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REVIEW PAPER

Late seed maturation: drying without dying

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Abstract

Besides the deposition of storage reserves, seed maturation is characterized by the acquisition of functional traits including germination, desiccation tolerance, dormancy, and longevity. After seed filling, seed longevity increases up to 30-fold, concomitant with desiccation that brings the embryo to a quiescent state. The period that we define as late maturation phase can represent 10–78% of total seed development time, yet it remains overlooked. Its importance is underscored by the fact that in the seed production chain, the stage of maturity at harvest is the primary factor that influences seed longevity and seedling establishment. This review describes the major events and regulatory pathways underlying the acquisition of seed longevity, focusing on key indicators of maturity such as chlorophyll degradation, accumulation of raffinose family oligosaccharides, late embryogenesis abundant proteins, and heat shock proteins. We discuss how these markers are correlated with or contribute to seed longevity, and highlight questions that merit further attention. We present evidence suggesting that molecular players involved in biotic defence also have a regulatory role in seed longevity. We also explore how the concept of plasticity can help understand the acquisition of longevity.

Key words: ABA, chlorophyll, desiccation tolerance, longevity, LEA proteins, raffinose family oligosaccharides.

Introduction

With the human population unlikely to stabilize before 2070, at around 9 billion, and with the prospect of climate changes that are shaping future agricultural practices and threatening natural habitats, seeds are at the centre stage of food security and conservation of genetic resources. The production of highly vigorous seeds is a key lever to improve and stabilize yield. Seed vigour is a concept used in the seed industry to define seed performance in the field, namely the rate and uniformity of seed germination and seedling growth, emergence ability under unfavourable environmental conditions, and the retention of these characteristics after storage (see Finch-Savage and Bassel, 2015, for a review). The latter of these characteristics is conferred by the longevity of the seed, which is defined as its capacity to remain viable for long periods

during storage in the quiescent dry state. Seed longevity is also pivotal to *ex situ* conservation of plant genetic resources as an 'insurance policy' against endangered biodiversity (Hay and Probert, 2013). Seed persistence in an *in situ* environment (i.e. the soil of a field or a forest) is equally important both in agricultural and ecological contexts, such as weed management and flora rehabilitation programmes and plant community dynamics (Long *et al.*, 2015). Thus, inherent seed longevity is seen as one of the adaptive mechanisms to allow a plant population to disperse in time and space.

For successful crop production, the stage of maturity at harvest is the primary factor in the seed production chain that influences seed vigour (Finch-Savage and Bassel, 2015). Harvesting too early may result in low longevity, low vigour,

and poor establishment because not all the seed vigour characteristics have been acquired yet. Delaying harvest, meanwhile, increases the risks of either shattering or seed deterioration in the field due to unfavourable weather conditions. Despite the central importance of longevity and seed vigour in agriculture, how they are acquired during seed development is still poorly understood. The stage of maturity is also of practical importance during collection of seeds of non-cultivated species for seed banking. It is often necessary to harvest seeds before they are fully ripe as this will secure collection prior to dispersal. However, this practice also leads to the collection of a significant proportion of immature seeds that are unsuitable for long-term storage (Hay and Probert, 2013).

Survival in the dry state is a result of a series of molecular and cellular processes during seed development that start with the acquisition of desiccation tolerance, defined as the ability to survive a loss of water that rapidly brings the seed moisture content to equilibration with that of ambient air [i.e. the relative humidity (RH) of the air]. In practice, this moisture content is around 10% on a fresh weight basis, corresponding to a water potential of around -150 MPa. Upon further maturation, mechanisms are put in place to confer seed longevity in the dry state. During this period, a progressive loss of water occurs, which needs to be precisely controlled to allow preparation for the incipient germination and the timing of fruit abscission from the parent plant. Whereas the definition of desiccation tolerance implies a threshold value of moisture content during drying, longevity involves a time component. Indeed, being able to survive the water loss is one thing; remaining alive in the dry state is another. This cannot be more powerfully illustrated than with the recent progress made on the preservation of human cells in the dry state. By increasing the intracellular concentration of trehalose and overexpressing two late embryogenesis abundant (LEA) proteins in human HepG2 cells, Li *et al.* (2012) were able to dry these transgenic cells, with 98% survival at 12% moisture, but were unable to maintain them alive in the dry state for more than a few minutes. The time component of survival in the dry state originates from the nature and speed of chemical and physical reactions that cause slow deterioration of seeds during storage, despite the extremely severe limitations in molecular diffusion and motion brought about by the glassy state. That the threshold (desiccation tolerance) and time (longevity) components are acquired separately during seed development implies the existence of different regulatory factors acting sequentially and/or different mechanisms of regulation if the molecular players are identical.

In this review, we describe the current knowledge on the so-called late seed maturation programme and present recent advances concerning the molecular determinism of seed longevity. For extensive reviews with regard to desiccation tolerance, the reader should refer to Hoekstra *et al.* (2001), Leprince and Buitink (2010), and Dekkers *et al.* (2015), and with regard to longevity, to Buitink and Leprince (2004), Walters *et al.* (2010), and Sano *et al.* (2016). Here we focus on the major events and regulatory pathways underlying late seed maturation, including the acquisition of seed longevity. We argue that, in contrast to embryogenesis and seed

maturation, the late seed maturation programme is poorly understood and deserves further attention.

Physiological and biochemical events of late seed maturation: more than just water loss

A useful starting place to illustrate the importance of the late maturation phase is a comparison of its relative length in time during seed development across species (Fig. 1). There is no consensus as to when late seed maturation starts, but the easiest criterion to use is the end of seed filling, when the embryo and/or endosperm ceases to expand and accumulates storage reserves. Fig. 1 shows that, using this criterion, the relative time span of late seed maturation varies tremendously among species, ranging from 78% of the total developmental time in rice to 10% in red cabbage. Arabidopsis exhibits a late maturation phase that is among the shortest, providing an explanation as to why this phase has not yet received a great deal of attention at the molecular level. Other markers associated with late maturation are the loss of chlorophyll and the accumulation of the raffinose family oligosaccharides (RFO). In *Medicago truncatula*, both processes start when seed filling has ceased (Fig. 2). In contrast, in Arabidopsis and other Brassicaceae, chlorophyll loss and RFO accumulation start when seed filling is still underway (Fig. 2; Baud *et al.*, 2002; Jolivet *et al.*, 2011; Righetti *et al.*, 2015). This suggests that the end of seed filling is not an appropriate marker of the start of the late maturation process, and that maturation *sensu stricto* (seed filling) and late seed maturation components strongly overlap in species such as Arabidopsis. This was confirmed by the expression of certain marker genes of seed filling and late maturation that show distinct expression profiles in developing seeds of *M. truncatula*, whereas they overlap in Arabidopsis (Righetti *et al.*, 2015). Another example illustrating that late seed maturation implies different regulatory controls compared with seed filling comes from [$1-^{14}\text{C}$]decanoate feeding experiments, which show that pathways of lipid metabolism during desiccation are clearly different from those during seed maturation (Chia *et al.*, 2005). In oily seeds, desiccation is accompanied by a 10–16% decrease in lipid reserves that are used for respiration, triacylglycerol turnover, and the production of malate and asparagine (Chia *et al.*, 2005). Species in which maturation *sensu stricto* and the late seed maturation programme are well separated are excellent models to distinguish the regulatory modules associated with late maturation from seed filling, and to decipher the regulatory networks that are involved in the acquisition of seed longevity (Chatelain *et al.*, 2012; Righetti *et al.*, 2015; Rosnoblet *et al.*, 2007; Verdier *et al.*, 2013).

From a physiological point of view, seeds entering the late maturation phase have already gained their germination capacity and desiccation tolerance, both occurring much earlier, when seeds are being filled (Fig. 2). During late maturation, longevity is acquired gradually, increasing 30- to 50-fold to reach a maximum just before or near the dry state, depending on the species, genotype, and environment

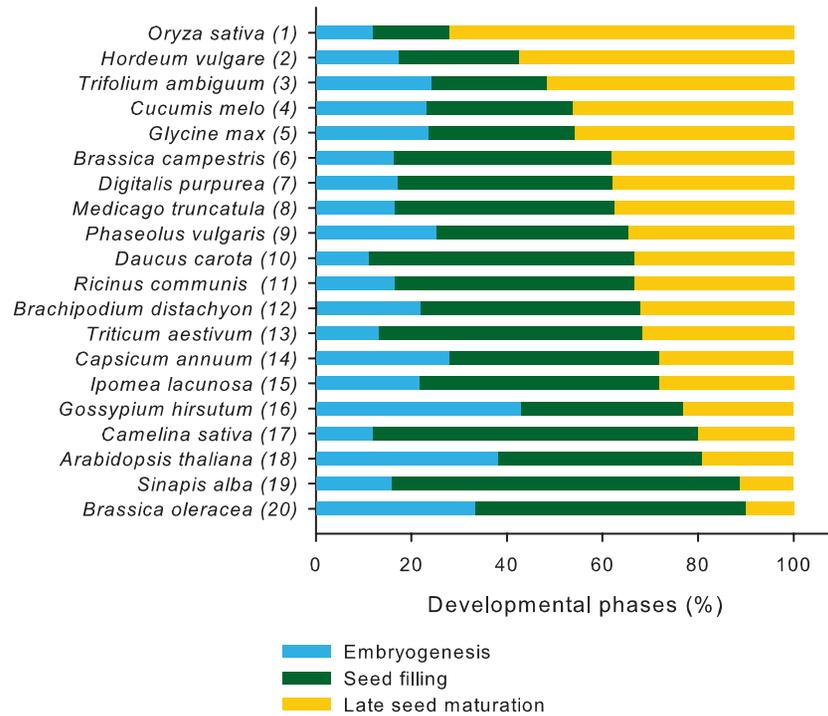


Fig. 1. Relative importance of the three phases of seed development. Data are taken from the literature showing the evolution of fresh and dry weight and water content during seed development. The switch from embryogenesis to seed filling is defined as the onset of the increase in dry weight. Late seed maturation starts when seed filling ceases. Seed development is considered to be terminated when seed moisture content is less than 20%. Data are from Ellis *et al.*, 1993 (1); Bartels *et al.*, 1988 (2); Hay *et al.*, 2010 (3); Welbaum and Bradford, 1988 (4); Zanakis *et al.*, 1994 (5); Sinniah *et al.*, 1998 (6); Hay and Probert, 1995 (7); Chatelain *et al.*, 2012 (8); Sanhewe and Ellis, 1996 (9); Gray *et al.*, 1988 (10); Kermode and Bewley 1985 (11); Guillon *et al.*, 2012 (12); Ellis and Pieta Filho, 1992 (13); Demir and Ellis, 1992 (14); Jayasuriya *et al.*, 2007 (15); Galau *et al.*, 1987 (16); Rodríguez *et al.*, 2013 (17); Baud *et al.*, 2002 (18); Fischer *et al.*, 1988 (19); Still and Bradford, 1998 (20). (This figure is available in colour at JXB online.)

(Hay *et al.*, 2015; Probert *et al.*, 2007; Righetti *et al.*, 2015). Upon abscission and final drying, seed water content decreases and reaches an equilibrium with ambient atmospheric conditions. When water content is below approximately 0.1 g H₂O g DW⁻¹ (equilibrium RH values below 65%), the cytoplasm transforms into a glassy state, where the molecular mobility and relaxation rates of molecules are severely slowed down, thereby imposing a state of quiescence (Ballesteros and Walters, 2011; Buitink and Leprince, 2004).

Below, we summarize the different molecular events that characterize the late seed maturation programme together with their underlying regulatory factors (Fig. 3). The regulation of seed coat components and their implications for longevity are thoroughly described in a recent review by Sano *et al.* (2016), and will not be discussed here further.

Longevity and the degradation of photosynthetic pigments: a continuing conundrum

The most visible change associated with late seed maturation is the degradation of photosynthetic pigments, including chlorophyll and carotenoids (Fig. 3; Delmas *et al.*, 2013; Gonzales-Jorge *et al.*, 2013; Nakajima *et al.*, 2012; Teixeira *et al.*, 2016). Taken together, this makes the presence of chlorophyll molecules in dry non-chlorophyllous seeds a characteristic of immaturity that can be used to discriminate highly vigorous seed in a seed lot (Jalink *et al.*, 1998).

In developing *Arabidopsis* and *M. truncatula* seeds, the degradation of chlorophyll is regulated by abscisic acid (ABA), acting through the transcription factor ABSCISIC ACID3 (ABI3), since seeds of *abi3* mutants remain green at maturity (Fig. 3; Delahaie *et al.*, 2013; Delmas *et al.*, 2013). ABI3 controls degreening by activating the expression of a series of genes encoding chlorophyll degradation enzymes, namely *NON-YELLOW COLORING1* (*NYC1*) and *NON-YELLOW COLORING1-like* (*NOL*), acting in the first steps of chlorophyll breakdown (Nakajima *et al.*, 2012), and *STAY-GREEN2* (*SGR2*, Delmas *et al.*, 2013). The *SGR* gene is the Mendel's I locus responsible for the colour of pea cotyledons and leaf greenness during senescence (Sato *et al.*, 2007), although its precise role in chlorophyll catabolism is still unclear. Seeds of the SnRK2 triple mutant (*snrk2.2/snrk2.3/snrk2.6*) also show chlorophyll persistence (Nakashima *et al.*, 2009). In *Arabidopsis*, several enhancers of the ABI3-dependent green phenotype have been identified, such as the GREEN SEED (*GRS*) and DOG1 (Clerckx *et al.*, 2003; Dekkers *et al.*, 2016). Their precise function in the regulation of chlorophyll catabolism and whether they act in parallel or upstream of one another remain to be established.

The physiological reasons why chlorophyll is degraded during late seed maturation are still unclear. Seeds of most species acquire their desiccation tolerance when they are still green, indicating that the presence of chlorophyll is not detrimental to their survival of drying. However, chlorophyll retention could be detrimental to longevity. Seeds of all but one mutant

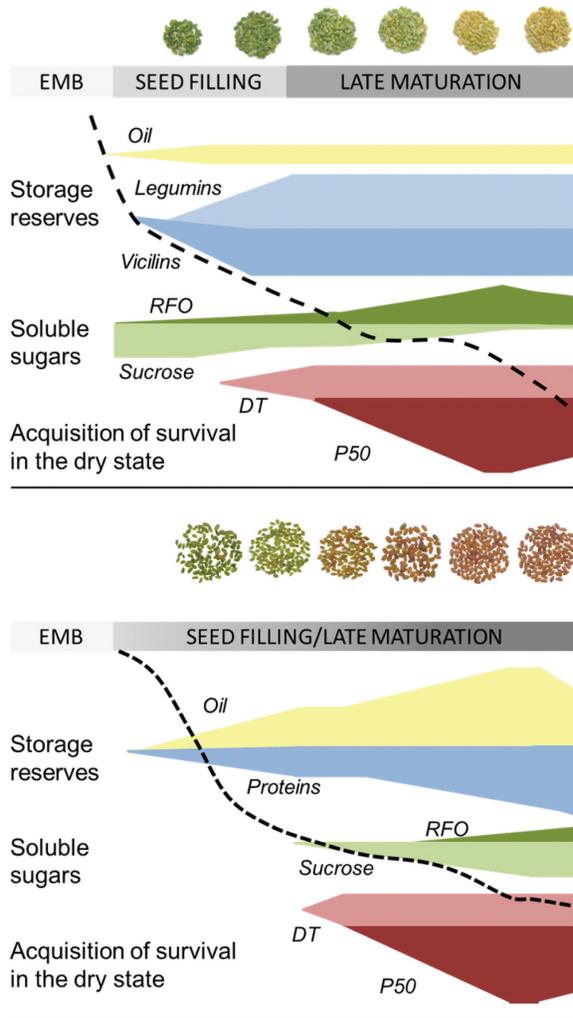


Fig. 2. Main physiological and biochemical events related to seed filling and the acquisition of longevity during late seed maturation are overlapping in *Medicago truncatula* (above) in contrast to *Arabidopsis thaliana* (below). The broken line indicates the loss of water during development. DT, desiccation tolerance; EMB, embryogenesis; P50, half-life of viability loss during storage; RFO, raffinose family oligosaccharide content. Adapted from Righetti *et al.* (2015).

affected in chlorophyll degradation and the regulation thereof that have been studied so far exhibit reduced longevity. Seeds of *abi3* and *snrk2.2/snrk2.3/snrk2.6* exhibit both chlorophyll retention and decreased longevity compared with wild type. Seeds of the *grs* mutant of *Arabidopsis* contain twice as much chlorophyll as wild type and show reduced shelf-life (Clerkx *et al.*, 2003). The *dog1-1 abi3-1* double mutant seeds also exhibit both a strongly reduced storability and higher chlorophyll content compared with *abi3-1* (Dekkers *et al.*, 2016). Direct evidence linking genes that specifically affect chlorophyll degradation to longevity comes from the characterization of *nyc1 nol* double mutants. These seeds contain 10-fold more chlorophyll than wild type and their longevity is strongly reduced (Nakajima *et al.*, 2012). The only gene for which a mutation leads to a green seed phenotype without apparent reduction of longevity is *SGR* (Delmas *et al.*, 2013; Sato *et al.*, 2007). To explain this discrepancy, Nakajima *et al.* (2012) proposed that, depending on the mutated gene in the

chlorophyll breakdown pathway, unbalanced degradation might occur, leading to the accumulation of unphysiological degradation products of chlorophyll. In leaves, *SGR* does not function as a chlorophyll catabolic enzyme but is a key regulator of this process (Christ and Hörtensteiner, 2014).

An alternative hypothesis to explain the function of degreening during late seed maturation could be that chlorophyll breakdown is necessary to release phytol tails to serve as a substrate for the synthesis of tocopherols, a known antioxidant involved in seed longevity (reviewed in Sano *et al.*, 2016). In support of this, seeds of the *Arabidopsis* mutant defective in *VITAMIN E DEFICIENT6* (VT6), a phytol-phosphate kinase that links phytol release from chlorophyll to tocopherol synthesis, exhibited reduced longevity and increased chlorophyll content (Fig. 3; Vom Dorp *et al.*, 2015). Thus, any mutation that leads to chlorophyll retention could affect the synthesis of tocopherols during maturation, thereby impacting the storability of the seed. Late seed maturation triggers not only chlorophyll degradation but also carotenoid catabolism. These pigments, which are accessory to photosynthesis, play crucial roles in photosystem assembly, protection against light-induced reactive oxygen species (ROS), and as substrates for the synthesis of ABA and strigolactones. The regulation of carotenoid metabolism is very well described in fruits and leaves (reviewed in Liu *et al.*, 2015), but only a few studies on seed maturation have been performed. Degradation of carotenoids is regulated by carotenoid cleavage dioxygenases (CDD). In seeds, they include 9-*cis*-epoxycarotenoid dioxygenases (NCED) that are involved in ABA synthesis, and several CCDs with broad substrate specificities. In *Arabidopsis*, four CCDs (CCD1, CCD4, CCD7, and CCD8) are active during development. The *CCD1* and *CCD4* genes are induced during the onset of the desiccation phase, and seeds of *ccd1* and *ccd4* mutants were impaired in the degradation of β -carotenes (Gonzales-Jorge *et al.*, 2013). Recent results showed that *ABI4* could act as a negative regulator of CCD gene expression (Yu *et al.*, 2016). Over-expression of *ABI4* in *Arabidopsis* resulted in an increase in transcript levels of *CCD7* and *CCD8* and a decrease in carotenoid contents in seeds. *ABI4* is mainly expressed during late seed maturation and early germination and appears to regulate after-ripening, a period of dry storage that releases dormancy (Shu *et al.*, 2013). It is also known to be involved in chloroplast–nucleus retrograde signalling in leaves (León *et al.*, 2012), although there is no evidence so far on whether this regulation takes place in late-maturing seeds.

The role of oligosaccharides in longevity remains elusive

Changes in soluble sugar content and composition are also a characteristic of late seed maturation. Typically, hexoses (Glc and Fru) and Fru6P and Glc6P progressively disappear, whereas Suc, Ara, Gal, and RFO increase (Fait *et al.*, 2006; Verdier *et al.*, 2013). The presence of reducing monosaccharides is negatively correlated with longevity. They are considered to be detrimental for seed shelf life because the carbonyl groups of these reducing sugars can react with amino acids

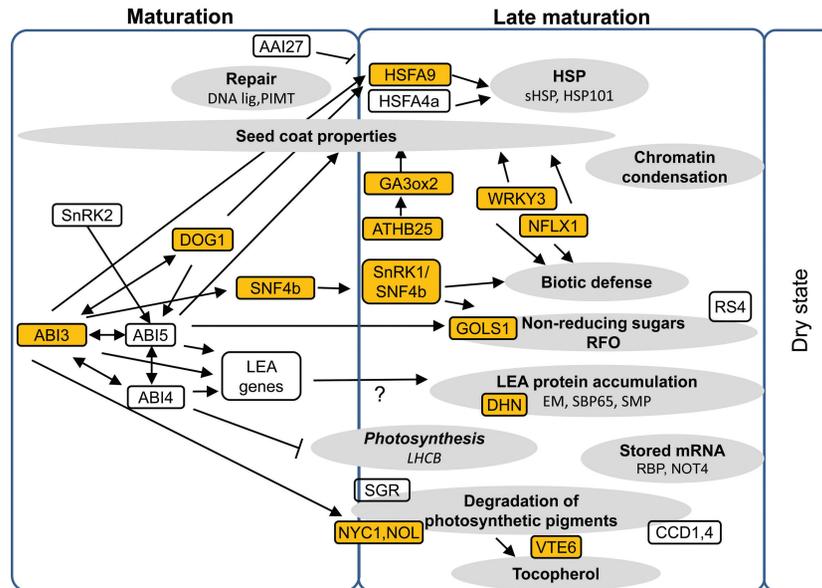


Fig. 3. Diagram of key events and players in the establishment of longevity during maturation and late maturation of a dicot seed. Most of the data are from *Arabidopsis* and *M. truncatula* as model plants. Grey ellipses indicate confirmed compounds, enzymes, or biochemical pathways that are activated or synthesized during late maturation. Orange boxes correspond to transcription factors, enzymes, or proteins whose mutants have been shown to exhibit reduced longevity. White boxes indicate players that participate indirectly in seed longevity or whose role remains to be evaluated. AAI27, AUXIN-RESPONSIVE PROTEIN27; ABI, ABA INSENSITIVE; CCD, CAROTENOID CLEAVAGE DIOXYGENASE; DHN, DEHYDRIN; DOG1, DELAY OF GERMINATION1; EM, EARLY METHIONINE; GA3ox2, GA3 OXIDASE2; GOLS1, GALACTINOL SYNTHASE1; HSF9A/4a, HEAT SHOCK FACTOR A9/4a; HSP, HEAT SHOCK PROTEINS; LEA, LATE EMBRYOGENESIS ABUNDANT; LHCB, LIGHT HARVESTING COMPLEX PROTEIN B; NFLX1, NUCLEAR FACTOR-X1-LIKE1; NOL, NYC1-LIKE; NYC1, NON YELLOW COLORING1; PIMT, PROTEIN REPAIR L-ISOASPARTYL METHYLTRANSFERASE1; RBP, RNA BINDING PROTEIN; RNF, RIBONUCLEOPROTEIN; RS4, RAFFINOSE SYNTHASE4; SBP65, SEED BIOTINYLATED PROTEIN 65; SGR, STAY-GREEN; SMP, SEED MATURATION PROTEIN; SNF4b, SUCROSE NONFERMENTING SUBUNIT4b; SNRK2, SUCROSE NONFERMENTING-RELATED KINASE 2; VTE6, VITAMIN E DEFICIENT6.

(the so-called Maillard reaction) and release toxic molecules during seed storage. In dry mature seeds, the most abundant soluble non-reducing sugars are either Suc, representing 60–80% of total soluble sugars (*Arabidopsis*, maize, *Acer* spp.) or RFO (legumes) (Fig. 2).

RFO are synthesized from Suc by the subsequent addition of galactinol, a reaction catalysed by raffinose (Raf) SYNTHASE (RafS) and stachyose (Sta) SYNTHASE (StaS), both of which exhibit broad substrate specificities, whereas galactinol is synthesized from UDP-Gal and myo-inositol by GALACTINOL SYNTHASE (GOLS), which is putatively the committed enzymatic step in the RFO synthesis (reviewed in Sengupta *et al.*, 2015). Regulation of RFO content appears to be controlled transcriptionally during late seed maturation, since there is a tight correlation between RFO accumulation and the increase in transcripts of *GOLS*, *StaS*, and *SEED IMBIBITION PROTEIN1* and 2 (*SIP*, a *RafS*; Downie *et al.*, 2003; Verdier *et al.*, 2013).

The regulation of RFO accumulation is under the transcriptional control of ABA and gibberellic acid (GA). *GOLS* transcripts remain high in the dry seeds of ABA- and GA-deficient mutants of tomato (Downie *et al.*, 2003). Expression of both *SIPs* is regulated by ABA, at least in part via ABI3 and ABI5 (Sengupta *et al.*, 2015). Both genes possess ABRE elements in their promoter region. In seeds of *Arabidopsis* *abi3* and *aba1 abi3* mutants, RFO levels are lower than wild type and accumulation could be re-induced by exogenous ABA (Dekkers *et al.*, 2016). In *M. truncatula*,

Mt-SIP2 is also a potential target of ABI3, as its transcripts are down-regulated in *Mt-abi3* mutants during late seed maturation, and up-regulated when *Mt-ABI3* is overexpressed in hairy roots (Verdier *et al.*, 2013). However, the increase in *Mt-SIP2* transcript level occurs later during development compared with increases in *Mt-ABI3* and *Mt-ABI5* transcripts, suggesting that additional downstream factors might be involved in their regulation. One possibility is the SUCROSE NONFERMENTING-RELATED KINASE COMPLEX (SnRK1), which is known for its central role in energy metabolism, linking stress, sugar, and developmental signals to regulate plant metabolism, energy balance, growth, and stress responses (Baena-Gonzalez and Sheen, 2008). Silencing *SNF4b* expression, a seed-specific regulatory subunit of the SnRK1, resulted in an increase of Suc and decrease of Sta contents during seed maturation, as well as a reduction in seed longevity (Rosnoblet *et al.*, 2007).

The functional role of Suc and RFO in dry seeds remains elusive. Seeds can become desiccation-tolerant without any accumulation of RFO (Black *et al.*, 1999; Hagely *et al.*, 2013). Rather, the increase of RFO during maturation is in parallel with the acquisition of seed longevity (Black *et al.*, 1999; Verdier *et al.*, 2013), although a genetic connection, if any exists, has not been discovered yet. A correlation between longevity and the Suc:RFO ratio or the amount of RFO has been reported, although this relationship does not always hold true (Buitink *et al.*, 2000; Gurusinghe and Bradford, 2001; Horbowicz and Obendorf, 1994; Verdier *et al.*, 2013).

Likewise, galactinol was found to be a marker for seed longevity in *Arabidopsis*, cabbage, and tomato (de Souza Vidal *et al.*, 2016). Yet, in the same study, *Arabidopsis* mutants of *StaS* showed a three-fold increase in galactinol levels, whereas germination after artificial ageing was not affected. The authors suggested that galactinol might reach a threshold level above which longevity no longer increases. However, in soybean and *M. truncatula*, galactinol accumulates transiently, showing that this correlation is not always valid. Further genetic evidence demonstrated a role for RFO metabolism in longevity, as seeds of *Arabidopsis alpha-galactosidase2* mutants exhibited reduced longevity, although only Suc levels were significantly higher in the mutants but not RFO levels (Righetti *et al.*, 2015).

Although the role of RFO metabolism in longevity remains elusive, several studies have made an attempt to understand its function. Protective roles for RFO in the dry state have been surmised from *in vitro* studies, but *in vivo* experimental data have shown that these hypotheses were not correct. One such hypothesized role, recurrently quoted in the literature, is that RFO prevents crystallization in the dry state. This hypothesis comes from the observation that a pure solution of Suc crystallizes when dried, whereas a mixture of different sugars does not. However, the composition of the cytoplasm is so complex that no crystallization of any molecule is likely to ever occur during drying; rather, the cytoplasm is transformed into a glassy state on drying, thereby preventing crystallization (Buitink and Leprince, 2004). A glass is defined as a supercooled liquid with an extremely high viscosity—typically in the order of 10^{14} Pa s⁻¹. The high viscosity and low molecular mobility and diffusion in the cytoplasmic glass play an important role in slowing down detrimental reactions, thus enhancing longevity (Ballesteros and Walters, 2011; Buitink and Leprince, 2004; Walters *et al.*, 2010).

A second hypothesis that was put forward to explain the role of RFO in protection in the dry state is related to oligosaccharides having a higher molecular weight than sucrose. The higher the molecular weight of the sugar, the higher the glass transition temperature (T_g), resulting in earlier glass formation during drying. Since Raf and Sta have a higher T_g than Suc, it was deduced that the RFO:Suc ratio might be linked to longevity. However, decreasing the oligosaccharide composition in primed seeds did not lead to a change in T_g or molecular mobility in the cytoplasm (Buitink *et al.*, 2000). In addition, cytoplasmic glasses do not have the same behaviour as simple sugar mixtures (Buitink and Leprince, 2004). Further studies need to focus on alternative roles for RFO during the establishment of seed longevity, including protection against oxidative damage (Nishizawa *et al.*, 2008). Interestingly, galactinol and RFO also act as signalling molecules following pathogen attack, suggesting a role in biotic stress signalling (Kim *et al.*, 2008). Both *GolS* and *RafS* contain W-box *cis*-elements in their promoters, regulated by ABA-inducible WRKY (Wang *et al.*, 2009). This links RFO metabolism during seed development to biotic defence pathways activated during late seed maturation (see ‘Biotic defence and longevity’).

Chromatin condensation: to protect or regulate?

During seed desiccation, the size of the nuclei markedly reduces and the chromatin undergoes a high but reversible condensation (Fig. 3; van Zanten *et al.*, 2011). This condensation is probably not induced by desiccation *per se* but by a regulated phenomenon controlled by ABI3 (van Zanten *et al.*, 2011). *In vitro*, chromatin extracted from nuclei of vegetative tissues readily decondenses in the absence of salts and denatures upon heating. However, chromatin extracted from dry maize embryo is poorly soluble, fails to decondense *in vitro*, and denatures at a much higher temperature during heating (Leprince *et al.*, 1995). Chromatin extracted from dried desiccation-sensitive germinated radicles also fails to decondense *in vitro*. However, it exhibits different physico-chemical characteristics compared with those of dry desiccation-tolerant seeds, such as increased solubility and decreased denaturation temperature, suggesting that the condensation/decondensation properties are lost in these desiccation-sensitive tissues (Leprince *et al.*, 1995). Taking this evidence together, we speculate that the chromatin acquires intrinsic properties during late seed maturation that enable a reversible condensation/decondensation upon desiccation and imbibition. Chromatin condensation does not prevent transcriptional activities during late seed maturation, since a significant amount of transcripts change during the late phase of desiccation, when the tissue water content falls below 0.4 g H₂O g DW⁻¹ (Verdier *et al.*, 2013). This suggests the existence of specific mechanisms enabling efficient transcription in very dense chromatin. Maintenance of genomic integrity is critical to seeds to prevent mutations before the onset of germination and is associated with seed vigour (reviewed by Waterworth *et al.*, 2015). Highly condensed chromatin that induces high genomic DNA compaction is known to offer increased tolerance against oxidative damage and radiation-induced double-strand breakage (Falk *et al.*, 2010 and references therein). Whether chromatin condensation contributes to seed longevity by protecting the genome integrity in the dry state remains to be investigated.

The developmental regulation of heat-shock proteins: no need for heat

Another aspect of the genetic programme of late maturation is the induction of *HSP* and *sHSP* genes (Fig. 3). Despite the absence of heat stress, *sHSP* transcripts typically accumulate during late maturation (Kotak *et al.*, 2007; Wehmeyer *et al.*, 1996; Wehmeyer and Vierling, 2000). By analogy with their chaperone function in response to heat stress, *sHSPs* are likely to have a protective role in seed longevity (Kaur *et al.*, 2015; Prieto-Dapena *et al.*, 2006). *HSP* gene expression is induced by heat shock factors (HSF), a large and highly diversified family of transcription factors that are the terminal components of the signalling pathway inducing *HSPs* upon stress. During maturation, *HSP* expression is upregulated by a seed-specific HEAT SHOCK TRANSCRIPTION FACTOR9 (HSFA9). HSFA9 is notable because it is exclusively expressed from maturation onwards

to the dry state (Kotak *et al.*, 2007). Overexpression of the sunflower *HaHSFA9* in tobacco seeds led to an increase in HSP101 and a subset of sHSPs. These seeds were more resistant to deterioration when incubated at 50 °C while their moisture content increased to 15%, a so-called controlled deterioration test used to assess seed vigour (Personat *et al.*, 2014; Prieto-Dapena *et al.*, 2006). Likewise, loss of function of *HaHSFA9* obtained by fusion with an SRDX motif, a potent active repressor, induces a drastic reduction in the accumulation of sHSPs and resistance against controlled deterioration. Dry viable transgenic seeds can still be obtained, suggesting that desiccation tolerance is not under the regulation of *HSFA9* (Tejedor-Cano *et al.*, 2010). HSFs can act with accessory HSFs and/or need additional HSFs for their activation during maturation. For instance, tobacco seeds that overexpress both *HaHSFA4a* and *HaHSFA9* show better resistance against controlled deterioration compared with seeds overexpressing *HSFA9* only (Personat *et al.*, 2014). *HaHSFA9* also interacts with the sunflower drought-responsive factor HaDREB2 (DEHYDRATION RESPONSIVE ELEMENT BINDING PROTEIN B2) in a seed-specific manner to enhance stability during controlled deterioration (Fig. 3; Almoguera *et al.*, 2009). During maturation, *HSFA9* is controlled by developmentally regulated ABI3 (Kotak *et al.*, 2007), in interaction with ABI5 and DOG1 (Dekkers *et al.*, 2016). *HSFA9* is also a target of Aux/IAA protein repression (Carranco *et al.*, 2010). The sunflower AUXIN-RESPONSIVE PROTEIN 27 (HaIAA27) directly interacts with *HaHSFA9*, and when expressed in transgenic seeds it represses the transcriptional activation of *sHSP* by *HaHSFA9*. Interestingly, overexpression of an auxin-resistant form of *HaIAA27* leads to increased activity of *HaHSFA9*, suggesting that auxin signalling pathway could be involved in seed longevity (Fig. 3, Carranco *et al.*, 2010). This hypothesis received further support by a gene coexpression network analysis of seed maturation (Righetti *et al.*, 2015). In this network, the genes forming a module associated with longevity are significantly enriched in the *cis*-element AUXIN RESPONSE FACTOR in their promoter region.

Late embryogenesis abundant proteins or late seed maturation proteins: calling a spade a spade

Accumulation of LEA proteins is a landmark of seed maturation (Fig. 4). Plants contain a large number of different LEA genes (up to 51 in Arabidopsis) that are divided into seven groups according to their PFAM domains (Bies-Ethève *et al.*, 2008; Hundertmark and Hincha, 2008). There have been considerable efforts to understand the regulation and function of LEA proteins, and we refer the reader to some of the excellent reviews (Banerjee and Roychoudhury, 2016; Battaglia *et al.*, 2008; Leprince and Buitink, 2010; Tunnacliffe *et al.*, 2010). In this section, we will highlight the role of those LEA proteins that accumulate during (late) seed maturation and discuss their regulation and possible functions.

In *M. truncatula*, 12 LEA transcripts appeared to be exclusively present in seeds, whereas a proteome study detected,

besides these 12 gene products, additional LEA polypeptides that were encoded by six other LEA genes (Chatelain *et al.*, 2012; Fig. 4). The 12 seed-specific LEA proteins belong to different LEA families: LEA_5 (EM1 and EM6), Seed Maturation Proteins (SMP) (D34I, II, and III, PM25), and several members of the LEA_4 group (LEA14-like c, LEAm, SBP65, PM18, PM12, PM10). Homologues of these LEA proteins in Arabidopsis localize in both cytosol and nucleus, whereas PM10 is localized in the vacuole and endoplasmic reticulum, and LEAm in the mitochondria (Candat *et al.*, 2014). A temporal study on the accumulation of the LEA polypeptides showed that a limited number of them accumulated at the acquisition of desiccation tolerance. Rather, their abundance increased further during late maturation (Fig. 4A). Strikingly, most of the LEA polypeptides appeared at the final stages of seed maturation, while their transcripts were detected 10–20 days earlier (Fig. 4B–D). Two interesting conclusions can be made from this analysis: (i) the abundance of most LEA proteins coincides with the final decrease in water content from 1 g H₂O g DW⁻¹ onwards; (ii) the considerable delay between transcripts and proteins suggests a post-transcriptional regulation of LEA protein abundance, coinciding with an over-representation of transcripts involved in post-transcriptional regulation (Verdier *et al.*, 2013). Possibly, this regulation ensures the timely production of LEA proteins to improve seed storability, considering that environmental conditions can rapidly accelerate or delay the time to abscission and final maturation drying. Post-transcriptional regulation was previously reported for EM proteins in Arabidopsis seeds (Bies *et al.*, 1998), but little is known about the underlying mechanisms.

The transcriptional regulation of a number of LEA genes during development has received more attention, and is in part governed by a network of transcription factors including ABI3, ABI4, ABI5, EEL, and DOG1 (Bies-Ethève *et al.*, 2008; Dekkers *et al.*, 2016; Delahaie *et al.*, 2013; Mönke *et al.*, 2012; Reeves *et al.*, 2011). In Arabidopsis, a number of LEA transcripts are reduced in mature mutant seeds of *abi3*, *abi5*, *leafy cotyledon1*, and *fusca3*, although the identity and number of the LEA genes that are deregulated varies for each mutant (Bies-Ethève *et al.*, 2008). A proteomic study of *abi3* mutants from *M. truncatula* showed that all the seed-specific LEA proteins were lower in abundance compared with wild-type seeds, whereas other LEA proteins were not affected (Delahaie *et al.*, 2013). Interestingly, the dehydrin MtDHN3 was more abundant in seeds of Mt-*abi3* mutants compared with wild type (Delahaie *et al.*, 2013), and dehydrin transcripts were also higher in *abi3* seeds of Arabidopsis (Bies-Ethève *et al.*, 2008), suggesting that an intricate regulatory network is set up during maturation to fine-tune the level of LEA proteins. It is noteworthy that a number of LEA genes expressed in seeds are not deregulated in any of the studied mutants (Bies-Ethève *et al.*, 2008), and their regulators remain to be elucidated. In this respect, Verdier *et al.* (2013) identified several candidate transcription factors coregulated with LEA transcripts during seed maturation that deserve further attention.

To our knowledge, only one study has demonstrated that LEA proteins are implicated in seed longevity. In Arabidopsis,

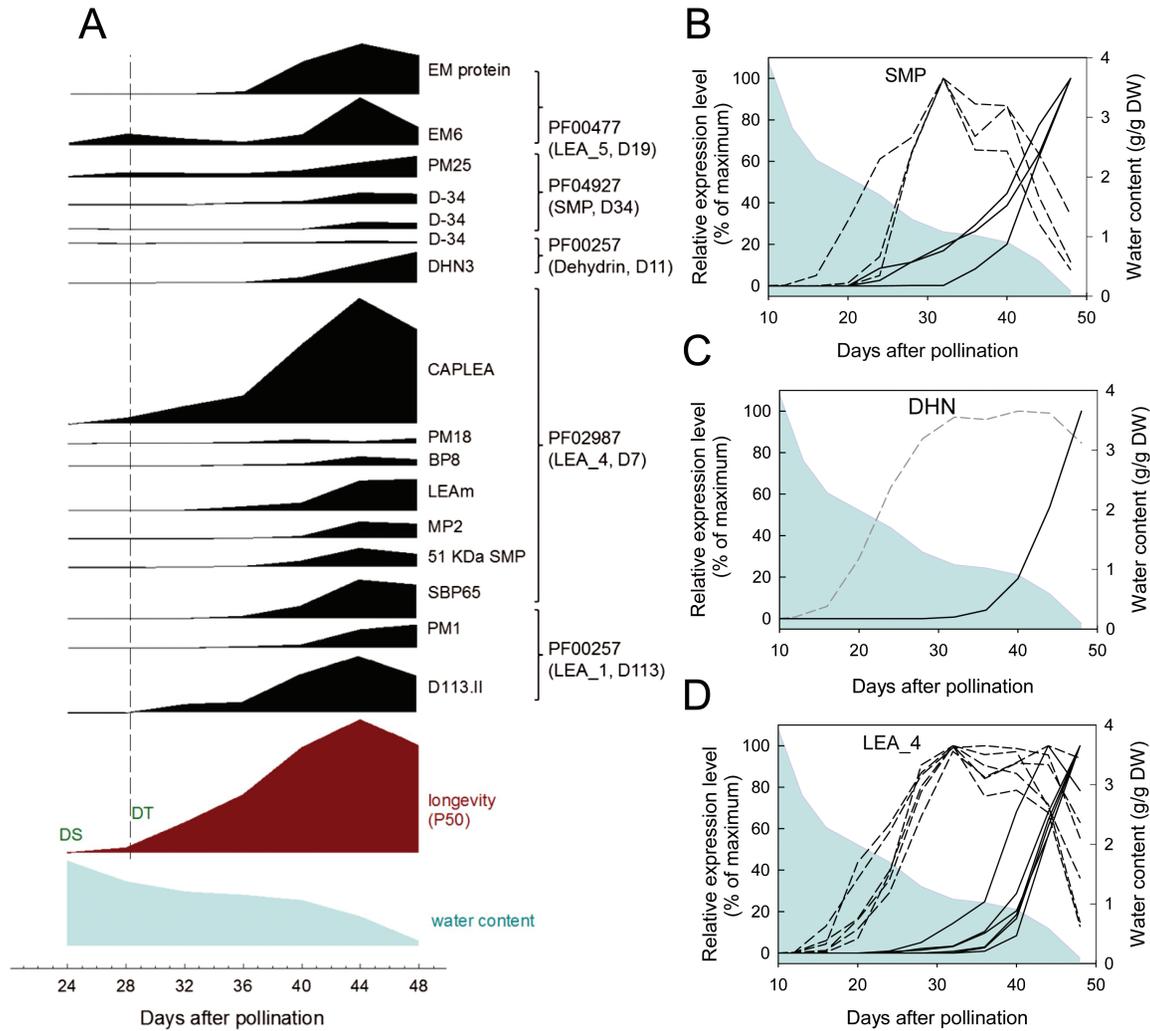


Fig. 4. Changes in the LEA proteome during (late) seed maturation of *M. truncatula* seeds. (A) Accumulation profiles of LEA polypeptides concomitant with the loss of water and increase in seed longevity during maturation. The acquisition of desiccation tolerance (DT) is indicated by a broken line. (B–D) Changes in transcript levels (broken lines) and protein abundance (solid lines) of different families of LEA proteins. The shaded area shows the change in water content during development. Data are from [Chatelain *et al.* \(2012\)](#) and [Verdier *et al.* \(2013\)](#).

the strong down-regulation of three seed-specific dehydrins resulted in a decrease in seed survival during storage ([Hundertmark *et al.*, 2011](#)). In *M. truncatula*, the four most abundant seed LEA proteins correlated with the increase in longevity during maturation ([Fig. 4](#); [Chatelain *et al.*, 2012](#)) and these are therefore putative candidates for further studies. The molecular functions of LEA proteins in seed longevity and why such a diversity exists in dry seeds remain speculative and can only be inferred from *in vitro* studies. LEA proteins are likely to have multiple functions during seed maturation. In the hydrated state they are mainly in an unstructured conformation, whereas upon drying, many LEA proteins readily adopt a more structured conformation. It has been speculated that they exert different functions depending on the water status of the cell ([Boudet *et al.*, 2006](#); [Tunnacliffe *et al.*, 2010](#)). Due to their hydrophilic nature and hydration buffer capacity, certain LEA proteins may participate in the control of water loss during maturation. *In vitro*, MtEM6 was found to bind water much more efficiently at 75% RH compared with the control lysozyme, acting as a molecular sponge. At

low RH, when MtEM6 has adopted a change in conformation, no difference in water absorption was found compared with the control ([Boucher *et al.*, 2010](#)). Consistent with this, *Arabidopsis em6* mutants showed defects in maturation drying ([Manfre *et al.*, 2009](#)). Upon progressive drying, LEA proteins may protect the cells from the resulting decrease in water potential by stabilizing proteins and preventing them from aggregation ([Chakrabortee *et al.*, 2007](#); [Li *et al.*, 2012](#)). They could also buffer an increase in ion concentration, notably Fe^{2+} , a catalyst of ROS production ([Hara *et al.*, 2005](#)). Based on *in vitro* studies showing that dehydrins bind to phospholipids and modulate membrane properties ([Eriksson *et al.*, 2016](#) and references therein), certain LEA proteins could also promote membrane fluidity and integrity. Upon further drying, they could participate in the glass formation in combination with Suc and oligosaccharides ([Ballesteros and Walters, 2011](#); [Buitink and Leprince, 2004](#); [Walters *et al.*, 2010](#); [Wolkers *et al.*, 2001](#)). In support of this hypothesis, combining the LEA proteins D7, MtEM6, or MtPM25 with Suc increased the density of the dry glassy matrix ([Tunnacliffe *et al.*, 2010](#);

Wolkers *et al.*, 2001). The advantage of a denser glass could be improved long-term stability in the dry state by restricting molecular mobility and relaxation.

Biotic defence and longevity: two sides of the same coin

Seed longevity relies on antioxidants, such as tocopherols, glutathione, and secondary metabolites such as flavonoids (see Sano *et al.*, 2016 for review). Defects in the testa structure and composition such as flavonoid pigmentation are known to affect seed storability (Sano *et al.*, 2016). These components also have an important role in defence against pathogens. Using a gene regulatory network of developing *M. truncatula* and Arabidopsis seeds, Righetti *et al.* (2015) found that defence process-related GO categories were highly enriched in a gene module co-regulated with the acquisition of longevity. These authors also showed that mutant seeds of *wrky3* and *nfx11* (*nuclear factor-X1-like1*), two biotic stress-related transcription factors present in the module, exhibit reduced longevity. WRKY3 is a positive regulator of defence against necrotrophic pathogen (Lai *et al.*, 2008). Furthermore, in the *wrky3* mutant, *WRK33*, a regulator of the jasmonate-related defence pathway, is also down-regulated (Righetti *et al.*, 2015). The NFXL1 is involved in salicylic acid-dependent defence responses (Asano *et al.*, 2008). These two mutants display a weakened physical barrier, reflected by increased seed coat permeability. These data suggest that during late maturation, developing seeds activate regulatory defence pathways to set up protective processes ensuring survival in the dry state.

Several molecular players in plant immunity have pleiotropic effects on plant growth and development, pointing to a link between both processes in terms of function and evolution. For example, the seed-specific regulatory subunit SNF4b of the SnRK1 complex plays a role in plant defence in dormant seeds of *M. truncatula* and was also shown to be involved in longevity (Bolingue *et al.*, 2010). The longevity module of *M. truncatula* contains not only *sHSP*, but also *HSP70* and *HSP90*, two chaperones assisting protein folding during abiotic stress responses (Jungkunz *et al.*, 2011; Wang *et al.*, 2016). The HSP90 chaperone machinery is also implicated in immunity, light signalling, and chloroplast biology, as well as in growth and development (Kadota and Shirasu 2012), making it an interesting candidate for connecting chloroplast dismantling and defence during late seed maturation. Mechanisms involved in passive defence also include physical barriers related to thickened cell walls, or substances such as cutin, suberin, or pectin. Interestingly, seeds of mutants with defects in cutin deposition exhibited reduced longevity (De Giorgi *et al.*, 2015). Likewise, the thick layer of β -glucans, which can act as a physical barrier against pathogen infection, also plays a key role in regulating seed coat-imposed dormancy (Leubner-Metzger, 2003).

The connection between plant development and defence has been highlighted by many studies (reviewed in Huot *et al.*, 2014; Whalen, 2005). In seeds, a recent study using the

pathosystem *M. truncatula* and *Xanthomonas campestris* pv. *campestris* (*Xcc*), a Brassicaceae pathogen, shows an active interaction between the presence of the bacterium and the developing seeds at different stages of maturation (Terrasson *et al.*, 2015). These contaminated seeds are able to mount a defence response through a transcriptional reprogramming of the maturation programme. Evidence for a dialogue was also observed in developing soybean seeds, where the expression of pathogenesis-related genes is induced upon infection with *Cercospora kikuchii* or *Diaporthe phaseolorum* (Upchurch and Ramirez, 2011). Interestingly, the activation of a defence response in the *Xcc*-infected developing seeds results in a down-regulation of genes related to seed maturation, such as genes involved in chlorophyll degradation and genes encoding seed storage proteins (Terrasson *et al.*, 2015). As a result, a higher chlorophyll content and lower weight is observed in mature infected seeds compared with the control. Transcripts of major ABA-related signalling transcription factors, *ABI3*, *ABI5*, *bZIP67*, and their target genes are also affected by this interaction. This shows that, like vegetative tissues, upon interaction with pathogens, developing seeds undergo a similar fine-tuning of resources, resulting in a trade-off between seed filling and defence activation.

As well as regulating seed development, ABA interacts in either an antagonistic or a synergistic manner with salicylic acid and jasmonate, two plant defence hormones (Anderson *et al.*, 2004; Mosher *et al.*, 2010). For instance, ABA accumulates in leaves upon infection with *Leptosphaeria maculans* in an ABI1- and ABI4-dependent manner, resulting in callose deposition and induction of *RLM1* (RESISTANCE to *L. maculans*) gene expression. ABI4 appears to have a dual role in this resistance by controlling a feedback loop through its upstream regulator ABI1 (Kaliff *et al.*, 2007). In response to bamboo mosaic virus infection, Arabidopsis mutants in ABA signalling show an opposite defence phenotype. Mutations in *ABA2* positively affect resistance, whereas mutations in the downstream components *ABI1*, *ABI3*, and *ABI4* affect it negatively (Alazem *et al.*, 2014). Whether upregulation of *ABI3* and *ABI4* during the late maturation phase confers immunity against pathogen attack on developing seed merits further investigation.

All these observations illustrate the link between the developmental programme of (late) seed maturation, longevity, and defence. The optimal defence hypothesis proposes that under biotic threat, a plant will defend best those tissues or organs that contribute most to its fitness and/or exhibit a high probability of being attacked by pathogens (Meldau *et al.*, 2012). Thus, the expression of defence-related components during seed maturation could be a programmed mechanism to install passive barriers as a pre-emptive strategy to protect the embryos from pathogen attack, as in the dry quiescent state the seed will not be able to activate defence responses. In turn, these mechanisms will also have an impact on the longevity of the seed. This raises the question as to whether seed longevity evolved by co-opting existing genetic pathways regulating the activation of defence against pathogens, to ensure proper protection during storage in the soil until they are ready to germinate.

The concepts of robustness and plasticity should help understanding of the acquisition of survival in the dry state

The concepts of robustness and the other extreme, sensitivity or plasticity, in developmental biology have been reviewed by Donohue (2003), Félix and Wagner (2008), Lempe *et al.* (2013), and Boukhibar and Barkoulas (2016). Robustness is the persistence of a trait under perturbation. In other words, robustness ensures an invariant phenotype in the presence of considerable ‘noise’, which can be due to environmental or genetic variations. Robustness is essential to keep processes and traits in any living organism within narrow limits (Lempe *et al.*, 2013). In contrast, plasticity is defined as the ability to adjust phenotypes predictably in response to specific environmental stimuli. Phenotypic plasticity, or high phenotypic variation, is a key means to cope with environmental challenge and spark evolutionary innovation. Recognition of the importance of robustness and plasticity will help with the identification of regulatory genes that are responsible for the amplification or restriction of seed traits, not only in relation to the survival in the dry state that is acquired during maturation, but also for other traits relevant for seed vigour (Boukhibar and Barkoulas, 2016).

Desiccation tolerance is a robust trait

In orthodox seeds, the acquisition of desiccation tolerance during development is resilient to both environmental and genetic perturbations. For example, in *Arabidopsis*, the percentage of desiccation tolerance was not altered in seeds obtained from 13 different environments and 12 different genotypes, whereas gene \times environment interactions were found for many other seed traits, such as seed weight, dormancy, or germination under unfavourable conditions (He *et al.*, 2014). The authors’ group also showed that the acquisition of desiccation tolerance occurs at the same developmental age in developing *M. truncatula* seeds regardless of the growth conditions of the parent plant, including drought or heat stress conditions (Righetti *et al.*, 2015). Considering that the acquisition of desiccation tolerance is a prerequisite for species dispersal, the robustness of this trait is probably not surprising, since the dry quiescent state offers a stable state that is tolerant to environmental extremes.

What the underlying molecular mechanisms are that explain how desiccation tolerance achieves robustness remains an open question. This robustness is likely to be due to a complex regulatory network where both redundancy and topology of interactions among genes play an important role (Righetti *et al.*, 2015). Comparison of transcriptomes obtained upon acquisition of desiccation tolerance in different genotypes of *M. truncatula* under several developmental conditions led to the identification of 49 transcription factors with expression profiles associated with the induction of desiccation tolerance (Terrasson *et al.*, 2013). However, except for certain alleles of *abi3*, none of these transcription factors, when mutated, are known to produce seeds that are desiccation-sensitive, suggesting a strong redundancy or the existence of compensation

mechanisms. Indirect evidence for the presence of redundant regulatory factors during maturation comes from studies on the re-induction of desiccation tolerance in desiccation-sensitive germinated radicles upon osmotic or ABA treatment. In *M. truncatula* and *Arabidopsis*, mutants of *abi5* produced desiccation-tolerant seeds during maturation, but desiccation tolerance could no longer be re-established in germinated *abi5* radicles upon osmotic or ABA treatment in contrast to wild type (Maia *et al.*, 2014; Terrasson *et al.*, 2013). Likewise, Costa *et al.* (2015) identified three genes from the ABI3 regulon whose mutants were affected in the re-establishment of desiccation tolerance in germinated seeds, while they produced dry, viable mature seeds. These genes encode a homolog of the LEA protein *ECP63*, *CBSX4*, a regulatory protein sensing adenosine-containing ligands, and *At4g25580*, whose function is unknown. Interestingly, these mutants exhibited a significant increase in their ability to re-establish desiccation tolerance upon osmotic treatment compared to wild type, suggesting the presence of repressors of desiccation tolerance. This redundancy complicates the dissection of the regulatory pathways leading to desiccation tolerance. The use of different physiological models and comparative analyses between sensitive and tolerant species that are closely related might help us move forward in the future (Delahaie *et al.*, 2013).

Longevity exhibits plasticity

In contrast to desiccation tolerance, longevity is increasingly recognized to be under the strong influence of environmental cues perceived by both the parent plant and zygote, showing that this trait exhibits plasticity towards the environment. Environmental cues include temperature, drought, nutrition, and light (Contreras *et al.*, 2008; Ellis *et al.*, 1993; He *et al.*, 2014; Nagel *et al.*, 2015; Righetti *et al.*, 2015). During maturation, they can speed or retard the acquisition of longevity, thereby inducing large variations in the interval between the end of seed filling (mass maturity) and maximum shelf-life (Ellis and Pieta Filho, 1992; Ellis *et al.*, 1993; Righetti *et al.*, 2015). Longevity can also be modulated by processing treatments after harvest before the seeds are totally dry (Probert *et al.*, 2007; Whitehouse *et al.*, 2015 and references therein).

Phenotypic plasticity can be quantified as the slope of the so-called reaction norms, a mathematical function relating the phenotype and the environment. Three examples are shown in Fig. 5. In rice, the conditions in which seeds were dried induced a stronger plasticity response in genotype IRGC117273 compared with genotype IRGC117276 (Fig. 5A, Whitehouse *et al.*, 2015). Environmental conditions can influence seed longevity even before meiosis and seed set, as reported by two fascinating studies on wild species (Kochanek *et al.*, 2011, Mondoni *et al.*, 2014). Fig. 5B illustrates the extent to which the prezygotic growing conditions of the parent plants of *Plantago cunninghamii* affect seed longevity. Parents were grown in suboptimal conditions, then moved to optimal conditions shortly before or after flowering. In each condition, the parental response was passed on to their offspring, whose longevity was affected. When plants were moved shortly before flowering, warm and wet prezygotic

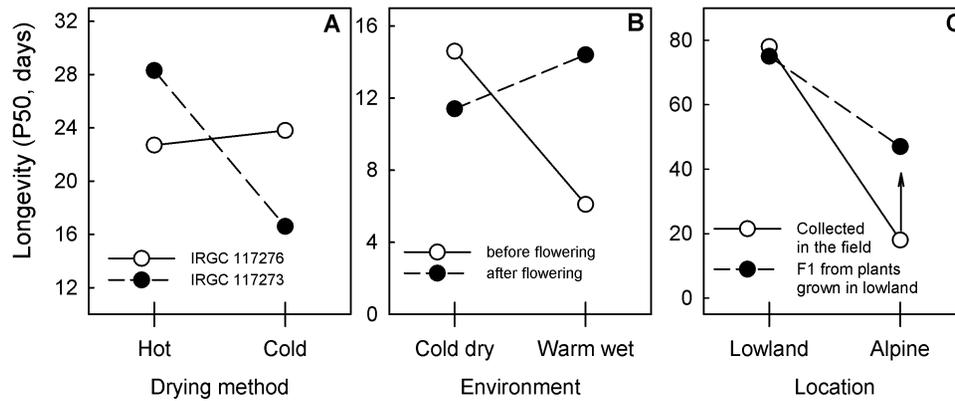


Fig. 5. Reaction norms revealing the plasticity of longevity. Longevity is expressed in P50, the time during storage for viability of the seed population to fall to 50%. (A) Effect of hot air-flow drying (hot) and cold still-air drying (cold) on longevity of harvested seeds of the indicated genotypes of rice. Data are from Whitehouse *et al.* (2015). (B) Effect of parental environment on longevity of *Plantago cunninghamii* seeds obtained from plants grown in cold dry or warm wet climates, then transferred to optimal conditions shortly before or after flowering. Data are from Kochanek *et al.* (2011). (C) Effect of parental habitat on longevity of *Silene vulgaris* seeds obtained from wild plants that were collected in the field in Italy (lowland and Alpine) and from their offspring (F1) that were sown and cultivated in lowland conditions. Data are from Mondoni *et al.* (2014).

conditions were associated with a two-fold decrease in longevity compared with cold and dry conditions, whereas the opposite response was obtained with plants moved after flowering (Fig. 5B). This indicates that the responses to the environment of both maternal and paternal plants contributed to seed longevity, suggesting a role for pollen quality in this process. Whether this suggestion also applies to crops deserves attention in the future. In *Silene vulgaris*, the parental environment also affects longevity with high plasticity (Mondoni *et al.*, 2014). However, adding a new twist to this influence, the authors showed functionally adaptive transgenerational effects on longevity (Fig. 5C). Using two wild populations from Alpine and lowland locations, the authors showed that the parental response to the growing conditions was translated into the longevity of their offspring (Fig. 5C, open symbols). However, when the seeds obtained from parents grown in Alpine conditions were sown and grown in lowland conditions to produce new seed lots, longevity of their offspring increased almost two-fold compared with the first generation (Fig. 5C, arrow). Interestingly, this adaptive response was associated with differential accumulation of *SvHSP17*, which encodes a small heat shock protein putatively associated with longevity (Mondoni *et al.*, 2014). In the future, the influence of the parental environment should be incorporated into studies aimed at deciphering the protective mechanisms conferring seed longevity.

Considering that longevity is essential for the plant cycle and exhibits a plasticity response, one can wonder about the trade-offs between longevity and other seed traits. Longevity trade-offs are suggested by the crossings in norm reactions between genotypes shown in Fig. 5. Recently, a negative correlation between seed longevity and dormancy was observed using six RIL populations of *Arabidopsis*, with low shelf-life correlating with high dormancy levels (Nguyen *et al.*, 2012). Interestingly, there is also a significant correlation between the presence of endosperm and longevity, with endospermic seeds being shorter lived than non-endospermic seeds (Probert *et al.*, 2009). Another interesting question pertains

to the molecular machinery that passes the parental response to the zygote and how it induces the expression of genes and biochemical pathways conferring long-term protection in the dry state. In *Arabidopsis*, *DELAY OF GERMINATION1 (DOG1)* is a likely candidate gene, as *dog1-1* mutant seeds exhibited reduced longevity and lower expression of *LEA* and *sHSP* genes (Dekkers *et al.*, 2016). *DOG1*, whose function is unknown, acts as a thermal sensing mechanism that translates the temperature during seed maturation into abundance of *DOG1* protein in freshly harvested seeds. The amount of *DOG1* protein will subsequently determine the after-ripening requirement to release seed dormancy during storage (Footitt *et al.*, 2015; Kendall *et al.*, 2011; Nakabayashi *et al.*, 2012). However, whether this mechanism is relevant to the regulation of the plasticity response of longevity remains to be investigated.

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