Chapter 2

Challenges Facing Seed Banks and Agriculture in Relation to Seed Quality

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Abstract

Seeds form a convenient vehicle for storage of germplasm, both for agricultural purposes and conservation of wild species. When required, seeds can be taken from storage and germinated, and plants can be propagated for the desired purpose, e.g., crop production or biome restoration. However, seed dormancy often interferes with stand establishment or industrial utilization in crops and germination of wild species. An anticipated termination of dormancy (i.e., before crop harvest) also occurs, with preharvest sprouting as a consequence. In order to overcome these problems, a better understanding of dormancy is required. This chapter is devoted to discuss the achievement of such understanding in problematic species.

Key words: Conservation, Crops, Seed deterioration, Seed dormancy, Preharvest sprouting, Seed storage, Wild species

1. Introduction

Seed dormancy has been a subject of intensive research during the last decades. Physiological and environmental control of dormancy has been studied in depth and, more recently, its molecular and genetic bases are starting to be elucidated. As in other areas of plant science, progress on the elucidation of the molecular basis of dormancy has been attained through the use of model organisms. Indeed, work with *Arabidopsis* has revealed the involvement of several genes controlling the different processes that converge to impose, maintain, or release dormancy. Beyond the fact that dormancy is a highly intriguing biological process and consequently moves the curiosity of scientists, the study of dormancy has strong practical implications. Both farmers and seed companies encounter problems associated with a lack of dormancy or conversely with profound dormancy. In the same way, industrial processes like malting, which depends on seed germination, can

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be largely hampered by the existence of dormancy or alternatively face important reductions in the quality of the received lots due to a precocious dormancy termination and pregermination in the field. Long-term seed conservation (i.e., seed banks) is also subjected to limitations imposed by dormancy and the paucity of knowledge that exists on the mechanisms behind the phenomenon.

Dormancy is a trait displayed by seeds of most temperate species. Since dormancy is highly undesirable for agricultural purposes, selection pressure against this trait must have been important throughout the domestication process, particularly in species of temperate origin. In some cases, this pressure must have gone too far, as in the case of cereals in which some crops are susceptible to preharvest sprouting (PHS) or pregermination, due to a loss of dormancy inception during seed development. Nevertheless, intraspecific variability for dormancy exists and "dormancy genes" are still present in some genotypes, although their positive selection through breeding has been difficult due to strong linkage to other undesired traits. In some other cases, selection pressure might have not succeeded in eliminating dormancy, so seeds are not germinable by the time of the next sowing or by the time they need to be "ready" by the malting industry. In any case, dormancy release should take place within a precise "time window." This rarely can be achieved without a solid knowledge of the mechanisms on which dormancy relies in each species. For this reason, a lot of research has been invested to elucidate dormancy mechanisms in problem crops (i.e., wheat, sorghum, sunflower, barley); some of these mechanisms are common to those of *Arabidopsis*, but others are not. With this in mind, most of the molecular information obtained from *Arabidopsis* and other model organisms has started to be "translated" into problematic species from a dormancy point of view. The latest achievements in the area come from this "translational" approach.

The role of seed dormancy is to spread germination across time, but in synchrony with the seasons to avoid unfavorable conditions for plant establishment (1). Different types of dormancy exist, first described by Nikolaeva (2) and more recently explored in detail by Baskin and Baskin (1). In seeds with *physiological dormancy*, a physiological mechanism prevents elongation of the axis, usually observed as radicle protrusion. Seeds with *morphological dormancy* are characterized by underdeveloped embryos that are either differentiated or not. Seeds with morphophysiological dormancy display a combination of morphological and physiological dormancy. In seeds with *physical dormancy*, the restricted permeability of water, typically caused by a palisade layer of lignified cells in the seed coat, interferes with imbibition and thus with germination. A combined dormancy of physiological and physical dormancy exists as well. This chapter focuses on physiological dormancy or the lack thereof.

In wild species that have not been cultivated, seed dormancy is often present (3). The type and degree of dormancy seem more pronounced in some families than in others. Dormancy forms a block to germination, which plays a role in the ecology of plant species. Dormancy is clearly an adaptive trait that contributes to the survival of plants by spreading germination over time, but in synchrony with the seasons, thus facilitating growth of the young plants and flowering when conditions are most favorable (1). Conservation of wild germplasm occurs by banking seeds under optimal storage conditions, often considered to be dry and at a subzero temperature, for long-term survival. However, only viable seeds should be stored under such conditions; dead seeds (for purposes other than conservation) are stored more cost-effectively at ambient conditions that require minimal infrastructure and maintenance costs. So far, a positive germination test has proven to form the most solid method to verify that stored seeds are viable. A germination test result is affected by any dormancy that is present, and any suspected dormancy must be released to get a proper estimate of the viability. These extra efforts require more resources: seed numbers, dormancy-releasing chemicals, space in germination chambers, a wider range of germination conditions, and more staff time to score germination and analyze viability. These are costs that cannot be entirely avoided, since wild species always display a degree of seed dormancy; but creating greater understanding of seed dormancy and the factors important for its termination can reduce them, and there is further a need for better tests to assess dormancy.

In this chapter, we point out the most important challenges facing agriculture and long-term seed conservation in relation to the presence or absence of dormancy. We present some examples of each type of problem, illustrating on the physiological mechanisms responsible for the expression of the character and commenting how the molecular information coming from studies on *Arabidopsis* and other model species have started to be used to attain the final aim of solving these dormancy problems.

2. Challenges Facing Agriculture

Seeds in their natural form are still the best way to preserve and propagate most crop species, and their capacity to germinate at the right moment (either in the field or under controlled conditions) is a most important feature to us. Therefore, any factors (internal or external) affecting the germination capacity of seeds and ultimately their agronomical performance should be understood in order to improve seed quality through breeding and/or crop

management strategies. Among the factors that may affect germination, dormancy is probably the most important, as it is a heritable trait that can inhibit germination of a viable seed.

Dormancy is the result of mechanisms operating within the seed that inhibit germination even when the seed is exposed to an environment known to be friendly for that species (i.e., in which water, oxygen, and adequate temperature are available). Although mechanisms behind dormancy seem quite diverse among different species, they converge at the point where cell expansion – that would result in embryo growth – is blocked. Primary dormancy is acquired during seed development. In most angiosperm species, primary dormancy is gradually lost after the seed is ready for dispersal (in wild species) or harvest during a postmaturation or after-ripening period. This process takes place in seeds during dry storage or in buried seeds in the soil as well, although these different environmental conditions can have great impact on the rate at which dormancy is terminated. Release of primary dormancy occurs at different rates depending mainly on the genotype and nothing else, but time is needed in many species for their seeds to be able to germinate (although some hard-coated seeds depend absolutely on chemical or mechanical scarification). Even in species that depend on the presence of light for germination (like many *Solanaceae*), sensitivity to light also varies with time after seed dispersal, and seed germination eventually becomes light independent. So the time lapse required by a seed population to lose dormancy and be able to undergo rapid germination immediately after sowing is a character that varies greatly not only between species, but also between different genotypes (i.e., cultivars, inbred lines, etc.) of the same species. The timing of the exit of dormancy for seeds of cultivated species can impact on agricultural systems in different ways.

2.1. The Persistence of Dormancy

For most cultivated species, the process of domestication has favored the abundance of genotypes that lose dormancy faster than their wild ancestors. A rapid and simultaneous germination of the seeds after sowing is required for a healthy and uniform stand of plants. A long-lasting dormancy (i.e., many months) would pose the problem of preventing a second sowing in the same season. Similarly, the process of malting relies on germination, and those genotypes that could be malted immediately after harvest are likely to be preferred. Nevertheless, among these reduced-dormancy genotypes, others with long-lasting dormancy have managed to survive up to the present and offer a source of variability for manipulating the level of dormancy through identification of dormancy QTL and its use in breeding programs. In species with a shorter history of domestication, like sunflower (*Heliantus annuus*), dormancy elimination has not been as dramatic as in cereal species and is frequently a problem in seed lots intended for sowing.

2.1.1. Sunflower: A Crop Species with Persistent Dormancy

Sunflower is a good example of a crop species with prolonged dormancy. Dormancy persistence in different sunflower genotypes can vary from several weeks up to almost a year. Also, important variation has been reported on this trait for the same genotype under different environments, indicating a strong interaction with the environment. Sunflower is cultivated in many areas around the globe, and is a summer crop. At harvest time, sunflower seeds are dormant and germinate poorly (4–6). This dormancy is the result of true embryo dormancy (4, 7) and the inhibitory action of the envelopes (4, 5, 7), including the seed coat and the pericarp since sunflower "seeds" are achenes. In the case of freshly harvested sunflower seeds, dormancy is expressed at temperatures lower and higher than 25°C (4). Dormancy expression at low temperatures is attributed to embryo dormancy which is not expressed at high temperatures (4); conversely, dormancy expressed at high temperatures results from coat-imposed dormancy (4). Consequently, a few weeks of dry after-ripening allow seed germination at low temperatures due to termination of embryo dormancy; the acquisition of the capacity to germinate at high temperatures, in contrast, may take several weeks of dry after-ripening (4).

The inception of embryo dormancy occurs relatively early during seed development. Sunflower embryos are germinable if isolated from the entire seed from as early as 7 days after pollination (DAP) and until approximately 12 DAP; the entire seed, however, germinates very poorly during this period showing that the coat (seed coat plus pericarp) imposes dormancy (Fig. 1) (4, 8).

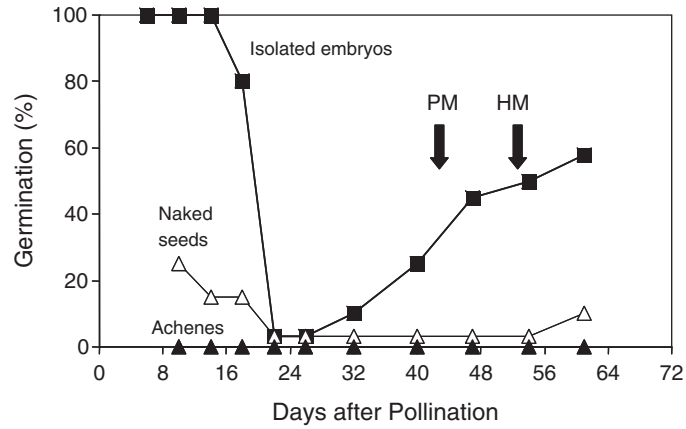

Fig. 1. Germination percentage of sunflower achenes (*solid triangles*), naked seeds (*open triangles*), and isolated embryos (*solid squares*) harvested at different days after pollination (DAP) and incubated at 25°C. Whole achenes are totally unable to germinate when incubated at any of the developmental stages displayed in the graph. The *arrows* associated with PM and HM indicate the approximate times during development when the crop reaches physiological maturity and harvest maturity, respectively. This figure has been redrawn with data originally published in LePage-Degivry and Garello (8) (Copyright American Society of Plant Biologists) and in Corbineau, et al. (4) (Permission of the Israel Journal of Botany). This Figure was first published in the "Handbook of Seed Physiology: Applications to Agriculture". Reprinted here with permission from the Haworth Reference Press of the Taylor and Francis Group.

From 12 DAP onward, embryo dormancy progressively develops and at 20–22 DAP, embryos are fully dormant (Fig. 1). This embryo dormancy is not eliminated if the axis is separated from the cotyledons, indicating that the axis itself is dormant (8). While the seed progresses toward maturation, embryos are slowly released from dormancy; by the time the grain has attained harvest maturity, some embryo dormancy still persists (Fig. 1). Therefore, the deep dormancy that sunflower grains present at harvest results from the coexistence of coat-imposed dormancy and some remnant embryo dormancy (4). Embryo dormancy is lost shortly after harvest if the seed is subjected to dry after-ripening, but coat-imposed dormancy persists for longer and may require several weeks of dry after-ripening to be overcome.

The plant growth regulator ABA appears to be involved in the imposition of embryo dormancy. The inclusion of fluridone (an inhibitor of ABA biosynthesis) in media upon which developing sunflower embryos are cultured prevents the induction of embryo dormancy (8, 9). Nevertheless, the pattern of accumulation of ABA in the developing embryo does not coincide with the physiological behavior of embryo: during seed development, embryos germinate well at the time when the endogenous ABA level is at its highest (7–12 DAP); thereafter, ABA decreases to a low value when embryo dormancy becomes established (9). It seems, then, that the ABA peak at early stages is responsible for the imposition of the dormant state that is established immediately after that peak has taken place.

Moreover, it appears that ABA needs to be present during a critical time period to induce dormancy. In an interesting study, LePage-Degivry and Garello (8) showed that when young (7 DAP), nondormant embryos are cultured in the presence of ABA, the hormone produces a temporary inhibition of germination but does not induce dormancy (i.e., embryos are able to germinate when transferred to a basal medium). In contrast, exogenous ABA becomes effective if applied immediately prior to the natural induction of dormancy. For example, a 5-day culture on a medium containing 5×10^{-5} M ABA results in dormancy of a proportion of 13 DAP embryos while induction of dormancy occurs in 100% of 17 DAP embryos. The authors concluded that either a change in sensitivity to ABA occurs during development or the existence of a second factor is necessary along with ABA to induce dormancy.

As mentioned before, embryo dormancy can be terminated by dry storage. Bianco, Garello, and LePage-Degivry (10) attempted to elucidate the mechanism through which dry storage terminates embryo dormancy by drying artificially dormant 17–26 DAP embryos and testing for germinability either immediately after drying or after leaving the embryos for 6 weeks in a desiccator (dry storage). A decrease in ABA content occurs immediately after the drying process which is not accompanied by a complete release

from dormancy and, on the other hand, additional dry storage does not affect the ABA content but promotes germination. In addition, the drying treatment also stimulates immature sunflower embryos and axes to respond to gibberellins (GAs) upon rehydration. Thus, although the drying treatment induces both a decline in ABA and an increase in sensitivity to GA, additional dry storage is necessary to obtain germination; the authors propose that dry storage suppresses the capacity of the embryo to synthesize ABA. The extent to which the drying treatment itself suppresses ABA biosynthesis was not examined. To identify the process by which dormancy is broken during after-ripening, Oracz et al. (11) focused on the role of reactive oxygen species (ROS) in this phenomenon. After-ripening is accompanied by a progressive accumulation of ROS, namely, superoxide anions and hydrogen peroxide, in cells of embryonic axes. This accumulation, which was investigated at the cellular level by electron microscopy, occurs concomitantly with lipid peroxidation and oxidation (carbonylation) of specific embryo proteins. Incubation of dormant seeds for 3 h in the presence of hydrogen cyanide (a compound that breaks dormancy) or methylviologen (a ROS-generating compound) also releases dormancy and causes the oxidation of a specific set of embryo proteins. In summary, the mechanism proposed by the authors involves ROS production and targeted changes in protein carbonylation patterns (11).

As with other cultivated species, such as *Lactuca sativa* (12) and *Arachis hypogea* (13), ethylene (C_2H_4) and ethephon strongly stimulate the germination of dormant sunflower seeds (4, 5, 14). In contrast, gibberellic acid (GA_3) and cold stratification (moist chilling) do not overcome dormancy in this species (15), although 1 mM GA_3 is effective for overcoming dormancy in some wild sunflowers (16). Corbineau et al. (4) showed that ethylene and its immediate precursor (1-aminocyclopropane-1-carboxylic acid) strongly stimulate germination of primary dormant sunflower seeds; on the contrary, inhibitors of ethylene (i.e., amino-oxyacetic acid and $CoCl_2$) or ethylene action (silver thiosulfate and 2.5 norbomadiene) inhibit germination of nondormant seeds. Beyond the evident practical implications, these results indicate that ethylene synthesized by the seeds themselves is involved in the regulation of sunflower seed germination.

Although endogenous ethylene is known to be involved in sunflower seed alleviation of dormancy, Oracz et al. (17) assessed the possible role of cyanide, which is produced by the conversion of 1-aminocyclopropane 1-carboxylic acid to ethylene, in this process. The beneficial HCN effect on germination of dormant embryos is associated with a marked increase in hydrogen peroxide and superoxide anion generation in the embryonic axes (17). The effect of HCN can be mimicked by the ROS-generating compounds, methylviologen and menadione, but suppressed by ROS

scavengers (18). The increase of ROS results from an inhibition of catalase and superoxide dismutase activities and also involves activation of NADPH oxidase. The expression of genes related to ROS production (NADPHox, POX, AO1, and AO2) and signaling (MAPK6, Ser/ThrPK, CaM, and PTP) is differentially affected by dormancy alleviation either during after-ripening or by HCN treatment; the effect of cyanide on gene expression may be mediated by ROS. Surprisingly, HCN and ROS both activate similarly ERF1, a component of the ethylene-signaling pathway (18).

The use of ethylene or its precursors appears as a promising technology to stimulate the germination of dormant sunflower lots. Possibly, seed companies have not adopted treatments based on these chemicals due to a lack of adequate devices to treat large amounts of seeds and increased costs associated with the treatments. Also, the correct doses may vary greatly with the genotype and the level of dormancy, and seeds can overreact to ethylene with adverse effects on the young plantlet known as the "triple response" (stunting of growth, twisting of plants, and abnormal thickening of stems). The effect of storage conditions is being explored as it has been observed that high temperatures (i.e., 25°C) accelerate dormancy release of sunflower seeds as compared to storage at 5°C.

2.2. Cereal Dormancy: A Two-Edged Sword

2.2.1. Persistence of Dormancy in Malting Barley

In addition to impairing a rapid and simultaneous germination after sowing, a persistent dormancy would prevent the utilization of a seed lot for industrial purposes when the process requires germination. This is the case for the malting process, which uses barley as the main grain. The malting process itself requires grain germination, so a low dormancy level at harvest is a desirable characteristic because the grain can be malted immediately after crop harvest, thus avoiding costs and deterioration resulting from grain storage until dormancy is terminated. Therefore, breeders have to solve the compromise between the desire to obtain genotypes with low dormancy at harvest, but without the worry that dormancy is so reduced as to risk sprouting (a phenomenon which is referred to in the next section). In other words, dormancy release in barley must be adjusted to occur within a narrow and precise "time window." The achievement of this aim requires a thorough knowledge of the mechanisms determining dormancy release in the maturing grain.

Dormancy of the barley grain is typically imposed by the seed-covering structures (lemma and palea, pericarp plus seed coat). Indeed, embryos can germinate well from the very early stages of development if they are isolated from the rest of the grains and incubated in water (19). Limitation of oxygen supply to the embryo by oxygen fixation as a result of oxidation of phenolic compounds in the lemma and palea (hereafter referred to as the glumellae or the hull) has been suggested to be responsible for the dormancy of dressed caryopses of cereals, such as barley (20) and oat (21).

In dormant grains of barley, for example, whole intact caryopses germinate with difficulty, even in the air, while dehulled caryopses are all able to germinate under oxygen tensions of at least 10%, suggesting that oxygen concentration under the covering structures might be less than 10% (20).

As in many other species, dormancy in barley can be regarded as a relative phenomenon, the expression of which depends on the incubation temperature. It is usually not expressed at relatively low temperatures (10–20°C) while its expression increases as the temperature rises (20, 22, 23). The amount of water in the incubation medium also allows differential expression of dormancy in barley grains. Indeed, most barley cultivars, which present some dormancy at harvest, do not germinate if the grains are incubated in a Petri dish at favorable temperatures but with 8 or even 6 mL instead of 4 mL of distilled water (24); the same does not occur in grains from cultivars with low dormancy or in those that have after-ripened, showing that it is truly an expression of dormancy. This phenomenon is known by the malting industry as "sensitivity to water" and is one of the quality parameters assessed upon reception of a grain lot. This sensitivity to water is likely related to oxygen deprivation (hypoxia) imposed by the presence of the hull, which may be enhanced by an excess of water in the incubation media.

Dormancy of the barley grain also appears to be under ABA control: termination of glumellae-imposed dormancy during grain development correlates with a sharp decline both in ABA embryonic content and sensitivity (19). A role for ABA in dormancy maintenance of the barley grain has also been suggested: the ABA content of embryos declines during the first hours of incubation of nondormant seeds, whereas it remains at high levels in embryos of dormant grains (25). In recent years, Benech-Arnold et al. (26) confirmed this role of ABA in dormancy maintenance and extended it to the differential expression of dormancy at different temperatures. The ABA level of the embryo largely decreases during the first hours of incubation and prior to any visible germination in both dormant and nondormant grains at 20°C, the temperature at which dormancy is not expressed, and in nondormant grains incubated at 30°C, suggesting that ABA catabolism and/or conjugation exceeds ABA biosynthesis. By contrast, at 30°C, dormancy expression is associated with maintenance of ABA at high levels: after an initial increase, ABA content decreases very smoothly and is always between two- and fourfold higher than in embryos from grains in which dormancy is not expressed (Fig. 2). Millar et al. (27) examined gene expression profiles in *Arabidopsis* focusing on the gene families corresponding to ABA biosynthetic and catabolic enzymes, i.e., the 9-cis epoxycarotenoid dioxygenasegene and ABA 8'-hydroxylase gene families (CYP707A), respectively. Of these, only the *AtCYP707A2* gene was differentially

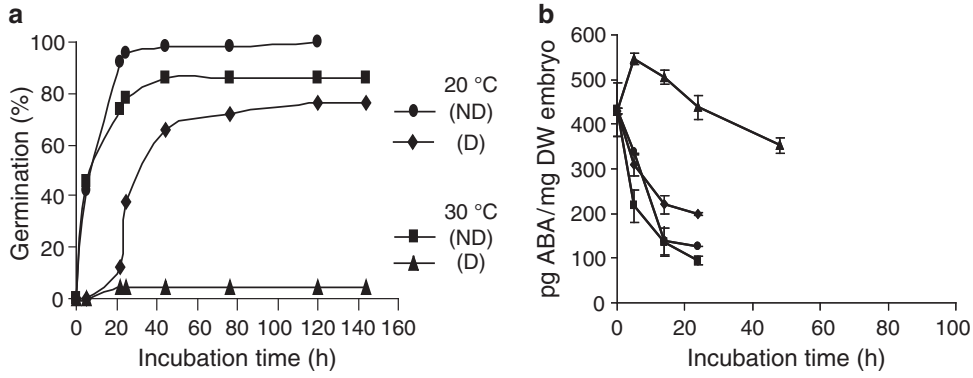

Fig. 2. Germination (**a**) and embryo ABA content (**b**) during incubation at 20°C (*circles, diamonds*) and 30°C (*triangles, squares*) of dormant (*circles, triangles*) and nondormant (*diamonds, squares*) grains. Grains were either freshly harvested (*dormant*, *D*) or were stored dry for at least 3 months at 25°C (*nondormant*, *ND*). Determinations are the means of two measurements ± arithmetical spread (germination) or of three measurements ± SD (ABA content). This figure has been redrawn with data originally published in Benech-Arnold et al. (26) and was printed with permission of Oxford University Press.

expressed between dormant and nondormant seeds, exhibiting higher expression in nondormant seeds. Similarly, a barley *CYP707* homologue (*HvABA8'OH-1*) was expressed to a much higher level in embryos from nondormant grains than from dormant grains (27). Consistent with this, in situ hybridization studies showed that *HvABA8'OH-1* mRNA expression was stronger in embryos from nondormant grains. Surprisingly, the signal was confined to the coleorhizae, suggesting that this tissue plays a key role in dormancy release (27). These results support a role for ABA in dormancy maintenance of the barley grain, and further indicate that dormancy release may be partly mediated by ABA catabolism via the 8'-hydroxylation pathway.

Both the removal of the glumellae and the incubation of dehulled grains under low oxygen concentrations modify grain germination behavior and ABA content evolution throughout incubation at 30°C (26). Thus, the glumellae is instrumental for dormancy maintenance because it imposes oxygen deprivation to the embryo, which, in turn, promotes ABA synthesis and/or inhibits ABA inactivation. Indeed, removal of the glumellae in dormant grains incubated at 30°C under 21% oxygen suppresses the initial increase in ABA content that occurs in dressed dormant grains incubated at the same temperature. By contrast, incubation under hypoxia (5% oxygen) of dehulled grains restores it and inhibits its germination (26). In a recent paper, Mendiondo et al. (28) investigated the effect of the glumellae on the expression of ABA synthesis and catabolism genes, and the extent to which artificially imposed hypoxia can mimic the effect of the glumellae on gene expression. The presence of the glumellae enhanced, in dormant grains incubated at 30°C, the expression of genes encoding enzymes involved in ABA synthesis (HvNCED1 and HvNCED2)

without altering substantially the expression of HvABA8′OH-1. These results indicate that the presence of the glumellae maintains dormancy through an enhancement of ABA synthesis regulated at the level of gene transcription. On the other hand, artificially imposed hypoxia does not mimic the effect of the presence of the glumellae in terms of alteration of gene expression, thus casting doubt on the contention that the glumellae effects (at least on the expression of ABA synthesis genes) are through oxygen deprivation. Only the expression of HvABA8'OH-1 is enhanced by hypoxia, possibly as a feedback effect that results from poor functioning of the enzyme at low oxygen tensions (28).

Artificially imposed hypoxia also enhances embryo sensitivity to ABA by several folds (26). These results suggest that, in addition to interference with ABA metabolism, the presence of the glumellae increases embryo responsiveness to the phytohormone. To explore this possibility, Mendiondo et al. (28) measured the expression of several components of the ABA-signaling pathway during incubation of entire and dehulled grains: the presence of the glumellae enhanced the expression of most of the investigated genes, suggesting that the hull increases the sensitivity to ABA of the enclosed embryos and that this enhancement is effected at the level of gene expression. Again, artificially imposed hypoxia is not able to mimic the effect of the glumellae on the expression of ABA-signaling components, indicating that the apparent enhancement of embryo sensitivity to ABA under low oxygen tensions is due to overaccumulation of ABA as a result of malfunctioning of the enzyme committed to ABA inactivation (26).

One of the most popular methods used by the malting industry, whenever allowed by the customer, is the addition of gibberellic acid to the incubation medium to promote the germination of dormant barley grains. Indeed, it is well-known that gibberellic acid at low concentrations (0.1–0.2 ppm) stimulates germination of these grains (29). Studies on the most appropriate point in the malting process at which to add gibberellic acid have concluded that it should be sprayed on soon after the grain is removed from the steep (29). Other methods to remove dormancy in barley include the use of dilute solutions of hydrogen sulfide and keeping the grains for 3 days at 40°C, either in the open air when their moisture content falls to about 8% or in closed vessels when moisture contents are unchanged at between 17 and 20% (24). However, when the customer allows none of these methods, time of after-ripening is the only way of obtaining a germinable lot. Unfortunately, the physiology and molecular biology of after-ripening in barley has not been assessed as it has been in sunflower. Only experiments comparing dormant and nondormant grains have been reported in the literature as commented on in the above paragraphs. Within this frame, Gubler et al. (30) found that after-ripening has no effect on expression of ABA biosynthesis genes,

but promotes expression of an ABA catabolism gene (*HvABA8'OH1*), a GA biosynthetic gene (*HvGA3ox2*), and a GA catabolic gene (*HvGA2ox3*) following imbibition. In the same paper, the authors report that blue light mimics the effect of white light (which promotes dormancy) on germination, increasing ABA levels, and expression of GA and ABA metabolism genes (30).

2.2.2. The Lack of Dormancy in Cereals: Preharvest Sprouting

The advantages of having a freshly harvested seed lot with the capacity to germinate rapidly and uniformly under a wide range of environmental conditions are related to the possibility of immediate sowing or malting, thus avoiding financial costs associated with delays and/or the need for storage until germination capacity is good enough. Nevertheless, genotypes that produce nondormant seeds at harvest may already be able to germinate to some degree even before harvest. The main problem related to an early loss of dormancy in crop species is PHS. This phenomenon is a characteristic of cereal species, like rice, barley, wheat, and sorghum. As these species all exhibit intraspecific variability for the rate of dormancy loss and PHS behavior, genotypes with contrasting sprouting behavior have proved useful for many comparative studies (31–34), in addition to QTL analysis to identify several loci related to dormancy (35–38). When low levels of dormancy during late grain maturation period are combined with rainy or damp conditions in the field, the process of germination is activated while the seeds are still attached to the mother plant, and the resulting emergence of the radicle from the seed coats is called PHS. Depending on the intended purpose for the seeds after harvest, PHS can have serious negative consequences on their quality and this is economically penalized by the industry. PHS in cereals is a problem in many parts of the world and in some wheat production areas, PHS occurs in 3–4 years out of 10. Total worldwide annual losses have been estimated to about US$1 billion, but precise statistics are lacking. Direct economic losses caused by PHS to producers occur in several ways: desiccation of a sprouted grain leads to its subsequent loss of viability because, together with the activation of metabolism implicated in embryo growth, tolerance to dehydration is lost rendering the sprouted grain useless for sowing or malting. Sprouting also promotes carbohydrate respiration that not only reduces grain yield, but also creates a favorable environment for the attack of grains by saprophytic fungi and bacteria that produce toxins.

Depending on the level of dormancy and environmental cues, such as water availability for imbibition and temperature, the germination process can advance to different extents, and grains do not always reach the completion of germination and sprouting (i.e., germination is complete when embryo growth begins, and postgerminative growth leads to visible radicle and/or coleoptile emergence through the seed-covering structures). Even when desiccation occurs before germination is complete, starch degradation

may have advanced partially. This phenomenon is known as pregermination. Pregerminated grains cannot be distinguished visually, but the level of starch degradation is correlated with a decrease in falling number (FN) values (31) and a reduction in seed lot longevity (39). Industrial processes based on wheat and barley (flour and malting) are particularly sensitive to sprouting and pregermination. Seed lots are commonly assessed with the FN test, with smaller FN values indicating a greater degree of starch degradation. Wheat and barley seed lots can be penalized or even rejected if FN values show moderate incidence of pregermination and sprouting.

The phenomena of PHS and pregermination are closely related with the process of domestication. Cereal crops have a brief period of dormancy as compared to their wild ancestors. Throughout many years, farmers have pressed toward the selection of low levels of dormancy along with other traits, such as nonshattering, increased grain size, and less pigmentation. The occurrence of PHS not only depends on morphological and physiological traits genetically controlled (such as infructescence structure and permeability of structures surrounding the seeds and seed dormancy), but also depends on environmental factors (water availability and temperature). A single genotype may express PHS when grown in some areas, but not in others. Seed dormancy is the main heritable factor that contributes to PHS resistance, but the many attempts to control it through breeding programs have shown that dormancy is tightly linked to other interesting traits. Breeding programs that attempted to separate characters associated with seed color, dormancy, and longevity suggest that these characteristics may not always be separable and are referred to as domestication block (40). For example, in rice, both loci sh-h (for shattering) and Rc (conferring red pericarp) are tightly linked together with a QTL qSDs-7-1 for seed dormancy, implying that this region might represent a domestication block in the evolutionary pathway of rice (41). A mutation in the R-gene in white wheat is required for low tannin content (higher flour quality), but is also responsible for lower levels of dormancy (low PHS resistance). However, grain dormancy is a complex trait that relies on numerous genes, and other QTLs contributing to dormancy that are not linked to seed color have been identified and are being considered in breeding for PHS tolerance in white wheat (42).

In addition to classical breeding techniques, elucidation of the mechanisms involved in the control of dormancy may open other possibilities for manipulation of dormancy through genetic engineering techniques. Cereal species, like barley and sorghum, have served as model systems to study the mechanisms behind dormancy with the objective to understand the hormonal basis of the repression of germination in barley and sorghum, especially related to hormone metabolism and signaling (19, 20, 26, 32–34, 43).

2.2.3. Problems Associated with Preharvest Sprouting in Sorghum

Sweet sorghum (*Sorghum bicolor*) domestication began in Africa and later spread to India and the rest of the world. As in other cereal species, sorghum has a great intraspecific variability for the duration of dormancy: in some genotypes, dormancy may last up to several months while in others dormancy release may begin even before physiological maturity is reached. Due to its C4 metabolism, sorghum is often cultivated in hot, dry regions where other crops would fail. But recent introduction of sorghum to more humid cultivation areas has made this crop susceptible to PHS. Studies using two inbred lines with contrasting sprouting behavior (IS9530, PHS resistant, and RedlandB2, susceptible) have shown that dormancy release in the susceptible line begins between 20 and 30 DAP (Fig. 3). Dormancy in these sorghum lines is coat-imposed, and isolated embryos are capable of germinating fully from early development regardless of the genotype and the level of dormancy of the whole grain (Fig. 3). Previous research with both lines has shown that dormancy is established during early stages of seed development and is regulated mainly by the antagonistic effects of plant hormones, ABA (which enhances dormancy and inhibits germination) and gibberellins (which reduce dormancy and promote germination) (32, 33). However, absolute levels of both hormones in developing sorghum embryos are not related to the different level of dormancy exhibited by both sorghum lines.

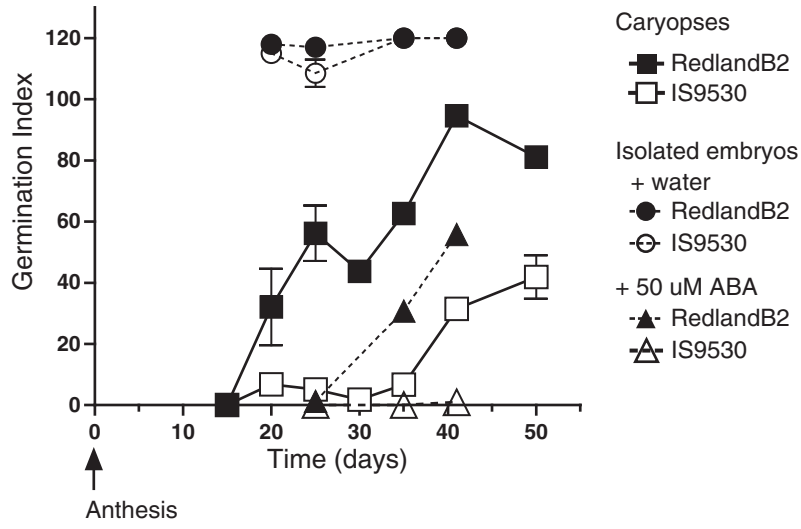

Fig. 3. Germination index for sorghum lines IS9530 and RedlandB2. Caryopses were harvested at different days after pollination (DAP) and incubated for 12 days in water at 25°C (*squares*). Embryos (axis plus scutellum) were dissected and incubated at 25°C in distilled water (*circles*) or 50 μM ABA (*triangles*). Each data point is the mean value of two biological replicates (i.e., two field plots) assessed in triplicate. *Bars* indicate standard error with $n = 2$. The approximate time of physiological maturity is indicated with an *arrow*. This figure has been redrawn with data originally published in Rodríguez et al. (34) and was printed with Permission of American Society of Agronomy, Inc., Madison, WI, the USA.

Nevertheless, different responses can be explained by sensitivity to these hormones and/or their postimbibition metabolism. Incubation of isolated embryos in a solution of ABA inhibits germination in a way that resembles the dormant grain, and an overall correlation between changes in grain dormancy and embryo "sensitivity" to ABA can be observed in both sorghum lines throughout development (32) (Fig. 3). In contrast to other species in which ABA accumulates in imbibed dormant seeds, ABA levels in these sorghum lines decrease at a similar rate and are not related to the level of dormancy.

In accordance to the observed contrast in embryo sensitivity to ABA, several sorghum genes known to be involved in ABA signaling in other species of reference are expressed differently in imbibed sorghum grains with contrasting levels of dormancy (34) (Fig. 4). Genes encoding several transcription factors (*SbVP1*, *SbABI4*, and *SbABI5*) and a protein kinase (*SbPKABA1*) that are positive regulators of ABA signaling are induced transiently in dormant IS9530 (PHS resistant) immature grains (just before physiological maturity), and may be involved in the higher sensitivity to ABA in this line. No induction of these genes is observed in the PHS susceptible line. These observations were also confirmed at the protein level for SbABI5. Nevertheless, expression of these genes (and ABI5 protein levels) fails to explain differences in grain dormancy and embryo sensitivity to ABA after physiological maturity, suggesting that mechanisms involved in the control of germination by ABA may differ with the developmental stage of the seed (before and after physiological maturity). This is in accordance with a biphasic pattern of dormancy release observed in sorghum and other species (i.e., barley, see next section).

On the other hand, ABA inhibition of germination is opposed by the germination-promoting effect of gibberellins. The ABA/GA balance governs imposition of dormancy during early development (33) as well as the expression of dormancy in imbibed grains. Quantification of embryonic GA in imbibed immature sorghum grains shows that embryos belonging to the PHS susceptible line RedlandB2 produce higher levels of active GA (GA1, GA3) as compared to those from the dormant line IS9530. This is correlated with a lower expression of a gene encoding a GA inactivation enzyme (GA 2-oxidase) in the nondormant line RedlandB2 while expression of this gene is higher in dormant IS9530 grains (Fig. 4). Thus, less-dormant grains are able to produce higher GA levels, in addition to the different ABA signaling activities. The observation that ABA inhibits an increase of GA levels in RedlandB2-isolated embryos suggests the existence of a mechanism by which ABA activity interferes with GA metabolism either by inhibiting GA biosynthesis or promoting GA catabolism. A possible link between enhanced ABA signaling and the promotion of GA catabolism activity through transcriptional activation of GA 2-oxidase genes is currently under investigation.

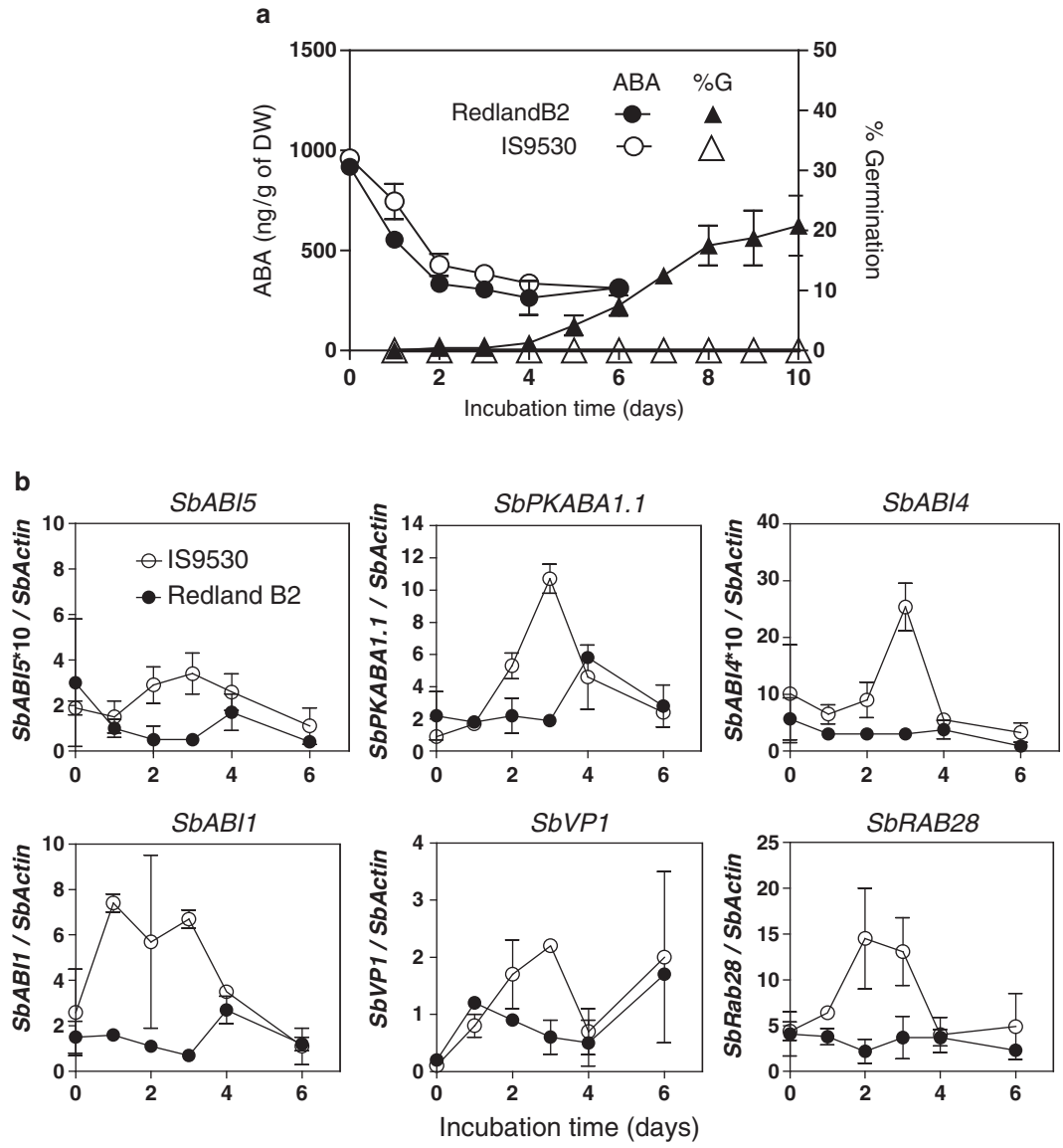

Fig. 4. (**a**) ABA embryonic content and germination response during incubation at 20°C of sorghum caryopses (30 days after pollination) from two inbred lines: sprouting resistant IS9530 and sprouting susceptible RedlandB2. (**b**) Expression (determined by RT-QPCR, relative to SbActin) of sorghum genes encoding homologues for ABA-signaling proteins: transcription factors SbABI4, SbABI5, SbVP1, protein kinase SbPKABA1, and a PP2C-type protein kinase, SbABI1. SbRAB28 was included as an ABA-responsive gene. Each value is the mean of two biological replicates coming from two independent field plots. This figure has been redrawn with data originally published in Rodríguez et al. (34) and was printed with permission of Oxford University Press.

2.3. Environmental Control of Dormancy

The duration of dormancy is determined mainly by the genotype but, as in many other species, it has been known for long that dormancy in grain crops can also be influenced by the environment experienced by the mother plant (44–48; see also 49 for references). Indeed, the effects of the parental environment on seed

dormancy have been reported for a wide range of species (for reviews, see 50, 51). Some well-defined patterns emerge, however, with certain environmental factors tending to have similar effects on different species. For example, low dormancy is generally associated with high temperatures, short days, drought, and nutrient availability during seed development (50, 52–55). The assessment and quantification of these effects might lead to the development of predictive models that could be of great help for reducing the incidence of problems associated with either reduced or persistent dormancy in crop production.

Among the different factors acting on the mother plant, temperature appears to be a major cause of year-to-year variation in grain dormancy of a same genotype. This is the case with barley, and different works have shown that temperature is effective in modulating the pattern of dormancy release only within a sensitivity period during grain filling (46, 56–58). A first predictive model was developed by Reiner and Loch (46), who determined that low temperatures during the first half of grain filling, combined with high temperatures during the second half, result in a low dormancy level of the barley grain and presumably in PHS susceptibility. The authors established a linear relationship between the ratio of the temperatures prevailing at both halves of the filling period and the dormancy level of the grains 3 weeks after harvest. This model was since used by the German malting industry to predict dormancy levels in the malting barley harvest lots. In a more recent work, Rodríguez et al. (58) identified a "time window" within the grain-filling period of cultivar Quilmes Palomar, with sensitivity to temperature for the determination of dormancy. The grain-filling period was divided in discrete intervals within a thermal timescale ranging from anthesis to physiological maturity (430°C days, accumulated over a base temperature of 5.5°C). This "time window" was found to go from 300 to 350°C days after heading. A positive linear relationship was established between the average temperature perceived by the crop during this "time window" and the germination index of the grains 12 days after physiological maturity (Fig. 5a, b). Twelve days after physiological maturity is approximately half way between physiological and harvest maturity; grain germination index measured at this stage is a good estimate of the rate with which the grains are being released from dormancy after physiological maturity. In addition, dormancy release shows a biphasic pattern with a short plateau between 10 and 14 DAP, in which the GI value remains relatively stable (Fig. 5a). According to this model, the higher the temperature experienced during the sensitivity "time window," the faster the rate with which grains are released from dormancy after physiological maturity and, consequently, the lower the dormancy level prior to crop harvest. Such a situation, combined with a forecast of heavy rains for the forthcoming days, implies a risk for the crop and the farmer could decide

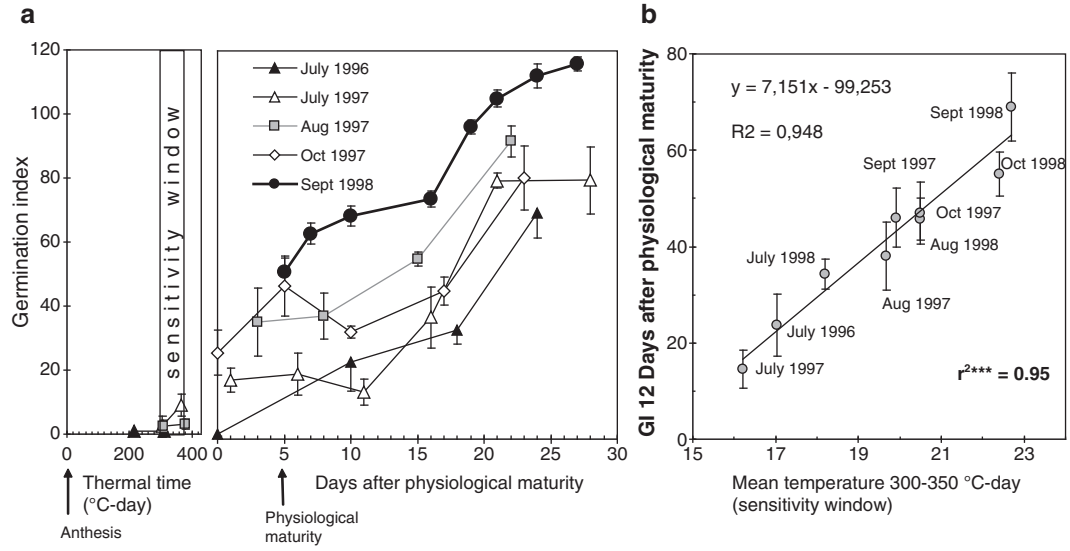

Fig. 5. (**a**) Germination index for barley grains (Quilmes Palomar CV) harvested at different moments after anthesis. A thermal timescale was used to describe the anthesis–physiological maturity period to compare grain-filling stages of plots grown under different thermal environments (base temperature for grain-filling period: 5.5°C). After physiological maturity, a chronological scale was used. Quilmes Palomar CV was sown on different dates during 1996, 1997, and 1998. Each value is the average of three subplots. *Vertical bars* indicate SE. (**b**) A linear equation was used to describe the relationship between the germination index of grains harvested 12 days after physiological maturity and incubated at 20°C and mean air temperature values obtained within the 300–350°C·day interval after anthesis for Q. Palomar cultivar sown on nine dates between 1996 and 1998. Regression equation: GI12DAPM = 7.14 × (Tm300–350) – 98.8 ($r^{2***} = 0.95$, $P < 0.0001$, $n = 9$). *Vertical bars* indicate SE. This figure has been redrawn with data originally published in Rodríguez et al. (58) and was printed with permission of Oxford University Press.

to anticipate the harvest. Conversely, low temperatures experienced by the crop during the sensitivity window would result in a high dormancy level prior to harvest, making the crop resistant to sprouting. This model was successfully validated against data collected from commercial plots 700 km away from the site, where the model was produced (58) and later validated with other commercial cultivars (59). However, it was also noted from validation that temperature explains only one dimension of the observed variability in dormancy. Indeed, there appear to be other unknown factor(s) that are responsible for displacing up or down the relationship between temperature and dormancy (58). Current efforts are directed to identify these factors and to quantify their effects. Notably, water stress promotes dormancy release in several barley cultivars (60), but this effect is noticeable only under moderate temperature conditions; in warmer environments, dormancy is low enough and masks any effect of water stress.

Sunflower is an exception to the general rule that low dormancy is associated with high temperatures experienced during grain filling. In this species, high temperatures during grain development result in an extended period of dormancy (61). In this case, grains that develop at high temperatures require a longer time

of dry after-ripening to acquire the capacity to germinate at low temperatures. Since embryo dormancy is expressed at low temperatures, it might be, then, that high temperatures during grain filling extend the duration of embryo dormancy. Germination at high temperatures, however, was not tested, and therefore it is not possible to say whether the duration of coat-imposed dormancy was also extended by high temperatures during grain development. When plants of sunflower commercial hybrids are exposed to 32°C (control) or 38°C (treated), seeds of the latter exhibit prolonged dormancy. In this case, germination was tested for both achenes (at 25°C) and isolated embryos (at 11°C) (Bodrone, unpublished results). Surprisingly, embryo dormancy was diminished by previous exposure to high temperature while the achenes behaved in the opposite manner. These results indicate that the enhancement of dormancy by high temperature during fruit development is related to coat-imposed dormancy, and the importance of this effect overrides the simultaneous reduction in embryo dormancy. Nevertheless, more trials are still needed to obtain a predictive model as in the case of barley.

3. Challenges Facing Seed Banks

Seed dormancy is usually lost in the process of cultivation. One likely explanation for this phenomenon is the removal of a selection mechanism by the farmer by controlling the time of germination through storing dry seeds and sowing in the soil at the appropriate time of year. However, as pointed out above, strong linkage with other traits can hamper the elimination of dormancy in selection and breeding. This ancient farm management tool has diminished the requirement for seeds from arable crops to sense the correct time of year for germination. Selection for reduced dormancy has occurred traditionally as only those seeds that germinate quickly and contribute to the seed crop are maintained in the farmer's seed bank. In this agricultural context, survival of the fittest seeds is established through a correct dose of dormancy, i.e., low enough to allow untroubled germination but high enough to prevent vivipary. For the correct timing of plant growth and survival in the field, dormancy is no longer required. Dormancy in most cultivated and bred crops is lost, which indicates that it is an expensive trait that is redundant if it no longer provides a function or advantage. On the other hand, this does not apply to many wild species that still require a sensing mechanism to complete germination in the correct time of year. Consequently, when working with wild species, all types of dormancy should be anticipated, particularly when these are members of families that are frequently troubled (3). The presence of dormancy imposes problems in the

correct assessment of seed quality, as it can cause an underestimate of the viability. Knowledge of dormancy is, therefore, important.

Seeds that do not complete germination are either dormant or dead. Usually upon maturity, seeds are shed with high viability. Seed viability decreases depending on the stage of maturation at harvest, the environmental conditions, and the time spent at these conditions. Temperature and water content play an important role in maintaining seed viability. Generally, for orthodox seeds, a lower temperature and lower water content help to keep high seed viability for a longer time. The seed longevity for each species influences how long high viability can be maintained. There are reports of unusually high seed longevity, e.g., for sacred lotus (62). However, many species display a much shorter longevity, and even longevity as short as 1 year has been reported for an orthodox species despite optimal storage conditions (63, 64). The intraspecies variation in genetic background contributes to seed longevity, and it is likely that interspecies genetic variation for these (and other) loci plays a similar role (65). Strongly reduced longevity is also reflected in recalcitrant storage behavior of seeds that are intolerant to desiccation and, therefore, cannot usually be stored for extended periods of time (66). Moist storage of these seeds leads either to germination or death within a fairly short time span. Cryostorage is an alternative to dry storage, but not all recalcitrant seeds are suitable for cryostorage. The need for development of protocols for both freezing, e.g., in the presence of cryoprotectants, and recovery from cryostorage combined with higher costs for cryopreservation makes this technique less suitable for widespread application. Vivipary is an extreme form of reduced longevity, since these seeds display precocious growth while still attached to the mother plant, making storage impossible. This phenomenon is the natural counterpart of dormancy represented in crops through PHS, as pointed out above. Similar to dormancy forming a challenge for seed banking, vivipary does so too. True vivipary has been mainly encountered in species populating shallow marine habitats with a highly reduced chance for offspring to disperse to a patch that is different from the parent's habitat, thus eliminating the need for a selection mechanism, like seed dormancy (67). Although true vivipary is distributed to a relatively limited extent in the plant kingdom, knowledge of the topic not only contributes to the understanding of dormancy, but also PHS in crops.

Seeds stored dry in seed banks inevitably lose viability as a result of storage time and/or suboptimal conditions. However, any dormancy that is present is not usually affected by these conditions. As a result, seed viability undergoes a reduction while dormancy is maintained during dry storage, leading to increasing difficulties with germination upon dry storage. Dry after-ripening releases dormancy of *Arabidopsis* seeds and a molecular pathway has been described (68). While dry after-ripening is typically

displayed at elevated temperatures and water contents, it is not at subzero temperatures and low water contents. Dry after-ripening is also a characteristic of nondeep physiological dormancy, but not of other types of dormancy (69). Therefore, during storage under optimal conditions, dormancy is not expected to be released, and seed quality is expected to decline as a result of a reduction in viability. Seed storage inevitably leads to an increase in problems with germination. A positive viability test becomes progressively harder to accomplish with longer storage time.

The purpose of seed banks is to store seeds with high viability as long as necessary. Testing the quality is essential to serve the higher goal of seed banks, which is to preserve the germplasm for future use. Such would be true for any seed bank; but in the case of wild germplasm seed banks, e.g., the Millennium Seed Bank, an additional goal is to conserve species and protect them from potential extinction through ex situ conservation. Dormancy interferes with viability testing, but it is necessary that the test discriminates between dormancy and viability. Limiting the number of seeds required for a test would also be desired to avoid wasting precious materials. When applying conventional germination tests, large seed numbers are often required for an accurate assessment since different dormancy release factors can be used. Dormancy in situ is often released by environmental factors, since it forms a mechanism that links germination to the correct season. For many species, temperature is an important factor that determines dormancy release. The temperature requirements for dormancy release often differ from those for germination. Seeds of many species with physiological dormancy require either a cold stratification (moist chilling) or warm stratification (warm moist conditions) before they can germinate at intermediate temperatures. Other dormancy-breaking factors include nitrogenous compounds that signal sufficient soil nutrients for subsequent plant growth and smoke that signals opportunities for rejuvenation of vegetation after fire. Thus, finding the optimal environmental conditions requires multiple germination tests and relatively large seed numbers. For conservation purposes, this is not desirable and an alternative test that requires smaller seed numbers would be appealing. Such a test ideally distinguishes dormancy from viability through positive noninterfering test scores for each of these qualities. Not only is a positive test desirable, but speed of the test is an important aspect as well. Dormancy release and completion of germination can take up to a few months, if not longer, in many wild species. A faster test would be of great benefit in those extreme cases. Creating understanding of various aspects of dormancy through fundamental research should eventually lead to a better dormancy test, useful for the agricultural industry and seed banks. Such a test would satisfy the need to accurately determine seed quality.

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