in hormone sensitivity in relation to the onset and breaking of sunflower embryo dormancy. In: Lang GA (ed) Plant dormancy: physiology, biochemistry and molecular biology. CAB International, Wallingford, pp 221–231

Lee SC, Cheng H, King KE, Wang WF, He YW, Hussain A, Lo J, Harberd NP, Peng JR (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

Leprince O, Vertucci CW (1995) A calorimetric study of the glass-transition behaviors in axes of bean-seeds with relevance to storage stability. Plant Physiol 109:1471–1481

Leubner-Metzger G (2002) Seed after-ripening and over-expression of class I β-1, 3-glucanase confer maternal effects on tobacco testa rupture and dormancy release. Planta 215: 959–968

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

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

Leubner-Metzger G, Meins F Jr (2000) Sense transformation reveals a novel role for class I β-1, 3-glucanase in tobacco seed germination. Plant J 23:215–221

Leymarie J, Bruneaux E, Gibot-Leclerc S, Corbineau F (2007) Identification of transcripts potentially involved in barley seed germination and dormancy using cDNA-AFLP. J Exp Bot 58:425–437

Liu P-P, Koizuka N, Homrichhausen TM, Hewitt JR, Martin RC, Nonogaki H (2005a) Large-scale screening of Arabidopsis enhancer-trap lines for seed germination-associated genes. Plant J 41:936–944

Liu P-P, Koizuka N, Martin RC, Nonogaki H (2005b) The BME3 (Blue Micropylar End 3) GATA zinc finger transcription factor is a positive regulator of Arabidopsis seed germination. Plant J 44:960–971

Liu Y, Koornneef M, Soppe WJJ (2007) The absence of histone H2B monoubiquitination in the Arabidopsis hub1 (rod4) mutant reveals a role for chromatin remodeling in seed dormancy. Plant Cell 19:433–444

Manz B, Müller K, Kucera B, Volke F, 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

Miura K, Lin SY, Yano M, Nagamine T (2002) Mapping quantitative trait loci controlling seed longevity in rice (Oryza sativa L.). Theor Appl Gen 104:981–986

Müller K, Tintelnot S, 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

Nakabayashi K, Okamoto M, Koshiba T, Kamiya Y, 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, Marion-Poll A (2003) ABA action and interactions in seeds. Trends Plant Sci 8:213–217

Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y, 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, 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

Ooms JJJ, Leon-Kloosterziel KM, Bartels D, Koornneef M, Karssen CM (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

Oracz K, Bouteau HE-M, Farrant JM, Cooper K, Belghazi M, Job C, Job D, Corbineau F, Bailly C (2007) ROS production and protein oxidation as a novel mechanism for seed dormancy alleviation. Plant J 50:52–465

Peeters AJM, Blankestijn-DeVries H, Hanhart CJ, Leon-Kloosterziel KM, Zeevaart JAD, 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

Penfield S, Josse E-M, Kannangara R, Gilday AD, Halliday KJ, Graham IA (2005) Cold and light control seed germination through the bHLH transcription factor SPATULA. Current Biol 15:1998–2006

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

Piskurewicz U, Jikumaru Y, Kinoshita N, Nambara E, Kamiya Y, Lopez-Molina L (2008) The gibberellic acid signaling repressor RGL2 inhibits Arabidopsis seed germination by stimulating abscisic acid synthesis and ABI5 activity. Plant Cell 20:2729–2745

Rajjou L, Gallardo K, Debeaujon I, Vandekerckhove J, Job C, 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

Richards DE, King KE, Aitali T, Harberd NP (2001) How gibberellin regulates plant growth and development: a molecular genetic analysis of gibberellin signaling. Ann Rev Plant Physiol Plant Mol Biol 52:67–88

Rohde A, Kurup S, Holdsworth M (2000) ABI3 emerges from the seed. Trends Plant Sci 5:418–419

Schwachtje J, Baldwin IT (2004) Smoke exposure alters endogenous gibberellin and abscisic acid pools and gibberellin sensitivity while eliciting germination in the post-fire annual, Nicotiana attenuata. Seed Sci Res 14:51–60

Shen-Miller J, Mudgett MB, Schopf JW, Clarke S, Berger R (1995) Exceptional seed longevity and robust growth: ancient sacred lotus from China. Am J Bot 82:1367–1380

Sliwinska E, Jendrzejczak E (2002) Sugar-beet seed quality and DNA synthesis in the embryo in relation to hydration-dehydration cycles. Seed Sci Technol 30:597–608

Stupnikova I, Benamar A, Tolleter D, Grelet J, Borovskii G, Dorne A-J, Macherel D (2006) Pea seed mitochondria are endowed with a remarkable tolerance to extreme physiological temperatures. Plant Physiol 140:326–335

Tamura N, Yoshida T, Tanaka A, Sasaki R, Bando A, Toh S, Lepiniec L, Kawakami N (2006) Isolation and characterization of high temperature-resistant germination mutants of Arabidopsis thaliana. Plant Cell Physiol 47:1081–1094

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

Toorop PE, van Aelst AC, Hilhorst HWM (2000) The second step of the biphasic endosperm cap weakening that mediates tomato (Lycopersicon esculentum) seed germination is under control of ABA. J Exp Bot 51:1371–1379

Tyler L, Thomas SG, Hu J, Dill A, Alonso JM, Ecker JR, Sun TP (2004) DELLA proteins and gibberellin-regulated seed germination and floral development in Arabidopsis. Plant Physiol 135:1008–1019

Walters C (2004) Temperature dependency of molecular mobility in preserved seeds. Biophys J 86:1253–1258

Wen CK, Chang C (2002) Arabidopsis RGL1 encodes a negative regulator of gibberellin responses. Plant Cell 14:87–100

White CN, Rivin CJ (2000) Gibberellins and seed development in maize. II. Gibberellin synthesis inhibition enhances abscisic acid signaling in cultured embryos. Plant Physiol 122:1089–1097

White CN, Proebsting WM, Hedden P, Rivin CJ (2000) Gibberellins and seed development in maize. I. Evidence that gibberellin/abscisic acid balance governs germination versus maturation pathways. Plant Physiol 122:1081–1088

Yamaguchi S, Kamiya Y (2002) Gibberellins and light-stimulated seed germination. J Plant Growth Regul 20:369–376

Yamaguchi S, Smith MW, Brown RG, Kamiya Y, Sun T (1998) Phytochrome regulation and differential expression of gibberellin 3beta-hydroxylase genes in germinating Arabidopsis seeds. Plant Cell 10:2115–2126

Yamaguchi S, Kamiya Y, Sun TP (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, Yamaguchi S (2004) Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of Arabidopsis thaliana seeds. Plant Cell 16:367–378

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