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RESEARCH PAPER

Seed comparative genomics in three coffee species identify desiccation tolerance mechanisms in intermediate seeds

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Abstract

In contrast to desiccation-tolerant 'orthodox' seeds, so-called 'intermediate' seeds cannot survive complete drying and are short-lived. All species of the genus *Coffea* produce intermediate seeds, but they show a considerable variability in seed desiccation tolerance (DT), which may help to decipher the molecular basis of seed DT in plants. We performed a comparative transcriptome analysis of developing seeds in three coffee species with contrasting desiccation tolerance. Seeds of all species shared a major transcriptional switch during late maturation that governs a general slow-down of metabolism. However, numerous key stress-related genes, including those coding for the late embryogenesis abundant protein EM6 and the osmosensitive calcium channel ERD4, were up-regulated during DT acquisition in the two species with high seed DT, *C. arabica* and *C. eugenioides*. By contrast, we detected up-regulation of numerous genes involved in the metabolism, transport, and perception of auxin in *C. canephora* seeds with low DT. Moreover, species with high DT showed a stronger down-regulation of the mitochondrial machinery dedicated to the tricarboxylic acid cycle and oxidative phosphorylation. Accordingly, respiration measurements during seed dehydration demonstrated that intermediate seeds with the highest DT are better prepared to cease respiration and avoid oxidative stresses.

Keywords: *Coffea*, dehydration, desiccation tolerance, intermediate seeds, late maturation, metabolic quiescence, respiration, seed development, transcriptome.

Introduction

Desiccation tolerance (DT) is the ability of an organism or tissue to withstand removal of intracellular water whilst retaining structural integrity and viability, and then to resume normal metabolism upon rehydration (Crowe *et al.*, 1992; Leprince and Buitink, 2015). The vast majority of flowering plants produce seeds that are desiccation tolerant. In a dry quiescent

Abbreviations: DEG, differentially expressed gene; DS, desiccation sensitivity; DT, desiccation tolerance; ST, stage.

state at maturity, these seeds are endowed with an exceptional capacity to endure extreme adverse environmental conditions after dispersal (Sano *et al.*, 2016). These so-called 'orthodox' seeds can survive *ex situ* storage for very long periods under conventional gene-bank conditions (Li and Pritchard, 2009). DT is a complex trait that is mostly genetically determined in the developing seed and is put in place during the late maturation program. This is achieved through the interplay of multiple cellular protectants and repair mechanisms designed to cope with the various desiccation-associated stresses including oxidation, hyperionicity, mechanical strain associated with cell shrinkage, protein misfolding/aggregation and phase transition of lipid membranes (Crowe *et al.*, 1992; Leprince and Buitink, 2015). Coordinated repression of metabolism, stabilization of membranes and proteins against conformational change by chemical and protein chaperones, and the accumulation of efficient antioxidant, detoxication and repair systems appear to be among the essential attributes that contribute to seed DT (Hoekstra *et al.*, 2001; Buitink and Leprince, 2008; Leprince and Buitink, 2010). Some of these protective mechanisms are shared across eukaryotic kingdoms since the accumulation of chemical and protein chaperones such as non-reducing sugars (e.g. trehalose), heat-shock proteins, and intrinsically disordered proteins (e.g. late embryogenesis abundant (LEA) proteins) have been recently confirmed to be of tremendous importance in conferring DT in unicellular yeasts, tardigrades, and nematodes (Erkut *et al.*, 2011; Tapia *et al.*, 2015; Boothby *et al.*, 2017). The repertoire of DT-related genes, including key transcriptional regulators, has been extensively studied in orthodox seeds through transcriptome profiling and co-expression analyses during the acquisition of desiccation tolerance (e.g. Verdier *et al.*, 2013; Righetti *et al.*, 2015; González-Morales *et al.*, 2016; for a review see Leprince *et al.*, 2017).

Estimates suggest that about 8% of the world's flowering plants (>40 000 species) produce desiccation-sensitive short-lived seeds (Tweddle *et al.*, 2003; Wyse and Dickie, 2017). Two other categories of seeds—intermediate and recalcitrant—have been defined with respect to their storability in gene banks (Roberts, 1973; Ellis *et al.*, 1990) and these terms are now used to describe their sensitivity to drying. Although desiccation tolerance may vary considerably within each of these two seed categories (Dussert *et al.*, 1999; Berjak and Pammenter, 2008), it is commonly acknowledged that recalcitrant seeds are extremely sensitive to dehydration and do not survive if dried to about 90% relative humidity (RH), while intermediate seeds are able to withstand enforced drying to about 30% RH (Black and Pritchard, 2002). Seeds of many major tropical crops are recalcitrant (e.g. cocoa, coconut, rubber tree) or intermediate (e.g. coffee, tea, oil palm, citrus), which represents a major constraint for growers, seed companies, and germplasm repositories (Li and Pritchard, 2009; Walters *et al.*, 2013).

Albuminous seeds of *Coffea* species are the model system for the 'intermediate seed' category (Ellis *et al.*, 1990; Dussert *et al.*, 1999). As in all fleshy fruits, coffee seeds are not subjected to dry ambient air during maturation (Dussert *et al.*, 2000). Partial DT is acquired without dehydration *in planta* during the late stage of maturation, well after the onset of reserve deposition in the cellular endosperm, a living tissue that represents more than 98% of the mature coffee seed mass (Dussert *et al.*, 2018). The desiccation-induced loss of seed viability is due to damage to the endosperm only, the embryo being much more tolerant to desiccation (Dussert *et al.*, 2006). Transcriptomic analysis of developing *C. arabica* seeds revealed that the cellular and regulation processes that occur during late maturation in the coffee seed are strikingly similar to those known or thought to be involved in orthodox seed DT (Dussert *et al.*, 2018). Briefly, acquisition of DT coincides with a dramatic transcriptional switch characterized by the repression of genes involved in the cell cycle, DNA processing, and primary metabolism, and the up-regulation of a large number of genes coding for proteins involved in cell protection and rescue, including reactive oxygen species (ROS)-scavenging enzymes, LEA proteins and heat-shock protein stress proteins, defense-, cold-, and drought-induced proteins, as well as many components of sugar and nutrient sensing systems. The DT-associated transcriptional switch also had the signature of a coordinated metabolic slow-down, with a marked decrease in the mRNA abundance of genes involved in protein fate and major energy processes such as photosynthesis, the tricarboxylic acid (TCA) cycle, respiratory electron transport, ATP synthase, and glycolysis. With regard to phytohormones, there was an up-regulation of genes for abscisic acid (ABA) synthesis, perception, and signaling during the late maturation and a drastic down-regulation of cytokinin biosynthetic genes associated with a large drop in cytokinin content. Finally, major transcription factors (TFs) homologous to those identified as associated with DT in orthodox seeds have been described as massively up-regulated during DT acquisition in coffee seeds, namely PLATZ, DOGL4, HSFA9, and DREB2G (Prieto-Dapena *et al.*, 2006, 2008; Righetti *et al.*, 2015; González-Morales *et al.*, 2016). The late maturation program described in coffee seed is therefore qualitatively comparable to that of orthodox seeds. This led to the hypothesis that the differences observed in DT between orthodox and intermediate seeds, and between intermediate seeds displaying different DT levels, could be due to a quantitative variation in gene expression and metabolite accumulation. In the present work we therefore compared the seed maturation transcriptomes of three closely related *Coffea* species (Cenci *et al.*, 2012) that were previously shown to differ in their DT (Dussert *et al.*, 1999): *C. arabica*, *C. eugenioides*, and *C. canephora*.

Material and methods

Plant material

Seeds at three different maturation stages known to span the acquisition of desiccation tolerance in *C. arabica* (ST5, ST6, and ST7, Dussert *et al.*, 2018) were collected from three trees of each species from the Biological Resource Center *Coffea*, Bassin Martin, Reunion Island. The average seed development duration being different for the three studied species, i.e. respectively ca. 7, 8, and 10 months for *C. arabica*, *C. eugenioides*, and *C. canephora*, respectively (Dussert *et al.*, 2000). The developmental stages were selected based on marked anatomical and morphological seed and fruit traits that are shared across coffee species, as defined and described previously for *C. arabica* (Joët *et al.*, 2009; Dussert *et al.*, 2018). Briefly, stage 5 is the peak of reserve deposition and corresponds to endosperm hardening due to massive deposition of galactomannans in cell walls, stage 6 coincides with fruit veraison, and stage 7 corresponds to mature

cherry fruits with red pericarp. After being cross-sectioned, the seed was separated from the pericarp and immediately frozen in liquid nitrogen and stored at −80 °C. The endosperm was separated from the perisperm and the embryo while frozen. To minimize the impact of genotypic effect and facilitate inter-species comparisons, developing seeds were collected (pools of ca. 50 seeds) on different wild accessions for each species (*C. arabica* accessions AR28-06, AR02-06, AR38b/05; *C. eugenioides* accessions DA71, DA78, DA78c; *C. canephora* accessions BD55, BD56, DAF71), and were considered biological replicates.

Desiccation tolerance assays and respiration measurement during desiccation

Mature seed lots (50 seeds) of *C. arabica*, *C. canephora*, and *C. eugenioides* were desiccated by equilibration over various saturated salt solutions (K-acetate (23% RH), K_2CO_3 (45% RH), NH_4NO_3 (62% RH), and $(NH_4)_2SO_4$ (81% RH)) for 20 d at 27 °C in the dark, as previously described (Dussert *et al.*, 2000). For germination tests, batches of nine seeds were placed on 18 g of vermiculite fully imbibed with 50 ml of sterile water in closed plastic boxes (Magenta, Chicago, IL, USA). After 6 weeks of culture at 27 °C in the dark, successful seed germination was scored (emergence of the hypocotyl and radicle geotropic growth). Desiccation sensitivity was quantified using the previously developed quantal response model (Dussert *et al.*, 1999). For respiration measurements, fresh ST7 mature seeds were desiccated at 25 °C by equilibration over a saturated K_2CO_3 solution. During 4 d of desiccation, subsamples of four to five seeds were taken every 12 h for respiration measurement. For each replicate, seeds (the fresh weight was recorded) were placed in individual 10 ml hermetically sealed vials at 25 °C. CO_2 release (µmol CO_2 min^{-1} g^{-1} DM) was measured at 5 min intervals for 25 min by gas chromatography with an Agilent M200 apparatus (SRA, Marcy l'Etoile, France). Variations in gas concentration were corrected using the volume of the seeds in the vial.

Transcriptome analysis

For each of the 27 samples of the experimental design, a mix of >20 endosperms was ground to a fine powder in an analytical grinder (IKA A10, Staufen, Germany) and total RNA was extracted from 70 mg using the Qiagen RNeasy Lipid Tissue kit (Qiagen, Stanford, CA, USA). cDNA libraries were constructed using the TruSeq™ Stranded mRNA sample preparation kit (Illumina, USA) then sequenced on an Illumina HiSeq 2500 (single reads, 100 nt) on the MGX platform (Montpellier Genomix, http://www.mgx.cnrs.fr/). After quality filtering using Cutadapt (quality score>Q30 and removal of reads shorter than 60 bp or higher than 140 bp), a total of 875 million reads were retained (average of 31.95 million reads per library) (Supplementary Table S1 at *JXB* online). The entire dataset has been deposited at the European Nucleotide Archive (ENA) under the project number PRJEB32533. Owing to the very low genetic divergence between the three *Coffea* species (average of 1.3% gene sequence difference; Cenci *et al.*, 2012), the trimmed reads (cutadapt, Genome Analysis Toolkit) of each library were mapped to the *C. canephora* coding transcriptome DNA reference sequence (25574 CDS) (Denoeud *et al.*, 2014) using BWA MEM (Li, 2013, Preprint) with the default parameters. Reads were counted using IDXstats in SAMtools (Li *et al.*, 2009) and counts normalized (RPKM; Supplementary Table S2). A total of 628 million reads were mapped (average of 23.3 million reads per library). Genes with extremely low expression (<20 counts in total) were removed for subsequent analyses (22 503 retained genes).

Differential expression analysis was performed using the DESeq2 package with default parameters (version 1.14.1, Love *et al.*, 2014) in R (version 3.3.2). Hierarchical clustering of species stages was calculated using the Euclidean method after read count normalization of each sample. Each species-stage was contrasted to each other (36 pairwise comparisons) and *P*-values were adjusted using the Benjamini–Hochberg method (Supplementary Table S3). Combinations of Boolean operators were used as filters of differentially expressed genes (DEGs) to define different categories of DT- and desiccation sensitivity (DS)-related candidate genes (Table 1). Each candidate gene was manually curated, taking into account recent relevant literature and the predicted conserved domains (www.ncbi.nlm.nih.gov/Structure/cdd), and assigned to a functional group. Gene ontology (GO) annotation was performed using Blast2GO software (Götz *et al.*, 2008) with default parameters and Mapman annotation (Klie and Nikoloski, 2012) was performed using Mercator (Lohse *et al.*, 2014). Fisher's exact test was used to evaluate the significance (*P*-value<0.05) of term enrichment in each category of candidates.

Proteome analysis

Soluble proteins were extracted from 500 mg of ground mature coffee endosperms by suspension and stirring in 10 ml buffer (50 mM HEPES pH 8.0, 1 mM EDTA, 5 mM DTT, 4% polyvinylpolypyrrolidone, 700 units of benzonase endonuclease, and 1% protease inhibitor cocktail) for 40 min at 4 °C. After centrifugation, proteins were precipitated from supernatant at 4 °C with trichloroacetic acid (10% v/v), washed twice with acetone, and air dried. Proteome analysis included protein fractionation by one-dimensional SDS-PAGE sliced into four bands, tryptic digestion, and analysis of samples by nano-LC-MS/MS using a QExactive Plus (ThermoFisher Scientific Inc), as described by Geiger *et al.* (2015). Raw files were analysed using Maxquant 1.5.5.1 on the predicted *C. canephora* peptide database (25 574 peptides, http://coffee-genome.org/) with a protein and peptide false discovery rate <1%, as described in Chen *et al.* (2019). Difference in protein abundance between species was tested by one-way ANOVA and *post hoc* Tukey test. Label-free data have been deposited at ProteomeXchange under the project number PXD015806.

Table 1. Categories of DT-related candidates and the filters used for each category

Cluster		Filters[a]						*n*	Uniques	Candidates
1	a	A5<A6	E5<E6	C6<A6	C6<E6	C5<A6[b]	C5<E6[b]	49	17	C1=150
1	b	A5<A7	E5<E7	C7<A7	C7<E7	C5<A7[b]	C5<E7[b]	133	101	
2	a	A5>A6	E5>E6	C6>A6	C6>E6	C5>A6[b]	C5>E6[b]	111	33	C2=292
2	b	A5>A7	E5>E7	C7>A7	C7>E7	C5>A7[b]	C5>E7[b]	259	181	
3	a	C5<C6	C6>A6	C6>E6	C6>A5	C6>E5		180	57	C3=571
3	b	C5<C7	C7>A7	C7>E7	C7>A5	C7>E5		514	391	

Cluster C1 is designed to comprise genes that are up-regulated during late maturation in both *C. arabica* and *C. eugenioides* desiccation-tolerant seeds, and whose expression is concomitantly lower in desiccation-sensitive *C. canephora* seeds (key positive effectors of DT). Cluster C2 groups genes that are down-regulated during late maturation in both *C. arabica* and *C. eugenioides* desiccation-tolerant seeds, and whose expression is concomitantly higher in desiccation-sensitive *C. canephora* seeds (negative effectors of seed DT). Cluster C3 is the opposite of C1; it includes genes that are up-regulated during late maturation in desiccation-sensitive *C. canephora* seeds, and whose expression is concomitantly lower in both *C. arabica* and *C. eugenioides* seeds. C3 thus comprises late maturation genes specific to a desiccation-sensitive context.

[a] An adjusted *P*-value cut-off of 0.05 was used during the filtering.

[b] For these filters a *P*-value cut-off was not imposed.

Hormone analysis

Hormones in freeze-dried powder of endosperms (50 mg) were analysed by National Research Council, Canada. ABA and ABA metabolites, cytokinins, auxins, and gibberellins were quantified by ultra-performance liquid chromatography–electrospray ionization–tandem mass spectrometry as previously described (Chiwocha *et al.*, 2003).

Promoter cis-element analysis

The 1000 bp sequences upstream of the start codon of the candidate genes and of a random selection of 500 *C. canephora* genes (control genes) were collected from the Coffee Genome Hub (coffee-genome.org) and submitted to the Genomatix MatInspector tool (Cartharius *et al.*, 2005). The presence of seed and stress-related plant *cis*-elements was determined using the default parameters of MatInspector. The resulting contingency tables were submitted to enrichment analysis (hypergeometric test in R) by comparing the candidate gene *cis*-elements by cluster with those found in the control genes.

Results

The transcriptional program associated with late seed maturation shares common essential features among the different coffee species

Mature seeds of *C. eugenioides* and *C. arabica* were tolerant to relatively intense dehydration, with almost no loss of viability noticed when dried up to 23% RH (Fig. 1A). For both species, the equilibrium relative humidity at which 50% of the initial viability was lost, RH_{50}, was lower than 10%. By contrast, *C. canephora* seeds displayed significantly higher desiccation sensitivity. They could only withstand mild drying without noticeable loss of viability, i.e. drying at 62% RH, and did not survive drying at 23% RH. The equilibrium RH_{50} estimated for *C. canephora* was around 50%. At stage (ST) 5, most seeds of the three species were already able to germinate and to develop into normal seedlings but displayed high mortality upon drying, at both 45% and 62% RH (Fig. 1B). The seeds of the three studied species acquired most of the capacity to be dried to 62% RH between ST5 and ST6 (Fig. 1B), demonstrating a conserved phenological sequence among the three coffee species for partial DT acquisition.

DT acquisition was concomitant with a major transcriptional switch observed during late maturation in all three species (Fig. 2A). When species were analysed separately, most transcriptional changes appear to occur in the transition between the ST5 and ST6 with 2837, 3622, and 4109 DEGs identified in *C. eugenioides*, *C. arabica*, and *C. canephora*, respectively (11.3–16% of the reference genes). The ST5–ST7 comparison, i.e. taking the whole late maturation phase into account, identified a larger number of DEGs than the ST5–ST6 transition, specifically 6285, 5506, and 5535, in *C. canephora*, *C. arabica*, and *C. eugenioides*, respectively (21.5–24.6% of the reference genes). By contrast, the late maturation stages ST6 and ST7 were transcriptionally similar, with very few DEGs between them (16, 8, and 82 DEGs in *C. canephora*, *C. arabica*, and *C. eugenioides*, respectively). It is worth noting that down-regulated genes represented a slightly higher fraction than the up-regulated genes in these comparisons. Hierarchical clustering analysis of the global expression dataset grouped the vast majority of ST5 samples on one major branch of the dendogram (group I, Fig. 2B), while late stages ST6 and ST7 grouped together, by species, on another branch (group II). This suggests a close transcriptional program between the three species, with 2533 genes being commonly up- or down-regulated in the three coffee species (Fig. 2C; Supplementary Table S4).

Mapman-based DEG enrichment analysis showed a clear overview of molecular processes involved in the late

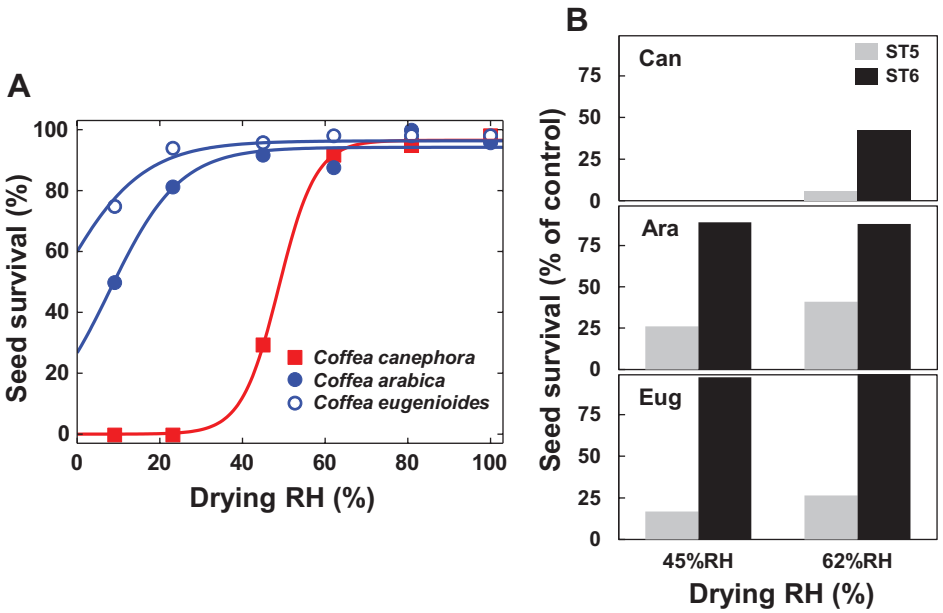

Fig. 1. Characterization of seed desiccation tolerance in the three coffee species, *Coffea arabica*, *C. canephora*, and *C. eugenioides*. (A) Survival rate of mature (stage 7) coffee seeds after equilibration drying at various relative humidity (RH) conditions. The curves correspond to the fitted patterns using the quantal response model described in Dussert *et al.* (1999). (B) Changes in viability (%) after equilibration drying at various RH conditions during seed development (stage 5–6).

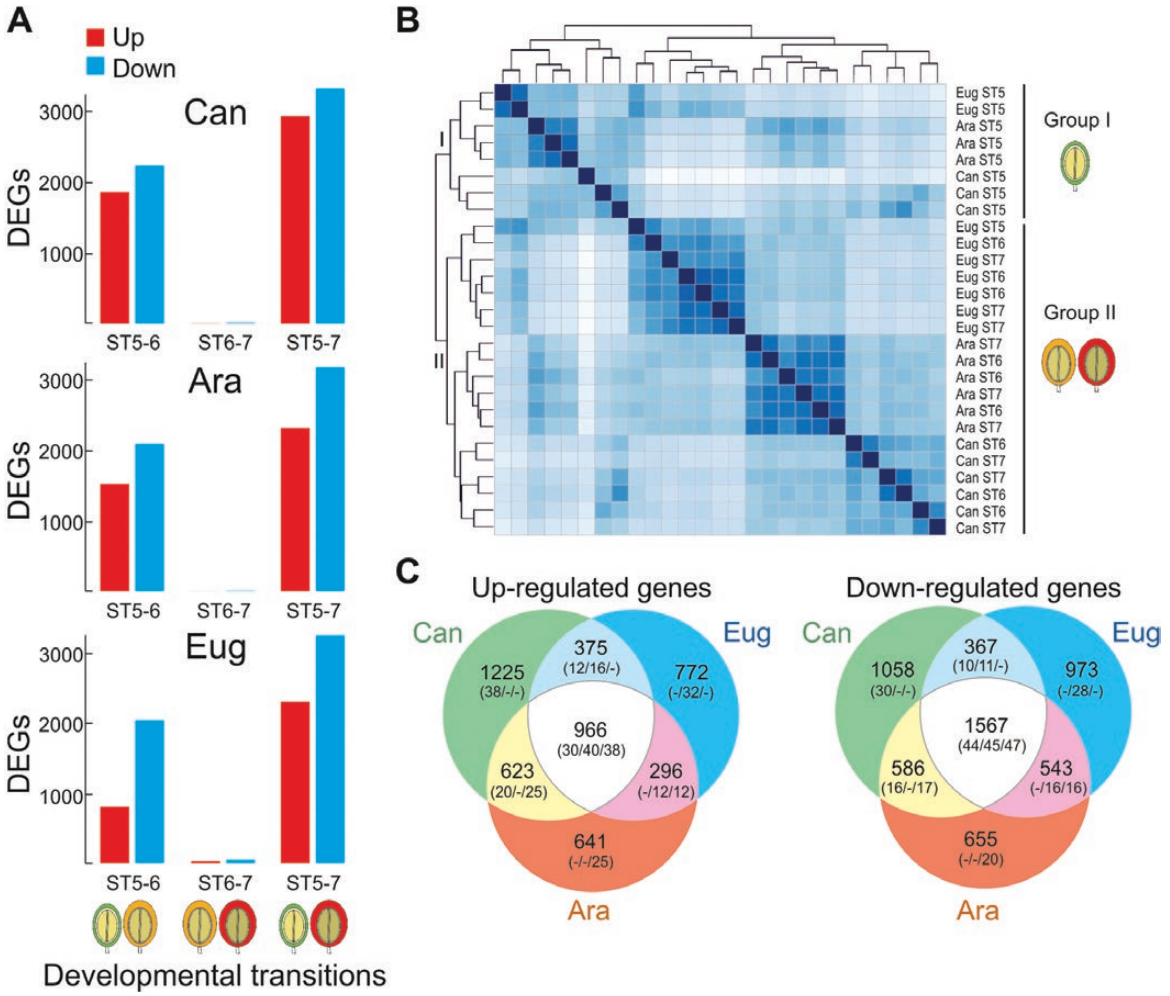

Fig. 2. Analysis of the transcriptional program associated with seed late maturation in the three coffee species *Coffea arabica*, *C. canephora*, and *C. eugenioides*. (A) Numbers of differentially expressed genes (adjusted *P*-value<0.05) in the three *Coffea* species between the consecutive developmental stages 5 and 6 (ST5–6 transition), stages 6 and 7 (ST6–7 transition), as well as over the entire late maturation period (ST5–7). Up- and down-regulated genes are labelled in red and blue, respectively. (B) Hierarchical clustering of normalized RNA-seq libraries based on Euclidian distance matrix; similar samples are darker blue. (C) Venn diagrams of the total up- and down-regulated genes between all stage comparisons, of each species studied (Ara, *C. arabica*; Eug, *C. eugenioides*; Can, *C. canephora*). Numbers in parentheses indicate the percentage of the DEGs that are found in each Venn category for a given species. The order is always Can, Eug, Ara.

maturation program, with a total of 94 functional categories detected as significantly enriched at least in one species (Fig. 3; Supplementary Tables S5, S6). More categories (62) were significantly over-represented among the down-regulated genes than among the up-regulated genes (30), and only two categories displayed contrasting behavior among different species (minor CHO metabolism and redox processes, mapman bin codes 3 and 21, respectively, which were enriched in *C. canephora* up-regulated genes and in *C. eugenioides* down-regulated genes). Most importantly, 24 functional categories (25.5%) were shared among the three species for at least one of the two comparisons (ST5–6 or ST5–7, labeled by red stars in Fig. 3) while each species also revealed a similar number (14–15) of specific categories. Transcriptional processes associated with *C. canephora* during the ST5–6 transition appear to be the most dissimilar compared with other stages and species, since only nine out of the 24 shared functional categories are represented during this early transition, whereas 23/24 were identified within the ST5–7 comparison. By contrast, most of these shared functional categories were already detected during the early ST5–6 transition among *C. arabica* and *C. eugenioides*. This important result indicates that the late maturation transcriptional program is delayed in the desiccation-sensitive *C. canephora* compared with species with desiccation-tolerant seeds.

The composition for the 18 common categories shared between species for down-regulated genes strongly suggests a transcriptional co-ordination of metabolic slow-down during late maturation, with a significant decrease in mRNA abundance of genes involved in key energetic processes such as respiration (including the TCA cycle, mitochondrial electron transport and ATP synthase) and photosynthesis (including light reactions and photosystem I components). Genes encoding components of cytoskeleton, membrane transporters, or components of the machinery for cell wall precursors were also found to be significantly down-regulated (Fig. 3). GO enrichment analysis revealed similar features, but also pointed out a general decrease of carbohydrate metabolic process, and

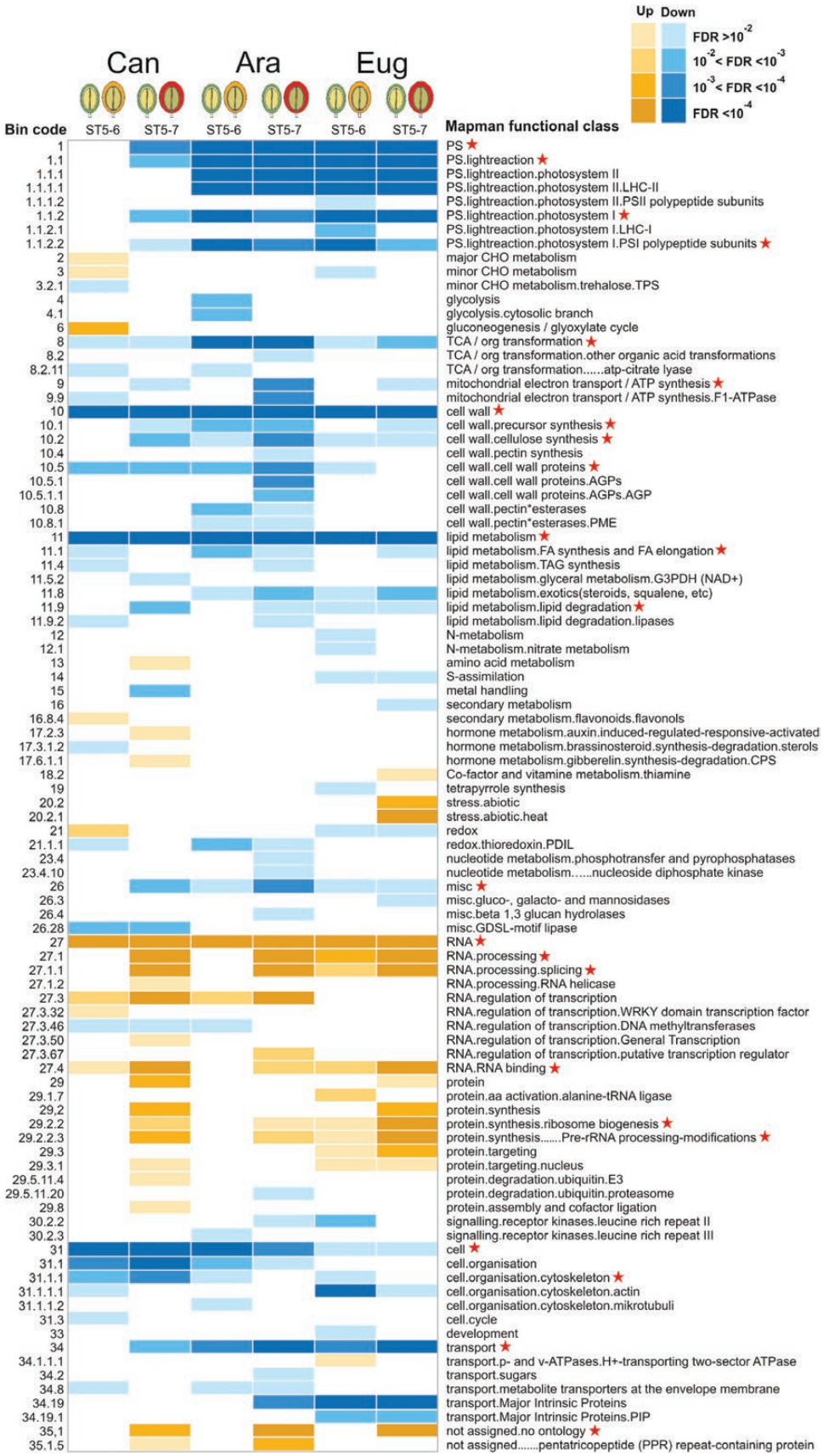

Fig. 3. Mapman functional class enrichment (Fisher enrichment test, adjusted *P*-value<0.05) for DEGs of ST5–6 and ST5–7 for each *Coffea* species. Up- and down-regulated genes are colored in shades of orange and blue, respectively; only significant enrichments are colored. Mapman functional classes which are found to be enriched in all three species are indicated by a red star. FDR, false discovery rate.

intracellular trafficking, including endomembrane systems and Golgi apparatus (Supplementary Table S7).

With regard to functional classes associated with late maturation up-regulated genes, only six were found to be shared among the three species. These mostly concerned RNA processing (splicing and RNA binding) and the translational machinery (i.e. ribosome biogenesis, including pre-rRNA processing and modifications). Surprisingly, functional enrichment analysis did not identify any functional class that would be specifically induced during late maturation in desiccation-tolerant seeds only. By contrast, it revealed several specific features of desiccation-sensitive *C. canephora* seeds. Indeed, functional processes such as gluconeogenesis and carbohydrate metabolism, flavonoid metabolism, redox homeostasis, and hormone metabolism (gibberellin synthesis and auxin-regulated genes) were significantly over-represented among the *C. canephora* genes up-regulated during late maturation (Fig. 3).

DT-related candidate genes and processes identified through inter-specific comparative transcriptomics

The DEGs between species/stages were filtered by using combinations of Boolean operators to identify candidate genes related to different categories of DT (Table 1; Fig. 4). The first cluster (C1; 150 genes), designed to encompass positive effectors of DT, groups genes that are up-regulated during late maturation in both *C. arabica* and *C. eugenioides* seeds, and whose expression is concomitantly lower in desiccation-sensitive *C. canephora* seeds (Fig. 4; Supplementary Tables S7, S8). Interestingly, the 1000 bp promoter regions of C1 candidate genes were significantly enriched in four *cis*-elements (Supplementary Table S9), including CGCG, which is bound by Calmodulin-binding transcription activator and known to play a key role in plant stress response (Galon *et al.*, 2008; Doherty *et al.*, 2009). Among the 120 genes with expert-assigned putative functions, the most-represented functional classes were transcriptional activities (19.2% of the 120 candidate genes),

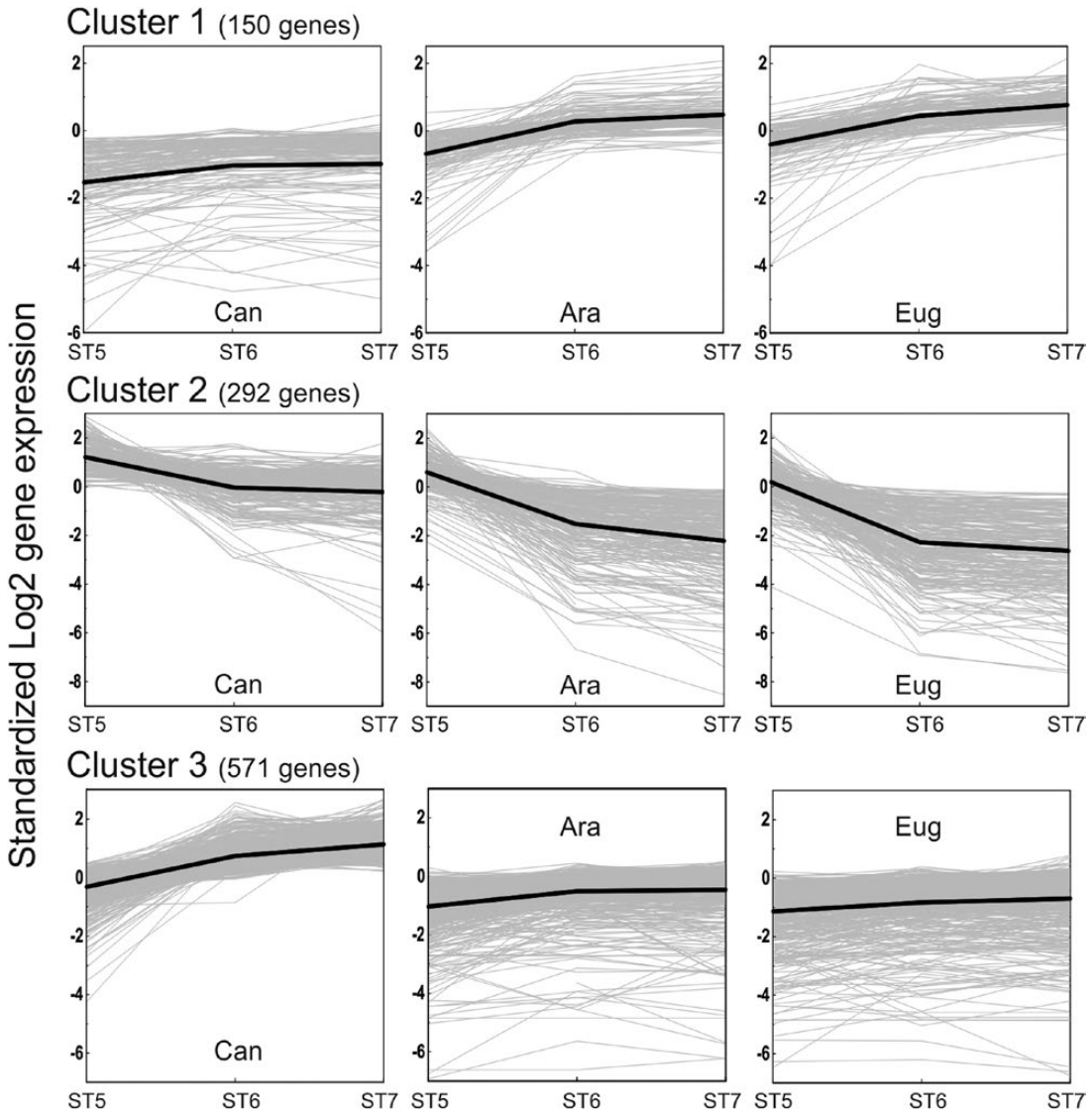

Fig. 4. Candidate gene clusters related to desiccation tolerance. The standardized log2 of the gene expression is plotted against the developmental stage of each *Coffea* species. The average gene expression for each cluster is traced in a dark line, while individual gene expressions are plotted in thin gray lines.

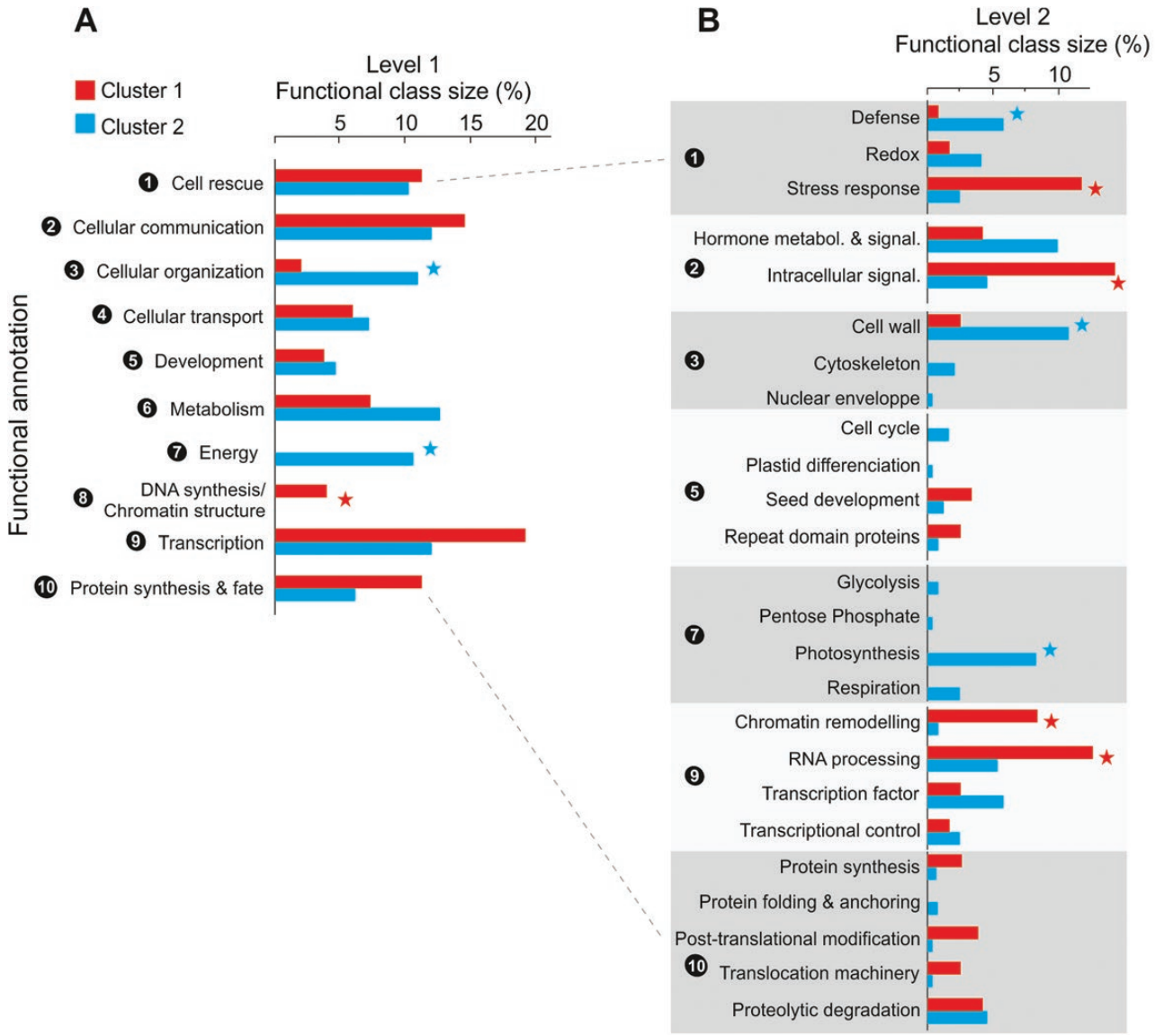

Fig. 5. The percentage of candidate genes in clusters 1 (red) and 2 (blue) that belong to each functional class. Class sizes that are significantly different between the two clusters (χ^2 test, *P*-value<0.05) are indicated with asterisks. (A) The first level of functional terms; (B) the second level of functional terms. Functional terms that are child terms of the first level are separated and numbered for ease of visualization.

cellular communication (14.6%), cell rescue (11.3%), and protein synthesis and fate (11.3%) (Fig. 5A).

The second cluster (C2), designed to comprise negative effectors of seed DT, was composed of genes that are down-regulated during late maturation in both *C. arabica* and *C. eugenioides* seeds, and whose expression is concomitantly higher in desiccation-sensitive *C. canephora* seeds (Fig. 4). Cluster C2 contains 292 genes, including 250 with a putative function assigned, with primary metabolism (12.7% of the candidate genes), cellular communication (12%), cellular organization (11%), transcriptional activities (11.6%), and energetic processes (10.6%) being the most represented functional classes in this group (Fig. 5A; Supplementary Table S7). Generation of precursor metabolites and energy, photosynthesis, and TCA cycle were among the biological processes significantly enriched using GO or Mapman ontologies (Supplementary Table S8). This result suggests that over the course of seed maturation differences in DT between species may be based on quantitative differences at the level of transcriptional control of the metabolic slow-down associated with seed quiescence.

The analysis of the relative size and composition of the different gene functional classes (Fig. 5B) revealed striking differences between DT-associated (C1) and DS-related genes (C2), with some functional classes being almost exclusively represented in one group. Specifically, C1 alone includes representatives of the functional class related to the synthesis and repair of DNA and chromatin structure (Fig 5A). Similarly, the relative size of the functional class related to transcriptional processes was highest for DT-associated genes, with many genes predicted to be implicated in RNA processing or encoding many chromatin remodeling factors involved in regulation of transcription, but with very few TFs (three) including GATA and MADS factors (Fig 5B). In this context, it is worth noting that C1 contains multiple genes encoding putative chromatin remodeling factors/complex subunits, including MRG1 (Cc07_g06470), SWC6 (Cc07_g10850), SWI3A (Cc07_g20270), and SWIB

complex BAF60b domain-containing protein (Cc02_g18450). In Arabidopsis, MRG1 has been shown to be involved in increasing the transcriptional levels of the flowering time genes *FLC* and *FT* (Bu *et al.*, 2014) while SWC6 is a component of the SWR1 complex that mediates transcriptional regulation of selected genes such as *FLC* (Choi *et al.*, 2007). By contrast, C2 does not contain chromatin remodeling factors; instead it contains many TFs (14) related to key developmental activities and cell differentiation, including homologs of HOX, B3, LOB, MADS, and MYB TFs. This result suggests DT acquisition could be associated with intense chromatin remodeling and repression of specific developmental TFs.

Moreover, C2 displays a higher number of genes involved in redox homeostasis and defense against biotic stress while C1 includes many genes with a direct role in the regulation of abiotic stress response (Fig 5B). C1 genes include key stress proteins such as LEA (EM6, Cc05_g00080), co-chaperone (JAC1, Cc10_g03210; Cc08_g03570), cold-stress (COR28, Cc02_g18840), and drought-stress proteins (ERD4, Cc11_g12570) (Supplementary Table S7). C1 also includes mediators of ABA and jasmonate stress response (AFP2, ABO5, NHL6, MKS1), a regulator of ROS homeostasis (APP1, Cc07_g09130; Yu *et al.*, 2016), a modulator of unfolded protein response (CPR5, Cc07_g16050), and proteins involved in plastid stress response mediated by effector nucleotides (ppGpp synthetase, Cc10_g06010)(Supplementary Table S7).

With regard to cellular communication processes, while C1 presents mainly genes implicated in signal transduction processes (Fig 5B), such as components of receptor-like kinase signaling pathways, protein kinase cascades, and protein phosphatases (subunits of PP2A and PP2C), C2 mostly contains genes implicated in hormone metabolism and signaling, a majority of them being putatively dedicated to the metabolism, transport, and perception of auxin, and, to a lesser extent, cytokinin and gibberellins (Supplementary Table S7).

Finally, functional classes related to energetic processes appear mostly restricted to DS-related genes (Fig 5). Many C2 genes are associated with key energetic processes such as respiration (including molecular components of the TCA cycle, respiratory electron transfer chain and ATP synthase), photosynthesis, and glycolysis, as well as key enzymes of the pentose phosphate pathway. These candidates point to the existence of a transcriptional orchestration of metabolic quiescence that differs between desiccation-tolerant and desiccation-sensitive seeds. This could lead to different levels of repression of energetic processes and basal cellular processes when entering quiescence during seed desiccation.

Desiccation-sensitive specific genes expressed during late maturation

Finally, a third cluster (C3) included 571 genes specific to a desiccation-sensitive context, i.e. genes that are up-regulated during late maturation in *C. canephora* seeds, and whose expression is concomitantly lower in both *C. arabica* and *C. eugenioides* desiccation-tolerant seeds (Fig. 4). The Mapman functional classes over-represented within this cluster are mainly related to nitrogen metabolism, tocopherol biosynthesis, and translational control (Supplementary Table S8). As shown for genes that were up-regulated during *C. canephora* late seed maturation (Fig. 3), auxin-regulated genes were also enriched in C3. Accordingly, the analysis of promoter regions for C3 candidate genes revealed significant enrichment for three *cis*-elements (Supplementary Table S9), including the auxin response factor ARF3 *cis*-element. This motif is bound by auxin-regulated TFs (Chandler, 2016), probably including the ARF homolog gene (Cc08_g16330) detected in C3. The *cis*-element GCCF (GCC-box bound by TF of the AP2/ERF family) was shown to be enriched in both C3 and C1 genes, suggesting this *cis*-element is an important driver of gene expression at the late maturation stage, independently of the DT level. The most common GCCF *cis*-element signatures found in these two clusters are those of the AtERF-1 and of the RAP2.6 TF, known to be involved in jasmonate- and ethylene-dependent stress response, and in ABA and salt stress response signaling, respectively (Lorenzo *et al.*, 2003; Pré *et al.*, 2008; Zhu *et al.*, 2010).

A comparison of the functional classes' relative size between C1 and C3 also highlighted striking differences in late maturation transcripts specific to *C. arabica* and *C. eugenioides* compared with the desiccation-sensitive *C. canephora* seeds (Fig. 6). Interestingly, genes of C1 are enriched in functions related to stress response, chromatin remodeling, and RNA processing when compared with C3, as seen in the C1–C2 comparison. By contrast, C3 contains many genes involved in defense against biotic stress or associated with energetic processes (cytosolic and plastidial glycolysis, control of the redox poise in plastid and mitochondrial respiratory electron transporters), and cell cycle or organelle differentiation (Fig. 6). In addition to many TFs, C3 also contains numerous genes encoding members of the Pentatricopeptide repeat-containing (PPR) protein family. To date, a majority of characterized PPRs are predicted or shown to localize to chloroplasts or mitochondria where they play essential roles in gene expression, mRNA editing, mRNA processing, and protein synthesis (Hayes *et al.*, 2015; Liu *et al.*, 2016).

Comparative proteomics validates processes and candidate genes inferred from transcriptome analysis

The soluble proteome of mature coffee seeds was analysed among the three species in order to ascertain the differential functions inferred from transcriptomic analysis (Supplementary Table S10; Supplementary Fig. S1). Among the 1389 proteins accurately detected in at least one species, quantification revealed 95 proteins whose abundance is higher in desiccation-tolerant *C. arabica* and *C. eugenioides* seeds compared with *C. canephora* (Supplementary Table S11). Mapman functional classes over-represented within this group are mainly related to calcium signaling, protein post-translational modification, and synthesis of amino acids of the serine–glycine–cysteine group (Supplementary Table S12). This proteomic analysis also revealed 99 proteins over-accumulated in desiccation-sensitive *C. canephora* mature seeds. Hormone metabolism related to auxin, secondary metabolism associated with flavonoids, and biotic stress-associated PR proteins were among the biological processes significantly enriched using Mapman ontologies (Supplementary Table S12). All these functional classes were

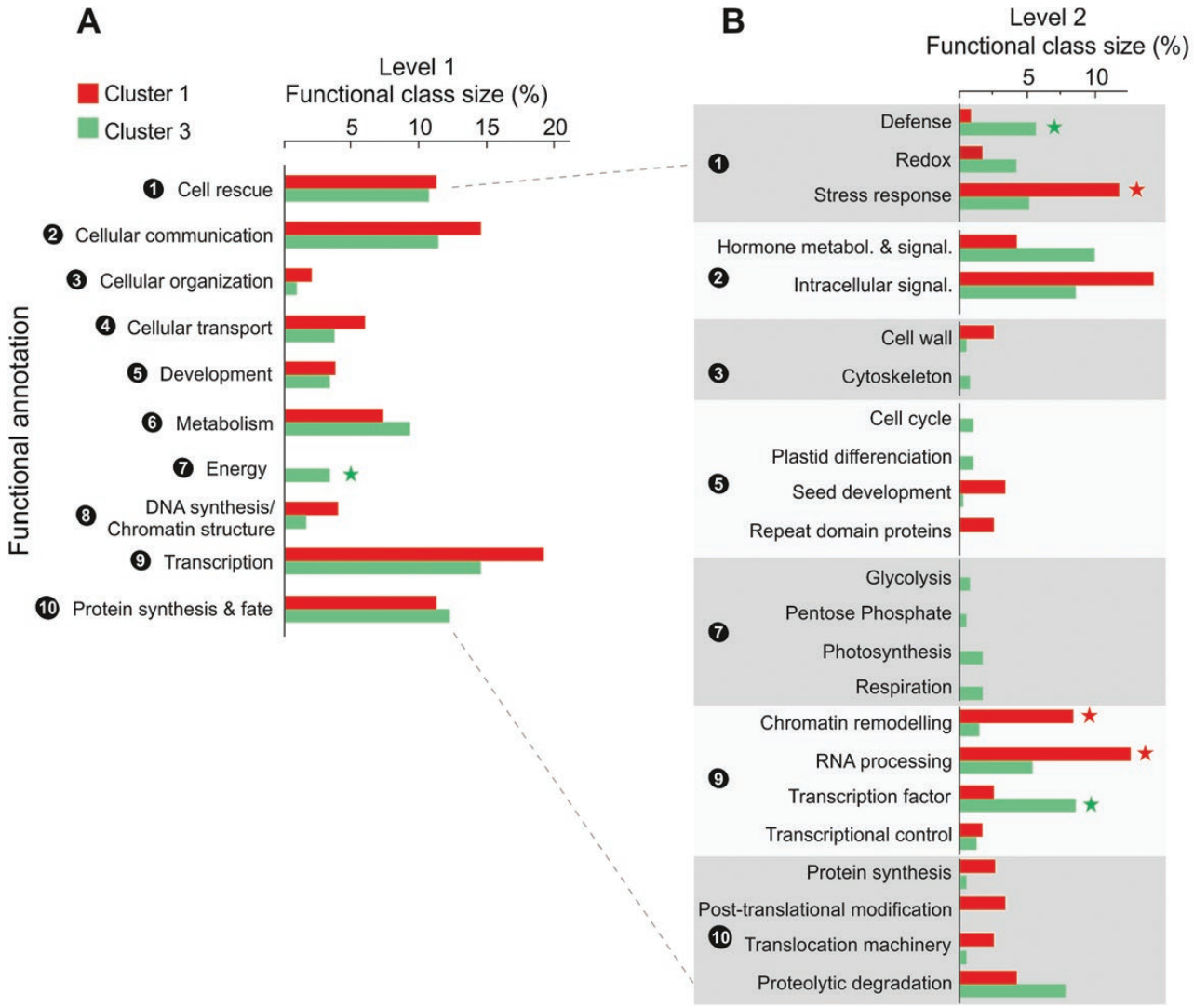

Fig. 6. The percentage of candidate genes in clusters 1 (red) and 3 (green) that belong to each functional class. Class sizes that are significantly different between the two clusters (χ^2 test, *P*-value<0.05) are indicated with asterisks. (A) The first level of functional terms; (B) the second level of functional terms. Functional terms that are child terms of the first level are separated and numbered for ease of visualization.

also detected at the transcriptome. Besides, the expert analysis of the relative size and composition of the different functional classes revealed striking differences between DT- and DS-associated proteins, with some functional classes such as those related to glycolysis, photosynthesis, hormone signaling, and defense against biotic stress being higher in desiccation-sensitive *C. canephora* seeds as observed with transcriptomic data (Supplementary Fig. S2). Finally, DT candidates detected at both transcript and protein levels included the LEA EM6 in desiccation-tolerant seeds and the glycolytic GAPC2c (Cc07_g00460) or the redox-involved NRX1 (Cc11_g14760) for desiccation-sensitive seeds (Supplementary Table S13).

Hormone sensing and signaling pathways are differentially expressed between desiccation-tolerant and -sensitive coffee species

Phytohormone profiling detected only faint amounts of active molecules in mature seeds and no significant differences among the three species studied (Supplementary Table S14). However, our survey highlighted key genes involved in hormone sensing, predominantly in desiccation-sensitive seeds (Supplementary Table S7). Indeed, in the DT-repressed genes (C2) we found homologs of major hormone transporters (auxin, PIN1, Cc06_g19880; ABA, ABCG40, Cc11_g01420; cytokinin, ABCG14, Cc08_g05640; Zhang *et al.*, 2014; Kang *et al.*, 2015), several predicted auxin response regulators (Phabulosa, Cc09_g08040; LBD16, Cc01_g18520; MIZU-KUSSEI-like protein, Cc04_g07700; Lee *et al.*, 2009; Moriwaki *et al.*, 2011; Müller *et al.*, 2016), and a cytokinin signaling inhibitor that mediates auxin–cytokinin crosstalk (AHP6, Cc04_g04370; Bishopp *et al.*, 2011). Furthermore, C3 (DS-specific late maturation genes) contained predicted hormone receptors (ABA, PYL8, Cc08_g15960; auxin, AFB5, Cc10_g15520; Prigge *et al.*, 2016), and other potential auxin response proteins (ARF9, Cc08_g16330, IAA1, Cc04_g03620). This gene composition fingerprint suggests increased hormone sensitivity in desiccation-sensitive seeds, especially for auxin and cytokinin, while desiccation-tolerant seeds displayed higher expression (C1) for NHL6 (Cc05_g06810), a mediator of ABA signaling in Arabidopsis

(Bao *et al.*, 2016). Differentially accumulated proteins associated with auxin signaling were also detected in the proteome. Similar levels of phytohormones in mature seeds do not exclude the possibility that desiccation-tolerant and -sensitive species integrate and respond to the hormone signals differently.

Desiccation-tolerant and -sensitive coffee seeds display differences in transcriptional and metabolic control of respiratory processes

The three coffee species shared a transcriptional slow-down for energy metabolism during seed maturation with down-regulation of numerous mitorespiration-related transcripts, such as those encoding several subunits of ATP synthase, many components of the electron transfer chain (NADH dehydrogenase complex I, cytochrome bc_1 complex, cytochrome *c* oxidase), as well as many enzymes of the TCA cycle (isocitrate dehydrogenase, pyruvate dehydrogenase, succinyl-CoA ligase, dihydrolipoyl dehydrogenase) (Supplementary Table S4). However, the interspecific transcriptome comparison revealed fundamental differences in the regulation of mitochondrial energy metabolism between desiccation-tolerant and desiccation-sensitive seeds. First, compared with *C. canephora*, desiccation-tolerant seeds displayed higher expression (C1 genes) of the translocase inner membrane subunit TIM44-2 (Cc01_g18340) and the mitochondrial splicing factor OTP439 (Cc01_g05040, Organelle transcript processing), involved in basal cellular processes such as protein import and organelle post-transcriptional processes, respectively (Murcha *et al.*, 2014; Colas des Francs-Small *et al.*, 2014). Secondly, there is a down-regulation in desiccation-tolerant seeds (C2 genes) of many genes encoding proteins pivotal for regulation of energetic processes, namely malate dehydrogenase MMDH1 (Cc02_g32320), a central enzyme in the TCA cycle (Sew *et al.*, 2016), the voltage-dependent anion channel VDAC1 (Cc11_g13460), and the ATP synthase delta subunit (Cc02_g04940). VDAC1, an abundant protein of the outer mitochondrial membrane, contributes to the exchange of small metabolites essential for respiration and plays a role in redox control (Tateda *et al.*, 2011; Robert *et al.*, 2012). This transcriptional fingerprint suggests a slow-down of the machinery dedicated to oxidative phosphorylation, i.e. ATP production, without necessarily affecting the electron transfer chain operability. By contrast, desiccation-sensitive *C. canephora* seeds displayed late maturation induction (C3 genes) of several respiratory electron transfer chain complex components or genes involved in their assembly, namely complex I accessory subunits 6 and 7 (Cc02_g25880, Cc08_g10410) and complex I α subcomplex assembly factor 3 (Cc02_g25880), as well as the ubiquinone biosynthesis protein COQ4 (Cc06_g15640) and the cytochrome *c*-type biogenesis protein CcmH (Cc04_g07670). Desiccation-sensitive *C. canephora* seeds also displayed late maturation induction of genes involved in mitochondrial post-transcriptional and translational activities, such as elongation factors EFTu and EFTs (Cc03_g02960 and Cc05_g09920), and editing factors MEF10 and MEF11 (Cc04_g00410 and Cc11_g07090, respectively), the latter also being involved in ABA signaling (Sechet *et al.*, 2015). The high expression level of these genes corresponds to the signature of organelles capable of maintaining high energy metabolism on demand. In this context, one may note the presence of a mitochondrial phosphate transporter, MPT3 (Cc07_g01200), which plays vital roles in phosphate homeostasis and ATP biosynthesis and whose expression levels modulate salt stress tolerance in Arabidopsis (Zhu *et al.*, 2012). Finally, C3 contains two genes encoding proteins that may play a role in regulating mitochondrial metabolism under stress conditions: formate dehydrogenase (FDH, Cc07_g02270) and prohibitin PHB3 (Cc07_g00470). The mitochondrial FDH is a positive regulator of cell death and defense response, and confers tolerance to hypoxia, metal toxicity and low pH in Arabidopsis (Choi *et al.*, 2014; Lou *et al.*, 2016) while PHB3 functions in nitric oxide-mediated plant stress responses (Wang *et al.*, 2010).

This transcriptional signature for differential energetic processes among desiccation-tolerant and desiccation-sensitive seeds led us to probe their respiratory activity during desiccation. Respiration rates, estimated as CO_2 release, were measured on mature seeds of the three coffee species at various moisture contents upon dehydration (Fig. 7). Within the 0.6–0.8 g H_2O g^{-1} DM hydration window, all seeds displayed roughly equivalent respiratory activities. Upon desiccation, *C. arabica* and *C. eugenioides* seeds displayed a smooth reduction in respiration rate until ceasing detectable respiration at approximately 0.3 g H_2O g^{-1} DM. Desiccation-sensitive *C. canephora* seeds, however, had a biphasic mode of respiration, initially displaying rather stable respiration rates between 0.8 and 0.4 g H_2O g^{-1} DM. Below this threshold *C. canephora* seeds experienced drastic reduction in respiration rate that remained significantly higher than that measured for *C. arabica* and *C. eugenioides* seeds (Fig. 7).

Discussion

A late maturation program also occurs in desiccation-sensitive coffee seeds

Performed on developing seeds of three coffee species, the present transcriptome survey reveals the shared existence of a major transcriptional switch during the ST5–ST6 transition

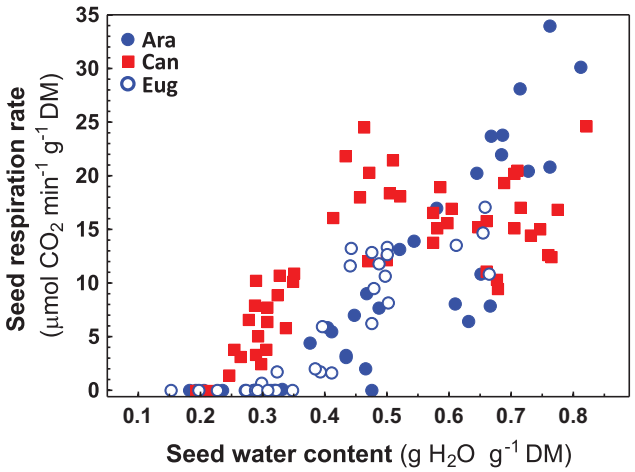

Fig. 7. Respiration rates, measured as release of CO_2 (µmol CO_2 min^{-1} g^{-1} DM) as a function of the hydration state (g H_2O g^{-1} DM) of fresh mature seeds (ST7) of the three coffee species during equilibrium drying.

(Fig. 2A). This switch coincides with partial DT acquisition in the three species (Fig. 1), and is a mark of a late maturation program since it occurs well after transcriptional events associated with storage reserve deposition, as already described in *C. arabica* (Dussert *et al*., 2018). A large number of processes associated with late maturation are conserved among the three species, as demonstrated through functional enrichment analysis. This suggests a late maturation phase has been conserved during the course of *Coffea* evolution independently of DT levels. Among the 6285 DEGs (24.6% of the reference genes) identified in *C. canephora*, 40.3% were found to be commonly up- or down-regulated in the other coffee species (Fig. 2C), suggesting the late maturation phase serves the acquisition of several major functional traits other than DT. Initially thought to be restricted to orthodox seeds (reviewed in Leprince *et al*., 2017), a late maturation phase is presumably conserved in intermediate seeds, even those that display very low DT such as those of *C. canephora*. Interestingly, a recent proteomic and metabolomic survey of the recalcitrant cocoa developing seed also revealed important metabolic changes during the late phases of development, including accumulation of stress-related proteins, which occurred after the peak stage of reserve deposition, thus resembling a late maturation phase (Wang *et al*., 2016).

Desiccation-tolerant seeds harbor key stress proteins and integrators of stress response

Analysis of the DEGs identified relatively few DT-specific up-regulated genes (C1, 150 genes) compared with DT-specific down-regulated genes (C2, 292 genes), and identified many genes specifically induced in maturing desiccation-sensitive *C. canephora* seeds (C3, 571 genes; Fig. 4). Although transcription factors were under-represented among DT candidate genes (Figs 5, 6), three putative TFs were identified, a MADS (Cc09_g07070) and two GATA (Cc02_g34640; Cc01_g21240). These deserve further attention and validation of their potential role in orchestrating the DT-associated transcriptome during late maturation in coffee seeds.

In addition to these TFs, our survey did detect multiple genes related to cell rescue and stress response. Most notably, our survey revealed one LEA protein, EM6 (Cc05_g00080), whose gene expression and protein levels are significantly higher in desiccation-tolerant coffee seeds. EM6 protein accumulation has been previously observed temporally associated with DT acquisition during seed development, as well as with re-induction of DT in germinated radicles of *Medicago truncatula* (Boudet *et al*., 2006; Chatelain *et al*., 2012). Due to their hydrophilic nature and hydration buffer capacity, certain LEA proteins may participate in the control of water loss during maturation. EM6 from *Medicago* was found *in vitro* to bind water much more efficiently at 75% RH compared with a control protein, acting as a molecular sponge (Boucher *et al*., 2010). Similarly, EM6 has been suggested to play a critical role in water binding during Arabidopsis maturation drying (Manfre *et al*., 2009). In addition to the EM6, two heat-shock protein 70 co-chaperones were identified among DT-associated genes, including JAC1 (Cc10_g03210), known to be involved in plastid movement in Arabidopsis (Takano *et al*., 2010). Since both heat-shock proteins and LEA proteins have been proposed to play important roles in DT through chaperone protection of membranes and/or proteins in plants and various other organisms (Tolleter *et al*., 2007; Chakrabortee *et al*., 2007; Erkut *et al*., 2011; Tapia *et al*., 2015; Boothby *et al*., 2017), it is tempting to attribute a quantitative role to these candidates in the seed DT level of coffee species. Furthermore, among stress-response genes with increased expression during DT acquisition, genes such as those for COR28, ERD4, and APP1 are worth noting, the latter playing a critical role in the control of H_2O_2 content and ROS homeostasis in Arabidopsis (Yu *et al*., 2016). ERD4 was shown to be an osmosensitive calcium-permeable cation channel (Hou *et al*., 2014), and therefore could play a central role in early salt and drought stress signaling through variation of cytosolic calcium fluxes. Because cytosolic calcium elevation is one of the earliest responses of plant cells to different stress stimuli (Knight *et al*., 1991), the presence in C1 of ERD4 and the calcium-binding protein Calmodulin-like38 (CML38, Cc07_g00660) is particularly attractive since they may act as sensors of early subtle variations of water potential upon dehydration and play a role in orchestrating metabolic quiescence. Interestingly, we also detected calcium signaling through proteomics, including calmodulin CAM7 (Cc04_g09200 and Cc06_g22690) involved in ABA responsiveness in Arabidopsis (Abbas and Chattopadhyay, 2014). Finally, concerning C1 genes involved in stress response, one may also note the presence of genes encoding DNA ligase (Cc00_g04310) and SMC5 (Cc02_g28540), which both have canonical functions in repair of damaged DNA and maintenance of genome integrity (Watanabe *et al*., 2009). Different DNA ligases were indeed shown to be involved in DNA repair during early imbibition, and were major determinants of Arabidopsis seed longevity (Waterworth *et al*., 2010).

Interestingly, C1 also included three genes coding for proteins that were shown to play important roles in ABA signaling, namely NHL6 (Cc05_g06810), AFP2 (Cc11_g11930) and ABO5 (Cc06_g12800). NHL6 has recently been demonstrated to act as positive regulator of ABA-mediated seed germination inhibition in Arabidopsis (Bao *et al*., 2016) while AFP2 acts epistatically to ABI5, a master regulator of ABA signaling and seed maturation in orthodox seeds (Garcia *et al*., 2008; Zinsmeister *et al*., 2016). Mutations in AFP2 result in increased sensitivity to ABA in Arabidopsis seeds (Garcia *et al*., 2008). Finally ABO5 mediates ABA response in organelles through splicing of key mitochondrial genes in Arabidopsis (Liu *et al*., 2010). Indeed, Arabidopsis *abo5* mutants expressed lower transcripts of stress-inducible genes as well as plastid-related genes involved in adaptation of photosynthesis to low ATP conditions (Liu *et al*., 2010). In genes specifically up-regulated in desiccation-sensitive seeds (C3, Fig. 6), we found *PYL8* (Cc08_g15960), an ABA sensor in Arabidopsis seeds (Saavedra *et al*., 2010). The presence of these hormone-sensing and modulation genes in our candidate clusters suggests that a differential fine-tuning of ABA perception could be implicated in the differential acquisition and regulation of DT. Similarly, modulation of ABA sensitivity, rather than ABA content, was recently proposed to regulate the ability of DT re-induction and DT

loss during Arabidopsis seed germination (Maia *et al.*, 2014). Our survey also revealed DT and DS differences in the expression of nuclear-encoded mitochondrial genes dedicated to energetic processes. Recent evidence suggests hormones and the mitochondrial metabolism could be more closely linked than initially thought as ABA was shown to induce typical transcripts of the mitochondrial retrograde response while auxin has the inverse effect (Ivanova *et al.*, 2014; Wagner *et al.*, 2018).

Metabolic shut-down is better coordinated in desiccation-tolerant seeds

In coffee seeds, the major transcriptional switch observed during late maturation included several nuclear-encoded mitochondrial proteins that are markers of large reorganization of energy metabolism (Fig. 3), mimicking what is observed in orthodox seeds, such as down-regulation of genes dedicated to the TCA cycle (Logan *et al.*, 2001; Howell *et al.*, 2006). Furthermore, our dataset revealed numerous quantitative differences in transcript accumulation for key mitochondrial proteins between coffee seeds displaying different DT levels. Among them, it is worth noting that desiccation-sensitive *C. canephora* seeds displayed enhanced accumulation of transcripts for subunits of the complexes I and III (NADH dehydrogenase and cytochrome bc_1), the major sites of superoxide production through one-electron reduction of molecular oxygen (Wagner *et al.*, 2018). These transcriptomic differences observed between desiccation-tolerant and -sensitive coffee seeds appear to reflect the potential to slow down metabolism and respiration during dehydration rather than an actual slow-down phase during maturation since measured respiration rates in non-dehydrated mature seeds were similar among the three coffee species (Fig. 7). However, during their dehydration we observed striking differences in respiration rates. A better preparation or coordination of down-regulation of metabolism in desiccation-tolerant seeds during drying could play an important role in avoiding or limiting oxidative stress conditions and/or accumulation of by-products of metabolism to toxic levels. Seed respiration is generally detectable around 0.25 g H_2O g^{-1} DM and respiration rates increase with increasing water contents above this threshold (Vertucci and Leopold, 1984; Vertucci, 1989). However, it is considered that at intermediate water contents the metabolism that occurs is unregulated, and there is evidence of damaging reactions that are probably free radical-mediated occurring in this water-content range (Leprince *et al.*, 1990; Hendry *et al.*, 1992; Hendry, 1993; Finch-Savage *et al.*, 1994; Vertucci and Farrant, 1995). Interestingly, differences in the respiratory activities we observed between intermediate coffee seeds displaying different DT levels upon dehydration were similar to those observed between intermediate tea seeds and orthodox pea seeds (Walters *et al.*, 2001), and between different recalcitrant *Castanea* seed tissues (axes and cotyledons) displaying differences in desiccation sensitivity (Leprince *et al.*, 1999). The lack of coordinated repression of metabolism during drying is thought to play a predominant role in the oxidative burst that triggers lipid oxidation, membrane disruption, and ultimately the death of recalcitrant seeds (Leprince *et al.*, 1999; Bailly, 2004; Kranner and Birtic, 2005). This mechanism could be shared with intermediate seeds displaying relatively low DT levels. Given that ROS-provoked mitochondria-dependent cell death has also been recently described for hydrated orthodox elm seeds under detrimental artificial ageing conditions (Wang *et al.*, 2015), it defines mitochondria and the regulation of respiratory processes as universal targets of seed sensitivity to stress.

Supplementary data

Supplementary data are available at *JXB* online.

Fig. S1. Protein abundance variation among mature coffee seeds of the three species.

Fig. S2. Functional categories of DT-related proteins.

Table S1. RNAseq statistics of samples used.

Table S2. Gene expression (RPKM) in each sample.

Table S3. Pairwise comparisons of gene expression.

Table S4. Genes commonly up- and down-regulated during late maturation.

Table S5. Mapman functional enrichment analysis of late seed maturation.

Table S6. GO-term enrichment analysis of late maturation.

Table S7. DT-related gene candidates of seed late maturation.

Table S8. Mapman and GO functional enrichment analysis of DT-related clusters.

Table S9. Promoter *cis*-element enrichment analysis.

Table S10. Proteome of mature coffee seeds.

Table S11. DT-related protein candidates.

Table S12. Mapman functional enrichment analysis of candidate proteins.

Table S13. Candidate genes detected at both transcript and protein levels.

Table S14. Phytohormone profiling in mature coffee seed.

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References

Abbas N, Chattopadhyay S. 2014. CAM7 and HY5 genetically interact to regulate root growth and abscisic acid responses. Plant Signaling & Behavior **9**, e29763.

Bailly C. 2004. Active oxygen species and antioxidants in seed biology. Seed Science Research **14**, 93–107.

Bao Y, Song WM, Pan J, *et al*. 2016. Overexpression of the *NDR1/HIN1-like* gene *NHL6* modifies seed germination in response to abscisic acid and abiotic stresses in *Arabidopsis*. PLoS ONE **11**, e0148572.

Berjak P, Pammenter NW. 2008. From *Avicennia* to *Zizania*: seed recalcitrance in perspective. Annals of Botany **101**, 213–228.

Black M, Pritchard H. 2002. Desiccation and survival in plants. Drying without dying. Wallingford: CABI Publishing.

Bishopp A, Help H, El-Showk S, Weijers D, Scheres B, Friml J, Benková E, Mähönen AP, Helariutta Y. 2011. A mutually inhibitory interaction between auxin and cytokinin specifies vascular pattern in roots. Current Biology **21**, 917–926.

Boothby TC, Tapia H, Brozena AH, Piszkiewicz S, Smith AE, Giovannini I, Rebecchi L, Pielak GJ, Koshland D, Goldstein B. 2017. Tardigrades use intrinsically disordered proteins to survive desiccation. Molecular Cell **65**, 975–984.e5.

Boucher V, Buitink J, Lin X, Boudet J, Hoekstra FA, Hundertmark M, Renard D, Leprince O. 2010. MtPM25 is an atypical hydrophobic late embryogenesis-abundant protein that dissociates cold and desiccation-aggregated proteins. Plant, Cell & Environment **33**, 418–430.

Boudet J, Buitink J, Hoekstra FA, Rogniaux H, Larré 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 Physiology **140**, 1418–1436.

Bu Z, Yu Y, Li Z, Liu Y, Jiang W, Huang Y, Dong AW. 2014. Regulation of *Arabidopsis* flowering by the histone mark readers MRG1/2 via interaction with CONSTANS to modulate FT expression. PLoS Genetics **10**, e1004617.

Buitink J, Leprince O. 2008. Intracellular glasses and seed survival in the dry state. Comptes Rendus Biologies **331**, 788–795.

Cartharius K, Frech K, Grote K, Klocke B, Haltmeier M, Klingenhoff A, Frisch M, Bayerlein M, Werner T. 2005. MatInspector and beyond: promoter analysis based on transcription factor binding sites. Bioinformatics **21**, 2933–2942.

Cenci A, Combes MC, Lashermes P. 2012. Genome evolution in diploid and tetraploid *Coffea* species as revealed by comparative analysis of orthologous genome segments. Plant Molecular Biology **78**, 135–145.

Chakrabortee S, Boschetti C, Walton LJ, Sarkar S, Rubinsztein DC, Tunnacliffe A. 2007. Hydrophilic protein associated with desiccation tolerance exhibits broad protein stabilization function. Proceedings of the National Academy of Sciences, USA **104**, 18073–18078.

Chandler JW. 2016. Auxin response factors. Plant, Cell & Environment **39**, 1014–1028.

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 & Environment **35**, 1440–1455.

Chen Y, Rofidal V, Hem S, *et al*. 2019. Targeted proteomics allows quantification of ethylene receptors and reveals SlETR3 accumulation in never-ripe tomatoes. Frontiers in Plant Science **10**, 1054.

Chiwocha SD, Abrams SR, Ambrose SJ, Cutler AJ, Loewen M, Ross AR, Kermode AR. 2003. A method for profiling classes of plant hormones and their metabolites using liquid chromatography-electrospray ionization tandem mass spectrometry: an analysis of hormone regulation of thermodormancy of lettuce (*Lactuca sativa* L.) seeds. The Plant Journal **35**, 405–417.

Choi DS, Kim NH, Hwang BK. 2014. Pepper mitochondrial FORMATE DEHYDROGENASE1 regulates cell death and defense responses against bacterial pathogens. Plant Physiology **166**, 1298–1311.

Choi K, Park C, Lee J, Oh M, Noh B, Lee I. 2007. *Arabidopsis* homologs of components of the SWR1 complex regulate flowering and plant development. Development **134**, 1931–1941.

Colas des Francs-Small C, Falcon de Longevialle A, Li Y, Lowe E, Tanz SK, Smith C, Bevan MW, Small I. 2014. The pentatricopeptide repeat proteins TANG2 and ORGANELLE TRANSCRIPT PROCESSING439 are involved in the splicing of the multipartite *nad5* transcript encoding a subunit of mitochondrial complex I. Plant Physiology **165**, 1409–1416.

Crowe JH, Hoekstra FA, Crowe LM. 1992. Anhydrobiosis. Annual Review of Physiology **54**, 579–599.

Denoeud F, Carretero-Paulet L, Dereeper A, *et al*. 2014. The coffee genome provides insight into the convergent evolution of caffeine biosynthesis. Science **345**, 1181–1184.

Doherty CJ, Van Buskirk HA, Myers SJ, Thomashow MF. 2009. Roles for *Arabidopsis* CAMTA transcription factors in cold-regulated gene expression and freezing tolerance. The Plant Cell **21**, 972–984.

Dussert S, Chabrillange N, Engelmann F, *et al*. 2000. Relationship between seed desiccation sensitivity, seed water content at maturity and climatic characteristics of native environments of nine *Coffea* L. species. Seed Science Research **10**, 293–300.

Dussert S, Chabrillange N, Engelmann F, Hamon S. 1999. Quantitative estimation of seed desiccation sensitivity using a quantal response model: application to nine species of the genus *Coffea* L. Seed Science Research **9**, 135–144.

Dussert S, Davey MW, Laffargue A, Doulbeau S, Swennen R, Etienne H. 2006. Oxidative stress, phospholipid loss and lipid hydrolysis during drying and storage of intermediate seeds. Physiologia Plantarum **127**, 192–204.

Dussert S, Serret J, Bastos-Siqueira A, Morcillo F, Déchamp E, Rofidal V, Lashermes P, Etienne H, Joët T. 2018. Integrative analysis of the late maturation programme and desiccation tolerance mechanisms in intermediate coffee seeds. Journal of Experimental Botany **69**, 1583–1597.

Ellis RH, Hong TD, Roberts EH. 1990. An intermediate category of seed storage behaviour? I. Coffee. Journal of Experimental Botany **41**, 1167–1174.

Erkut C, Penkov S, Khesbak H, Vorkel D, Verbavatz JM, Fahmy K, Kurzchalia TV. 2011. Trehalose renders the dauer larva of *Caenorhabditis elegans* resistant to extreme desiccation. Current Biology **21**, 1331–1336.

Finch-Savage WE, Hendry GAF, Atherton NM. 1994. Free radical activity and loss of viability during drying of desiccation-sensitive tree seeds. Proceedings of the Royal Society of Edinburgh. Section B. Biological Sciences **102**, 257–260.

Galon Y, Nave R, Boyce JM, Nachmias D, Knight MR, Fromm H. 2008. Calmodulin-binding transcription activator (CAMTA) 3 mediates biotic defense responses in *Arabidopsis*. FEBS Letters **582**, 943–948.

Garcia ME, Lynch T, Peeters J, Snowden C, Finkelstein R. 2008. A small plant-specific protein family of ABI five binding proteins (AFPs) regulates stress response in germinating *Arabidopsis* seeds and seedlings. Plant Molecular Biology **67**, 643–658.

Geiger A, Hamidou Soumana I, Tchicaya B, Rofidal V, Decourcelle M, Santoni V, Hem S. 2015. Differential expression of midgut proteins in *Trypanosoma brucei* gambiense-stimulated vs. non-stimulated *Glossina palpalis gambiensis* flies. Frontiers in Microbiology **6**, 444.

González-Morales SI, Chávez-Montes RA, Hayano-Kanashiro C, Alejo-Jacuinde G, Rico-Cambron TY, de Folter S, Herrera-Estrella L. 2016. Regulatory network analysis reveals novel regulators of seed desiccation tolerance in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences, USA **113**, E5232–E5241.

Götz S, García-Gómez JM, Terol J, Williams TD, Nagaraj SH, Nueda MJ, Robles M, Talón M, Dopazo J, Conesa A. 2008. High-throughput functional annotation and data mining with the Blast2GO suite. Nucleic Acids Research **36**, 3420–3435.

Hayes ML, Dang KN, Diaz MF, Mulligan RM. 2015. A conserved glutamate residue in the C-terminal deaminase domain of pentatricopeptide repeat proteins is required for RNA editing activity. The Journal of Biological Chemistry **290**, 10136–10142.

Hendry GAF. 1993. Oxygen, free radical processes and seed longevity. Seed Science Research **3**, 141–153.

Hendry GAF, Finch-Savage WE, Thorpe PC, Atherton NM, Buckland SM, Nilsson KA, Seel WE. 1992. Free radical processes and loss of seed viability during desiccation in the recalcitrant species *Quercus robur* L. New Phytologist **122**, 273–279.

Hoekstra FA, Golovina EA, Buitink J. 2001. Mechanisms of plant desiccation tolerance. Trends in Plant Science **6**, 431–438.

Hou C, Tian W, Kleist T, He K, Garcia V, Bai F, Hao Y, Luan S, Li L. 2014. DUF221 proteins are a family of osmosensitive calcium-permeable cation channels conserved across eukaryotes. Cell Research **24**, 632–635.

Howell KA, Millar AH, Whelan J. 2006. Ordered assembly of mitochondria during rice germination begins with pro-mitochondrial structures rich in components of the protein import apparatus. Plant Molecular Biology **60**, 201–223.

Ivanova A, Law SR, Narsai R, *et al*. 2014. A functional antagonistic relationship between auxin and mitochondrial retrograde signaling regulates alternative oxidase1a expression in *Arabidopsis*. Plant Physiology **165**, 1233–1254.

Joët T, Laffargue A, Salmona J, Doulbeau S, Descroix F, Bertrand B, de Kochko A, Dussert S. 2009. Metabolic pathways in tropical dicotyledonous albuminous seeds: *Coffea arabica* as a case study. New Phytologist **182**, 146–162.

Kang J, Yim S, Choi H, Kim A, Lee KP, Lopez-Molina L, Martinoia E, Lee Y. 2015. Abscisic acid transporters cooperate to control seed germination. Nature Communications **6**, 8113.

Klie S, Nikoloski Z. 2012. The choice between mapman and gene ontology for automated gene function prediction in plant science. Frontiers in Genetics **3**, 115.

Knight MR, Campbell AK, Smith SM, Trewavas AJ. 1991. Transgenic plant aequorin reports the effects of touch and cold-shock and elicitors on cytoplasmic calcium. Nature **352**, 524–526.

Kranner I, Birtic S. 2005. A modulating role for antioxidants in desiccation tolerance. Integrative and Comparative Biology **45**, 734–740.

Lee HW, Kim NY, Lee DJ, Kim J. 2009. LBD18/ASL20 regulates lateral root formation in combination with LBD16/ASL18 downstream of ARF7 and ARF19 in *Arabidopsis*. Plant Physiology **151**, 1377–1389.

Leprince O, Bronchart R, Deltour R. 1990. Changes in starch and soluble sugars in relation to the acquisition of desiccation tolerance during maturation of *Brassica campestris* seed. Plant, Cell & Environment **13**, 539–546.

Leprince O, Buitink J. 2010. Desiccation tolerance: From genomics to the field. Plant Science **179**, 554–564.

Leprince O, Buitink J. 2015. Introduction to desiccation biology: from old borders to new frontiers. Planta **242**, 369–378.

Leprince O, Buitink J, Hoekstra FA. 1999. Axes and cotyledons of recalcitrant seeds of *Castanea sativa* Mill. exhibit contrasting responses of respiration to drying in relation to desiccation sensitivity. Journal of Experimental Botany **50**, 1515–1524.

Leprince O, Pellizzaro A, Berriri S, Buitink J. 2017. Late seed maturation: drying without dying. Journal of Experimental Botany **68**, 827–841.

Li DZ, Pritchard HW. 2009. The science and economics of ex situ plant conservation. Trends in Plant Science **14**, 614–621.

Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv, 1303.3997 [Preprint].

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R; **1000 Genome Project Data Processing Subgroup**. 2009. The sequence alignment/map format and SAMtools. Bioinformatics **25**, 2078–2079.

Liu Y, He J, Chen Z, Ren X, Hong X, Gong Z. 2010. *ABA overly-sensitive 5* (*ABO5*), encoding a pentatricopeptide repeat protein required for *cis*-splicing of mitochondrial *nad2* intron 3, is involved in the abscisic acid response in Arabidopsis. The Plant Journal **63**, 749–765.

Liu JM, Zhao JY, Lu PP, Chen M, Guo CH, Xu ZS, Ma YZ. 2016. The E-subgroup pentatricopeptide repeat protein family in *Arabidopsis thaliana* and confirmation of the responsiveness PPR96 to abiotic stresses. Frontiers in Plant Science **7**, 1825.

Logan DC, Millar AH, Sweetlove LJ, Hill SA, Leaver CJ. 2001. Mitochondrial biogenesis during germination in maize embryos. Plant Physiology **125**, 662–672.

Lohse M, Nagel A, Herter T, May P, Schroda M, Zrenner R, Tohge T, Fernie AR, Stitt M, Usadel B. 2014. Mercator: a fast and simple web server for genome scale functional annotation of plant sequence data. Plant, Cell & Environment **37**, 1250–1258.

Lorenzo O, Piqueras R, Sánchez-Serrano JJ, Solano R. 2003. ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. The Plant Cell **15**, 165–178.

Lou HQ, Gong YL, Fan W, Xu JM, Liu Y, Cao MJ, Wang MH, Yang JL, Zheng SJ. 2016. A formate dehydrogenase confers tolerance to aluminum and low pH. Plant Physiology **171**, 294–305.

Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biology **15**, 550.

Maia J, Dekkers BJ, Dolle MJ, Ligterink W, Hilhorst HW. 2014. Abscisic acid (ABA) sensitivity regulates desiccation tolerance in germinated Arabidopsis seeds. New Phytologist **203**, 81–93.

Manfre AJ, LaHatte GA, Climer CR, Marcotte WR Jr. 2009. Seed dehydration and the establishment of desiccation tolerance during seed maturation is altered in the *Arabidopsis thaliana* mutant *atem6-1*. Plant & Cell Physiology **50**, 243–253.

Moriwaki T, Miyazawa Y, Kobayashi A, Uchida M, Watanabe C, Fujii N, Takahashi H. 2011. Hormonal regulation of lateral root development in Arabidopsis modulated by MIZ1 and requirement of GNOM activity for MIZ1 function. Plant Physiology **157**, 1209–1220.

Müller CJ, Valdés AE, Wang G, Ramachandran P, Beste L, Uddenberg D, Carlsbecker A. 2016. PHABULOSA mediates an auxin signaling loop to regulate vascular patterning in Arabidopsis. Plant Physiology **170**, 956–970.

Murcha MW, Wang Y, Narsai R, Whelan J. 2014. The plant mitochondrial protein import apparatus – the differences make it interesting. Biochimica et Biophysica Acta **1840**, 1233–1245.

Pré M, Atallah M, Champion A, De Vos M, Pieterse CM, Memelink J. 2008. The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense. Plant Physiology **147**, 1347–1357.

Prieto-Dapena P, Castaño R, Almoguera C, Jordano J. 2006. Improved resistance to controlled deterioration in transgenic seeds. Plant Physiology **142**, 1102–1112.

Prieto-Dapena P, Castaño R, Almoguera C, Jordano J. 2008. The ectopic overexpression of a seed-specific transcription factor, HaHSFA9, confers tolerance to severe dehydration in vegetative organs. The Plant Journal **54**, 1004–1014.

Prigge MJ, Greenham K, Zhang Y, Santner A, Castillejo C, Mutka AM, O'Malley RC, Ecker JR, Kunkel BN, Estelle M. 2016. The *Arabidopsis* auxin receptor F-box proteins AFB4 and AFB5 are required for response to the synthetic auxin picloram. G3 **6**, 1383–1390.

Righetti K, Vu JL, Pelletier S, *et al*. 2015. Inference of longevity-related genes from a robust coexpression network of seed maturation identifies regulators linking seed storability to biotic defense-related pathways. The Plant Cell **27**, 2692–2708.

Robert N, d'Erfurth I, Marmagne A, *et al*. 2012. Voltage-dependent-anion-channels (VDACs) in *Arabidopsis* have a dual localization in the cell but show a distinct role in mitochondria. Plant Molecular Biology **78**, 431–446.

Roberts E. 1973. Predicting the storage life of seed. Seed Science and Technology **1**, 499–514.

Saavedra X, Modrego A, Rodríguez D, González-García MP, Sanz L, Nicolás G, Lorenzo O. 2010. The nuclear interactor PYL8/RCAR3 of *Fagus sylvatica* FsPP2C1 is a positive regulator of abscisic acid signaling in seeds and stress. Plant Physiology **152**, 133–150.

Sano N, Rajjou L, North HM, Debeaujon I, Marion-Poll A, Seo M. 2016. Staying alive: molecular aspects of seed longevity. Plant & Cell Physiology **57**, 660–674.

Sechet J, Roux C, Plessis A, *et al*. 2015. The ABA-deficiency suppressor locus HAS2 encodes the PPR protein LOI1/MEF11 involved in mitochondrial RNA editing. Molecular Plant **8**, 644–656.

Sew YS, Ströher E, Fenske R, Millar AH. 2016. Loss of mitochondrial malate dehydrogenase activity alters seed metabolism impairing seed maturation and post-germination growth in Arabidopsis. Plant Physiology **171**, 849–863.

Takano A, Suetsugu N, Wada M, Kohda D. 2010. Crystallographic and functional analyses of J-domain of JAC1 essential for chloroplast photorelocation movement in *Arabidopsis thaliana*. Plant & Cell Physiology **51**, 1372–1376.

Tapia H, Young L, Fox D, Bertozzi CR, Koshland D. 2015. Increasing intracellular trehalose is sufficient to confer desiccation tolerance to *Saccharomyces cerevisiae*. Proceedings of the National Academy of Sciences, USA **112**, 6122–6127.

Tateda C, Watanabe K, Kusano T, Takahashi Y. 2011. Molecular and genetic characterization of the gene family encoding the voltage-dependent anion channel in *Arabidopsis*. Journal of Experimental Botany **62**, 4773–4785.

Tolleter D, Jaquinod M, Mangavel C, Passirani C, Saulnier P, Manon S, Teyssier E, Payet N, Avelange-Macherel MH, Macherel D. 2007. Structure and function of a mitochondrial late embryogenesis abundant protein are revealed by desiccation. The Plant Cell **19**, 1580–1589.

Tweddle JC, Dickie JB, Baskin CC, Baskin JM. 2003. Ecological aspects of seed desiccation sensitivity. Journal of Ecology **91**, 294–304.

Verdier J, Lalanne D, Pelletier S, *et al*. 2013. A regulatory network-based approach dissects late maturation processes related to the acquisition of desiccation tolerance and longevity of *Medicago truncatula* seeds. Plant Physiology **163**, 757–774.

Vertucci CW. 1989. The effects of low water contents on physiological activities of seeds. Physiologia Plantarum **77**, 172–176.

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

Vertucci CW, Leopold AC. 1984. Bound water in soybean seed and its relation to respiration and imbibitional damage. Plant Physiology **75**, 114–117.

Wagner S, Van Aken O, Elsässer M, Schwarzländer M. 2018. Mitochondrial energy signaling and its role in the low-oxygen stress response of plants. Plant Physiology **176**, 1156–1170.

Walters C, Berjak P, Pammenter N, Kennedy K, Raven P. 2013. Plant science. Preservation of recalcitrant seeds. Science **339**, 915–916.

Walters C, Pammenter NW, Berjak P, Crane J. 2001. Desiccation damage, accelerated ageing and respiration in desiccation tolerant and sensitive seeds. Seed Science Research **11**, 135–148.

Wang L, Nägele T, Doerfler H, *et al*. 2016. System level analysis of cacao seed ripening reveals a sequential interplay of primary and secondary metabolism leading to polyphenol accumulation and preparation of stress resistance. The Plant Journal **87**, 318–332.

Wang Y, Li Y, Xue H, Pritchard HW, Wang X. 2015. Reactive oxygen species-provoked mitochondria-dependent cell death during ageing of elm (*Ulmus pumila* L.) seeds. The Plant Journal **81**, 438–452.

Wang Y, Ries A, Wu K, Yang A, Crawford NM. 2010. The *Arabidopsis* prohibitin gene *PHB3* functions in nitric oxide-mediated responses and in hydrogen peroxide-induced nitric oxide accumulation. The Plant Cell **22**, 249–259.

Watanabe K, Pacher M, Dukowic S, Schubert V, Puchta H, Schubert I. 2009. The STRUCTURAL MAINTENANCE OF CHROMOSOMES 5/6 complex promotes sister chromatid alignment and homologous recombination after DNA damage in *Arabidopsis thaliana*. The Plant Cell **21**, 2688–2699.

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

Wyse SV, Dickie JB. 2017. Predicting the global incidence of seed desiccation sensitivity. Journal of Ecology **105**, 1082–1093.

Yu Q, Tian H, Yue K, Liu J, Zhang B, Li X, Ding Z. 2016. A P-Loop NTPase regulates quiescent center cell division and distal stem cell identity through the regulation of ROS homeostasis in *Arabidopsis* root. PLoS Genetics **12**, e1006175.

Zhang K, Novak O, Wei Z, Gou M, Zhang X, Yu Y, Yang H, Cai Y, Strnad M, Liu CJ. 2014. *Arabidopsis* ABCG14 protein controls the acropetal translocation of root-synthesized cytokinins. Nature Communications **5**, 3274.

Zhu Q, Zhang J, Gao X, Tong J, Xiao L, Li W, Zhang H. 2010. The *Arabidopsis* AP2/ERF transcription factor RAP2.6 participates in ABA, salt and osmotic stress responses. Gene **457**, 1–12.

Zhu W, Miao Q, Sun D, Yang G, Wu C, Huang J, Zheng C. 2012. The mitochondrial phosphate transporters modulate plant responses to salt stress via affecting ATP and gibberellin metabolism in *Arabidopsis thaliana*. PLoS ONE **7**, e43530.

Zinsmeister J, Lalanne D, Terrasson E, *et al*. 2016. ABI5 is a regulator of seed maturation and longevity in legumes. The Plant Cell **28**, 2735–2754.