BURLEIGH DODDS SERIES IN AGRICULTURAL SCIENCE

Advances in seed science and technology for more sustainable crop production

Edited by Dr Julia Buitink, INRAE, France
Professor Olivier Leprince, L'Institut Agro Rennes Angers, France

burleigh dodds
SCIENCE PUBLISHING

Contents

Introduction

With the continued effects of climate change threatening the security of the global food system, there is a greater emphasis on ensuring successful crop establishment as a means of optimising agricultural production.

This volume considers how an improved understanding of seed quality, germination and seedling emergence can address this challenge. The book is split into two parts: Part 1 covers understanding seeds, from biology to the field, and includes chapters that examine ways to improve seed vigour. Part 2 chapters review how seed health and vigour can be controlled and tested, drawing attention specifically to seed phenotyping, testing and monitoring, preservation as well as seed conditioning and priming techniques.

Part 1 Understanding seeds: from biology to the field

The book opens with a chapter that provides an overview of seed dormancy and germination. Chapter 1 begins by introducing the five most frequently occurring types of dormancy: morphophysiological, morphological, physical, physiological and nondormancy before going on to examine the acquisition of dormancy, with special emphasis on the transcriptional network and the importance of compartmentation. The chapter then goes on to discuss the concept of ABA-GA balance which influences the seed's potential to germinate. Sections on breaking primary dormancy and secondary dormancy and dormancy cycling are also provided. The chapter next reviews the regulation of seed-to-seedling phase transition and biological variability, before concluding with an analysis of potential future research trends in seed biology.

Expanding on topics previously touched upon in Chapter 1, the next chapter examines the effects of the maternal environment in controlling seed dormancy. Chapter 2 first reviews dormancy response patterns to the effect of the maternal environment in seeds displaying both physiological and physical dormancy. It then goes on to analyse the physiological and molecular bases underlying the modulation of dormancy by the environment experienced by the mother plant during reproductive development. Sections on the ecological and agricultural implications of dormancy modulation by the maternal environment are also included.

Chapter 3 focuses on applying population-based threshold models to quantify and predict seed vigour attributes. The chapter begins by emphasising the importance of seed quality and behaviour in crop management and yield, weed control and transplant production. After a review of the different methods to analyse seed behaviour in population terms, the next section teaches the basic concepts of population-based threshold models and how they can

describe seed behaviour in response to environmental factors that influence germination. The chapter also highlights the use of population-based models for physiological factors affecting seed germination and behaviour, such as dormancy, aging, seed vigour, respiration and hormones. A section on applying population-based threshold models is also provided.

The next chapter of Part 1 examines biotic sources of seed losses influencing germination and emergence success in crop plants and agricultural weeds. Chapter 4 first reviews the contribution of pathogens and predators to seed losses, distinguishing events that occur before and after seed dispersal. The chapter includes both weeds and crop plants because the greatest yield improvements can often result from targeting weed seed survival after crops are harvested. The chapter also highlights the strong potential for management practices to enhance predation losses of weed seeds relative to seed pathogen losses, particularly for species with long-lived dormant seeds.

The final chapter of Part 1 focuses on advances in understanding the genetic and environmental factors determining seed longevity. Chapter 5 begins by examining the distribution of seed life spans amongst species, then moves on to review how genetic diversity can be used to understand longevity. A section on the environmental effects of longevity is also provided, focusing specifically on plasticity and adaptation. The final section reviews aging protocols and the limits of extrapolating accelerated ageing conditions.

Part 2 Seed quality control and treatment

The first chapter of Part 2 examines advances in seed phenotyping using X-ray imaging. Chapter 6 begins by reviewing seed sample preparation which is then followed by an analysing of projection acquisition, drawing specific attention to radiography and tomography. With these two acquisitions in mind, the chapter moves on to review image reconstruction and how radiography and tomography can be used to do this. A section on image processing is also included, highlighting how visual assessment is crucial for successful seed analysis in phenotyping. The chapter concludes by emphasising the importance of developing current and new X-ray imaging techniques for the purpose of seed phenotyping.

The subject of Chapter 7 deals with testing seed health. The chapter discusses several methods for seed health testing which are broken down into four sections: traditional detection methods, serological detection techniques, nucleic acid-based detection methods and spectroscopy-based methods. The chapter also evaluates the use of next-generation sequencing for the detection of the most important seed transmitted pathogens. These methods are compared with TaqMan polymerase chain reaction methods.

Chapter 8 focuses on advances in preservation of seed vigour during storage. The chapter begins by posing the question how conditions during seed production can affect seed quality. The next sections examine post-harvest treatments to improve seed lot quality, maintaining seed viability and vigour during storage as well as the ways to predict viability and vigour during storage, drawing attention to monitoring and different testing methods. The chapter concludes with an overview of the challenges in seed longevity research and potential areas of development for future research.

The next chapter explores advances in enhancing seed defence mechanisms against pathogens. Chapter 9 reviews defence priming against plant pathogens when the priming agent is associated with seeds, concentrating on microbial endophytes and volatile organic compounds, and, where known, the mechanisms involved in defence responses. The chapter discusses the microbial and chemical priming agents that can be used to enhance seed vigour then goes on to consider transgenerational defence priming. The last section examines the use of beneficial microbe delivery for defence priming.

The final chapter of the book considers advances in seed priming techniques. Chapter 10 focuses on recent developments in priming from the point of view of the seed industry, based on patents and applications. It assesses the strengths and weaknesses of new techniques such as oxygen controlled priming, vapour heat priming, pulsed radio frequency, ultrasonics, cold plasma compared to established priming methods: drum, osmotic and solid matrix priming.

Acknowledgement

We wish to acknowledge the following for their help in reviewing particular chapters:

- Chapter 5: Dr Julia Zinsmeister, Institute of Biochemistry and Biophysics, Poland
- Chapter 7: Dr Valerie Grimault, GEVES, France

Part 1

Understanding seeds: from biology to the field

Chapter 1

Seed dormancy and germination: a critical update

Henk W. M. Hilhorst, Wageningen University & Research, The Netherlands and University of Cape Town, South Africa

1 Introduction

Seed dormancy and germination are adaptive traits that play a key role, together with flowering, in the timing of emergence of plants and their subsequent fitness. These developmental transitions can be seen as the basis for the establishment and maintenance of plant communities (Auge et al., 2018). Dormancy is defined as a physiological state resulting in the absence of germination of a viable seed under favourable conditions. Dormancy allows seeds to avoid germination during periods that are only transiently favourable.

Seed dormancy and (delayed) flowering time may have co-evolved to allow the colonization of habitats with seasonal fluctuations in the environment (Ritland, 1983). The most ancient signs of dormancy have been found in mature seeds of a Palaeozoic fossil conifer; a significant delay between fertilization and seed germination was interpreted as post-zygotic quiescence, a first step in the evolution of seed dormancy (Mapes et al., 1989). The evolutionary history of dormancy is complex as there exists a rich diversity in types and intensities of dormancy. This has resulted in various classifications of dormancy (Nikolaeva, 1999; Baskin and Baskin, 2004).

http://dx.doi.org/10.19103/AS.2022.0105.02

The five most frequently occurring types of dormancy are:

1 **Morphophysiological dormancy:** a combination of morphological and physiological dormancy. This is considered the ancestral state of dormancy.
2 **Morphological dormancy:** dormancy imposed by underdevelopment of the embryo which needs to complete development and grow inside the seed before radicle emergence.
3 **Physical dormancy:** dormancy imposed by dedicated physical structures of the embryo-surrounding tissues which require very specific environmental cues to allow water uptake and radicle emergence.
4 **Physiological dormancy:** dormancy imposed by one or more metabolic blocks in the seed that are removed by specific environmental cues. This type of dormancy is reversible.
5 **Nondormancy:** absence of dormancy in species in (near) constant environments.

It has been argued that the latter four dormancy types have evolved from morphophysiological dormancy, each adapting to specific habitats. Physiological dormancy is by far the most frequently occurring type of dormancy and often shows a transition to nondormancy, probably when a niche is occupied that does not demand seasonal dormancy (Willis et al., 2014). However, the intensity of dormancy is strongly dependent on the maternal environment. The observed transitions between physiological- and nondormancy may therefore not be permanent but rather dependent on the year of observation.

This chapter will mainly focus on the most dominant dormancy class, namely physiological dormancy, as well as germination, and review new insights into their regulation and mechanisms. For the other types of dormancy, the reader is referred to excellent reviews by, for example, Baskin et al. (2000) and Hudson et al. (2015) on physical dormancy and Baskin and Baskin (2014) on the other types of dormancy. Excellent reviews on seed dormancy in combination with germination have been published by Finch-Savage and Leubner-Metzger (2006), Graeber et al. (2012), Chahtane et al. (2017), Penfield (2017), Nonogaki (2018), Tuan et al. (2018) and Carrera-Castaño et al. (2020).

Despite numerous studies on physiological dormancy, predominantly in *Arabidopsis thaliana*, a comprehensive understanding of the phenomenon and its regulation is largely lacking. Current gene by gene discoveries, using mutants, and initial evidence from gene/protein regulatory networks, using whole-genome approaches, have not yet led to a comprehensive dormancy model. An important obstacle to progress in this field is a lack of non-destructive methodology to assess dormancy (or viability) of single seeds in a population.

Experiments in seed biology necessarily resort to seed lots (i.e. populations) for the assessment of quantitative biological properties such as dormancy and longevity. Germination is a qualitative, all-or-nothing phenomenon. However, dormancy is a quantitative trait described by such terms as shallow or deep, referring to the reluctance of a seed population to respond to a dormancy-breaking treatment. It therefore remains problematic to assign experimental output variables to inherent seed traits, unless comparing seed lots that germinate at 0% or 100%.

Nevertheless, studies on dormancy and germination continue, new technologies and approaches are developed, and a critical update seems timely. This review aims to (subjectively) pinpoint important and promising recent developments in seed science. In addition, it will draw attention to the huge complexity of seeds as biological systems. It will also briefly explore more holistic approaches to 'understand' the system (Nurse, 2008).

2 The acquisition of dormancy

Like various other functional seed attributes, such as germinability, desiccation tolerance and deposition of food reserves, primary dormancy is acquired during seed development, predominantly during the maturation and drying phases. A set of highly conserved transcription factors (TFs) with a B3 DNA-binding domain includes the master regulators of seed development and maturation (Carbonero et al., 2017):

- ABA-INSENSITIVE 3/ Viviparous-1 (ABI3/VP1);
- FUSCA3 (FUS3); and
- LEC2.

In addition, LEC1, encoding an atypical subunit of the nuclear transcription factor Y (NF-Y) CCAAT-binding transcription factor, is also considered a central regulator of many aspects of seed development (Pelletier et al., 2017). These 'LAFL' transcription factors not only control the thousands of genes involved in the seed development and maturation programs but may also have more specific functions. LEC1, for example, initiates the resetting or re-activation of *FLOWERING LOCUS C* (*FLC*) during early embryogenesis in *A. thaliana* so as to reset the parental 'memory of winter cold' (Tao et al., 2017).

2.1 Targets of transcription factors in the acquisition of dormancy

Experimental systems, such as yeast one-hybrid and two-hybrid screening techniques used to study interactions between TFs and their targets, have

made it possible to map a multitude of (potential) physical DNA–protein interactions. This has been done for some of the master regulators. For example, LEC1 potentially interacts with some 1500 targets, as identified by chromatin immunoprecipitation (ChIP) and differential gene-expression analyses (Pelletier et al., 2017). Here we focus on ABI3 and its targets as this TF contributes to the abscisic acid (ABA) response which is strongly associated with dormancy as well as responses to abiotic stress (Hilhorst, 1995).

The first ABI3-regulon was identified by a combination of genome-wide ChIP-chip, transcriptome analysis, real-time quantitative reverse transcription-polymerase chain reaction and a transient promoter activation assay (Mönke et al., 2012). This regulon contained 98 ABI3 target genes, including FUS3 and LEC2, confirming that the LAFL genes also regulate each other (To et al., 2006). Two over-represented promoter binding motifs for bZIP and bHLH TFs were identified:

- the well-known G-box-derived ABRE (ABA-responsive element) motif ACGTG(T/G)C); and
- the RY/Sph motif CATGCA.

Not surprisingly, members of this ABI3 regulon appeared to be involved in such biological processes as embryonic development ending in seed dormancy, response to ABA and response to the stimulus. An important finding was that many of the ABI3 targets require ABA for their activation (Box 1).

Box 1

It has long been known that mutations in these TFs may lead to severe seed phenotypes, including loss of desiccation tolerance, loss of dormancy, no degradation of chlorophyll and impaired reserve food synthesis, reflecting a change of embryo identity to a vegetative developmental mode (Meinke et al., 1994; Lepiniec et al., 2018). Studies with these mutants underscore the status of these TFs as 'master regulators' of seed development. However, the question arises as to how this higher tier of regulation is translated into more specific downstream responses, by multitudes of genes, to regulate these distinct developmental processes, including dormancy. These various regulatory tiers include hormone signalling, (post)-transcriptional regulation and (post)-translational regulation, as well as epigenetic control (Fig. 1). In addition, intracellular compartmentation, tissue specificity and biophysical status of the cell add more layers of complexity to understanding the ultimate responses of the seed. With this complexity in mind, the common practice of gene by gene discovery, mainly by using mutants of a

(limited) number of model species, seems to lack sufficient context to understand seed function. It is, however, encouraging that more and more studies recognize this complexity and make attempts to study genes in the context of cell identity, compartmentation and regulatory networks. New technologies create the possibilities to generate the data of this complexity, by *inter alia* (comparative) genomics, (single-cell) transcriptomics, ChIP-sequencing, translatomics and sophisticated imaging at high resolution. The role of bioinformatics in this is evident.

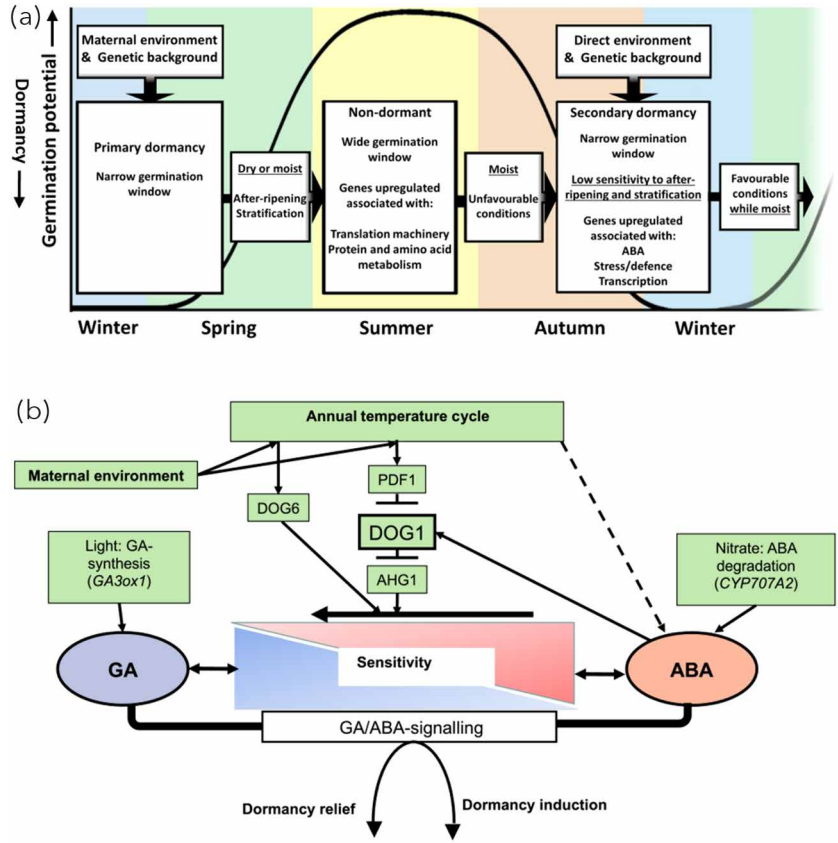

Figure 1 In the soil, seeds go through multiple dormancy cycles until they either germinate or lose viability. The driving force of the cycling are (seasonal) temperature shifts. (a) Genetic and environmental factors determine dormancy status which is reflected in transcriptomic changes dominated by transcripts associated with the translation machinery and protein synthesis. (b) Molecular model with key genes like *DOG1* controlling the cycling. Figure based on Buijs et al. (2020) (a) and Footitt et al. (2019) (b). With permission.

A more extensive attempt was made to identify ABI3 targets by ChIP-tiling array experiments to map binding sites for ABI3 genome wide (Tian et al., 2020). This approach yielded 317 directly activated genes and 87 directly repressed genes, also with overrepresented ACGTG(T/G)C) and RY/Sph motifs binding sites for B3 domain proteins. Among the directly activated genes, both by ABI3 and FUS3, were *DELAY OF GERMINATION1* (*DOG1*) and *REDUCED DORMANCY5* (*RDO5*), both positive regulators of dormancy. These genes had been identified by quantitative trait loci (QTL) analysis and, thus, are subject to natural variation (Bentsink et al., 2006, 2010). Extensive studies on DOG1 have shown its conserved role in the regulation of dormancy in various species besides Arabidopsis, including lettuce (Huo et al., 2016), wheat (Ashikawa et al., 2014) and Brassica spp. (Graeber et al., 2010). The severity of the dormancy phenotype of the *dog1* mutant in Arabidopsis has led to the conclusion that DOG1 is one of the central dormancy regulators. The DOG1 protein binds to specific members of clade A PP2C phosphatases which play an important role in ABA-signalling. This central role of DOG1 places it just downstream of ABI3 and FUS3 although its suggested function as a transcriptional factor remains to be shown (Soppe and Bentsink, 2020).

2.2 Network inference

The next question is whether a relatively few master regulators control the expression of many downstream genes. Transcriptome analysis combined with mutagenesis has revealed the existence of hundreds of genes associated with dormancy (e.g. Finch-Savage et al., 2007). Of these genes, some give a dormancy phenotype when knocked out but many show no direct effect on seed performance. Transcriptomes have revealed groups of genes with similar expression behaviour under varying conditions. Analysis of these patterns is facilitated by co-expression analysis, where genes of similar behaviour are clustered (Serin et al., 2016). A genome-wide network model based on co-expression correlations has been inferred from all available data on dormancy and germination of *A. thaliana* (Bassel et al., 2011). The topology of this network made it possible, for the first time, to identify clusters of genes associated with germination, dormancy, hormone activation and dormancy breaking. This study opened the way to further exploration of gene co-expression networks in relation to seed maturation processes, including dormancy.

A developmental study in seeds of *Medicago truncatula*, employing RNAseq and regulatory network inference, has resulted in the identification of several distinct clusters of genes closely associated with developmental intervals and, hence, with the related acquisition of seed traits such as longevity, desiccation tolerance and dormancy (Verdier et al., 2013). The dormancy and desiccation tolerance modules of the network could not be

distinguished as these traits were acquired in the same interval. The module has been enriched with genes related to stress responses to desiccation, heat and temperature. These included genes encoding late embryogenesis abundant (LEA) proteins, heat shock and small heat shock proteins, as well as ABA-induced genes.

ABA is clearly the common denominator in the acquisition of dormancy and responses to stress (Hilhorst, 1995) and also explains the approximately 50% proportion of stress-associated genes in the dormancy gene set, identified by Finch-Savage et al. (2007). One of the genes identified in *M. truncatula* seeds by Verdier et al. (2013), initially predicted to play a role in seed longevity, encodes the seed-specific heat shock factor A9 (MtHSFA9) but appears to act as a negative regulator of the depth of dormancy during seed development via the modulation of ABA biosynthesis and sensitivity (Zinsmeister et al., 2020). This outcome underscores the intertwining of dormancy- and stress-associated regulation by ABA, presumably, but not exclusively, through ABI3. It is clear that network inference is a good approach to clarify a whole tier of regulation of distinct developmental processes, including dormancy (see Box 1).

2.3 Compartmentation

Seeds (and seed lots) are often considered incorrectly as a homogenous entity. Seed extracts of compounds like RNA, proteins and metabolites consist of a mixture derived from seed compartments like testa, endosperm, cotyledons and embryonic axis. When studying regulatory principles in seeds, this may lead to the masking of the essential processes and possibly erroneous conclusions (Box 2).

Box 2:

While more and more components of the regulation of dormancy and germination are being discovered, still largely through the use of (Arabidopsis) mutants, the question arises as to how the accumulated knowledge may provide us with insight in the dynamics and plasticity of seed performance and in interaction with the environment. Interpretation of results from studies with mutants has largely aimed at acquiring mechanistic insights in molecular interactions. However, if we adopt the idea that genes or proteins do not act in an isolated way but rather as entities within networks, a mutation, or a change in the environment, must be seen as a perturbation of that network. Hence, the question now is how such a perturbation may result in an altered phenotype or not at all. In other words, how is the flow of information changed by this perturbation?

Index

http://dx.doi.org/10.19103/AS.2022.0105.06