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REVIEW

Acquisition and loss of desiccation tolerance in seeds: from experimental model to biological relevance

Bas J. W. Dekkers · Maria Cecilia D. Costa · Julio Maia · Leónie Bentsink · Wilco Ligterink · Henk W. M. Hilhorst

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Abstract
Main conclusion **Besides being an important model to study desiccation tolerance, the induction of desiccation tolerance in germinated seeds may also play an ecological role in seedling establishment.**

Desiccation tolerance (DT) is the ability of certain organisms to survive extreme water losses without accumulation of lethal damage. This was a key feature in the conquering of dry land and is currently found in all taxa including bacteria, fungi, roundworms and plants. Not surprisingly, studies in various fields have been performed to unravel this intriguing phenomenon. In flowering plants, DT is rare in whole plants (vegetative tissues), yet is common in seeds. In this review, we present our current understanding of the evolution of DT in plants. We focus on the acquisition of DT in seeds and the subsequent loss during and after germination by highlighting and comparing research in two model plants *Medicago truncatula* and *Arabidopsis thaliana*. Finally, we discuss the ability of seeds to re-establish DT during post-germination, the possible ecological meaning of this phenomenon, and the hypothesis that DT, in combination with dormancy, optimizes seedling establishment.

B. J. W. Dekkers, M. C. D. Costa and J. Maia contributed equally to this work.

B. J. W. Dekkers (✉) · M. C. D. Costa · J. Maia · L. Bentsink · W. Ligterink · H. W. M. Hilhorst
Wageningen Seed Lab, Laboratory of Plant Physiology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands
e-mail: bas.dekkers@wur.nl

B. J. W. Dekkers
Department of Molecular Plant Physiology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

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Introduction

Life without water

Water is the most limiting resource in living systems. Water molecules constitute most of the cellular volume of plants, as well as of most other organisms. Due to their properties, water molecules are critical components of chemical reactions and contribute to the stability of proteins, DNA, lipids and membranes. How different organisms survive in the absence or under very limited amounts of water is still an open question. The first observations of such a phenomenon were made by Antonie van Leeuwenhoek, a Dutch tradesman and scientist, who recorded them in his letter 'On certain Animalcules found in the sediment in gutters of the roofs of houses'. In this letter, he describes how certain 'animalcules' (today's microorganisms) would contract themselves into an oval shape when dehydrated and unfold their bodies upon re-watering to regain life (Keilin 1959). He repeated these experiments many times with the same success and even 'animalcules' that were in a dry sediment that was kept in his study for months were competent to regain life. Insightfully, van Leeuwenhoek also hypothesized that if such organisms could stay so long in a dry state and regain life, this should be the way of survival in places where water bodies dry up during summer time or the dry season (e.g. in deserts). He also suggested that these 'animalcules' were likely transported from one place to another in the dried mud adhered to the feet or feathers of aquatic birds (Keilin 1959). These two examples already illustrate important functions, in an

ecological sense and in the life history of an organism, that can be attributed to the possibility of surviving in a dry state (anhydrobiosis) by tolerating desiccation. What van Leeuwenhoek did not mention, however, was that after the 'animalcules' were contracted in a ball shape they had minute amounts of water inside of them and were living in an anhydrobiotic quiescent state which we now know depends on the activation of a series of protective mechanisms including genes, proteins and metabolites, collectively referred to as 'desiccome' (Leprince and Buitink 2010; Potts et al. 2005).

Desiccation tolerance (DT) is defined as the ability of certain organisms to deal with extreme water loss to levels below 0.1 g H_2O per gram dry weight and subsequent rehydration without accumulation of lethal damage (Alpert 2005; Leprince and Buitink 2010; Oliver et al. 2005). Thus, desiccation-tolerant organisms usually do not avoid water losses; instead, they deal with water removal by equipping themselves with protective molecules and by entering into a quiescent, metabolically inactive state (Alpert 2005). To date, a vast body of knowledge has been built around the understanding of anhydrobiosis, or DT (Alpert 2005). Since the late 70s, studies of the physiology, physics, biophysics, and most recently, genetics of dry living systems have burst into bloom both on fundamental and applied aspects (reviewed by Alpert 2006; Leprince and Buitink 2010; Farrant and Moore 2011; Gechev et al. 2012). Since the first observations by van Leeuwenhoek, the ability to withstand desiccation has been found in a wide array of organisms, including bacteria, yeast, fungi, roundworms, arthropods and plants (Alpert 2006).

DT research has diverse (potential) applications, such as improvement of drought tolerance in crop species, improvement of ex situ preservation of germplasm, stabilization of biomolecules and eukaryotic cells, and extension of the shelf-life of vaccines and biological materials, such as blood cells for transfusion and tissues for transplantation (Garwe et al. 2006; Kumar et al. 2013; Loi et al. 2013; Potts et al. 2005; Satpathy et al. 2004). Understanding the mechanisms of DT is an important step towards a multitude of plant and non-plant applications.

In plants, DT is rare in shoots and roots; however, it is common in seeds and pollen. Here, we present our current understanding of the evolution and mechanisms underlying DT in seeds. This review mainly deals with DT sensu stricto, i.e. the ability to survive extreme water loss, and the biological role of DT, which is also dependent on storability in the dry state (seed longevity). Desiccation-tolerant seeds are not by definition long-lived, as seeds acquire longevity in a gradual fashion, late during development (Verdier et al. 2013). However, DT is a prerequisite for seeds to acquire longevity, implying strong interdependency between both traits. We describe the acquisition of DT in seeds and the subsequent loss during and after germination by highlighting and comparing studies in the two model plants *Medicago truncatula* and *Arabidopsis thaliana*. Finally, we discuss the ability of seeds to re-establish DT during and after germination, the possible biological/ecological meaning of this phenomenon, and the hypothesis that DT in germinated seeds, in combination with dormancy, optimizes seedling establishment. Although we will briefly introduce strategies to deal with water limitation as well the challenges of withstanding desiccation, this is not the focus of this review and more details on this topic can be found elsewhere (e.g. Berjak and Pammenter 2008; Dinakar and Bartels 2013; Farrant and Moore 2011; Gechev et al. 2012; Leprince and Buitink 2010; Moore et al. 2008; Verslues and Juenger 2011).

Different ways to deal with water limitation

Dehydration is a common stress and plants evolved in various ways to cope with it. Plants differ in their level of tolerance to dehydration and can be roughly divided into extremely tolerant, moderately tolerant and lowly tolerant (Fig. 1). Extremely tolerant plants are desiccation tolerant and tolerate nearly complete dehydration. Plants with this extreme capacity are also known as resurrection plants (Gaff 1971). These desiccation-tolerant plants do not avoid water loss, but protect themselves against water removal by shutting down metabolism and activating protective mechanisms (reviewed by Farrant et al. 2007). Unlike resurrection plants, plants with a moderate tolerance remain hydrated, are metabolically active, and use the available water efficiently under water-restricted conditions (Verslues and Juenger 2011). Many plants, however, have a low tolerance to water loss and depend for their survival on drought tolerance mechanisms (Verslues and Juenger 2011). Drought tolerance (which is different from desiccation tolerance) denotes the capacity to tolerate moderate dehydration down to $\sim$0.3 g H_2O per gram dry weight. Usually, drought refers to a temporary type of stress which is dealt with via the continuation of most of the physiological functions of the organism while preventing water loss, for example by limiting growth, stomata closure, and/or the accumulation of solutes (Moore et al. 2008). If the drought stress is too severe or the period of drought is too long, these drought-tolerant organisms will eventually perish.

Challenges and mechanisms to deal with desiccation

Cells that undergo desiccation have to cope with significant changes in turgor pressure, which generate cell shrinkage

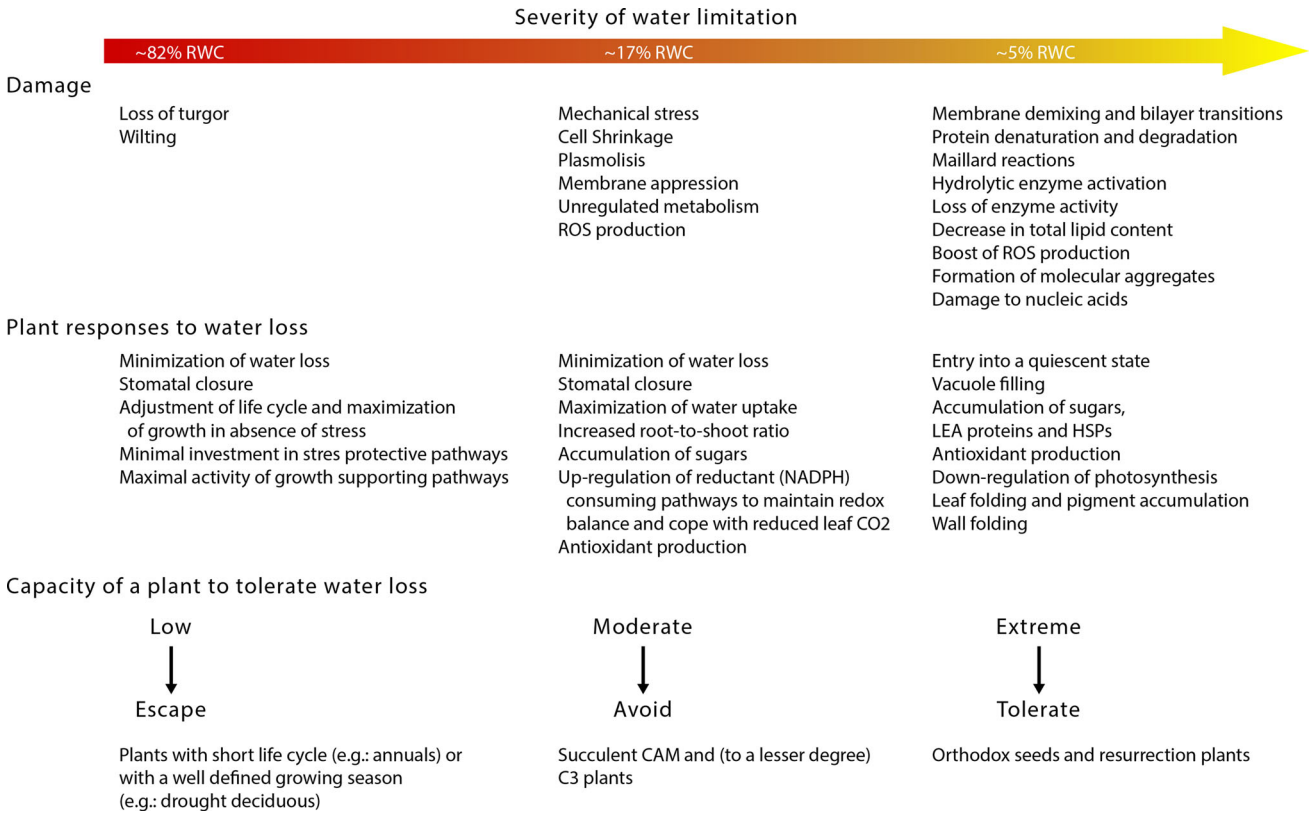


Fig. 1 Plant responses and adaptation strategies to different levels of water limitation. Plant adaptation strategies to water limitation can range from escape (e.g.: annuals and drought deciduous) to tolerance (orthodox seeds and resurrection plants) (Verslues and Juenger 2011)

and mechanical stress (Farrant 2000; Scoffoni et al. 2014). Other challenges faced by desiccating cells are the prevention of denaturation of large molecules, loss of enzyme activity, formation of molecular aggregates, and cellular damage caused by reactive oxygen species (ROS) (reviewed by Kranner and Birtic 2005). Protective measures can either be (partly) present or induced by signalling and activation of transcription factors (Gechev et al. 2012). The effects of desiccation and the stresses it causes are as multifaceted as the response to desiccation. The response to desiccation comprises a complex array of protective mechanisms against mechanical stress caused by water removal, including modification of the cell wall and membranes, of the cytoskeleton, and of chromatin compaction. To prevent damage by ROS, metabolic activity is reduced and protective molecules (antioxidants, ROS scavengers) are produced. Additionally, deposition of insoluble storage compounds and non-reducing sugars, late embryogenesis abundant proteins (LEAs) and heat shock proteins (HSPs) can act as fillers and are thought to prevent protein aggregation by acting as molecular shields (for protective mechanisms see, e.g. Berjak and Pammenter 2008; Dinakar and Bartels 2013; Farrant et al. 2007; Farrant and Moore 2011; Gechev et al. 2012; Hoekstra et al. 2001; Terrasson et al. 2013; van Zanten et al. 2011).

The role of DT in the evolution of plants

Terrestrial organisms are constantly confronted with the desiccation stress imposed by air dryness. Consequently, during the evolution of plant life on land, adaptations that allow surviving and/or avoiding desiccation were required (Oliver et al. 2005). The ability to tolerate near complete desiccation was an important evolutionary step that played a key role in dry land colonization. Likely, DT was primitively present in chlorophytic algae that were precursors of the basal land plants (Bryophytes, i.e. liverworts, mosses and hornworts) (Farrant and Moore 2011). Bryophytes evolved mechanisms to limit water loss (cuticle and/or stomata) but the majority of bryophyte species are desiccation tolerant, which is an essential feature for life in habitats where water is not always available (Proctor et al. 2007; Proctor and Pence 2002). Interestingly, genes responsible for synthesis and signalling of the dehydration stress hormone abscisic acid (ABA) are found in basal land plants and were likely important in the acquisition of DT and drought tolerance during plant evolution (Hauser et al. 2011; Umezawa et al. 2010). For example, a pre-treatment of the basal land plant *Marchantia polymorpha* with ABA induced protectants and resulted in morphological alterations which enhanced survival after desiccation (Akter

et al. 2014). Additionally, ABA was found to regulate stomatal aperture in the model moss *Physcomitrella patens* and the lycophyte *Selaginella uncinata* (Chater et al. 2011; Ruszala et al. 2011). Further, in *P. patens*, knockouts of the *ABA INSENSITIVE 3* (*ABI3*) gene or in both class A PP2Cs (orthologs to the Arabidopsis PP2Cs that regulate ABA signalling) were shown to affect the acquisition of DT (Khandelwal et al. 2010; Komatsu et al. 2013). In contrast to the Bryophytes, higher (vascular) plants rarely have desiccation-tolerant vegetative tissues. Currently, only some 330 resurrection plant species are known (<0.15 % of the total number vascular plant species), which have been reported as desiccation tolerant in their vegetative parts (Proctor and Pence 2002).

In gymnosperms (e.g. conifers), DT is completely absent from vegetative tissues, which could be explained by the ecophysiological constraint that excludes trees from being desiccation tolerant (Lüttge et al. 2011; Oliver et al. 2000). Although DT is rare in vegetative organs of angiosperms, it is present in most seeds (~95 %) and pollen (~87 %) of the investigated spermatophyte species (Gaff and Oliver 2013). Basal lineages of angiosperms do not contain any desiccation-tolerant plants (with DT in vegetative tissues), but they are found in later lineages, suggesting that DT in vegetative tissues was lost early during plant evolution and regained later. In fact, DT re-evolved multiple times (at least 10) in the history of angiosperms, mostly within herbaceous lineages (Oliver et al. 2000, 2005; Porembski 2011). It is hypothesized that in these plants the activation of already present DT mechanisms (from seeds and/or pollen) was the source of genetic reprogramming for DT acquisition rather than effective adaptation of abiotic stress responses (Farrant and Moore 2011; Gaff and Oliver 2013; Illing et al. 2005; Oliver et al. 2000).

In Spermatophytes, DT is mainly confined to seeds and pollen

It has been suggested that the rarity of DT in vegetative tissues of seed plants is related to the trade-off between DT and growth rate. Alpert (2006) discussed this trade-off along three lines of evidence: (1) from a genetic and evolutionary point of view, (2) from ecological studies that are consistent with the idea that desiccation-tolerant species are poor competitors, and (3) from the hypothesis that DT mechanisms constrain growth (for details see Alpert (2006) and references therein). This trade-off is visible, for example, in the moss *P. patens*. When Komatsu et al. (2013) disabled two PP2Cs that are present in the *P. patens* genome, the moss became desiccation tolerant, indicating that the PP2Cs acted as negative regulators of an intrinsically present DT pathway. Interestingly, this double mutant was compromised in growth. Based on their data, the authors hypothesized that the PP2Cs were recruited to inhibit the DT response, allowing cells to relocate the energy once spent for DT and use it for growth and reproduction. ABA acts as environmental response signal that relieves this inhibition, releasing DT on demand upon dehydration stress.

It is likely that, as plants evolved to fill diverse niches available on dry land, the selection pressure for faster growth, plant height and dry-mass productivity (likely in combination with other water-related adaptations such as the formation of cuticle and stomata, and modifications of the root and vascular system) favoured the loss of DT in vegetative tissues (Alpert 2005, 2006; Illing et al. 2005; Oliver et al. 2000, 2005). Although DT was lost from vegetative tissues, it was retained in reproductive propagules (seeds and pollen) (Alpert 2006; Gaff and Oliver 2013; Oliver et al. 2000). The confinement of DT to seeds meant an important advantage, as it enabled plants to evade a stressful season or drought period through a short life cycle or seasonal growth and survive the unfavourable period by wrapping the next generation in a stress-tolerant seed.

Two traits are important for such a strategy, i.e. seed dormancy and DT. Dormancy is an important mechanism for controlling the timing of germination (to prevent plant growth during an unfavourable season/period) and increasing time for dispersal (Baskin and Baskin 2014; Bewley et al. 2013; Finch-Savage and Leubner-Metzger 2006). In addition, by dispersing seeds with different levels of dormancy, a plant is able to spread germination of its offspring in time, reducing the risk of losing an entire generation by a catastrophic event (Hilhorst 2007). At the same time, desiccation-tolerant seeds can remain in the soil seed bank and survive a wide range of environmental conditions. Together with dormancy, DT represents an important trait for seed survival, allowing seeds to withstand severe dehydration, as air dry tissues are very stable and able to tolerate a wide range of stressful conditions that would be detrimental to adult plants, such as extreme temperatures (Fenner and Thompson 2005; Gaff and Oliver 2013). There is evidence that ancestral seeds largely possessed morphophysiological dormancy (Willis et al. 2014). Whether or not the ancestral state of seeds had DT is unclear and both possibilities have been put forward (discussed by Tweddle et al. (2003)). Both traits are related to seed survival and are acquired during seed maturation, and are controlled by the plant hormone ABA. Further, the combination of dormancy and desiccation sensitivity in seeds is counterintuitive (although exceptions exist), since such seeds are generally badly storable and may die before they are able to germinate. Therefore, it seems conceivable that DT and dormancy may have evolved simultaneously. Whatever the case may be, the seed habit is an evolutionary

success, to which both DT and dormancy are important contributors, as shown by the tremendous increase and diversification within seeded plants during the Cretaceous era (144-65 MY ago) and their dominance in the world's vegetation today (Linkies et al. 2010; Steeves 1983).

DT in seeds: acquisition, loss and re-establishment

The vast majority of angiosperm species produce seeds that tolerate desiccation and long-term dry storage and are termed 'orthodox' (Roberts 1973). Our most cultivated crops, such as rice, wheat, corn, barley, soybean and beans, produce desiccation-tolerant seeds. However, a significant number of wild species, particularly from wet climate areas, produce desiccation-sensitive seeds. Desiccation-sensitive or 'recalcitrant' seeds do not tolerate drying and are hardly storable (Roberts 1973). Consequently, the use and conservation of recalcitrant-seeded species, which include some economically important crops, remain a challenge (Berjak and Pammenter 2013).

Acquisition of desiccation tolerance during seed development

Seed development consists of two main phases, i.e. embryogenesis and maturation (Bewley et al. 2013). Embryogenesis comprises tissue specification and patterning, which is obtained via a well-organized series of cell divisions and cell differentiation. After its completion, seed development switches to the maturation phase that can be divided into early and late maturation. During early maturation, the seed acquires DT and accumulates storage compounds, including proteins, oils and carbohydrates (Vertucci and Farrant 1995). Storage of proteins in protein storage vacuoles, and lipids in oil bodies fill up the cells and offer resistance against cellular collapse upon drying (Leprince et al. 1998).

During late maturation, seeds dry out while considerable changes occur at both transcriptome and metabolome levels (Fait et al. 2006; Angelovici et al. 2010). Changes at this stage coincide with a gradual increase in seed longevity (Chatelain et al. 2012; Verdier et al. 2013). In this period, LEA proteins and non-reducing sugars, such as sucrose and raffinose family oligosaccharides, have been shown to accumulate to relatively high levels in Arabidopsis seeds (Angelovici et al. 2010; Baud et al. 2002; Hoekstra et al. 2001). LEA proteins may protect cellular structures, membranes and other proteins by acting as a hydration buffer, sequestering ions and renaturing unfolded proteins (reviewed by Tunnacliffe and Wise 2007). Non-reducing sugars fill the free volume between large molecules, created during dehydration, allowing less molecular mobility in the matrix (Buitink and Leprince 2004, 2008). Other structural adaptations that occur during this stage are chromatin compaction and nuclear size reduction (the latter is also observed in dried leaves of the resurrection plant *Craterostigma plantagineum*), which are reversed during germination (van Zanten et al. 2011).

Furthermore, metabolic activity is reduced towards the end of seed maturation, which minimizes the production of ROS (Pammenter and Berjak 1999). The excessive production of ROS and a limited action of antioxidant defences can induce oxidative stress. To alleviate it, many antioxidants such as ascorbate, glutathione, polyols, tocopherols, quinones, flavonoids and phenolics are believed to operate (Kranner and Birtic 2005). However, to effectively limit the extent of ROS production, photosynthesis has to be down-regulated (Farrant 2000). In seeds, the photosynthetic apparatus is usually dismantled during maturation (Bewley et al. 2013).

The expression of such protective mechanisms can be observed during both early and late seed maturation. A correct execution of the seed maturation programme is dependent on a transcriptional network referred to as the LAFL developmental network [reviewed by Jia et al. (2014)]. This transcriptional network consists of master regulators that interact in a complex manner and include *LEAFY COTYLEDON* (*LEC) 1*, *LEC2*, *FUSCA* (*FUS*) *3* and *ABI3*. *LEC1* is an HAP3 family CCAAT-box binding factor, whereas *LEC2*, *FUS3* and *ABI3* are B3 domain-containing transcription factors (Giraudat et al. 1992; Lotan et al. 1998; Luerssen et al. 1998; Stone et al. 2001). Mutations in any of these genes result in severe seed maturation phenotypes. The severe *abi3-5* mutant is defective in chromatin compaction and nuclear size reduction (van Zanten et al. 2011), while a lower expression of LEA proteins has been reported for *Mtabi3* mutants (Delahaie et al. 2013). Chlorophyll breakdown is impaired in the seed maturation mutants, particularly the severe alleles of *abi3* in which chlorophyll degradation does not take place and mature seed possesses green cotyledons (Delmas et al. 2013; Nambara et al. 1992, 1995; Ooms et al. 1993). A lack of chlorophyll degradation was also found in two *Mtabi3* mutants in Medicago (Delahaie et al. 2013). These seed maturation mutants failed to acquire DT and were barely storable. For example, *abi3-5* and *lec1-3* were shown to have a severely reduced number of germinating seeds at harvest (due to a lack of DT) and displayed a strongly reduced longevity (Sugliani et al. 2009). Besides the important roles of *ABI3* and *LEC1,* the strong seed maturation phenotypes of these mutant alleles were shown to be affected by the genetic background. Introgressions of the accessions of Seis am Schlern (Sei-0) and Shahdara (Sha-0) partially suppressed the *abi3-5* and *lec1-3* phenotypes and thus allowed the identification of genetic loci that

could improve seed longevity (Sugliani et al. 2009). It is not possible to establish such improved longevity in seeds that do not tolerate desiccation, indicating that these loci also control the acquisition of DT (at least to a certain extent) in these severe maturation mutants. Thus, the identified loci are important for the acquisition of DT as well as seed longevity.

A window of desiccation tolerance in germinated seeds

DT is usually fully established just before the drying phase, towards the end of seed maturation and is generally lost during germination (Bewley et al. 2013). Drying back of seeds at different intervals along the germination–time curve has shown that seeds were killed already before or quickly after visible germination (Buitink et al. 2003; Daws et al. 2007; Lin et al. 1998; Maia et al. 2011; Vertucci and Farrant 1995). It should be noted that these analyses were based on fast drying treatments in which seeds lose most of their water within ~2 h. However, germinated seeds have actually a longer window in which they tolerate desiccation. The existence of this window can be observed when a mild osmotic stress (by a polyethylene glycol (PEG) treatment) is applied before fast drying. This has been demonstrated for a number of species, including *Cucumis sativus*, *Impatiens walleriana*, Medicago, *Tabebuia impetiginosa* (Brazilian tree species), and recently, Arabidopsis (Bruggink and van der Toorn 1995; Buitink et al. 2003; Maia et al. 2011, 2014; Vieira et al. 2010). For this reason, the mild osmotic stress is said to re-induce DT in germinated seeds. Nevertheless, at a certain developmental stage after germination, orthodox seeds completely lose the ability to tolerate extreme drying (even after application of a mild osmotic stress) and become desiccation sensitive (Fig. 2). Thus, this ability is strictly dependent on the developmental stage of the germinated seeds (Buitink et al. 2003; Leprince et al. 2000; Maia et al. 2011; Vieira et al. 2010). For example, Medicago seeds with radicle length up to 1 mm survive fast drying. When the radicles are up to 2.7 mm in length, their ability to become fully desiccation tolerant is dependent on a mild osmotic stress treatment using PEG (Buitink et al. 2003).

To determine the developmental window in which germinated Arabidopsis seeds could be triggered to re-induce DT, four clearly distinct developmental stages were defined (Maia et al. 2011, 2014): (I) at testa rupture, (II) at radicle protrusion, (III) with a primary root of 0.3–0.5 mm in length, and (IV) at the appearance of the first root hairs (Fig. 2). In the first three stages, seeds were able to withstand desiccation after a PEG treatment and seedling survival rates were close to 100 %. At stage IV, this number dropped to ~20–40 % indicating that this ability was largely lost at this stage. Another noticeable feature was that different seed parts displayed variable levels of re-induction of DT. In Arabidopsis, the cotyledons were the most tolerant tissue followed by hypocotyls and roots. Also in Medicago, the cotyledons are more tolerant to desiccation as compared to the radicles (Buitink et al. 2003).

These experiments revealed a developmental window in which germinated seeds have the capacity to tolerate desiccation, helping to differentiate between stages in which desiccation is tolerated (the DT window) or is completely lost (as is shown for Arabidopsis in Fig. 2). This DT window represents an extreme stress tolerance mechanism that could be ecologically relevant for seedling establishment. The acquisition of DT is an active process, which takes some time to become effective. Interestingly, the observation that DT can be re-induced in germinated seeds by PEG treatment resembles observations made in mosses. Of 62 moss species studied, 22 % showed a DT phenotype, but this was increased to 71 % in case these same moss species were hardened before desiccation (Gaff and Oliver 2013).

The loss of DT has been correlated with the cell cycle (Boubriak et al. 2000; Faria et al. 2005; Osborne and Boubriak 1994). The switch from desiccation tolerant to desiccation sensitive coincides with radicle cells entering the G_2 phase of the cell cycle, which contains the double amount of DNA (Faria et al. 2005; Saracco et al. 1995). This could potentially affect the induction of DT. In tomato seeds, DNA synthesis precedes germination, which does not support such correlation. Thus, whether DNA duplication or cell cycle activation is the key trigger for the loss of DT remains questionable. In tomato for example, seed priming results in DNA synthesis in non-germinated seeds (Bino et al. 1992) and reduction of seed longevity, but does not lead to loss of DT. Furthermore, Arabidopsis mutants affected in DNA repair (DNA ligase enzymes, *atlig4* and *atlig6*) displayed reduced seed longevity (Waterworth et al. 2010). Also microtubular dynamics and integrity are affected by dehydration (Sargent et al. 1981) and could be related to the loss of DT. Interestingly, the ability of yeast to tolerate desiccation is related to growth rate of the culture (and thus cell division). Within the cell population that is growing exponentially, only one in a million survives desiccation whereas 1 in 5 survives desiccation in the stationary phase (Calahan et al. 2011; Welch et al. 2013). Survival can be enhanced in exponentially growing cells by exposure to heat stress or nutrient limitation before desiccation. These treatments alter growth rate which is probably correlated with the reduction of 60S ribosomal subunit biogenesis (Welch et al. 2013). Yeast mutants that are affected in 60S biogenesis have an increased DT. Whether DT in plants is also regulated at the level of ribosomal subunit biogenesis remains to be shown.

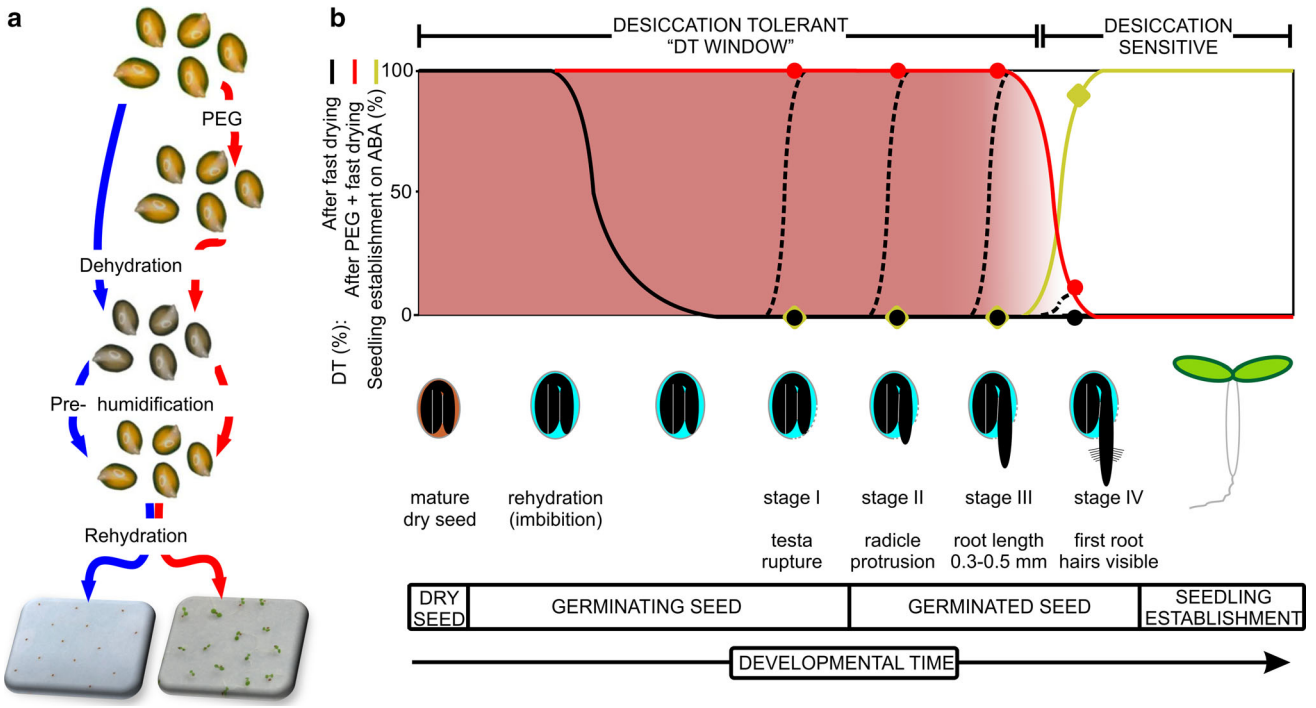


Fig. 2 Loss of DT in germinating seeds. **a** Schematic representation of the re-induction of DT by PEG treatment. When germinated seeds at the stage of radicle protrusion (stage II) are fast dried (*blue arrows*), they do not survive, as shown by the lack of seedling growth after pre-humidification (to reduce imbibitional damage) and rehydration. In case, the drying treatment is preceded by a 3d PEG treatment, seeds survive, as shown by further growth and seedling establishment. **b** Schematic representation of the DT window. Arabidopsis seeds at testa rupture stage (stage I) do not survive fast desiccation treatment, and resistance against this treatment is already lost earlier during imbibition (*black solid line*). However, a PEG treatment is able to re-induce tolerance against this desiccation treatment in stages I–III but this is largely lost at stage IV (as indicated by the *dashed lines*). The *red line* shows DT after PEG treatment and indicates the DT window after germination. This window strongly correlates with ABA sensitivity (*green line*) which is also restricted to a limited time window after germination (Lopez-Molina et al. 2001; Maia et al. 2014)

The ability to withstand drying coincides with structural changes in chromatin compaction and nuclear size reduction (van Zanten et al. 2011) and these structural adaptations are not reverted upon rehydration alone. The nuclear size in rehydrated dormant seeds remains small. In non-dormant seeds, the reversion to a larger nuclear size seems related to germination and the largest increase is observed between 2 and 3 days after sowing (van Zanten et al. 2011). Although several processes are correlated with the loss of DT in germinated seeds, its genetic regulation and the molecular mechanisms involved are still poorly understood.

Regulation of DT in seeds

Transcriptional regulation of desiccation tolerance

The re-induction of DT in Medicago radicles was studied using transcriptome profiling. Three-millimetre-long radicles are sensitive to fast drying but this can be reverted by a PEG treatment. A time course of gene expression profiling revealed ~1300 differentially expressed genes during the re-induction of DT in PEG-treated radicles of Medicago (Buitink et al. 2006). Most of these genes (~720) were down-regulated and related to cell cycle, biogenesis, and primary metabolism. Sucrose accumulates in desiccation-tolerant radicles and a combination of transcriptome and metabolite measurements indicates that sucrose is produced by mobilizing lipids and starch (Buitink et al. 2006). Also, LEAs are rapidly induced transcriptionally and their accumulation was confirmed by analysis of the heat stable proteome (Boudet et al. 2006). Interestingly, a significant overlap was found between genes that are differentially expressed during seed maturation (between 14 and 20 DAP) and during the re-induction of DT in radicles (Buitink et al. 2006; Terrasson et al. 2013). Based on these transcriptome data, it appeared that during the re-induction of DT, germinated seeds (partially) revert to an earlier developmental stage (Buitink et al. 2006). In Arabidopsis, the top 50 DT up-regulated genes during re-induction of DT were down-regulated during germination, while the top 50 DT down-regulated showed generally an increased expression during germination, supporting this 'reversion' theory (Maia et al. 2011).

Also other transcriptome data show that the largest set of differentially expressed genes is down-regulated during re-induction of DT (2,829 down vs 740 up, Terrasson et al. (2013) and 414 down vs 263 up, Maia et al. (2011) for Medicago and Arabidopsis, respectively). This might indicate the importance of shutting down certain processes as part of the ability to induce DT. Although the specific overrepresented GO terms differed between both species, the genes in both down-regulated sets related to cellular metabolic processes, biogenesis and growth. Additionally, in Arabidopsis also photosynthesis-related genes were down-regulated. The up-regulated gene classes revealed a larger overlap between the GO terms found in Medicago and Arabidopsis, and include response to stress, response to abiotic stimulus, response to water deprivation, response to abscisic acid stimulus, lipid localisation, seed development, and embryonic development ending in seed dormancy (Maia et al. 2011; Terrasson et al. 2013).

Another powerful approach to obtain insights into the regulation and downstream processes involved in DT was taken by the construction of a coexpression network (Verdier et al. 2013). In this network, several modules that linked gene expression to different processes of seed development, including embryogenesis, seed filling, DT, and final maturation drying, were identified. The DT module contained genes related to stress responses, LEAs and ABA-induced genes. Another gene regulatory network containing 22 seed-specific transcription factors and seed-specific probe sets indicated genes that correlate strongly with DT, LEAs and longevity (Verdier et al. 2013). Four transcription factors, *MtABI3* (Giraudat et al. 1992; Koornneef et al. 1984; McCarty et al. 1989), *MtABI4* (Finkelstein 1994; Finkelstein et al. 1998), *MtABI5* (Finkelstein 1994; Finkelstein and Lynch 2000; Lopez-Molina and Chua 2000) and an *MtAP2/EREBP* gene, were found to be highly connected with DT genes, and are therefore good candidates as DT regulators. *MtABI3* is one of the most connected transcription factors. Interestingly, a large proportion of the genes connected to *MtABI3* in the network were identified as direct targets of ABI3 in Arabidopsis (Mönke et al. 2012; Verdier et al. 2013).

Role for ABA signalling in desiccation tolerance

The phytohormone ABA is a central regulator of plant development and responses to environmental stresses. Currently, over 100 loci have been identified as being involved in ABA perception and downstream signalling (Cutler et al. 2010). ABA controls seed developmental processes, including accumulation of food reserves, as well as acquisition of dormancy and DT (Kermode and Finch-Savage 2002). In Arabidopsis, DT is acquired slightly later during seed maturation in the ABA-deficient mutant *aba1-1* as compared to the wild type (Koornneef et al. 1989). It is likely that *aba* mutants in Arabidopsis are not complete null mutants, as discussed by Barrero et al. (2005) based on their analysis of several mutant alleles of *ABA1* as well as double mutant analysis with other ABA-deficient mutants (*aba2* and *aba3*). Therefore, they suggested that an alternative route might produce minor amounts of ABA (Barrero et al. 2005). Possibly, such low levels of ABA are enough to evoke DT. This is supported by the finding that seed-specific expression of an ABA antibody in tobacco (*Nicotiana tabacum*) resulted in much stronger seed phenotypes including desiccation-sensitive seeds (Phillips et al. 1997). In Arabidopsis, the triple mutant of SnRK2 genes (*snrk2.2/3/6*) is severely distorted in ABA signalling. Although these mutant seeds germinate just after harvest, following drying, their germinability is already reduced after 1 week and lost after 2 weeks of dry storage, indicating a severe seed longevity lesion (Nakashima et al. 2009).

As mentioned before, in germinated seeds, DT can be re-induced by a mild osmotic treatment using PEG. This response can be mimicked by applying ABA instead, as has been shown in *C. sativus*, Medicago and Arabidopsis (Buitink et al. 2003; Lin et al. 1998; Maia et al. 2014). In germinated seeds, the induction of DT depends on ABA. Fluridone (an ABA biosynthesis inhibitor) treatment of Medicago radicles and the use of the Arabidopsis *aba2-1* mutant (disrupted in ABA biosynthesis) compromised the re-induction of DT (Buitink et al. 2003; Maia et al. 2014). In germinated Arabidopsis seeds the osmotic treatment did not appear to change the ABA content of the seeds, but, likely, influenced sensitivity to ABA. Interestingly, two ABA receptors (i.e. *PYL7* and *PYL9*) were highly induced upon PEG treatment (Maia et al. 2014).

In agreement with a role of ABA, several mutants in ABA signalling were shown to be compromised in their ability to re-induce DT in germinated seeds. For example, two *abi5* mutants, *Mtabi5-1* and *Mtabi5-2*, lacked the ability to re-induce DT in Medicago radicles by osmotic stress (Terrasson et al. 2013). Maia et al. (2014) reported phenotypes in the re-induction of DT in germinated seeds for several mutants in ABA signalling, such as *abi3-8*, *abi3-9*, *abi4-3* and *abi5-7*. In spite of being compromised for ABA sensitivity or synthesis, all of the mutants tested produced desiccation-tolerant seeds at the end of seed maturation. However, all five mutants showed a reduced capacity to re-induce DT in germinated seeds. These observations imply that the acquisition of DT during seed development is different from the re-induction of DT in geminated seeds. There are two hypotheses to explain these differences. First, except for ABI3 (that is clearly involved in both) largely distinct pathways are involved in the induction of DT during seed development and following

germination. Second, the pathways that induce DT involve ABA, *ABI4* and *ABI5*, but their function remains unnoticed when testing the mutant alleles, because of additional redundant factors present during seed development but absent after germination. In the gene regulatory network presented by Verdier et al. (2013), *ABI3*, *ABI4* and *ABI5* are strongly linked to DT genes supporting the latter hypothesis.

Lopez-Molina et al. (2001) have identified a small developmental window of ABA sensitivity after germination, in which seedling growth could be arrested. Such arrested seedlings were resistant to a drying treatment. It has been suggested that during this phase, the young plantlets monitor the environmental osmotic status. In case of dehydration stress, ABA, via ABI3 and ABI5, induces a developmental arrest of germinated embryos, thereby protecting young seedlings from the loss of water (Lopez-Molina et al. 2001, 2002). Interestingly, the DT window overlaps with this ABA sensitivity window: DT could be re-induced in Arabidopsis seeds at developmental stages I–III, when ABA sensitivity was high, whereas low ABA sensitivity levels at stage IV correlated with a reduced ability to re-induce DT (Fig. 2) (Maia et al. 2014). This supports the hypothesis above and confirms the importance of ABA in DT induction (Buitink et al. 2003; Maia et al. 2014; Terrasson et al. 2013; Verdier et al. 2013). Thus, seedling establishment might not only be regulated by control of germination, but by an additional post-germination checkpoint as well (Lopez-Molina et al. 2001, 2002).

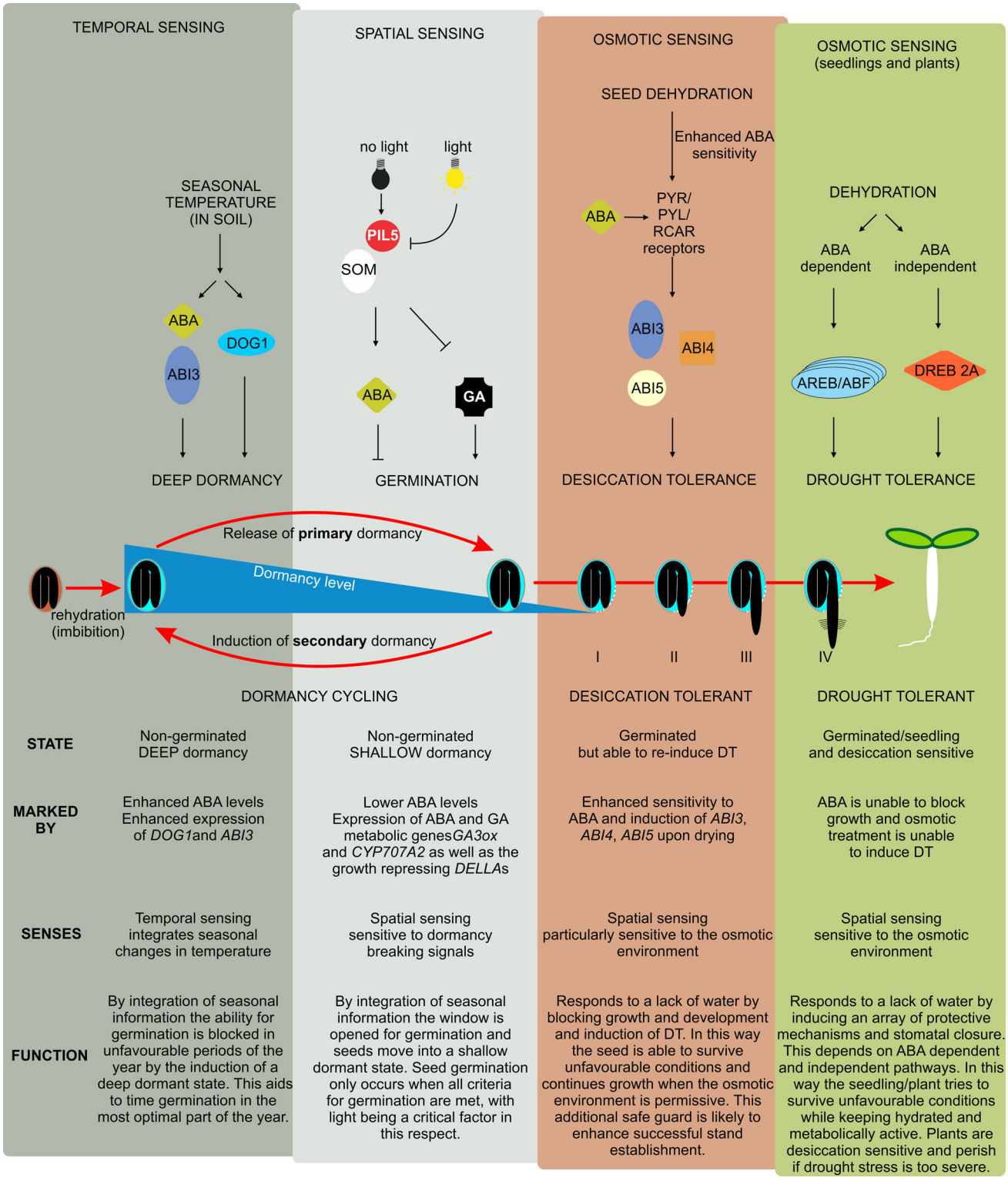
A role for sugar signalling in desiccation tolerance?

In addition to the role of sugars as protectants, high exogenous levels of sugars are able to arrest seedling establishment in an ABA-dependent manner (Dekkers and Smeekens 2007). Both ABA- and sugar-arrested seedlings have an increased resistance to a drying treatment (Dekkers et al. 2008; Lopez-Molina et al. 2001). Germinated seeds are sensitive to sugar-induced arrest during an approximately 2- to 3-day time window following germination (Gibson et al. 2001). In this developmental window, embryonic marker genes like *ABI3*, *ABI5* and several *LEA* genes can be induced by sugars (Dekkers et al. 2008). In the liverwort *M. polymorpha,* ABA-induced survival after a desiccation treatment was strongly promoted in the presence of sugars (Akter et al. 2014). The addition of glucosamine (an inhibitor of hexokinase) early during the PEG treatment negatively affected the ability to re-induce DT in Medicago and *C. sativus* radicles (Leprince et al. 2004). The HEXOKINASE 1 protein in Arabidopsis was shown to possess a dual function, acting in signalling and as an enzyme (Moore et al. 2003). Currently, it is unclear whether the effect of glucosamine in the re-induction of DT is caused by the repression of enzymatic activity or sugar signalling. Thus, possibly, sugars play a dual role by acting both as structural protectants (as mentioned before) and signalling intermediates. With having Arabidopsis as an experimental model for studies on the re-induction of DT, all the genetic resources available for this species can be used to address these questions.

Combinatorial roles of dormancy and the DT window in optimizing seedling establishment?

Obviously, the establishment of seedlings is strongly affected by the control of germination via dormancy mechanisms that control when, and the conditions in which, germination occurs. The DT/ABA window might represent an additional layer of control. It capacitates germinated seeds to arrest development and survive a period of water limitation by complete desiccation and thereby optimizes seedling establishment. Thus, seedling establishment is regulated at least at three different levels: at the germination level via temporal and spatial sensing and at the level of the ABA/DT window (see Fig. 3). These regulation mechanisms are discussed in more detail below.

To achieve germination in the proper period of the year and under favourable conditions, seeds sense their environment at two levels, which are referred to as *temporal* and *spatial sensing*. Within a year, seeds can alternate between the deep and shallow dormancy which, basically, opens and closes the window in which seed germination can occur (Bouwmeester and Karssen 1993; Derkx and Karssen 1994; Footitt et al. 2011; Hilhorst and Karssen 1988). Seeds achieve this by integrating slow seasonal changes, via temporal sensing of, most likely, temperature (Bouwmeester and Karssen 1993), which sets the depth of dormancy. During shallow dormancy, when seeds are able to germinate if the environmental conditions permit it, seeds can respond fast (by spatial sensing) to favourable germination conditions (Footitt et al. 2011). The presence of light and its spectrum are critical signals in this respect and likely act as a gap-detection mechanism or as sensor to ensure that the seed, once germinated, is close enough to the soil surface for successful seedling establishment (Bewley et al. 2013; Footitt et al. 2011; Silvertown 1980). In the absence of light, the activity of SOMNUS (SOM) and of the bHLH transcription factor PIF3-LIKE (PIL) 5 inhibits germination (Kim et al. 2008; Oh et al. 2004). These proteins affect genes involved in ABA and gibberellin metabolism and signalling and both *som* and *pil5* mutants germinate in the absence of light (Kim et al. 2008; Oh et al. 2006). If during this stage the proper environmental conditions are not met to complete germination, the



window for germination closes by the induction of a deeper state of (secondary) dormancy through temporal sensing (Fig. 3). However, light relieves PIL5 repression which interrupts the germination repressing circuitry and thereby promotes germination (see Fig. 3 for a simplified scheme).

Dormancy, through temporal sensing, ensures germination in the right time of the year and, through spatial sensing, ensures that germination only occurs under the right circumstances. Although these are very important mechanisms, the decision (upon spatial sensing) of a seed

◀**Fig. 3** Seedling establishment is regulated on different levels by seed dormancy and DT. Low temperatures over winter (by temporal sensing of seasonal changes) induce strong dormancy and close the germination window in Cvi seeds (Footitt et al. 2011). This is linked with enhanced levels of ABA, and higher expression of *ABI3* and *DOG1*. Release of primary dormancy occurs gradually and results in a more shallow state of dormancy. At different stages, seeds become sensitive to dormancy breaking (Finch-Savage et al. 2007; Finch-Savage and Leubner-Metzger 2006). Sensing of environmental conditions during this shallow dormant state is referred to as spatial sensing in which light is a critical factor for germination (Footitt et al. 2011). In the absence of light, germination is inhibited by the activity of *PIL5* (Oh et al. 2004). *PIL5* stimulates the expression of a CCCH-type zinc finger gene called *SOMNUS* (*SOM*) (Kim et al. 2008). Upon a light signal *PIL5* repression is relieved which allows seed germination to occur. After germination, in stages I–III, seeds are able to survive complete dehydration which represents an important mechanism to an otherwise deadly stress and likely helps to optimize successful seedling establishment. The induction of DT at this stage relies on ABA and three transcription factors that play an important role in ABA signalling (ABI3, ABI4, and ABI5). From stage IV onwards, germinated seeds largely lost the ability to tolerate desiccation. Likely, the response switches to drought tolerance instead, which is regulated by ABA-dependent as well as -independent pathways (for a recent review see Yoshida et al. 2014)

to germinate is based on a snapshot of the environment (light, water and temperature, for example) that is favourable. However, the environmental conditions (weather) can be unpredictable and seeds can encounter serious stresses once germinated, including lack of water. The fact that germinated seeds have a window in which they cope with dehydration by tolerating the stress is likely beneficial. Thus, in response to the lack of water, germinating or germinated seeds are able to stop growth and remain in a quiescent desiccated state only to resume development when water is in ample supply again. This response could represent an ecologically important stress tolerance mechanism that optimizes successful seedling establishment in conjunction with dormancy, as explained above (Fig. 3). When this ability is lost and seedlings become irreversibly desiccation sensitive, Arabidopsis roots have formed root hairs already. Root hairs grow from specialized epidermal cells (trichoblasts) and are important structures for water uptake and anchoring in the soil (Gilroy and Jones 2000). This observation suggests that the switch from desiccation tolerant to desiccation sensitive in Arabidopsis is made around the point when the germinated seed is capacitated for anchoring and active water uptake from its environment by these specialized structures.

The loss of DT may underlie a change in the response to dehydration, which is switched from *desiccation* tolerance (in stages I–III in Arabidopsis) to *drought* tolerance (from stage IV onwards). Although several factors have been related to the loss of DT (like start of cell division), this switch is not well understood. Since the ability of Arabidopsis seeds to re-induce DT during germination is tightly linked with ABA sensitivity, this switch could be linked with a change in ABA response or sensitivity. The ABA mode of action might change during this developmental switch from a DT to a drought tolerance-inducing agent. During the first three stages (Maia et al. 2011), ABA induces a stress-tolerant state by blocking growth and inducing quiescence and essential mechanisms to re-induce DT. However, from stage IV onwards the seedling attempts to limit the loss of water and thereby staying hydrated and metabolically active, employing the ABA signal to induce cellular protective and water-saving mechanisms (drought tolerance). In Arabidopsis, ABA re-induces late embryogenesis-related genes (*AtEM1*, *AtEM6*, *RAB18*) within the ABA-sensitive window (DT window). Beyond this window, ABA fails to block growth and re-induction of these late embryogenesis-related genes. Instead, other stress-related genes such as *COR47* and *RD29A* are activated (Lopez-Molina et al. 2002). In Medicago, radicles that are 5 mm in length are unable to regain DT after a PEG treatment. However, such PEG-treated radicles tolerate dehydration much better compared to untreated radicles, as was shown by a survival curve. It showed that 50 % survival was obtained at 0.8 gr H_2O/gr DW for PEG-treated radicles compared to 3.6 gr H_2O/gr DW for the untreated ones (Boudet et al. 2006). Combined, these data suggest that during the progression of radicle elongation the response towards water limitation shifts from DT to drought tolerance. Since ABA does not block growth and does not induce quiescence as it does during the induction of DT, this could explain the 'ABA insensitive' phenotype of germinated seeds in stage IV. This developmental switch likely involves chromatin remodelling factors like PICKLE and histone deacetylases, that repress either embryonic or seedling traits (Perruc et al. 2007; Tanaka et al. 2008; van Zanten et al. 2014). Moreover, given the essential role of ABA in DT induction, a reduction in ABA sensitivity after visible germination could be a critical factor to reduce the ability to re-induce DT. Rubio et al. (2009) described a triple mutant of three PP2C genes (which are negative regulators of ABA signalling) that showed a strong growth reduction, extreme sensitivity to ABA, delayed germination, and a partially constitutive ABA responsive transcriptome. Analysis of such a genotype could uncover whether desensitizing of ABA signalling is involved in the regulation of this developmental switch.

Conclusion and future perspectives

DT has been a critical trait during the evolution of plants on land. In most angiosperm species, vegetative DT has been lost, although the majority of species maintained this trait in their seeds. This already indicates the important role

of DT in seed function. Medicago has been used as a model for over a decade in the study of DT in seeds. The system in which DT is lost in germinated seeds and re-induced by an osmotic treatment has proven to be a powerful approach. More recently, germinated Arabidopsis seeds have also emerged as a strong model to investigate the re-induction of DT. Recent results confirmed the role of ABA and ABI3 in the acquisition of DT and supported a role for ABI4 and ABI5 in this process. The results of both model systems are complementary and suggest at least a certain level of conservation in the re-induction of DT in germinated seeds.

An ABA sensitivity window was proposed as a post-germination checkpoint of the osmotic environment during germination towards seedling establishment and was suggested to be related to DT (Lopez-Molina et al. 2001, 2002). The observation that the DT window overlaps with this ABA sensitivity window strongly supports this hypothesis. Thus, seedling establishment is controlled, on the one hand by dormancy (by timing germination through temporal and spatial sensing) and on the other hand by a post-germination window in which growth can be blocked in the absence of water and resumed when osmotic conditions become favourable (Fig. 3).

Whether this DT window is indeed important for seedling establishment and reproductive success remains to be proven and several issues remain to be clarified. E.g. to be an effective stress tolerance mechanism during and after seed germination, DT should be able to be induced under natural drying conditions that occur in the soil and the dried seeds should be viable for a certain period of time. Importantly, recent research in Medicago and Arabidopsis identified several genotypes that are disturbed in re-induction of DT in germinated seeds, and perhaps these might be useful to answer the question whether this DT window plays an important role in seedling establishment under suboptimal conditions. Alternatively, such genotypes can be sown in the field to assess the importance of this DT window in seedling establishment and reproductive success under field conditions. Research in these directions may provide a further understanding of the ABA/DT developmental window present in germinated seeds.

Furthermore, the regulation underlying the developmental switch from DT (ABA sensitive) to drought tolerance (ABA "insensitive") is not well understood and needs further study. Also, there is little information whether natural variation exists for this trait. Perhaps variation exists for the sensitivity to induce DT in germinated seeds or in the length of the window in which this is possible. Such variation (if present), together with a better understanding of this ABA/DT window, and its regulation, may offer future possibilities to improve stand establishment in field grown crops.

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Conflict of interest The authors declare that they have no conflict of interest.

References

Akter K, Kato M, Sato Y, Kaneko Y, Takezawa D (2014) Abscisic acid-induced rearrangement of intracellular structures associated with freezing and desiccation stress tolerance in the liverwort *Marchantia polymorpha*. J Plant Physiol 171:1334–1343

Alpert P (2005) The limits and frontiers of desiccation-tolerant life. Integr Comp Biol 45:685–695

Alpert P (2006) Constraints of tolerance: why are desiccation-tolerant organisms so small or rare? J Exp Biol 209:1575–1584

Angelovici R, Galili G, Fernie AR, Fait A (2010) Seed desiccation: a bridge between maturation and germination. Trends Plant Sci 15:211–218

Barrero JM, Piqueras P, Gonzalez-Guzman M, Serrano R, Rodriguez PL, Ponce MR, Micol JL (2005) A mutational analysis of the *ABA1* gene of *Arabidopsis thaliana* highlights the involvement of ABA in vegetative development. J Exp Bot 56:2071–2083

Baskin CC, Baskin JM (2014) Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego

Baud S, Boutin JP, Miquel M, Lepiniec L, Rochat C (2002) An integrated overview of seed development in *Arabidopsis thaliana* ecotype WS. Plant Physiol Bioch 40:151–160

Berjak P, Pammenter NW (2008) From Avicennia to Zizania: seed recalcitrance in perspective. Ann Bot 101:213–228

Berjak P, Pammenter NW (2013) Implications of the lack of desiccation tolerance in recalcitrant seeds. Front Plant Sci 4:478

Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H (2013) Seeds: physiology of development, germination and dormancy. Springer, New York

Bino RJ, de Vries JN, Kraak HL, van Pijlen JG (1992) Flow cytometric determination of nuclear replication stages in tomato seeds during priming and germination. Ann Bot 69:231–236

Boubriak I, Dini M, Berjak P, Osborne DJ (2000) Desiccation and survival in the recalcitrant seeds of *Avicennia marina*: DNA replication, DNA repair and protein synthesis. Seed Sci Res 10:307–315

Boudet J, Buitink J, Hoekstra FA, Rogniaux H, Larre C, Satour P, Leprince O (2006) Comparative analysis of the heat stable proteome of radicles of *Medicago truncatula* seeds during germination identifies late embryogenesis abundant proteins associated with desiccation tolerance. Plant Physiol 140:1418–1436

Bouwmeester HJ, Karssen CM (1993) Annual changes in dormancy and germination in seeds of *Sisymbrium officinale* (L) Scop. New Phytol 124:179–191

Bruggink T, van der Toorn P (1995) Induction of desiccation tolerance in germinated seeds. Seed Sci Res 5:1–4

Buitink J, Leprince O (2004) Glass formation in plant anhydrobiotes: survival in the dry state. Cryobiology 48:215–228

Buitink J, Leprince O (2008) Intracellular glasses and seed survival in the dry state. C R Biol 331:788–795

Buitink J, Vu BL, Satour P, Leprince O (2003) The re-establishment of desiccation tolerance in germinated radicles of *Medicago truncatula* Gaertn. seeds. Seed Sci Res 13:273–286

Buitink J, Leger JJ, Guisle I, Vu BL, Wuilleme S, Lamirault G, Le Bars A, Le Meur N, Becker A, Kuester H, Leprince O (2006) Transcriptome profiling uncovers metabolic and regulatory processes occurring during the transition from desiccation-sensitive to desiccation-tolerant stages in *Medicago truncatula* seeds. Plant J 47:735–750

Calahan D, Dunham M, DeSevo C, Koshland DE (2011) Genetic analysis of desiccation tolerance in *Saccharomyces cerevisiae*. Genetics 189:507–519

Chatelain E, Hundertmark M, Leprince O, Le Gall S, Satour P, Deligny-Penninck S, Rogniaux H, Buitink J (2012) Temporal profiling of the heat-stable proteome during late maturation of *Medicago truncatula* seeds identifies a restricted subset of late embryogenesis abundant proteins associated with longevity. Plant Cell Environ 35:1440–1455

Chater C, Kamisugi Y, Movahedi M, Fleming A, Cuming AC, Gray JE, Beerling DJ (2011) Regulatory mechanism controlling stomatal behavior conserved across 400 million years of land plant evolution. Curr Biol 21:1025–1029

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR (2010) Abscisic acid: emergence of a core signaling network. Annu Rev Plant Biol 61:651–679

Daws MI, Bolton S, Burslem DFRP, Garwood NC, Mullins CE (2007) Loss of desiccation tolerance during germination in neotropical pioneer seeds: implications for seed mortality and germination characteristics. Seed Sci Res 17:273–281

Dekkers BJW, Smeekens SCM (2007) Sugar and abscisic acid regulation of germination and transition to seedling growth. In: Bradford K, Nonogaki H (eds) Annual Plant Reviews, vol 27., Seed development, dormancy and germination. Blackwell Publishing Ltd, Oxford, pp 305–327

Dekkers BJW, Schuurmans JAMJ, Smeekens SCM (2008) Interaction between sugar and abscisic acid signalling during early seedling development in Arabidopsis. Plant Mol Biol 67:151–167

Delahaie J, Hundertmark M, Bove J, Leprince O, Rogniaux H, Buitink J (2013) LEA polypeptide profiling of recalcitrant and orthodox legume seeds reveals ABI3-regulated LEA protein abundance linked to desiccation tolerance. J Exp Bot 64:4559–4573

Delmas F, Sankaranarayanan S, Deb S, Widdup E, Bournonville C, Bollier N, Northey JGB, McCourt P, Samuel MA (2013) ABI3 controls embryo degreening through Mendel's *I* locus. Proc Natl Acad Sci USA 110:E3888–E3894

Derkx MPM, Karssen CM (1994) Are seasonal dormancy patterns in *Arabidopsis thaliana* regulated by changes in seed sensitivity to light, nitrate and gibberellin? Ann Bot 73:129–136

Dinakar C, Bartels D (2013) Desiccation tolerance in resurrection plants: new insights from transcriptome, proteome and metabolome analysis. Front Plant Sci 4:482

Fait A, Angelovici R, Less H, Ohad I, Urbanczyk-Wochniak E, Fernie AR, Galili G (2006) Arabidopsis seed development and germination is associated with temporally distinct metabolic switches. Plant Physiol 142:839–854

Faria JMR, Buitink J, van Lammeren AAM, Hilhorst HWM (2005) Changes in DNA and microtubules during loss and re-establishment of desiccation tolerance in germinating *Medicago truncatula* seeds. J Exp Bot 56:2119–2130

Farrant JM (2000) A comparison of mechanisms of desiccation tolerance among three angiosperm resurrection plant species. Plant Ecol 151:29–39

Farrant JM, Moore JP (2011) Programming desiccation-tolerance: from plants to seeds to resurrection plants. Curr Opin Plant Biol 14:340–345

Farrant JM, Brandt W, Lindsey GG (2007) An overview of mechanisms of desiccation tolerance in selected angiosperm resurrection plants. Plant Stress 1:72–84

Fenner M, Thompson K (2005) The ecology of seeds. Cambridge University Press, Cambridge

Finch-Savage WE, Leubner-Metzger G (2006) Seed dormancy and the control of germination. New Phytol 171:501–523

Finch-Savage WE, Cadman CS, Toorop PE, Lynn JR, Hilhorst HW (2007) Seed dormancy release in Arabidopsis Cvi by dry after-ripening, low temperature, nitrate and light shows common quantitative patterns of gene expression directed by environmentally specific sensing. Plant J 51:60–78

Finkelstein RR (1994) Mutations at two new Arabidopsis ABA response loci are similar to the *abi3* mutations. Plant J 5:765–771

Finkelstein RR, Lynch TJ (2000) The Arabidopsis abscisic acid response gene *ABI5* encodes a basic leucine zipper transcription factor. Plant Cell 12:599–609

Finkelstein RR, Wang ML, Lynch TJ, Rao S, Goodman HM (1998) The Arabidopsis abscisic acid response locus *ABI4* encodes an APETALA 2 domain protein. Plant Cell 10:1043–1054

Footitt S, Douterelo-Soler I, Clay H, Finch-Savage WE (2011) Dormancy cycling in Arabidopsis seeds is controlled by seasonally distinct hormone signaling pathways. Proc Natl Acad Sci USA 108:20236–20241

Gaff DF (1971) Desiccation-tolerant flowering plants in Southern Africa. Science 174:1033–1034

Gaff DF, Oliver M (2013) The evolution of desiccation tolerance in angiosperm plants: a rare yet common phenomenon. Funct Plant Biol 40:315–328

Garwe D, Thomson JA, Mundree SG (2006) *XvSAP1* from *Xerophyta viscosa* improves osmotic-, salinity- and high-temperature-stress tolerance in Arabidopsis. Biotechnol J 1:1137–1146

Gechev TS, Dinakar C, Benina M, Toneva V, Bartels D (2012) Molecular mechanisms of desiccation tolerance in resurrection plants. Cell Mol Life Sci 69:3175–3186

Gibson SI, Laby RJ, Kim DG (2001) The *sugar-insensitive1* (*sis1*) mutant of Arabidopsis is allelic to *ctr1*. Biochem Biophys Res Commun 280:196–203

Gilroy S, Jones DL (2000) Through form to function: root hair development and nutrient uptake. Trends Plant Sci 5:56–60

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

Hauser F, Waadtl R, Schroeder JI (2011) Evolution of abscisic acid synthesis and signaling mechanisms. Curr Biol 21:R346–R355

Hilhorst HWM (2007) Definitions and Hypotheses of Seed Dormancy. In: Bradford K, Nonogaki H (eds) Annual Plant Reviews, vol 27., Seed development, dormancy and germination. Blackwell Publishing Ltd, Oxford, pp 50–71

Hilhorst HWM, Karssen CM (1988) Dual effect of light on the gibberellin-stimulated and nitrate-stimulated seed-germination of *Sisymbrium officinale* and *Arabidopsis thaliana*. Plant Physiol 86:591–597

Hoekstra FA, Golovina EA, Buitink J (2001) Mechanisms of plant desiccation tolerance. Trends Plant Sci 6:431–438

Illing N, Denby KJ, Collett H, Shen A, Farrant JM (2005) The signature of seeds in resurrection plants: a molecular and physiological comparison of desiccation tolerance in seeds and vegetative tissues. Integr Comp Biol 45:771–787

Jia HY, Suzuki M, McCarty DR (2014) Regulation of the seed to seedling developmental phase transition by the LAFL and VAL transcription factor networks. Wires Dev Biol 3:135–145

Keilin D (1959) The Leeuwenhoek Lecture—the problem of anabiosis or latent life—history and current concept. Proc R Soc Ser B-Bio 150:149–191

Kermode AR, Finch-Savage WE (2002) Desiccation sensitivity in orthodox and recalcitrant seeds in relation to development. In: Black M, Pritchard HW (eds) Desiccation and survival in plants: drying without dying. CAB International, Wallingford, pp 149–184

Khandelwal A, Cho SH, Marella H, Sakata Y, Perroud PF, Pan A, Quatrano RS (2010) Role of ABA and ABI3 in desiccation tolerance. Science 327:546–546

Kim DH, Yamaguchi S, Lim S, Oh E, Park J, Hanada A, Kamiya Y, Choi G (2008) SOMNUS, a CCCH-type zinc finger protein in Arabidopsis, negatively regulates light-dependent seed germination downstream of PIL5. Plant Cell 20:1260–1277

Komatsu K, Suzuki N, Kuwamura M, Nishikawa Y, Nakatani M, Ohtawa H, Takezawa D, Seki M, Tanaka M, Taji T, Hayashi T, Sakata Y (2013) Group A PP2Cs evolved in land plants as key regulators of intrinsic desiccation tolerance. Nature Commun 4:2219

Koornneef M, Reuling G, Karssen CM (1984) The isolation and characterization of abscisic-acid insensitive mutants of *Arabidopsis thaliana*. Physiol Plant 61:377–383

Koornneef M, Hanhart CJ, Hilhorst HW, Karssen CM (1989) *In vivo* inhibition of seed development and reserve protein accumulation in recombinants of abscisic acid biosynthesis and responsiveness mutants in *Arabidopsis thaliana*. Plant Physiol 90:463–469

Kranner I, Birtic S (2005) A modulating role for antioxidants in desiccation tolerance. Integr Comp Biol 45:734–740

Kumar D, Singh P, Yusuf MA, Upadhyaya CP, Roy SD, Hohn T, Sarin NB (2013) The *Xerophyta viscosa* aldose reductase (ALDRXV4) confers enhanced drought and salinity tolerance to transgenic tobacco plants by scavenging methylglyoxal and reducing the membrane damage. Mol Biotechnol 54:292–303

Leprince O, Buitink J (2010) Desiccation tolerance: from genomics to the field. Plant Sci 179:554–564

Leprince O, van Aelst AC, Pritchard HW, Murphy DJ (1998) Oleosins prevent oil-body coalescence during seed imbibition as suggested by a low-temperature scanning electron microscope study of desiccation-tolerant and -sensitive oilseeds. Planta 204:109–119

Leprince O, Harren FJM, Buitink J, Alberda M, Hoekstra FA (2000) Metabolic dysfunction and unabated respiration precede the loss of membrane integrity during dehydration of germinating radicles. Plant Physiol 122:597–608

Leprince O, Satour P, Vu BL, Buitink J (2004) The role of sugars and hexose phosphorylation in regulating the re-establishment of desiccation tolerance in germinated radicles of *Cucumis sativa* and *Medicago truncatula*. Physiol Plant 122:200–209

Lin TP, Yen WL, Chien CT (1998) Disappearance of desiccation tolerance of imbibed crop seeds is not associated with the decline of oligosaccharides. J Exp Bot 49:1203–1212

Linkies A, Graeber K, Knight C, Leubner-Metzger G (2010) The evolution of seeds. New Phytol 186:817–831

Loi P, Iuso D, Czernik M, Zacchini F, Ptak G (2013) Towards storage of cells and gametes in dry form. Trends Biotechnol 31:688–695

Lopez-Molina L, Chua NH (2000) A null mutation in a bZIP factor confers ABA-insensitivity in Arabidopsis thaliana. Plant Cell Physiol 41:541–547

Lopez-Molina L, Mongrand S, Chua NH (2001) A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the AB15 transcription factor in Arabidopsis. Proc Natl Acad Sci USA 98:4782–4787

Lopez-Molina L, Mongrand B, McLachlin DT, Chait BT, Chua NH (2002) ABI5 acts downstream of ABI3 to execute an ABA-dependent growth arrest during germination. Plant J 32:317–328

Lotan T, Ohto M, Yee KM, West MA, Lo R, Kwong RW, Yamagishi K, Fischer RL, Goldberg RB, Harada JJ (1998) Arabidopsis LEAFY COTYLEDON1 is sufficient to induce embryo development in vegetative cells. Cell 93:1195–1205

Luerssen H, Kirik V, Herrmann P, Misera S (1998) FUSCA3 encodes a protein with a conserved VP1/AB13-like B3 domain which is of functional importance for the regulation of seed maturation in *Arabidopsis thaliana*. Plant J 15:755–764

Lüttge U, Beck E, Bartels D (2011) Plant desiccation tolerance. Springer-Verlag, Berlin Heidelberg

Maia J, Dekkers BJ, Provart NJ, Ligterink W, Hilhorst HWM (2011) The re-establishment of desiccation tolerance in germinated *Arabidopsis thaliana* seeds and its associated transcriptome. PLoS One 6:e29123

Maia J, Dekkers BJW, Dolle MJ, Ligterink W, Hilhorst HWM (2014) Abscisic acid (ABA) sensitivity regulates desiccation tolerance in germinated Arabidopsis seeds. New Phytol 203:81–93

McCarty DR, Carson CB, Stinard PS, Robertson DS (1989) Molecular analysis of Viviparous 1—an abscisic acid-insensitive mutant of maize. Plant Cell 1:523–532

Mönke G, Seifert M, Keilwagen J, Mohr M, Grosse I, Hahnel U, Junker A, Weisshaar B, Conrad U, Baumlein H, Altschmied L (2012) Toward the identification and regulation of the *Arabidopsis thaliana* ABI3 regulon. Nucleic Acids Res 40:8240–8254

Moore B, Zhou L, Rolland F, Hall Q, Cheng WH, Liu YX, Hwang I, Jones T, Sheen J (2003) Role of the Arabidopsis glucose sensor HXK1 in nutrient, light, and hormonal signaling. Science 300:332–336

Moore JP, Vicre-Gibouin M, Farrant JM, Driouich A (2008) Adaptations of higher plant cell walls to water loss: drought vs desiccation. Physiol Plant 134:237–245

Nakashima K, Fujita Y, Kanamori N, Katagiri T, Umezawa T, Kidokoro S, Maruyama K, Yoshida T, Ishiyama K, Kobayashi M, Shinozaki K, Yamaguchi-Shinozaki K (2009) Three Arabidopsis SnRK2 protein kinases, SRK2D/SnRK2.2, SRK2E/SnRK2.6/OST1 and SRK2I/SnRK2.3, involved in ABA signaling are essential for the control of seed development and dormancy. Plant Cell Physiol 50:1345–1363

Nambara E, Naito S, McCourt P (1992) A mutant of Arabidopsis which is defective in seed development and storage protein accumulation is a new *abi3* allele. Plant J 2:435–441

Nambara E, Keith K, McCourt P, Naito S (1995) A regulatory role for the *ABI3* gene in the establishment of embryo maturation in *Arabidopsis thaliana*. Development 121:629–636

Oh E, Kim J, Park E, Kim JI, Kang C, Choi G (2004) PIL5, a phytochrome-interacting basic helix–loop–helix protein, is a key negative regulator of seed germination in *Arabidopsis thaliana*. Plant Cell 16:3045–3058

Oh E, Yamaguchi S, Kamiya Y, Bae G, Chung WI, Choi G (2006) Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in Arabidopsis. Plant J 47:124–139

Oliver MJ, Tuba Z, Mishler BD (2000) The evolution of vegetative desiccation tolerance in land plants. Plant Ecol 151:85–100

Oliver MJ, Velten J, Mishler BD (2005) Desiccation tolerance in bryophytes: a reflection of the primitive strategy for plant survival in dehydrating habitats? Integr Comp Biol 45:788–799

Ooms JJJ, Leon-Kloosterziel KM, Bartels D, Koornneef M, Karssen CM (1993) Acquisition of desiccation tolerance and longevity in seeds of *Arabidopsis thaliana* (A comparative study using abscisic acid-insensitive *abi3* mutants). Plant Physiol 102:1185–1191

Osborne DJ, Boubriak II (1994) DNA and desiccation tolerance. Seed Sci Res 4:175–185

Pammenter NW, Berjak P (1999) A review of recalcitrant seed physiology in relation to desiccation tolerance mechanisms. Seed Sci Res 9:13–37

Perruc E, Kinoshita N, Lopez-Molina L (2007) The role of chromatin-remodeling factor PKL in balancing osmotic stress responses during Arabidopsis seed germination. Plant J 52:927–936

Phillips J, Artsaenko O, Fiedler U, Horstmann C, Mock HP, Muntz K, Conrad U (1997) Seed-specific immunomodulation of abscisic acid activity induces a developmental switch. EMBO J 16:4489–4496

Porembski S (2011) Evolution, diversity, and habitats of poikilohydrous vascular plants. In: Lüttge U, Beck E, Bartels D (eds) Plant desiccation tolerance. Springer-Verlag, Berlin

Potts M, Slaughter SM, Hunneke FU, Garst JF, Helm RF (2005) Desiccation tolerance of prokaryotes: application of principles to human cells. Integr Comp Biol 45:800–809

Proctor MCF, Pence VC (2002) Vegetative tissues: Bryophytes vascular resurrection plants and vegetative propagules. In: Black M, Pritchard HW (eds) Desiccation and survival in plants: drying without dying. CAB International, Wallingford, pp 207–238

Proctor MCF, Oliver MJ, Wood AJ, Alpert P, Stark LR, Cleavitt NL, Mishler BD (2007) Desiccation-tolerance in bryophytes: a review. Bryologist 110:595–621

Roberts EH (1973) Predicting the storage life of seeds. Seed Sci Technol 1:499–514

Rubio S, Rodrigues A, Saez A, Dizon MB, Galle A, Kim TH, Santiago J, Flexas J, Schroeder JI, Rodriguez PL (2009) Triple loss of function of protein phosphatases type 2C leads to partial constitutive response to endogenous abscisic acid. Plant Physiol 150:1345–1355

Ruszala EM, Beerling DJ, Franks PJ, Chater C, Casson SA, Gray JE, Hetherington AM (2011) Land plants acquired active stomatal control early in their evolutionary history. Curr Biol 21:1030–1035

Saracco F, Bino RJ, Bergervoet JHW, Lanteri S (1995) Influence of priming-induced nuclear replication activity on storability of pepper (*Capsicum annuum* L) seed. Seed Sci Res 5:25–29

Sargent JA, Mandi SS, Osborne DJ (1981) The loss of desiccation tolerance during germination—an ultrastructural and biochemical approach. Protoplasma 105:225–239

Satpathy GR, Torok Z, Bali R, Dwyre DM, Little E, Walker NJ, Tablin F, Crowe JH, Tsvetkova NM (2004) Loading red blood cells with trehalose: a step towards biostabilization. Cryobiology 49:123–136

Scoffoni C, Vuong C, Diep S, Cochard H, Sack L (2014) Leaf shrinkage with dehydration: coordination with hydraulic vulnerability and drought tolerance. Plant Physiol 164:1772–1788

Silvertown J (1980) Leaf-canopy-induced seed dormancy in a grassland flora. New Phytol 85:109–118

Steeves TA (1983) The evolution and biological significance of seeds. Can J Bot 61:3550–3560

Stone SL, Kwong LW, Yee KM, Pelletier J, Lepiniec L, Fischer RL, Goldberg RB, Harada JJ (2001) LEAFY COTYLEDON2 encodes a B3 domain transcription factor that induces embryo development. Proc Natl Acad Sci USA 98:11806–11811

Sugliani M, Rajjou L, Clerkx EJ, Koornneef M, Soppe WJ (2009) Natural modifiers of seed longevity in the Arabidopsis mutants *abscisic acid insensitive3-5* (*abi3-5*) and *leafy cotyledon1-3* (*lec1-3*). New Phytol 184:898–908

Tanaka M, Kikuchi A, Kamada H (2008) The Arabidopsis histone deacetylases HDA6 and HDA19 contribute to the repression of embryonic properties after germination. Plant Physiol 146:149–161

Terrasson E, Buitink J, Righetti K, Vu BL, Pelletier S, Zinsmeister J, Lalanne D, Leprince O (2013) An emerging picture of the seed desiccome: confirmed regulators and newcomers identified using transcriptome comparison. Front Plant Sci 4:497

Tunnacliffe A, Wise MJ (2007) The continuing conundrum of the LEA proteins. Die Naturwissenschaften 94:791–812

Tweddle JC, Dickie JB, Baskin CC, Baskin JM (2003) Ecological aspects of seed desiccation sensitivity. J Ecol 91:294–304

Umezawa T, Nakashima K, Miyakawa T, Kuromori T, Tanokura M, Shinozaki K, Yamaguchi-Shinozaki K (2010) Molecular basis of the core regulatory network in ABA responses: sensing, signaling and transport. Plant Cell Physiol 51:1821–1839

van Zanten M, Koini MA, Geyer R, Liu Y, Brambilla V, Bartels D, Koornneef M, Fransz P, Soppe WJ (2011) Seed maturation in *Arabidopsis thaliana* is characterized by nuclear size reduction and increased chromatin condensation. Proc Natl Acad Sci USA 108:20219–20224

van Zanten M, Zoll C, Wang Z, Philipp C, Carles A, Li Y, Kornet NG, Liu Y, Soppe WJ (2014) HISTONE DEACETYLASE 9 represses seedling traits in *Arabidopsis thaliana* dry seeds. Plant J 80:475–488

Verdier J, Lalanne D, Pelletier S, Torres-Jerez I, Righetti K, Bandyopadhyay K, Leprince O, Chatelain E, Vu BL, Gouzy J, Gamas P, Udvardi MK, Buitink J (2013) A regulatory network-based approach dissects late maturation processes related to the acquisition of desiccation tolerance and longevity of *Medicago truncatula* seeds. Plant Physiol 163:757–774

Verslues PE, Juenger TE (2011) Drought, metabolites, and Arabidopsis natural variation: a promising combination for understanding adaptation to water-limited environments. Curr Opin Plant Biol 14:240–245

Vertucci CW, Farrant JM (1995) Acquisition and loss of desiccation tolerance. In: Kigel J, Galili G (eds) Seed development and germination. Marcel Dekker, New York, pp 237–271

Vieira CV, da Silva EAA, de Alvarenga AA, de Castro EM, Toorop PE (2010) Stress-associated factors increase after desiccation of germinated seeds of *Tabebuia impetiginosa* Mart. Plant Growth Regul 62:257–263

Waterworth WM, Masnavi G, Bhardwaj RM, Jiang Q, Bray CM, West CE (2010) A plant DNA ligase is an important determinant of seed longevity. Plant J 63:848–860

Welch AZ, Gibney PA, Botstein D, Koshland DE (2013) TOR and RAS pathways regulate desiccation tolerance in S*accharomyces cerevisiae*. Mol Biol Cell 24:115–128

Willis CG, Baskin CC, Baskin JM, Auld JR, Venable DL, Cavender-Bares J, Donohue K, Rubio de Casas R, Group NEGW (2014) The evolution of seed dormancy: environmental cues, evolutionary hubs, and diversification of the seed plants. New Phytol 203:300–309

Yoshida T, Mogami J, Yamaguchi-Shinozaki K (2014) ABA-dependent and ABA-independent signaling in response to osmotic stress in plants. Curr Opin Plant Biology 21C:133–139