

# Seed desiccation: a bridge between maturation and germination

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**The development of orthodox seeds concludes by a desiccation phase. The dry seeds then enter a phase of dormancy, also called the after-ripening phase, and become competent for germination. We discuss physiological processes as well as gene expression and metabolic programs occurring during the desiccation phase in respect to their contribution to the desiccation tolerance, dormancy competence and successful germination of the dry seeds. The transition of developing seeds from the phase of reserve accumulation to desiccation is associated with distinct gene expression and metabolic switches. Interestingly, a significant proportion of the gene expression and metabolic signatures of seed desiccation resemble those characterizing seed germination, implying that the preparation of the seeds for germination begins already during seed desiccation.**

## Seed development and desiccation

Seed development initiates by a stage of embryogenesis in which the mature embryo develops from a single fertilized cell by a series of processes that are conclusively termed ‘morphogenesis’. Following morphogenesis, developing seeds enter a ‘maturation’ stage, which is also termed the period of ‘reserve accumulation’, and essentially includes reorganization of metabolism and synthesis of storage compounds including starch, storage proteins and oil. Following the period of reserve accumulation, seeds enter the last period of maturation, which has been classically termed ‘maturation drying’ [1] but in more recent studies was more suitably termed ‘desiccation’. This stage is associated with a major loss of water, leading to a dry seed in preparation for a quiescent period and thereafter for germination [2]. The stages of morphogenesis, reserve accumulation and after ripening (also called post-harvest) have been extensively discussed in recent reviews [3–7]. Here we focus on the desiccation stage, which has recently gained renewed attention due to the application of modern genomics and analytical approaches [8,9]. Seed desiccation appears to be more than drying of the seed, but rather an active stage in terms of gene expression and metabolism. This is also reflected in the multiple functions of this state in respect to seed development, acquirement of dormancy, after-ripening and germination. We start by evaluating published genomics and metabolomics data

indicating that the transition from late reserve accumulation into the desiccation stage is associated with transcriptional and metabolic switches. We go on to discuss the biological processes associated with seed desiccation and their influential interconnection with early seed germination.

## Physiology of seed desiccation and the acquirement of desiccation tolerance

The definition of recalcitrant and orthodox seeds was coined in the early 1970s by Roberts [10]. He described recalcitrant seeds are those that are shed with high water content and active metabolism (they deteriorate upon dehydration) and cannot be stored for long periods of time. In contrast, orthodox seeds, which are the focus of the present review, are those that tolerate desiccation and are storable in a dry state for a long period of time, called the ‘after-ripening’ period. Following shedding, the desiccation tolerance of orthodox seeds allows the embryo to: (i) maintain viability for long periods at the dormant stage; and (ii) switch from a dormant to a non-dormant state to allow germination under favorable conditions and in response to endogenous (e.g. hormonal balance and active oxygen species) and exogenous signals (e.g. nitrate, nitric oxide, chilling and light), for review of seed dormancy see [11]. After ripening is species specific and depends on parameters such as moisture, oil *vs* starch content, seed covering structures, and temperature ([12] and references therein). During after-ripening, the seed maintains low level of metabolic activity, which preserves seed viability. In addition, during this period there is a decrease of germination inhibitors, alteration of membranes and protein degradation that improve germination vigor [13]. From an evolutionary perspective, seed desiccation tolerance has been found associated with seeds of plants grown in drier environments, whilst desiccation sensitivity is most common in moist environments. It was also shown that desiccation tolerance in seeds is highly associated with dormancy [14]. The implication of seed desiccation tolerance on plant fitness still remains an open question [14]. It is likely that plants growing in dry habitats have developed a mechanism to delay germination until a colder wet season arrive, which enables efficient germination. The acquisition of desiccation tolerance in orthodox seeds is associated with multiple cellular processes. The main processes, which were also broadly discussed in previous

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**Table 1. Representative mutations negatively affecting desiccation tolerance acquisition.**

Gene name	Gene annotation	Biological process	Phenotype	Refs
<i>LEC1</i> ( <i>Arabidopsis</i> )	LEAFY COTYLEDON	Transcriptional activator of genes required for both embryo maturation and cellular differentiation. <i>LEC1</i> encodes a CBF transcription factor	Reduced sensitivity to abscisic acid (ABA) an intolerance to desiccation	[63,79,80]
<i>LEC2</i> ( <i>Arabidopsis</i> )	LEAFY COTYLEDON	<i>LEC2</i> encode related plant-specific transcription factors containing the conserved B3 DNA binding domain. Regulates FUS and ABI3.	Strong phenotypic variability Chlorophyll accumulation in dry seed (in sectors)	[81–83]
<i>FUS3</i> ( <i>Arabidopsis</i> )	FUSCA	Transcriptional factor with high similarity to the B3 region of the VP1/ABI3-like proteins. Positive regulation of ABA biosynthesis and negative regulation of GA biosynthesis	Intolerance to desiccation No accumulation of anthocyanins. No dormancy	[79,84,85]
<i>ABI3</i> ( <i>Arabidopsis</i> )	ABA INSENSITIVE3	Putative seed-specific transcriptional activator.	Reduced sensitivity to (ABA) An intolerance to desiccation Chlorophyll accumulation in dry seed	[63,79]
<i>ATEM6</i> ( <i>Arabidopsis</i> )	<i>Arabidopsis</i> <i>Thaliana</i> group 1 LEA protein	Water binding at maturation	Premature dehydration of seeds	[86]
<i>AtMYB118</i> ( <i>Arabidopsis</i> )	R2R3-MYB transcription factor	Regulation of genes associated with LEA proteins, storage proteins, and proteins related with seed desiccation and ABA signaling pathway	Premature dehydration of seeds	[87]
<i>OsALDH7</i> ( <i>Oryza sativa</i> )	Rice Aldehyde Dehydrogenase7	Detoxification of ROS induced-(lipid peroxidation associated) aldehydes.	Accumulation of brown pigments at desiccation and storage	[88]
<i>ERA</i> ( <i>Arabidopsis</i> )	ENHANCED RESPONSE TO ABA	Homologous to the maize transcription factor Viviparous-1	Vivipary	[89,90]
<i>VP –class 1</i> ( <i>Zea mays</i> )		Unknown (not ABA)	Vivipary	[91,92]
<i>VP –class 2</i> ( <i>Zea mays</i> )		Early biosynthetic steps of ABA biosynthesis	Mutable aleurone in colored background Reduced or suppressed carotenoid accumulation in seeds	[93]
<i>VP –class 3</i> ( <i>Zea mays</i> )		Synthesis of molybdenum, required in last steps of ABA biosynthesis Oxidative cleavage of carotenoids	Vivipary	[94–96]

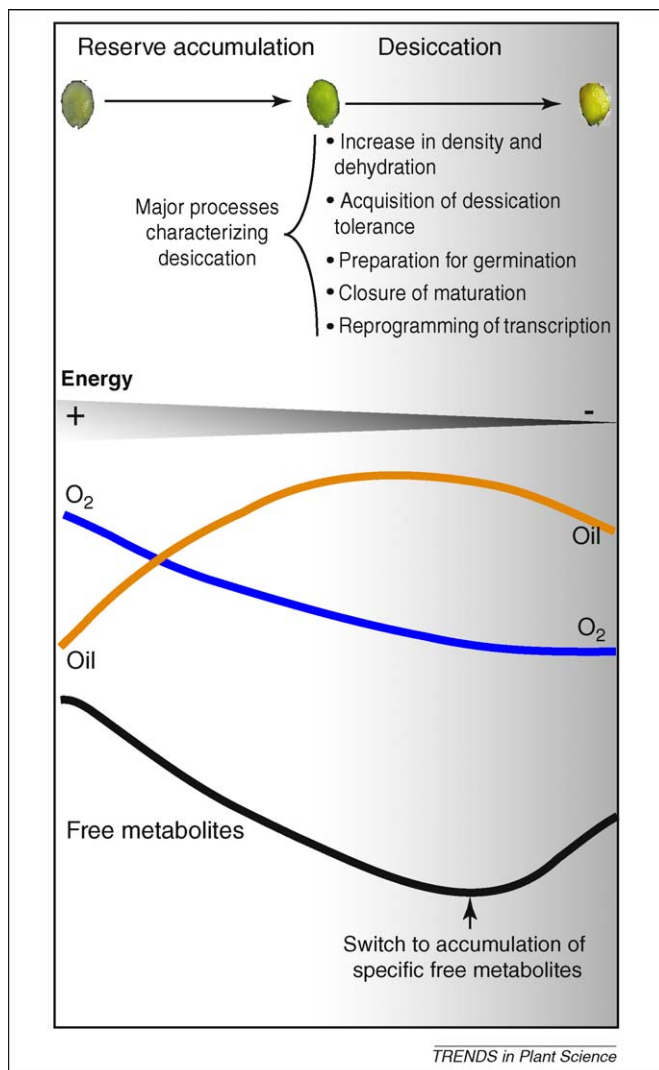
reviews, include the accumulation of disaccharides and oligosaccharides [15,16], synthesis of storage proteins [17], late embryogenesis abundant (LEA) proteins [18–20] and heat-shock proteins [21], activation of antioxidative defenses [16,22,23], changes in the physical structure of the cell [24,25], and a gradual and steady increase in density [26]. Table 1 depicts representative mutations that influence desiccation, and their supposed functions and the phenotypes. It has also been suggested that the variability in desiccation tolerance between different plant species is attributed to the physical structure of the seed internal matrix, which apparently involves interactions between sugar and protein complexes with salts, organic acids and amino acids. [19,26]. Seed desiccation is likely regulated by interplay of hormonal balance, sugar signaling and chromatin remodeling [6,11,27–32].

### Energy and oxygen status during seed desiccation

The struggling for energy and oxygen continues from the period of reserve accumulation into desiccation. Seeds have no stomata, but many have thick seed coats; two factors that significantly limit the uptake of oxygen (O<sub>2</sub>) and its availability for mitochondrial energy production (Figure 1). These hypoxic conditions become quite limiting during the period of reserve accumulation because of the large energy demand to support the synthesis of reserve compounds. In addition anatomical changes of the seed

matrix, due to the accumulation of protein and oil bodies, prevent O<sub>2</sub> penetration [33]. Photosynthesis in green seeds partially relieves the drop in energy in the internal seed layers [34–38], nevertheless, the energy status remains limiting [39,40] due to the bulky structure of the internal part of the seed, limiting oxygen movement, and high metabolic demand. It has been suggested that the contribution of the TCA cycle to ATP biosynthesis varies among green seeds of different plant species [37,41] and also between green and non-green seeds, such as soybean and sunflower seeds [42]. However, the consequence of this variation on seed maturation and plant fitness requires additional studies.

The energy limitation during the period of reserve accumulation becomes even more problematic during seed desiccation because the viscosity and molecular packing density of the cells increase gradually and transform the tissue into a glassy matrix [26,43]. Exposure of soybean seeds to high oxygen shows that late maturing seeds can recover from the low energy status, supporting the notion that desiccating seeds operate a number of energy demanding processes [35]. However, while massive synthesis of storage reserves provides a major demand for energy during the period of reserve accumulation, it is still not clear whether the high demands for energy during seed desiccation are mostly due to the process of drying or other processes. Low internal oxygen levels may be advantageous



**Figure 1.** A summary of major processes that characterize seed desiccation, including a reprogramming of transcription (top section) as well as the trends of changes in energy, oxygen, oil accumulation and the levels of free metabolites that characterize the entire stages of seed maturation (bottom part). With special notes in the bottom part are: (i) the significant decline of O<sub>2</sub> and energy during the period of reserve accumulation and desiccation; (ii) the switch from oil accumulation to oil degradation during seed desiccation; and (iii) the switch from a general reduction of metabolite levels during reserve accumulation to accumulation of some metabolites, which are apparently important to prime germination, during seed desiccation.

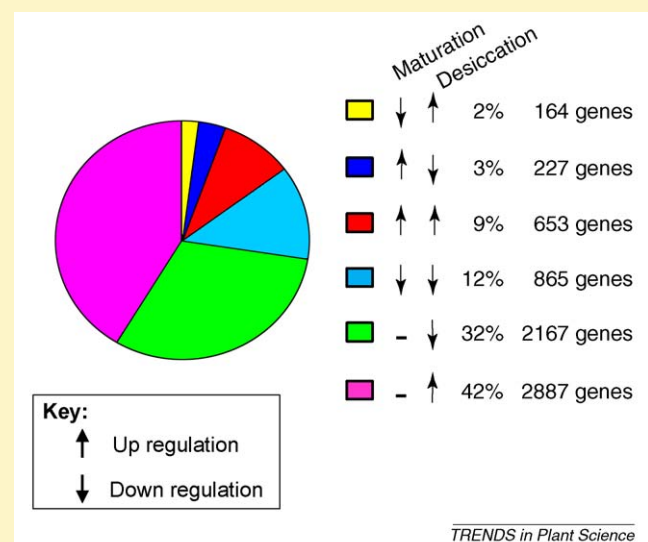
during seed desiccation by alleviating oxidative damage of membranes and enzymes, which would otherwise lead to defects in seed vitality and longevity [33]. Yet, desiccating seeds have an active metabolism and transcription machinery (Figure 1; see next section), and likely require energy for these operations. In addition, the questions of what the sources of energy are that fuel seed desiccation, remain open and require further studies.

### Seed desiccation is associated with distinct transcriptome and metabolome patterns

Even though the term seed desiccation implies a physical process of drying, the transition from the period of reserve accumulation to seed desiccation is associated with massive gene expression changes, which indicate that seed desiccation is also a very active stage in respect to transcription. Transcript profiling analysis of developing *Ara-*

### Box 1. Interpretation of publicly available transcript data with respect to transcriptional switches associated with the transition from the stage of reserve accumulation to the stage of seed desiccation

To review the transcriptional switch associated with the transition from the late stage of reserve accumulation to desiccation stage as well as the characteristics of the desiccation stage itself, we screened an available dataset of transcription profiles of maturing *Arabidopsis* seeds (Series accession number: GSE18112). In this dataset, we identified 6963 genes whose expression level is significantly altered (more than 1.5-fold; after False Discovery Rate (FDR) correction, using the Partek Genomics Suite tool) during the desiccation period by comparing changes in transcript levels occurring between 16 days after flowering (DAF) (approaching the termination of the maturation stage) and dry seeds. This indicates a major activity of gene expression programs in this stage. Next, we attempted to elucidate whether the transition from the period of reserve accumulation to desiccation is associated with a transcriptional switch involving these 6963 genes. To address this, we also analyzed the trends of changes in transcript levels of these 6963 genes occurring during the period of mid-late maturation (14–16 DAF) as a measure of their activity during this period, and further compared it with the trends of changes observed during desiccation (transcript levels in dry seeds versus 16 DAF seeds). Interestingly, as shown in Figure I, only 21% of these 6963 genes were regulated in the same manner in the two stages (12% upregulated and 9% downregulated in both stages; light blue and red squares, respectively). The remaining 79% of the genes were either unchanged during late reserve accumulation and then upregulated or downregulated during desiccation (42 and 32% of the 6963 genes; pink and green squares, respectively) or either suppressed or stimulated during late reserve accumulation and then showing an opposite expression pattern during seed desiccation (2 and 3% of the 6963 genes; yellow and dark blue squares, respectively). These results indicate that the transition from late reserve accumulation to desiccation is associated with a major transcriptional switch.



**Figure I.** A pie chart distribution of genes whose expression is altered during seed desiccation. Genes whose expression level was significantly upregulated or downregulated by more than 1.5-fold during desiccation were grouped according to their expression behavior in the two stages of mid-late maturation (14–16 DAF) and desiccation (16 DAF to dry seeds). Arrows facing up or down represent upregulated or downregulated genes, respectively, at the stages of late reserve accumulation and maturing drying as indicated on the top. The results were retrieved, using the online GEO database (Series accession number: GSE18112).

*bidopsis* (*Arabidopsis thaliana*) seeds at the stage of seed desiccation (Box 1) indicates that the expression of 6963 genes (~30% of the *Arabidopsis* genome) significantly change at this stage. Moreover, the expression change of

only ~21% of these genes already begins during late reserve accumulation and continues the same trend during seed desiccation (Box 1). Nearly 43% of these early-expressed genes are upregulated and include the classical heat shock and Late Embryogenesis Associated (LEA) genes that are associated with quiescence and desiccation tolerance as well as some of the maturation (MAT) genes that are generally associated with seed maturation [31]. The transcriptional suppression of at least some of these MAT genes is mediated by the RY/Sph element repression system, which may also involve the ABI3 transcription factor [44]. Interestingly, suppression of the MAT genes also continues during early seed germination by the VAL1/HSI2 family repressors [44], indicating the presence of regulatory links connecting seed desiccation and early germination.

The transcription profiling analysis (Box 1) indicates that the expression change of the major part of the 6963 genes is specifically associated with the period of seed desiccation. Interestingly, a significant amount of the genes, whose expression declines during desiccation, are associated with biological processes that are generally stimulated during germination. For example, genes associated with the biological processes of glycolysis, some aspects of the TCA cycle, cell wall metabolism, DNA synthesis and transport of amino acids and nucleotides are downregulated during seed desiccation and then upregulated during early germination (Box 2) [45–47]. The biology behind these patterns is still unclear and requires further studies. However, it can be speculated that these processes may have a negative effect on the seed desiccation process, while positively effecting seed germination and are thus

repressed during seed desiccation to enable its optimal operation. Despite the significant transcriptome changes occurring during seed desiccation, it is important to note that parallel transcriptome and proteome studies in *Medicago truncatula* seeds [48] suggest that seed desiccation is also associated with post-transcriptional processes.

Intriguingly, many of the genes that are either upregulated during seed desiccation and/or highly abundant in the stored mRNA population of dry seeds, are associated with biological processes, which may not necessarily contribute to the functions of seed desiccation *per se* (Box 2) [49,50]. These include for example genes encoding: (i) components of the translation machinery, such as ribosomal proteins and translation initiation factors (Box 2) [49,51,52]; (ii) proteases and/or peptidases and proteins related to the ubiquitine and/or proteasome machinery [49]; (iii) proteins associated with energy metabolism; and (iv) proteins associated with DNA repair and lipid degradation [53,54]. Notably, many of the genes associated with these categories were also shown to be either upregulated or essential to seed germination in different species [45,47,53]. It can therefore be speculated that many of the biological processes occurring during seed desiccation serve for seed germination. This hypothesis is also supported by published evidence showing that: (i) the desiccation process plays an important role in switching seeds from the developmental to germination regimes [55,56]; (ii) radicle protrusion during germination requires *de novo* synthesis of proteins, but not *de novo* synthesis of mRNAs, which are likely presented in the stored mRNA population of dry seeds [52,57]; (iii) there is a smooth transition of transcription programs between late seed

## Box 2. Interpretation of publicly available transcript data with respect to biological processes occurring during seed desiccation

To review the biological processes associated with the six groups of genes whose expression is upregulated or downregulated during desiccation (Box 1, Figure 1), we performed an overrepresentation analysis, using the PageMan tool (<http://mapman.mpimp-golm.mpg.de/general/ora/ora.shtml>) on each of them (Figure-Table 1, below). As expected from previous studies, processes associated with desiccation tolerance (heat shock and LEA proteins) are strongly upregulated already during late reserve accumulation and continue to be upregulated (up to ~300-fold) during desiccation. This indicates that the acquisition of desiccation tolerance is a prolonged process that begins before the onset of desiccation and is strongly enhanced along the entire stage of desiccation. Additional processes that are upregulated during desiccation are associated with energy metabolism (TCA cycle/organic acid transformation; tetrapyrrole synthesis; oxidative pentose phosphate pathway; degradation of branch chain amino acids; photosynthesis), nucleic acid metabolism and repair, as well as protein metabolism and targeting (mainly ribosomal proteins of mitochondria and plastids). Interestingly, many of the enriched processes occurring during desiccation seem to be related to biological processes occurring during germination. This implies that some transcription programs of germination may already be initiated during desiccation, exposing a major functional link between these two stages. Whether these processes are essential for germination vigor is an interesting question for future studies. The PageMan analysis also indicates that many genes are also downregulated during seed desiccation. These genes are associated with processes involving primary and secondary metabolism, hormone metabolism and synthesis of storage compounds (storage proteins and oil). For example, genes encoding biosynthetic enzymes of fatty acids are severely downregulated by up to 50-folds during seed desiccation. Many of these downregulated processes occurring during desiccation

apparently represent the termination of seed maturation. Yet, we also hypothesize that the attenuation of at least some of these metabolic processes may promote seed germination.

Interestingly some categories appear in more than one section and those apparently contain either different sub categories or different genes of the same categories. Three interesting examples are given below. The category 'Abiotic stress' appears in both sections of 'Downregulated in desiccation' and 'Upregulated during maturation & desiccation'. Yet, these categories include different sub-categories, namely the sub-categories of drought and salt stresses are downregulated and the sub-category of heat stress is upregulated. The increased expression of heat shock genes during desiccation is important for the physiology of this stage. The reason for the downregulation of genes associated with drought and salt stress during this period is yet unknown, but may be related to the desiccation tolerance of the tissue. The category of 'Amino acids' appears in the three sections of 'Downregulated only during seed desiccation', 'Downregulated during seed maturation and desiccation' and 'Downregulated and upregulated during seed maturation and desiccation, respectively'. However, its sub-categories reveal a differential regulation of amino acid degradation. It seems that the degradation of Gly and Pro is downregulated, whereas degradation of branch chain amino acids is upregulated during desiccation. It is possible that Gly and Pro are important for germination and are therefore retained during desiccation, while the enhanced degradation of branch chain amino acids during desiccation may contribute to the energy status of the desiccating seed as well as early germination periods. Finally, the category 'Protein synthesis' appears in the two sections of 'Upregulated only during seed desiccation' and 'Downregulated and upregulated during seed maturation and desiccation', and may be associated with different genes.





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**Figure 1.** Functional categories that are overrepresented in the six groups described in Box 1, Figure 1. Overrepresentation analysis was performed by PageMan tool (<http://mapman.mpimp-golm.mpg.de/general/ora/ora.shtml>). Only significant functional categories with more than two genes are shown. Abbreviations: CHO, Carbohydrate; FA, Fatty acids; LEA, Late embryogenesis abundant; OPP, Oxidative pentose phosphate; Misc, Miscellaneous; and PGM, phosphoglycerate and/or bisphosphoglycerate mutase.

maturation and germination within the embryo, but not in the endosperm and/or aleurone of barley (*Hordeum vulgare*) seeds, indicating that the initiation of some germination programs begin already in the desiccating embryos [47]; and (iv) post-translational processes occurring during germination, such as oxidation of disulfide bonds in metabolic enzymes, have been suggested to play a major regulatory role in the activation of proteins that were accumulated already during seed desiccation [48].

In addition to the massive transcriptome changes, the transition from late reserve accumulation to seed desiccation is also associated with a distinct metabolome switch [46]. The levels of several free metabolites, including sugars (e.g. sucrose, raffinose, galactinol and trehalose), secondary metabolites (tocopherols and flavonoids),  $\gamma$ -aminobutyric acid (GABA), the TCA-cycle intermediates fumarate and succinate, some amino acids and free fatty acids, accumulate specifically during seed desiccation

[3,23,46,54,58,59]. Among the sugars, raffinose-family oligosaccharides (RFO) have been traditionally associated with desiccation tolerance and seed longevity [25,48,59,60]. RFO are suggested to protect cellular integrity during desiccation by stabilizing membranes during dehydration [61] and provide substrates for energy generation during germination [62], and are also part of the scavenging machinery of hydroxyl radicals [58]. However, RFO composition in desiccating seeds changes significantly in nature [59] and its role in contributing to the glassy matrix and to sustaining germination has recently been challenged [63,64]. Increase in trehalose has also been associated with acquiring of desiccation tolerance [65] as well as being involved in the regulation of germination [66]. The flavonoids provide a chemical barrier against infection by fungi due to their antimicrobial properties [67] and also decrease permeability to solutes, thus limiting damage during early germination [68]. Flavonoids in

legume seeds have also been related to the establishment of symbiotic interactions upon germination [69]. Tocopherols are lipophilic antioxidants that limit non-enzymatic lipid oxidation during seed desiccation, storage, and early germination stages [70–73]. Desiccating seeds also accumulate several amino acids, including the non-protein amino acid GABA, whose levels decline rapidly during very early seed germination. The rapid decline of GABA may assist in fueling the TCA cycle through the GABA shunt [74]. The accumulation of amino acids during seed desiccation is associated with upregulation of specific transcripts involved in amino acid biosynthesis [46]. The evidence for an active biosynthesis of free amino acids during desiccation is also supported by a proteome study of dry *Lotus japonicus* seeds [75], where the authors show an enrichment in the functional category of amino acid biosynthesis. A number of the amino acids that are synthesized during seed desiccation decrease during early seed germination [46] apparently to support translational processes occurring during early germination [52,56,76]. Another piece of evidence for the high potential of desiccating seeds to synthesize amino acids is provided by the analysis of a seed-specific high-Lys mutant [77]. Although the expression of the Lys biosynthesis enzyme in this mutant already begins during the early stages of seed maturation, the major boost of free Lys accumulation occurs during seed desiccation [77].

Another interesting characteristic of seed desiccation is a degradation of ~15% of the oil that is synthesized during the period of reserve accumulation (Figure 1) [3,54]. Oil degradation during the desiccation period apparently occurs by  $\beta$ -oxidation because seed desiccation is also associated with induction of genes associated with  $\beta$ -oxidation (isocitrate lyase) as well as increased levels of potential products of  $\beta$ -oxidation, succinate and fumarate (Box 2) [46]. It has been hypothesized that oil degradation provides fatty acid degradation products to support energy production during seed desiccation [78] as well as during early germination [46].

### Conclusions and future prospects

The processes that characterize seed desiccation reflect the diverse functions of this stage, such as (i) conclusion of seed maturation; (ii) buildup of protection mechanisms to sustain dehydration and quiescence; and (iii) preparation for germination (Figure 1). The commitment of seed desiccation to prepare for germination initiates multiple changes in transcriptional, post-transcriptional and metabolic processes. Some of the transcriptional and metabolic processes associating seed desiccation with germination already commence during seed desiccation, while others are initiated during germination.

Elucidation of the magnitude of these processes and their relative contribution to germination needs further research. Among other issues awaiting future studies are the integration of all the transcriptome, proteome and metabolome data during seed desiccation. In addition, many of the physiological aspects of seed desiccation, such as its energy demand and the energy sources fueling this stage, are still unclear and require further research.

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