Plant, Cell & Environment

Plant, Cell and Environment (2012) doi: 10.1111/j.1365-3040.2012.02542.x

Molecular mechanisms of seed dormancy

KAI GRAEBER[1]*, KAZUMI NAKABAYASHI[2]*, EMMA MIATTON[2], GERHARD LEUBNER-METZGER[1,3] & WIM J. J. SOPPE[2]

[1]University of Freiburg, Faculty of Biology, Institute for Biology II, Botany/Plant Physiology, 79104 Freiburg, Germany, [2]Department of Plant Breeding & Genetics, Max Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, 50829 Cologne, Germany and [3]School of Biological Sciences, Plant Molecular Sciences, Royal Holloway, University of London, Egham, Surrey TW20 0EX, UK

ABSTRACT

Seed dormancy is an important component of plant fitness that causes a delay of germination until the arrival of a favourable growth season. Dormancy is a complex trait that is determined by genetic factors with a substantial environmental influence. Several of the tissues comprising a seed contribute to its final dormancy level. The roles of the plant hormones abscisic acid and gibberellin in the regulation of dormancy and germination have long been recognized. The last decade saw the identification of several additional factors that influence dormancy including dormancy-specific genes, chromatin factors and non-enzymatic processes. This review gives an overview of our present understanding of the mechanisms that control seed dormancy at the molecular level, with an emphasis on new insights. The various regulators that are involved in the induction and release of dormancy, the influence of environmental factors and the conservation of seed dormancy mechanisms between plant species are discussed. Finally, expected future directions in seed dormancy research are considered.

Key-words: abscisic acid (ABA); dormancy; gibberellin (GA); germination; reactive oxygen species (ROS); seed maturation.

INTRODUCTION

Plants are bound to the location where they have established themselves and require developmental adaptations to survive unfavourable environmental conditions. Most plants cycle between different developmental states, starting with the seed, followed by the seedling, the vegetative phase, and finally the reproductive phase. The duration of these different states widely varies between species and the timing of the transitions between them is highly regulated. This regulation ensures that the most vulnerable phases of the life cycle occur during favourable seasonal and environmental conditions. Two of the systems that control developmental transitions depend on dormancy mechanisms. Bud dormancy prevents the outgrowth of buds, for instance in perennial plants during winter (Rohde & Bhalerao 2007; Cooke, Eriksson & Junttila 2012) or in potato tubers (Suttle 2004; Rentzsch *et al*. 2012), whereas seed dormancy prevents the germination of intact viable seeds during (temporary) favourable conditions in an otherwise unfavourable season (Bewley 1997). Both types of dormancy are characterized by very low metabolic activities and a temporary insensitivity to growth-promoting signals. In comparison to the analysis of another important developmental transition mechanism, flowering induction, the study of the molecular mechanisms of dormancy started later and is less advanced. In this review, our present knowledge about the molecular mechanisms of seed dormancy will be discussed, whereas the accompanying review by Cooke *et al*. (2012) deals with bud dormancy in trees.

Seed dormancy is an innate seed property that defines the environmental conditions in which the seed is able to germinate (Finch-Savage & Leubner-Metzger 2006). It is determined by genetic factors with a substantial environmental influence and provides adaptation to a diversity of habitats. Seed dormancy is therefore an important component of plant fitness (Donohue *et al*. 2005; Huang *et al*. 2010). Too low seed dormancy levels can lead to germination before the start of a favourable growth season, risking seedling mortality. In contrast, too high seed dormancy levels delay germination and reduce the length of the growing season (Donohue *et al*. 2010). Exposure to favourable germination conditions in crop plants is determined by the moment when the farmer sows the seeds. These seeds should germinate immediately, making seed dormancy an unwanted trait. As a result, selection for reduced seed dormancy levels occurred during the domestication process and most crop plants germinate uniformly and fast after sowing in contrast to their wild ancestors (Kilian *et al*. 2009). On the other hand, too low seed dormancy levels reduce the quality of seeds for sowing and trigger pre-harvest sprouting, causing yield losses in cereals (Gubler, Millar & Jacobsen 2005). Therefore, seeds of crop plants require a well-balanced level of seed dormancy.

Different seed dormancy classes exist among plant species, which can be divided in physiological dormancy, morphological dormancy, morphophysiological dormancy,

Correspondence: W. J. J. Soppe. Fax: +49 221 506 2413; e-mail: soppe@mpipz.mpg.de

*Equal contribution.

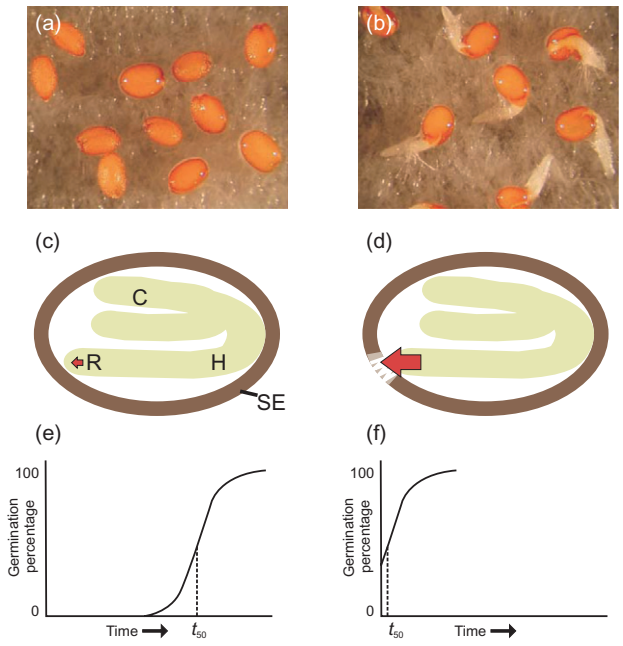

Figure 1. A comparison of dormant and non-dormant seeds. (a,b) Freshly harvested seeds of the *Arabidopsis* accession Cape Verde Islands are dormant and do not germinate after 3 days imbibition in the light (a), whereas after-ripened seeds have lost dormancy and germinate under the same conditions (b). (c,d) The growth force of the radicle (indicated by the red arrow) does not overcome the strength of the surrounding tissues in dormant seeds (c), but weakening of the surrounding tissues (seed envelopes) and an increased growth force of the radicle enables germination (d). The embryo is shown in green and the seed envelopes in brown. C, cotyledons; H, hypocotyl; R, radicle; SE, seed envelopes (testa and endosperm). (e,f) Germination-over-time diagrams of a dormant seed batch with a high t_{50} value (e) and a non-dormant seed batch with a low t_{50} value (f).

physical dormancy and combinational dormancy (Baskin & Baskin 2004; Finch-Savage & Leubner-Metzger 2006). Physiological dormancy is most common and can be separated into deep and non-deep, of which the latter is the most prevalent and also the major form of dormancy in plant model species (Fig. 1a,b). Embryos excised from seeds with non-deep physiological dormancy produce normal seedlings (Baskin & Baskin 2004). This review focuses on the molecular mechanisms of non-deep physiological dormancy.

Dormancy is a complex trait because it is influenced by both environmental and endogenous factors. Moreover, the final level of dormancy is determined by the contributions of the different tissues that comprise a seed. Different plant species show a variety of seed structures (Linkies *et al.* 2010), but as a general rule in angiosperms, the embryo surrounding tissues or seed envelopes (e.g. testa and endosperm) prevent germination by providing a physical barrier for the elongating radicle (Debeaujon, Léon-Kloosterziel & Koornneef 2000). In several species, including the model plant *Arabidopsis thaliana*, the endosperm has a pivotal role as a regulatory barrier (Müller, Tintelnot & Leubner-Metzger 2006; Bethke *et al.* 2007). Germination and dormancy depend on the balance between the growth force of the elongating radicle and the resistance strength of the surrounding tissues (Fig. 1c,d). The activities of cell wall remodelling proteins influence the strength of the surrounding tissues (Leubner-Metzger 2005; Endo *et al.* 2012), whereas the force of the radicle is determined by elongation of cells in the transition zone and lower hypocotyl (Sliwinska, Bassel & Bewley 2009). The vast majority of molecular and genetic studies on seed dormancy have been conducted on complete seeds and did not take the individual contributions of these tissues into account. More recently, several studies have started to address the roles of separate tissues in dormancy control.

Dormancy is a quantitative trait whose depth varies over time. Primary dormancy is induced during the seed maturation phase and reaches a high level in freshly harvested seeds. During subsequent dry storage of seeds (after-ripening), dormancy slowly reduces (Holdsworth, Bentsink & Soppe 2008). When the dormancy level of a seed batch gradually decreases, the window of environmental conditions that enable germination is widening. Seeds can also rapidly loose dormancy during imbibition at specific conditions. For instance, high or low temperatures for a few days (Finch-Savage & Leubner-Metzger 2006) or compounds present in smoke (Flematti *et al.* 2004) release seed dormancy of imbibed seeds. Dormancy does not only decrease with time, but it can also be re-induced in non-dormant seeds when conditions for germination (for instance light) are lacking. This is called secondary dormancy. Under natural conditions in the seed bank, the dormancy level of seeds usually cycles throughout the year enabling seeds to have the highest germination potential at the start of the growth season (Footitt *et al.* 2011). The level of dormancy of a seed batch cannot be directly assessed, but can only be indirectly measured by germination tests. Complete germination-over-time curves will give a good estimate of the level of dormancy (Fig. 1e,f), and a parameter that describes the dormancy level is the time until 50% of the seeds in a seed batch have germinated (t_{50}; Hilhorst 2011).

This review gives an overview of the mechanisms that control seed dormancy at the molecular level with an emphasis on new insights obtained in the last few years. We will not focus on the germination process itself, and several recent reviews can be consulted for more information on this topic (Kucera, Cohn & Leubner-Metzger 2005; Holdsworth *et al.* 2008; Weitbrecht, Müller & Leubner-Metzger 2011). Here, the induction and release of dormancy will be discussed followed by the influence of environmental factors and the conservation of seed dormancy mechanisms between plant species. Finally, we will point to expected breakthroughs in the field and future research directions.

INDUCTION OF SEED DORMANCY

The induction of seed dormancy is controlled by a diverse group of regulators that act at various levels and that show

Table 1. Genes involved in dormancy regulation

Gene	Encoded protein	Biological function	Species
Maturation regulators			
ABI3/VP1	B3 TF	Maturation regulation	*Arabidopsis*, rice
FUS3	B3 TF	Maturation regulation	*Arabidopsis*
LEC1	HAP3 subunit of NF-Y TF	Maturation regulation	*Arabidopsis*
LEC2	B3 TF	Maturation regulation	*Arabidopsis*
VP8/PLA3/GO/AMP1	Glutamate carboxypeptidase	–	*Arabidopsis*, rice, maize
SUA	Splicing factor	Regulation of alternative splicing of *ABI3*	*Arabidopsis*
Hormone regulators			
PYR, PYL/RCAR	ABA receptors	ABA perception	*Arabidopsis*
ABI1, ABI2, HAB1, AHG3	Protein phosphatase 2C	Negative regulators of ABA signalling	*Arabidopsis*
SnRK2.2, 2.3, 2.6	Protein kinase	Positive ABA signalling	*Arabidopsis*
KAI1/MAX2	F-Box protein	Strigolactone/karrikin signalling	*Arabidopsis*
Other dormancy genes			
DOG1	Unknown protein	–	*Arabidopsis*
Sdr4	Unknown protein	–	Rice
qSD7-1/qPC7/Rc/TT8	bHLH TF	Flavonoid synthesis	*Arabidopsis*, rice
DEP	C3HC4 RING finger	–	*Arabidopsis*
AtHB20	Homeobox TF	–	*Arabidopsis*
CBF	AP2 TF	Cold response	*Arabidopsis*
MFT	Phosphatidylethanolamine-binding protein	–	*Arabidopsis*, wheat
FLC	MADS-box TF	Repressor of floral transition	*Arabidopsis*
Epigenetic regulators			
HUB1, HUB2	C3HC4 RING finger	Histone H2B monoubiquitination	*Arabidopsis*
RDO2	Transcription elongation factor SII	Transcription elongation	*Arabidopsis*
VIP4, VIP5, ELF7, ELF8, ATXR7	PAF1 components	Transcription	*Arabidopsis*
EFS	Histone H3 methyltransferase	H3K9 methylation	*Arabidopsis*
FIE	Component of PRC2	H3K27 trimethylation	*Arabidopsis*
KYP/SUVH4, SUVH5	Histone methyltransferase	H3K9 methylation	*Arabidopsis*
Release from dormancy			
SPT	bHLH TF	Integration of cold signal into GA signalling	*Arabidopsis*
PIL5	bHLH TF	Integration of light signal into GA signalling	*Arabidopsis*
AtrbohB	NADPH-oxidase	ROS production	*Arabidopsis*
PRT6, ATE	Targeted proteolysis	Inactivating components of ABA signalling	*Arabidopsis*

TF, transcription factor; ROS, reactive oxygen species.

different degrees of specificity. In this section, we divided these regulators into four groups involved in seed maturation, hormonal action, dormancy and chromatin regulation. An overview of all described genes involved in the induction and release of seed dormancy is given in Table 1.

Seed maturation regulators

Seed development comprises the two major phases embryogenesis and seed maturation. Seed dormancy is induced during the seed maturation phase simultaneously with the accumulation of storage compounds, the acquisition of desiccation tolerance and, finally, the quiescence of metabolic activity. Concerted actions of four transcription factors, namely ABSCISIC ACID INSENSITIVE 3 (ABI3), FUSCA 3 (FUS3), LEAFY COTYLEDON 1 (LEC1) and LEC2, play a central role in the regulation of seed maturation and the phase transition from embryo to seedling. Mutations in any of these transcription factors result in aberrant seed maturation leading to heterochronic phenotypes including reduced dormancy. The distinct functions of these regulators as well as their complex interactions were reviewed recently (Holdsworth *et al*. 2008).

Several factors, which control seed dormancy indirectly by regulating ABI3, FUS3, LEC1 and LEC2, have recently been identified. For instance, maize *VIVIPAROUS 8* (*VP8*) has been shown to regulate these transcription factors and a mutation in this gene causes a viviparous seed phenotype with pleiotropic developmental changes (Suzuki *et al*. 2008). In addition, mutants of its rice homologue *PLASTOCHRON 3/GOLIATH* (*PLA3/GO*) and *Arabidopsis* homologue *ALTERED MERISTEM PROGRAM 1* (*AMP1*) also show altered dormancy levels (Kawakatsu *et al*. 2009; Griffiths *et al*. 2011), suggesting a conserved mechanism across

dicots and monocots. These homologous genes encode a putative glutamate carboxypeptidase (Helliwell *et al*. 2001; Suzuki *et al*. 2008; Kawakatsu *et al*. 2009), which opens the possibility that a peptide signal is involved in seed maturation and the induction of seed dormancy.

Hormonal regulation

Numerous genetic studies using abscisic acid (ABA) and gibberellin (GA) biosynthesis and signalling mutants have demonstrated that these two hormones have essential and antagonistic roles in dormancy and germination (Fig. 2). In particular, the balance between the levels of these two hormones and their respective signalling pathways are important in regulating both induction and maintenance of dormancy, and promotion of germination (reviewed in Finkelstein *et al*. 2008). The importance of ABA biosynthesis and signalling in dormancy in diverse species is detailed in the section about conservation of seed dormancy mechanisms.

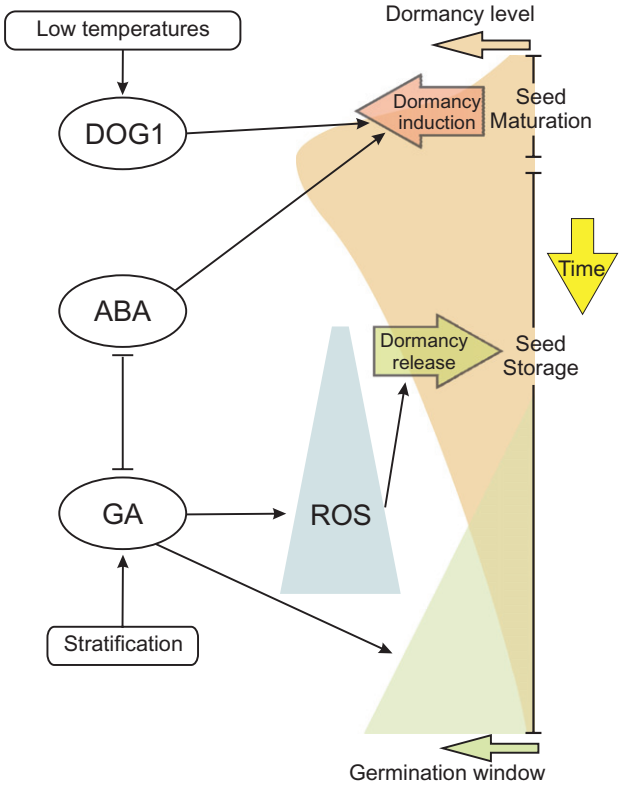

Figure 2. A hypothetical model showing the mechanisms of seed dormancy induction and release. The seed dormancy level (indicated in beige) increases during seed maturation and decreases during seed storage (after-ripening), leading to a widening of the germination window (indicated in green). Major factors for the induction of seed dormancy are the plant hormone ABA and the dormancy factor DOG1. *DOG1* transcription levels are enhanced by low temperatures during seed maturation. The plant hormone GA is required for germination and the ABA and GA pathways have an antagonistic relation. Stratification enhances germination by enhancing GA levels. Increasing ROS levels during seed storage reduce seed dormancy. The relative passage of time is indicated by a yellow arrow, other arrows indicate positive effects.

A recent epoch-making finding concerning ABA was the identification of PYR/PYL/RCAR ABA receptors (Ma *et al*. 2009; Park *et al*. 2009). Fourteen members of this protein family in *Arabidopsis* function redundantly in mediating the ABA response by interacting with type 2C protein phosphatase (PP2C) negative regulators and antagonizing their action. However, it is not known yet whether any of these PYR/PYL/RCAR proteins are specifically involved in ABA signalling during the seed maturation stage. The PP2Cs ABA-INSENSITITVE 1 (ABI1) and ABI2 were originally identified in an ABA-insensitive mutant screen (Koornneef, Reuling & Karssen 1984; Leung *et al*. 1994; Meyer, Leube & Grill 1994; Leung, Merlot & Giraudat 1997). The *abi1-1* and *abi2-1* mutants show reduced dormancy phenotypes (Koornneef *et al*. 1984) and are caused by dominant-negative mutations that lead to abi proteins that are unable to bind to the ABA receptors (Ma *et al*. 2009; Park *et al*. 2009). Consequently, in the presence of ABA, these abi-PP2Cs remain active and repress downstream ABA-activated protein kinases belonging to the SNF1-related protein kinase subfamily 2 (SnRK2). Three *Arabidopsis* SnRK2s (SnRK2.2, SnRK2.3 and SnRK2.6) have been shown to act redundantly in the transmission of an ABA signal during seed development and dormancy induction (reviewed by Nambara *et al*. 2010). The triple mutant of these kinases is nearly blind to ABA and exhibits abnormal seed development, produces ABA-insensitive green seeds similar to severe alleles of *abi3* and germinates precociously under high humidity conditions (Nakashima *et al*. 2009). Major targets of these kinases have been shown to be a group of bZIP-type transcription factors including ABI5 and ABSCISIC ACID RESPONSIVE ELEMENTS-BINDING PROTEIN 3 (AREB3). Surprisingly, mutants of these transcription factors generally do not show strong dormancy phenotypes. This can be partly explained by their redundant function, but there might also be additional important targets that act as regulators of ABA responses during dormancy induction.

Antagonistic to ABA action, GA, ethylene and other hormones have been shown to promote germination (reviewed in Holdsworth *et al*. 2008; Matilla & Matilla-Vázquez 2008; Linkies & Leubner-Metzger 2012). Environmental signals such as light and temperature during imbibition and germination are integrated into GA biosynthesis and signalling by transcription factors like PHYTOCHROME INTERACTING FACTOR 3-LIKE 5 (PIL5) and SPATULA (SPT) (Penfield *et al*. 2005; Oh *et al*. 2009). Ethylene has been shown to regulate endosperm cap weakening and rupture in *Lepidium sativum*, counteracting the action of ABA (Linkies *et al*. 2009). Very recent reports have demonstrated the roles of strigolactones and karrikins (germination-promoting compounds in smoke) in dormancy and germination. Strigolactone signalling is mediated by the F-box protein KARRIKIN INSENSITIVE 1 (KAI1), which is allelic to MORE AXILLARY BRANCHES 2 (MAX2).

The *kai1*/*max2* mutant shows increased primary dormancy (Nelson *et al*. 2011). It has recently been shown that strigolactones modulate the ABA/GA ratio in secondary dormancy control (Toh *et al*. 2012). These observations further reinforce the importance of the coordinated interaction of various hormones in the regulation of dormancy and germination.

Seed dormancy-specific genes

Studies of natural variation have led to the identification of several quantitative trait loci (QTL) controlling seed dormancy (Bentsink *et al*. 2010). Most of these do not co-locate with known dormancy regulators and the molecular identification of the first few QTLs indeed revealed novel dormancy genes. The first cloned dormancy QTL in *Arabidopsis*, *DELAY OF GERMINATION 1* (*DOG1*), encodes a protein of unknown function (Bentsink *et al*. 2006). The absence of dormancy with no obvious pleiotropic phenotypes in the *dog1* mutant indicates that *DOG1* is a key player specific for the induction of seed dormancy (Fig. 2). Extensive QTL mapping has also been performed for dormancy/pre-harvest sprouting traits in crop species. Recently, *Seed dormancy 4* (*Sdr4*) has been identified as one of the major determinants for dormancy in rice. It was shown that Sdr4 is localized in the nucleus and that it affects the expression of several *DOG1-LIKE* genes (rice genes similar to *Arabidopsis DOG1*). Yet, its mechanism of action is still not understood because *Sdr4* encodes a novel protein with unknown function (Sugimoto *et al*. 2010). Characterization of the function of these novel factors and the molecular identification of additional dormancy QTLs will provide us with more clues on the mechanisms that control the induction and maintenance of dormancy.

Candidate gene approaches by reverse genetics have become increasingly feasible during the last decade by utilizing large transcriptome and proteome data sets. Recently, two new seed dormancy factors have been identified in *Arabidopsis* using high-throughput quantitative RT-PCR. These are *DESPIERTO* (*DEP*), which is a C3HC4 RING finger protein and the HDZip gene *ATHB20* (Barrero *et al*. 2010). A mutation in the *DEP* gene causes lack of dormancy, whereas the *athb20-1* insertion mutant shows increased dormancy compared with the wild type. Interestingly, both genes modulate ABA sensitivity. It is noteworthy that the *dep* mutant is completely non-dormant, similar to the *dog1* mutant. The biochemical properties of DEP and identification of its downstream targets will be of great interest.

Regulation of dormancy at the chromatin level

The organization of chromatin influences gene expression and is therefore important for all (developmental) processes in the plant, including seed dormancy and bud dormancy (Cooke *et al*. 2012). However, mutations in chromatin factors do not influence all plant processes similarly. The induction of dormancy during seed maturation occurs independent from the simultaneous reduction in nuclear size and compaction of chromatin (van Zanten *et al*. 2011). Nevertheless, genetic and biochemical studies have identified a number of chromatin factors that are required for a proper regulation of seed dormancy and germination. *REDUCED DORMANCY 4* (*RDO4*)/*HISTONE MONOUBIQUITINATION 1* (*HUB1*) and its homologue *HUB2* encode C3HC4 RING finger proteins necessary for histone H2B monoubiquitination. The *RDO4*/*HUB1* gene was originally identified based on its reduced dormancy phenotype (Liu, Koornneef & Soppe 2007). The *RDO2* gene was found in the same mutagenesis screen for reduced dormancy and encodes transcription elongation factor SII (TFIIS). The HUB and RDO2 proteins are predicted to interact with the RNA polymerase II-associated factor 1 complex (PAF1C) and influence seed dormancy by regulating transcription elongation during seed maturation at a time when transcriptional efficiency is likely to be reduced due to desiccation (Liu *et al*. 2011). In accordance, mutants in other components of PAF1C (Table 1) also showed reduced dormancy. Among others, *DOG1* and ABA-related genes are differentially regulated in these mutants, which is a potential cause for their reduced dormancy phenotype.

The *EARLY FLOWERING IN SHORT DAYS* (*EFS*) gene has been selected as a phase transition regulator during seed germination in a transcriptional network modelling study (Bassel *et al*. 2011b). *EFS* codes for a histone H3 methyltransferase involved in histone H3 lysine 4 trimethylation (H3K4me3), which is a transcription activating histone mark. The *efs* mutant was initially identified by its altered flowering time (Soppe, Bentsink & Koornneef 1999). Interestingly, mutant *efs* seeds also show a variety of seed phenotypes including precocious germination (Bassel *et al*. 2011b). Direct targets of EFS associated with dormancy have not been explored yet, but a potential target is *FLOWERING LOCUS C* (*FLC*) because its expression was shown to be modulated by EFS in the control of flowering time and it has been implicated to be involved in germination regulation (Kim *et al*. 2005; Chiang *et al*. 2009).

Evidence for the involvement of the repressive histone mark H3K27me3 in dormancy induction comes from a study of null mutants for *FERTILIZATION INDEPENDENT ENDOSPERM (FIE)*, which is an essential component of the polycomb repressive complex 2 (PRC2) (Bouyer *et al*. 2011). The *fie* mutant is globally defective in H3K27 trimethylation, and lack of this histone modification caused increased seed dormancy, as the *fie* mutant is more dormant than the wild type. A microarray analysis showed that many of the maturation regulators, namely *ABI3*, *FUS3*, *LEC2* as well as ABA/GA signalling factors and *DOG1*, are repressed by PRC2.

Finally, the repressive histone mark H3K9me influences seed dormancy. The *KRYPTONITE (KYP)/SUVH4* and *SUVH5* genes encode histone methyltransferases that mediate H3K9 dimethylation (Jackson *et al*. 2002). The *kyp-2* and *suvh5* mutants show enhanced dormancy and increased expression of several dormancy genes, including *DOG1* and *ABI3* (Zheng *et al*. 2012). Since *KYP/SUVH4* expression is down-regulated by ABA and up-regulated by

GA, KYP/SUVH4 is likely to mediate, at least partially, downstream signalling of the ABA/GA balance on seed dormancy.

RELEASE OF SEED DORMANCY

Dormancy can either be quickly released in imbibed seeds (within a couple of days) or relatively slow in dry seeds (within weeks or months). The molecular mechanisms controlling dormancy release are less well understood compared to those controlling dormancy induction. The fast release of dormancy requires imbibition at species-specific temperatures and is called stratification. In general, imbibition at low temperatures releases dormancy in seeds of summer annuals, while high temperatures release dormancy in seeds of winter annuals (Probert 2000). It is largely unclear how stratification drives the release of seed dormancy, and, especially, the temperature sensing mechanism is unknown, but a few genes with a role in this process have been identified. The basic helix-loop-helix transcription factors SPT and PIL5 have a role in cold stratification (Penfield *et al*. 2005). SPT is a negative regulator of germination that looses its repressive activity after stratification, whereas PIL5 is not responding to low temperatures, but represses germination in the dark after a cold treatment. Both transcription factors act by inhibiting the GA biosynthesis genes *GA3 OXIDASE 1* (*GA3OX1*) and *GA3OX2* expression, thereby preventing germination (Fig. 2; Penfield *et al*. 2005).

Dormancy can be artificially released by removing constraints (i.e. embryo surrounding tissues) that prevent germination (scarification) or by storing seeds at room temperature under dry conditions (after-ripening). Increased time of after-ripening is associated with a widening of the conditions required for germination, resembling gradual dormancy loss (Fig. 2; Finch-Savage & Leubner-Metzger 2006). The time required for a complete release of dormancy shows high inter- and intra-species variation. For example, in *Arabidopsis*, the accessions Landsberg *erecta* (L*er*) and Cape Verde Islands (Cvi) have very different after-ripening requirements. L*er* needs 12 to 17 d of dry storage to achieve 50% germination, while Cvi needs 74 to 185 d (Alonso-Blanco *et al*. 2003). After-ripening is effective at low moisture contents (MC) of about 5–15%, but is prevented in very dry seeds (Probert 2000). It is not well understood whether the changes that occur within the seed during after-ripening are predominantly happening at the transcript or protein level, but recent findings have started to shed some light on this issue.

Several transcriptome analyses showed that after-ripening affects transcript abundances in dry seeds, resulting in the selective change of specific transcripts (Bove *et al*. 2005; Finch-Savage *et al*. 2007; Leymarie *et al*. 2007). An increase in transcript abundance during dry storage of seeds seems counterintuitive, but could be explained by the occurrence of 'humid pockets' whose existence has been proposed in dry seeds of tobacco. Such local areas with higher moisture levels within the seeds could allow transcriptional activities. Transient transcription and translation changes in dry tobacco seeds were shown for β-1,3-glucanase (Leubner-Metzger 2005). However, the presence of active transcription in dry seed has to be proven yet.

It is also possible that the quantity and quality of stored mRNAs is changed within the dry seed by mechanisms that do not require an active metabolism. A recent study showed that the selective oxidation of a subset of stored mRNAs is associated with dormancy release in sunflower seeds. Oxidation of mRNA can prevent their translation and lead to changes in the proteome after translation has been restarted during seed imbibition. Interestingly, there seems to be a selective oxidation of mRNAs corresponding to genes involved in stress response (Bazin *et al*. 2011).

Oxidative processes within the dry seed also influence proteins. Proteomic approaches have been used as a tool to study the dynamics of posttranslational modifications (PTMs) during after-ripening. PTMs have a major role in the regulation of seed development and maturation (Arc *et al*. 2011). Carbonylation is an irreversible PTM that occurs in response to oxidative stress and that leads to a change in the enzymatic and binding properties of the protein or to its degradation due to a higher sensitivity to proteolytic attack. After-ripening results in an accumulation of reactive oxygen species (ROS; Fig. 2), which is associated with the carbonylation of specific proteins in sunflower (Oracz *et al*. 2007) and in *Arabidopsis* (Job *et al*. 2005). It was suggested that the specific carbonylation of seed storage protein helps their mobilization during germination by promoting their proteolytic attack (Job *et al*. 2005). In mammals, carbonylation is mainly associated with aging and diseases (Stadtman 1992; Agarwal & Sohal 1994), whereas *Arabidopsis* seeds still germinate and produce healthy plantlets when accumulating carbonylated proteins.

Further support for the important role of ROS in dormancy release comes from wheat, for which it was shown that the antioxidant defence pathway is associated with the maintenance of dormancy (Bykova *et al*. 2011). The importance of the ROS-dependent pathway in after-ripening was highlighted by the finding that the signal transduction of hydrogen cyanide (HCN), a compound used to break dormancy artificially, is ROS-dependent and results in an enhanced expression of genes involved in ethylene signalling (Oracz *et al*. 2009). Moreover, Müller *et al*. (2009a) showed that the ROS-producing NADPH oxidase AtrbohB promotes seed after-ripening in *Arabidopsis*. Interestingly, it has been shown that DELLA repressor proteins, which are negative regulators of GA signalling that are degraded by GA, repress ROS accumulation, leading to an enhanced tolerance to abiotic and biotic stress (Achard *et al*. 2008). Although this mechanism has not been demonstrated in seeds, it opens the possibility that GA can accelerate after-ripening by indirectly increasing ROS (Fig. 2).

As mentioned earlier, ABA and GA have essential roles in dormancy and germination. Differences between dormant and non-dormant seeds in the levels of and sensitivities to these two hormones are likely to be established downstream of the dormancy release mechanism. Some

recent findings in *Arabidopsis* highlighted the importance of the endosperm in dormancy release. Germination of the embryo upon imbibition is repressed by ABA which is actively produced and released by the dormant endosperm (Lee *et al*. 2010). The ubiquitous signalling molecule nitric oxide (NO) releases seed dormancy in many species and has been proposed to be an endogenous dormancy regulator (Bethke *et al*. 2004; Šírová *et al*. 2011). NO has the endosperm of imbibed *Arabidopsis* seeds as a major target (Sarath *et al*. 2006; Bethke *et al*. 2007) and seems to act upstream of GA in a signalling pathway leading to vacuolation of aleurone cells, which is associated with storage compound degradation (Bethke, Libourel & Jones 2006). NO might act by decreasing ABA sensitivity of imbibed seeds (Bethke *et al*. 2006) and it has been proposed by Holman *et al*. (2009) that NO achieves this through the N-end rule pathway. Two components of this pathway, PROTEOLYSIS 6 (PRT6) and arginyl-tRNA:protein arginyltransferase (ATE), have been shown to regulate after-ripening and to reduce ABA sensitivity, implicating a role of targeted proteolysis in dormancy release (Holman *et al*. 2009).

Seed dormancy release occurs during after-ripening, but extended periods of seed storage and high oxidative stress, especially under unfavourable conditions, lead to a gradual breakdown of proteins and nucleic acids resulting in a loss of viability. The *Arabidopsis* gene *DNA LIGASE VI* is involved in the control of seed aging, and mutations in this gene cause reduced seed longevity and a delayed germination (Waterworth *et al*. 2010). The link between seed dormancy and seed longevity still remains an open question. Seeds from the non-dormant *Arabidopsis dog1* mutant have a reduced longevity compared to wild-type seeds during dry storage (Bentsink *et al*. 2006), suggesting that dormancy positively correlates with longevity. However, QTLs detected for dormancy (Bentsink *et al*. 2010) and longevity (Clerkx *et al*. 2004) do not always co-locate, suggesting that natural variation for these two traits is under the control of different genetic mechanisms. This does not exclude the possibility that dormancy and longevity are connected at a basic level.

THE ENVIRONMENTAL REGULATION OF SEED DORMANCY

Seeds act as environmental sensors and adjust their dormancy status as a response to a range of environmental factors (Finch-Savage & Leubner-Metzger 2006; Footitt *et al*. 2011; Kendall *et al*. 2011). This response tunes dormancy cycles with the seasons and provides the optimum timing for seed germination and seedling establishment. Key environmental factors like temperature, nitrate, light, water, oxygen, smoke and allelochemicals influence dormancy levels either during seed development on the mother plant or in the soil seed bank. Germination requires specific environmental conditions and Finch-Savage & Leubner-Metzger (2006) state that the sensitivity of seeds to environmental factors changes continuously as a function of variable ambient conditions. The nature and scale of these changes may be a species- or ecotype-specific adaptation to their habitat. Thus, a clearly defined dormant state does not exist, and there are only different requirements for germination. Some of these germination requirements can be so extreme that they do not normally occur in the species' natural habitat. Exposure to specific environmental conditions is usually required to bring germination sensitivities back into a range that matches potential environmental exposure (Finch-Savage & Leubner-Metzger 2006). Thus, drawing a theoretical line between dormancy and germination should be regarded with great care since experimentally derived conclusions might be biased depending on where this artificial line is drawn considering the continuous nature of the transition from dormancy to non-dormancy and germination. Following up on these conclusions, we summarize here recent work on the environmental control of seed dormancy.

Temperature and light perceived during seed maturation have been shown to influence the dormancy level (Donohue *et al*. 2008; Chiang *et al*. 2009). In particular, temperature is a major environmental factor controlling primary dormancy that acts through several identified dormancy regulators controlling ABA and GA contents, as well as *DOG1* gene expression (Fig. 2; Chiang *et al*. 2011; Footitt *et al*. 2011; Kendall *et al*. 2011). Kendall *et al*. (2011) showed that transcription factors of the C-repeat binding factor (CBF) group are necessary for regulation of dormancy caused by low seed-maturation temperatures. CBFs also seem to play a role in the light-mediated induction of bud dormancy (Cooke *et al*. 2012). Interestingly, although CBFs are required for dormancy, their transcript abundances are not temperature regulated in seeds. CBF, DOG1 and ABA/GA metabolism have been proposed as central components of a pathway mediating the effect of seed-maturation temperature on dormancy (Kendall *et al*. 2011). In addition, both phytochrome and FLC seem to play important roles in the interaction with seed-maturation temperature (Chiang *et al*. 2009; Donohue *et al*. 2010; Penfield & Springthorpe 2012). Another gene involved in the low temperature response during seed maturation in wheat is *MOTHER OF FT AND TFL1* (*MFT*). Interestingly, *MFT* is a candidate for the gene underlying a QTL for pre-harvest sprouting on wheat chromosome 3 (Nakamura *et al*. 2011).

Once shed from the mother plant, seed dormancy in the soil bank is altered by environmental cues, with soil temperature and moisture being the main factors (Batlla & Benech-Arnold 2010; Footitt *et al*. 2011). Population-based threshold models can be utilized as a framework to quantify changes in seed sensitivity to soil temperature and moisture regulating dormancy loss and/or induction (Benech-Arnold *et al*. 2000; Bradford 2005; Batlla & Benech-Arnold 2010). For many wild species, germination requires additional permissive conditions like light and/or alternating temperatures (Juroszek & Gerhards 2004; Oh *et al*. 2004; Batlla & Benech-Arnold 2005; Penfield *et al*. 2005; Batlla, Nicoletta & Benech-Arnold 2007; Pinto *et al*. 2007; Heschel *et al*. 2008; Chao *et al*. 2011). An ecological interpretation of these

conditions has been related to the possibility of detecting canopy gaps, the light flash during tillage operations and depth of burial under field conditions (Casal & Sanchez 1998; Batlla & Benech-Arnold 2010). When it comes to interpreting persistence of seeds in soil banks, the germination responses to environmental cues, and phenological adaptation to environmental change, it is important to bear in mind that results obtained from ecological experiments may represent a combination of effects on dormancy as well as on germination (*per se*) mechanisms.

Some environmental factors, for example soil temperature and moisture, are related to slow seasonal changes that indicate when a suitable time of the year and climate space exists (temporal window). These signals are integrated over time to alter the depth of dormancy and therefore the sensitivity to a second set of environmental factors. These include light, nitrate and alternating temperatures, and indicate in a more immediate way that conditions are suitable to terminate dormancy and induce germination (spatial window). This spatial window includes appropriate soil depth, temperature, moisture and lack of competing plants. If the correct spatial window does not occur, the temporal window will close for another year (Footitt *et al*. 2011). Using a targeted investigation of gene expression over the dormancy cycle of seeds from the *Arabidopsis* accession Cvi in the field, Footitt *et al*. (2011) investigated how these mechanisms are seasonally coordinated. Depth of dormancy and gene expression patterns were correlated with seasonal changes in soil temperature. ABA signalling was found to be linked to deep dormancy in winter and is repressed in spring when depth of dormancy decreased. Seed dormancy increased during winter as soil temperature declined and expression of ABA biosynthesis and GA catabolism genes increased. This was linked to an increase in endogenous ABA that plateaued, although dormancy and *DOG1* and *MFT* expression continued to increase. The expression of SNF1-related protein kinases also increased, which is consistent with enhanced ABA signalling and sensitivity. Dormancy then declined in spring and summer. Endogenous ABA decreased along with positive ABA signalling, whereas ABA catabolism and GA synthesis gene expression increased. However, during the low-dormancy phase in the summer, expression of transcripts for the DELLA germination repressors RGA and RGL2 increased. Unlike deep winter dormancy, this repression can be removed on exposure to light, enabling the completion of germination at the correct time of year (Footitt *et al*. 2011). This unique study presented a comprehensive overview of transcript abundance changes related to dormancy during the year and can serve as a reference point to identify the regulators of these changes and their relation with environmental changes. One of these regulators could be the circadian clock, which was shown to be required for the response to signals that release seed dormancy (Penfield & Hall 2009).

Apart from temperature and humidity, allelochemicals represent another environmental factor affecting seed dormancy levels in the soil. Allelopathy is defined as a direct or indirect interaction, whereby allelochemicals released by one organism influence the physiological processes of another neighbouring organism. *Nicotiana attenuata* (wild tobacco) is a post-fire annual plant that germinates from seed banks in response to smoke cues from wildfires. On the other hand, ABA and four terpenes leaching from the litter of the dominant vegetation can induce dormancy of *N. attenuata* seeds (Krock *et al*. 2002; Preston, Betts & Baldwin 2002; Linkies & Leubner-Metzger 2012). ABA leaching from litter into the soil has been proposed to be an allelochemical that affects germination and determines species composition of forests (Zhao *et al*. 2011). Environmentally and hormonally controlled production of ROS can act directly by cell-wall polysaccharide scission or interact with ABA signalling to mediate seed dormancy release and after-ripening (e.g. Oracz *et al*. 2007; El-Maarouf-Bouteau & Bailly 2008; Müller *et al*. 2009b; Graeber *et al*. 2010; Bazin *et al*. 2011; Leymarie *et al*. 2012). The putative allelochemical myrigalone A (MyA) of *Mygrica gale* interferes with GA biosynthesis and apoplastic ROS production required for embryo expansion (Oracz *et al*. 2012). MyA also inhibits endosperm weakening, demonstrating that allelochemicals may have several targets to prevent germination and seedling establishment.

CONSERVATION OF SEED DORMANCY MECHANISMS BETWEEN SPECIES

Physiological dormancy is present among species distributed over the entire phylogenetic tree of gymnosperms, basal angiosperms, monocots and eudicots (Baskin & Baskin 2004; Finch-Savage & Leubner-Metzger 2006). Recent advances in unravelling the molecular mechanisms underlying dormancy have mainly been based on the model plant *Arabidopsis* (belonging to the rosid clade of the core eudicots). In this section, we will compare the knowledge of dormancy mechanisms between species with the aim to identify evolutionary conserved mechanisms.

The conserved role of seed structure in dormancy mechanisms

It has been proposed that the seed envelopes and especially the endosperm play pivotal roles in regulating dormancy and that the seed envelopes resistance can be seen as a dormancy mechanism in many species (Finch-Savage & Leubner-Metzger 2006; Linkies *et al*. 2010). Testa structure, colour and permeability can influence dormancy status and germination behaviour as revealed by testa mutant studies in *Arabidopsis* (Debeaujon *et al*. 2000) and heterogeneous *Sisymbrium officinale* (Brassicales) seeds with different testa properties (Iglesias-Fernández *et al*. 2007). In *Arabidopsis*, the testa (Debeaujon *et al*. 2000) and the endosperm (Bethke *et al*. 2007) were shown to be the primary determinant tissues of seed dormancy, and, also, for *Medicago trunculata* (Fabales), the importance of the endosperm as a

main factor controlling dormancy is evident (Bolingue *et al*. 2010).

The influence of ABA on dormancy could be at least partially mediated by seed envelope tissues such as the endosperm since it was shown that endosperm rupture is inhibited by ABA in the related Brassicales species *Arabidopsis* and *L. sativum.* In the latter, the actual mechanical weakening of the endosperm is regulated by ABA (Müller *et al*. 2006; Linkies *et al*. 2009; Graeber *et al*. 2010) as is the case with the Gentianales species *Coffea arabica* (coffee; Silva *et al*. 2004). In the Solanales *Solanum lycopersicum* (tomato), it was shown that endosperm weakening is inhibited in dormant seeds but weakening occurs in non-dormant as well as ABA-deficient seeds (Groot & Karssen 1992). Endosperm weakening is also inhibited by ABA in the wild tomato relative *Solanum lycocarpum* (Wolf Apple) (Pinto *et al*. 2007).

Seed envelope properties such as pericarp colour are associated with seed dormancy in monocots like wheat (Gfeller & Svejda 1960; Himi *et al*. 2002) and weedy rice (Gu, Chen & Foley 2003). Recently, a weedy red rice dormancy QTL was identified (*SD7-1/Rc*) as a basic helix-loop-helix transcription factor which is likely orthologous to the *Arabidopsis* gene *TRANSPARENT TESTA 8*. *SD7-1/Rc* controls ABA synthesis, thereby influencing red pericarp colour and seed dormancy (Gu *et al*. 2011). The role of monocot seed envelopes in controlling dormancy and germination was highlighted by a study of Barrero *et al*. (2009) who showed that changes in ABA catabolism as well as ABA sensitivity in the barley coleorhiza (a radicle covering layer) are responsible for dormancy breaking by after-ripening. They suggested a role of the coleorhiza in dormancy regulation similar to that of the dicots' endosperm. Further evidence for the importance of seed envelopes in the regulation of germination and dormancy in monocots came from rice where Fujino *et al*. (2008) showed that a low-temperature germination QTL (qLTG3-1), encoding a protein with unknown function, shows promoter activity in embryo-covering tissues during germination. The expression of qLTG3-1 is highly correlated with the vacuolation of this tissue, which has been suggested to be involved in its weakening. Transcriptomic analysis of this rice QTL suggested a role for programmed cell death (PCD)-related genes in vacuolation and weakening of seed tissues during germination (Fujino & Matsuda 2010). Strikingly, a recent tissue-specific transcriptomic study of germinating *Arabidopsis* seeds by Endo *et al*. (2012) also identified a cell death-related gene to be strongly expressed in the endosperm and associated with vacuolation prior to germination (Bethke *et al*. 2007). Also in the tomato endosperm PCD events have been observed during seed imbibition (DeBono & Greenwood 2006).

The phylogenetically broadly conserved role of seed envelopes in dormancy control is underscored by the association between weakening of embryo surrounding tissues and dormancy breaking in the gymnosperm *Chamaecyparis nootkatensis* (yellow-cedar) (Ren & Kermode 1999).

ABA

ABA is a positive regulator of dormancy in many species (Kucera *et al*. 2005). The involvement of ABA metabolism in dormancy regulation of a phylogenetically broad range of species was already evident from early studies on rosids (Koornneef *et al*. 1982), asterids (Groot & Karssen 1992) and monocots (Tan *et al*. 1997) where mutations of ABA biosynthesis genes showed reduced dormancy of freshly harvested seeds. These initial findings were further supported by studies in a broad range of species as depicted below. Especially, the 9-cis-epoxycarotenoid dioxygenase (NCED) genes involved in ABA biosynthesis and the ABA-8′-hydroxylase (CYP707A) genes involved in ABA degradation seem to have universal roles (Nambara *et al*. 2010).

Asterids

Well-studied Solanales seeds include tobacco (*Nicotiana* spp.) and tomato, which differ in their seed structure and germination morphology (Petruzzelli *et al*. 2003). In these species, manipulation of seed ABA content by genetic modification can affect dormancy as shown in tobacco where overexpression of an endogenous zeaxanthin epoxidase (encoding an enzyme involved in ABA biosynthesis) resulted in increased dormancy, whereas down-regulation led to a less dormant phenotype (Frey *et al*. 1999). Furthermore, changes in endogenous ABA content during imbibition of tobacco seeds have been shown to be important for their dormancy status (Grappin *et al*. 2000). In agreement with this, induced expression of the *Phaseolus vulgaris* (common bean, Fabales) *PvNCED1* gene in imbibed tobacco seeds delayed seed germination (Qin & Zeevaart 2002). Also in tomato *NCED* genes play a role in dormancy induction since overexpression of endogenous *NCED1* led to enhanced dormancy due to elevated ABA levels (Thompson *et al*. 2000), whereas the ABA-deficient *sitiens* mutant of tomato is non-dormant and has a thinner testa (Hilhorst & Downie 1996). ABA synthesis is also necessary for the imposition and maintenance of embryo dormancy in the Asterales species *Helianthus annuus* (sunflower) (Le Page-Degivry & Garello 1992).

Monocots

Identification of the *Zea mays* (maize) viviparous mutants indicated an involvement of ABA synthesis in control of dormancy in cereals (McCarty 1995). Tan *et al*. (1997) showed that a maize *NCED* gene is affected by the viviparous mutation *vp14*. In the related Poales species *Hordeum vulgare* (barley), a high ABA content in dormant imbibed seeds contrasts the low ABA content in non-dormant (after-ripened) seeds (Jacobsen *et al*. 2002). Furthermore, Leymarie *et al*. (2008) found that in barley, the *HvNCED1* and *HvNCED2* genes are involved in ABA-mediated primary and secondary dormancy. In *Oryza sativa* (rice), a transposon-induced mutation in a zeaxanthin epoxidase

gene showed a viviparous (non-dormant) phenotype indicating that ABA synthesis is also important for rice dormancy (Agrawal *et al*. 2001). Also in the grass model system *Brachypodium distachyon* (Poales) higher ABA levels were observed in dormant compared to after-ripened imbibed grains and the endogenous *BdNCED1* gene showed higher expression in dormant compared to after-ripened imbibed grains (Barrero *et al*. 2012). ABA degradation was also found to play an important role in dormancy regulation in cereals. Jacobsen *et al*. (2002) showed that ABA content decreased and was metabolized to phaseic acid upon imbibition in non-dormant barley grains, whereas ABA content remained high in dormant imbibed grains. Millar *et al*. (2006) showed that a barley CYP707A gene (*HvABA8'OH-1*) was expressed much higher in non-dormant compared to dormant grains during imbibition and, strikingly, the expression was localized to the coleorhiza, highlighting the importance of seed tissue interactions in dormancy regulation. In agreement with this, transgenic manipulation of ABA catabolism by RNAi-mediated down-regulation of *HvABA8'OH-1* increased ABA levels and dormancy in barley (Gubler *et al*. 2008). ABA leaching of the embryo also appears to be an important mechanism of cereal dormancy and germination (Visser *et al*. 1996; Suzuki *et al*. 2000) and is also known from the Caryophyllales species *Beta vulgaris* (sugar beet) (Hermann *et al*. 2007).

Rosids

ABA metabolism genes like *NCED* and *CYP707A* have been extensively studied in the Brassicales model plant *Arabidopsis* (Nambara & Marion-Poll 2005). Similar to other species, *Arabidopsis NCED* genes have been found to be involved in seed dormancy (Nambara *et al*. 2010; Frey *et al*. 2011). Recently, also in *Arabidopsis*, it could be confirmed that transgenic manipulation of ABA synthesis by up-regulating endogenous *NCED6* during seed imbibition leads to increased dormancy (Martinez-Andujar *et al*. 2011). Changes in ABA metabolism during dormancy loss resulting in a low ABA content in imbibed seeds have also been described for the Fagales species *Fagus sylvatica* (beech) (Le Page-Degivry, Garello & Barthe 1997).

Gymnosperms

ABA metabolism is also known to be involved in dormancy control in gymnosperm species (Kermode 2005). Appropriate dormancy-breaking treatments (such as cold stratification) were accompanied by decreased ABA levels in seeds of *Pinus monticola* (western white pine; Feurtado *et al*. 2004), *Pseudotsuga menziesii* (Douglas fir; Corbineau *et al*. 2002) and yellow-cedar (Schmitz, Abrams & Kermode 2002).

The above-mentioned studies showed that ABA is a necessary and universal seed dormancy factor in many plant species. However, the role of ABA as a sufficient factor in the maintenance of physiological dormancy seems controversial since no clear relationship between the ABA content of mature dry seeds and dormancy intensity exists in barley (Dunwell 1981; Boivin, Kohl & Clamagirand 1995), *Avena* spp. (oats) (Berrie *et al*. 1979) and *Triticum aestivum* (wheat) (Walker-Simmons 1987; Walker-Simmons & Sesing 1990). Furthermore, Goggin *et al*. (2009) showed for *Lolium rigidum* (annual ryegrass, Poales) that the dormancy state of the imbibed seed did not correlate with ABA content but with ABA sensitivity. Moreover, Gianinetti & Vernieri (2007) found no direct correlation of ABA content with the dormancy status of seeds from the weedy species *Oryza sativa f. spontanea* (red rice) but a higher sensitivity of dormant seeds to ABA. A role for ABA sensitivity in dormancy regulation was also evident for gymnosperm seeds of Douglas fir (Corbineau *et al*. 2002) and yellow cedar (Schmitz *et al*. 2002).

Thus, ABA signalling as well as ABA metabolism seems important for dormancy regulation in diverse species. Highlighting a role for ABA signalling, both mutant (Kawakami, Miyake & Noda 1997) and QTL mapping approaches (Noda *et al*. 2002) in hexaploid bread wheat have shown that ABA sensitivity is important for the acquisition of dormancy. In addition, Nakamura, Komatsuda & Miura (2007) showed that the position of wheat homologues of *Arabidopsis* ABA signalling genes in a diploid wheat genome correlates with identified wheat dormancy QTL positions, and, in a comparative study of seed dormancy QTLs of wheat and rice, an ABA perception-related candidate gene was identified (Somyong *et al*. 2011). PP2C family members are known factors involved in ABA signalling and have been shown to play a role in dormancy in *Arabidopsis* (reviewed in Finkelstein *et al*. 2008). Also in beech evidence has been gathered for the involvement of ABA signal transduction via PP2C in the regulation of dormancy (reviewed in Rodríguez-Gacio, Matilla-Vázquez & Matilla 2009).

In agreement with ABA sensitivity regulating dormancy, early studies in *Arabidopsis* (Koornneef *et al*. 1984) and maize (Hattori *et al*. 1992) provided evidence that ABA signal transduction factors may be involved in a conserved dormancy mechanism. Various studies have been conducted on the ABI3/VP1 transcription factor which is highly conserved among plants and plays a role in dormancy regulation from Brassicaceae to gymnosperms (reviewed in Kermode 2005; Graeber *et al*. 2010). Recently, the *Arabidopsis* gene *SUPPRESSOR OF ABI-3* (*SUA*) has been shown to encode a splicing factor that controls alternative splicing of *ABI3* (Sugliani *et al*. 2010). Alternative splicing of *ABI3* homologues was also reported in monocots, where it leads to the production of non-functional truncated protein from mis-spliced transcripts, which is often linked to reduced seed quality including shallow dormancy (McKibbin *et al*. 2002; Fan *et al*. 2007). Although there is yet no proof for any developmental regulation of splicing in these examples, it is tempting to speculate that regulated alternative splicing can play a conserved role in seed maturation and dormancy. This hypothesis is supported by the splicing

variants of *ABI3* whose ratio appears to be developmentally regulated (Sugliani *et al*. 2010).

Dormancy-specific genes

The spatio-temporal regulation of ABA metabolism and signalling as a dormancy mediator acting through the control of seed envelope properties seems to be of utmost importance for dormancy and germination control in many species as outlined above. Seeds integrate developmental and environmental signals to determine their dormancy status. Recently, several dormancy influencing genes have been identified in different species which might act at this level of integration. The functions of these genes as well as their possible participation in a conserved dormancy mechanism yet remain to be determined. The *DOG1* gene has been identified as a major dormancy gene in *Arabidopsis* (Bentsink *et al*. 2006). Besides evidence for the presence of putative orthologous *DOG1* genes in the Brassicaceae species *L. sativum* and *Brassica rapa* (Graeber *et al*. 2010), it is still unclear whether *DOG1* is part of a conserved dormancy mechanism in a broad phylogenetic spectrum of species. Interestingly, both *Arabidopsis* and *B. rapa DOG1* promoter regions contain a RY repeat (Graeber *et al*. 2010) required for ABI3/VP1-mediated expression (Nambara *et al*. 2010) indicating a possible *DOG1* regulation by a highly conserved dormancy factor.

The *Sdr4* gene was identified as a dormancy QTL in rice (Sugimoto *et al*. 2010). Although putative homologues of *Sdr4* were identified in *Arabidopsis* based on sequence similarity, none of them was correlated with a dormancy function. Strikingly, the *Sdr4* promoter also contains RY repeats and its expression is controlled by OsVP1 highlighting its putative involvement in a conserved dormancy mechanism.

MFT has been implicated in regulation of germination and dormancy in diverse species as mentioned earlier. MFT belongs to the plant phosphatidylethanolamine-binding protein (PEBP) family which forms three clades, FLOWERING LOCUS T (FT)-like, TERMINAL FLOWER1 (TFL1)-like and MFT-like (Fig. 3a; Chardon & Damerval 2005). MFT-like genes are present in a broad phylogenetic spectrum from mosses to angiosperms (Fig. 3b) and seem ancestral to FT-like and TFL1-like (Hedman, Källman & Lagercrantz 2009; Pin & Nilsson 2012). In *Arabidopsis*, MFT has been identified as an ABA-induced negative regulator of ABA signalling that promotes embryo growth in germinating seeds (Xi *et al*. 2010). In addition, *MFT* expression is highly correlated with dormancy cycling in *Arabidopsis* (Footitt *et al*. 2011). As described above, a wheat *MFT* homolog has been proposed to be involved in dormancy induction (Nakamura *et al*. 2011). Moreover, MFT-like genes have been associated with seed development since they were found specifically expressed in seeds in different cereals (Danilevskaya *et al*. 2008) and *Populus nigra* (poplar, Malpighiales) (Igasaki *et al*. 2008). In gymnosperms, *MFT* has been associated with seed maturation and dormancy induction due to its expression being ABA inducible and confined to the embryo (Karlgren *et al*. 2011). Although the precise action of MFT needs to be clarified, it will be of great interest to determine its involvement in dormancy and germination regulation in different species due to its high conservation among plant species (Fig. 3b) and its close phylogenetic relationship to genes controlling developmental phase transition like FT, which is also known to be involved in the control of bud dormancy (Cooke *et al*. 2012; Pin & Nilsson 2012)

CONCLUSIONS AND OUTLOOK

The last years have seen a marked progress in our understanding of the molecular mechanisms that regulate the induction, maintenance and release of seed dormancy. Major advances have been achieved in the unravelling of hormonal pathways determining dormancy and in the interactions between these pathways. In addition, the existence of important dormancy regulators that are not directly part of the hormonal pathways is becoming increasingly clear. A number of essential and specific dormancy genes have been identified, as well as chromatin organizers that influence seed dormancy induction. The release of dormancy has long been a black box, but the recent recognition of the important contribution of non-enzymatic processes, like oxidation, in the after-ripening process is starting to reveal the mechanisms behind this process. The last decade has also seen intense research efforts on changes that occur during dormancy at the transcript, protein and metabolite level. These 'omic' approaches have not yet led to major novel insights, but they enriched and supported our understanding of the dormancy mechanisms. In addition, they generated a wealth of data that will be very helpful for further studies.

Despite the progress of the last years, several major questions remain unanswered. It is for instance largely unknown how temperatures are sensed during stratification. Even more importantly, the true molecular identity of dormancy is not understood yet. Many factors influencing dormancy have been described, but it is not known for most of them how they interact and relate with each other. It is also not known whether the molecular nature of dormancy consists of a combination of many different factors or whether a central agent exists that functions downstream of all these factors. Such a hypothetical central agent, or dormancy molecule, could be named 'dormagen' analogous to the flowering-inducing molecule 'florigen' (Turck, Fornara & Coupland 2008).

The research on seed dormancy is moving fast at the moment and major 'break-throughs' in dormancy research can be expected within the coming years. Although the final answers to the above-mentioned questions will probably still take time, more immediate progress is expected in several areas. Recently identified major dormancy genes like *DOG1* and *Sdr4* encode genes with unknown function. The revealing of these functions as well as the understanding of the role of MFT can soon be expected and will lead to better insights in the dormancy mechanism. In addition, we

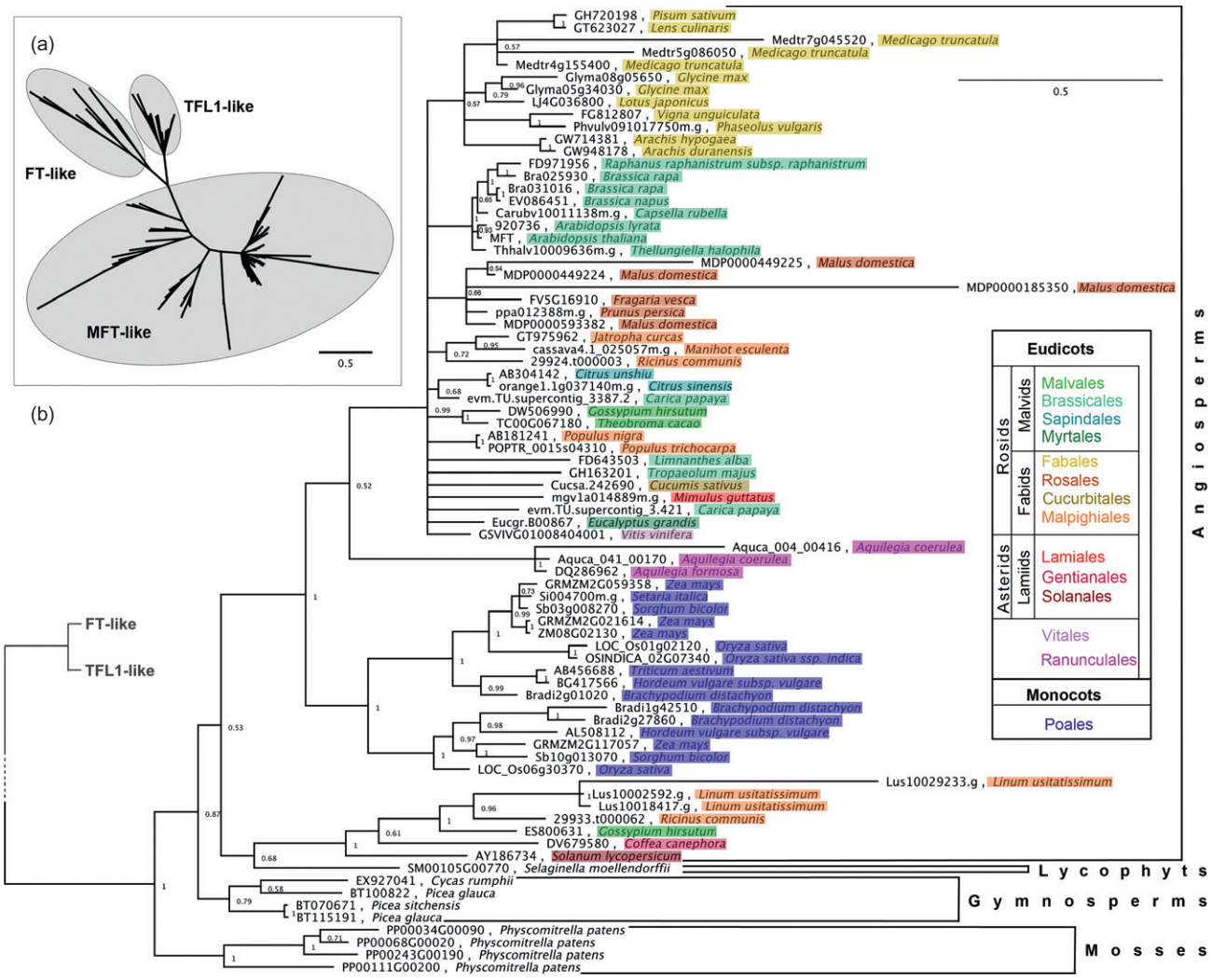

Figure 3. Phylogenetic analysis of MFT-like proteins. (a) Unrooted Bayesian tree topology of plant PEBP proteins indicating the three main clades FT-like, TFL1-like and MFT-like. (b) Detailed sub-tree of the MFT-like clade shown in (a) illustrating the broad phylogenetic distribution of MFT-like proteins among plants. Given are sequence accession numbers/gene names (Phytozome, PLAZA, Genebank) and the respective species names. Angiosperm species are colour coded to the order level as indicated. Node labels depict posterior probabilities from the Bayesian inference analysis. Scale bar shows substitutions per site. Sequence data were obtained by querying comparative genomic databases Phytozome and PLAZA with *Arabidopsis* MFT sequence and combined with previously identified MFT-like sequences for species not included in these databases from Hedman *et al.* (2009), Karlgren *et al.* (2011) and Nakamura *et al.* (2011). This data set (Phytozome family #31632823, PLAZA orthogroup ortho002920 and literature derived MFT-like sequences) contained 99 sequences from 52 species. The deduced amino acid sequences were aligned together with *Arabidopsis* FT and TFL1 sequences [to be able to clearly identify MFT-like genes as depicted in (a)] using MUSCLE (Edgar 2004). Phylogenetic analysis by Bayesian Inference was performed using MrBayes 2.0.3 (Huelsenbeck & Ronquist 2001). Analysis was based on Jones rate matrix allowing heterogeneity between site (four gamma rate categories) and priors set to defaults. Two parallel Markov runs each with four heated chains starting from random trees were analysed for 1.5 million generations, sampling every 1000th generation disregarding the first 150 000 steps as burn in.

expect that the after-ripening process will soon be further unravelled at the molecular level. This progress should also lead to the development of molecular markers that constitute a reliable readout for seed dormancy levels.

We can also expect an increasing role for systems approaches in the dissection of the mechanisms controlling dormancy. Bassel *et al.* (2011b) recently developed a network model of global transcriptional interactions during dormancy and germination (Seednet). This work demonstrated a possible evolutionary adaptation of existing transcriptional pathways (regulating cellular phase transition and abiotic stress) to effect seed dormancy. Further investigations in this direction promise novel insights. Furthermore, improved methods to extract information from existing large-scale data sets will lead to the identification of new genes and mechanisms as has for instance been recently shown by Bassel *et al.* (2011a), who identified novel regulators of seed germination using

co-prediction to compute a functional gene interaction network (ScoPNet).

The general mechanisms of seed dormancy, in particular the role of the envelopes and ABA, are highly conserved among plant species, but it is still not known whether dormancy-specific genes show a similar level of conservation or if they constitute species-specific adaptations. We expect increasing activities at the cross-species level, since dormancy is a highly complex and adaptive trait, and, thus, analysis of only one species might not lead to the discovery of the essential underlying mechanisms that control dormancy. Advances in molecular technologies, especially next-generation sequencing, will make it possible to study non-model species in-depth at the molecular level. The choice of the species to study may not depend anymore on their establishment as model species but rather whether it contains a dormancy mechanism of scientific interest.

ACKNOWLEDGMENTS

We thank Maarten Koornneef for critical reading of the manuscript and Klaus Mummenhoff for helpful advise on phylogenetic analysis. The work in our labs was supported by the Deutsche Forschungsgemeinschaft (grants no. LE720/7 to GLM and SO691/3-1 and SFB 572 to WJJS) and the Max Planck Society (to WJJS), which is gratefully acknowledged.

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Received 14 February 2012; received in revised form 8 May 2012; accepted for publication 14 May 2012