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Seed Germination and Dormancy

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INTRODUCTION

Seeds are a vital component of the world's diet. Cereal grains alone, which comprise ~90% of all cultivated seeds, contribute up to half of the global per capita energy intake. Not surprisingly then, seed biology is one of the most extensively researched areas in plant physiology. Even in relation to the topics reviewed here, a casual perusal of the Agricola database reveals that well over 5000 publications on seed germination and 700 on seed dormancy have appeared in the last decade. Yet we still cannot answer two fundamental questions: how does the embryo emerge from the seed to complete germination, and how is embryo emergence blocked so that seeds can be maintained in the dormant state? Obviously, with such a large literature on the subject, this review is far from comprehensive. Nevertheless, it provides both an overview of the essential processes that are associated with germination and a description of the possible impediments thereto that may result in dormancy.

With the seed, the independence of the next generation of plants begins. The seed, containing the embryo as the new plant in miniature, is structurally and physiologically equipped for its role as a dispersal unit and is well provided with food reserves to sustain the growing seedling until it establishes itself as a self-sufficient, autotrophic organism. Because the function of a seed is to establish a new plant, it may seem peculiar that dormancy, an intrinsic block to germination, exists. But it may not be advantageous for a seed to germinate freely, even in seemingly favorable conditions. For example, germination of annuals in the spring allows time for vegetative growth and the subsequent production of offspring, whereas germination in similar conditions in the fall could lead to the demise of the vegetative plant during the winter. Thus, dormancy is an adaptive trait that optimizes the distribution of germination over time in a population of seeds.

Seed dormancy is generally an undesirable characteristic in agricultural crops, where rapid germination and growth are required. However, some degree of dormancy is advantageous, at least during seed development. This is particularly true for cereal crops because it prevents germination of grains while still on the ear of the parent plant (preharvest sprouting), a phenomenon that results in major losses to the agricultural industry. Extensive domestication and breeding of crop species have ostensibly removed most dormancy mechanisms present in the seeds of their wild ancestors, although under adverse environmental conditions, dormancy may reappear. By contrast, weed seeds frequently mature with inherent dormancy mechanisms that allow some seeds to persist in the soil for many years before completing germination.

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What Is Germination?

By definition, germination incorporates those events that commence with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis (Bewley and Black, 1994). The visible sign that germination is complete is usually the penetration of the structures surrounding the embryo by the radicle; the result is often called visible germination. Subsequent events, including the mobilization of the major storage reserves, are associated with growth of the seedling. Virtually all of the cellular and metabolic events that are known to occur before the completion of germination of nondormant seeds also occur in imbibed dormant seeds; indeed, the metabolic activities of the latter are frequently only subtly different from those of the former. Hence, a dormant seed may achieve virtually all of the metabolic steps required to complete germination, yet for some unknown reason, the embryonic axis (i.e., the radicle) fails to elongate.

What Is Dormancy?

Despite the fact that many researchers study dormancy, there is no unambiguous definition of the phenomenon, perhaps because it is manifest and broken in different ways in different species (Bewley and Black, 1994; Vleeshouwers et al., 1995; Lange, 1996). For the sake of simplicity, seed dormancy is regarded here as the failure of an intact viable seed to complete germination under favorable conditions. The seeds of some species are prevented from completing germination because the embryo is constrained by its

surrounding structures. This phenomenon is known as coat-enhanced dormancy; embryos isolated from these seeds are not dormant. In other species, a second category of dormancy is found in which the embryos themselves are dormant (embryo dormancy).

Before considering some of the cellular and metabolic aspects of dormancy, its breaking, and the resultant completion of germination, it is worthwhile pondering why so little progress has been made toward understanding dormancy. Undoubtedly, one contributing factor is that we do not know the defining events in germination. Without this information, there are no "baseline" data with which to compare observations made on dormant seeds that exhibit a block to germination. However, studying germination is also difficult because populations of seed do not complete the process synchronously; release from dormancy can be even more erratic because the threshold stimulus required to promote germination varies widely among individual seeds. Recently, a "biotime" concept has been introduced, which incorporates a mathematical model to characterize and predict seed germination behavior with respect to dormancy and the factors that influence it (Bradford, 1996).

There are other inherent difficulties in working with seeds. Events essential for the release from dormancy and the completion of germination may occur only within a relatively few cells associated with the embryonic root axis. Thus, the presence in experimental material of nonresponding cells in the axis or the presence of other seed parts, such as cotyledons and endosperm, that do not behave similarly can mask or dilute the changes sought. In seeds with coat-enhanced dormancy, the use of isolated embryos or axes is unsatisfactory because they are no longer dormant! Some interesting observations have been made using isolated dormant embryos, but again, these are composed of several tissue types; moreover, the cellular bases for the imposition and breaking of their dormancy may be different from those for seeds with coat-enhanced dormancy.

Is dormancy the result of a deficiency in some vital cellular event of germination, or is there is some dormancy-imposed event that must be negated before germination can be completed? A broader issue is whether release from dormancy, which can be triggered by a variety of environmental and chemical stimuli, is mediated through a common signal transduction chain that coordinates diverse cellular responses but that may differ between the seeds of different species and dormancy types. It has been suggested that there are related or common receptors for dormancy-breaking agents within the plasma membrane of the responsive embryonic cells. When triggered, these receptors then initiate a signal transduction cascade, perhaps involving synthesis of or sensitization to germination-promoting gibberellins (GAs), that leads to the completion of germination (Hilhorst, 1993; Vleeshouwers et al., 1995). Changes in the phosphorylating activity of membrane-associated, Ca^{2+}-dependent protein kinases that lead to dormancy or germination have been proposed as well (Trewavas, 1988). However, in the absence of any corroborating evidence, these suggestions must be regarded only as stimulating bases for future research.

GERMINATION

Before considering dormancy, which imposes a block to the completion of germination, it is appropriate first to consider the processes that comprise germination. Germination commences with the uptake of water by the dry seed—imbibition—and is completed when a part of the embryo, usually the radicle, extends to penetrate the structures that surround it.

Imbibition and the Resumption of Metabolism

Uptake of water by a mature dry seed is triphasic (Figure 1), with a rapid initial uptake (phase I) followed by a plateau phase (phase II). A further increase in water uptake occurs only after germination is completed, as the embryonic axes elongate. Because dormant seeds do not complete germination, they cannot enter phase III.

The influx of water into the cells of dry seeds during phase I results in temporary structural perturbations, particularly to membranes, which lead to an immediate and rapid leakage of solutes and low molecular weight metabolites into the surrounding imbibition solution. This is symptomatic of a transition of the membrane phospholipid components from the gel phase achieved during maturation drying to the normal, hydrated liquid-crystalline state (Crowe and Crowe, 1992). Within a short time of rehydration, the membranes return to their more stable configuration, at which time solute leakage is curtailed.

How repair to desiccation- and rehydration-induced damage to membranes and organelles is achieved is unknown. However, during the imbibition of cotton seeds, the amount of *N*-acetylphosphatidylethanolamine, a phospholipid with membrane-stabilizing properties, increases, as does that of the corresponding synthase. These molecules may be involved in maintaining or enhancing membrane integrity (Sandoval et al., 1995).

Upon imbibition, the quiescent dry seed rapidly resumes metabolic activity. The structures and enzymes necessary for this initial resumption of metabolic activity are generally assumed to be present within the dry seed, having survived, at least partially intact, the desiccation phase that terminates seed maturation. Reintroduction of water during imbibition is sufficient for metabolic activities to resume, with turnover or replacement of components occurring over several hours as full metabolic status is achieved (Figure 1).

One of the first changes upon imbibition is the resumption of respiratory activity, which can be detected within minutes. After a steep initial increase in oxygen consumption, the rate declines until the radicle penetrates the surrounding

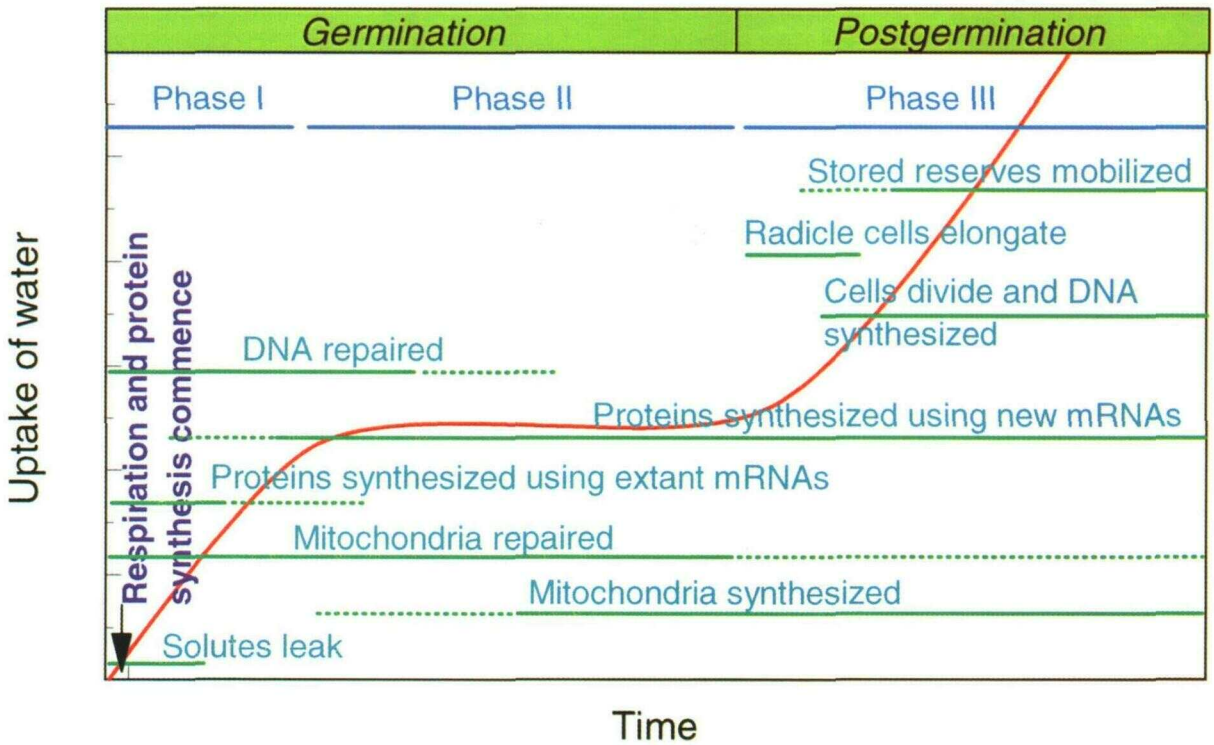

Figure 1. Time Course of Major Events Associated with Germination and Subsequent Postgerminative Growth.

The time for events to be completed varies from several hours to many weeks, depending on the plant species and the germination conditions.

structures. At this time, another burst of respiratory activity occurs (Botha et al., 1992; Bewley and Black, 1994). The glycolytic and oxidative pentose phosphate pathways both resume during phase I, and the Kreb's cycle enzymes become activated (Nicolás and Aldasoro, 1979; Salon et al., 1988). Germinating seeds of many species frequently produce ethanol (Morohashi and Shimokoriyama, 1972). This is often the result of an internal deficiency in oxygen that is caused by restrictions to gaseous diffusion by the structures that surround the seed and by the dense internal structure of most seeds. This oxygen deficiency may result in more pyruvate production than utilization for activities of the Kreb's cycle and electron transport chain.

Tissues of the mature dry seed contain mitochondria, and although these organelles are poorly differentiated as a consequence of maturation drying, they contain sufficient Kreb's cycle enzymes and terminal oxidases to provide adequate amounts of ATP to support metabolism for several hours after imbibition (Ehrenshaft and Brambl, 1990; Attucci et al., 1991).

During germination of embryos, there appear to be two distinct patterns of mitochondrial development. These patterns, which are particularly obvious in cotyledons, depend on the nature of the stored reserves. In starch-storing seeds, repair and activation of preexisting organelles predominate, whereas oil-storing seeds typically produce new mitochondria (Morohashi and Bewley, 1980; Morohashi, 1986). For example, the biogenesis of mitochondria in germinating maize embryos (which store oil in the scutellum, although starch is the major endosperm reserve) involves the synthesis of cytochrome *c* oxidase subunits encoded by the organellar genome, which is followed within hours by the synthesis of nuclear-encoded subunits (Ehrenshaft and Brambl, 1990). This observation also implies that the coordinated regulation of mitochondrial and nuclear genomes in plants begins during the early stages of germination.

Protein Synthesis during Germination

All of the components necessary for the resumption of protein synthesis upon imbibition are present within the cells of mature dry embryos, although polysomes are absent. However, within minutes of rehydration there is a decline in the number of single ribosomes as they become recruited into polysomal protein-synthesizing complexes. Initial protein synthesis is dependent on extant ribosomes, but newly synthesized ribosomes are produced and used within hours of initial polysome assembly (Dommes and Van der Walle, 1990).

Preformed mRNAs are also present within the dry embryo. Some of these are residual messages associated with previous developmental processes (Comai and Harada, 1990; Lane, 1991) and may be used transiently during early germination (Figure 1). Messages encoding proteins that are important during seed maturation and drying, such as late embryogenesis abundant (LEA) proteins, are likely to be degraded rapidly upon imbibition (Jiang and Kermode, 1994; Han et al., 1996). Conversely, those encoding proteins required during early germination (e.g., ribosomal protein messages; Beltrán-Peña et al., 1995) are replaced by identical messages at later times, with protein synthesis becoming more dependent on the new transcripts with time (Figure 1; Bewley, 1982). The nature of the stored messages in seeds has not been studied extensively, although it is known that they are likely to be present in association with proteins as mRNP complexes (Ajtkhozhin et al., 1976; Peumans et al., 1979). An alternative possibility is that stored transcripts remain sequestered within the nucleus (Hammett and Katterman, 1975).

New mRNAs are transcribed as germination proceeds. The majority of these are likely to encode proteins essential for the support of normal cellular metabolism, that is, "growth maintenance" reactions that are not restricted to germination (Bewley and Marcus, 1990). Indeed, no specific protein markers exclusive to germination have been found. Therefore, it is cautioned here that many so-called germination-specific mRNAs reported in the literature encode enzymes integral to the mobilization and conversion of the major stored reserves; these are postgerminative events that are important during seedling growth, but they are unrelated to germination per se (Figure 1). Nevertheless, some changes in embryo mRNA populations and synthesized proteins do occur during germination of several species of monocots (e.g., maize; Sánchez-Martinez et al., 1986), dicots (e.g., peas; Lalonde and Bewley, 1986), and conifers (e.g., loblolly pine; Mullen et al., 1996). The importance of these newly synthesized proteins to the completion of germination of the embryo remains to be elucidated.

One promising candidate for a "germination-specific" gene could be the one that encodes the protein germin (Lane, 1991), which is an oxalate oxidase. However, by its nature, the timing of its synthesis, and its potential metabolic functions (Lane, 1994), germin is more likely to be involved with postgerminative cell elongation. Thus, the search for specific protein markers with an exclusive role in germination must continue.

Radicle Extension and the Completion of Germination

With few exceptions, radicle extension through the structures surrounding the embryo is the event that terminates germination and marks the commencement of seedling growth. This extension may or may not be accompanied by cell division. Two discrete phases of DNA synthesis occur in the radicle cells after imbibition (Figure 1). The first takes place soon after imbibition and probably involves the repair of DNA damaged during maturation drying and rehydration as well as the synthesis of mitochondrial DNA. DNA synthesis associated with postgerminative cell division accounts for the second phase (Figure 1; Zlatnova et al., 1987; Osborne and Boubriak, 1994).

Extension of the radicle is a turgor-driven process that requires yielding of walls in those cells of the embryonic root axis that lie between the root cap and the base of the hypocotyl (see Cosgrove, 1997, in this issue, for a review of cell expansion). There are three possible reasons for the commencement of radicle growth. One possibility is that late during germination, the osmotic potential ($\psi\pi$) of the radicle cells becomes more negative because of the accumulation of solutes, perhaps as a result of the hydrolysis of polymeric reserves present within the radicle cells themselves. The decrease in $\psi\pi$ would lead to increased water uptake, and the resulting increase in turgor would drive cell extension. However, there is no consistent evidence for changes in cellular $\psi\pi$ during germination (Welbaum and Bradford, 1990; Bradford, 1995).

A second possibility is that extensibility of the radicle cell walls allows for their elongation. Whether the mechanisms by which cells of the radicle become more extensible differs from those in other tissues is not known. Cell wall loosening may result from the cleavage and rejoining of xyloglucan molecules that tether adjacent cellulose microfibrils, which would permit expansion by microfibril separation. The activity of xyloglucan endotransglycosylase (XET), an enzyme capable of reversibly cleaving xyloglucan molecules, increases in the apical region of maize seedling roots during their elongation (Wu et al., 1994), but this increase occurs after germination is completed. Alternative candidates for cell wall–loosening proteins are the expansins, which have the ability to disrupt the hydrogen bonds between cell wall polymers (e.g., matrix polysaccharides and cellulose microfibrils). Expansins have been strongly implicated in the expansion of cucumber hypocotyls (McQueen-Mason and Cosgrove, 1995; see Cosgrove, 1997, in this issue). However, neither of these cell wall–loosening proteins has been reported in germinating seeds. Moreover, both XET and expansin activities in seedlings appear to be enhanced by auxin, which is generally regarded as ineffective in promoting seed germination, and XET activity in maize seedling roots is also enhanced by abscisic acid (ABA), a potent inhibitor of embryo radicle elongation!

A third possibility is that the seed tissues surrounding the radicle tip weaken, thus allowing the tip to elongate. Because there are no changes in cell $\psi\pi$ before radicle growth commences, it is axiomatic that the turgor potential (ψp) of the radicle cells is sufficient to drive their elongation if there is little or no restraint exerted by the surrounding structures. In many germinating seeds, including those of rape, the testa splits during imbibition, and it is only the rigidity of the radicle cell walls that restrains growth (Schopfer and Plachy,

1985). As the walls yield during the initial stages of radicle elongation, there is a decline in cell $\psi\rho$. Conversely, in other seeds, $\psi\rho$ alone is insufficient to drive wall extension, and there is a severe constraint on radicle cell growth imposed by the surrounding structures. In lettuce, tobacco, and tomato seeds, the endosperm is the constraining structure, whereas in muskmelon it is the perisperm. A reduction in the resistance of these enclosing structures is necessary for germination to be completed. Measurements have revealed a decline in the mechanical resistance of the structures covering the embryo root cap at the time of radicle emergence in the endosperm of pepper seeds (Watkins and Cantliffe, 1983) and the perisperm of muskmelon seeds (Welbaum et al., 1995). This decline in resistance is likely to be achieved by cell wall hydrolases, such as hemicellulases, produced within and secreted by the endosperm itself, a subject that is considered in the next section.

DORMANCY

There is considerable circumstantial evidence that ABA is involved in regulating the onset of dormancy and in maintaining the dormant state. How this regulation is achieved is unknown; moreover, there is a paucity of unequivocal evidence that ABA is in fact an important controlling factor in the dormancy of most seeds.

ABA, Seed Development, and Developmental Mutants

Seeds are said to exhibit primary dormancy (commonly abbreviated, as here, to dormancy) when they are dispersed from the parent plant in a dormant state. Thus, primary dormancy is initiated during development. Developing seeds rarely germinate, and when precocious germination does occur, it is frequently associated with deficiencies in ABA synthesis or sensitivity (Black, 1991; Hilhorst, 1995; Karssen, 1995).

Typically, ABA accumulation in developing seeds is low during the early stages, is greatest during mid-development, when storage reserves are being synthesized, and declines as the seed undergoes maturation drying. Prevention of germination during development may be due to the endogenous ABA content of the seed, the osmotic environment surrounding the seed, or both (Berry and Bewley, 1992). Maturation drying and shedding from the parent plant are sufficient to relieve these constraints in nondormant seeds. In these instances, there may be an associated decline in the ABA content of the seed, and the sensitivity of the embryo to ABA is much reduced (Xu and Bewley, 1991).

Support for the hypothesis that the absence of, or insensitivity to, ABA during seed development results in the production of viviparous or precociously germinating seeds has come from the isolation of mutants deficient in ABA content or responsiveness. These include maize *viviparous* (*vp*), tomato *sitiens* (*sit*), and Arabidopsis *ABA-deficient* (*aba*) and *ABA-insensitive* (*abi*) mutants (Koornneef and Karssen, 1994; McCarty, 1995). There are also *reduced dormancy* (*rdo*) mutants of Arabidopsis, in which neither ABA synthesis nor sensitivity is altered (Léon-Kloosterziel et al., 1996). It is possible that in *rdo* seeds there is a block to some dormancy-inducing process that is normally controlled by ABA but is "downstream" from ABA synthesis and perception.

At least 10 *vp* mutants of maize are known, most of which are deficient in ABA because of defects in its biosynthetic pathway (Neill et al., 1986; McCarty, 1995), although the *vp1* mutant is ABA insensitive and does not respond to the appreciable amount of ABA it produces. The *sit* mutant of tomato germinates viviparously in overripe fruits, and its ABA content is ~10% of that of the dormant wild type (Groot et al., 1991). Single *aba* and *abi* mutants of Arabidopsis exhibit reduced seed dormancy but do not germinate precociously, whereas double mutants (e.g., *aba/abi3-1*) do (Koornneef et al., 1989).

Reciprocal crosses between *aba* mutants and wild-type plants show that dormancy is initiated only when the embryo itself produces ABA; ABA-deficient seeds (*aba/aba*) on ABA-producing parent plants (*Aba/aba*) are not dormant. Maternal ABA does not play a role in preventing germination in developing maize kernels either, and embryos of *vp5* and *vp8* mutants are even viviparous in the presence of a normal endosperm (McCarty, 1995). That in situ ABA synthesis in the embryo is necessary for the imposition of dormancy has been demonstrated in sunflower as well (LePage-Degivry and Garello, 1992). Although applied ABA prevented germination of isolated developing sunflower embryos, this inhibition was overcome upon their transfer to water; only ABA synthesized within the embryo imposed a lasting dormancy (LePage-Degivry and Garello, 1992).

These observations raise the concern that there may be temporary cellular and metabolic responses of embryos or seeds to exogenous ABA that are spatially and/or temporally distinct from the more permanent responses to its endogenous counterpart. An increase in the synthesis of some proteins occurs, for example, in oat embryos incubated in ABA; removal of ABA results in a cessation of their synthesis but does not trigger an increase in germination (Corbineau et al., 1991).

ABA-deficient mutant seeds are a useful demonstration that ABA can prevent germination and impose at least an equivalent to dormancy during seed development. An interesting secondary characteristic of developing seeds of the *sit* mutant of tomato is that they produce a thinner testa. Thus, not only is the ABA content of the mature embryo reduced, but one of the surrounding structures it must penetrate to complete germination is weaker as well (Hilhorst and Downie, 1996). Similarly, in the *aberrant testa shape* (*ats*) and *transparent testa glabrous* (*ttg*) seed shape mutants of Arabidopsis, the testa contributes to the degree of dormancy exhibited by the seed (Léon-Kloosterziel et al., 1994).

The ABA-insensitive mutants *vp1* and *abi3* are programmed to germinate even in the presence of the inhibitor, and this has been taken to indicate that the corresponding wild-type gene products are involved in mediating dormancy. So, can we identify any products of maize *VP1* and Arabidopsis *ABI3* gene expression that could explain why wild-type seeds are sensitive to ABA and fail to germinate?

The two structurally similar *VP1* and *ABI3* genes encode proteins that include domains characteristic of transcriptional coactivators (McCarty et al., 1991; Giraudat et al., 1992), and their failure to be synthesized in the *vp1* and *abi3* mutants leads to a number of pleiotropic responses. These include reduced synthesis of several maturation-specific proteins (including Em, a LEA protein; Paiva and Kriz, 1994) and enzymes of the anthocyanin biosynthesis pathway. However, none of these can be specifically regarded as being essential for germination. The *ABI1* gene of Arabidopsis encodes a calcium-modulated, serine/threonine protein phosphatase (Leung et al., 1994; Meyer et al., 1994). This protein could play a role in a signal transduction pathway involving Ca^{2+}, protein kinases, and phosphatases, which may respond to ABA and somehow prevent germination (see Trewavas and Malhó, 1997, in this issue, for a discussion of signal transduction in plants).

ABA and "Dormancy Genes"

The presence of and differential sensitivity of embryos to ABA may be important in the maintenance of mature seeds in a dormant state, although there are surprisingly few species for which this has been clearly demonstrated (Bewley and Black, 1994; Hilhorst, 1995). However, in wheat embryos, an interesting positive correlation has been shown between sensitivity to ABA and both resistance to germination during development and dormancy after maturation (Walker-Simmons, 1987). This has led to a search for ABA-responsive and dormancy-related genes in cereal embryos. Differences in patterns of protein synthesis between dormant and nondormant embryos of cereals (e.g., wild oat and wheat) have been demonstrated (Kawakami et al., 1992; Dyer, 1993; Li and Foley, 1994). The synthesis of some proteins is higher in nondormant than in dormant embryos, and that of others is less. However, despite differences in in vivo and in vitro protein synthesis profiles, no cause-and-effect relationship can be made between the presence or absence of a particular protein and dormancy.

A similar approach has been attempted at the mRNA level. Several cDNA clones have been isolated from wheat, barley, wild oat, and *Bromus secalinus* grains, and the messages with which they hybridize are expressed in higher amounts and for longer periods of time in imbibed dormant embryos than in nondormant embryos (afterripened, i.e., stored in the dry state until dormancy is lost; Morris et al., 1991; Goldmark et al., 1992; Hong et al., 1992; Johnson et al., 1995; Li and Foley, 1995, 1996; Stacy et al., 1996). The expression of many of the corresponding genes can also be enhanced by ABA, which raises the possibility that they are "dormancy genes," encoding proteins that act to suppress germination. However, some of the transcripts that are more prevalent in the dormant and ABA-treated embryos encode LEA-type proteins, which are typically synthesized during the later stages of seed development, or proteins associated with the survival of stress (Reid and Walker-Simmons, 1990; Li and Foley, 1995). Moreover, several of these proteins are also known to be synthesized in vegetative tissues in response to ABA. Thus, their synthesis in the embryo may not be associated with the imposition of dormancy per se but rather with the adaptive ability of the dormant grains to survive periods of wetting and drying in the soil. The gene for one stress-regulated protein in wild oat embryos exhibits a higher transcriptional activity in dormant embryos than it does in afterripened embryos, and its transcript is more stable in the former (Li and Foley, 1996).

The continued transcription of developmentally regulated genes in the dormant embryos of mature seeds may also indicate that their metabolism remains partially arrested in a prior developmental mode. In this mode, the genes are responsive to ABA, a known development-regulating factor. In addition, because the seeds may not have switched fully to a germinative mode, some genes could remain unable to respond to germination cues. Many of the transcripts produced by dormant and ABA-treated cereal embryos remain to be identified, and perhaps when this is achieved, some progress will be made toward linking the synthesis of specific proteins with the onset and maintenance of dormancy; currently, the link is tenuous at best.

ABA and Radicle Extension

Prevention of embryo radicle extension can be achieved by incubating mature seeds in solutions of ABA. This inhibition can occur even when ABA is introduced late during germination, an hour or so before radicle extension would be expected to occur. This raises the possibility that ABA acts to prevent a late event during germination, such as radicle cell wall loosening. Indeed, ABA inhibits radicle extension in *Brassica napus* embryos, thus preventing them from completing germination (Schopfer and Plachy, 1985). Moreover, an analysis of the water relations of the embryonic axes indicates that neither their osmotic potential nor their ability to take up water is affected by the presence of ABA, but rather the cell wall loosening that is associated with radicle extension is prevented. How this occurs is unknown. It should be noted that *B. napus* seeds are nondormant, and inhibition of their germination can only be achieved by the application of ABA. Dormant seeds of many species contain ABA, and in sunflower embryos the continuous synthesis of ABA is required for the expression of embryo dormancy (LePage-Degivry and Garello, 1992). Temporary application of ABA to embryos only prevents radicle extension while this inhibitor

is present; its removal leads to the completion of germination. Thus, the modes or sites of action of endogenous ABA, which is required continuously, and exogenous ABA, which is applied only temporarily, in preventing germination could be rather different.

GAs and Dormancy

GAs appear not to be involved in the control of dormancy per se but rather are important in the promotion and maintenance of germination, that is, they act after the ABA-mediated inhibition of germination has been overcome (Figure 2). The activities of ABA and GA may be linked, because in the *aba* and *rdo2* mutants of Arabidopsis, reduced dormancy is accompanied by a lowered requirement for GA to achieve germination (Léon-Kloosterziel et al., 1996). GAs are known to obviate the requirement of seeds for various environmental cues, promote germination, and counteract the inhibitory effects of ABA, frequently in combination with cytokinins (Bewley and Black, 1982, 1994). In seeds of a very few species, there is an increase in GA content in response to an external stimulus, but there is no evidence that this increase is important for breaking dormancy. On the other hand, sensitivity to GA may be a key factor (Hilhorst et al., 1986; Karssen et al., 1989). GA-deficient mutants of tomato (*gib1*) and Arabidopsis (*ga1-3*) require an exogenous supply of GA to complete germination (Koornneef and Van der Veen, 1980; Groot and Karssen, 1987). Embryos of GA-deficient tomato will germinate if removed from their surrounding structures. Thus, the role of GA in these seeds is probably restricted to the induction of endosperm-weakening enzymes (see below).

Metabolism of Dormancy Maintenance and Termination

Some seeds lose their dormancy while still in the dry state (during afterripening), when their rate of metabolism is very low. However, imbibed, dormant seeds are metabolically very active and in this state can receive an external signal (e.g., light, chilling, alternating temperatures, and chemical or hormonal treatment) that can stimulate germination. The primary events in the release from dormancy are the reception of the stimulus by the embryo and the immediate signal transduction chain that leads to the secondary events, which could involve metabolic and hormonal changes (Figure 2). The final result is emergence of the embryonic axis from the seed, that is, the completion of germination. Of the primary events, the role of phytochrome is well known, although what occurs after the active form, Pfr, is produced is

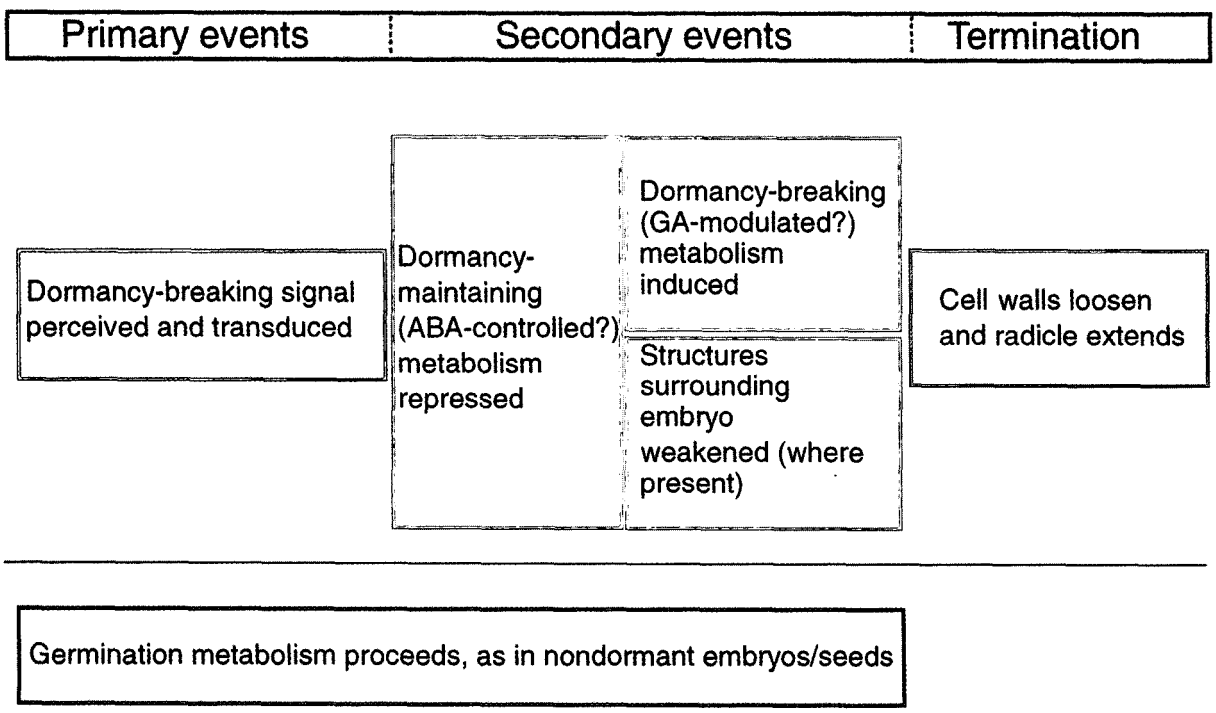

Figure 2. An Overview of the Major Events That Have Been Associated with the Breaking of Seed Dormancy.

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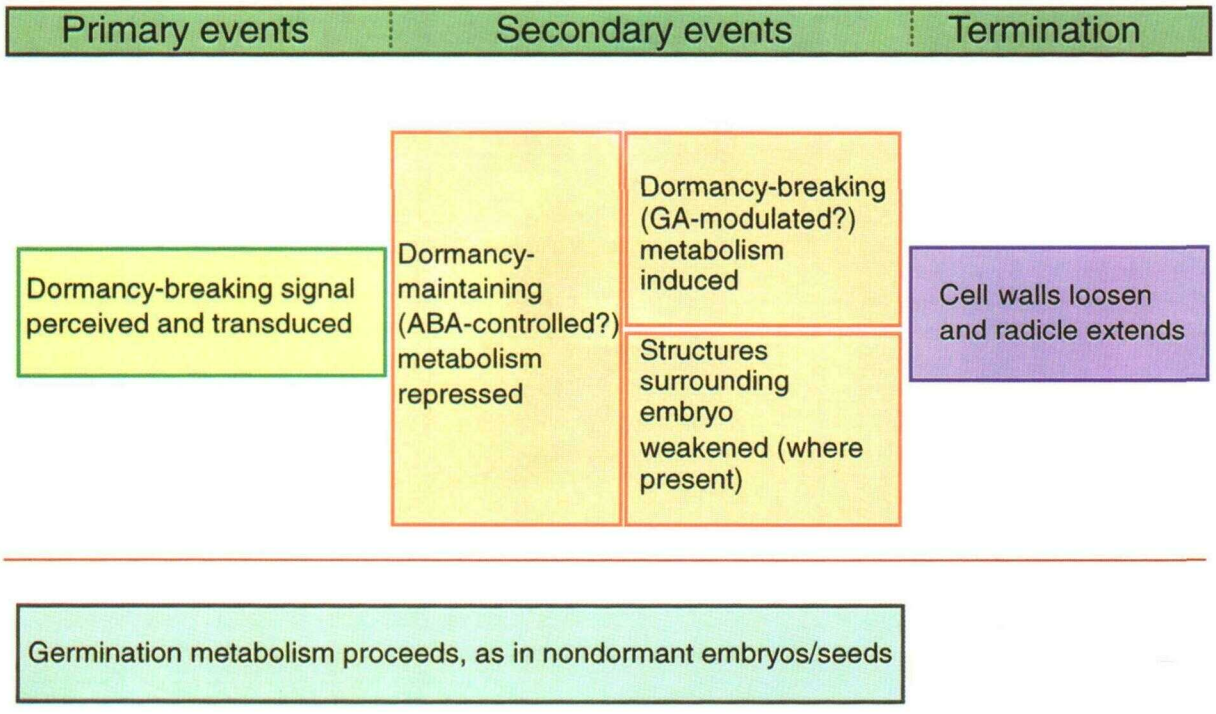

Figure 2. An Overview of the Major Events That Have Been Associated with the Breaking of Seed Dormancy.

not (see Chory, 1997, in this issue). The receptors of the other external stimuli are unknown, but it is suspected that they are membrane associated.

The secondary events in the release from dormancy were critically reviewed more than a decade ago (Bewley and Black, 1982), with the following conclusions: (1) The metabolism of imbibed dormant seeds is not appreciably different from that of their nondormant counterparts, either quantitatively or qualitatively. (2) Environmental and chemical stimuli factors that break dormancy may act at the level of transcription, but there is no evidence for the production of proteins that are essential for or associated with the induction of germination (see above). (3) There is no compelling evidence that changes in respiration rate, respiratory pathways, or their enzymes are essentially linked to the regulation of dormancy. (4) Although the state of membranes may be involved in the regulation of dormancy (primary and secondary events), it is not known how. A plea was also made for more additional and critical studies.

Now, a decade and a half later, and despite the best efforts of many researchers, more or less the same conclusions can be drawn and the same plea can be made. More advanced techniques have been applied, including new analytical, biochemical, and molecular approaches (see previous section), but the outcome has largely been to advance our ignorance of dormancy to a higher level of sophistication. This research is not elaborated on here because no definitive conclusions can be reached; however, the reader is directed to two recent publications as literature sources (Bewley and Black, 1994; Lang, 1996) and to review articles (e.g., Hilhorst, 1995; Karssen, 1995; Cohn, 1996).

Mature primary dormant seeds can enter into a state of secondary dormancy in response to unfavorable germination conditions and can remain fully imbibed for long periods without loss of viability (Bewley and Black, 1994). ABA does not appear to be involved. Secondary dormant lettuce seeds conduct <30% of the respiration and protein synthesis that occur in primary dormant seeds and use stored reserves to maintain themselves (Powell et al., 1983). Upon release from secondary dormancy, there is an increase in respiration, but this is lower than in seeds emerging from primary dormancy (Powell et al., 1984). These observations raise the question as to how much or how little of the respiration (or any other metabolic process) measured in primary dormant seeds is really essential for its maintenance and breakage, and how much is excess, resulting in a high "background" that masks any subtle metabolic changes taking place.

Facilitating Radicle Emergence

Embryos that are constrained by a mechanical barrier, such as the surrounding endosperm, perisperm, or megagametophyte (i.e., those that exhibit coat-enhanced dormancy), appear to require a weakening of these structures to permit radicle protrusion (Figure 2). This weakening involves partial enzymatic degradation of the cell walls (e.g., of the endosperm of tomato, lettuce, tobacco, and *Datura ferox*). In germinating wild-type tomato seeds, lowering of the puncture force required by the radicle to penetrate the mannan-rich endosperm cell walls has been attributed to endo-β-mannanase, an enzyme produced within the endosperm itself at about the time of radicle emergence (Groot et al., 1988). The enzyme is first produced in the micropylar region and later, after germination, in the rest of the endosperm; each region produces different isoforms (Nonogaki and Morohashi, 1996; Toorop et al., 1996; Voigt and Bewley, 1996).

Thus, in the same tissue, there may be germination-related isoforms of the enzyme and other isoforms associated with postgermination mannan cell wall reserve mobilization. Whether endo-β-mannanase is in itself sufficient for germination to be completed has been questioned, however, because the enzyme can accumulate in the micropylar region of the endosperm in the absence of germination, for example, when embryo growth potential is not limiting (Toorop et al., 1996; Still and Bradford, 1997). Endo-β-mannanase is also produced in the endosperm of lettuce seeds when the seeds are released from dormancy by GA or red light, but this also occurs postgerminatively (Halmer et al., 1976). Moreover, as in tomato, endo-β-mannanase activity can increase in the absence of radicle protrusion (J.D. Bewley, unpublished results), although the reciprocal relationship has also been reported (Powell et al., 1984).

Perhaps the production of other hemicellulases is more critical for endosperm dissolution (Dutta et al., 1994). Indeed, by contrast to the situation in germinating seeds of lettuce and tomato, seeds of *D. ferox* exhibit increased endo-β-mannanase and β-mannosidase activities in the micropylar region of the endosperm after red light stimulation and many hours before the radicle protrudes through it (Sánchez and de Miguel, 1997). An increase in cellulase activity in the whole seed also occurs at about the same time as red light–induced radicle elongation (Sánchez et al., 1986). Thus, although no cause-and-effect relationships have been established between the increases in activity of these hydrolytic enzymes and the completion of germination in *D. ferox*, the possibility certainly exists. Cellulase activity is not important for the germination of tomato or lettuce seeds. In the former, cellulase activity increases after germination (Leviatov et al., 1995), whereas in the latter, cellulase activity is barely detectable in dormant or nondormant seeds (Bewley et al., 1983). Tobacco endosperm cell walls may instead be weakened by β-1,3-glucanase to permit germination. Activity of this enzyme increases before emergence of the radicle, and ABA retards both enzyme accumulation and endosperm rupture (Leubner-Metzger et al., 1995).

As with other facets of dormancy breaking, the unsatisfactory conclusion has to be drawn that although endosperm weakening is likely to be essential for some seeds to complete germination, how this is achieved remains a mystery.

CONCLUSION

Much more needs to be learned about the key processes involved in germination and dormancy. Both germinating and dormant seeds must undergo many cellular and metabolic changes in common after imbibition, and yet only the embryos of the former emerge from their surrounding structures. The real block to germination in dormant seeds may occur at the very last stage: radicle cell wall extension. Even so, there may still be many steps that must be completed between the perception of the signal for dormancy breaking and the final emergence of the radicle (Figure 2). In the past decade, most research on the cellular aspects of dormancy has focused on the secondary events, the metabolism of seeds during and after release from dormancy, but to little avail. New approaches that can be or are being tried in an attempt to identify germination- and dormancy-associated genes include T-DNA mutagenesis, differential display, subtractive cDNA hybridization, and the use of nondestructive reporter gene technology. Perhaps it is time to focus also on the primary events: perception and transduction of the dormancy-breaking signal. Finally, we need to determine how radicle extension occurs, the ultimate manifestation of germination!

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REFERENCES

Ajtkhozhin, M.A., Doschanov, K.J., and Akhanov, A.J. (1976). Informosomes as a stored form of mRNA in wheat embryos. FEBS Lett. **66,** 124–126.

Attucci, S., Carde, J.P., Raymond, P., Saint Gès, V., Spiteri, A., and Pradet, A. (1991). Oxidative phosphorylation by mitochondria extracted from dry sunflower seeds. Plant Physiol. **95,** 390–398.

Beltràn-Peña, E., Ortiz-López, A., and Sànchez de Jiménez, E. (1995). Synthesis of ribosomal proteins from stored mRNAs early in seed germination. Plant Mol. Biol. **28,** 327–336.

Berry, T.A., and Bewley, J.D. (1992). A role for the surrounding fruit tissues in preventing germination of tomato (*Lycopersicon esculentum*) seeds. A consideration of the osmotic environment and abscisic acid. Plant Physiol. **100,** 951–957.

Bewley, J.D. (1982). Protein and nucleic acid synthesis during germination and early seedling growth. In Encyclopaedia of Plant Physiology, Vol. 14A, D. Boulter and B. Parthier, eds (New York: Springer-Verlag), pp. 61–81.

Bewley, J.D., and Black, M. (1982). Physiology and Biochemistry of Seeds in Relation to Germination. 2. Viability, Dormancy and Environmental Control. (Berlin: Springer-Verlag).

Bewley, J.D., and Black, M. (1994). Seeds: Physiology of Development and Germination. (New York: Plenum Press).

Bewley, J.D., and Marcus, A. (1990). Gene expression in seed development and germination. Prog. Nucleic Acid Res. Mol. Biol. **38,** 165–193.

Bewley, J.D., Leung, D.W.M., and Ouellette, F.B. (1983). The cooperative role of endo-β-mannanase, β-mannosidase and α-galactosidase in the mobilization of endosperm cell wall hemicellulases in germinated lettuce seed. Recent Adv. Phytochem. **17,** 137–152.

Black, M. (1991). Involvement of ABA in the physiology of developing and maturing seeds. In Abscisic Acid: Physiology and Biochemistry, W.J. Davies and H.G. Jones, eds (Oxford, UK: BIOS Scientific), pp. 99–124.

Botha, F.C., Potgieter, G.P., and Botha, A.-M. (1992). Respiratory metabolism and gene expression during seed germination. J. Plant Growth Regul. **11,** 211–224.

Bradford, K.J. (1995). Water relations in seed germination. In Seed Development and Germination, J. Kigel and G. Galili, eds (New York: Marcel Dekker), pp. 351–396.

Bradford, K.J. (1996). Population-based models describing seed dormancy behaviour: Implications for experimental design and interpretation. In Plant Dormancy, G.A. Lang, ed (Oxford, UK: CAB International), pp. 313–339.

Chory, J. (1997). Light modulation of vegetative development. Plant Cell **9,** 1225–1234.

Cohn, M.A. (1996). Operational and philosophical decisions in seed dormancy research. Seed Sci. Res. **6,** 147–153.

Comai, L., and Harada, J.J. (1990). Transcriptional activities in dry seed nuclei indicate the timing of the transition from embryogeny to germination. Proc. Natl. Acad. Sci. USA **87,** 2671–2674.

Corbineau, F., Poljakoff-Mayber, A., and Côme, D. (1991). Responsiveness to abscisic acid of embryos of dormant oat (*Avena sativa*) seeds. Involvement of ABA-inducible proteins. Physiol. Plant. **83,** 1–6.

Cosgrove, D.J. (1997). Relaxation in a high-stress environment: The molecular bases of extensible cell walls and cell enlargement. Plant Cell **9,** 1031–1041.

Crowe, J.H., and Crowe, L.M. (1992). Membrane integrity in anhydrobiotic organisms: Toward a mechanism for stabilizing dry seeds. In Water and Life, G.N. Somero, C.B. Osmond, and C.L. Bolis, eds (Berlin: Springer-Verlag), pp. 87–103.

Dommes, J., and Van de Walle, C. (1990). Polysome formation and incorporation of new ribosomes into polysomes during germination of the embryonic axis of maize. Physiol. Plant. **79,** 289–296.

Dutta, S., Bradford, K.J., and Nevins, D.J. (1994). Cell-wall autohydrolysis in isolated endosperms of lettuce (*Latuca sativa L.*). Plant Physiol. **104,** 623–628.

Dyer, W.E. (1993). Dormancy-associated embryonic mRNAs and proteins in imbibing *Avena fatua* caryopses. Physiol. Plant. **88,** 201–211.

Ehrenshaft, M., and Brambl, R. (1990). Respiration and mitochondrial biogenesis in germinating embryos of maize. Plant Physiol. **93,** 295–304.

Giraudat, J., Hauge, B.M., Valon, C., Smalle, J., Parcy, F., and Goodman, H.M. (1992). Isolation of the *Arabidopsis ABI3* gene by positional cloning. Plant Cell **4,** 1251–1261.

Goldmark, P.J., Curry, J., Morris, C.F., and Walker-Simmons, M.K. (1992). Cloning and expression of an embryo-specific mRNA up-regulated in hydrated dormant seeds. Plant Mol. Biol. **19,** 433–441.

Groot, S.P.C., and Karssen, C.M. (1987). Gibberellins regulate seed germination in tomato by endosperm weakening: A study with gibberellin-deficient mutants. Planta **171,** 525–531.

Groot, S.P.C., Kieliszewska-Rokicka, B., Vermeer, E., and Karssen, C.M. (1988). Gibberellin-induced hydrolysis of endosperm cell walls in gibberellin-deficient tomato seeds prior to radicle emergence. Planta **174,** 500–504.

Groot, S.P.C., Van Yperen, I.I., and Karssen, C.M. (1991). Strongly reduced levels of endogenous abscisic acid in developing seeds of tomato mutant *sitiens* do not influence *in vivo* accumulation of dry matter and storage proteins. Physiol. Plant. **81,** 73–78.

Halmer, P., Bewley, J.D., and Thorpe, T.A. (1976). An enzyme to degrade lettuce endosperm cell walls. Appearance of a mannanase following phytochrome- and gibberellin-induced germination. Planta **130,** 189–196.

Hammett, J.R., and Katterman, F.R. (1975). Storage and metabolism of poly(adenylic acid)-mRNA in germinating cotton seeds. Biochemistry **17,** 3250–3256.

Han, B., Hughes, D.W., Galau, G.A., Bewley, J.D., and Kermode, A.R. (1996). Changes in late embryogenesis abundant (*Lea*) messenger RNAs and dehydrins during maturation and premature drying of *Ricinus communis* L. seeds. Planta **201,** 27–35.

Hilhorst, H.W.M. (1993). New aspects of seed dormancy. In Fourth International Workshop on Seeds, Basic and Applied Aspects of Seed Biology, D. Côme and F. Corbineau, eds (Paris: ASFIS), pp. 571–579.

Hilhorst, H.W.M. (1995). A critical update on seed dormancy. I. Primary dormancy. Seed Sci. Res. **5,** 61–73.

Hilhorst, H.W.M., and Downie, B. (1996). Primary dormancy in tomato (*Lycopersicon esculentum* cv. Moneymaker): Studies with the *sitiens* mutant. J. Exp. Bot. **47,** 89–97.

Hilhorst, H.W.M., Smitt, I., and Karssen, C.M. (1986). Gibberellin biosynthesis and sensitivity mediated stimulation of seed germination of *Sysimbrium officinale* by red light and nitrate. Physiol. Plant. **67,** 285–290.

Hong, B., Barg, R., and Ho, T.-h.D. (1992). Developmental organ-specific expression of an ABA- and stress-induced protein in barley. Plant Mol. Biol. **18,** 663–674.

Jiang, L., and Kermode, A.R. (1994). Role of desiccation in the termination of expression of genes for storage proteins. Seed Sci. Res. **4,** 149–173.

Johnson, R.R., Cranston, H.J., Chaverra, M.E., and Dyer, W.E. (1995). Characterization of cDNA clones for differentially expressed genes in embryos of dormant and nondormant *Avena fatua* L. caryopses. Plant Mol. Biol. **28,** 113–122.

Karssen, C.M. (1995). Hormonal regulation of seed development, dormancy, and germination studied by genetic control. In Seed Development and Germination, J. Kigel and G. Galili, eds (New York: Marcel Dekker), pp. 333–350.

Karssen, C.M., Zagórski, S., Kepczyński, J., and Groot, S.P.C. (1989). Key role for endogenous gibberellins in the control of seed germination. Ann. Bot. **63,** 71–80.

Kawakami, N., Kawabata, C., and Kuzuhiko, N. (1992). Differential changes in levels of mRNAs during maturation of wheat seeds that are susceptible and resistant to preharvest sprouting. Plant Cell Physiol. **33,** 511–517.

Koornneef, M., and Karssen, C.M. (1994). Seed dormancy and germination. In *Arabidopsis,* E.M. Meyerowitz and C.R. Somerville, eds (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press), pp. 313–334.

Koornneef, M., and Van der Veen, J.H. (1980). Induction and analysis of gibberellin sensitive mutants in *Arabidopsis thaliana* (L.) Heynh. Theor. Appl. Genet. **58,** 257–263.

Koornneef, M., Hanhart, C.J., Hilhorst, H.W.M., and Karssen, C.M. (1989). *In vivo* inhibition of seed development and reserve protein accumulation in recombinants of abscisic acid biosynthesis and responsiveness mutants in *Arabidopsis thaliana* (L.) Heynh. Theor. Appl. Genet. **58,** 257–263.

Lalonde, L., and Bewley, J.D. (1986). Desiccation during germination and its effects on the messenger RNA population. J. Exp. Bot. **37,** 754–764.

Lane, B.G. (1991). Cellular desiccation and hydration: Developmentally regulated proteins, and the maturation and germination of seed embryos. FASEB J. **5,** 2893–2901.

Lane, B.G. (1994). Oxalate, germin, and the extracellular matrix of higher plants. FASEB J. **8,** 294–301.

Lang, G.A. (1996). Plant Dormancy. (Oxford, UK: CAB International).

Léon-Kloosterziel, K.M., Keijzer, C.J., and Koornneef, M. (1994). A seed shape mutant of Arabidopsis that is affected in integument development. Plant Cell **6,** 385–392.

Léon-Kloosterziel, K.M., Van de Bunt, G.A., Zeevaart, J.A.D., and Koornneef, M. (1996). Arabidopsis mutants with reduced seed dormancy. Plant Physiol. **110,** 233–240.

LePage-Degivry, M.T., and Garello, G. (1992). *In situ* abscisic acid synthesis. A requirement for induction of embryo dormancy in *Helianthus annus.* Plant Physiol. **98,** 1386–1390.

Leubner-Metzger, G., Fründt, C., Vögeli-Lange, R., and Meins, F., Jr. (1995). Class I β-1,3-glucanases in the endosperm of tobacco during germination. Plant Physiol. **109,** 751–759.

Leung, J., Bouvier-Durand, M., Morris, P.C., Guerrier, D., Chefdor, F., and Giraudat, J. (1994). *Arabidopsis* ABA response gene *ABI1*: Features of a calcium-modulated protein phosphatase. Science **264,** 1448–1452.

Leviatov, S., Shoseyov, O., and Wolf, S. (1995). Involvement of endomannanase in the control of tomato seed germination under low temperature conditions. Ann. Bot. **76,** 1–6.

Li, B., and Foley, M.E. (1994). Differential polypeptide patterns in imbibed dormant and afterripened *Avena fatua* embryos. J. Exp. Bot. **45,** 275–279.

Li, B., and Foley, M.E. (1995). Cloning and characterization of differentially expressed genes in imbibed dormant and afterripened *Avena fatua* embryos. Plant Mol. Biol. **29,** 823–831.

Li, B., and Foley, M.E. (1996). Transcriptional and posttranscriptional regulation of dormancy-associated gene expression by afterripening in wild oat. Plant Physiol. **110,** 1267–1273.

McCarty, D.R. (1995). Genetic control and integration of maturation and germination pathways in seed development. Annu. Rev. Plant Physiol. Plant Mol. Biol. **46,** 71–93.

McCarty, D.R., Hattori, T., Carson, C.B., Vasil, V., and Vasil, I.K. (1991). The *viviparous 1* developmental gene of maize encodes a novel transcriptional activator. Cell **66,** 895–905.

McQueen-Mason, S., and Cosgrove, D.J. (1995). Expansin mode of action on cell walls. Analysis of wall hydrolysis, stress relaxation, and binding. Plant Physiol. **107,** 87–100.

Meyer, K., Leube, M.P., and Grill, E. (1994). A protein phosphatase 2C involved in ABA signal transduction in *Arabidopsis thaliana*. Science **264,** 1452–1455.

Morris, C.F., Anderberg, R.J., Goldmark, P.J., and Walker-Simmons, M.K. (1991). Molecular cloning and expression of abscisic-acid responsive genes in embryos of dormant wheat seeds. Plant Physiol. **95,** 814–821.

Morohashi, Y. (1986). Patterns of mitochondrial development in reserve tissue of germinated seeds: A survey. Physiol. Plant. **66,** 653–658.

Morohashi, Y., and Bewley, J.D. (1980). Development of mitochondrial activities in pea cotyledons during and following germination of the axis. Plant Physiol. **66,** 70–73.

Morohashi, Y., and Shimokoriyama, M. (1972). Physiological studies on germination of *Phaseolus mungo* seeds. II. Glucose and organic-acid metabolisms in the early phases of germination. J. Exp. Bot. **23,** 54–61.

Mullen, R.T., King, J.E., and Gifford, D.J. (1996). Changes in mRNA populations during loblolly pine (*Pinus taeda*) seed stratification, germination and postgerminative growth. Physiol. Plant. **97,** 545–553.

Neill, S.J., Horgan, R., and Parry, A.D. (1986). The carotenoid and abscisic acid content of viviparous kernels and seedlings of *Zea mays* L. Planta **169,** 87–96.

Nicolás, G., and Aldasoro, J.J. (1979). Activity of the pentose phosphate pathway and changes in nicotinamide nucleotide content during germination of seeds of *Cicer arietinum* L. J. Exp. Bot. **30,** 1163–1170.

Nonogaki, H., and Morohashi, Y. (1996). An endo-β-mannanase develops exclusively in the micropylar endosperm of tomato seeds prior to radicle emergence. Plant Physiol. **110,** 555-559.

Osborne, D.J., and Boubriak, I.I. (1994). DNA and desiccation tolerance. Seed Sci. Res. **4,** 175–185.

Paiva, R., and Kriz, A.L. (1994). Effect of abscisic acid on embryo-specific gene expression during normal and precocious germination in normal and *viviparous* maize (*Zea mays*) embryos. Planta **192,** 332–339.

Peumans, W.J., Caers, L.I., and Carlier, A.R. (1979). Some aspects of the synthesis of long-lived messenger ribonucleoproteins in developing rye embryos. Planta **144,** 485–490.

Powell, A.D., Leung, D.W.M., and Bewley, J.D. (1983). Long-term storage of dormant Grand Rapids lettuce seeds in the imbibed stage: Physiological and metabolic changes. Planta **159,** 182–188.

Powell, A.D., Dulson, J., and Bewley, J.D. (1984). Changes in germination and respiratory potential of embryos of dormant Grand Rapids lettuce seeds during long-term imbibed storage, and related changes in the endosperm. Planta **162,** 40–45.

Reid, J.L., and Walker-Simmons, M.K. (1990). Synthesis of abscisic-acid responsive, heat-stable proteins in embryonic axes of dormant wheat grain. Plant Physiol. **93,** 662–667.

Salon, C., Raymond, P., and Pradet, A. (1988). Quantification of carbon fluxes through the tricarboxylic acid cycle in early germinating lettuce embryos. J. Biol. Chem. **263,** 12278–12287.

Sánchez, R.A., and de Miguel, L. (1997). Phytochrome promotion of mannan-degrading enzymes in the micropylar endosperm of *Datura ferox* seeds requires the presence of the embryo and gibberellin synthesis. Seed Sci. Res. **7,** 27–33.

Sánchez, R.A., de Miguel, L., and Mercuri, O. (1986). Phytochrome control of cellulase activity in *Datura ferox* L. seeds and its relationship with germination. J. Exp. Bot. **37,** 1574–1580.

Sánchez-Martinez, D., Puigdomènech, P., and Pagès, M. (1986). Regulation of gene expression in developing *Zea mays* embryos. Protein synthesis during embryogenesis and early germination of maize. Plant Physiol. **82,** 543–549.

Sandoval, J.A., Huang, Z.-H., Garrett, D.C., Gage, D.A., and Chapman, K.D. (1995). *N*-Acylphosphatidylethanolamine in dry and imbibing cotton seeds. Amounts, molecular species, and enzymatic synthesis. Plant Physiol. **109,** 269–275.

Schopfer, P., and Placy, C. (1985). Control of seed germination by abscisic acid. III. Effect of embryo growth potential (minimum turgor pressure) and growth coefficient (cell wall extensibility) in *Brassica napus* L. Plant Physiol. **77,** 676–686.

Stacy, R.A.P., Murnthe, E., Steinum, T., Sharma, B., and Aalen, R.B. (1996). A peroxiredoxin antioxidant is encoded by a dormant-related gene, Per1, expressed during late development in the aleurone and embryo of barley grains. Plant Mol. Biol. **31,** 1205–1216.

Still, D.W., and Bradford, K.J. (1997). Endo-β-mannanase activity from individual tomato endosperm caps and radicle tips in relation to germination rates. Plant Physiol. **113,** 21–29.

Toorop, P.E., Bewley, J.D., and Hilhorst, H.W.M. (1996). Endo-β-mannanase isoforms are present in the endosperm of tomato seeds, but are not essentially linked to the completion of germination. Planta **200,** 153–158.

Trewavas, A.J. (1988). Timing and memory processes in seed embryo dormancy—A conceptual paradigm for plant development questions. Bioessays **6,** 87–93.

Trewavas, A.J., and Malhó, R. (1997). Signal perception and transduction: The origin of the phenotype. Plant Cell **9,** 1181–1195.

Vleeshouwers, L.M., Bouwmeester, H.J., and Karssen, C.M. (1995). Redefining seed dormancy: An attempt to integrate physiology and ecology. J. Ecol. **83,** 1031–1037.

Voigt, B., and Bewley, J.D. (1996). Developing tomato seeds when removed from the fruit produce multiple forms of germinative and post-germinative endo-β-mannanase. Responses to desiccation, abscisic acid and osmoticum. Planta **200,** 71–77.

Walker-Simmons, M. (1987). ABA levels and sensitivity in developing wheat embryos of sprouting resistant and susceptible cultivars. Plant Physiol. **84,** 61–66.

Watkins, J.T., and Cantliffe, D.J. (1983). Mechanical resistance of the seed coat and endosperm during germination of *Capsicum annuum* at low temperature. Plant Physiol. **72,** 146–150.

Welbaum, G.E., and Bradford, K.J. (1990). Water relations of seed development and germination in muskmelon (*Cucumis melo* L.). V. Water relations of imbibition and germination. Plant Physiol. **92,** 1046–1062.

Welbaum, G.E., Muthui, W.J., Wilson, J.H., Grayson, R.L., and Fell, R.D. (1995). Weakening of muskmelon perisperm envelope tissue during germination. J. Exp. Bot. **46,** 391–400.

Wu, Y., Spollen, W.G., Sharp, R.E., Hetherington, P.R., and Fry, S.C. (1994). Root growth maintenance at low water potentials. Increased activity of xyloglucan endotransglycosylase and its possible regulation by abscisic acid. Plant Physiol. **106,** 607–615.

Xu, N., and Bewley, J.D. (1991). Sensitivity to abscisic acid and osmoticum changes during embryogenesis of alfalfa (*Medicago sativa*). J. Exp. Bot. **42,** 821–826.

Zlatanova, J.S., Ivanov, P.V., Stoilov, L.M., Chimshirova, K.V., and Stanchev, B.S. (1987). DNA repair precedes replicative synthesis during early germination of maize. Plant Mol. Biol. **10,** 139–144.