
Preface

This book covers analytical methods and approaches, which have led to significant advances in our understanding of seed dormancy and germination and have the potential to lead to new avenues of research. The methods detailed in this book are of value for both applied and fundamental seed research, and range from whole seed batch treatments to the analysis of tissues or even single seeds at the DNA, protein, and metabolite levels. Methods are described in detail and critical steps explained so that several of the chapters of this book can serve both as a protocol and a guideline for their adaptation and optimization to new applications or species.

The viability and vigor of seeds are the very basis for sustainable agriculture and forestry, and understanding the molecular and cellular events underlying these processes will become increasingly important to many economical sectors and for species that provide the world's food supply. Seeds are also the focus of environmental conservation efforts aimed at preserving the floral biodiversity of vanishing habitats. Understanding seed-related processes is thus of major social, environmental, and economical concern. The main foci of seed-related research are the regulation of lifecycle transitions, such as the dormancy-to-germination and germination-to-growth transitions; seed viability, quality, and vigor are central as well, and are influenced by events that take place during seed development.

Some orthodox seeds enter a state of dormancy during their development. Like their nondormant counterparts, they become quiescent during the final stages of seed development associated with desiccation and a cessation of the vascular supply from the mother plant, in which seed metabolism is strongly reduced and meristematic activity ceases. However, unlike the mature, quiescent, nondormant seed, the dormant seed fails to complete germination under favorable conditions (e.g., adequate moisture and oxygen), even though it is viable. Thus, dormancy ensures the proper temporal (and in some cases spatial) distribution of seed germination and is critical for a species' survival in its natural habitat. Dormant seeds must be exposed to species-specific environmental cues for dormancy to be terminated; these cues include moist chilling, light of a certain wavelength, or even smoke. Dormancy release also occurs in some species during seed after-ripening, i.e., air-dry storage of seeds at room temperature.

The first step toward an understanding of the processes leading to the induction, maintenance, and termination of seed dormancy is a classification of different dormancy types, as described in Chapter 3. The dormancy classification system explained in this chapter has been detailed by Baskin and Baskin and is now widely accepted and used throughout this book. Chapter 3 also deals with a major challenge in dormancy research, namely, the fact that dormancy in laboratory practice usually assessed by the absence of germination in a seed batch within a defined period of time, while a seed batch that germinates to 100% is classified as non-dormant. This can lead to some inaccuracies because seed viability within a population may not be 100%.

A proper balance of seed dormancy is crucial for agricultural crop production: seeds must not germinate on the mother plant, a process known as preharvest sprouting, which leads to major losses in crop production due to a decline in seed quality. On the other hand,

deeper dormancy can interfere with the ability of seeds to germinate readily when sown. Huge efforts go into optimizing the level of dormancy in cereals and other cultivated crops with these challenges in mind, and understanding the various external and internal factors that influence the depth of seed dormancy is thus critical. Chapter 2 discusses this point in detail focusing on agricultural crops, as well as the challenges faced by seed banks in the storage and preservation of a large variety of seeds with differing characteristics.

Similar issues are relevant to the forest industry, as explained in Chapter 1. As examples, some of the conifer species that are particularly important to the forest industry of British Columbia, Canada are discussed, including yellow-cedar (*Callitropsis nootkatensis*), western white pine (*Pinus monticola*), and true fir (*Abies*) species. Many species-specific protocols have been developed to break seed dormancy, as the agricultural and forest industries rely upon seeds that exhibit high germinability and vigorous, synchronous growth after germination. This work is critical to the forest industry, where certain conifer species of economic value exhibit profound dormancy, which is only terminated by a prolonged protocol – sometimes up to 4–5 months in duration. For species with profound dormancy, effective termination of dormancy appears not only to promote synchronous germination of seeds, but also to promote vigorous growth after germination. An added complication for forest species is the large genetic variability, meaning that protocols must be tested on several different seedlots and clones of a given conifer species. Chapter 4 gives an overview of protocols used for conifer species exhibiting deep dormancy, including traditional protocols and alternative protocols that are shorter in duration. Although not directly addressed in this book, understanding the optimal conditions for long-term storage of conifer seeds, as well as the genetic/environmental basis for different rates of seed deterioration during long-term storage is another issue of critical importance to the forest industry. The chapters on crop species of value to the forest and agriculture industries and the greatest challenges that impact these industries underscore the importance of conducting basic seed biology research on economically relevant species, rather than excluding these because of a preference for the more readily studied established model species (e.g., *Arabidopsis thaliana*).

While the agricultural industry may consider deep dormancy to be an undesirable trait, this is not necessarily the case for conifer species as it pertains to their regeneration at natural stands. Yellow-cedar is an example of a conifer species whose natural habitat is typically at high elevation. After dispersal from the tree, the seeds of this species must rely on experiencing external cues (including prolonged cold temperatures at high moisture) to time its germination such that seedling survival is optimized. It is not clear whether such a species will be able to adapt to climate change (warming) for regeneration at natural stands, especially considering the seeds' requirement of prolonged moist chilling for effective dormancy breakage.

Obtaining relevant data from seed dormancy research conducted in the laboratory is undoubtedly a challenge. This challenge becomes even greater when one is characterizing seed behavior in the natural environment. Chapter 5 deals with some of the key factors that require careful consideration when one is embarking on molecular studies of seeds in their natural environment such that the most informative interpretation of the data is fostered.

Research into the role of hormones in the control of seed dormancy and germination has long focused on two key players: abscisic acid (ABA) and gibberellins (GAs). Exciting discoveries are still being made regarding these two antagonists, for which the ultimate downstream targets responsible for their effects on germination are only very partially known. The horizon is now widening to include the effects of other hormones, such as

ethylene. The ability to profile a range of hormones and hormone metabolites simultaneously in seed and embryo tissues, and to monitor enzymes involved in hormone catabolism (Chapters 7–9) has contributed to a more broad perspective of hormone action and has also led to significant advances in our understanding of the roles of hormone flux in the control of dormancy and germination. We also know more about the localization of hormones and hormone metabolites in plant cells and tissues, and other regulatory mechanisms that involve hormone metabolites as “storage” forms of hormones (i.e., those able to “release” the free and physiologically active hormone at a later time). This book emphasizes methods important for ABA analyses, partly because of the role of this hormone as a key regulator of dormancy and germination; this includes methods that have been important for receptor identification (Chapter 6), analyses of ABA-catabolizing enzymes (the 8'-hydroxylases) (Chapters 8 and 9), and identification of novel signal transduction components, interacting partners and/or response factors (e.g., Chapter 12). Some of the approaches can serve as a general guideline to the analysis of other hormones involved in the control of seed dormancy and germination.

Plant hormones can interact with or cause changes in the concentration of reactive oxygen species and nitric oxide. In addition to the traditional view of reactive oxygen species as damaging compounds, reactive oxygen and nitrogen species can play roles as signaling molecules or as components of signal transduction pathways (Chapter 22). For example, reactive oxygen species produced in select cells of the micropylar seed tissues may signal these cells to undergo programmed cell death and/or weaken cell walls, thus promoting radicle protrusion. On another level, the redox state of seeds has also been correlated with their longevity and storage characteristics, and protein oxidation caused by reactive oxygen species during storage has been shown to play a role in seed deterioration. Methodological approaches to these issues are described in Chapters 20–22.

Epigenetic changes occur during stress responses and lifecycle transitions in plants, including the dormancy to germination transition, and this represents a new and exciting area. It has been known for some time that the conditions under which the mother plant sets seeds influence the dormancy and germination characteristics as well as the seedling performance of its offspring. These processes, including epigenetic control of gene expression, and relative dominance of maternal versus paternal genetic contributions due to selective gene silencing, are in some instances critical to seed development and viability, seed dormancy/germination, and seedling growth and stress resilience. Thus, epigenetic studies are an integral part of seed biology, and seeds of angiosperms, with their maternal seed coat, triploid endosperm, and diploid embryo present a unique system to study epigenetic and imprinting processes. The evolutionary aspects of these processes are also of great interest. MicroRNAs (Chapter 13) are involved in the attenuation of hormone signaling, and they also underlie some epigenetic processes.

Methods have been developed to analyze some key mechanistic features of epigenetic processes, including DNA methylation and histone modifications, the latter representing a highly complex pattern of posttranslational modifications. The tools for analyses of some of these modifications are covered in a comprehensive manner by Chapter 16, which also describes a novel NMR-based approach for the analysis of histone modifications. This allows the identification of a range of the modifications present on a particular type of histone. Chapter 15 focuses on methods used to analyze specific modifications to histones and the associated DNA, as well as DNA methylation. The results of these types of analyses, particularly as they pertain to lifecycle transitions and stress resilience of seedlings,

along with the identification of the genes/enzymes that mediate the epigenetic changes, are awaited with interest.

The genetic basis of seed dormancy has been investigated largely in the model species *Arabidopsis thaliana*, and Chapter 11 focuses on the methodological aspects of these genetic analyses (identification of quantitative trait loci) while Chapter 14 focuses on proteomics approaches. DNA and RNA isolation is no easy task in seeds because of their high concentrations of phenolic substances and other inhibitors; the relevant extraction procedures are described in Chapter 10.

As noted earlier, the attention of seed biologists on non-model plant species is an encouraging trend that is widening the range of seed anatomies, dormancy types, and germination control mechanisms that are being investigated. Progress in genome sequencing techniques has strongly contributed to this development, and the methods available will be useful tools for the study of other species that are not established models.

Seed dormancy has parallels to other dormancy processes in plants, such as bud dormancy. In photoperiodic trees exposed to short days in autumn, internode elongation ceases and the apex is transformed to a bud, which acquires desiccation and cold tolerance and enters a state of dormancy. Bud formation is controlled by ABA, and genes that are differentially regulated during *Arabidopsis* seed dormancy induction and apical bud development in the angiosperm model, poplar, show many parallels, suggesting similar mechanisms.

The current trends in seed biology include a heightened emphasis on the analysis of processes beyond the “level” of the whole seed, i.e., at the tissue, cellular, or even subcellular levels. This has become possible with refinements allowing for a “down-scaling” of longer standing methods as well as the development of new technologies, a selection of which are included in Chapters 17–19. This includes spectroscopic methods (some of which allow for nondestructive sampling) as well as highly effective tissue-printing methods for seed dormancy research. Imaging techniques, such as MRI and NMR, have seen huge advances during the last few years, and their use on seeds has led to important discoveries related to seed structure, water uptake and distribution (including the route of water uptake during seed imbibition), seed oil content, and selected secondary metabolites, and the relation of these parameters to seed dormancy, germination, stress resilience/vigor, and storability (resistance to deterioration). The use of these imaging techniques to elucidate additional aspects of seed biology is part of the larger trend toward using techniques traditionally associated with biophysics or engineering in biology research.

Mining the huge amount of transcriptomic and proteomic data that has been made publicly available is still a challenge, and the development of easily accessible interfaces has greatly helped those that are not adept in bioinformatics to make use of this valuable resource. A seed-specific database for the eFP-browser and a seed-specific gene ontology system are explained in Chapter 23. In connection with these bioinformatic efforts, a very recent approach to seed biology is centered on interdisciplinary collaborations with mathematicians, statisticians, and computer scientists: modeling of dormancy and germination at the whole seed, tissue or molecular levels will reveal as yet overlooked connections and insights into these highly complex processes.

The last 10 years have led to significant advances in the realm of seed dormancy and germination research and research on various other aspects of seed biology that control the viability and vigor of the next generation. All seed biologists should look forward to the next 10 years of advancement with great excitement.



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