

Supplementary materials legends

Figure S1.

Pyramid plots showing numbers of up-regulated and down-regulated DEGs in the comparison of *gi-2* vs. *aba1-6* at matched time-points (ZT1 to ZT16) under "normal irrigation" (left) or "water deficit" conditions (right). Right (blue) and left (red) bars represent upregulated or downregulated genes, respectively.

Figure S2.

Expression profiles of selected genes. Scatterplots of log₂ normalized total reads (log₂ CPM) for a selection of "diagnostic genes", across time points, genotypes and conditions. Time points are represented on the x-axis, log₂(CPM) on the y-axis. Genotypes are indicated by different colours.

Figure S3.

Left, pyramid plots showing numbers of up-regulated and down-regulated DEGs in the comparison of *aba1-6* vs. wild type at matched time-points (ZT1 to ZT16) under "normal irrigation" (top) or "water deficit" conditions (bottom). Right (blue) and left (red) bars represent upregulated or downregulated genes, respectively.

Right, intersection of DEGs in *gi-2* and *aba1-6* with respect to the wild type. Heatmaps illustrate the total number of DEGs and numbers of common DEGs between *gi-2* and *aba1-6* mutants compared with the wild-type background at ZT1 and ZT12 under different irrigation schemes. Upper panel, comparison of *aba1-6* under "normal irrigation" and "well-watered" conditions. Middle panel, comparison of *aba1-6* and *gi-2* under "well-watered" conditions. Bottom panel, comparison of *aba1-6* and *gi-2* under water deficit conditions. Colours indicate the overlap (%) for each pairwise comparison according to the colour scale positioned on the right. The horizontal bar chart on the right represents the total number of DEGs detected at each ZT/condition combination (grey) and those which are in common with any the other ZTs/conditions (black).

Figure S4.

Volcano plots showing a selection of ABA/Cold and circadian-related DEGs in *gi-2* (top) or *aba1-6* mutants (bottom panel) at ZT1 under water deficit. LogFC is reported on the X-axis. FDR on the Y axis. Differentially expressed genes (FDR for differential expression ≤ 0.05)

are represented in purple, non-differentially expressed genes (FDR > 0.05) in green. Selected genes are displayed in yellow.

Figure S5.

Levels of ABA accumulation (expressed as ng per grams of dry weight) in different genotypes undergoing well-watered or water deficit conditions. Bars represent the standard deviation. n = 2-3 per genotype/condition

Figure S6.

Lollipop chart of the top 10 most enriched terms in the Gene Ontology molecular function class in *gi-2* under “well-watered” and “drought” conditions at ZT1 and ZT12. Colours indicate significance levels of enrichment (-log₁₀ FDR) according to the colour scale on the right. Size of the dots indicated the fold enrichment.

Figure S7.

Proportion of DEGs (in *gi-2* and *aba1-6* vs. WT, top and bottom panels, respectively) classified as circadian genes according to the ‘CCEE’ dataset (Covington *et al.* 2008), at different time points and conditions. Left and right bars represent down or up regulated genes, respectively. Asterisks denote a significant over-representation of up or down regulated genes based on Fisher’s exact test (corresponding p-values are shown in the table below).

Figure S8.

Left, Real Time qPCR of *Cor15a* (Top panel) and *ERD7* (Bottom panel) transcripts in the wild-type compared to *35S::GI* (*35S::HA-GI*) plants treated with ABA or mock. Samples were analysed at ZT1 and ZT8 coinciding with the light phase in a long day of 16h. Reaction to norm plots represent the mean of four biological replicates and associated standard error. Transcript levels are expressed as -dCt values (relative to *ACT2* expression). Asterisks indicate statistically significant effect (*p<0.05, **p<0.01) for the genotype and, when detected, for the Genotype x Treatment effects (G XT) after factorial ANOVA analysis.

Right, as above, except that bars indicate mean fold change variations of *GI* levels relative to wild-type expression detected under mock conditions (n = 4 biological replicates). *ACT2* expression was used for normalization; error bars represent SD.

Figure S9.

Real-time qPCR of *ABI1* and *ABI2* transcripts in 3-week-old wild-type (WT) Col-0, *gi-2*, *aba1-6* seedlings. Plants were subject to normal-watering (grey lines, control) or water deficit (black lines, treatment) regimes and harvested at the indicated time points coinciding with the light phase (white bar) or in the dark (black bar) during an SD-to-LD shift. At each time point, values represent fold change variations of *ABI1* (top) and *ABI2* (bottom) transcript levels relative to the wild type under normal watering. *ACT2* expression was used for normalization; error bars represent SD of two technical replicates.

Table S1.

List of differentially expressed genes. Gene IDs, according to the TAIR10 annotation of the *A. thaliana* genome, AGI codes and a brief description of the genes (if/where available) are reported in the first 3 columns. FDR for differential expression in every pairwise comparison performed in the study are reported in the subsequent columns. Conditions that were compared are indicated in the headers.

Table S2.

The table reports p-values for the significant over-representation of the complete collection of *A. thaliana* transcription factor binding sites, as available in the Jaspar database in distinct groups of differentially expressed genes. The first column indicates the name of the transcription factor, and the identifier of its consensus binding sites in the Jaspar database. Subsequent columns indicate the criteria used to form different selection of genes and report the complete list of p-values according to p-scan.

Table S3.

Intersection of differentially expressed genes with putative targets of a selection of TFs. The table reports p-values for the significance of the intersection of target genes of a selection of TFs, as determined by ChIP-seq experiments under different experimental conditions, and the differentially expressed genes observed in this study. Target genes of a specific TF were determined as those having a ChIP-seq peak in their promoter, here defined as the genomic interval comprising 1000 bp upstream and 100 bp downstream of an annotated TSS. TFs and corresponding ChIP-seq experiments are indicated in the first column, by the same syntax used in Table S4. p-values for the significance of the intersection are reported in the subsequent columns.

Table S4.

Intersection of ChIP-seq peaks reveals a significant coincidence between GI and multiple TFs binding at target genes. The table reports p-values for the significance of the intersection of genomic coordinates between ChIP-seq peaks of GI and ChIP-seq experiments for a selection of TFs under different experimental conditions. TFs are indicated in the first column, along with a brief description of the experimental conditions and GEO database identifiers. For ChIP-seq peaks reported by Song et al. (GSE80564) mock indicates no treatment, while ABA indicates treatment with ABA in the second column. The third column reports the total number of peaks in common (i.e that overlap by at least 1 nucleotide). Total number of GI peaks is indicated in the third column, while the fifth and sixth columns indicate, respectively, the total number of ChIP-seq peaks for any given TF, and the number of genomic intervals that could be formed by segmenting the genome into random intervals of matched size. The last 2 columns report the p-value for the intersection, and the fold change (observed/expected) for the over-representation of overlapping peaks.

Table S5.

Total Number of Read and mapped reads. Summary statistics regarding the total number of reads, total number of mapped reads and total number of uniquely mapped reads on the reference assembly of *Arabidopsis thaliana* genome TAIR vs.10, are reported for every biological replicate.

Table S6.

List of primers used in this study.