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Running head: Transcriptional Dynamics in Two Seed Compartments

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Title: Transcriptional Dynamics of Two Seed Compartments with Opposing Roles in Arabidopsis Seed Germination

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One sentence summary: Gene expression profiling in two seed compartments revealed two transcriptional phases during seed germination which are separated by testa rupture.

Footnotes:

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ABSTRACT

Seed germination is a critical stage in the plant life cycle and the first step towards successful plant establishment. Understanding germination is therefore of important ecological and agronomical relevance. Previous research revealed that different seed compartments (testa, endosperm and embryo) control germination, but little is known about the underlying spatial and temporal transcriptome changes that lead to seed germination. We analyzed genome-wide expression in germinating *Arabidopsis thaliana* seeds with both temporal and spatial detail and provide web accessible visualizations of the data reported (vseed.nottingham.ac.uk). We show the potential of this high resolution data set for the construction of meaningful co-expression networks, which provide insight into the genetic control of germination. The data set reveals two transcriptional phases during germination that are separated by testa rupture. The first phase is marked by large transcriptome changes as the seed switches from a dry, quiescent state to a hydrated and active state. At the end of this first transcriptional phase the number of differentially expressed genes between consecutive time points drop. This increases again at testa rupture, the start of the second transcriptional phase. Transcriptome data indicates a role for mechano-induced signalling at this stage, and subsequently highlights the fates of the endosperm and radicle; senescence and growth respectively. Finally, using a phylotranscriptomic approach we show that expression levels of evolutionary young genes drop during the first transcriptional phase and increase during the second phase. Evolutionary old genes show an opposite pattern, suggesting a more conserved transcriptome prior to the completion of germination.

INTRODUCTION

Seeds are important in the plant life cycle since they represent the link between two successive generations. They are stress resistant structures that help to bridge unfavorable periods and allow dispersal. Seed formation starts with a double fertilization event and in Arabidopsis it takes approximately 20 days to form a mature dry seed (Debeaujon et al., 2007; Ohto et al., 2007). At maturity three major seed compartments can be distinguished (Holdsworth et al., 2008a; Belmonte et al., 2013), i.e. the testa (seed coat), a dead tissue that forms a protective outer layer; the endosperm, a single cell layer of tissue positioned directly underneath the testa; the embryo (enclosed by the testa and endosperm), which emerges to become the future plant (Rajjou et al., 2012) (Fig. 1A). A dry seed is a unique structure in the sense that it allows severe dehydration (desiccation tolerance) and enters a phase of quiescence bringing processes occurring in "living" organisms to halt without affecting viability (Farrant and Moore, 2011; Rajjou et al., 2012). Upon imbibition of water, the dry mature seed swells and metabolic activity resumes; marking the start of seed germination and the end of the quiescent state. Arabidopsis germination consists of two visible sequential events (Holdsworth et al., 2008a; Weitbrecht et al., 2011). First, the testa splits (testa rupture, TR) due to underlying expansion of the endosperm and embryo. Thereafter, the radicle (embryonic root) protrudes through the endosperm (endosperm rupture, ER), completing germination *sensu stricto* (Fig. 1B). There are two non-exclusive mechanisms proposed to explain seed germination (Nonogaki, 2006; Nonogaki et al., 2007). The first involves the increase in embryo growth potential leading to elongation of the proximal embryonic axis (hypocotyl and radicle) that overcomes the restraint of the covering tissues. The second involves the weakening of these covering layers (including the micropylar endosperm, positioned over the radicle tip, Fig. 1A) to ease the protrusion of the radicle (for review (Finch-Savage and Leubner-Metzger, 2006)). The endosperm has been shown to affect germination even in species with a thin endosperm layer such as Arabidopsis (Muller et al., 2006; Bethke et al., 2007; Lee et al., 2010). Genome-wide expression studies have been previously applied to gain insight into several aspects of seed biology (Holdsworth et al., 2008a; Holdsworth et al., 2008b; Le et al., 2010) including temporal changes during Arabidopsis germination (Nakabayashi et al., 2005; Preston et al., 2009; Narsai et al., 2011) and in spatial differences between embryo and endosperm (Penfield et al., 2006; Endo et al., 2012). Nevertheless, a detailed knowledge of the temporal changes in gene expression in the different compartments of the Arabidopsis seed is thus far missing, but is essential to understanding the control of the timing of germination as well as the underlying molecular processes contributed by these different seed compartments. Therefore, we have analyzed the Arabidopsis transcriptome by sampling 11 points along the germination time course, including those that allow an analysis of gene expression changes at the key events of germination (TR and ER), with a focus on the micropylar endosperm and the radicle.

RESULTS & DISCUSSION

Arabidopsis seed imbibition, germination kinetics and transcriptome analyzes. We characterized Arabidopsis seed germination by scoring TR and ER over time. TR started around 20 hours after sowing (HAS) and at 31 HAS almost all seeds were fully ruptured. From 31 HAS onwards ER was observed, which was completed in the entire seed population by 45 HAS (Fig. 1C). Microarray experiments were performed using dry seeds and seeds at nine time points along the germination time course until the completion of germination (Fig. 1C). The time points 25 and 38 HAS showed a mixture of non-ruptured (NR) & TR seeds and TR & ER seeds respectively; at these time points both classes were separated and collected as distinct samples, which enabled us to map the transcriptome changes induced by TR and ER. To capture spatial dynamics, imbibed seeds were dissected into four parts. The key compartments for germination, the radicle (including a large part of the hypocotyl to ensure that it encompasses the region that elongates (Sliwinska et al., 2009), RAD) and the micropylar end of the endosperm (which is a combination of micropylar and chalazal endosperm, MCE) were sampled at all time points. At three time points (3, 16 and 31 HAS), the cotyledons (COT) and the remainder of the endosperm (peripheral endosperm, PE) were collected (for details see Fig. 1A,C,D and SI). The 29 samples, with four replicates for each sample, were analyzed using Affymetrix ATH1 gene chips. Plotting probe set values in a histogram showed clearly distinguishable peaks for noise and signal and revealed that an appropriate cutoff for considering a gene as potentially expressed as 5 on a $\log_2$ scale (Supplemental Fig. S1). The percentage of genes detected in the different seed compartments are within the same range that were described for other Arabidopsis seed transcriptome analyses (Nakabayashi et al., 2005; Penfield et al., 2006; Belmonte et al., 2013). In total 14,317 genes (67.2% of the 21,313 genes on the chip) were found to be expressed at least once in the 29 samples, of which 11,298 (78.8%) were shared between all compartments (Supplemental Fig. S2A).

At the start of the time course a lower number of genes were found to be expressed and this number increased during the time course in all tissues, most notably during the first 12 to 16 HAS (Supplemental Fig. S2B). We identified gene sets that were tissue specifically expressed, by considering genes as specifically expressed in one tissue when expressed above 6 in that tissue and expressed below 5 (on a $\log_2$ scale) in all the other tissues (which is therefore in the noise region). This resulted in 415 genes specific to the endosperm and 546 genes specific to the embryo in our data set (Supplemental Fig. S2, Supplemental Dataset S1) which overlap with previously published data sets (Penfield et al., 2006; Le et al., 2010) (Supplemental Data, Supplemental Fig. S3). In total 12,856 genes are expressed >6 in either tissue, with 10,801 expressed >6 in both tissues. Thus according to this definition, 84.01% of the genes are shared between both tissues whilst 3.22% are specific to the embryo and 4.24% are specific to the endosperm. The remaining genes (8.53%) are expressed over 6 in one tissue but between 5 and 6 in another tissue, and so are not classed as being highly specific to any one tissue. Interrogation using overrepresentation analysis (ORA) revealed that the endosperm

gene set was overrepresented for genes related to response to ABA, defense response, cell wall macromolecule metabolism/catabolism and cell death as well as genes associated with the regulation of transcription (Supplemental Fig. S2D), in agreement with recent findings (Endo et al., 2012). In the embryo the largest class was related to plant development. Other GO classes that were overrepresented included cell division, hormone metabolic process, protein amino acid phosphorylation, signalling and regulation of transcription (Supplemental Fig. S2E). Thus, different GO classes were found to be overrepresented in each tissue, with regulation of transcription/gene expression appearing in both. Both tissue specific gene sets are enriched for transcription factors (Supplemental Fig. S2F). In the endosperm transcription factors of NAC, WRKY and C3H classes and in the embryo transcription factors of bHLH, G2-like and HB classes are particularly enriched (Supplemental Fig. S2F). Compartment specific gene sets containing 106, 47, 21 and 2 genes were identified for the RAD, COT, MCE and PE (Supplemental Data, Supplemental Fig. S2, Supplemental Dataset S1) respectively and RT-qPCR confirmed the compartment specific expression of 20 genes (Supplemental Fig. S4).

In order to globally compare gene expression between the samples, all 116 arrays were plotted using Principal Component Analysis (PCA) (Fig. 2A). In general, the largest transcriptome differences were observed between the endosperm and embryo (MCE vs RAD) followed by the comparison between both embryo parts (RAD vs COT). The smallest differences were found between both endosperm (MCE vs PE) parts (Fig. 2). The quality controls (Supplemental Fig. S1), the high correlation between the replicates (Supplemental Table S1), and the confirmation by RT-qPCR of compartment specific expression (Supplemental Fig. S4) indicate that this is a robust dataset revealing transcriptome changes during seed rehydration and the developmental switch from a quiescent dry seed to germination in both temporal and spatial detail.

Generation of co-expression networks and data visualization tools. We generated co-expression networks (Bassel et al., 2011) for the endosperm (EndoNet) and the RAD samples (RadNet). We identified compact clusters of genes in the networks (Supplemental Dataset S1) that were further scrutinized with the network topological analyzer, TopoGSA (available at http://www.topogsa.net/, Glaab et al., 2010) (Supplemental Data, Supplemental Fig. S5). Interactive visualizations of both networks are available online at http://vseed.nottingham.ac.uk. Compared to our previous visualization tool (Bassel et al., 2011), these visualizations offer improved performance and more advanced gene selection options; such as the highlighting of individual genes or entire clusters, searching for genes by name or descriptive keywords and visualization of gene expression using our new Electronic Fluorescent Pictograph (eFP) browser (Winter et al., 2007).

The EndoNet shows a ring-like display, a result of the scarcity of genes with constant expression (Fig.

3A). This indicates that regulation of gene expression is very dynamic in the endosperm during germination. The largest 30 EndoNet clusters are spread around the network and thus represent the major gene expression profiles. Overrepresentation analysis (ORA) revealed cluster-specific overrepresentation of specific biological processes (Supplemental Fig. S6). These clusters consist of 26 to 195 genes and contain at least 99.7% of all possible edges within them (Fig. 3A,B), indicating that genes within such clusters have very similar expression patterns. Genes of some clusters (e.g. EndoNet cluster 1) are also co-expressed in the RadNet (81% of the edges in cluster 1 are also found in the RadNet at a 0.85 correlation) and show similar expression patterns in both compartments while other genes (such as EndoNet cluster 27) show an endosperm specific expression pattern and have few edges in common with the RadNet (Fig. 3B). On the other hand, almost all connections in EndoNet clusters 7 and 14 (98% and 88% respectively) are also present in the RadNet (Fig. 3B). Despite strong co-expression between both networks the expression profile in these clusters are different between the two compartments, being induced in both but subsequently repressed in the endosperm.

Arabidopsis seed germination is comprised of two transcriptional phases. Analyzing the transcriptional dynamics between consecutive time points of the germination time course revealed two transcriptional phases (Fig. 4). The first phase runs from 1-25 HAS non-ruptured (NR) and is characterized by large transcriptional changes, in both up- and down-regulated genes. At the end of this first phase the number of differentially expressed genes was reduced (Fig. 4). The second phase, which runs from TR to the completion of germination, was marked by resumption of differential gene expression, most notably at TR. During the second phase the majority of the differentially expressed genes are induced rather than repressed, in contrast to the first phase.

The first transcriptional phase is characterized by an inversion of the seed maturation transcriptional programme. Between 1 and 3 HAS differential gene expression was observed, particularly in the MCE (Fig. 4). In comparison, the response of the RAD is delayed, which could be due to its slower imbibition kinetics compared to the more outward positioned MCE (Fig. 4). Large transcriptional changes occurred in the first 16 HAS. ORA of this phase suggests a large overlap in the functional classes that are activated in the MCE and RAD (i.e. genes related to cell wall function, nucleotide metabolism, amino acid metabolism and protein translation, Fig. 5). A major difference is the activation of classes related to transport and energy metabolism (lipid metabolism, glycolysis, TCA and mitochondrial electron transport) that are specifically activated in the MCE from 20 HAS, in agreement with findings that storage lipids are more rapidly mobilized in the endosperm compared to the embryo (Penfield et al., 2005).

We compared gene expression during seed germination to gene expression during seed development and identified two gene sets containing 602 and 907 genes (Supplemental Dataset S1) that were strongly up- and down-regulated, respectively, between the embryo cotyledon phase (early seed maturation) and the post mature green stage (PMG, late maturation) from a publicly available data set (Le et al., 2010). The expression of the two gene sets was analyzed during germination and the majority of the genes of both sets showed inverse expression patterns during seed germination (Fig. 6). The largest overlap (75%) was found between genes that were up-regulated during seed maturation and those down-regulated during germination. Additionally, 67% of the genes from the set that were down-regulated during seed maturation showed an inverse expression pattern (are induced) during germination. The re-induction of these seed maturation down-regulated genes during germination is slower than the removal of the seed maturation induced genes. Nevertheless, the majority of the seed maturation repressed genes were re-activated in the first transcriptional phase rather than the second transcriptional phase.

TR is marked by high transcriptional activity that overlaps in part with a response to touch-induced signalling. TR is characterized by a large number of differentially expressed genes when compared to non-ruptured seeds at 25 HAS, mostly genes that are up-regulated in the MCE (Fig. 7A). At TR, 104 genes were over 5-fold up-regulated in the MCE (Supplemental Dataset S1), 30 of which are related to cell wall function. Other classes induced by TR in the MCE include genes related to biotic stress, hormone metabolism, regulation of transcription, signalling (receptor kinases) and transport (Fig. 7B). Possible reasons for these large transcriptional changes between NR and TR seeds include an enhanced access to oxygen, light signalling and/or a touch (mechano)-sensing response (Fig. 7C). ORA did not reveal a clear indication of the involvement of either oxygen or light. However, the gene set included *TOUCH3* and *TOUCH4* (both >8 fold induced), which are known to respond rapidly to touch (Braam, 2005) (Fig. 7D). To investigate whether the transcriptional up-regulation at TR resembles touch-sensing we compared our MCE TR up data set to genes up-regulated upon touch in aerial parts of plants (Lee et al., 2005). We re-analyzed a published touch data set (Lee et al., 2005) (Supplemental Data) and found a 30% overlap with our TR induced set in the MCE and the touch up-regulated genes, with a lower overlap between the touch data set and the TR induced genes in the RAD (Fig. 7E).The overlap between the gene sets induced by TR in the MCE and touch was more striking when the gene classes were considered. Touch-induced signalling resulted in a relatively higher abundance of genes related to the GO classes cell wall associated, calcium binding, disease resistance, kinase and transcription factor (Lee et al., 2005) which match well with the classes identified at TR (Fig. 7B). We also observed that gene expression associated with jasmonate (JA) biosynthesis was activated upon TR in the MCE; this plant hormone was recently shown to be a key regulator of plant morphogenesis and enhanced pest resistance upon touch (Chehab et al., 2012). It has been hypothesized that gene expression in the endosperm during germination

might be affected by touch/mechano-sensing (Martinez-Andujar et al., 2012) and this transcriptome study provides a strong suggestion that touch-signalling is indeed, at least in part, responsible for induction of gene expression in the endosperm.

The second transcriptional phase highlights distinct fates for the embryo and the endosperm. The second transcriptional phase starts at TR and includes gene expression changes related to the completion of germination. Using ORA, we analyzed the temporal changes in the MCE and the RAD (Fig. 5) as well as gene sets that are higher expressed within the MCE or RAD along the time course (Supplemental Fig. S7). This revealed that, in the MCE genes related to secondary metabolism, amino acid metabolism and protein synthesis are overrepresented transiently (Fig. 5). Genes higher expressed in the MCE than the RAD are enriched for protein degradation, transport and stress related genes (although the latter is overrepresented in the MCE over the whole time course) (Supplemental Fig. S7). The RAD, particularly at the later stage, is enriched for cellular metabolism related to DNA, RNA and proteins compared to the MCE (Supplemental Fig. S7). ORA suggests that energy metabolism (lipid metabolism, glycolysis, TCA and mitochondrial electron transport), is activated by 38 HAS. At this stage genes for cell wall biosynthesis, transport and secondary metabolism are activated notably just prior to ER (Fig. 5). In addition, genes related to the cell cycle, lipid and amino acid metabolism are overrepresented within genes higher expressed in the RAD than the MCE (Supplemental Fig. S7), which are all classes supporting tissue growth. The GO gene class 'aging' becomes overrepresented in the latter part of the germination time course in the MCE (Fig. 5, Supplemental Fig. S7). This is in agreement with the down regulation of key cellular metabolic pathways and the induction of gene classes related to remobilization, reminiscent of transcriptional changes described for senescence (Lim et al., 2007; Breeze et al., 2011).

The transition from a dry quiescent to a hydrated and germinating seed coincides with increased transcriptional differences between seed compartments. From the PCA of all 116 arrays (Fig. 2A) we conclude that the transcriptome differences between seed compartments are small during early germination and increase with time. This is in agreement with the observation that the number of endosperm and embryo specific genes expressed increased along the time course from approximately 40 to 400 (Supplemental Fig. S8A). This may be explained by the fact that the majority of genes induced in seed maturation and which are subsequently removed during germination are shared by the MCE and RAD (72%) and that seed maturation-repressed genes (re-activated during germination) are, in contrast, mostly specific to either the RAD or the MCE (Fig. 6). Presumably, repression of genes related to development and differentiation is a more general response for an organism passing through a desiccated state, as is shown for the expression of genes involved in stomatal development (in the cotyledon samples) and root development (in the RAD samples). Many of these genes are induced

(sometimes transiently) during germination, with low or no expression initially (Supplemental Fig. S8B).

Differential gene expression in the endosperm is concentrated at the micropylar end.

The observation that the transcriptional differences increase with time between seed compartments is, besides the PCA, also shown in the number of differentially expressed genes between the seed compartments. The number of differentially expressed genes was the least between both endosperm compartments. At 31 HAS about 200 genes were differentially expressed (>3 fold difference), with the majority of these (95%) being up-regulated in the MCE (Fig. 2B) compared to the PE. Such a skewed division was not observed for other comparisons (Fig. 2B). The micropylar endosperm is hypothesized to possess an inhibitory role in germination and endosperm changes, in particular of cell wall properties, are suggested to be important for germination control (Nonogaki et al., 2007). Recently, using in situ cell wall epitope detection, showed that Arabidopsis endosperm cell walls have a different structure compared to the embryo cell wall and that the endosperm walls contain cellulose, unesterified homogalacturonan, arabinan and xyloglucan polymers (Lee et al., 2012). However, no spatial or temporal heterogeneity in cell wall polymers was observed prior to germination (Lee et al., 2012). This could indicate that cell wall changes leading to germination are modifications that are not detectable by in situ analysis and/or occur very locally. We compared both endosperm samples and found many differentially expressed genes between the MCE and PE (Supplemental Dataset S1). The largest differences were found close to the point of germination (31 HAS) in the MCE and this set was investigated for candidates that are potentially involved in ER. Several transcription factors were found to be highly expressed in the MCE compared to the PE which may function in gene regulation in this particular compartment. Genes related to cell wall function, including peroxidases, a pectin lyase-like superfamily protein, chitinase family protein and *ARABINOGALACTAN PROTEIN31* (*AGP31*) were identified in this set and these could be potential candidates for affecting cell wall properties to enable seed germination. It is notable that one of the highest differentially expressed (>20 fold) genes in the MCE is *INFLORESCENCE DEFICIENT IN ABSCISSION-LIKE1* (*IDL1*). This encodes a putative ligand that promotes cell separation and floral organ abscission via the interaction with receptor-like kinases (Stenvik et al., 2008). Recently, it has been reported that the *INFLORESCENCE DEFICIENT IN ABSCISSION* (*IDA*) peptide and its receptors *HAESA* (*HAE*) and *HAESA-LIKE2* (*HSL2*) are also important for cell separation during lateral root emergence (Kumpf et al., 2013), suggesting that Arabidopsis seed germination may occur via a cell separation event which is potentially regulated by the *IDA*/*IDL-HAE*/*HSL* signalling module. This detailed dataset allowed the identification of transcription factors, cell wall related genes, and genes related to cell separation although further research is needed to investigate their potential role in seed germination.

Seed germination is characterized by coordinated expression of evolutionarily old and young genes. Recently it has been shown that, like animal embryogenesis, plant embryogenesis involves a passage through a conserved and evolutionarily old transcriptional stage (Quint et al., 2012). This so-called phylotypic stage is mainly caused by repression of evolutionarily young genes and is proposed to help the spatio-temporal organization and differentiation of multi-cellular life (Quint et al., 2012). Since we observed a largely inverse expression pattern during germination of gene sets that are up- and down-regulated during seed development (Fig. 6), we asked whether i) seed germination is also characterized by coordinated expression of evolutionarily old and young genes and ii) if so, whether these patterns are linked to the two transcriptomic phases we observed. To answer these questions we first applied the phylostratigraphic approach (Domazet-Loso et al., 2007; Domazet-Loso and Tautz, 2010; Quint et al., 2012) in which we ordered the Arabidopsis genome into 12 evolutionary age classes (phylostrata, PS). Each Arabidopsis gene is blasted against all genomes underlying the twelve PS and is sorted in its phylostratum (PS) defined as the most distant phylogenetic node containing at least one species with detectable homologue (Quint et al., 2012). This resulted in the phylostratigraphic map in which PS1 (cellular organisms) contains the evolutionarily oldest genes and PS12 (*Arabidopsis thaliana*) contains the youngest genes that are specific to *Arabidopsis thaliana*, with no homologues detected in any of the other species (Supplemental Fig. S9A).

Next we interrogated the gene expression data of the MCE and plotted the relative expression values of i) genes that arose before plant evolution (PS1 and 2 combined), ii) genes that arose during early plant evolution (algae and non-seed bearing plants, PS3-5) and iii) the evolutionarily youngest genes (which evolved in seed bearing plants, PS6-12). The analysis shows that in the MCE the relative expression of evolutionarily young genes is high shortly after imbibition but drops during the first transcriptional phase followed by an increase in the second transcriptional phase (Fig. 8A, Supplemental Fig. S9). Interestingly, the oldest genes (PS1 and 2) showed an inverse behaviour, starting low at the beginning of germination, peaking at the end of the first transcriptional phase followed by a decrease in the second transcriptional phase. Genes of PS3-5 show a different pattern, starting low and increasing during the course of germination. Comparable results were obtained for the RAD during germination (Fig. 8B, Supplemental Fig. S9). The patterns in both seed parts, particularly the inverse patterns of the evolutionarily old and young genes, suggest that seeds not only pass through an evolutionary conserved stage during seed development but also during the successive germination phase. Coordinated expression of evolutionarily old and young genes (and in this way passage through a conserved transcriptional state) may help to channel large physiological transitions.

CONCLUSION

This study revealed two separate transcriptional phases for seed germination that are separated by TR and provides a strong indication that mechano-induced signalling affects gene expression at TR in the

MCE. It also shows that time is an important determinant for spatial expression differences. Surprisingly, we found similar patterns of expression of evolutionarily old and young genes in seed development and seed germination, suggesting that in plants passing through a transcriptional old and conserved stage may not be limited to embryogenesis. In addition to the novel biological insight, we are convinced that this detailed transcriptome data including the tools developed for data visualization and mining provides a powerful resource to gain further understanding in the role of different seed compartments in germination, novel regulators and gene networks underlying seed germination.

MATERIAL & METHODS

Plant material, sampling and microarray analysis. For this experiment the *Arabidopsis thaliana* accession Columbia-0 (Col-0, N60000) was used. Seeds were sown on 0.7% water agarose (Eurogentec) and incubated in a germination cabinet at 22°C with continuous light. Germination curves (for both testa and endosperm rupture) were assessed by scoring germination in time. After the indicated hours after sowing seeds were harvested and dissected using forceps and a scalpel knife. For the isolation of RNA a commercial kit (Agilent Technologies, Absolutely RNA Nanoprep kit) was used. In total 100ng of RNA was used to synthesize Biotin-labeled cRNA (using the Affymetrix 3' IVT-Express Labeling Kit) which was hybridized on the Affymetrix GeneChips Arabidopsis ATH1 Genome Array. The raw .cel files were background corrected and normalized using the Robust Microarray Averaging (RMA) procedure (Irizarry et al., 2003). The microarray data used in this article has been deposited in NCBI's Gene Expression Omnibus GEO Series accession number 41212. A detailed version of the material & methods is available in Supplemental Data file.

SUPPLEMENTAL MATERIAL

Supplemental Data MaterialandMethods Figures and Tables.pdf

Supplemental Dataset S1.xlsx

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FIGURE LEGENDS

Fig. 1. Seed compartments and seed germination kinetics of Arabidopsis seeds. A, A section through an Arabidopsis seed depicting the different seed compartments. B, Different stages during seed germination including TR (which exposes the underlying endosperm layer) and ER (also known as radicle protrusion or germination *sensu stricto*). C, Arabidopsis seed germination is analyzed by measuring TR (grey line), ER (black line) and seed water content (WC, blue diamonds). Underneath the graph the time points and physiological stage (dry, non-ruptured, TR and ER) are indicated for each sample. The 29 samples that were analyzed are schematically shown underneath the germination graph by the yellow pictograms. D, The four seed sections that were used for transcriptome analysis. MCE = micropylar and chalazal endosperm, HAS = hours after sowing.

Fig. 2. Transcriptional differences between seed compartments. A, Principle component analysis of the 116 samples. The four replicates of all 29 samples are indicated by colour. B, Tissue differences are represented by the number of differentially expressed genes at three time points during imbibition (3, 16 and 31 HAS, the time points in which all four tissues were sampled). Comparisons were made between endosperm and embryo (MCE vs RAD), between embryo tissues (RAD vs COT) and between both endosperm samples (MCE vs PE). The bars show the number of differentially expressed genes at a 2, 3, 5 and 10-fold cutoff. The pie diagrams underneath the graph indicate the fraction of the total number of differentially expressed genes (at a 3-fold cutoff level) in either of the two tissues that were compared at 31 HAS.

Fig. 3. The endosperm co-expression network, EndoNet. A, Sample lay-out of EndoNet, the nodes (genes) are indicated by grey circles and edges (grey lines) are drawn between two nodes if their correlation of expression is above 0.932. The 30 largest clusters are indicated by different colors. To visualise the gene expression profiles captured in the network the expression profiles of exemplar genes are shown around the network. B, Details of the largest 30 clusters are shown including the number of nodes, edges and the percentage of edges that are shared with RadNet (at a cutoff of 0.85). The gene expression profiles of genes in the EndoNet clusters 1, 7, 12 and 27 are shown (the position of these clusters in the EndoNet is shown A). The right side of the graph depicts the expression profiles of the same set of genes in the RAD samples.

Fig. 4. Arabidopsis seed germination is characterized by two transcriptional phases. The number of differentially expressed genes (both UP and DOWN) between consecutive time points (3 was

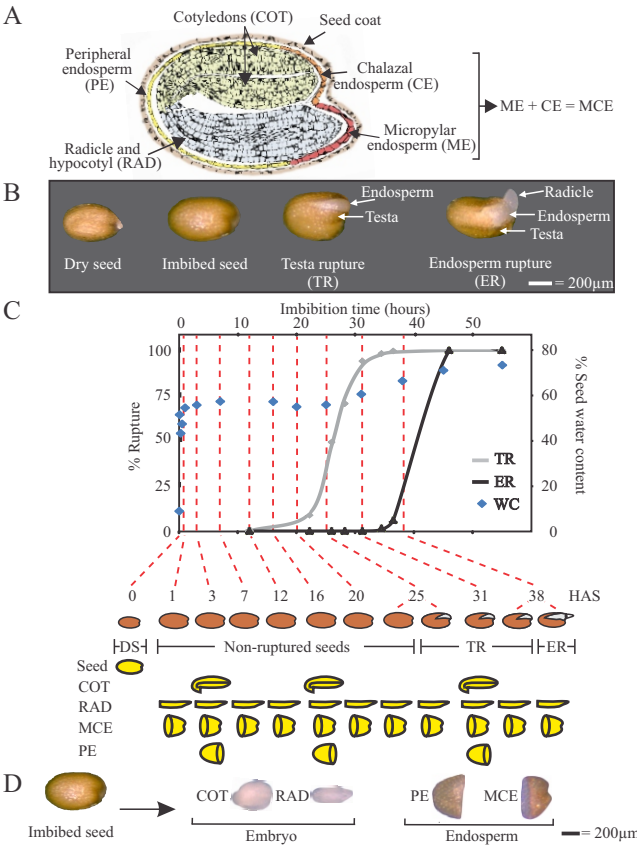
compared to 1, 7 vs. 3, 12 vs. 7 etc.) in the MCE (white bars) and RAD (brown bars) with a reasonable fold change (taking a 3 fold difference as cutoff) are presented. The two transcriptional phases, phase I from 1 to 25 HAS NR and phase II from 25 HAS NR to 38 HAS ER are indicated by the red arrows.

Fig. 5. Temporal differences between endosperm and embryo using ORA. The overrepresented gene categories of the up-regulated genes of the germination time course (all time points were compared to 1 HAS) were identified in the MCE (upper graph) and the RAD (bottom graph) using Pageman (Usadel et al., 2006). Selected categories are summarized in the graphs and black bars show the time points during germination at which the indicated gene categories are overrepresented.

Fig. 6. Inverse expression of seed maturation genes during germination in temporal and spatial detail. The upper panel shows the percentage of up-regulated genes during germination, of a set of 907 genes that are down-regulated during seed maturation. The lower panel shows the percentage of down-regulated genes during germination, of a set of 602 genes that are up-regulated during seed maturation. Genes expressed specifically in the MCE (in brown), in the RAD (in white) and in both (in black).

Fig. 7. Genes induced with respect to TR show a large overlap with touch-induced signalling. A, Number of differentially expressed genes at 25 HAS TR (compared to 25 HAS NR) in the MCE and RAD at different fold change cutoffs. B, Gene classes overrepresented in the TR-induced gene sets in the MCE and RAD. C, Schematic presentation of effectors that could be responsible for the large gene expression changes observed at TR. D, Expression behaviour of four *TOUCH* genes at TR in the MCE. E, Table shows the percentage of the TR up-regulated genes in the MCE and the RAD (at 2, 3 and 5 fold cutoff) that overlap with the 934 touch up-regulated genes. Percentage expected by chance is indicated using number of genes present on chip, genes expressed in the germination time course, genes expressed in the MCE and genes expressed in the RAD. Met = metabolism, synt = synthesis, degr = degradation, misc = miscellaneous, reg = regulation

Fig. 8. Relative expression of evolutionary old and young genes across the Arabidopsis germination time course. Plotted are the relative expression levels (± SEM) of genes of PS1-2, PS3-5, PS6-12 across the Arabidopsis germination time course in the A, MCE and B, RAD compartments. The significance between the relative expression levels between the groups is indicated at each time point by asterisks; * = p-value <0.05, ** = p-value <0.01, *** = p-value <0.001. See Supplemental Fig. S9 for the phylostratigrapic map and the mean relative expression of the individual phylostrata.



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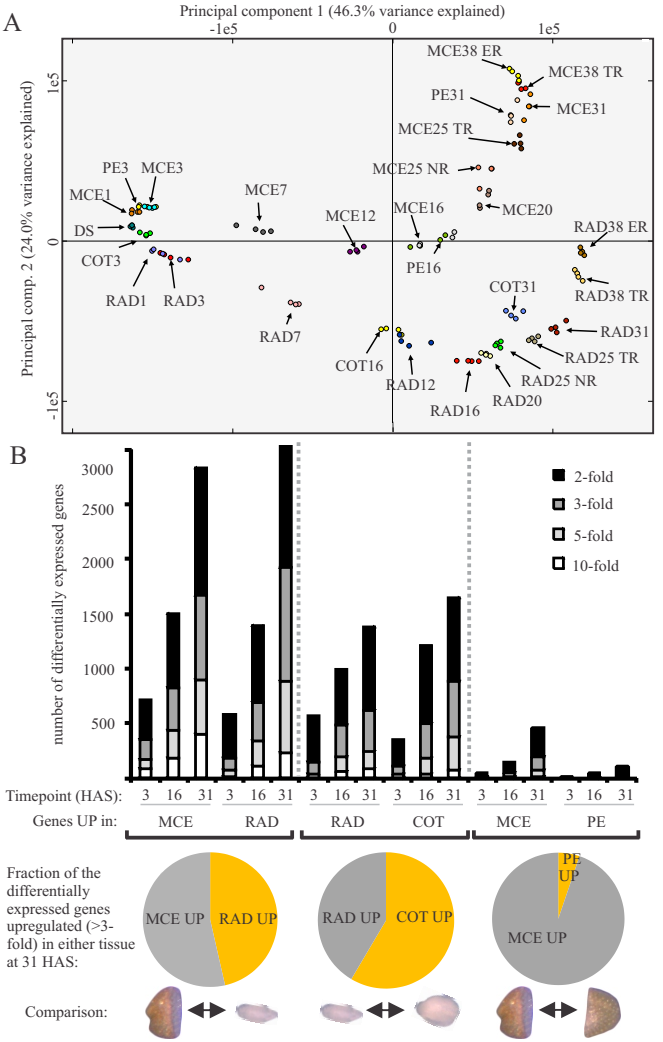

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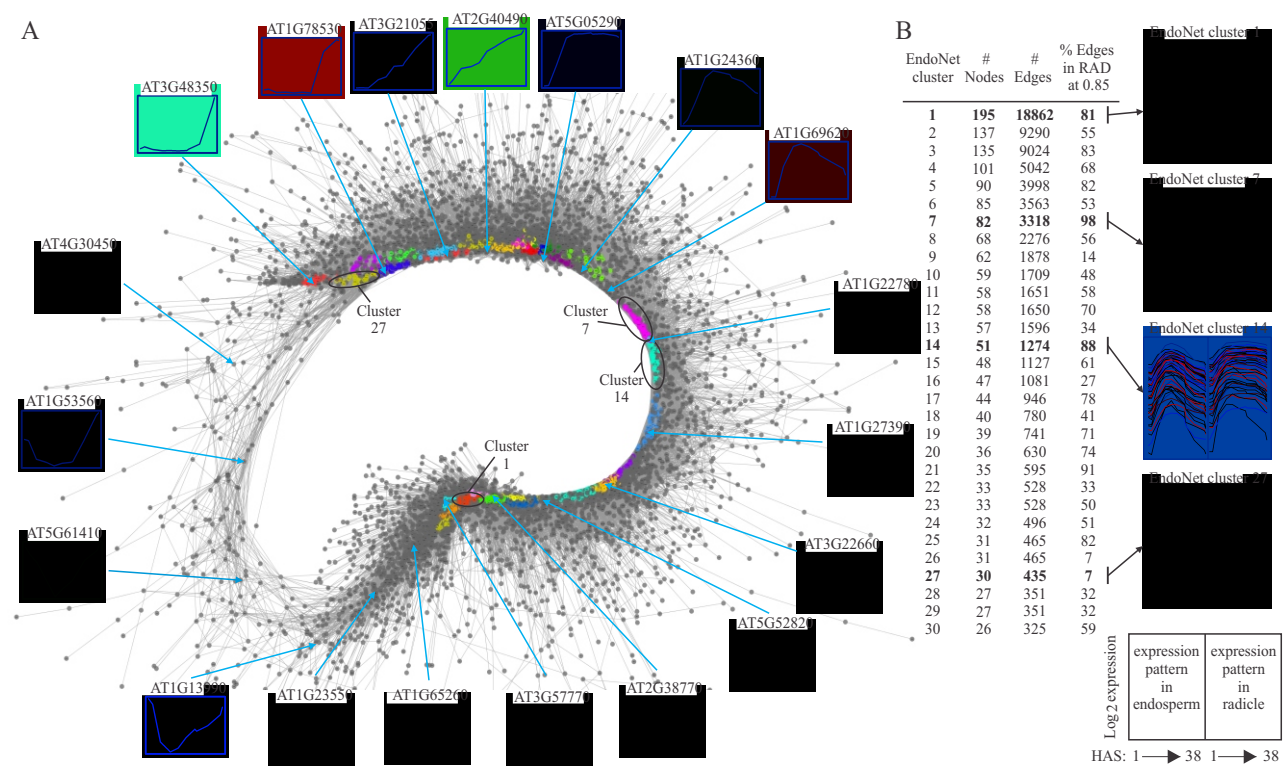

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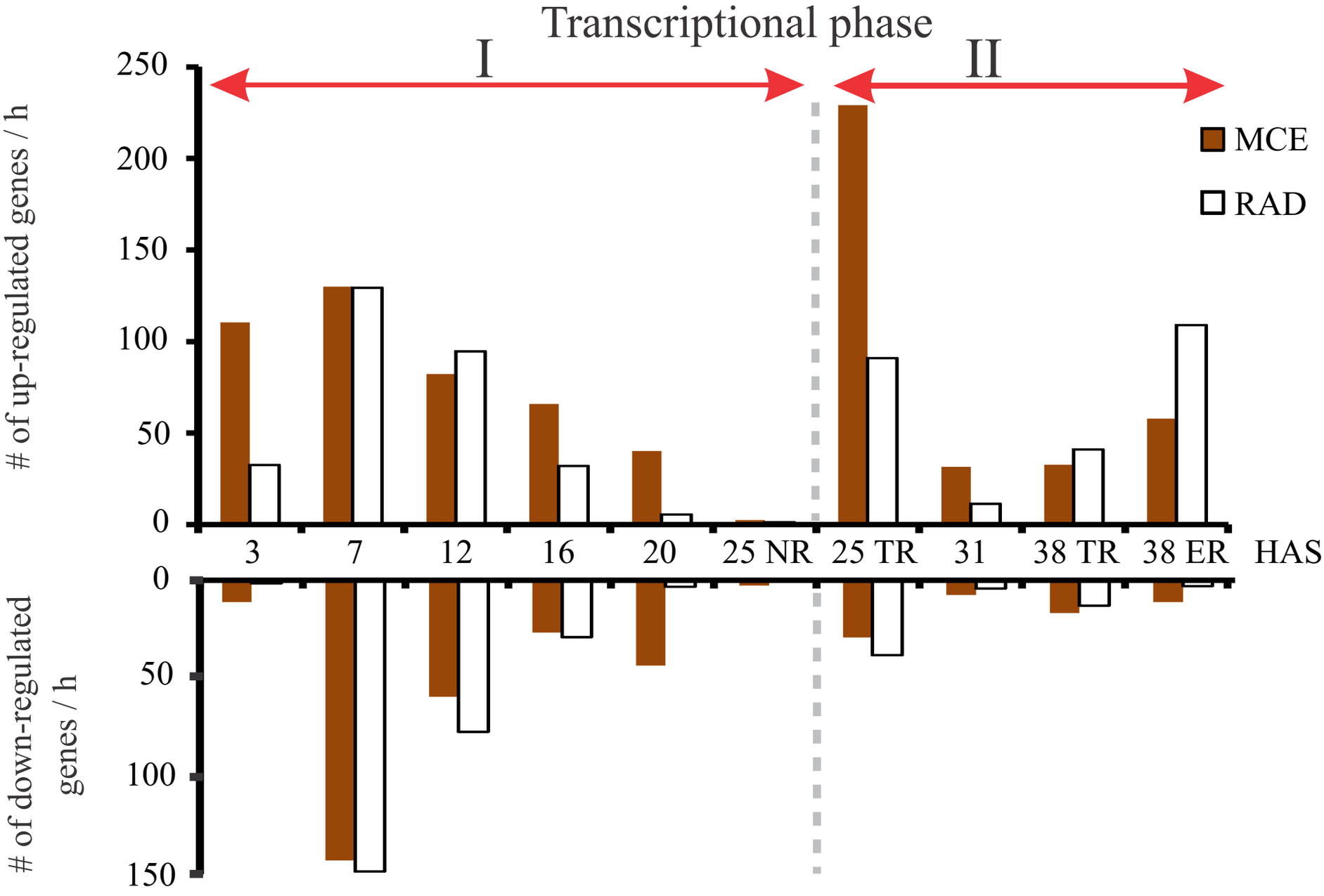

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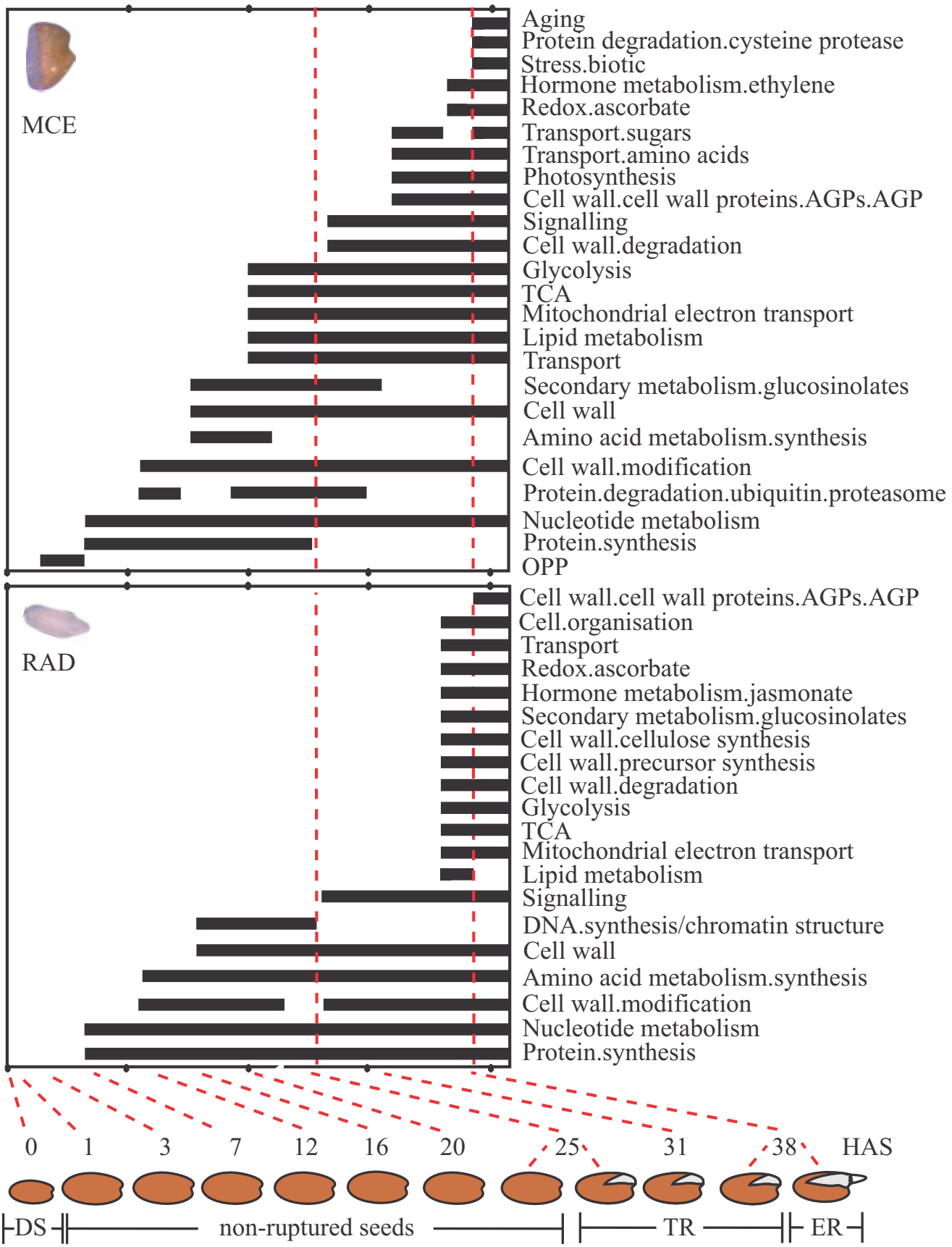

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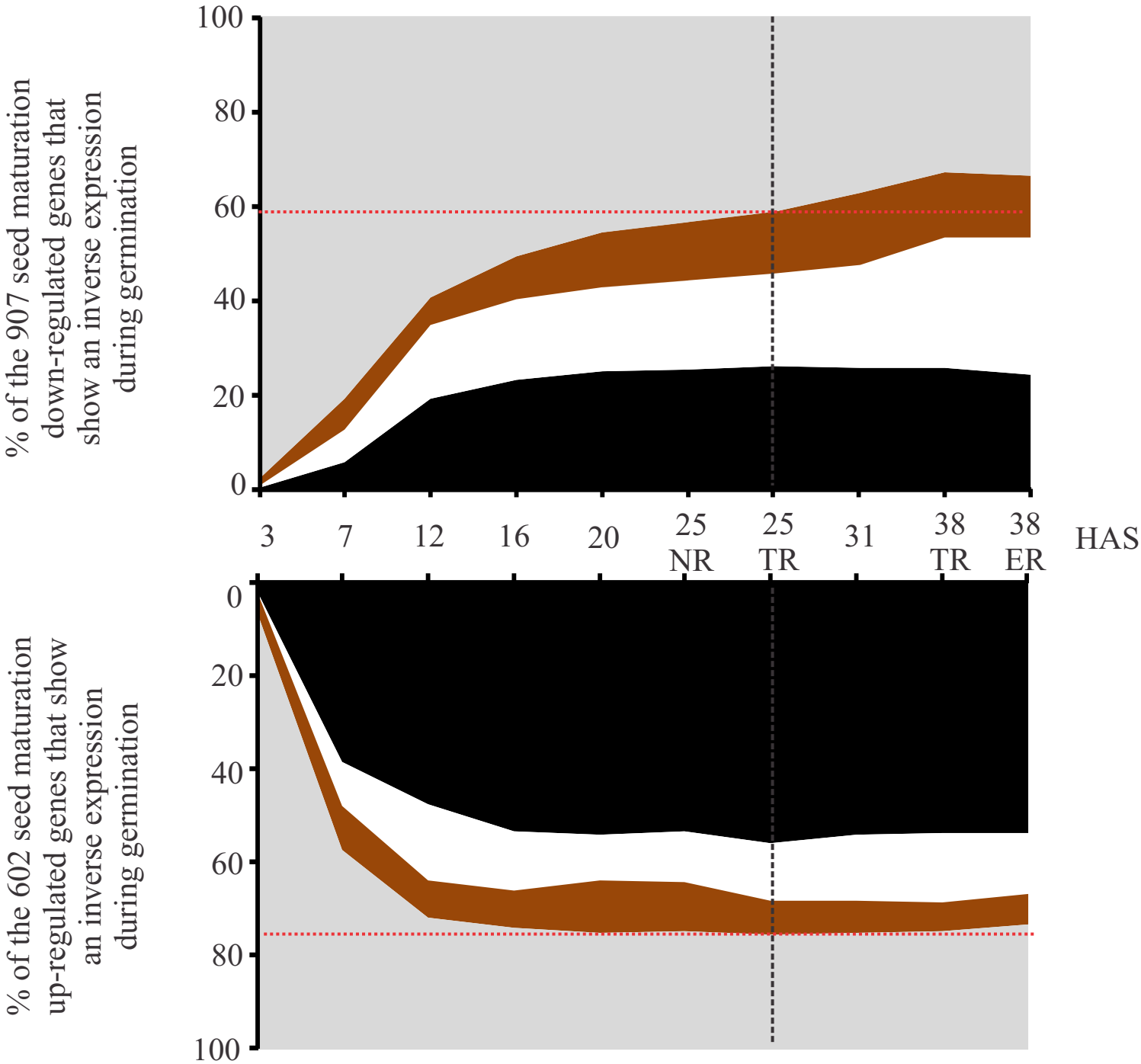

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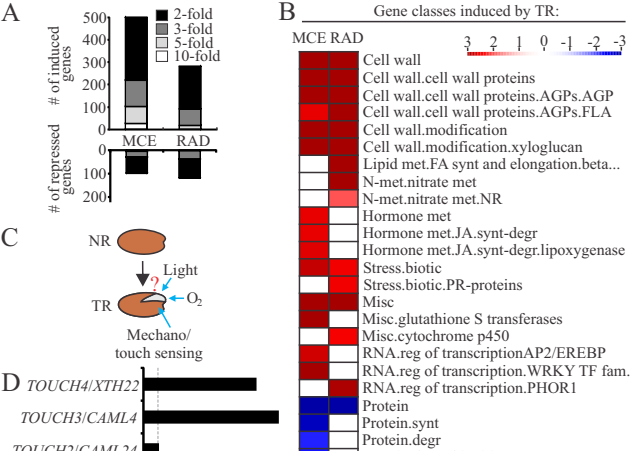

E Percentage of TR-induced genes that overlap with touch-induced gene set.

	Fold change 2	3	5
MCE	**23.11%** (116/502)	**29.26%** (67/229)	**30.40%** (31/102)
RAD	**21.99%** (62/282)	**18.68%** (17/91)	**5.56%** (1/18)

Percentage expected by chance considering:

All genes on chip (21,313)	All genes expressed in time-course (14,317)	All genes expressed in MCE (13,085)	All genes expressed in RAD (13,279)
4.11%	**5.38%**	**5.33%**	**5.46%**

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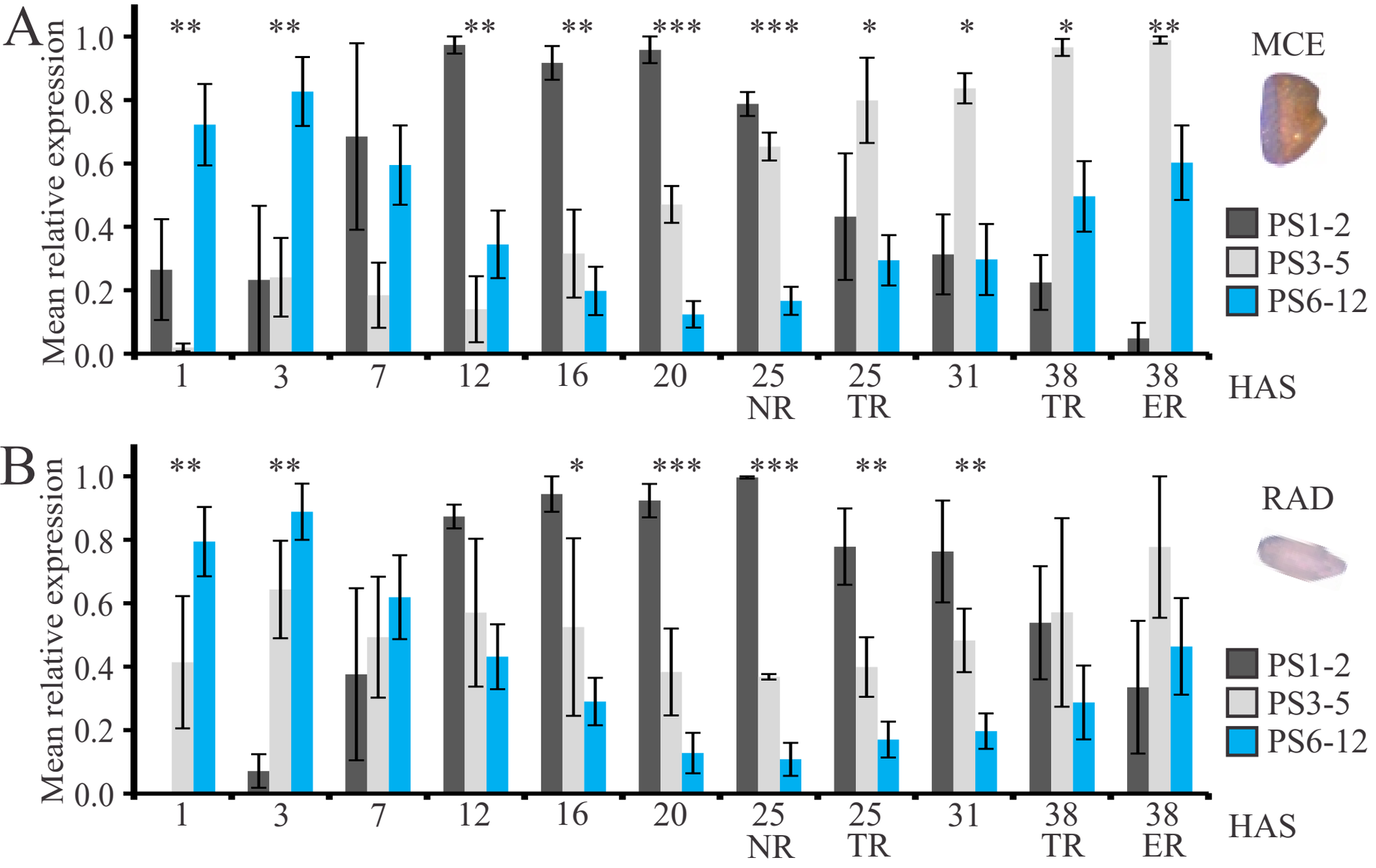

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