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Review

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Seed dormancy and the control of germination

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Summary

Seed dormancy is an innate seed property that defines the environmental conditions in which the seed is able to germinate. It is determined by genetics with a substantial environmental influence which is mediated, at least in part, by the plant hormones abscisic acid and gibberellins. Not only is the dormancy status influenced by the seed maturation environment, it is also continuously changing with time following shedding in a manner determined by the ambient environment. As dormancy is present throughout the higher plants in all major climatic regions, adaptation has resulted in divergent responses to the environment. Through this adaptation, germination is timed to avoid unfavourable weather for subsequent plant establishment and reproductive growth. In this review, we present an integrated view of the evolution, molecular genetics, physiology, biochemistry, ecology and modelling of seed dormancy mechanisms and their control of germination. We argue that adaptation has taken place on a theme rather than via fundamentally different paths and identify similarities underlying the extensive diversity in the dormancy response to the environment that controls germination.

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I. Introduction

Seed dormancy could be considered simply as a block to the completion of germination of an intact viable seed under favourable conditions, but earlier reviews concluded that it is one of the least understood phenomena in the field of seed biology (Hilhorst, 1995; Bewley, 1997a). In the decade since these reviews, there has been a large volume of published work and significant progress has been made in understanding seed dormancy, which we overview below. However, there have also been some potential sources of confusion that have been reported in the literature. In ecological studies, there has been confusion reported between seed dormancy and persistence in soil (Thompson *et al.*, 2003; Fenner & Thompson, 2005; Walck *et al.*, 2005). This has resulted in part from different views on dormancy, such as whether light terminates dormancy or induces germination. In the physiological literature much of the research has adopted a molecular genetic approach using model species such as *Arabidopsis thaliana*, Solanaceae and cereals (reviewed in Koornneef *et al.*, 2002; Gubler *et al.*, 2005; Kucera *et al.*, 2005), but these model species and the ecotypes used often have only a shallow dormancy (discussed by Hilhorst, 1995; Cohn, 1996). A further potential source of confusion is that a clear description/definition of the type of dormancy studied has not always been provided (Baskin & Baskin, 2004). It has also been observed that, although physiologists and ecologists have both studied dormancy, there has been 'little cross fertilization between disciplines' (Krock *et al.*, 2002). In this review we discuss these issues, and present an integrated view across the evolution, molecular genetics, physiology, biochemistry, modelling and ecophysiology of the control of seed germination by dormancy in an attempt to draw together these linked, but often separate disciplines.

Note that, in addition, a section on applied aspects of the control of seed germination by dormancy is available as supplementary material.

II. What is Dormancy and How is it Related to Germination?

1. Seed structure and seed germination

A completely nondormant seed has the capacity to germinate over the widest range of normal physical environmental factors possible for the genotype (Baskin & Baskin, 1998, 2004). Besides the basic requirement for water, oxygen and an appropriate temperature, the seed may also be sensitive to other factors such as light and/or nitrate. Germination commences with the uptake of water by imbibition by the dry seed, followed by embryo expansion. The uptake of water is triphasic with a rapid initial uptake (phase I, i.e. imbibition) followed by a plateau phase (phase II). A further increase in water uptake (phase III) occurs as the embryo axis elongates and breaks through the covering layers to complete germination (e.g. Schopfer & Plachy, 1984; Manz *et al.*, 2005). In typical angiosperm seeds (Figs 1, 2) the embryo is surrounded by two covering layers: the endosperm and testa (seed coat). Cell elongation is necessary and is generally accepted to be sufficient for the completion of radicle protrusion (visible germination) (Bewley, 1997a; Kucera *et al.*, 2005).

2. Definition of seed dormancy

In the Introduction we stated as a simple operational definition that seed dormancy is a block to the completion of germination of an intact viable seed under favourable conditions (Hilhorst, 1995; Bewley, 1997a; Li & Foley, 1997). This block to germination has evolved differently across species through adaptation to the prevailing environment, so that germination occurs when conditions for establishing a new plant generation are likely to be suitable (Hilhorst, 1995; Vleeshouwers *et al.*, 1995; Bewley, 1997a; Li & Foley, 1997; Baskin & Baskin, 2004; Fenner & Thompson, 2005). Therefore, a diverse range of blocks (dormancy mechanisms) have evolved, in keeping with the diversity of climates and habitats in which they operate. A more sophisticated and experimentally useful definition of dormancy has recently been proposed by Baskin & Baskin (2004): a dormant seed does not have the capacity to germinate in a specified period of time under any combination of normal physical environmental factors that are otherwise favourable for its germination, i.e. after the seed becomes nondormant. However, definitions of dormancy are difficult because dormancy can only be measured by the absence of germination. We can observe completion of germination of a single seed as an all-or-nothing event, whereas dormancy of a single seed can have any value between all (maximum dormancy) and nothing (nondormancy).

Dormancy should not just be associated with the absence of germination; rather, it is a characteristic of the seed that determines the conditions required for germination (Vleeshouwers *et al.*, 1995; Thompson, 2000; Fenner & Thompson, 2005). When dormancy is considered in this way, any environmental cue that alters the conditions required for germination is by definition altering dormancy. Also, by extension, when the seed no longer requires specific environmental cues it is nondormant. It is said by some researchers that only temperature can alter physiological dormancy (PD, defined in section II.3) following dispersal as dormancy cycles in the seed bank (reviewed by Probert, 2000). However, Krock *et al.* (2002) have demonstrated the induction of secondary dormancy in *Nicotiana attenuata* seeds by a naturally occurring chemical signal [abscisic acid (ABA) and four other terpenes] in leachate from litter that covers the seeds in their habitat. In addition, exogenous nitrate can affect the requirement for light to promote *A. thaliana* seed germination (Batak *et al.*, 2002), and their initial level of dormancy is influenced by the nitrate regime fed to the mother plant (Aloresi *et al.*, 2005). Therefore, nitrate affects the requirements

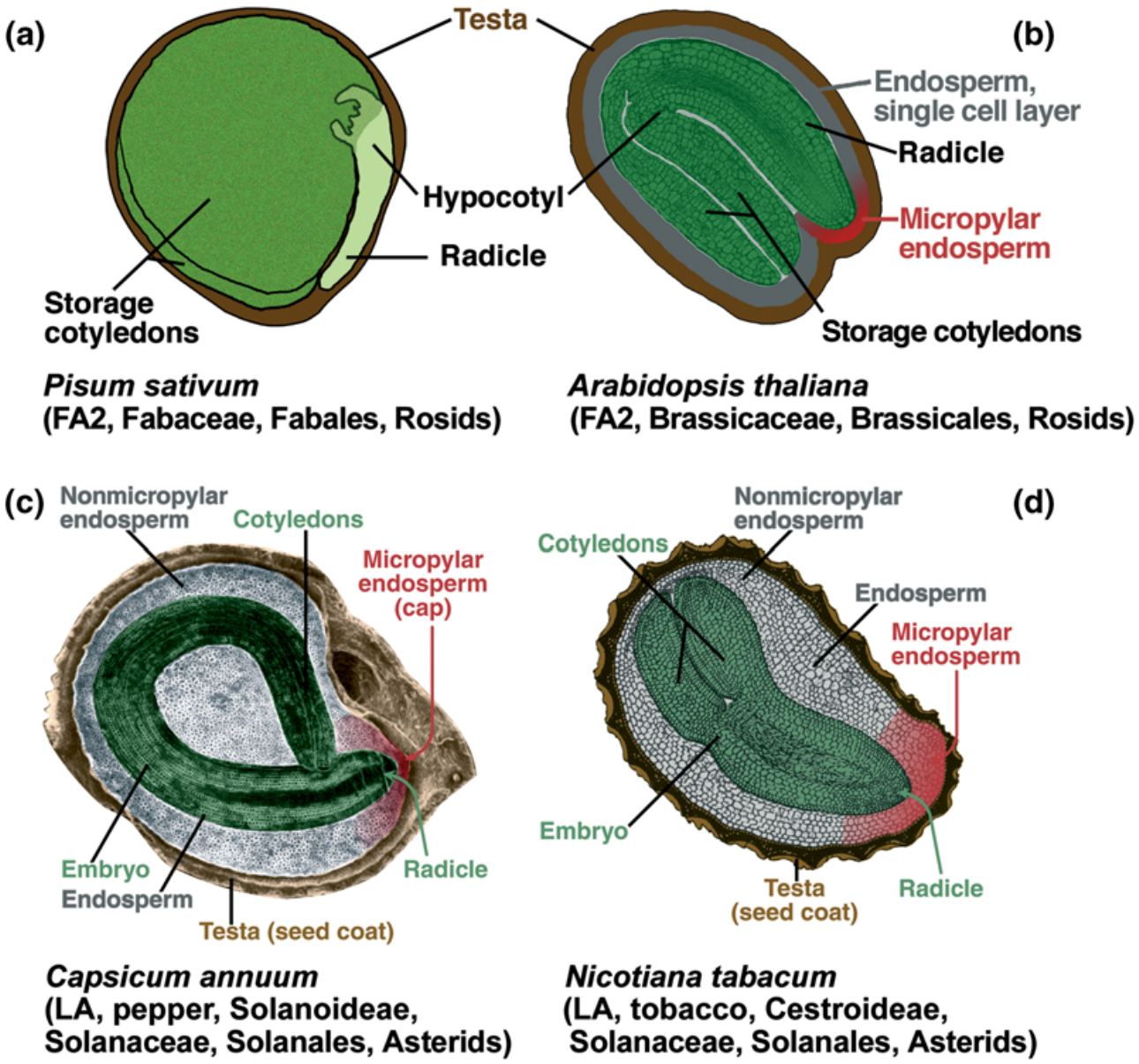

Fig. 1 Biodiversity of the structure of mature seeds of angiosperms and the importance of the seed-covering layers. The diploid embryo is surrounded by two covering layers: the triploid (in most species) endosperm (nutritive tissue; mostly living cells) and the diploid testa (seed coat; maternal tissue; mostly dead cells). In several species the endosperm is completely obliterated during seed development and the nutrients are translocated to storage cotyledons. Mature seeds of (a) pea (*Pisum sativum*) (without endosperm) and (b) *Arabidopsis thaliana* (single cell layer of endosperm) are characterized by embryos with storage cotyledons. The micropylar endosperm (several cell layers) is known to be a germination constraint of Solanaceae seeds (c, d). FA2 and LA are seed types (see Fig. 3). Part (c) is modified from Watkins & Cantliffe (1983) and reprinted with permission from the American Society of Plant Biologists. Parts (a), (b) and (d) are modified from 'The Seed Biology Place' (http://www.seedbiology.de).

for germination and so could be said to directly affect dormancy rather than just promote germination.

It is widely accepted that temperature regulates both dormancy and germination and that light regulates germination; however, it is a matter of debate whether light is also a regulator of dormancy (Bewley & Black, 1994; Vleeshouwers *et al.*, 1995; Casal & Sanchez, 1998; Pons, 2000; Baskin & Baskin, 2004; Fenner & Thompson, 2005; Kucera *et al.*, 2005). Light has been considered both to stimulate germination (e.g. Vleeshouwers *et al.*, 1995) and to terminate dormancy (e.g. Benech-Arnold *et al.*, 2000; Batlla *et al.*, 2004). To some extent, this depends on where one chooses to draw the line between the processes of dormancy and germination. In this review, we have used the definition above, that dormancy is a seed characteristic that defines the conditions required for germination, and therefore any cue that widens the environmental requirements for germination should be regarded as a dormancy release factor. Following this argument, exposure to

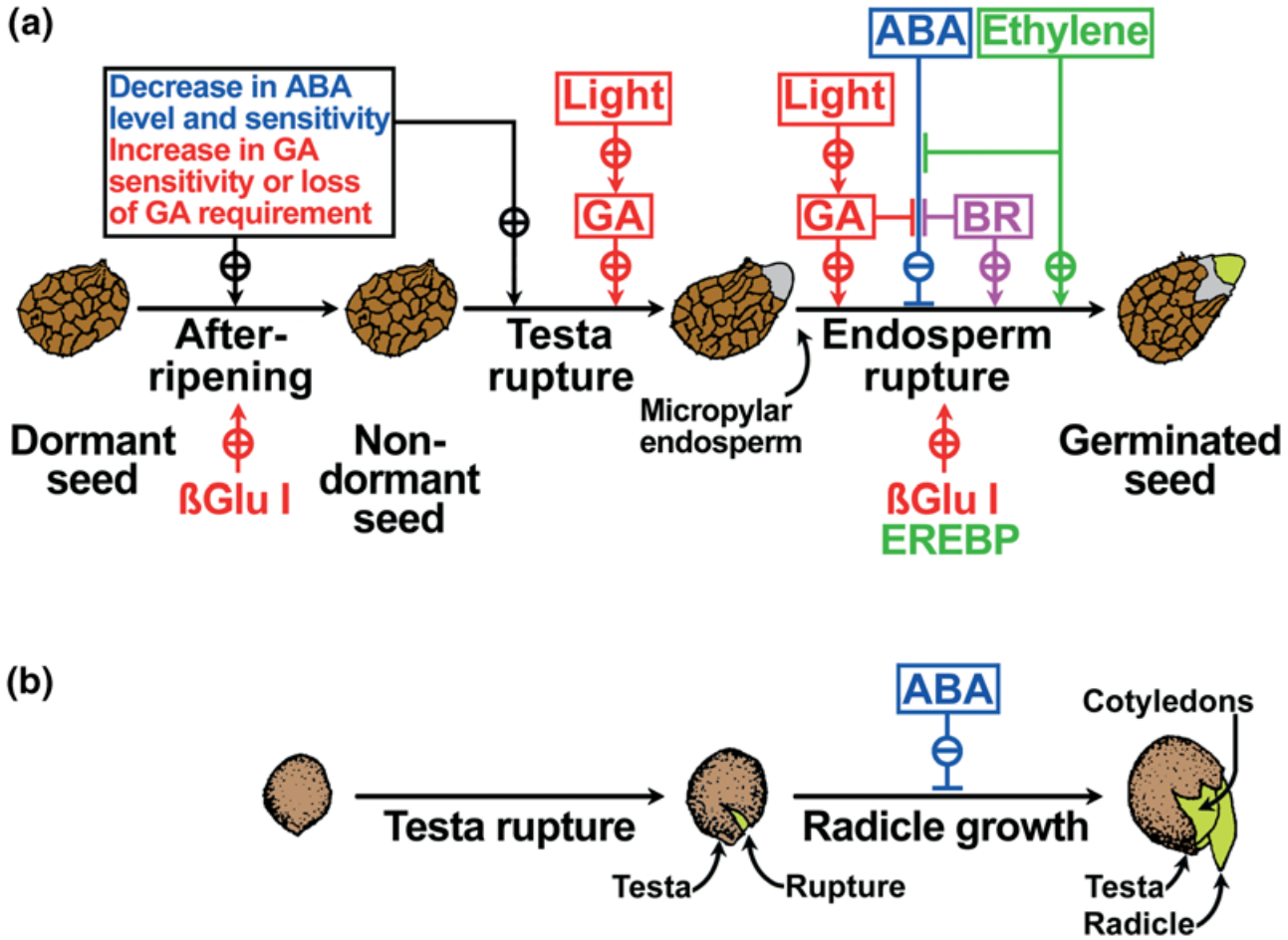

Fig. 2 Hormonal interactions during the regulation of seed dormancy release and germination of *Nicotiana* (a) and *Brassica* (b) model species. (a) *Nicotiana* sp. seed germination is two-step: testa rupture followed by endosperm rupture. Dormancy release and germination promotion occur during seed after-ripening (dry storage at room temperature for several months) or via the light-gibberellin (GA) pathway during imbibition. Abscisic acid (ABA) inhibits endosperm rupture but not testa rupture. GA, ethylene and brassinosteroids (BRs) promote endosperm rupture and counteract the inhibitory effects of ABA. The ABA-inhibited class I β-1,3-glucanase genes (*βGlu I*) are transcriptionally induced in the micropylar endosperm just before endosperm rupture (reviewed in Leubner-Metzger, 2003). This induction is highly localized in the micropylar endosperm at the site of radicle emergence. Light, GA and ethylene promote and ABA, osmotica and darkness inhibit βGlu I expression and endosperm rupture. This inhibition is partially reversed in transgenic tobacco (*Nicotiana tabacum*) seeds that over-express βGlu I in the seed-covering layers under the control of an ABA-inducible transgene promoter. βGlu I is therefore causally involved in the promotion of endosperm rupture. EREBP, ethylene-responsive element binding protein transcription factor. Part (a) is modified from Leubner-Metzger (2006) and reprinted with permission from The Haworth Press, Inc.; article copies are available from The Haworth Document Delivery Service: 1-800-HAWORTH; Email: docdelivery@haworthpress.com. (b) *Brassica napus* seed germination is one-step. The mature seeds of these species are without an endosperm and so testa rupture plus initial radicle elongation result in the completion of germination. ABA does not inhibit testa rupture, but inhibits subsequent radicle growth (Schopfer & Plachy, 1984; for a review, see Kucera *et al*., 2005).

light changes the seed so that it can germinate in darkness and is therefore the last step in the dormancy-breaking process, rather than the first step in the germination process (Bewley & Black, 1994; Pons, 2000; Leubner-Metzger, 2003). This light effect (red light via phytochrome) can also be reversed in some cases by far-red light, until the seed is committed to the process of germination (Casal & Sanchez, 1998; Sanchez & Mella, 2004). In seeds with coat dormancy, it is thought that light and gibberellins (GA) can both release (coat) dormancy and promote germination (e.g. Casal & Sanchez, 1998; Leubner-Metzger & Meins, 2001; Leubner-Metzger, 2001; Sanchez & Mella, 2004; Kucera *et al*., 2005).

A wide range of factors can therefore alter dormancy in PD seeds. However, there is an important distinction in the seed response to these factors. (1) There are factors that are related to slow seasonal change. These factors (e.g. temperature) are integrated over time to alter the depth of dormancy, and the sensitivity to other factors (e.g. light). (2) There are other factors that indicate in a more immediate way that conditions are suitable for germination (e.g. light), which could be considered to terminate dormancy and therefore induce germination. Each of these factors therefore removes successive blocks to germination, but this process usually needs to be carried out in a set order for it to work, i.e. in the process

described light must come last to be effective. There is recent evidence from global transcriptional analysis of dormant states that the successive blocks are associated with both quantitative and qualitative changes in gene expression programmes (Cadman *et al.*, 2006 and pers. comm.).

A generally accepted distinction made in dormancy studies is that of primary vs secondary dormancy. Freshly harvested mature water-permeable dormant seeds are said to have primary dormancy, which has been induced with the involvement of ABA during seed maturation on the mother plant (see section III.1; Hilhorst, 1995; Kucera *et al.*, 2005). Subsequent dormancy release 'in the field', following dispersal, may involve the same factors that are commonly used 'in the laboratory': either after-ripening in the relatively dry state (section III.2) or dormancy-release treatments in the imbibed state. These imbibed seed treatments include chilling (cold stratification), warm stratification, light, gibberellins and other hormones (Kucera *et al.*, 2005), smoke substances such as butenolide (Krock *et al.*, 2002; Flematti *et al.*, 2004) and compounds such as nitric oxide (Bailly, 2004; Bethke *et al.*, 2006). In contrast to primary dormancy, secondary dormancy can be induced in seeds with nondeep physiological dormancy (see section II.3) after seed dispersal, and is often associated with annual dormancy cycles in the seed bank (Baskin & Baskin, 1998, 2004; Hilhorst, 1998; Fenner & Thompson, 2005). Once primary dormancy is lost in response to prevailing environmental conditions, secondary dormancy will soon start to be induced if the conditions required to terminate dormancy and induce germination are absent (e.g. light and/or nitrate). Secondary dormancy can be lost and re-introduced repeatedly as seasons change until the required germination conditions become available (e.g. through soil disturbance). These changes in the depth of dormancy are gradual. Seeds in the intermediate states are said to have conditional or relative dormancy because the range of environmental factors that are permissive to germination is limited. Thus as dormancy is lost the germination window (permissive range of environments) progressively opens and then closes as dormancy is induced to a deeper state. This is a fluid process, and induction to a deeper state can occur at any point during dormancy loss in response to a change in the environment. In summary, seed dormancy is an innate seed property, which defines the environmental conditions that must be met before the seed can germinate. The intrinsic molecular mechanisms that determine dormancy have an embryo and/or a coat component (see section III). However, dormancy itself is a 'whole seed' characteristic that controls germination and can be classified as described in the following section (section II.3).

3. A classification system for seed dormancy

Marianna G. Nikolaeva devised a dormancy classification system reflecting the fact that dormancy is determined by both morphological and physiological properties of the seed (Nikolaeva, 1967, 2004). Based on this scheme, Baskin & Baskin (1998, 2004) have proposed a comprehensive classification system which includes five classes of seed dormancy: physiological (PD), morphological (MD), morphophysiological (MPD), physical (PY) and combinational (PY + PD). The system, which is summarized below, is hierarchical, with these five classes further divided into levels and types.

(1) **Physiological dormancy (PD)** PD is the most abundant form and is found in seeds of gymnosperms and all major angiosperm clades (Figs 3, 4). It is the most prevalent dormancy form in temperate seed banks and the most abundant dormancy class 'in the field'. PD is also the major form of dormancy in most seed model species 'in the laboratory', including *A. thaliana*, *Helianthus annuus*, *Lactuca sativa*, *Lycopersicon esculentum*, *Nicotiana* spp., *Avena fatua*, and several cereals. PD is therefore the major focus of this review. PD can be divided into three levels: deep, intermediate and nondeep (Baskin & Baskin, 2004).

PD deep. Embryos excised from these seeds either do not grow or will produce abnormal seedlings; GA treatment does not break their dormancy, and several months of cold (subtype a) or warm (subtype b) stratification are required before germination can take place (Baskin & Baskin, 2004; Baskin *et al.*, 2005). Examples: *Acer platanoides* (PD deep) and *Acer pseudoplatanus* (PD intermediate) (Aceraceae, Finch-Savage *et al.*, 1998).

PD nondeep. The great majority of seeds have nondeep PD (Baskin & Baskin, 2004). Embryos excised from these seeds produce normal seedlings; GA treatment can break this dormancy and, depending on species, dormancy can also be broken by scarification, after-ripening in dry storage, and cold or warm stratification. Based on patterns of change in physiological responses to temperature, five types of nondeep PD can be distinguished. Most seeds belong to type 1 or 2, in which the temperature range at which seed germination can occur increases gradually during the progression of nondeep dormancy release from low to higher (type 1, e.g. *A. thaliana*) or from high to lower temperature (type 2). In addition, the sensitivity of the seeds to light and GA increases as nondeep PD is progressively released.

(2) **Morphological dormancy (MD)** MD is evident in seeds with embryos that are underdeveloped (in terms of size), but differentiated (e.g. into cotyledons and hypocotyl-radical). These embryos are not (physiologically) dormant, but simply need time to grow and germinate. Example: celery (*Apium graveolens*) (Fig. 3, Apiaceae; Jacobsen & Pressman, 1979).

(3) **Morphophysiological dormancy (MPD)** MPD is also evident in seeds with underdeveloped embryos, but in addition they have a physiological component to their dormancy (Baskin & Baskin, 2004). These seeds therefore require a dormancy-breaking treatment, for example a defined combination of warm and/or cold stratification which

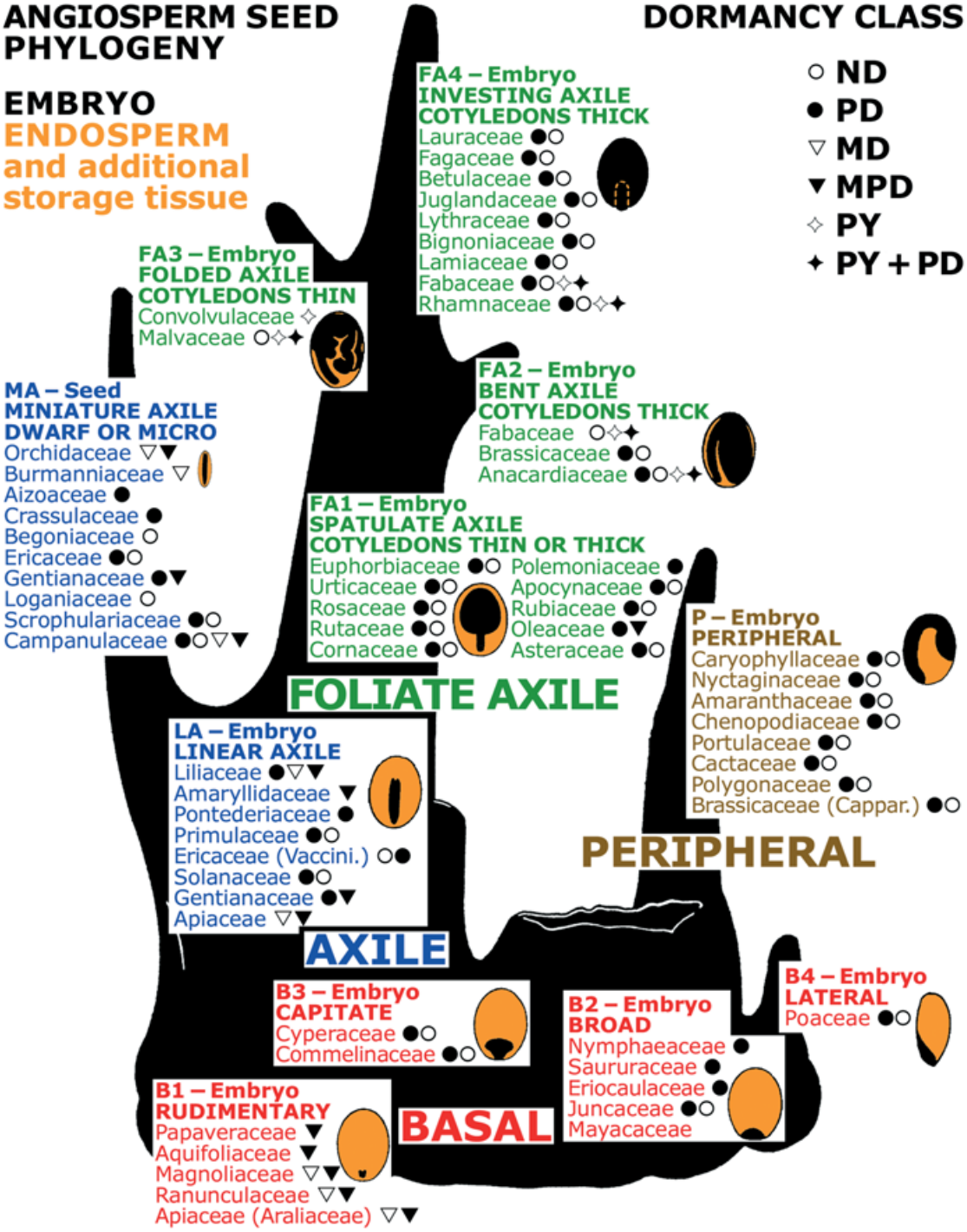

Fig. 3 Seed phylogenetic tree based on the internal morphology of the embryo and endosperm in mature angiosperm seeds. Martin (1946) investigated the embryo (form, size and position) and endosperm (plus additional storage tissue) in 1287 species and proposed seed types (B1 to B4, LA, P, MA, and FA1 to FA4). Seed types with abundant endosperm (orange or grey) and a tiny embryo (black) are basal (B1, B2, B3 and B4). In the more advanced endospermic LA-type seeds, the embryo is linear axile. From this developed the FA-type seeds (FA1, FA2, FA3 and FA4) where the embryo is foliate axile and, depending on the subtype, differs in shape and occupies more or less the entire seed. Mature FA-type seeds have little or no endosperm, and the nutrients are stored in the cotyledons. The evolution of whole seed size (e.g. dwarf seeds; MA) is beyond the scope of this review and is discussed elsewhere (e.g. Baskin & Baskin, 1998, 2005; Fenner & Thompson, 2005). Seed dormancy classes are indicated next to each family name: nondormancy (ND, open circles), physiological dormancy (PD, closed circles), morphological dormancy (MD, open triangles), morphophysiological dormancy (MPD, closed triangles), physical dormancy (PY, open diamonds), and combinational dormancy (PY + PD, closed diamonds) are explained in the text. The figure is updated and modified from Martin (1946) based on work by Baskin & Baskin (1998, 2004, 2005, and pers. comm).

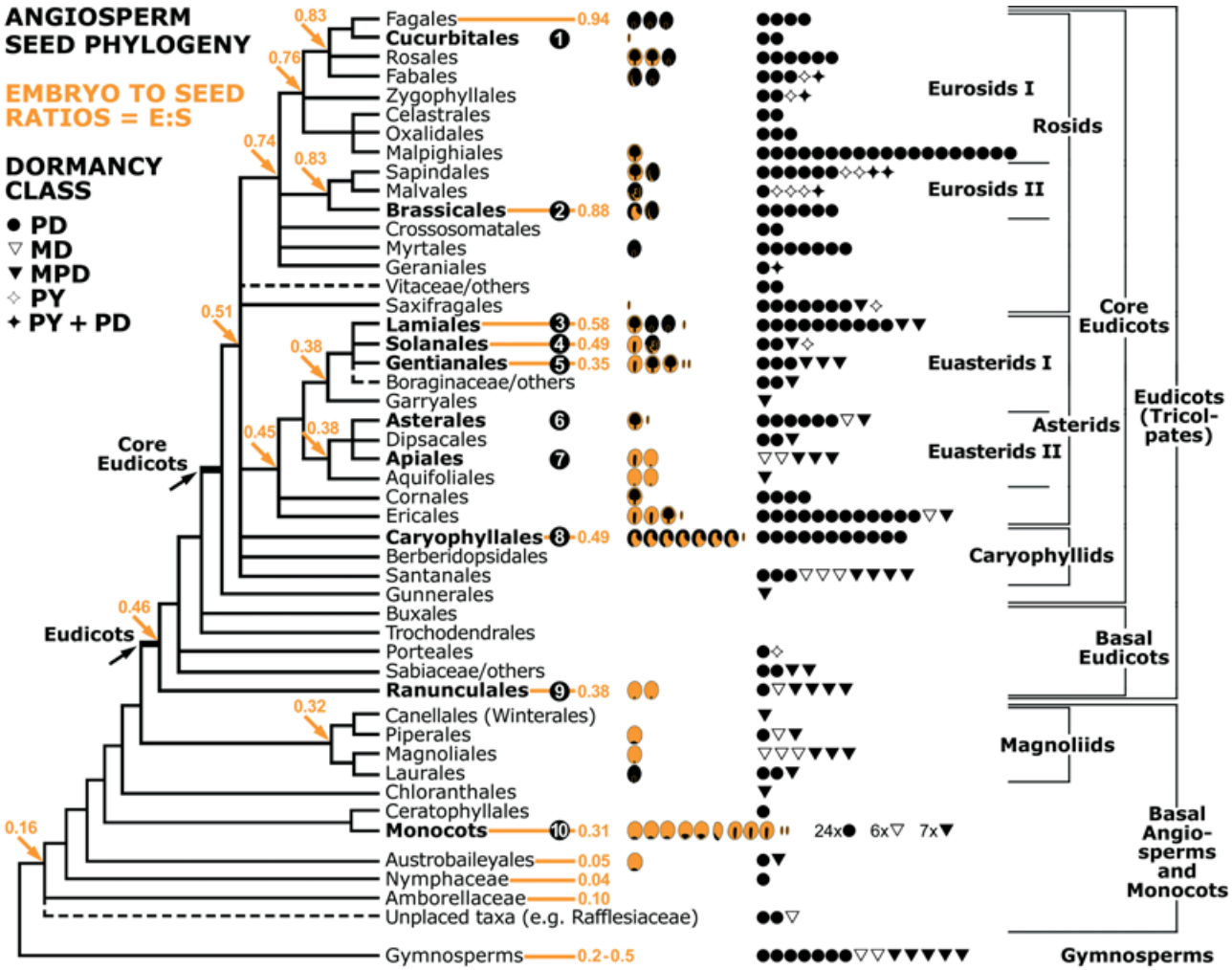

Fig. 4 Angiosperm seed evolution depicted in a phylogenetic tree constructed by the Angiosperm Phylogeny Group II (2003). For each family, pictographs of the seed type and symbols for the dormancy class are placed next to the corresponding clade (see Fig. 3 for symbols, abbreviations and data source). The number of pictographs or symbols is equal to the number of families with the corresponding seed type or dormancy class, respectively. Numbers in the phylogenetic tree represent 'embryo to seed' (E:S) ratios expressed as generalized least squares (GSL) values from Forbis *et al*. (2002, and pers. comm.). Clades with experimental evidence for endosperm-limited germination and endosperm weakening are shown in bold and numbered 1 to 10. The experimental evidence can be summarized as follows: Rosid clade: (1) Cucurbitaceae: *Cucumis* P↓ (perisperm weakening); (2) Brassicaceae: *Lepidium* C↓GA↓ABA↑ (Müller *et al*., 2006), *Arabidopsis*; (3) Oleaceae: *Syringa* C↓GA↓ (Junttila, 1973), *Fraxinus* C↓GA↓ (Fig. 6; Finch-Savage & Clay, 1997); (4) Solanaceae (Solanoideae): *Lycopersicon* C↓GA↓ABA↑ (Groot & Karssen, 1987, 1992; Toorop *et al*., 2000; Wu *et al*., 2000), *Capsicum* C↓GA↓ (Watkins & Cantliffe, 1983; Petruzzelli *et al*., 2003), *Datura* (Sanchez & Mella, 2004), Solanaceae (Cestroideae): *Nicotiana* (reviewed in Leubner-Metzger, 2003), *Petunia* (Petruzzelli *et al*., 2003); (5) Rubiaceae: *Coffea* C↓GA↓ABA↑ (da Silva *et al*., 2004; da Silva *et al*., 2005); (6) Asteraceae: *Lactuca* C↓GA↓ (Tao & Khan, 1979); (7) Apiaceae: *Apium* (Jacobsen & Pressman, 1979); other clades: (8) Amaranthaceae: *Chenopodium* (Karssen, 1976); (9) Ranunculaceae: *Trollius* (Hepher & Roberts, 1985); (10) Iridaceae: *Iris* C↓ (Blumenthal *et al*., 1986), Poaceae: *Triticum* C↓ (Benech-Arnold, 2004). ABA, abscisic acid; GA, gibberellin; C, control. Puncture force measurements: C↓, endosperm weakening before endosperm rupture; GA↓, endosperm weakening promoted by GA; ABA↑, endosperm weakening inhibited by ABA. The phylogenetic tree is modified from Judd *et al*. (2002); Angiosperm Phylogeny Group II (2003); Soltis & Soltis (2003); Stevens (2005).

in some cases can be replaced by GA application. There are eight known levels of MPD. Examples: *Trollius* (Fig. 3, Ranunculaceae; Hepher & Roberts, 1985), *Fraxinus excelsior* (Fig. 3, Oleaceae; Finch-Savage & Clay, 1997).

(4) **Physical dormancy (PY)** PY is caused by water-impermeable layers of palisade cells in the seed or fruit coat that control water movement. Mechanical or chemical scarification can break PY dormancy. Examples: *Melilotus* and *Trigonella* (Fabaceae, Fig. 3; Baskin & Baskin, 1998; Baskin, 2003).

(5) **Combinational dormancy (PY + PD)** PY + PD is evident in seeds with water-impermeable coats (as in PY) combined with physiological embryo dormancy (Baskin & Baskin, 2004). Examples: *Geranium* and *Trifolium* (Fig. 3).

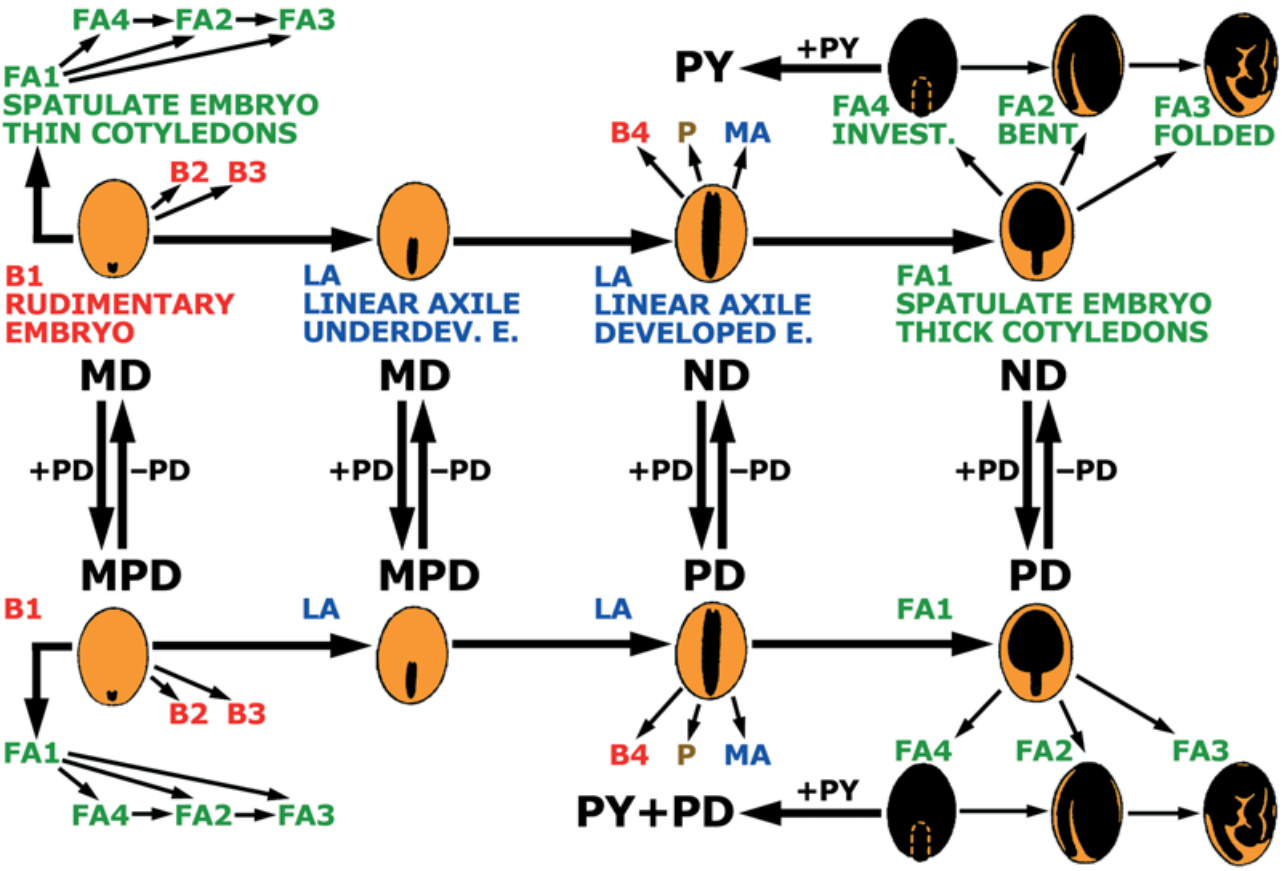

Fig. 5 Summary of general trends in angiosperm seed evolution with respect to seed type and dormancy class. Seeds with a small embryo and abundant endosperm (B1 seed type) are primitive and evolution shows a general trend (indicated by arrows) towards mature seeds with little or no endosperm in which the embryo occupies most of the seed (through LA to FA seed types). Gain or loss of physiological dormancy (PD) in each of these seed types (B1, LA and FA1) alters the dormancy class as indicated. Similar changes can also occur in the other seed types. Addition of physical dormancy (PY) to FA4-type nondormancy (ND) or PD seeds is indicated as a side branch occurring in specialized species. This addition can be reversed and PY can also be added to other seed types (not shown). Seed types and dormancy classes are described in Fig. 3. This diagram is based on information given in figs 12.21 and 12.22 of Baskin & Baskin (1998).

4. Evolution of seed structure and seed dormancy

The classification system above shows that a great diversity of morphological and physiological features have evolved to control dormancy in response to different environments (Vleeshouwers *et al.*, 1995; Li & Foley, 1997; Baskin & Baskin, 2004; Donohue, 2005). The most obvious morphological difference in mature angiosperm seeds is their 'embryo to seed' size ratios resulting from the extent to which the endosperm is obliterated during seed development by incorporating the nutrients into the storage cotyledons. The evolution of these differences has been analysed with respect to its implications for the evolution of seed dormancy (Martin, 1946; Baskin & Baskin, 1998, 2004; Forbis *et al.*, 2002; Nikolaeva, 2004). Based on the internal morphology of mature seeds, Martin (1946) defined seed types with distinct embryo to endosperm ratios, arranged them in a seed phylogenetic tree and proposed evolutionary seed trends. This has been revised and extended in Fig. 3.

Figure 4 shows the distribution of Martin's seed types in the modern angiosperm phylogenetic tree. Forbis *et al.* (2002) calculated 'embryo to seed' (E:S) values for the different seed types. These E:S values show a clear trend increasing from low E:S values up the phylogenetic tree to high E:S values. Although there is a lack of evidence for functional differences among embryo shapes, the results of Forbis *et al.* (2002) strongly support the view that the relative embryo size is an important determinant of seed dormancy evolution. Together with additional arguments (Baskin & Baskin, 1998, 2004; Forbis *et al.*, 2002), these data support the following general evolutionary seed trends (Figs 4, 5). (1) In mature seeds of primitive angiosperms a small embryo is embedded in abundant endosperm tissue. Such seed types (e.g. B1) prevail among basal angiosperms. (2) The general evolutionary trend within the higher angiosperms is via the LA seed type (embryo linear axile and developed, endosperm abundance medium to high) towards the FA seed types (embryo foliate axile and developed, often storage cotyledons, endosperm abundance low or endosperm obliterated) with storage cotyledons. The LA seed type is typical for many Asterids, for example the endospermic Solanceae seeds. Further embryo dominance

and endosperm reduction lead via the FA1 seed type to the diverted seed types FA2, FA3 and FA4. The FA seed types are typical for many Rosids, for example Brassicaceae seeds with more or less no endosperm at maturity. (3) In addition to these general seed trends there are clade-specific seed type differences ('exceptions'), for example within the basal angiosperms (Laurales) and the Asterids (Aquifoliales). (4) A small embryo is also found in primitive gymnosperms and an increase in the E:S values is also evident within the gymnosperms. An increase in relative embryo size appears therefore to be a general evolutionary trend within the angiosperms and the gymnosperms.

The evolutionary trend of an increase in the relative embryo size of seeds has functional importance for the evolution of dormancy of angiosperms and gymnosperms (Baskin & Baskin, 1998, 2004; Forbis *et al.*, 2002; Nikolaeva, 2004). MD and MPD are present in B-type seeds, but can also be typical of some specialized species (Figs 3, 4). MD is thought to be the ancestral dormancy type among seed plants and is the most primitive dormancy class. The dispersal of seeds with an underdeveloped embryo that need time to grow might have evolved as an ancient strategy to disperse germination over time. MD and MPD are typical not only for primitive angiosperms (Figs 3, 4) but also for primitive gymnosperms such as the Zamiaceae, Ginkgoaceae, Podocarpaceae and Taxaceae. Evolution of larger embryo size in MD seeds resulted in nondormant (ND) seeds. Concurrently, the gain of physiological dormancy mechanism(s) led from seeds with MD to seeds with MPD, which upon gain in embryo size led to PD seeds (Fig. 5).

PD is the most phylogenetically widespread dormancy class. This class of dormancy is distributed over the entire phylogenetic tree, from gymnosperms and the basal angiosperms to the higher core eudicot Rosid clade (Fig. 4). As ND is also distributed over the entire phylogenetic tree it has been proposed that gain and loss of PD occurred at several times and at several levels of seed evolution (Fig. 5). The most phylogenetically restricted and derived dormancy classes are PY and PY + PD (Figs 3, 4, 5). The occurrence of an impermeable seed or fruit coat combined with a ND embryo (PY) or a PD embryo (PY + PD) is probably an adaptation to specialized life strategies or habitats. PY and PY + PD are the only dormancy classes not found in gymnosperms (Baskin & Baskin, 2004).

III. How is Nondeep Physiological Dormancy Regulated within the Seed at the Molecular Level?

The use of different terms and definitions of seed dormancy have lead to confusion between ecologists and physiologists. Baskin & Baskin (2004) proposed that seed scientists state the class, level and type of dormancy based on their new classification system (see section II.3). This hierarchical system accurately reflects the 'whole-seed' ecologists' view of the control of germination by dormancy and should be widely used in future work. At the molecular level, very little is known about MD, MPD, PY, PY + PD and deep PD. In contrast, recent physiological and molecular work has provided insight into mechanisms of nondeep PD, which are the focus of this section. These studies show that the intrinsic molecular mechanisms determining dormancy can have an embryo and/or a coat component (Hilhorst, 1995; Bewley, 1997a; Kucera *et al.*, 2005). The terms 'embryo' and 'coat dormancy' will therefore be used here to distinguish between these two mechanisms, but it should be emphasized that they are not used as part of a classification system for 'whole seeds' *sensu* Baskin & Baskin (2004). Embryo dormancy and coat dormancy are components of PD; their sum and interaction determine the degree of 'whole-seed' PD.

Embryo dormancy is characterized by a block that inhibits extension growth, and therefore excised embryos do not grow. Coat dormancy is characterized by a block that is conferred by the covering layers. Nondormant embryos excised from coat-dormant seeds will therefore extend and grow. 'Coat' is used in a loose sense and can be any embryo-covering structure, for example the testa, endosperm and/or pericarp, and terms such as 'testa dormancy' and 'endosperm dormancy' will be used to specify these. For example, mechanical resistance from combined testa and endosperm dormancy, which is greater than the embryo growth potential opposing it, appears to be the cause of nondeep PD in seed model systems such as *A. thaliana* and Solanaceae species (Hilhorst, 1995; Bewley, 1997b; Koornneef *et al.*, 2002; Leubner-Metzger, 2003a).

1. Induction, maintenance and release of physiological dormancy by plant hormones and environmental signals

There is considerable evidence that ABA is an important positive regulator of both the induction of dormancy and the maintenance of the dormant state in imbibed seeds following shedding. As this evidence for ABA involvement has been described in detail in a very recent review (Kucera *et al.*, 2005) and in several earlier reviews (Hilhorst, 1995; Bewley, 1997a; Li & Foley, 1997; Koornneef *et al.*, 2002) only an update is provided here.

- ABA deficiency during seed development is associated with absence of primary dormancy in the mature seed, whereas overexpression of ABA biosynthesis genes can increase seed ABA content and enhance seed dormancy or delay germination (e.g. Finkelstein *et al.*, 2002; Nambara & Marion-Poll, 2003; Kushiro *et al.*, 2004).
- ABA produced by the seed itself during seed development can impose a lasting dormancy, whereas maternal ABA or ABA application during seed development fails to induce lasting seed dormancy, but has other functions (Kucera *et al.*, 2005).
- In *A. thaliana*, the members of the *AtNCED* gene family encode g-cis-epoxycarotenoid dioxygenases catalysing the key

regulatory step in ABA biosynthesis (Lefebvre *et al.*, 2006). This work suggests that ABA synthesis in the embryo and that in the endosperm both contribute to the induction of seed dormancy.

- High ABA contents are present in the imbibed seeds of the strongly dormant *A. thaliana* ecotype Cape Verde Island (Cvi) and decrease as dormancy is lost (Ali-Rachedi *et al.*, 2004). A recent transcriptome analysis with this ecotype strongly supports the view that increased ABA biosynthesis is associated with the dormant state (Cadman *et al.*, 2006).
- *De novo* ABA biosynthesis during imbibition of dormant, but not nondormant, seeds has been demonstrated in the *A. thaliana* ecotype Cvi (Ali-Rachedi *et al.*, 2004), as well as in other species including *Nicotiana plumbaginifolia* (Grappin *et al.*, 2000), *Helianthus annuus* (Le Page-Degivry & Garello, 1992), and *Hordeum vulgare* (Wang *et al.*, 1995). This *de novo* ABA biosynthesis has been interpreted as a mechanism for dormancy maintenance.

Work with the strongly dormant *A. thaliana* ecotype Cvi shows that dormancy may depend on an intrinsic balance of GA and ABA biosynthesis and catabolism, which will determine the dominance of either of the hormones (Ali-Rachedi *et al.*, 2004; Cadman *et al.*, 2006). While PD release of Cvi seeds occurs effectively by after-ripening, stratification or inhibition of ABA biosynthesis, the addition of GA appears less effective. GA treatment of dormant Cvi seeds caused a transient increase in ABA concentration (Ali-Rachedi *et al.*, 2004), suggesting that in dormant seeds a feedback mechanism exists that maintains a high ABA:GA ratio.

Thus, the net result of the dormant state is characterized by increased ABA biosynthesis and GA degradation. 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 'seed life'. ABA induces dormancy during maturation, and GA plays a key role in dormancy release and in the promotion of germination. Newer evidence suggests that it is likely that this revision went too far. Experiments with sorghum (*Sorghum bicolor*) (Steinbach *et al.*, 1997), and with ABA-deficient and -insensitive mutants of maize (*Zea mays*) (White & Rivin, 2000; White *et al.*, 2000) demonstrated that GA and ABA can act at the same time on dormancy and germination. Inhibition of GA biosynthesis during seed development mimics the effects of exogenous ABA, for example in suppressing vivipary. It appears to be the ABA:GA ratio, and not the absolute hormone contents, that controls germination. Thus, it seems that GA directly antagonizes ABA signalling during dormancy induction of cereal grains. Experiments with other species are needed to determine whether this is a general phenomenon.

While dormancy maintenance also depends on high ABA:GA ratios, dormancy release involves a net shift to increased GA biosynthesis and ABA degradation resulting in low ABA:GA ratios (e.g. Ali-Rachedi *et al.*, 2004; Cadman *et al.*, 2006). This supports the proposal of Le Page-Degivry *et al.* (1996) that ABA is the primary hormone involved in any step during dormancy maintenance and release, and that GAs are present at sufficient concentrations to promote germination as soon as ABA biosynthesis is inhibited. There is further support from genetic work with *Avena fatua* (Fennimore & Foley, 1998) showing that GA itself, although its addition to the medium can cause germination of dormant seeds, is not involved in (embryo) dormancy loss, but in stimulating seed germination. Thus, dormancy release is characterized by the capacity for enhanced ABA degradation and increased GA biosynthesis, which is followed by GA promotion of seed germination.

We assume that these conclusions regarding the role of ABA and GA concentrations/synthesis in dormancy and germination are valid for the regulation of embryo dormancy. However, the emerging picture is incomplete without considering coat dormancy (see sections III.3 and III.4) 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). In addition to hormone content and synthesis, the transition from the dormant to the nondormant state of many seeds is characterized 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). The seed phenotypes of the *A. thaliana ABA-insensitive* (*abi*) response mutants *abi1* to *abi5* demonstrate that ABI1 to ABI5 are involved in seed dormancy and/or germination (Finkelstein, 2004; Kucera *et al.*, 2005). Transcript expression of *ABI1* to *ABI5* is regulated in a complex manner during dormancy induction and release of *A. thaliana* ecotype Cvi (Cadman *et al.*, 2006). An important finding of this study is that the dormant state is characterized by the transcription of genes with an overrepresentation of ABA-responsive elements (ABRE) in their promoters and of genes for transcription factors that bind to the ABRE. Such an overrepresentation 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.

Among the genes that are induced in *A. thaliana* ecotype Landsberg erecta (*Ler*) and Columbia (Col) seeds during imbibition are many GA-responsive genes, but GA also causes down-regulation of many ABRE-containing genes (Yamaguchi & Kamiya, 2002; Ogawa *et al.*, 2003; Yamauchi *et al.*, 2004). Bioactive GAs accumulate in the embryo just before radicle protrusion, and light is one of the environmental factors that induces this GA biosynthesis, which occurs in two separate embryo tissues during germination: (1) the provascular tissue, where *ent*-copalyl diphosphate synthase 1 (*AtCPS1*) gene promoter activity is localized, has the early biosynthetic pathway, including the geranylgeranyl diphosphate cyclization reaction

catalysed by CPS; (2) the cortex and endodermis of the root, where GA 3-oxidase 1 (*AtGA3ox1*) and *AtGA3ox2* transcripts accumulate and *AtGA3ox2* gene promoter activity is localized, have the late biosynthetic pathway, including the formation of bioactive GA by GA3ox. This physical separation of the early and late GA biosynthetic pathway implies that intercellular transport of an intermediate (probably *ent*-kaurene) is required for the production of bioactive GA by the embryo.

Cold and light responses are mediated, at least in part, by promoting GA biosynthesis via enhanced expression of *AtGA3ox* (Yamaguchi & Kamiya, 2002; Oh *et al.*, 2004; Yamauchi *et al.*, 2004; Liu *et al.*, 2005b; Penfield *et al.*, 2005). The Blue Micropylar End 3 (BME3) GATA zinc finger transcription factor is expressed in the radicle and seems to be involved as a positive regulator of seed germination and GA biosynthesis in response to cold stratification (Liu *et al.*, 2005b). The recent model by Penfield *et al.* (2005) explains the control by these two environmental factors (cold and light) through the interaction of the basic helix-loop-helix (bHLH) transcription factors Spatula (SPT) and Phytochrome-Interacting-Factor-Like5 (PIL5). In the dark, SPT and PIL5 are both active as repressors of germination, while in 'light plus cold' their repressive activities are low. The regulation of PIL5 activity is controlled at the level of protein stability by light, which causes its repressive activity to decrease. In dark stratified seeds, SPT activity appears to be dependent on PIL5. Tsiantis (2006) speculates whether natural allelic variants of these transcription factors are responsible for determining some of the observed differences in dormancy behaviour in response to these environmental variables (temperature and light) between different ecotypes of the same species and indeed between species.

A number of GA-responsive genes are found to be differentially expressed when global transcript abundances are compared among seeds with different depths of dormancy and nondormant seeds of *A. thaliana* ecotype Cvi (Cadman *et al.*, 2006). This study also suggests that there is active biosynthesis of GA precursors in all states: *AtGA20ox1* transcripts are always present at high abundance, suggesting that biologically inactive GA_9 and GA_{20} are always produced. There is also a high abundance of *AtGA2ox1* transcripts present in all states, suggesting that any biologically active GAs formed (e.g. GA_4 and GA_1) are degraded rapidly. In seeds that require only light to germinate and those exposed to light, the transcript expression of *AtGA3ox2* increases dramatically, presumably completing the final step of the biosynthesis of biologically active GA (e.g. GA_4 from GA_9, and GA_1 from GA_{20}). Therefore, a dynamic balance of biosynthesis and degradation of ABA and GA may exist that determines a state-specific equilibrium in the ABA:GA ratio (Cadman *et al.*, 2006). High ABA signalling is associated with dormancy and high GA signalling with germination, while the transition between the two programmes is controlled by shifting the signalling between the two hormones.

Two functions for GA during seed germination have been proposed (reviewed in Kucera *et al.*, 2005). First, GA increases the growth potential of the embryo and promotes germination. Secondly, GA is necessary to overcome the mechanical restraint conferred by the seed-covering layers by weakening of the tissues surrounding the radicle (see sections III.3 and III.4). Other plant hormones are involved in regulating gene expression during the induction, maintenance and release of PD (reviewed in Kucera *et al.*, 2005). Further key information about the control of germination may come from the study of natural allelic variation at loci linked to dormancy and germination. Quantitative trait loci (QTL) mapping approaches for *A. thaliana* (Alonso-Blanco *et al.*, 2003; Clerkx *et al.*, 2004; Koornneef *et al.*, 2004), *Brassica oleracea* (Bettey *et al.*, 2000; Finch-Savage *et al.*, 2005a) and cereals (Koornneef *et al.*, 2002; Gu *et al.*, 2004) are being used to identify germination and dormancy-related genes. Such QTL have been identified, but the cloning of the corresponding genes has not yet been reported.

2. Seed after-ripening: dormancy release and promotion of germination

After-ripening, i.e. a period of usually several months of dry storage at room temperature of freshly harvested, mature seeds, is a common method used to release dormancy (Bewley, 1997a; Probert, 2000; Leubner-Metzger, 2003; Kucera *et al.*, 2005; Bair *et al.*, 2006). Seed after-ripening can be characterized by: (1) a widening of the temperature range for germination; (2) a decrease in ABA concentration and sensitivity and an increase in GA sensitivity or loss of GA requirement (Fig. 2); (3) a loss of light requirement for germination in seeds that do not germinate in darkness; (4) an increase in seed sensitivity to light in seeds that do not germinate even with light; (5) a loss of the requirement for nitrate; (6) an increase of germination velocity.

The parameters that determine seed after-ripening are moisture and oil contents, seed-covering structures, and temperature (Manz *et al.*, 2005 and references therein). After-ripening is prevented in very dry seeds; it requires seed moisture contents above a threshold value. This threshold moisture content is species-specific and lower in oilseeds compared with starchy seeds because they contain less bound water when equilibrated at any given relative humidity. After-ripening is also prevented during storage at very high air humidity (higher equilibrium moisture content). For several species, the conditions that generate optimal low-hydration values for after-ripening have been determined (e.g. Probert, 2000; Hay *et al.*, 2003; Steadman *et al.*, 2003; Leubner-Metzger, 2005; and references therein). The molecular mechanisms of after-ripening are not known. Nonenzymatic reactions that remove germination inhibitors, reactive oxygen species and antioxidants (Bailly, 2004), membrane alterations (Hallett & Bewley, 2002), and specific protein degradation via the

proteasome (Skoda & Malek, 1992; Borghetti *et al.*, 2002) have been proposed. Using cDNA-amplified fragment length polymorphism (cDNA-AFLP) gene expression analysis, Bove *et al.* (2005) provide evidence that *Nicotiana* seed after-ripening generates a developmental switch at the transcript level. This is in agreement with the *A. thaliana* Cvi transcriptome work (Cadman *et al.*, 2006). In wild oat (*Avena fatua*), transcriptional regulation and post-transcriptional regulation are both important for the expression of dormancy-associated genes (Li & Foley, 1997).

Two recent publications provide evidence for gene expression in air-dry *Nicotiana* seeds during after-ripening (Bove *et al.*, 2005; Leubner-Metzger, 2005). A rapid promotion of testa rupture of *Nicotiana tabacum* seeds occurred after *c.* 60 days of dry storage (Leubner-Metzger, 2005). This was associated with transient β-1,3-glucanase gene expression in the covering layer during tobacco after-ripening. Bove *et al.* (2005) found that at least eight specific mRNAs accumulated in air-dry, low-hydrated seeds of *Nicotiana plumbaginifolia* during after-ripening. Thus, while degradation of mRNAs and proteins for positive regulators of dormancy and for negative regulators of germination appears to be part of the molecular mechanisms of seed after-ripening, the possibility of *de novo* gene expression during seed after-ripening should also be considered.

3. Control of germination by the seed coat: testa mutant studies

In PD seeds, the embryo-covering layers can confer mechanical constraint (coat dormancy) that must be overcome by the growth potential of the embryo (Bewley, 1997b; Koornneef *et al.*, 2002; Leubner-Metzger, 2003a; Kucera *et al.*, 2005). Note that this condition is different from really hard-coated seeds (PY). Completion of germination in seeds with PD-type coat dormancy requires that the embryo growth potential increases to overcome the mechanical constraint, and/or the mechanical constraint associated with the seed-covering layer(s) is reduced. Two forms of mechanical constraints and release mechanisms can be distinguished: (1) living seed-covering layers where a regulated tissue weakening occurs before germination and the tissue itself can produce enzymes for this process, for example the endosperm (section III.4) or inner testa; (2) mostly dead seed-covering layers where predetermined breaking points facilitate tissue rips before germination, for example the outer testa (this section) or pericarp. Enzymes that facilitate testa rupture might be released by the endosperm and/or the radicle.

The testa is 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 & 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 embryonic ABA.

4. Control of germination by the endosperm: endosperm weakening

The endosperm acts as a mechanical barrier to the germination of seeds in several angiosperm clades (Fig. 4). 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 (for reviews, see Hilhorst, 1995; Bewley, 1997b; Leubner-Metzger, 2003; Sanchez & Mella, 2004; Kucera *et al.*, 2005). This endosperm weakening (C↓ in Fig. 4) can be promoted by GA and, at least in part, inhibited by ABA (GA↓ and ABA↑ in Fig. 4). Solanaceae species such as tomato (*Lycopersicon esculentum*), tobacco (*Nicotiana* spp.), pepper (*Capsicum annuum*) and *Datura* have become model species for endosperm weakening. Although freshly harvested mature seeds of these species have nondeep PD, endosperm weakening has been studied using these seeds in the nondormant (e.g. after-ripened) or conditionally dormant state. Possibly as a consequence of this, in most of these cases, it has been proposed that the endosperm-weakening mechanism is part of the germination process of nondormant seeds and is not part of a dormancy release process *per se* (Baskin & Baskin, 2004). There are some exceptions to this in gymnosperm seeds, where weakening of the embryo-covering layer (megagametophyte) occurred during dormancy-breaking treatments, well separated from the germination process (see References in Baskin & Baskin, 2004).

In Fig. 6 we present unpublished work with ash (*Fraxinus excelsior*, Oleaceae; W. E. Finch-Savage, unpublished results) seeds that clearly shows that endosperm weakening can be part of the dormancy release process in angiosperms. The fruits of ash are deeply dormant and require prolonged warm followed by cold periods of stratification to break dormancy (optimally 16 wk warm, 16 wk cold; Nikolaeva, 1969; Finch-Savage & Clay, 1997). Warm stratification is required for the release of dormancy in the small but fully differentiated embryo and is associated with a decline in ABA concentration (Fig. 6a). Subsequent cold stratification is required for germination and is associated with an increase in GA concentration (Fig. 6c,d). If seeds are exposed to constant cold conditions after full stratification germination will continue slowly, but if seeds are exposed to alternating temperatures of 3 and 25°C germination will proceed more quickly. However, if the warm period exceeds the cold period in the 24-h cycle the seeds will not germinate and they become secondarily dormant. This implies that dormancy breaking continues during this regime, as only a limited proportion of seeds in the population germinate if transferred to 15°C. Within the dormant seed population there is a distribution of forces required to puncture the endosperm layer covering the radicle, which moves to lower puncture forces (Fig. 6f) during stratification,

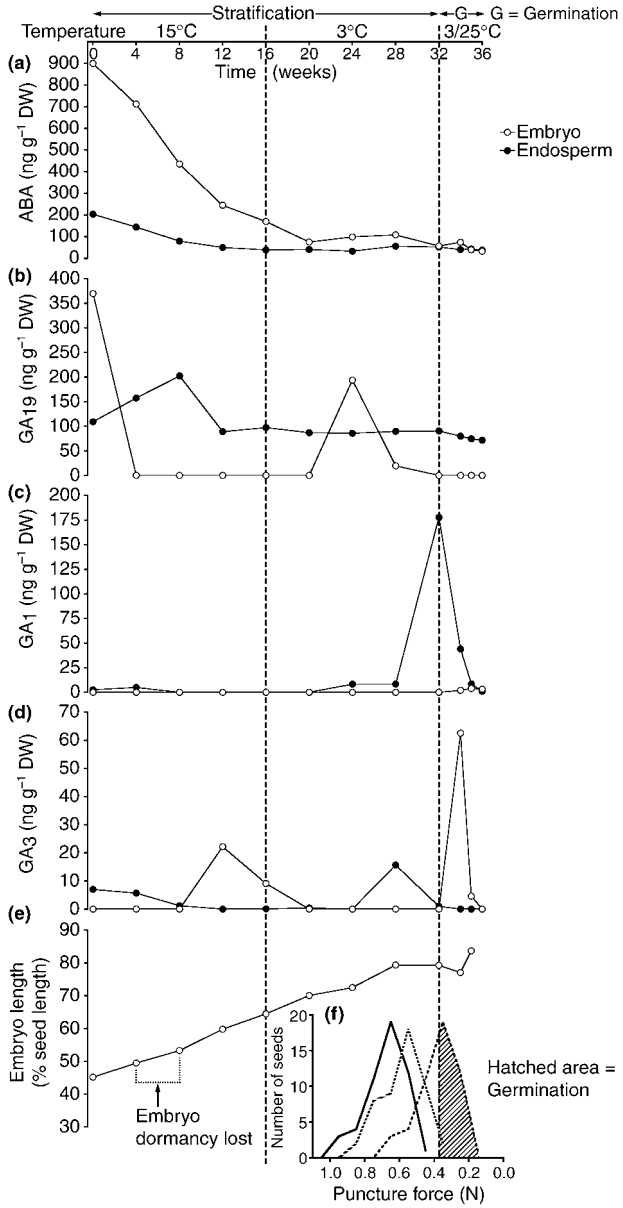

Fig. 6 Dormancy release during stratification in *Fraxinus excelsior* includes endosperm weakening that is mediated by gibberellins. The stratification treatment was 16 wk at 15°C followed by 16 wk at 3°C. Seeds were then transferred to dormancy breaking/germination conditions of 16 h at 3°C followed by 8 h at 25°C. (a–d) Original endogenous hormone data [abscisic acid (ABA) and gibberellins GA_{19}, GA_1 and GA_3, respectively] sampled and measured by methods beccribed in Blake *et al*. (2002). Closed circles, endosperm; open circles, embryo. (b, c, d) The inactive precursor of GA_1, GA_{19}, accumulates in the endosperm during stratification, but significant GA_1 concentrations do not occur for several weeks after the start of cold treatment when the ABA concentration is minimal. GA_1 is not present in the embryo, but GA_3 accumulates later in the embryo, coincident with radicle extension growth leading to germination. (e) Embryo growth within the seed. Initially the embryo will not grow when excised from the seed, but this physiological dormancy is progressively lost in the first half of the warm period as the endogenous ABA concentration (a) declines. The embryo will then grow to the full length of the endosperm cavity and will continue to grow if excised from the seed coat (e). However, germination of the whole seed will not reach completion without cold. If provided only with a cold treatment the embryo remains dormant and will not grow. (f) The distribution of forces in the population required to puncture the endosperm/seed coat layer which constrains the embryo (Finch-Savage & Clay, 1997). Radicle emergence was possible as the puncture force declined below 0.4 N. Solid dark line, dormant seed; dotted grey line, stratified seed; dotted dark line, hypothetical data following continued exposure to the dormancy breaking/germination conditions; the hatched area designates germination. Data in (e) and (f) are from Finch-Savage & Clay (1997). DW, dry weight.

suggesting that these changes begin to occur while the seed is still dormant. Thus, the localized weakening of the enclosing tissues should be considered part of dormancy loss, rather than part of germination following dormancy loss in this species. Further evidence for this view is that dormancy is reinstated after stratification by constant warm conditions, but these same conditions do not prevent continued growth of the excised embryo. Based on current knowledge, it is not always possible to unambiguously assign endosperm weakening to either dormancy release or germination promotion. This apparent confusion is consistent with the proposal that a continuum appears to exist between dormancy and germination (Cohn, 1996). Thus, considering the fact that many of the molecular processes of endosperm weakening have been studied in more or less nondormant seeds, it seems reasonable to consider all the known molecular mechanisms of endosperm weakening as putative coat dormancy release mechanisms. The available evidence suggests that control of germination through GA-promoted endosperm weakening is a general phenomenon, associated with seeds that differ considerably in endosperm abundance. We propose, therefore, that at least some of the molecular mechanisms of endosperm weakening are widespread and constitute evolutionarily ancient traits.

The germination of intact tomato seeds is inhibited by ABA, but surgical removal of the micropylar cap permits germination even in the presence of ABA (Liptay & Schopfer, 1983). Endosperm rupture has commonly been observed to be inhibited by ABA, and in the established model systems of the Asterid clade this occurs, at least in part, by inhibition of changes in the micropylar endosperm (e.g. Ni & Bradford, 1993; Leubner-Metzger, 2003a; da Silva *et al*., 2004). Puncture-force experiments investigating the effect of ABA on coffee (*Coffea arabica*) and tomato seeds (ABA↑ in Fig. 4) have shown that endosperm weakening is biphasic. The first phase is ABA-insensitive, and this is followed by the second phase which is inhibited by ABA. The ABA-inhibited second phase accounts for *c*. 53% of the total decrease in puncture force required for germination in coffee (da Silva *et al*., 2004), and *c*. 6% (Wu *et al*., 2000) or *c*. 24% (Toorop *et al*., 2000) in tomato. 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).

Testa rupture and endosperm rupture are temporally separate events during the germination of many seeds of the Cestroideae subfamily of the Solanaceae, for example *Nicotiana* and *Petunia* (Fig. 2, Krock *et al.*, 2002; Leubner-Metzger, 2003; Petruzzelli *et al.*, 2003). These events are also mechanistically distinct processes, because the testa is dead and the endosperm is living tissue in these species. Testa rupture of tobacco occurs at predetermined breaking points and depends on water uptake and swelling of the embryo and the endosperm. It is associated with an additional increase in seed water content in the late part of phase II water uptake (Manz *et al.*, 2005). In contrast, water uptake in dormant tobacco seeds is blocked before testa rupture and no additional phase II water uptake occurs (Mohapatra & Johnson, 1978). Following testa rupture, storage reserves in the micropylar endosperm cells are degraded and the radicle emerges through a hole in the endosperm which has a smooth outline. This hole always forms at the micropylar end of germinating tobacco seeds and results from tissue dissolution rather than increased growth potential of the emerging radicle (reviewed in Leubner-Metzger, 2003a).

Ikuma & Thiman (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 (for reviews, see Hilhorst, 1995; Bewley, 1997b; Koornneef *et al.*, 2002; Leubner-Metzger, 2003; Bailly, 2004; Kucera *et al.*, 2005). Taken together, the current findings support the view that germination control by the seed-covering layers is achieved through the combined or successive actions of several cell-wall-modifying proteins. One intriguing issue arising from these studies is that there seem to be evolutionarily conserved molecular mechanisms as well as species-specific adaptations for endosperm weakening and/or coat dormancy release.

β-1,3-Glucanases are proposed to be involved in coat dormancy release, after-ripening and endosperm weakening (Fig. 2) and we use them here to illustrate how cell-wall-modifying proteins may act in the control of germination. These enzymes regulate symplastic trafficking, for example of cell-to-cell movement of GA, by controlling the strategically localized callose (β-1,3-glucan) deposition in the neck regions of plasmodesmata (Rinne *et al.*, 2001; Leubner-Metzger, 2003). Increased callose deposition is associated with bud dormancy of trees, and release of bud dormancy by GA or chilling seems to involve callose degradation by β-1,3-glucanase. Expression of β-1,3-glucanase in the micropylar endosperm, its inhibition by ABA and the inhibition of endosperm rupture by ABA are widespread among the Solanaceae (Fig. 2, Leubner-Metzger, 2003; Petruzzelli *et al.*, 2003). ABA inhibition of β-1,3-glucanase expression is also evident in perisperm weakening of Cucurbitaceae seeds (Welbaum *et al.*, 1998; Yim & Bradford, 1998; Amritphale *et al.*, 2005; Ramakrishna & Amrithhale, 2005). Proteomic analysis of *A. thaliana* showed that β-1,3-glucanases are glycosylphosphatidylinositol-anchored membrane proteins (GPI-APs; Elortza *et al.*, 2003). GPI-APs are proposed to be involved as enzymes and receptors in cell adhesion, cell separation and differentiation processes. Karssen *et al.* (1989) proposed that the second step of tomato endosperm weakening resembles a cell separation process. β-1,3-Glucanase induction in the micropylar endosperm of tomato is associated with this second step (Toorop *et al.*, 2000; Wu *et al.*, 2000; Petruzzelli *et al.*, 2003). Based on this, we speculate that β-1,3-glucanases facilitate endosperm rupture of seeds by breaking intercellular adhesion and causing cell separation.

The evolutionary trend towards cotyledon storage and seeds without endosperm at maturity is taken to the extreme in the Rosid clade (Fig. 4) and can be represented by the Brassicaceae. For example, the mature seeds of *Raphanus* and *Brassica* are without endosperm (Schopfer & Plachy, 1984; Schopfer *et al.*, 2001), those of *A. thaliana* retain a single cell layer of endosperm (Pritchard *et al.*, 2002; Liu *et al.*, 2005a) and the mature seeds of *Lepidium* spp. have a thin endosperm layer (Nguyen *et al.*, 2000; Müller *et al.*, 2006). Endosperm weakening has recently been demonstrated in Brassicaceae seeds, indicating that the endosperm is also a constraint to germination in seeds of the Rosid clade (Müller *et al.*, 2006). In this work, seeds of both *A. thaliana* and its much larger-seeded relative *Lepidium sativum* (garden cress) were studied. Both species belong to the Brassicoideae subfamily of the Brassicaceae (Hall *et al.*, 2002; Koch *et al.*, 2003) and are very similar in seed structure (FA2 type) and physiology. Testa rupture and endosperm rupture are separate events and only the latter is inhibited by ABA in both species (Liu *et al.*, 2005a; Müller *et al.*, 2006). Direct biomechanical measurement of the puncture force required to rupture the endosperm showed that the *L. sativum* micropylar endosperm weakened before 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.

IV. How is Nondeep Physiological Seed Dormancy Regulated by the Environment? Ecophysiology and Modelling

1. Response to the environment

Physiological dormancy has a very wide biogeographical distribution (Baskin & Baskin, 1998; Fenner & Thompson,

2005). As a consequence, the induction and loss of physiological dormancy following seed dispersal can be triggered by divergent environmental cues activated through many apparently different physiological mechanisms. These cues can be seasonally characteristic (usually temperature) and integrated by the seed over time. For example, dormancy may be broken by higher temperatures or lower temperatures, depending on species, in order for germination to occur in the correct season (autumn or spring, respectively) for subsequent growth. However, additional environmental conditions may be required to end dormancy and initiate germination (e.g. light or nitrate). In this way, flushes of germination are strongly promoted by disturbance of the soil, which terminates dormancy and induces germination of seeds by exposing them to light, but the timing of flushes is largely governed by temperature-driven changes in the depth of seed dormancy.

Even within the same species, several cues and responses may operate with differing importance in distinct ecotypes that may also have different depths of dormancy. For example, in *A. thaliana* these differences in response can result in different seasonal patterns of germination, so that different populations can express either a winter or spring annual life history and in some cases both (reviewed by Donohue, 2005). These very different behaviours are therefore not necessarily species-specific and are unlikely to be fundamentally different, but are probably adaptations of the same mechanisms through natural allelic variation in key regulatory genes.

It is not just the postdispersal environment that influences germination time; conditions during seed maturation (photoperiod, temperature and light quality) can also be crucial and interact with postdispersal environmental factors, further fine-tuning germination behaviour. This will also provide further variation in response from the same underlying dormancy mechanism. Predispersal conditions may also influence time of dispersal and therefore the postdispersal conditions experienced by the seed (Donohue, 2005). These dynamic interactions with the environment have a genetic basis and can therefore have a major influence on plant demography and evolution.

2. Dormancy cycling in the seed bank

In species adapted to regions of seasonal drought and dry soils, physiological changes recorded during dry after-ripening storage may reflect a natural mechanism, which can control the annual germination timing in the wild (Probert, 2000). In addition, there can be an annual rhythm of germination, which in *Mesembryanthemum nodiflorum*, a desert plant of the Aizoaceae (Caryophyllid clade), is retained after more than 30 years of dry storage (Gutterman & Gendler, 2005). When seeds are in the imbibed state, dormancy may be broken by exposure to high summer temperatures.

In temperate regions, seeds may cycle through different depths of dormancy in the seed bank for prolonged periods, but may also persist in a state that only requires light to terminate dormancy and induce germination (Thompson *et al.*, 2003; Fenner & Thompson, 2005). Dormancy is therefore a moving target continuously reacting to the environment and adjusting the conditions required for germination. Induction and breaking of nondeep PD in the variable natural environment are therefore continuous, and often gradual, processes where the temperature range (window) permissive for germination, and/or sensitivity to light and nitrate, etc, widens then narrows on a continuous scale. This conditional dormancy is recognized in the classification of five types of nondeep PD (section II.4; Baskin & Baskin, 2004). Whether the window opens from low to high or high to low temperatures (types 1 and 2, respectively) determines not only the classification type but also the environment to which it is adapted. The former is adapted to germination in the autumn (winter annual) and the latter to germination in the spring (summer annual); however, these changes in permissive temperatures and rate of germination with loss of dormancy will occur at different rates in individual seeds within the population (see sections 4 and 5 below and Fig. 7). Some species may also have seeds that are physiologically heterogeneous (Baskin & Baskin, 1998). Thus, as the ambient temperatures continue to change, secondary dormancy can be induced to close the window and prevent inappropriate late germination of seeds that are slower to lose dormancy (Vleeshouwers *et al.*, 1995; Baskin & Baskin, 1998; Probert, 2000; Vleeshouwers & Bouwmeester, 2001; Fenner & Thompson, 2005). In some cases, the mechanism can be further complicated as diurnal temperature variation (alternating temperatures) is required to terminate dormancy and promote germination and this may be further influenced by other environmental factors, particularly light (reviewed by Probert, 2000; Batlla *et al.*, 2004). Both these signals may, for example, indicate a position close enough to the soil surface to enable seedling emergence. Fluctuations in soil water content can also affect the dormancy status of seed banks (Batlla & Benech-Arnold, 2006). In all cases, the completion of germination of the nondormant seed is dependent on the availability of sufficient moisture. In dryer climates, water may also fulfil other roles such as diluting salinity, which would otherwise inhibit germination (e.g. Gutterman & Gendler, 2005).

3. Population based threshold models may provide a common framework to explain ecophysiological observations of seed dormancy and the control of germination by the environment

Population-based threshold models appear to have the potential to provide a universal approach to quantifying the array of ecophysiological responses described in sections 1 and 2. The models use biological time, in which germination

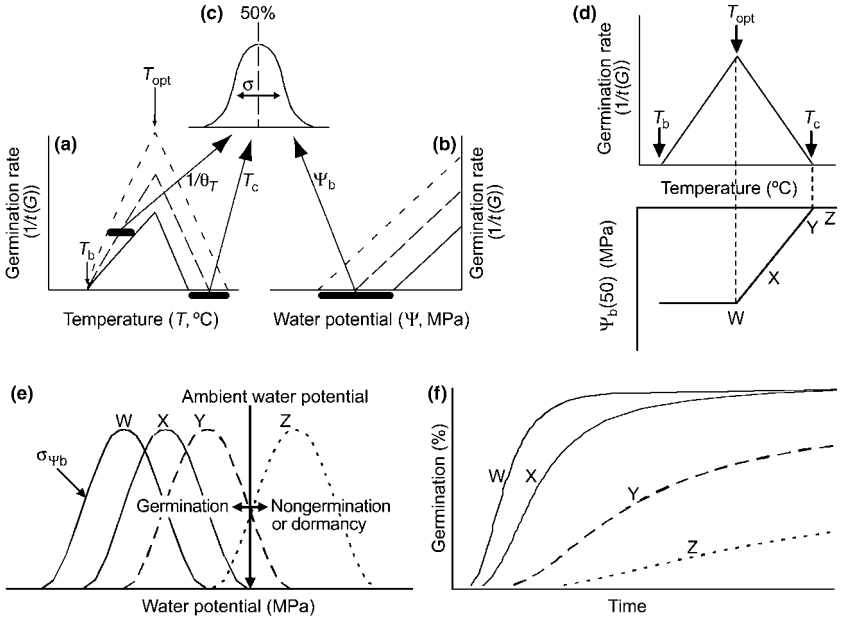

Fig. 7 Schematic illustration of the effects of temperature (a) and water potential (b) on germination rate. G_{10} (dotted lines), G_{50} (dashed lines) and G_{90} (solid lines) represent individual seeds in the population at percentiles 10, 50 and 90, respectively. The germination rate increases linearly with temperature above a base (T_b). The slopes of these lines are the reciprocal of the thermal times to germination ($1/\theta_T$). As temperature increases above an optimum (T_{opt}), the rate of germination decreases to a ceiling temperature (T_c). The rate of germination also decreases linearly with water potential (Ψ) to a base (Ψ_b). T_b is often the same for all seeds in the population, but $1/\theta_T$, T_c and Ψ_b vary among seeds in a normal distribution (c, σ). (d) Schematic representation of the relationship between the water potential of the fiftieth percentile (Ψ_b50) and temperature and their effect on the germination rate of percentile G_{50}. Parts (a) to (d) are modified from Finch-Savage (2004) and reprinted with permission from the Haworth Press, Inc. (e, f) An illustration of the effect of Ψ_b values changing at higher temperatures on cumulative germination. Each seed in the distribution will respond by the extent to which ambient conditions exceed their individual threshold sensitivity (Alvarado & Bradford, 2002). The black line (W) shows the distribution of thresholds around Ψ_b50 ($\sigma_{\Psi b}$) for a nondormant seed population at optimum temperature (e) with rapid germination of all seeds (f). As temperature increases above optimum, the distribution (W) shifts to the right (grey line; X) and seeds germinate more slowly. Further increases in the temperature (dashed line, Y; then dotted lines, Z), further reduce the germination rate, and the percentage of seeds that can germinate is also progressively reduced as Ψ_b exceeds ambient water potential. These seeds at high temperature are not dormant, but limited by their environment. However, the same illustration shows how other environmental conditions can also increase and decrease sensitivity (shift the distribution of Ψ_b) in the same way to induce or break dormancy (movement to the right or left, respectively). Ψ_b values greater than 0 MPa cannot be measured, but are an extrapolation based upon those seeds that can germinate (Bradford, 1996).

progresses at different rates according to the ambient conditions. These rates are determined by incrementing progress towards germination as a function of the difference between ambient conditions and a threshold (base) below which germination is not completed. The models, which were developed from thermal time (e.g. Garcia-Huidobro *et al.*, 1982) through to hydrothermal time (HTT; Gummerson, 1986) and separately to hydrotime (Bradford, 1990), describe the effects of temperature and/or water potential on the germination rate by using linear relationships (Fig. 7a,b), but are no longer constrained to only this.

These threshold models, as they are used in the laboratory with nondormant seeds, are described briefly in the next section. We then go on to review their application to dormancy cycling and the prediction of germination in the field. More detailed descriptions of these models with examples of their use, and the parallel development of the virtual osmotic potential (VOP) model, are available elsewhere (Bradford, 1990, 1995, 1996, 2002; Allen, 2003; Finch-Savage, 2004).

4. Threshold models and the description of germination responses in the laboratory

Over a certain range of temperature, germination will speed up as temperature increases and slow down as it decreases. When other environmental factors are nonlimiting, biological time can therefore be quantified by the amount by which temperature (T; in °C) exceeds a minimum temperature for germination (threshold or base; T_b in Fig. 7a). When this value is added each day to accumulate degree-days (thermal time), progress towards the completion of germination can be measured. This relationship holds when there is a linear relationship between increasing germination rate and increasing temperature ($< T_{opt}$ in Fig. 7a), so that for any individual seed:

$$\text{Thermal time} = (T - T_b) \times \text{time}$$

When the temperature remains constant, but water is suboptimal, progress towards the completion of germination

can be quantified in the analogous hydrotime, where progress is a function of water potential (Ψ). As germination rate is linearly related to water potential (Fig. 7b), biological time can be calculated by the amount by which water potential exceeds the base water potential (Ψ_b in Fig. 7b), below which germination will not reach completion, so that for any individual seed:

$$\text{Hydrotime} = (\Psi - \Psi_b) \times \text{time}$$

When both temperature and water potential vary, thermal time and hydrotime can be combined into hydrothermal time (HTT). In the HTT model, the germination time of a given seed $t(G)$ is quantified by the extent to which the water potential (Ψ) and suboptimal temperature (T) of each seed (G) exceed thresholds (bases; Ψ_b, T_b) so that:

$$\text{HTT} = [\Psi - \Psi_b(G)]\ (T - T_b)t(G)$$

HTT is assumed to be constant and in many cases the base temperature (T_b) is constant for all seeds (Fig. 7a), whereas the base water potential (Ψ_b; Fig. 7b) varies between seeds (G) and so the distribution of the germination times of individual seeds within the population is determined by the distribution of this parameter ($\sigma_{\Psi\,b}$; Fig. 7c). When these relationships exist, it is possible to describe the response of the whole seed population in a single equation by incorporation of this distribution (usually a normal distribution) of base water potentials within the population.

5. Threshold models and the prediction of dormancy and germination responses in the field

The models described in the previous section have been extended to simulate germination of nondormant seeds (and subsequent seedling emergence) in the field from these simple parameters describing the seed response to ambient soil conditions (e.g. Finch-Savage & Phelps, 1993; Batlla *et al.*, 2004; Finch-Savage, 2004; Finch-Savage *et al.*, 2005b). However, seeds of many species are dormant and temperature is the major factor that drives change in the depth of dormancy, and separate factors may then be required to terminate dormancy and induce germination. The threshold modelling approach can also be used to quantify how seeds integrate these various environmental signals to alter sensitivity thresholds (therefore dormancy level) and determine when to germinate (Batlla *et al.*, 2004). For example, changes in both the temperature threshold (T_b; del Monte & Tarquis, 1997; Steadman & Pritchard, 2004; Batlla & Benech-Arnold, 2005) and the median water potential threshold (Ψ_b50; Christensen *et al.*, 1996; Bauer *et al.*, 1998; Meyer *et al.*, 2000; Bradford, 2002; Alvarado & Bradford, 2005; Batlla & Benech-Arnold, 2005; Huarte & Benech-Arnold, 2005; Bair *et al.*, 2006) have been used to predict dormancy breaking or induction in both the laboratory and the field. The distribution of thresholds around Ψ_b50 ($\sigma_{\Psi b}$) tends to change little in most studies, and so predicting change in Ψ_b50 can indicate changes in the whole population of seeds. When temperature is the driver for change in dormancy status, changes in these thresholds (T_b; Ψ_b50) and/or the temperature range permissive for germination can be modelled by using thermal time approaches to integrate the time exposed to either constant or alternating temperatures that influence dormancy (Batlla *et al.*, 2004). This general modelling approach can again be visualized in Fig. 7(e,f). Changing thresholds to light (Bradford, 1996; Alvarado & Bradford, 2005; Batlla & Benech-Arnold, 2005) or other environmental factors required for germination (e.g. nitrate) could be similarly modelled as they act either simultaneously or separately in time (e.g. Bradford, 1995, 1996, 2002). Thus a wide range of seed germination and dormancy phenotypes can be accounted for by this method of calculating biological time through systematically shifting the distribution of sensitivities to environmental factors of individual seeds in relation to the nature and time of exposure to dormancy-breaking conditions. These sensitivities are species- and/or environment-specific as a result of evolution and/or adaptation to the locality so that dormancy appears bewilderingly complex in its variation, but the underlying mechanism itself may be more universal, as implied by the model.

6. Do threshold models have biological meaning?

Bradford and coworkers have argued that these models may be more than empirically descriptive, and that their parameters, in particular Ψ_b, may have biological significance (Bradford, 1995, 2002; Welbaum *et al.*, 1998). It is reasonable to speculate that the value of Ψ_b relates to endogenous and/or exogenous physical constraints to embryo growth depending on the species. We have reviewed in section III how these constraints, which are likely to differ in individual seeds (i.e. have a distribution), are relieved by molecular and biophysical changes made in response to ambient environmental conditions. Ψ_b also changes in response to exogenous applications of plant hormones; in general, gibberellins promote germination and decrease Ψ_b (more negative; Ni & Bradford, 1993; Alvarado & Bradford, 2005) whereas ABA inhibits germination and increases Ψ_b, but also has an independent effect so that there is a synergistic effect of ABA and reduced Ψ (Ni & Bradford, 1992; Ni & Bradford, 1993; Alvarado & Bradford, 2005). These same studies have shown that distributions of sensitivity to these hormones occur and the population-based threshold modelling approach can be applied to show the effect of exogenous applications of hormones on germination. Fluridone inhibits ABA biosynthesis and therefore promotes germination and also decreases Ψ_b (Alvarado & Bradford, 2005). This inhibitor would reduce endogenous ABA (e.g.

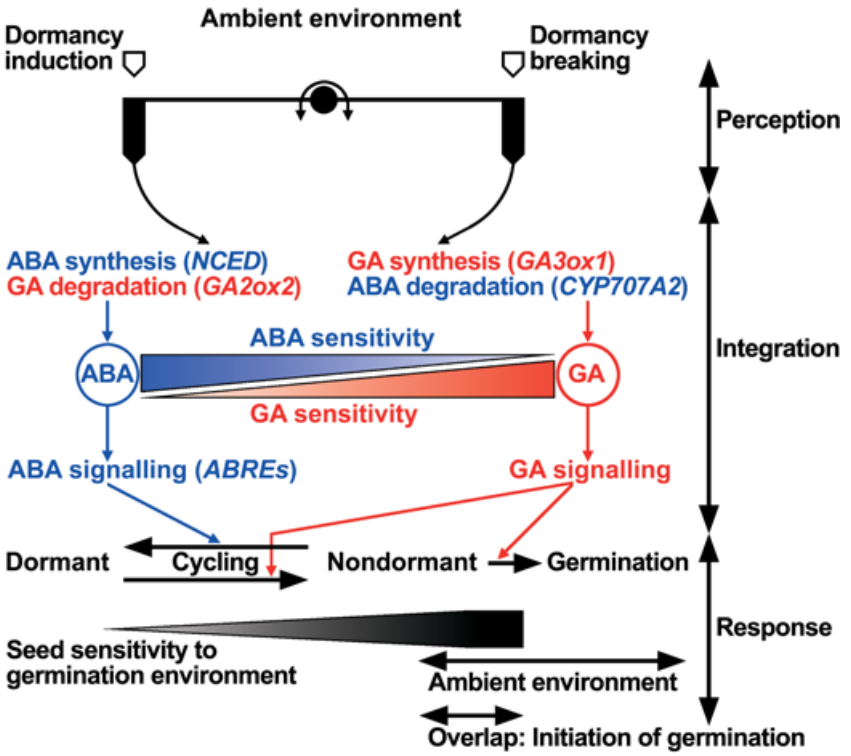

Fig. 8 Model for the regulation of dormancy and germination by abscisic acid (ABA) and gibberellins (GA) in response to the environment. According to this model, ambient environmental factors (e.g. temperature) affect the ABA:GA balance and the sensitivity to these hormones. ABA synthesis and signalling (GA catabolism) dominate the dormant state, whereas GA synthesis and signalling (ABA catabolism) dominate the transition to germination. The complex interplay among 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. The model is based on work with *Arabidopsis thaliana* ecotype Cvi, modified from Cadman *et al*. (2006) and reprinted with permission from Blackwell Publishing. Key target genes are in parentheses (see text for definitions).

Ali-Rachedi *et al*., 2004), suggesting that endogenous hormone concentrations similarly influence Ψ_b and its distribution and therefore the germination characteristics of the seed population. This view is supported by threshold modelling of tomato hormone mutants (Ni & Bradford, 1993). Although the detailed biochemical mechanisms by which Ψ_b values are determined have yet to be identified, these studies indicate that this approach could provide a way to link ecological observation of germination and dormancy to laboratory-based molecular studies.

V. Conclusions and Perspectives

Physiological seed dormancy (PD) is the most widespread dormancy class of the new ecological classification system proposed by Baskin & Baskin (2004) which provides a comprehensive ecological description of the 'whole-seed' dormancy response. In this review, we have tried to look beyond the enormous variation reported for underlying theories and mechanisms of PD in molecular, physiological and ecophysiological studies and found similarities. There appears to be a common evolutionary path for dormancy, and field observations which show that single species can have different life histories suggest that adaptations have taken place on a theme rather than via fundamentally different paths. Similarity is also suggested by a mathematical modelling approach, using threshold (sensitivity) distributions, which can describe many of these field observations.

At the molecular level, recent work supports the common view that PD 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. 8). There is active transcription, which has strong similarities in both the primary and secondary dormant states, but there is reduced capacity for protein synthesis (Cadman *et al*., 2006). A dynamic balance of hormone synthesis and catabolism operates which establishes a controlling balance of ABA:GA ratio to direct signalling pathways that regulate dormancy/germination by altering seed sensitivity (thresholds) to the ambient germination environment (Fig. 8). This process will occur at different rates in individual seeds, so that not all seeds of a population will have the same response. Thus, dormancy induction and release are probably mediated through this conserved signalling mechanism to coordinate diverse cellular responses. The latter may differ both within and among species (dormancy levels/types) as a consequence of natural allelic variation in key regulatory genes resulting from selection during adaptation to their specific environments.

When embryo and coat dormancy are considered as components of nondeep PD, the influence of ABA and GA on dormancy and the control of germination can be summarized as follows.

- Embryo dormancy (embryo component of nondeep PD). A dormant embryo is characterized by a high ABA:GA ratio,

high ABA sensitivity and low GA sensitivity. Embryo dormancy release involves remodelling of hormone biosynthesis and degradation towards a low ABA:GA ratio, a decrease in ABA sensitivity and an increase in GA sensitivity. Thus, ABA dominates the embryo dormancy programme, and GA the embryo germination programme. A nondormant embryo is characterized by increased growth potential, the capacity for cell extension growth and the ability to induce the release of coat dormancy.

• Coat dormancy (coat component of nondeep PD). The combination of an embryo with low growth potential and mechanical constraint from the seed-covering layers can result in dormancy. GA can release this coat dormancy by increasing the embryo growth potential and/or by reducing the mechanical constraint. There are two forms which are distinguished based on whether the covering layer consists of dead (e.g. outer testa) or living (e.g. endosperm) tissue.

• Testa dormancy: ABA during seed development determines the subsequent GA requirement for germination, as ABA influences the testa characteristics (e.g. thickness) and GA the embryo growth potential.

• Endosperm dormancy: endosperm weakening can be either part of the coat dormancy release or part of the germination programme. As the endosperm is in most cases living tissue, it can actively participate in regulating embryo constraint by influencing both the ABA:GA ratio and sensitivity to these hormones. GA acts by increasing the embryo growth potential and by promoting endosperm weakening, which is achieved though ABA-independent and ABA-inhibited mechanisms.

When considering the environmental control of germination (dormant and nondormant seeds), it seems that the sensitivity of seeds (thresholds for germination) to the full range of environmental factors that influence germination (temperature, nitrate, light, water, oxygen, smoke, allelopathic compounds …) is not fixed; even at the point of dispersal it is dependent on the previous maternal environment. The sensitivities change continuously as a function of variable ambient conditions (Fig. 8), and the nature and scale of the change may be a species- or ecotype-specific adaptation to the habitat. The sensitivities to different environmental factors may alter independently, but they are more likely to interact to provide variation. Thus a clearly defined dormant (PD) state does not exist in many cases; there are only different requirements for germination. The resulting germination requirements may be so extreme as to not normally exist, at least not in the natural habitat of the species, and act as a block to the completion of germination (e.g. PD deep seeds at shedding). Exposure to specific conditions, characteristic of the natural habitat of the species, may be required to bring sensitivities back into a range that matches potential environmental exposure.

For germination to occur, the window defined by the current sensitivities of the seeds has to overlap with ambient conditions for sufficient time to allow the completion of germination (Fig. 8). Only one environmental factor needs to be unfulfilled to prevent germination. In almost, if not all studies, for good practical reasons, only a limited number of these factors are studied, and so the picture remains confused. Even within a constant environment or in the dry state, the passage of time can alter dormancy, and so direct comparison between studies even within a single species will continue to provide controversy, especially where the depth of dormancy is significant. However, therein lies the fascination and challenge of dormancy.

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References

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.

Allen PS. 2003. When and how many? Hydrothermal models and the prediction of seed germination. *New Phytologist* **158**: 1–9.

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.

Aloresi A, Gestin C, Leydecker M-T, Bedu M, Meyer C, Truong H-N. 2005. Nitrate, a signal relieving seed dormancy in Arabidopsis. *Plant, Cell & Environment* **28**: 500–512.

Alvarado V, Bradford KJ. 2002. A hydrothermal time model explains the cardinal temperatures for seed germination. *Plant, Cell & Environment* **25**: 1061–1069.

Alvarado V, Bradford KJ. 2005. Hydrothermal time analysis of seed dormancy in true (botanical) potato seeds. *Seed Science Research* **15**: 77–88.

Amritphale D, Yoneyama K, Takeuchi Y, Ramakrishna P, Kusumoto D. 2005. The modulating effect of the perisperm-endosperm envelope on ABA-inhibition of seed germination in cucumber. *Journal of Experimental Botany* **56**: 2173–2181.

Angiosperm Phylogeny Group II. 2003. An updated classification of the angiosperms. *Botanical Journal of the Linnean Society* **141**: 399–436.

Bailly C. 2004. Active oxygen species and antioxidants in seed biology. *Seed Science Research* **14**: 93–107.

Bair NB, Meyer SE, Allen PS. 2006. A hydrothermal after-ripening time model for seed dormancy loss in *Bromus tectorum* L. *Seed Science Research* **16**: 17–28.

Baskin CC. 2003. Breaking physical dormancy in seeds – focussing on the lens. *New Phytologist* **158**: 227–238.

Baskin CC, Baskin JM. 1998. *Seeds – ecology, biogeography, and evolution of dormancy and germination*. San Diego, CA, USA: Academic Press.

Baskin JM, Baskin CC. 2004. A classification system for seed dormancy. *Seed Science Research* **14**: 1–16.

Baskin CC, Baskin JM. 2005. Underdeveloped embryos in dwarf seeds and implications for assignment to dormancy class. *Seed Science Research* **15**: 357–360.

Baskin CC, Baskin JM, Yoshinaga A, Thompson K. 2005. Germination of drupelets in multi-seeded drupes of the shrub *Leptecophylla tameiameiae* (Ericaceae) from Hawaii: a case for deep physiological dormancy broken by high temperatures. *Seed Science Research* **15**: 349–356.

Batak I, Devic M, Giba Z, Grubisi D, Poff KL, Konjevic R. 2002. The effects of potassium nitrate and NO-donors on phytochrome A- and phytochrome B-specific induced germination of *Arabidopsis thaliana* seeds. *Seed Science Research* **12**: 253–259.

Batlla D, Benech-Arnold RL. 2005. Changes in the light sensitivity of buried *Polygonum aviculare* seeds in relation to cold-induced dormancy loss: development of a predictive model. *New Phytologist* **165**: 445–452.

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 Science Research* **16**: 47–59.

Batlla D, Kruk BC, Benech-Arnold RL. 2004. Modelling changes in dormancy in weed soil seed banks: Implications for the prediction of weed emergence. In: Benech-Arnold RL, Sanchez RA, eds. *Handbook of seed physiology: applications to agriculture*. New York, NY, USA: Food Product Press and the Haworth Reference Press, 245–270.

Bauer MC, Meyer SE, Allen PS. 1998. A simulation model to predict seed dormancy loss in the field for *Bromus tectorum* L. *Journal of Experimental Botany* **49**: 1235–1244.

Benech-Arnold RL. 2004. Inception, maintenance, and termination of dormancy in grain crops: Physiology, genetics, and environmental control. In: Benech-Arnold RL, Sanchez RA, eds. *Handbook of seed physiology: applications to agriculture*. New York, NY, USA: Food Product Press and the Haworth Reference Press, 169–198.

Benech-Arnold RL, Sanchez RA, Forcella F, Kruk BC, Ghersa CM. 2000. Environmental control of dormancy in weed seed banks in soil. *Field Crops Research* **67**: 105–122.

Bethke PC, Libourel IGL, Jones RL. 2006. Nitric oxide reduces seed dormancy in *Arabidopsis*. *Journal of Experimental Botany* **57**: 517–526.

Bettey M, Finch-Savage WE, King GJ, Lynn JR. 2000. Quantitative genetic analysis of seed vigour and pre-emergence seedling growth traits in *Brassica oleracea*. *New Phytologist* **148**: 277–286.

Bewley JD. 1997a. Seed germination and dormancy. *Plant Cell* **9**: 1055–1066.

Bewley JD. 1997b. Breaking down the walls – a role for endo-β-mannanase in release from seed dormancy? *Trends in Plant Science* **2**: 464–469.

Bewley JD, Black M. 1994. *Seeds – physiology of development and germination, 2nd edn*. New York, NY, USA: Plenum Press.

Blake PS, Taylor JM, Finch-Savage WE. 2002. Identification of abscisic acid, indol-3-acetic acid, jasmonic acid, indole-3-acetonitrile, methyl jasmonate and gibberellins in developing, dormant and stratified seeds of ash (*Fraxinus excelsior*). *Plant Growth Regulation* **37**: 119–125.

Blumenthal A, Lerner HR, Werker E, Poljakoff-Mayber A. 1986. Germination preventing mechanisms in *Iris* seeds. *Annals of Botany* **58**: 551–561.

Borghetti F, Noda FN, de Sa CM. 2002. Possible involvement of proteasome activity in ethylene-induced germination of dormant sunflower embryos. *Brazilian Journal of Plant Physiology* **14**: 125–131.

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 Molecular Biology* **57**: 593–612.

Bradford KJ. 1990. A water relations analysis of seed germination rates. *Plant Physiology* **94**: 840–849.

Bradford KJ. 1995. Water relations in seed germination. In: Kigel J, Galili G, eds. *Seed development and germination*. New York, NY, USA: Marcel Dekker, 351–396.

Bradford KJ. 1996. Population-based models describing seed dormancy behaviour: implications for experimental design and interpretation. In: Lang GA, ed. *Plant dormancy: physiology, biochemistry and molecular biology*. Wallingford, Oxon, UK: CAB International, 313–339.

Bradford KJ. 2002. Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. *Weed Science* **50**: 248–260.

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 Journal*. **46**: 805–822.

Casal JJ, Sanchez RA. 1998. Phytochromes and seed germination. *Seed Science Research* **8**: 317–329.

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 Journal* **42**: 35–48.

Christensen M, Meyer SE, Allen PS. 1996. A hydrothermal time model of seed after-ripening in *Bromus tectorum*. *Seed Science Research* **6**: 155–163.

Clerkx EJM, El-Lithy ME, Vierling E, Ruys GJ, Blankestijin-DeVries H, Groot SPC, 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 Physiology* **135**: 432–443.

Cohn MA. 1996. Operational and philosophical decisions in seed dormancy research. *Seed Science Research* **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. *Physiologia Plantarum* **114**: 313–319.

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

Donohue K. 2005. Seeds and seasons: interpreting germination timing in the field. *Seed Science Research* **15**: 175–187.

Elortza F, Nuhse TS, Foster LJ, Stensballe A, Peck SC, Jensen ON. 2003. Proteomic analysis of glycosylphosphatidylinositol-anchored membrane proteins. *Molecular and Cellular Proteomics* **2**: 1261–1270.

Fenner M, Thompson K. 2005. *The ecology of seeds*. Cambridge, UK: Cambridge University Press.

Fennimore SA, Foley ME. 1998. Genetic and physiological evidence for the role of gibberellic acid in the germination of dormant *Avena fatua* seeds. *Journal of Experimental Botany* **49**: 89–94.

Finch-Savage WE. 2004. The use of population-based threshold models to describe and predict the effects of seedbed environment on germination and seedling emergence of crops. In: Benech-Arnold RL, Sánchez RL, eds. *Seed physiology: applications to agriculture*. New York, NY, USA: Haworth Press, 51–95.

Finch-Savage WE, Bergervoet JHW, Bino RJ, Clay HA, Groot SPC. 1998. Nuclear replication activity during seed-dormancy breakage and germination in the three tree species: Norway maple (*Acer platanoides* L.), sycamore (*Acer pseudoplatanus* L.) and cherry (*Prunus avium* L.). *Annals of Botany* **81**: 519–526.

Finch-Savage WE, Clay HA. 1997. The influence of embryo restraint during dormancy loss and germination of *Fraxinus excelsior* seeds. In: Ellis RH, Black M, Murdoch AJ, Hong TD, eds. *Basic and applied aspects of seed biology*. Dordrecht, the Netherlands: Kluwer Academic Publishers, 245–253.

Finch-Savage WE, Côme D, Lynn JR, Corbineau F. 2005a. Sensitivity of *Brassica oleracea* seed germination to hypoxia: a QTL analysis. *Plant Science* **169**: 753–759.

Finch-Savage WE, Phelps K. 1993. Onion (*Allium cepa* L.) seedling emergence patterns can be explained by the influence of soil temperature and water potential on seed germination. *Journal of Experimental Botany* **44**: 407–414.

Finch-Savage WE, Rowse HR, Dent KC. 2005b. Development of combined imbibition and hydrothermal threshold models to simulate maize (*Zea mays*) and chickpea (*Cicer arietinum*) seed germination in variable environments. *New Phytologist* **165**: 825–838.

Finkelstein RR. 2004. The role of hormones during seed development and germination. In: Davies PJ, ed. *Plant hormones – biosynthesis, signal transduction, action!*. Dordrecht, the Netherlands: Kluwer Academic Publishers, 513–537.

Finkelstein RR, Gampala SSL, Rock CD. 2002. Abscisic acid signaling in seeds and seedlings. *Plant Cell* **14**: S15–S45.

Flematti GR, Ghisalberti EL, Dixon KW, Trengove RD. 2004. A compound from smoke that promotes seed germination. *Science* **305**: 977.

Forbis TA, Floyd SK, deQueiroz A. 2002. The evolution of embryo size in angiosperms and other seed plants: Implications for the evolution of seed dormancy. *Evolution* **56**: 2112–2125.

Garcia-Huidobro J, Monteith JL, Squire GR. 1982. Time, temperature and germination of pearl millet (*Pennisetum typhoides* S.H.). 1. Constant temperature. *Journal of Experimental Botany* **33**: 288–296.

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.

Groot SPC, Karssen CM. 1987. Gibberellins regulate seed germination in tomato by endosperm weakening: a study with gibberellin-deficient mutants. *Planta* **171**: 525–531.

Groot SPC, Karssen CM. 1992. Dormancy and germination of abscisic acid-deficient tomato seeds. *Plant Physiology* **99**: 952–958.

Gu X-Y, Kianian SF, Foley ME. 2004. Multiple loci and epistases control genetic variation for seed dormancy in weedy rice (*Oryza sativa*). *Genetics* **166**: 1503–1516.

Gubler F, Millar AA, Jacobsen JV. 2005. Dormancy release, ABA and pre-harvest sprouting. *Current Opinion in Plant Biology* **8**: 183–187.

Gummerson RJ. 1986. The effect of constant temperatures and osmotic potential on the germination of sugar beet. *Journal of Experimental Botany* **37**: 729–958.

Gutterman Y, Gendler T. 2005. Annual rhythm of germination of seeds of *Mesembryanthemum nodiflorum* 32 years after collection. *Seed Science Research* **15**: 249–253.

Hall AE, Fiebig A, Preuss D. 2002. Beyond the Arabidopsis genome: Opportunities for comparative genomics. *Plant Physiology* **129**: 1439–1447.

Hallett BP, Bewley JD. 2002. Membranes and seed dormancy: beyond the anaesthetic hypothesis. *Seed Science Research* **12**: 69–82.

Hay FR, Mead A, Manger K, Wilson FJ. 2003. One-step analysis of seed storage data and the longevity of *Arabidopsis thaliana* seeds. *Journal of Experimental Botany* **54**: 993–1011.

Hepher A, Roberts JA. 1985. The control of seed germination in *Trollius ledebouri*: The breaking of dormancy. *Planta* **166**: 314–320.

Hilhorst HWM. 1995. A critical update on seed dormancy. I. Primary dormancy. *Seed Science Research* **5**: 61–73.

Hilhorst HWM. 1998. The regulation of secondary dormancy. The membrane hypothesis revisited. *Seed Science Research* **8**: 77–90.

Huarte R, Benech-Arnold RL. 2005. Incubation under fluctuating temperatures reduces mean base water potential for seed germination in several non-cultivated species. *Seed Science Research* **15**: 89–97.

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

Jacobsen JV, Pressman E. 1979. A structural study of germination in celery (*Apium graveolens* L.) seed with emphasis on endosperm breakdown. *Planta* **144**: 241–248.

Judd WS, Campbell CS, Kellog EA, Stevens PF, Donoghue MJ. 2002. *Plant systematics: a phylogenetic approach*. Sunderland, MA, USA: Sinauer Associates, Inc., 217–480.

Junttila O. 1973. The mechanism of low temperature dormancy in mature seeds of *Syringa* species. *Plant Physiology* **29**: 256–263.

Karssen CM. 1976. Uptake and effect of abscisic acid during induction and progress of radicle growth in seeds of *Chenopodium album*. *Physiologia Plantarum* **36**: 259–263.

Karssen CM, Haigh A, van der Toorn P, Weges R. 1989. Physiological mechanisms involved in seed priming. In: Taylorson RB, ed. *Recent advances in the development and germination of seeds*. New York, NY, USA: Plenum Press, 269–280.

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*. Berlin, Germany: Springer-Verlag, 315–323.

Koch M, Al-Shehbaz IA, Mummenhoff K. 2003. Molecular systematics, evolution, and population biology in the mustard family (Brassicaceae). *Annals of the Missouri Botanical Garden* **90**: 151–171.

Koornneef M, Alonso-Blanco C, Vreugdenhil D. 2004. Natrually occurring genetic variation in *Arabidopsis thaliana*. *Annual Review of Plant Biology* **55**: 141–172.

Koornneef M, Bentsink L, Hilhorst H. 2002. Seed dormancy and germination. *Current Opinion in Plant Biology* **5**: 33–36.

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 Science Research* **12**: 239–252.

Kucera B, Cohn MA, Leubner-Metzger G. 2005. Plant hormone interactions during seed dormancy release and germination. *Seed Science Research* **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 Journal* **23**: 1647–1656.

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*. Wallingford, UK: CAB International, 221–231.

Le Page-Degivry MT, Garello G. 1992. In situ abscisic acid synthesis: a requirement for induction of embryo dormancy in *Helianthus annuus*. *Plant Physiology* **98**: 1386–1390.

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

Leubner-Metzger G. 2001. Brassinosteroids and gibberellins promote tobacco seed germination by distinct pathways. *Planta* **213**: 758–763.

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 Science Research* **13**: 17–34.

Leubner-Metzger G. 2006. Hormonal interactions during seed dormancy release and germination. In: Basra A, ed. *Handbook of seed science and technology*. Binghamton, NY, USA: The Haworth Press, 303–342.

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 Journal* **41**: 133–145.

Leubner-Metzger G, Meins F Jr. 2001. Antisense-transformation reveals novel roles for class I β-1,3-glucanase in tobacco seed after-ripening and photodormancy. *Journal of Experimental Botany* **52**: 1753–1759.

Li BL, Foley ME. 1997. Genetic and molecular control of seed dormancy. *Trends in Plant Science* **2**: 384–389.

Liptay A, Schopfer P. 1983. Effect of water stress, seed coat restraint, and abscisic acid upon different germination capabilities of two tomato lines at low temperature. *Plant Physiology* **73**: 935–938.

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 Journal* **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 Journal* **44**: 960–971.

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 Physiology* **138**: 1538–1551.

Martin AC. 1946. The comparative internal morphology of seeds. *American Midland Naturalist* **36**: 513–660.

Meyer SE, Debaene-Gill SB, Allen PS. 2000. Using hydrothermal time concepts to model seed germination response to temperature, dormancy loss, and priming effects in *Elymus elymoides*. *Seed Science Research* **10**: 213–223.

Mohapatra SC, Johnson WH. 1978. Development of the tobacco seedling. 1. Relationship between moisture uptake and light sensitivity during seed germination in a flue-cured variety. *Tobacco Research* **4**: 41–49.

del Monte JP, Tarquis AM. 1997. The role of temperature in the seed germination of two species of the *Solanum nigrum* complex. *Journal of Experimental Botany* **48**: 2087–2093.

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 and Cell Physiology*. doi: 10.1093/pcp/pcj059.

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 Journal* **41**: 697–709.

Nambara E, Marion-Poll A. 2003. ABA action and interactions in seeds. *Trends in Plant Science* **8**: 213–217.

Nguyen H, Brown RC, Lemmon BE. 2000. The specialized chalazal endosperm in *Arabidopsis thaliana* and *Lepidium virginicum* (Brassicaceae). *Protoplasma* **212**: 99–110.

Ni B-R, Bradford KJ. 1992. Quantitative models characterizing seed germination responses to abscisic acid and osmoticum. *Plant Physiology* **98**: 1057–1068.

Ni BR, Bradford KJ. 1993. Germination and dormancy of abscisic acid-deficient and gibberellin-deficient mutant tomato (*Lycopersicon esculentum*) seeds – Sensitivity of germination to abscisic acid, gibberellin, and water potential. *Plant Physiology* **101**: 607–617.

Nikolaeva MG. 1967. *[Physiology of deep dormancy in seeds.]* Leningrad, Russia: Izdatel'stvo 'Nauka' (in Russian). [Translated from Russian by Z. Shapiro (1969), National Science Foundation, Washington, DC, USA: 219.]

Nikolaeva MG. 2004. On criteria to use in studies of seed evolution. *Seed Science Research* **14**: 315–320.

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.

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 Biology* **15**: 1998–2006.

Petruzzelli L, Müller K, Hermann K, Leubner-Metzger G. 2003. Distinct expression patterns of β-1,3-glucanases and chitinases during the germination of Solanaceous seeds. *Seed Science Research* **13**: 139–153.

Pons TL. 2000. Seed responses to light. In: Fenner M, ed. *Seeds – the ecology of regeneration in plant communities*. Wallingford, UK: CAB International, 237–260.

Pritchard SL, Charlton WL, Baker A, Graham IA. 2002. Germination and storage reserve mobilization are regulated independently in Arabidopsis. *Plant Journal* **31**: 639–647.

Probert RJ. 2000. The role of temperature in the regulation of seed dormancy and germination. In: Fenner M, ed. *Seeds: the ecology of regeneration in plant communities*. Wallingford, UK: CAB International, 261–292.

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

Ramakrishna P, Amrithhale D. 2005. The perisperm-endosperm envelope in *Cucumis*: Structure, proton diffusion and cell wall hydrolysing activity. *Annals of Botany* **96**: 769–778.

Rinne PLH, Kaikuranta PM, van der Schoot C. 2001. The shoot apical meristem restores its symplasmic organization during chilling-induced release from dormancy. *Plant Journal* **26**: 249–264.

Sanchez RA, Mella RA. 2004. The exit from dormancy and the induction of germination: Physiological and molecular aspects. In: Benech-Arnold RL, Sanchez RA, eds. *Handbook of seed physiology: applications to agriculture*. New York, NY, USA: Food Product Press and the Haworth Reference Press, 221–243.

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

Schopfer P, Plachy C, Frahry G. 2001. Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid. *Plant Physiology* **125**: 1591–1602.

da Silva EAA, Toorop PE, Nijsse J, Bewley JD, Hilhorst HWM. 2005. Exogenous gibberellins inhibit coffee (*Coffea arabica* cv. Rubi) seed germination and cause cell death in the embryo. *Journal of Experimental Botany* **413**: 1029–1038.

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.

Skoda B, Malek L. 1992. Dry pea seed proteasome. *Plant Physiology* **99**: 1515–1519.

Soltis DE, Soltis PS. 2003. The role of phylogenetics in comparative genetics. *Plant Physiology* **132**: 1790–1800.

Steadman KJ, Crawford AD, Gallagher RS. 2003. Dormancy release in *Lolium rigidum* seeds is a function of thermal after-ripening time and seed water content. *Functional Plant Biology* **30**: 345–352.

Steadman KJ, Pritchard HW. 2004. Germination in *Aesculus hippocastanum* seeds following cold-induced dormancy loss can be described in relation to temperature-dependent reduction in base temperature (T_b). *New Phytologist* **161**: 415–425.

Steinbach HS, Benech-Arnold R, Sanchez RA. 1997. Hormonal regulation of dormancy in developing sorghum seeds. *Plant Physiology* **113**: 149–154.

Stevens PF. 2005. *Angiosperm phylogeny website*, version 6. http://www.mobot.org/MOBOT/research/ABweb/

Tao K-L, Khan AA. 1979. Changes in the strength of lettuce endosperm during germination. *Plant Physiology* **63**: 126–128.

Thompson K. 2000. The functional ecology of soil seed banks. In: Fenner M, ed. *Seeds: the ecology of regeneration in plant communities*. Wallingford, UK: CAB International, 215–235.

Thompson K, Ceriani RM, Bakker JP, Bekker RM. 2003. Are seed dormancy and persistence in soil related? *Seed Science Research* **13**: 97–100.

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. *Journal of Experimental Botany* **51**: 1371–1379.

Tsiantis M. 2006. Plant development: Multiple strategies for breaking seed dormancy. *Current Biology* **16**: R25–R27.

Vleeshouwers LM, Bouwmeester HJ. 2001. A simulation model for seasonal changes in dormancy and germination of weed seeds. *Seed Science Research* **11**: 77–92.

Vleeshouwers LM, Bouwmeester HJ, Karssen CM. 1995. Redefining seed dormancy: an attempt to integrate physiology and ecology. *Journal of Ecology* **83**: 1031–1037.

Walck JL, Baskin JM, Baskin CC, Hidayati S. 2005. Defining transient and persistent seed banks in species with pronounced seasonal dormancy and germination patterns. *Seed Science Research* **15**: 189–196.

Wang M, Heimovaara-Dijkstra S, Van Duijn B. 1995. Modulation of germination of embryos isolated from dormant and nondormant barley grains by manipulation of endogenous abscisic acid. *Planta* **195**: 586–592.

Watkins JT, Cantliffe DJ. 1983. Mechanical resistance of the seed coat and endosperm during germination of *Capsicum annuum* at low temperatures. *Plant Physiology* **72**: 146–150.

Welbaum GE, Bradford KJ, Yim K-O, Booth DT, Oluoch MO. 1998. Biophysical, physiogical and biochemical processes regulating seed germination. *Seed Science Research* **8**: 161–172.

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 Physiology* **122**: 1081–1088.

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

Wu C-T, Leubner-Metzger G, Meins F Jr, Bradford KJ. 2000. Class I β-1,3-glucanase and chitinase are expressed in the micropylar endosperm of tomato seeds prior to radicle emergence. *Plant Physiology* **126**: 1299–1313.

Yamaguchi S, Kamiya Y. 2002. Gibberellins and light-stimulated seed germination. *Journal of Plant Growth Regulation* **20**: 369–376.

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.

Yim KO, Bradford KJ. 1998. Callose deposition is responsible for apoplastic semipermeability of the endosperm envelope of muskmelon seeds. *Plant Physiology* **118**: 83–90.

Supplementary Material

The following supplementary material is available for this article online:

Section III.5 Applied aspects of the control of germination by seed dormancy

This material is available as part of the online article from http://www.blackwell-synergy.com