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REVIEW PAPER

Seed dormancy cycling and the regulation of dormancy mechanisms to time germination in variable field environments

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Abstract

Many molecular mechanisms that regulate dormancy have been identified individually in controlled laboratory studies. However, little is known about how the seed employs this complex suite of mechanisms during dormancy cycling in the variable environment of the soil seed bank. Nevertheless, this behaviour is essential to ensure germination takes place in a favourable habitat and climate space, and in the correct season for the resulting plant to complete its life cycle. During their time in the soil seed bank, seeds continually adjust their dormancy status by sensing a range of environmental signals. Those related to slow seasonal change (e.g. temperature) are used for temporal sensing to determine the time of year and depth of dormancy. This alters their sensitivity to signals related to their spatial environment (e.g. light, nitrate, and water potential) that indicate that conditions are suitable for germination, and so trigger the termination of dormancy. We review work on the physiological, molecular, and ecological aspects of seed dormancy in Arabidopsis and interpret it in the context of dormancy cycling in the soil seed bank. This approach has provided new insight into the co-ordination of mechanisms and signalling networks, and the multidimensional sensing that regulates dormancy cycling in a variable environment.

Key words: Annual life cycle, Arabidopsis, DOG1, dormancy cycling, germination, nitrate signalling, PHYA, seed dormancy.

Introduction

Many genes and molecular mechanisms that can regulate seed dormancy and germination have been identified individually in controlled laboratory studies (Finch-Savage and Leubner-Metzger, 2006; Holdsworth *et al.*, 2008; North *et al.*, 2010; Graeber *et al.*, 2012; Dekkers and Bentsink, 2015; Rodriguez *et al.*, 2015). For good experimental reasons these studies minimize variation and usually consider only one gene and a single environmental variable, such as light, temperature, or nitrate. However, little is known about how the seed employs this complex suite of mechanisms to regulate dormancy in the variable field environment. Nevertheless, this behaviour is essential to ensure that germination takes place in a favourable habitat and climate space, and in the correct season for the resulting plant to complete its life cycle. Dormancy cycling is therefore also central to the competitiveness of weeds in crop production practice; and understanding it is crucial to the future development of more environmentally benign cultural weed management practices.

When shed from the mother plant in the field environment, seeds that do not germinate immediately enter the soil seed bank where they may remain in an imbibed dormant state for considerable periods (Baskin and Baskin, 1998; Fenner and Thompson, 2005; Long *et al.*, 2015). During their time in the soil, seed bank seeds repair their DNA to maintain genetic fidelity (Waterworth *et al.*, 2016), and they also continually adjust their dormancy status by sensing and integrating a

range of environmental signals (Fig. 1). These signals inform the seed whether it is in an appropriate habitat, climate space, and time of the year suitable for the resulting plant to survive, be competitive, and reproduce. Dormancy cycling coupled to seed longevity represents a bet-hedging strategy through persistence in the soil seed bank (Evans and Dennehy, 2005; Walck *et al.*, 2011; Footitt *et al.*, 2014). Subtle differences in this behaviour result in local adaptation and ecotypic differences.

In this review, we develop a molecular ecophysiological view of the involvement of seed dormancy and its role in the natural and agricultural environment. We then consider its regulation by signals from these environments through current knowledge of molecular mechanisms identified for seeds in the laboratory. We focus on *Arabidopsis thaliana* since most of these molecular mechanisms have been identified in this model species and because of its proven relevance in ecological studies. Furthermore, although not a competitive weed, it is a relevant model for the seed dormancy cycling behaviour of many dicot weed species.

Dormancy, dormancy cycling, and the concept of a dormancy continuum

Mature dry seeds are termed quiescent; they generally have a low moisture content (5–15%) and almost stationary metabolic activity; in this state, seeds can survive for decades (Long *et al.*, 2015). It is only when seeds are hydrated and placed under conditions suitable for germination that dormancy can be assessed. Dormancy is then recognized as an innate property (physical or physiological) of the seed that blocks the capacity to germinate over a specified time period under any combination of environmental conditions (adequate water, temperature, oxygen, and light) that will support the germination process (Baskin and Baskin, 2004). A diverse range of 'blocks' or dormancy mechanisms has evolved, in line with the diversity of climates and habitats that plant species have been able to colonize (Willis *et al.*, 2014). These mechanisms can be used to define five classes of seed dormancy (Baskin and Baskin, 2004). Of these classes 'physiological' dormancy (PD) is the most abundant form occurring across all major angiosperm clades and the class present in most seed model species including Arabidopsis (Finch-Savage and Leubner-Metzger, 2006).

In order to interpret seed responses to the environment, it is necessary to have a common general understanding of dormancy beyond its basic definition. It is agreed by many that dormancy exists as a continuum with a number of layers (blocks to germination completion) that are successively removed by appropriate environmental signals; the removal of the final layers or layer (often in response to light) is synonymous with the stimulation/induction of germination completion (radicle emergence through the layers surrounding the embryo) (Finch-Savage and Leubner-Metzger, 2006). There is a contrasting view that dormancy relief and stimulation of germination are separate processes so that non-dormant seeds can remain in the soil awaiting stimulation of germination by a change in the environment (Thompson and Ooi, 2010). Initially this distinction may seem trivial, but it is central to an agreed understanding of dormancy and dormancy cycling in the soil as a negatively regulated and dynamic process of changes in the seed, rather than a passive response to a change in the environment. A comprehensive argument has been provided for the former approach (dormancy continuum) based on advances in both the physiological and

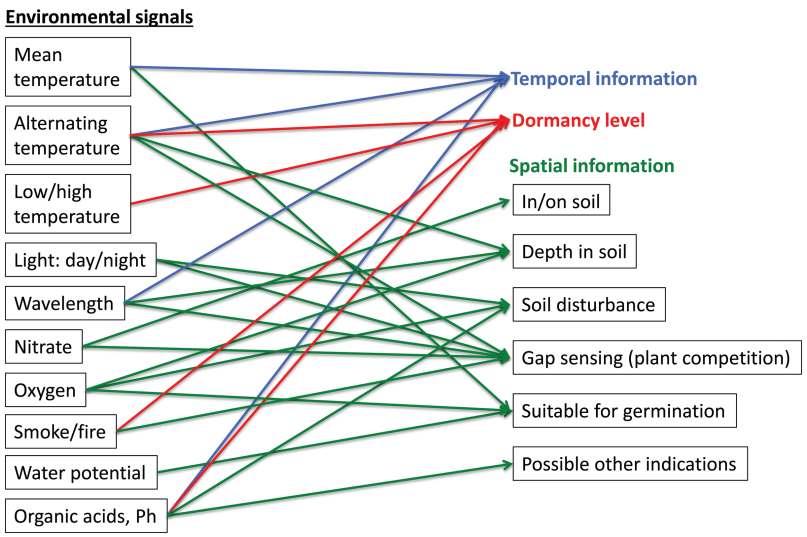

Fig. 1. Environmental signals in the soil seed bank. The schematic shows a range of potential signals that could influence dormancy directly; inform the seed about the time of year (temporal information), and/or the suitability of the immediate environment for the completion of germination (spatial information). The precise nature of the signals differs depending on the soil type and the modifying impact of the many other organisms that occupy the soil; in particular, soil microorganisms as their activity is temperature related, and they use oxygen and otherwise modify the gaseous atmosphere, mineralize nutrients, and help release many phytoactive chemicals including organics acids. The figure is based on Finch-Savage WE and Footitt S. 2015. Regulation of seed dormancy cycling in seasonal field environments. In: Anderson JV, ed. Advances in plant dormancy, 35–47, and is used with permission of Springer.

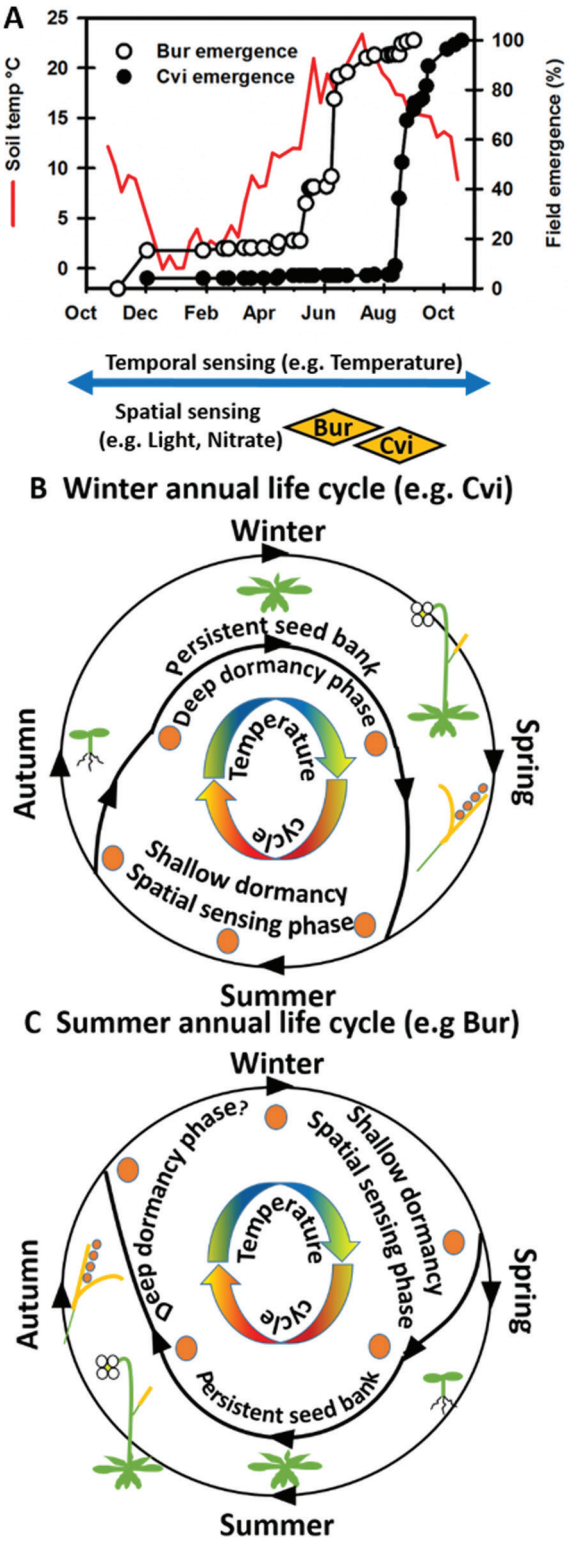

Fig. 2. Seed response to the environment initiates winter and summer annual life cycles. (A) In temperate zones, mean soil temperature follows a clear annual cycle (temporal signal) that drives changing sensitivity to spatial signals informing the seed of the immediate environmental suitability for germination. Yellow diamonds indicate increasing and decreasing sensitivity; maximum height of the diamond is when maximum germination occurred in exhumed seeds. Adaptation of this response leads to different patterns of dormancy cycling and subsequent life cycles. This is illustrated here using the Bur and Cvi ecotypes (B and C, respectively). Data redrawn from Footitt *et al.*, 2013. (B) Seedlings of winter annual Arabidopsis ecotypes such as Cvi emerge in the autumn. The rosettes are cold vernalized over winter to induce flowering and shed their seeds in spring. On entering the soil, seed dormancy (primary dormancy) slowly declines through the impact of warming soil temperature (temporal signal) and the spatial sensing phase of shallow dormancy begins. If signals are received in the correct order, the seed will germinate, resulting in seedling establishment in autumn. In the absence of these spatial signals, the window closes and falling soil temperature cycles dormancy (secondary dormancy) into the deep dormancy phase that represents the persistent seed bank. (C) Seedlings of summer annual Arabidopsis ecotypes such as Bur (Evans and Ratcliffe, 1972; Ratcliffe, 1976) emerge in the spring. The rosettes are vernalization insensitive and require a long rosette phase before flowering over the summer and shedding their seeds in autumn. On entering the soil, seed dormancy (primary dormancy) initially declines through the impact of low soil temperature, but prolonged low winter soil temperature (temporal signal) causes dormancy to increase (secondary dormancy). It then declines with increasing soil temperature in spring, entering the spatial sensing phase at which point seedling emergence is possible. If appropriate spatial signals are not received, seeds enter the persistent seed bank. At this point, high soil temperature may induce a deep dormancy phase of secondary dormancy.

molecular understanding of dormancy and germination (Finch-Savage and Footitt, 2012); this view is adopted in the rest of this review.

Environmental signals related to slow seasonal change, principally temperature (Probert, 2000), are used for temporal sensing to determine the time of year and depth of dormancy (Fig. 2). Response to temperature differs between species, resulting in characteristic germination timings (Batlla and Benech-Arnold, 2015). This response alters the depth of dormancy and therefore the sensitivity of the seeds to signals related to their spatial environment, henceforth termed spatial signals (Fig. 1; e.g. light, nitrate, and water potential). These signals indicate when conditions are suitable for germination, and so trigger the termination of dormancy if these conditions are present at that time (Finch-Savage and Leubner-Metzger, 2006). The process usually needs to be carried out in a set order for it to work, namely spatial signals only have an effect if temporal sensing has enhanced sensitivity to them. In an obvious example, deeply dormant seeds are not responsive to light, but as deep dormancy is relieved sensitivity and response to different signals (e.g. nitrate and light) occur progressively (Finch-Savage *et al.*, 2007). Thus, a dormancy continuum has been proposed that is driven in both directions by environmental signals and, when all layers are removed, germination occurs. In the annual dormancy cycle, if the correct spatial signal is not sensed during the spatial sensing phase, the seed becomes increasingly dormant.

Although spatial signals can have a temporal pattern, they appear to have little impact outside the spatial sensing phase. Once in the soil seed bank, the physical position of the space in which seeds find themselves is not likely to change, except by disturbance, but the nature of that space can alter either slowly or rapidly. For example, if competing plants die or are otherwise removed, light and nitrate signals to the seed are altered; or, if it rains, water potential and nitrate are altered. Although these are temporal changes to spatial signals, the effect is not integrated over time, but the suitability for germination completion is altered and within the spatial sensing phase the seed response to this is rapid.

Dormancy cycling: adaptation to climate as a driver of winter and summer annual life histories

Within Arabidopsis, both winter annual (e.g. Cvi) and summer annual (e.g. Bur) behaviour has been identified based on the requirement for vernalization-induced flowering (Effmertova, 1967; Des Marais *et al.*, 2012). The annual weather patterns in the regions of origin of Cvi and Bur indicate that this behaviour is driven by adaptation to climate (Footitt *et al.*, 2013) in agreement with the observations of Cetl *et al.,* (1965) (see Supplementary Fig. S1 at *JXB* online). When sown and compared in a common temperate environment, as illustrated in Fig. 2, they retain their winter or summer annual behaviour; and seedling emergence patterns reflect the adaptive positioning of the spatial sensing phase in response to soil temperature. Their contrasting behaviours make them ideal for studying the differential adaptation of dormancy cycling and germination mechanisms, and we return to this at the end of the review.

Soil temperature is the dominant environmental factor controlling depth of dormancy during cycling in imbibed seeds (Probert, 2000; Finch-Savage and Leubner-Metzger, 2006). Seasonal changes in soil temperature control the rate of increase and decrease in seed dormancy throughout the year. Many other signals also provide the seed with spatial information (Fig. 1). Furthermore, seasonal cycles in soil microbial activity (also temperature driven) drive the soil nitrogen (nitrous oxide) and CO_2 cycles and the release of organic compounds. These can also have a positive impact on seed germination potential as dormancy declines through changing sensitivity to soil nitrate and CO_2 (see nitrate section below; Yoshioka *et al.*, 1998).

Contribution of the mother plant to subsequent dormancy cycling

Depth of dormancy at shedding is genetically determined, but environmental conditions experienced by the mother plant significantly influence the characteristics and performance of the seeds produced (Fenner, 1991; Baskin and Baskin, 1998; Fenner and Thompson, 2005). As in the soil, temperature is the major factor during seed maturation that affects the depth of seed dormancy (Fenner, 1991; Chiang *et al.*, 2011; Kendall *et al.*, 2011; Huang *et al.*, 2014; Springthorpe and Penfield, 2015), for example via the quantitative expression of *DOG1* (*DELAY OF GERMINATION 1*) in Arabidopsis (Chiang *et al.*, 2011; Kendall *et al.*, 2011; Nakabayashi *et al.*, 2012). DOG1 protein levels increase during seed development, but appear to remain constant even in after-ripened (AR) seeds that subsequently germinate. However, modification of DOG1 in AR seeds indicated that protein inactivation was involved in reduced dormancy levels (Nakabayashi *et al.*, 2012); we return to this in describing regulation of dormancy following shedding.

Lower temperatures to the mother plant tend to enhance depth of dormancy (Fenner, 1991; Fenner and Thompson, 2005; Huang *et al*, 2014; Springthorpe and Penfield, 2015). Higher and lower dormancy at maturity appear to occur either side of a critical temperature in the region of 15 °C experienced during seed development (Springthrope and Penfield, 2015). Other environmental factors experienced by the mother plant during seed maturation such as water stress (e.g. Peters, 1982) and nutrient supply, in particular nitrate (Alboresi *et al.*, 2005; Matakiadis *et al.*, 2009; Huang *et al.*, 2014), also influence the depth of dormancy. At one extreme, maternal effects can result in minimal dormancy and preharvest sprouting; principally a problem in grain crops and reviewed elsewhere (Rodriguez *et al.*, 2015). These behaviours impact on the proportion of seeds that germinate immediately or enter the soil seed bank each year.

Dormancy in the freshly shed seed

Despite the obvious importance of dormancy cycling in the whole life cycle of plants very little is known about its regulation at the molecular level. In contrast, a great deal is known about mechanisms that influence dormancy loss in short-term laboratory experiments, many of which involve the screening of mutants for altered dormancy and germination (Finch-Savage and Leubner-Metzger, 2006; Baskin and Baskin, 1998; Nambara *et al.*, 2010; Bassel *et al.*, 2011; Graeber *et al.*, 2012; Dekkers *et al.*, 2013). This laboratory-based work has largely used seeds from accessions of the model species Arabidopsis that naturally have limited dormancy. In addition, the seeds used for study have been produced under optimal conditions, with temperatures sufficiently high to minimize dormancy (Kendall *et al.*, 2011). Many of the genes identified have subsequently been found to be involved in the abscisic acid (ABA) and gibberellin (GA) metabolism and signalling pathways (Fig. 3: Kucera *et al.*, 2005; Graeber *et al.*, 2012). This has confirmed the central involvement of the ABA/GA balance hypothesis in the ability of the seeds to interpret the environment and thereby regulate dormancy and germination (Fig. 4; Kucera *et al.*, 2005; Finch-Savage and Leubner-Metzger, 2006). This balance appears to operate as a central integration point for upstream incoming environmental signals (Fig. 5; Bassel, 2016). Downstream signalling is becoming well documented, but the critical control points remain unclear (Finch-Savage and Bassel, 2016). This signalling ultimately drives changes in turgor generation, altered mechanical properties of the cell wall, and sensitivity to external water potential, resulting in growth and the completion of germination. The key questions now are related to what exists upstream to influence and regulate this ABA/GA balance in response to environmental signals. We consider this below, but first discuss this central integrating hormone balance in the context of dormancy cycling in the field.

Temporal separation of mechanisms during dormancy cycling in the soil seed bank

As discussed above, most often genes/mechanisms have been considered in isolation, in constant and therefore simple environments. From these experiments, it is not obvious why so many different mechanisms are required and there is an apparent duplication of function and redundancy. However, in the field, seeds have to operate in the complex and variable

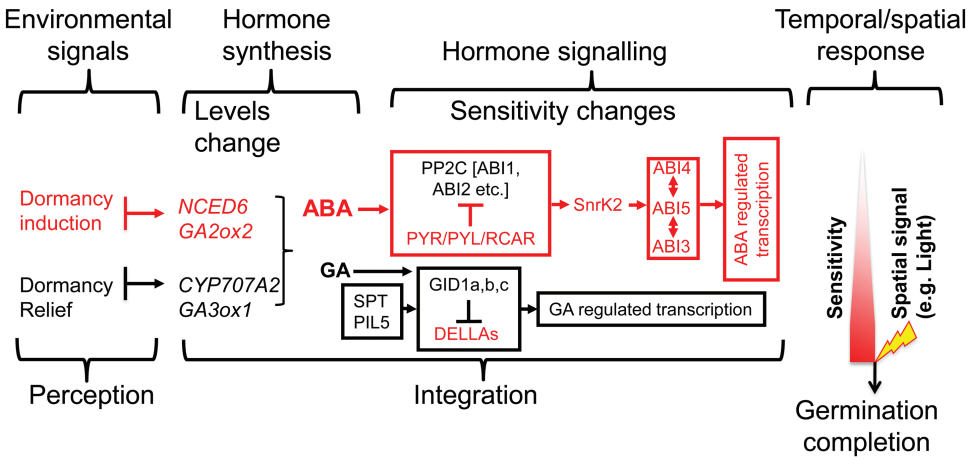

Fig. 3. Schematic model for the regulation of dormancy and germination by ABA and GA in response to the environment. According to this model ambient environmental signals affect the ABA/GA balance and the sensitivity to these hormones. On the ABA side of the balance, the ABA receptors PYR/PYL/RCAR bind to ABA to remove the repression of ABA responses by PP2Cs (protein phosphatase 2C; Cutler *et al.*, 2010; Nambara *et al.*, 2010). Removal of PP2C repression allows downstream signalling via SnRK2s to ABRE (ABA-response element) binding transcription factors (ABI3, ABI4, ABI5). On the other side of this balance, DELLA proteins (Bassel *et al.*, 2004; Lee *et al.*, 2012) repress GA responses and therefore germination potential (Sun and Gubler, 2004). DELLAs are degraded in the presence of GA (Hartweck, 2008). The repression activity of DELLA is therefore relieved upon GA binding its receptor GID1 and the F-box protein SLEEPY. Removal of DELLA proteins in seeds leads to a de-repression of cell wall remodelling gene expression and in turn growth of the embryo (Cao *et al.*, 2006). A further checkpoint in seedling establishment is mediated by ABA-INSENSITIVE5 (ABI5) in Arabidopsis, which acts to promote ABA-mediated growth arrest during a late stage of seed germination (Lopez-Molina *et al.*, 2003). ABA synthesis and signalling and GA catabolism dominate the induction and deepening of the dormant state (pathway indicated in red), whereas GA synthesis and signalling and ABA catabolism dominate the relief of dormancy and the transition to germination completion (pathway indicated in black). Change in the depth of dormancy alters sensitivity to spatial signals. When sensitivity overlaps with changing ambient conditions, germination will proceed to completion. The figure is adapted from Footitt *et al.* (2011).

conditions of the soil seed bank that may require a complexity of subtle dormancy regulation for its interpretation. Footitt *et al.*, (2011) began a series of field experiments to investigate how molecular mechanisms identified as controlling dormancy in the laboratory could be seasonally co-ordinated in seeds buried in field soil. They used the deeply dormant ecotype Cvi and initially approached this through gene expression studies targeted at the dynamic ABA/GA balance and key dormancy-regulating genes identified in the laboratory. The relative importance of these genes for dormancy cycling had previously been identified using full genome arrays of laboratory-derived samples of Cvi that built up the components of dormancy cycling (Cadman *et al.*, 2006; Finch-Savage *et al.*, 2007).

They found that depth of dormancy and gene expression patterns were correlated with seasonal changes in soil temperature. Dormancy and the expression of dormancy-related genes were highly sensitive to the soil environment, and molecular and physiological changes could be equated to changes in sensitivity to soil temperature history, nitrate, light, and GAs. This was consistent with dormancy as a continuum, with layers of dormancy being progressively removed by environmental signals until only light is required, in the absence of which seeds remain dormant and enter into another dormancy cycle as the seasons change (Footitt *et al.*, 2011, 2013, 2014; Finch-Savage and Footitt, 2012). The temporal patterns of gene expression were consistent with ABA signalling linked to deep dormancy in winter being repressed in spring concurrent with enhanced DELLA repression of GA signalling and germination as depth of dormancy decreased to a shallow dormancy phase (Fig. 4).

As soil temperature declined in winter, dormancy increased as expression of ABA synthesis (*NCED6*) and GA catabolism (*GA2ox2*) genes increased (Fig. 4). This was linked to an increase in endogenous ABA that plateaus, but dormancy and *DOG1* and *MFT* expression continued to increase. The expression of SNF1-related protein kinase genes, *SnrK2.1* and *2.4*, also increased, consistent with enhanced ABA signalling and sensitivity being modulated by seasonal soil temperature. Temperature then increased in spring and summer, and dormancy declined. Concurrent with this was a decrease in endogenous ABA along with positive ABA signalling as expression of *ABI2*, *ABI4*, and ABA catabolism (*CYP707A2*) and GA synthesis (*GA3ox1*) genes increased. However, during the low dormancy phase in the summer, expression of transcripts for the germination repressors *RGA* and *RGL2* increased.

Therefore, temporal separation of mechanisms exists, with deep dormancy in winter promoted by ABA signaling, and this contrasted with shallow dormancy in spring and summer controlled by repression of GA signalling. Thus seeds remain dormant throughout, but crucially the deep, ABA-regulated dormancy is unresponsive to spatial signals such as light (and GA), whereas the shallow dormancy due to DELLA repression is rapidly removed by exposure to light. That is to say the switch to shallow dormancy enables a response to spatial signals such as light. Before discussing this response further, we consider the deep dormancy phase in more detail.

Deep dormancy and DOG1

ABA has been linked to depth of dormancy in Cvi (Al-Rachedi *et al.*, 2004). However, during dormancy cycling in the soil, following an initial rise in the amount of ABA with dormancy, it reached a plateau while depth of dormancy

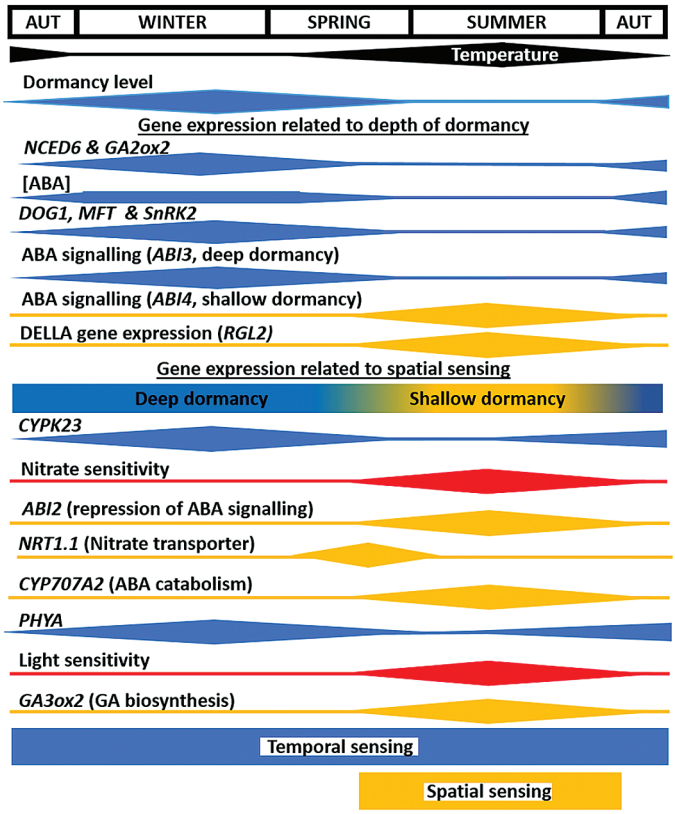

Fig. 4. Seasonal patterns of physiological measures and gene expression in Cvi seeds over an annual cycle in field soil. The height of the bars indicates the extent of changing soil temperature (seed depth), the amplitude of physiological response, or expression of the genes indicated over the seasons shown in the top panel. Changing dormancy level in buried seeds expressed as AR50 (dry after-ripening time required to achieve 50% germination) is shown. Temporal sensing represents this slow seasonal change in dormancy for the selection of time of year, climate space, and timing of the spatial sensing phase (blue bars). Sensitivity is demonstrated by germination of exhumed seeds at 20 °C/light with and without nitrate (red bars). Spatial sensing represents the period when seeds become sensitive to conditions suitable for germination completion (yellow bars). Completion occurs when sensitivity overlaps with suitable ambient conditions; if suitable ambient conditions do not occur at this time, seeds return to deep dormancy. The function of the genes shown is described in the text. (Data are redrawn from Footitt *et al.* (2011, 2013).

continued to increase (Fig. 4), showing that the final depth of dormancy is not set during seed maturation (Footitt *et al.*, 2011). This indicated that ABA signalling and sensitivity are more likely to be regulators of dormancy than the absolute amount of ABA.

In the laboratory, functional analysis shows that both DOG1 and ABA are essential for establishing primary dormancy. However, DOG1 can act independently of ABA to delay germination of less dormant seeds (Graeber *et al.*, 2014). Although ABA promotes *DOG1* expression (Graeber *et al.*, 2010), reduced dormancy was seen both in an ABA-deficient background (*aba1*) in the presence of the strong Cvi *DOG1* allele and in a high ABA content background in the absence of *DOG1* (*dog1-2 cyp707a2-1*) (Bentsink *et al.*, 2006; Nakabayashi *et al.*, 2012), indicating that both are required for induction of primary dormancy. In contrast, thermoinhibition of germination was DOG1 dependent and not reliant on an increased amount of ABA, indicating that they operate in parallel interacting pathways (Huo *et al.*, 2016).

In the field, Footitt *et al.* (2011) show that ABA is not quantitatively related to depth of dormancy during cycling. Therefore, once seeds enter deep dormancy, DOG1 expression may be the dominant factor by influencing ABA sensitivity so that dormancy can be enhanced without an increase in ABA. Postma and Agren (2016) show that the major quantitative trait locus (QTL) for seedling establishment was collocated with the QTL *DOG1* and that selection during this phase had a significant role in the fitness advantage of local genotypes. This indicates the importance of seed dormancy and the *DOG1* QTL in explaining variation in fitness across the whole life cycle. In other field studies, there was also co-location of a QTL at *DOG1* in both germination and seedling emergence (Huang *et al.*, 2010; Postma and Agren, 2016). Furthermore, annual seedling emergence pattern traits in a Cvi×Bur recombinant inbred line (RIL) mapping population also show that the principle QTL for emergence timing co-locates with *DOG1* (S. Footitt, P.G. Walley, J.R. Lynn, A.J. Hambidge, and W.E. Finch-Savage, unpublished). Co-location of these QTLs is presumably related to the influence of DOG1 on miRNA156, which regulates phase transitions (see below). Thus DOG1 is of central importance to dormancy cycling in the field in addition to its importance in determining the extent of primary seed dormancy (Bentsink *et al.*, 2006; Chiang *et al.*, 2011).

Overall, during the annual dormancy cycle, expression of *DOG1* is positively correlated with expression of genes that are positive regulators of dormancy and negatively correlated with negative regulators (Footitt *et al.*, 2011, 2013, 2014, 2015). In the spatial sensing phase of the dormancy cycle, germination only occurs in the light if *DOG1* expression is low as a result of chromatin remodelling (see below) and, based on the observations of Nakabayashi *et al.* (2012, 2015), the level of active DOG1 protein is reduced.

Is DOG1 part of a thermal sensing mechanism?

The strong relationship between *DOG1* expression, temperature, and dormancy described above may constitute part of a thermal sensing mechanism for the setting of dormancy levels in response to the prevailing environment during seed maturation and during dormancy cycling in the soil seed bank. This response may be regulated at the chromatin level. When Arabidopsis seeds lose dormancy, H3K4me3 marks on *DOG1* chromatin decrease while H3K27me3 marks increase, and *DOG1* expression decreases (Müller *et al.*, 2012). Footitt *et al.* (2015) investigated the deposition of these specific histone modifications (activating H3K4me3; repressing H3K27me3) to *DOG1* and its expression during a complete laboratory-induced dormancy cycle. They had previously suggested that DOG1 accumulation may represent accumulated thermal time (temporal sensing) to regulate the depth and persistence of dormancy (Footitt *et al.*, 2014). This more recent work by Footitt *et al.* (2015) led to the additional proposal that the changing proportions of H3K4me3 and H3K27me3 marks

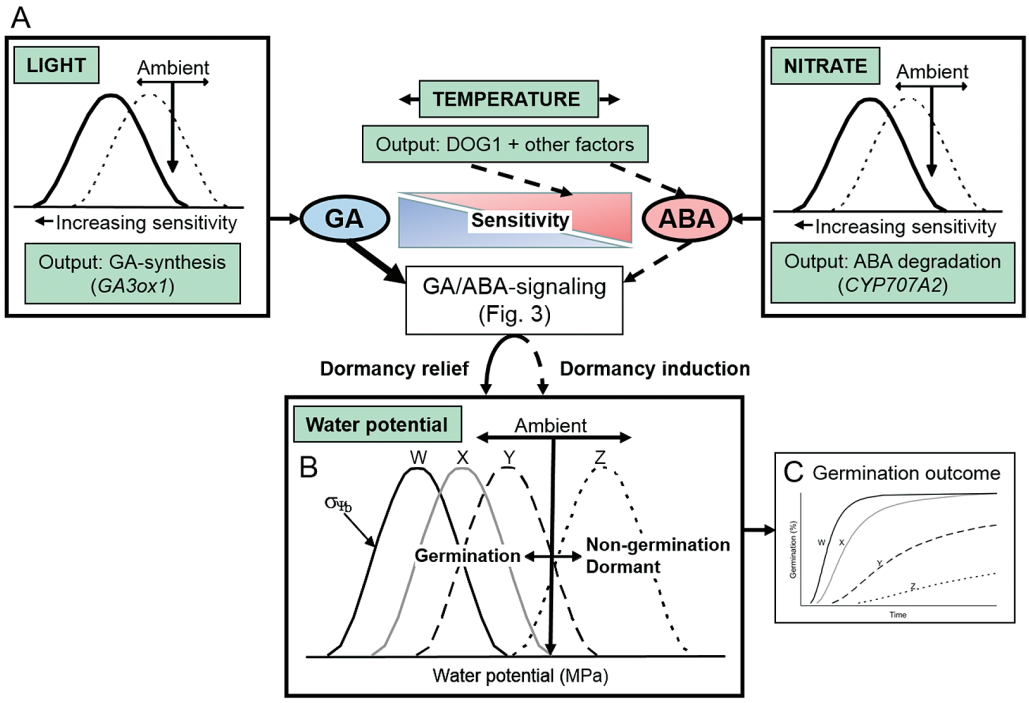

Fig. 5. Response to spatial signals during shallow dormancy. The schematic illustrates changes in seeds as they are relieved from ABA-dominated deep dormancy and enter DELLA-repressed shallow dormancy. (A) The ABA/GA balance acts as a central integration system accommodating the response to ambient signals that vary. Entry to shallow dormancy is marked by a reduced temperature- (DOG1) driven emphasis on ABA and sensitivity to it. In this phase, the ABA/GA balance is influenced by the ambient level of nitrate and exposure to light as a function of the sensitivity of the seeds (normally distributed in the seed population) to them. Changing sensitivity is illustrated as a shift in this normal distribution with the resulting output for light of enhanced *GA3ox1* expression (Cadman *et al.*, 2006) and for nitrate of enhanced *CYP707A2* expression (Matakiadis *et al.*, 2009). These increase GA and reduce ABA content and signalling, respectively (see Fig. 3). (B) The schematic uses the hydrothermal time model (Bradford, 1995, 2002) to illustrate the dynamic impact of changes in the ABA/GA balance on the potential to germinate. In the model, progress towards germination is proportional to the extent by which ambient water potential (Ψ) exceeds the threshold (base; Ψ_b) below which progress ceases. Thresholds differ between individuals in the population, giving a distribution of sensitivities ($\sigma_{\Psi b}$). The Ψ_b distribution is shown for a partially dormant population of seeds (Z); in the proportion where Ψ_b is greater than ambient water potential, germination completion does not occur. As dormancy is progressively relieved (Z>Y>X>W), Ψ_b of individuals in the population becomes more negative so the difference to ambient water potential is greater and their progress to germination completion speeds up. The resulting germination curves for W–Z at the same ambient water potential are shown in (C). In general, gibberellins decrease Ψ_b to enhance germination, whereas ABA increases Ψ_b to inhibit germination increasingly (Ni and Bradford, 1993; Alvarado and Bradford, 2005). In practice, ABA can act independently so that there is a synergistic effect of ABA and reduced water potential. The overall process is complex, with multidimensional sensitivity to a range of signals. For clarity, here only these three example inputs (temperature, light, and nitrate) to the hormone balance and their consequences are illustrated. The threshold model approach could be used to explain all the responses illustrated and probably other environmental signals (Bradford, 2002, 2005; Donohue *et al.*, 2015). However, continued work is required to understand fully the inputs to the hormone balance to build upon this general framework.

act as part of a thermal sensing mechanism in the regulation of *DOG1* transcription in line with seasonally changing soil temperature to provide another layer of regulation.

The mechanism by which DOG1 operates is complex and is only partially understood (Nakabayashi *et al.*, 2012, 2015; Cyrek *et al.*, 2016) so the question remains as to how DOG1 alters dormancy and the potential to germinate. Recently it was shown that DOG1 regulates seed dormancy by influencing levels of the miRNAs miR156 and miR172 in both lettuce and Arabidopsis (Huo *et al.*, 2016). These miRNAs govern the progression through the transition from dormancy to germination and indicate a potential mechanism for DOG1 action. In Arabidopsis, higher miR156 levels resulted in enhanced seed dormancy (Huo *et al.*, 2016). It is interesting to note that sequencing of a small RNA library of field seed samples collected in mid-winter (high dormancy) and mid-summer (low dormancy, requiring only light) identified highly abundant levels of miR156 at both stages (S. Footitt, O. Smith, and W.E. Finch-Savage, unpublished). This indicates that in the soil seed bank DOG1 maintains high levels of miR156 even during the spatial sensing phase until the final layer of dormancy is removed. Overall the data suggest that accumulation of DOG1 can transduce the environmental effect during maturation and that subsequent changes in its regulation at the chromatin level are closely linked to environmental signals in the soil seed bank. This is consistent with the hypothesis that DOG1 largely affects the sensitivity of the process to environmental signals rather than directly determining the resulting phenotype (Murphey *et al.*, 2015)

Are there other mechanisms that inform about the passage of time (thermal time) and result in a seasonal response?

Oxygen availability in the soil can have a temporal pattern and impacts dormancy status with hypoxia-inducing secondary

dormancy (Benvenuti and Macchia, 1995). Oxygen is also important in the guise of reactive oxygen species (ROS) in further modulating dormancy and relaying environmental signals (Bailly *et al.*, 2008; Kranner *et al.*, 2010). For example, seed dry after-ripening is associated with the accumulation of ROS, resulting in targeted mRNA oxidation and protein carbonylation of transcripts and proteins associated with cell signalling (mRNA; Bazin *et al.*, 2011) and protein storage (Oracz *et al.*, 2007). These modifications have been linked to dormancy changes during after-ripening (El-Maarouf-Bouteau *et al.*, 2013) and could underpin a mechanism indicating the passage of time. Recently the possibility of a further role for ROS to inform the seasonal response of the seeds through ultra-weak photon emission (UPE) has been suggested by Footitt *et al.* (2016). They hypothesize that beneath the soil surface the attenuation of light (virtual darkness: low background noise) enables seeds to exploit UPE for transducing key environmental variables in the soil (temperature, humidity, and oxygen) to inform them of seasonal and local temperature patterns.

Underlying the suggested potential mechanisms indicating the passage of time/thermal time it is likely that there is a background reference annual rhythm using components of the circadian clock. The circadian clock plays a role in the setting of primary seed dormancy and dormancy relief, as well as in tree bud dormancy (Penfield and Hall, 2009; Foley *et al.*, 2010; Cooke *et al.*, 2012). On an annual basis, the existence of a circannual rhythm in dormancy has been observed in both dry and hydrated seeds at constant temperature (Gutterman and Gendler, 2005; Duarte and Garcia, 2015), consistent with that seen elsewhere (Matrai *et al.*, 2005).

Shallow dormancy and sensitivity to spatial signals (soil water potential, light, and nitrate)

In contrast to those in deep dormancy, seeds in shallow dormancy, resulting largely from germination repression by DELLAs, can respond rapidly to spatial signals that indicate favourable germination conditions (spatial sensing). For example, exposure to light dramatically enhances *GA3ox* expression to remove DELLA repression (Cadman *et al.*, 2006). Nitrate sensitivity is also related to the enhancement of germination in the light in shallow dormancy (Hilhorst and Karssen, 1988), and so could complement light sensitivity during the spatial sensing phase (Pons, 1989). Although there are a wide range of other spatial signals (Fig. 1), for brevity we will consider only light, nitrate, and the presence of adequate soil moisture. In Fig. 5 we link the change to shallow dormancy and the response to these spatial signals with the central integrating function of the ABA/GA balance. In the following text, we add detail to this schematic.

Soil moisture content

The impact of moisture availability on germination has been extensively studied in the laboratory and can be described using hydro- and hydrothermal time analysis (Fig. 5; reviewed by Bradford, 1995), and extended to the field environment (Finch-Savage, 2004; Finch-Savage and Bassel, 2016). Conditions in the soil can be very different from those in the Petri dish, and this has been described elsewhere (Whalley and Finch-Savage, 2006). Seeds are not sensitive to the water content of soil *per se*, but the availability of water measured as water potential (MPa)—the sum of matric potential (adhesion of water to soil structure) and osmotic potential (influence of solutes). It is this potential that is referred to in the hydrothermal time model for seed germination. In the model, rate of progress towards germination is proportional to the extent by which ambient water potential exceeds the threshold (base) water potential (Ψ_b) below which progress ceases (Fig. 5). Ψ_b is a key unifying parameter relating germination performance to moisture stress that is probably determined by the physical restraint to germination of surrounding tissues and cell wall extensibility (Welbaum *et al.*, 1998). In the context of dormancy cycling, it is notable that Ψ_b changes as primary dormancy is relieved (Bradford, 2002; Fig. 5). Furthermore, Ψ_b increases and decreases as seed dormancy changes (primary and secondary dormancy) during the annual dormancy cycle (Footitt *et al.*, 2013) and therefore so does sensitivity to this spatially and temporally variable parameter.

Light and nitrate

Footitt *et al.* (2013) argue that during dormancy cycling the response (sensitivity) to nitrate alters via the phosphorylation and dephosphorylation of NITRATE TRANSPORTER 1 (NRT1.1) now known to involve both CBL-INTERACTING PROTEIN KINASE 23 (CIPK23) and the PP2C phosphatase ABI2; and the response (sensitivity) to light alters via PHYTOCHROME A (PHYA). Fig. 4 shows co-ordinated annual expression patterns in Cvi for *DOG1*, *PHYA*, and *CIPK23* with low expression at the point where germination/seedling emergence occurs. Thus all three act in a temporal pattern and appear to promote dormancy. However, preliminary mutant analyses show that CIPK23 and PHYA act as negative regulators of secondary dormancy during simulated dormancy cycling (S. Footitt, H. Ölçer-Footitt, A.J. Hambidge, and W.E. Finch-Savage, unpublished). Further work will be required to resolve fully the observations made on seeds exhumed from field soil and results obtained in the laboratory, but we consider current understanding of these signals and the responses to them below.

Light

Light is a key spatial signal, and phytochromes play a dominant role in its perception in seeds. In laboratory experiments, as seeds become increasingly light sensitive, regulation of germination by phytochromes A and B (PHYA and PHYB) is under hierarchical and temporal regulation. For example, under a low R/FR ratio (red/far-red, e.g. under a canopy of competing plants), PHYB in the endosperm promotes ABA biosynthesis (Lee *et al.*, 2012), and as seeds do not germinate

this probably maintains dormancy (positive regulation). As the signal declines, PHYA in the embryo removes the final layer of dormancy, enabling germination (Lee *et al.*, 2012), revealing PHYA as a negative regulator of dormancy and the final sensor in the removal of dormancy by light. PHYA is the most abundant phytochrome in seeds with high protein levels accumulating in the dark (Sharrock and Clack, 2002) that photoirreversibly result in germination in monochromatic light from 300 nm to 770 nm (Shinomura *et al.*, 1996). However, in tomato, PHYA can both positively and negatively regulate germination depending on the fluence rate of red light; in a low fluence rate, PHYA can relieve dormancy, whereas at a high fluence rate PHYA maintains dormancy (Appenroth *et al.*, 2006). Array data from laboratory experiments show that during Arabidopsis dormancy cycling of the PHYA and PHYB, only PHYA has a strong dormancy-associated expression pattern (Cadman *et al.*, 2006; Finch-Savage *et al.*, 2007).

In the soil seed bank, seeds are effectively in perpetual darkness at depths of ≥5 mm depending upon soil type and vegetation cover (Tester and Morris, 1987). During the spatial sensing phase, the final layer of dormancy can be removed by millisecond flashes of low fluence sunlight as the soil is disturbed (the very low fluence response: VLFR). Seeds therefore are extremely light sensitive. The mechanism for this is PHYA mediated and saturated by <1% of active phytochrome (Batlla and Benech-Arnold, 2014). Dark incubation of seeds sensitized them to dormancy breaking by PHYA-mediated low fluence red light in the range 1–100 nmol m^{-2} s^{-1} at wavelengths from 300 nm to 560 nm (Shinomura *et al.*, 1996). With seed coat attenuation of transmitted light in the phytochrome range of ≥95% (Scopel *et al.*, 1991) the effective fluence rate under the seed coat required to remove the final layer of dormancy in the embryo must be exceptionally low. Finally, the potential involvement of heterotrimeric G-proteins in PHYA-mediated signalling and germination (Botto *et al.*, 2009) provides a mechanism for signal amplification similar to that in retinal rod photoreceptors where heterotrimeric G-proteins enable signal amplification from single photons into a response (Kolesnikov *et al.*, 2011).

PHYA is implicated in the positive regulation of dormancy in seeds matured at low, but not warm temperature (Donohue *et al.*, 2008). This effect was lost as dormancy declined through dry after-ripening and stratification potentially related to increased GA levels/sensitivity (Donohue *et al.*, 2008, and references therein). This is consistent with field observations of PHYA expression (Fig. 4). However, the response was dependent upon the conditions under which seeds were produced (Donohue *et al.*, 2008; Dechaine *et al.*, 2009). Furthermore, regulation by PHYA could appear positive or negative depending on the wavelength and fluence rate used in experiments (Appenroth *et al.*, 2006). The cause of this PHYA effect is unclear, although PHYA overexpression represses GA levels (Jordan *et al.*, 1995). For dormancy cycling, it should also be considered that such differences probably occur during the continuous process of change in dormancy level in the soil seed bank. The response can also differ with ecotype (Dechaine *et al.*, 2009), consistent with observed differences in Cvi and Bur. Such differences in PHYA expression may represent adaptations to climate affecting fitness, as found by Donohue *et al.* (2012).

Nitrate

Nitrate, especially in conjunction with light, is another important spatial signal that has been studied in both the laboratory and field. Nitrate concentration in soil solution fluctuates and can vary from almost 0 to 50 mmol l^{-1} (Bouwmeester *et al.*, 1994), covering the range provoking a response from seeds in the laboratory. However, although annual variations in soil nitrate (Bouwmeester and Karssen, 1993, Derkx and Karssen, 1993) and *Sysmbrium officinale* seed nitrate content (Derkx and Karssen, 1993) were observed, changes in dormancy appeared driven by temperature, and not influenced by soil moisture or soil nitrate. In Arabidopsis, similar conclusions were reached, and temperature-driven seasonal dormancy patterns appeared to be regulated by changes in sensitivity to light (Derkx and Karssen, 1994). Nevertheless, seed nitrate content in Arabidopsis affected the maintenance of dormancy in the laboratory (Alboresi *et al.*, 2005). A reason for this apparent contradiction is provided by Hilhorst (1990) who showed that most endogenous nitrate is leached from seeds in the first 24 h of imbibition on water in the laboratory. Thus high nitrate content will relieve dormancy, but only temporally when placed in soil, and therefore nitrate concentration may have little ecological importance (Bouwmeester *et al.*, 1994). In contrast, seed sensitivity to nitrate is likely to have a significant ecological role in response to soil nitrate that varies both spatially and temporally.

In Arabidopsis, nitrate is thought to have a direct regulatory role and promotes germination by reducing the light requirement (Hilhorst and Karssen, 1988). Based on field studies, Derkx and Karssen (1994) suggested a model where temperature results in reversible changes in sensitivity to light and nitrate, which occur at the level of receptors. This was consistent with the model and earlier conclusions of Hilhorst (1990) in the laboratory studying secondary dormancy. It was later suggested that the nitrate receptor could be NRT1.1 (Alboresi *et al.*, 2005; Footitt *et al.*, 2013). Furthermore, nitrate release of seed dormancy acts by accelerating the decrease in ABA during germination (Ali-Rachedi *et al.* 2004) via induction of the catabolic ABA gene *CYP707A2* (Matakiadis *et al.*, 2009). This response is therefore separate from the GA response to light, consistent with nitrate acting to enhance the effect of light.

Alboresi *et al.* (2005) questioned whether nitrate acts *per se* on seed germination or through the production of N-related signals. NRT1.1 is a dual affinity nitrate transceptor (transporter/receptor), having high or low affinity functions depending on its phosphorylation status (Ho *et al.*, 2009). It acts as part of a complex with the kinase CIPK23 and the calcium sensor CBL9 (CALCINEURIN B-LIKE PROTEIN 9). The high affinity complex is produced by CBL9 phosphorylating CIPK23, which in turn phosphorylates Thr101 of NRT1.1 This form has repressed transport activity and reduced signalling, resulting in reduced expression of a second high affinity (<1 mM) nitrate transporter NRT2.1 (Ho *et al.*, 2009).

When this complex is dephosphorylated by ABI2 it is converted to the low affinity form in which nitrate transport and signalling are higher (Léran *et al.*, 2015). In seeds this would be expected to relieve dormancy, leading to germination. However, nitrate signalling via NRT1.1 irrespective of its phosphorylation state activates the protein NIN-LIKE PROTEIN 8 (NLP8), which binds the *CYP707A2* promoter inducing its expression. The resulting decrease in ABA levels results in the removal of the final level of dormancy proportional to the external nitrate concentration (Yan *et al.*, 2016). In the field, during the spatial sensing phase, there is a transient increase in *NRT1.1* expression followed by increased expression of *CYP707A2* and *ABI2*, and nitrate sensitivity (Fig. 4; Footitt *et al.*, 2013). Thus nitrate transport/signalling is occurring at this point as *CYP707A2* expression is induced by external nitrate (Matakiadis *et al.*, 2009). Collectively this suggests that the level of NRT1.1 limits nitrate signalling in seeds outside of the spatial sensing phase before the transient rise in its gene expression. At this time, a switch between high and low affinity forms of the transceptor will further increase sensitivity to nitrate. This switch may also be linked to the control of the primary nitrate response, known to regulate downstream expression of genes (Krapp *et al.*, 2014) involved in events important in cellular repair and readiness for germination.

Adaptation to local environments

There can be substantial variation in both genetic and phenotypic plasticity for seed dormancy and germination within Arabidopsis and other species over elevational and latitudinal gradients (Baskin and Baskin, 1998; Cavieres and Arroyo, 2000; Chiang *et al.*, 2011). Genetically identical cohorts of seeds can adapt to contrasting life cycles (Montesinos-Navarro *et al.*, 2012), and both spring and autumn germination windows have been described in coastal but not montane Spanish populations (Montesinos *et al.*, 2009), supporting the predictions of Springthorpe and Penfield (2015) that winter and summer annual life cycles can arise in the same population depending on the environments encountered.

DOG1 is thought to have an important role in the adaptation of dormancy to climate (Kronholm *et al.*, 2012) and to local environments (Postma and Agren (2016). When Cvi (winter annual) and Bur (summer annual) were put through a summer annual dormancy cycle (Fig. 6; Footitt *et al.*, 2011, 2013), some intriguing adaptive differences were revealed. In the case of *DOG1*, transcription profiles were negatively correlated with the soil temperature cycle in both ecotypes. However, although the dormancy level correlates with the *DOG1* profile in Cvi, it did not in Bur. This may reflect differences between transcript and protein profiles, but also suggests that the relationship between thermal sensing and dormancy is plastic as a result of allelic variation in *DOG1*; hence contributing to adaptation (e.g. Chiang *et al.*, 2011; Kronholm *et al.*, 2012). Differences in the spatial sensing phase also become apparent, with the transcript profiles of genes associated with spatial sensing being highly correlated

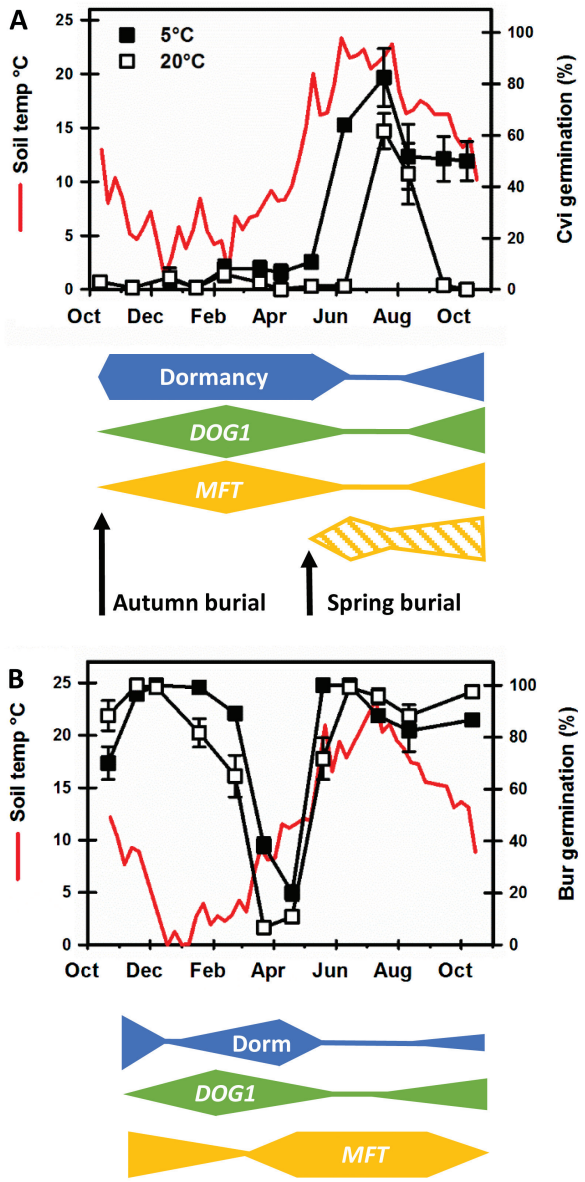

Fig. 6. Dormancy and gene expression patterns in winter (Cvi) and summer (Bur) annual ecotypes. All data are from seeds exhumed at intervals during the annual dormancy cycle, and for each ecotype show *DOG1* and *MFT* transcript profiles, soil temperature, dormancy levels, and germination at 5 °C and 20 °C/light. The height of the bars indicates the relative levels of gene expression. (A) Data are shown for seeds buried in the autumn to mimic Cvi in the persistent seed bank (i.e. not germinated following shedding) and (B) Bur undergoing its natural summer annual dormancy cycle following shedding (refer to Fig. 2B and C). In (A) data are also shown for Cvi seeds buried in spring to mimic its natural winter annual dormancy cycle following shedding. In this case, depth of dormancy, germination timing, and *DOG1* expression are the same as autumn buried seeds; however, *MFT* expression is significantly different as shown. Data are redrawn from Footitt *et al.* (2011, 2013, 2014).

with one another in the shallow dormant Bur ecotype compared with Cvi (Footitt *et al.*, 2011, 2013). This implies that in a background not dominated by the strong Cvi *DOG1* allele there is a greater role for dormancy regulation involving increased ABA signalling/sensitivity.

Of the genes examined, two had reversed transcript profiles in relation to temperature, highlighting this enhanced

role (Fig. 6; Footitt *et al.*, 2013). In Bur, transcription of the SNF1-related protein kinase *SnRK2.1* (positive regulator of ABA signalling) and *MFT* is positively correlated with temperature, but negatively correlated in Cvi (Footitt *et al.*, 2013). *MFT* transcription is high in Bur during the spatial sensing phase of the cycle prior to seedling emergence, indicating that MFT contributes to shallow dormancy maintenance (Fig. 6B). On the other hand, in Cvi it positively correlates with *DOG1* and dormancy level, but has low expression during the spatial sensing phase (Fig. 6A). Crucially, this changes when the deeply dormant Cvi ecotype undergoes its natural winter annual dormancy cycle with newly shed seed in spring spending the summer in the soil seed bank (compare Autumn and Spring burial in Fig. 6A). Here, in the absence of a low temperature winter phase, *DOG1* is not highly induced therefore bypassing induction of deep dormancy. Possibly as a result, *MFT* transcription increases in the spatial sensing phase, implying that *MFT* now has a more dominant role in dormancy maintenance in this phase similar to that seen in the summer annual Bur. Nevertheless, in both situations, maximum germination in Cvi coincides with the lowest *MFT* transcription. This is consistent with laboratory results; MFT has a role in signalling by the oxylipin, 12-oxo-phytodienoic acid (OPDA), which acts through MFT to induce ABA biosynthesis and sensitivity, with MFT and ABA then acting via a feedback loop to enhance OPDA levels (Dave *et al.*, 2016) to enhance low dormancy.

The implication is that when seeds are shed to the soil seed bank at their natural time, only a shallow dormancy cycle is required to position the spatial sensing phase at the appropriate time of year for seedling emergence. If seeds are shed outside of this period or do not receive appropriate spatial signals to remove the final layer of dormancy, they enter the persistent soil seed bank (Figs 2, 6). Then seeds enter a *DOG1*-dominated deep dormancy phase in order to position the spatial sensing phase correctly in the following year. This may represent events in the persistent seed bank and highlights the innate plasticity of dormancy cycling.

Concluding perspective

In recent years significant advances have been made in understanding the mechanistic underpinning of primary seed dormancy through the use of mutants, which have elucidated the pathways involved in the ABA/GA balance system. The natural variation of Arabidopsis exploited by mapping populations has led to the identification of DOG1 and showed its apparently overarching dominance of dormancy, germination timing (dormancy cycling), and seedling establishment. Natural variation has also led to advances in understanding of adaptation to climate and how dormancy and flowering times are linked to determine life cycle patterns. Nevertheless, we need a more detailed understanding of the regulation of dormancy cycling, in particular interaction at the molecular level between deep and shallow dormancy. Studying dormancy cycling in the field is a long-term undertaking, and ethical and regulatory reasons can preclude the use of seeds from genetically modified plants to dissect the role of individual genes. Progress in understanding is therefore likely to be slow. However, recent laboratory studies show that cycling can be simulated in Col-0 and Ler by enhancing their primary dormancy during production and by manipulating temperature and water stress to cycle them through secondary dormancy (S. Footitt and W.E. Finch-Savage, unpublished). Future use of such dormancy cycling screens to compare ecotypes and mutants should more rapidly enhance understanding.

Supplementary data

Supplementary data are available at *JXB* online.

Fig. S1. Climate of origin of the winter and summer annual Arabidopsis ecotypes Cvi and Bur, respectively.

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