Supplementary Information

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Supplementary Experimental Procedures

Calculation of relative and absolute expansion rates

For each stage, the absolute rate (AR) of leaf processes (P, leaf area expansion, leaf thickness expansion, cell division and cell expansion) were calculated as the local slope of the relationship between the value of the variable (leaf area, leaf thickness, cell number or cell area, respectively) and time t:

$AR_j = [dP/dt]_j$. (Eq. 1)

AR is calculated on the sigmoid fitting by linear regression on three values of P and t corresponding to sampling dates j–1, j and j+1.

The relative rate (RR) was also calculated for each process as followed:

$RR_j = [d(LnP)/dt]_j$ (Eq. 2)

Transcript profiling with AGRONOMICS1 microarrays

RNA extraction was done using a Qiagen (Qiagen, Hilden, Germany) QiaCube robot and the Qiagen RNA plant extraction kit. RNA was amplified and labelled with the GeneChip® IVT Express Labelling kit (Affymetrix, Santa Clara, CA). Labelled RNA was hybridized to AGRONOMICS1 microarrays. The AGRONOMICS1 array is a custom-made *Arabidopsis thaliana* Col-0 tiling array that contains the complete paths of both genome strands with on average one 25mer probe per 35bp genome sequence window. The microarray enables reliable expression profiling of more than 30,000 Arabidopsis genes and gives very similar results to the widely used ATH1 microarray for the set of common probes (Rehrauer *et al.,* 2010). The arrays were scanned using an Affymetrix 3000 7G confocal scanner. All data processing was performed using R (R Development Core Team, 2010). Background correction, normalisation, and calculation of probe set summaries were based on custom-made CDF files and RMA (Irizarry *et al.,* 2003) implemented in the Aroma.Affymetrix package (Bengtsson *et al.,* 2008). Nonperforming probes were dynamically masked during the analysis as described previously (Rehrauer *et al.,* 2010).

Transcript profiling with RT-qPCR

Plastid transcripts were measured by RT-qPCR from the same RNA samples as used for microarray profiling. cDNA synthesis and qPCR using a Roche Lightcycler 480 followed the same protocols and employed the same sets of primers as in (Chateigner-Boutin *et al.,* 2008). Standard curves were established for each primer pair using PCR product templates of known concentration such that the final values obtained are proportional to the quantity of template in the sample, allowing relative quantification of transcripts to each other. Values were normalised assuming equal total amounts of plastid RNA (including rRNAs) in each sample; i.e. the value for each plastid transcript was divided by the sum of values for all plastid transcripts in the same sample.

Sample preparation for proteomics

Proteins were solubilized by adding extraction buffer (20 mM Tris base, 5 mM $MgCl_2$, 8 M urea, 1x protease inhibitor cocktail (Roche, Basel, Switzerland)) and incubation for 30 min at room temperature. The supernatant fraction obtained after centrifugation at 16,100 x g for 10 min at 25°C was ultracentrifuged at 100,000 x g for 45 min at 25°C. Protein concentrations in the supernatants of the ultracentrifugation step were determined with the Pierce BCA Protein Assay Kit (Pierce Biotechnology, Rockford, USA). For each sample, 100 µg protein were subjected to an in-solution tryptic digest according to a modified protocol from Kinter and Sherman (Kinter and Sherman, 2000). For this, the volumes of the different samples were first adjusted, and the urea concentration was lowered to 6 M by adding 50 mM Tris-HCl pH 8.0. Disulfide bridges were reduced by adding 200 mM DTT in 50 mM Tris-HCl pH 8.0 to a DTT concentration of 9.5 mM and incubation for 1 h at room temperature. The thiol groups were then derivatised by the addition of 200 mM iodoacetamide in 50 mM Tris-HCl pH 8.0 to a final concentration of 32 mM iodoacetamide and incubation for 1 h at room temperature in the dark. Excess iodoacetamide was then reacted by anew addition of 200 mM DTT in 50 mM Tris-HCl to a total final concentration of 37 mM DTT and incubation for 1 h at room temperature in the dark. The urea concentration was then reduced to 0.6 M with 1 mM $CaCl_2$ in 50 mM Tris-HCl pH 8.0 before the addition of trypsin in a trypsin:protein ratio of 1:25 (w/w) and incubation at 30°C for at least 16 h. After tryptic digest, the peptides were purified using Sep-Pak reverse-phase cartridges (Waters, Milford, USA). Extraction, tryptic digest and Sep-Pak purification were performed in parallel for all samples in a reaction. Peptides were then labeled with 8-plex iTRAQ tags (Applied Biosystems, Foster City, USA) according to the labeling schemes in Supplementary Tables 24, 25 following the manufacturer's instructions. The labeled peptides were then combined and afterwards fractionated with strong cation-exchange (SCX) chromatography. For this, 1.5 ml buffer A (10 mM KH_2PO_4, pH 2.8, 25% v/v acetonitrile) were added to the peptides, the pH was adjusted with phosphoric acid to below pH 2.8, and the solution was loaded onto a Polysulfoethyl A (200 X 2.1 mm, 5 µm) column (PolyLC, Columbia, USA) connected to an Agilent HP1100 HPLC system. Peptides were eluted at a flow rate of 0.3 ml/min with an increasing KCl gradient (0-10 min 0% buffer B (0.35 M KCl, 10 mM KH_2PO_4, pH 2.8, 25% v/v acetonitrile), 10-15 min 0-10% buffer B, 15-50 min 10-40% buffer B, 50-60 min 40-100% B, 60-80 min 100% buffer B). Fractions of 0.8 ml were collected, combined into 8 pools (pool I: fractions 1-10, pool II: fraction 11, pool III: fraction 12, pool IV: fraction 13, pool V: fraction 14, pool VI: fraction 15, pool VII: fraction 16, pool VIII: fractions 17-30) and desalted with Sep-Pak reverse-phase cartridges (Waters, Milford, USA).

Mass spectrometry measurements

iTRAQ experiments were performed on a hybrid LTQ Orbitrap XL mass spectrometer (Thermo Scientific, Bremen, Germany) coupled to an Eksigent nanoLC system (Eksigent Technologies) and

analysed by reversed-phase liquid chromatography nanospray tandem mass spectrometry (nanoLC-MS/MS). Peptides were resuspended in 3% ACN and 0.2% formic acid, loaded from a cooled (10°C) Spark Holland autosampler (Emmen, Holland) and separated using an ACN/water solvent system containing 0.2% formic acid with a flow rate of 200 nl/min. Separation of the peptides was performed on a 10 cm long fused silica column (75 µm i.d.; BGB Analytik) in-house packed with 3 µm, 200 Å pore size C_{18} resin (MichromBioResources, CA). Elution was achieved using a gradient of 3−48% ACN in 50 min, 48−80% ACN in 3 min and 80% ACN for 7 min.

iTRAQ labelled peptides were analysed by applying spectral merging of CID and HCD of two consecutive scans from the same precursor. One scan cycle was comprised of a survey full MS scan of spectra from m/z 300 to m/z 2000 acquired in the FT-Orbitrap with a resolution of R= 60,000 at m/z 400, followed by up to six sequential data- dependent CID and HCD MS/MS scans. CID was done with a target value of 1e4 in the linear trap. Collision energy was set to 35%, Q value to 0.25 and activation time to 30 ms. HCD fragmentation ions including reporter ions were detected in the Orbitrap with a target value of 5e5, a collision energy of 43%. For all experiments dynamic exclusion was used with one repeat count, 30 s repeat duration and 90 s exclusion duration. The instrument was calibrated externally according to the manufacturer's instructions. The samples were acquired using internal lock mass calibration on m/z 429.088735 and 445.120025.

Interpretation of MS/MS spectra and quantification

MS/MS spectra were searched with Mascot (Matrix Science, London, UK) version 2.3.02 against TAIR10 (The Arabidopsis Information Resource) protein database (download on January17th, 2011) with concatenated decoy database supplemented with contaminants (71,032 entries). The search parameters were: mass = monoisotopic, requirement for tryptic ends, 2 missed cleavages allowed, precursor ion tolerance = +/- 10 ppm, fragment ion tolerance = +/- 0.8 Da, variable modifications of methionine (M, PSI-MOD name: oxidation, mono Δ = 15.994919) and tyrosine (Y, PSI-MOD name: iTRAQ8plex reporter+balance reagent derivatised residue, mono Δ = 304.2053539), and static modifications of cysteine (C, PSI-MOD name: iodoacetamide derivative, mono Δ = 57.021464), lysine and the N-terminus (K and N-term, PSI-MOD name: iTRAQ8plex reporter+balance reagent derivatised residue, mono Δ = 304.205360). Peptide spectrum assignments with ionscore > 24 and expect value < 0.05, except those of known contaminants, were filtered for ambiguity. Peptides matching to several proteins were excluded from further analyses. This does not apply to different splice variants of the same protein or to different loci sharing exactly the same amino acid sequence. All remaining spectrum assignments were inserted into the pep2pro database (Baerenfaller *et al.*, 2011). Mascot quantification parameters were: protein ratio type = average, normalization = median ratio, outlier removal = none, report peptide ratios = 1, min. # of peptides = 1, min. precursor charge = 2, peptide threshold = minimum score of 10. Ratios were calculated with reporter ion 121 as base, and ratios were corrected as specified by the supplier. From the resulting quantification .xml file, the

reporter ion ratios of those spectrum assignments that had been entered into the database were read out. If all 7 ratios had a positive value (neither ####, nor --, nor negative value) they were written into the pep2pro database, if not, the ratios in that spectrum were given a value of NULL. In addition, the ion intensity values of the reporter ions of those spectrum assignments that had been entered into the database were read from the .mgf files. Including only peptide spectrum assignments into the quantitative analysis that had an expect value < 0.05, an ion score > 24, and for which all seven ratios had a positive value excluded a considerable amount of low signal data. This solved the issue of high variability in the low signal range (Supplementary Figure 2).

As in each reaction two reference samples were included (Supplementary Tables 24, 25) the spectrum ratio is calculated by averaging the two sample/reference ratios (e.g. the spectrum ratio for sample PE48_1 labeled with reporter ion 113 in reaction ae1_5 is determined by calculating $(113/121 * (118/121)^{-1} + 113/121 * (119/121)^{-1}) / 2$, which is the same as calculating (113/118 + 113/119) / 2). The protein sample/reference ratio is then calculated by averaging all the spectrum ratios of that protein in a sample.

Statistical analysis and grouping of the protein and transcript data

In the statistical analysis of the individual datasets the log2-transformed sample/reference ratios of each dataset were subjected to an analysis of variance (ANOVA) treating stage (S) and day-time (ND) as main effects. The corresponding formula is $Y_{ijk} = \mu + S_i + ND_j + \varepsilon_{ijk}$, where Y_{ijk} is the expression of the k-th replicate of a gene or protein in stage i and at daytime j, μ the mean expression of the gene or protein and ε_{ijk} the corresponding normal distributed error. This formula was chosen because pre-analysis had shown that the interaction between the main effects was not significant and therefore was not considered in the presented model. The resulting p-values for the global F-test, the stage dependent level changes and the day-time dependent level changes were adjusted for multiple testing with the Benjamini-Hochberg method (Benjamini *et al.*, 1995) controlling the false discovery rate to give pGlobal (p-value for an overall global change), pS (p-value for a change between stages) and pND (p-value for the diurnal change). The effect size of the individual stages and the significance of the level changes were computed with the Tukey Honest Significant Differences (TukeyHSD) post-hoc test followed by correction with the Benjamini-Hochberg method. In addition to the significance testing we also included a minimum fold-change cut-off to exclude significant but spurious small changes from further analyses. The approach of combining significance testing with a fold-change cut-off has been recommended in a recent article validating differential gene expression algorithms (Yanofsky and Bickel, 2010). Especially for the proteomics data the technical variance in the measurements does not allow for the reliable detection of very small abundance changes. For that purpose the maximum difference in the mean values for each of the eight time points was computed. Only proteins and transcripts passing the p-value and fold-change cut-offs were used in the classification. For proteins we additionally required that they had at least one value in each of the eight time points leaving 1673

proteins in the SOW dataset and 1184 in the SWD dataset. For the SOW experiment, the RT-qPCR data for 80 transcripts of plastid genes were added to the transcript data. In total, 11,341 transcripts and 569 proteins in SOW, and 12,153 transcripts and 370 proteins in SWD had a global p-value < 0.05 and a fold change > 1.5 and were subjected to clustering by a decision tree.

Alternative experimental validation of a subset of the transcript and protein patterns

For DPE2 (AT2G40840), GSTF2 (AT4G02520), PORB (AT4G27440) and PORC (AT1G03630) we quantified transcript levels with qRT-PCR and assessed the protein levels qualitatively with western blotting. qRT-PCR analyses were done using cDNA generated from RNA from the SWD samples and for data analysis the relative mRNA abundance was determined using the formula

$$\text{Efficiency}_{\text{Ref}}{}^{\text{avg(CT)}}/\text{Efficiency}_{\text{GOI}}{}^{\text{avg(CT)}}$$

where GOI is the gene of interest, Ref the internal control and avg(CT) the average CT over two technical replicates (Ruijter *et al.* 2009). As internal control AT3G13530 was used, which displayed very small fold-changes and high pGlobal-values in the SOW and SWD tiling array data. The qRT-PCR data were log-transformed, subjected to statistical testing as described above, except for the correction for multiple testing, and afterwards clustered. The results in Supplementary Figure 13 show that the variation patterns between EOD and EON were consistent in the tiling array and qRT-PCR datasets. The variation over the developmental stages was identical for PORC and generally corresponded for DPE2, PORB and GSTF2.

For Western blotting we used the antibodies for POR (Agrisera), GSTF2 (Agrisera) and DPE2 (kindly provided by Sam Zeeman) to probe plots derived from 14% SDS-PAGE gels loaded with 20 µg of each SWD sample (Supplementary Figure 14). Probing the membrane with the antibody detecting the POR protein (PORA, 43.9 kDa; PORB, 43.4 kDa; PORC, 43.8 kDa) we detected three bands. In agreement with the iTRAQ data for PORB and PORC (PORA was not detected) these bands are decreasing across developmental stages. Also the signal for GSTF2 corresponds well with the iTRAQ data as it is increasing across developmental stages. Less clear, but still visible, also the signal for DPE2 is stronger in stage 4 as compared to stage 1.

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Supplementary Table 1

Leaf expansion rates. Leaf absolute expansion rate (AR) and relative expansion rate (RR) calculated from the data presented in Figure 1 at the four key stages of leaf development in SOW and SWD conditions and for leaf area, leaf thickness, cell number and cell area.

		SOW				SWD			
	Stages of growth	1	2	3	4	1	2	3	4
AR	leaf area [(1)]	1.58	7.39	4.72	0.23	0.92	3.19	2.68	0.63
	leaf thickness [(2)]	7.37	7.77	5.44	1.92	7.87	5.61	1.99	0.44
	cell number [(3)]	2359	1005	148	6.06	1746	246	20.6	1.13
	cell area [(4)]	71.0	205	199	31.9	67.2	207	96.4	13.1
RR	leaf area [(1)]	0.361	0.252	0.065	0.003	0.227	0.160	0.062	0.011
	leaf thickness [(2)]	0.100	0.064	0.034	0.010	0.118	0.045	0.013	0.003
	cell number [(3)]	0.191	0.043	0.006	0.000	0.143	0.013	0.001	0.000
	cell area [(4)]	0.253	0.189	0.082	0.010	0.257	0.147	0.038	0.005

Units are: (1) $mm^2\ day^{-1}$, (2) $\mu m\ day^{-1}$, (3) number of cell day^{-1}, (4) $\mu m^2\ day^{-1}$, (5) day^{-1}

Supplementary Table 2:

Leaf cell densities. Cell density in each tissue of the leaf during the four stages of leaf growth in SOW **A)** and SWD **B)**. Data are mean and SD values.

A) SOW								
	Stage 1		Stage 2		Stage 3		Stage 4	
Cell density	mean	± SD	mean	± SD	mean	± SD	mean	± SD
Adaxial epidermis	6639	2992	892	229	411	88	358	37
Palisade mesophyll	21379	10061	2967	826	1278	264	1136	101
Spongy mesophyll	10482	5496	1320	265	574	95	509	78
Abaxial epidermis	5936	1768	1060	242	464	56	420	58
B) SWD								
	Stage 1		Stage 2		Stage 3		Stage 4	
Cell density	mean	± SD	mean	± SD	mean	± SD	mean	± SD
Adaxial epidermis	4269	914	828	119	419	57	328	82
Palisade mesophyll	14140	2364	2492	234	1268	296	974	343
Spongy mesophyll	7170	1141	1555	164	877	178	798	34
Abaxial epidermis	4847	469	1149	130	591	94	496	32

Supplementary Table 3: The number of transcripts and proteins in SOW and SWD populating the different patterns

Pattern	# transcripts SOW	# proteins SOW	# transcripts SWD	# proteins SWD
D-D-D-E	64	31	52	6
D-D-D-ED	121		45	
D-D-D-EN	73		31	
D-D-E-E	40	28	43	24
D-D-E-ED	33		20	
D-D-E-EN	35		34	
D-D-U-E	2		2	
D-D-U-EN	1		1	
D-E	448	116	771	92
D-E-D-E	133	15	62	7
D-E-D-ED	541		124	
D-E-D-EN	103		52	
D-E-E-E	654	116	609	75
D-E-E-ED	1566	1	1232	
D-E-E-EN	395		224	
D-E-U-E	86	14	25	6
D-E-U-ED	125		25	
D-E-U-EN	179		41	
D-ED	558		917	
D-EN	318		539	
D-U-E-E	1			1
D-U-E-ED	2			
D-U-U-E	5	1		
D-U-U-ED	9			
D-U-U-EN	4			
E-D-D-E	51	5	37	3
E-D-D-ED	32		20	
E-D-D-EN	62		35	
E-D-E-E	27	6	24	4
E-D-E-ED	9		8	
E-D-E-EN	33		16	
E-D-U-EN	2			
E-E	925	60	583	45
E-E-D-E	484	18	502	10
E-E-D-ED	583		515	
E-E-D-EN	466		462	
E-E-U-E	682	44	622	20
E-E-U-ED	378		345	
E-E-U-EN	1358	1	900	
E-ED	1096		1561	1
E-EN	1548		1792	
E-U-D-E	1	1	1	
E-U-D-ED	1		2	
E-U-D-EN	1		1	
E-U-E-E	21	1	16	4
E-U-E-ED	40		22	
E-U-E-EN	21		8	
E-U-U-E	69	11	40	7
E-U-U-ED	72		33	
E-U-U-EN	114		47	
U-D-D-E	4		4	
U-D-D-ED	2		2	
U-D-D-EN	21		1	
U-D-E-E	20	1		
U-D-E-ED	1			
U-D-E-EN	12			
U-E	439	36	497	43
U-E-D-E	186	5	73	
U-E-D-ED	117		104	
U-E-D-EN	203		135	
U-E-E-E	578	31	275	16
U-E-E-ED	201		319	
U-E-E-EN	574		563	
U-E-U-E	81	7	102	3
U-E-U-ED	56		81	
U-E-U-EN	268		301	
U-ED	303		450	
U-EN	663		701	
U-U-D-E	3	1	1	
U-U-D-ED	9		4	
U-U-D-EN	3		2	
U-U-E-E	26	11	35	
U-U-E-ED	62		63	
U-U-E-EN	42		43	
U-U-U-E	80	8	49	3
U-U-U-ED	51		48	
U-U-U-EN	133		76	
Total	17710	569	16370	370

Supplementary Table 8:

Transcript and protein changes between stages and stage markers. A) The number of transcripts and proteins that are changing between two adjacent stages with the direction of change, and **B)** the number of stage-specific marker transcripts and proteins with their respective patterns not taking into account the diurnal changes (X indicates 'EN', 'ED' or 'E' for the differences between EOD and EON). A stage-specific marker is significantly different in one specific stage compared to all other stages while it is not significantly different between the other stages. A stage 2 – stage 3 marker is different in stage 2 and stage 3 compared to stage 1 and stage 4, but not different between stage 1 and stage 4.

A) Changes between adjacent changes	Transcripts		Proteins	
	Down	Up	Down	Up
Stage 1 - Stage 2	4172	2733	206	64
Stage 2 - Stage 3	645	770	71	34
Stage 3 - Stage 4	3264	3755	76	86
B) Stage-specific markers				
	D-E-E-X	U-E-E-X	D-E-E-X	U-E-E-X
Stage 1 Marker	1522	412	69	11
	D-U-E-X	U-D-E-X		U-D-E-X
Stage 2 Marker	2	27		1
		E-U-D-X		E-U-D-X
Stage 3 Marker		3		1
	E-E-D-X	E-E-U-X	E-E-D-X	E-E-U-X
Stage 4 Marker	859	1534	3	24
	D-E-U-X	U-E-D-X	D-E-U-X	U-E-D-X
Stage 2-Stage 3 Marker	177	190	3	2

Supplementary Table 9:

Expansion stage markers. A) Transcript expansion stage markers in GO categories *positive regulation of catalytic activity* and *photosynthetic electron transport in photosystem I* **B)** Protein expansion stage markers in pattern U-E-D-E.

A) Transcript expansion stage markers

Positive regulation of catalytic activity

AGI	Pattern	Symbol	Description
AT1G03680	U-E-D-ED	ATHM1	thioredoxin M-type 1
AT1G50320	U-E-D-EN	ATHX	thioredoxin X
AT3G02730	U-E-D-ED	TRXF1	thioredoxin F-type 1
AT3G15360	U-E-D-ED	ATHM4	thioredoxin M-type 4
AT5G50210	U-E-D-EN	QS	quinolinate synthase

Photosynthetic electron transport in photosystem I

AGI	Pattern	Symbol	Description
AT3G54050	U-E-D-E	HCEF1	high cyclic electron flow 1
AT2G05620	U-E-D-EN	PGR5	proton gradient regulation 5
AT1G70760	U-E-D-EN	CRR23	inorganic carbon transport protein-related

B) Protein expansion stage markers

AGI	Pattern	Symbol	Description
AT3G03780	U-E-D-E	ATMS2	methionine synthase 2
AT5G38430	U-E-D-E	RBCS	Ribulose bisphosphate carboxylase (small chain) family protein

Supplementary Table 11:

Response to abiotic stimulus and gravity. The transcripts in GO categories *cellular response to abiotic stimulus* and *response to gravity* that lead to the over-representation of these categories in the different sub-groups of diurnally regulated transcripts.

Cellular response to abiotic stimulus			
AGI	**Pattern**	**Symbol**	**Description**
AT1G09570	E-E-U-EN	PHYA,FHY2,FRE1,HY8	phytochrome A
AT1G22280	E-E-U-EN	PAPP2C	phytochrome-associated protein phosphatase type 2C
AT1G31480	E-E-U-EN	SGR2	shoot gravitropism 2 (SGR2)
AT1G52740	D-E-U-EN	HTA9	histone H2A protein 9
AT2G20180	E-EN	PIL5,PIF1	phytochrome interacting factor 3-like 5
AT2G24790	E-EN	COL3,ATCOL3	CONSTANS-like 3
AT2G37970	E-U-U-EN	SOUL-1	SOUL heme-binding family protein
AT2G46340	E-E	SPA1	SPA (suppressor of phyA-105) protein family
AT3G59060	E-E-U-EN	PIL6,PIF5	phytochrome interacting factor 3-like 6
AT4G02440	U-EN	EID1	F-box family protein
AT4G08920	U-EN	CRY1,BLU1,HY4,OOP2,ATCRY1	cryptochrome 1
AT4G16780	U-E-E-EN	ATHB-2,HAT4,ATHB2,HB-2	homeobox protein 2
AT5G04190	E-EN	PKS4	phytochrome kinase substrate 4
AT5G11260	E-EN	HY5,TED5	Basic-leucine zipper (bZIP) transcription factor family protein
AT5G15840	E-EN	CO,FG	B-box type zinc finger protein with CCT domain
AT5G49230	E-EN	HRB1	Drought-responsive family protein

Response to gravity			
AGI	**Pattern**	**Symbol**	**Description**
AT1G09570	E-E-U-EN	PHYA,FHY2,FRE1,HY8	phytochrome A
AT1G31480	E-E-U-EN	SGR2	shoot gravitropism 2 (SGR2)
AT1G70940	D-EN	PIN3,ATPIN3	Auxin efflux carrier family protein
AT2G01940	U-EN	SGR5,ATIDD15	C2H2-like zinc finger protein
AT2G18390	D-EN	TTN5,HAL,ARL2,ATARLC1	ADP-ribosylation factor family protein
AT2G38120	E-E-D-EN	AUX1,WAV5,PIR1,MAP1	Transmembrane amino acid transporter family protein
AT3G23050	E-E-D-EN	IAA7,AXR2	indole-3-acetic acid 7
AT5G06140	D-E-E-EN	SNX1,ATSNX1	sorting nexin 1

Supplementary Table 12:

Growth-stage specific changes between EON and EOD. The number of transcripts that change significantly in a pair-wise comparison of the EON and EOD samples are given for each growth stage and for SOW and SWD conditions. The number of transcripts repressed or induced by sugar as reported by Usadel *et al.* (2008) is given for each subset together with the p-value for over-representation as assessed with Fisher's exact test.

SOW	total	C repressed	% C repressed	pvalue	C induced	% C induced	p-value
sow_en	5154	362	7.0				
sow_ed	5376				464	8.6	
sow_1_en	1739	62	3.6	**6.57E-08**			
sow_1_ed	1451				34	2.3	**1.01E-19**
sow_2_en	1976	126	6.4	3.46E-01			
sow_2_ed	2487				213	8.6	9.66E-01
sow_3_en	492	7	1.4	**3.06E-08**			
sow_3_ed	456				8	1.8	**2.98E-09**
sow_4_en	485	25	5.2	1.30E-01			
sow_4_ed	531				25	4.7	**1.21E-03**
SWD							
swd_en	4556	362	7.9				
swd_ed	4861				464	9.5	
swd_1_en	1467	84	5.7	2.15E-01			
swd_1_ed	1915				170	8.9	4.05E-01
swd_2_en	1655	29	1.8	**1.60E-14**			
swd_2_ed	1380				35	2.5	**5.63E-21**
swd_3_en	1193	40	3.4	**7.73E-07**			
swd_3_ed	1087				42	3.9	**5.67E-11**
swd_4_en	1709	102	6.0	**7.88E-03**			
swd_4_ed	2526				186	7.4	**1.57E-03**

Supplementary Table 13:

Increasing protein and decreasing transcript levels. Transcript-protein pairs with decreasing transcript and increasing protein accumulation and their localisation.

AGI	Transcript pattern	Protein pattern	Symbols	Description	Localisation
AT1G08550	E-E-D-ED	U-E	NPQ1, AVDE1	non-photochemical quenching 1	Chloroplast thylakoid
AT1G12900	E-E-D-E	U-E-E-E	GAPA-2	glyceraldehyde 3-phosphate dehydrogenase A subunit 2	Chloroplast
AT1G32060	E-E-D-E	U-U-E-E	PRK	phosphoribulokinase	Chloroplast/ Membrane
AT1G42970	E-E-D-E	U-U-E-E	GAPB	glyceraldehyde-3-phosphate dehydrogenase B subunit	Chloroplast/ Membrane
AT1G72610	E-E-D-E	U-E-U-E	GLP1, ATGER1, GER1	germin-like protein 1	Extracellular matrix
AT1G76100	E-E-D-EN	E-E-U-E	PETE1	plastocyanin 1	Chloroplast thylakoid
AT1G80380	E-E-D-E	U-E-E-E		P-loop containing nucleoside triphosphate hydrolases superfamily protein	Chloroplast
AT3G01500	E-E-D-E	U-U-E-E	CA1, ATBCA1, SABP3, ATSABP3	carbonic anhydrase 1	Chloroplast/ Membrane
AT3G01510	E-E-D-ED	U-E	LSF1	like SEX4 1	Chloroplast
AT3G08580	D-E-D-ED	U-E-E-E	AAC1	ADP/ATP carrier 1	Mitochondrion inner membrane
AT3G12780	E-E-D-E	U-U-E-E	PGK1	phosphoglycerate kinase 1	Membrane/ Chloroplast / Mitochondrion /Nucleus
AT3G14310	E-E-D-ED	E-U-U-E	ATPME3, PME3	pectin methylesterase 3	Cell wall/ Plasma membrane /Cytoplasm
AT3G53110	D-E-E-EN	E-E-U-E	LOS4	P-loop containing nucleoside triphosphate hydrolases superfamily protein	Nuclear envelope/ Plasma membrane
AT3G63160	E-E-D-ED	E-E-U-E		unknown function	Chloroplast
AT4G02770	E-E-D-ED	E-E-U-E	PSAD-1	photosystem I subunit D-1	Chloroplast thylakoid
AT4G04640	E-E-D-ED	U-E-E-E	ATPC1	ATPase, F1 complex, gamma subunit protein	Chloroplast thylakoid
AT4G09650	E-E-D-ED	U-U-U-E	ATPD	ATP synthase delta-subunit gene	Chloroplast thylakoid
AT4G21280	E-E-D-EN	U-U-U-E	PSBQ, PSBQA, PSBQ-1	photosystem II subunit QA	Chloroplast thylakoid
AT5G03650	E-E-D-ED	U-E	SBE2.2	starch branching enzyme 2.2	Chloroplast
AT5G17020	D-E-E-ED	U-E-E-E	XPO1A, ATCRM1, ATXPO1, XPO1, HIT2	exportin 1A	Nuclear pore
AT5G60210	D-E-E-ED	E-E-U-E	RIP5	ROP interactive partner 5	Plasma membrane
ATCG00120	D-E	U-U-U-E	ATPA	ATP synthase subunit alpha	Chloroplast thylakoid
ATCG00130	D-E	U-E-E-E	ATPF	ATPase, F0 complex, subunit B/B', bacterial/chloroplast	Chloroplast thylakoid
ATCG00470	D-E	U-E-E-E	ATPE	ATP synthase epsilon chain	Chloroplast thylakoid
ATCG01110	D-E	U-E	NDHH	NAD(P)H dehydrogenase subunit H	Chloroplast thylakoid

Supplementary Table 14:

Exceptional transcript patterns of ribosomal proteins. Transcript patterns for ribosomal components increasing across the stages and for L18a ribosomal proteins.

Proteins that are upregulated during development and never down		
agi	**pattern**	**Description**
AT1G17080	U-E-U-EN	Ribosomal protein L18ae family
AT1G29970	U-E-U-EN	60S ribosomal protein L18A-1
AT1G53560	U-E-E-EN	Ribosomal protein L18ae family
AT1G68660	U-E-E-E	Ribosomal protein L12/ ATP-dependent Clp protease adaptor protein ClpS family protein
AT3G01170	U-E-U-EN	Ribosomal protein L34e superfamily protein
AT3G01740	E-E-U-E	Mitochondrial ribosomal protein L37
AT3G04230	U-U	Ribosomal protein S5 domain 2-like superfamily protein
AT3G14595	U-U-U-EN	Ribosomal protein L18ae family
AT4G01790	E-E-U-ED	Ribosomal protein L7Ae/L30e/S12e/Gadd45 family protein
AT4G26060	U-U-U-EN	Ribosomal protein L18ae family
AT4G38090	U-E-U-EN	Ribosomal protein S5 domain 2-like superfamily protein
AT5G15260	E-E-U-EN	Ribosomal protein L34e superfamily protein
AT5G39850	E-E-U-ED	Ribosomal protein S4
ATMG00080	U-E	ribosomal protein L16
Additional L18a ribosomal proteins		
agi	**pattern**	**Description**
AT1G29965	D-ED	Ribosomal protein L18ae/LX family protein
AT2G34480	D-D-E-ED	Ribosomal protein L18ae/LX family protein
AT3G14600	D-E-E-E	Ribosomal protein L18ae/LX family protein
AT1G54217	E-ED	Ribosomal protein L18ae family

Supplementary Table 15:

Promoter motifs of ribosomal proteins. The motifs over-represented with p-value < 1e-10 in the promoter elements of the nuclear-encoded transcripts of ribosomal proteins.

Motif enrichment	Enrichment fold	Number of transcripts with motif	p-value	Description
GGCCCANN	5.94	112	4.81E-46	similar to TGTGGCCCATTAT (tgtGGCCCATTat (PLACE)); similar to ACCGGCCCACTT (accGGCCCACTt (PLACE)); NCS-motif
AAACCCTA	2.75	181	2.44E-41	UP2ATMSD similar to AAACCCTAA (AAACCCTAa (PLACE)); similar to CTTAAACCCTAGGG (cttAAACCCTAggg (AGRIS)); similar to AAACCCTAAAA (AAACCCTAaaa (PLACE)); Up2 motif found in 193 of the 1184 up-regulated genes after main stem decapitation in Arabidopsis; W=A/T; See also S000470, S000471 NCS-motif; PLACE
CTAGGGTN	9.4	61	1.21E-40	ACCCTNAT NCS-motif
AAACCCTAA	2.51	110	7.20E-21	TELOBOXATEEF1AA1;AAACCCTAA telo-box (telomere motif) found in the Arabidopsis (A.t.) eEF1AA1 gene promoter; Conserved in all known plant eEF1A gene promoters; Found in the 5' region of numerous genes encoding components of the translational apparatus; Required for the activation of expression in root primordia; Acts co-operatively with tef-box; Binding site of AtPur alpha-1; See S000309, S000474;TELO-box promoter motif AGRIS;PLACE
GGCCCAWWW	2.34	119	1.81E-19	UP1ATMSD Up1 motif found in 162 of the 1184 up-regulated genes after main stem decapitation in Arabidopsis; W=A/T; See also S000470, S000472 PLACE
GGGCC	1.69	181	1.03E-16	SORLIP2AT;GGGCC one of Sequences Over-Represented in Light-Induced Promoters (SORLIPs)
TGGGCY	1.56	202	2.13E-15	SITEIIATCYTC Site II element found in the promoter regions of cytochrome genes (Cytc-1, Cytc-2) in Arabidopsis; Located between -147 and -156 from the translational starts sites (Welchen et al., 2005); Y=C/T; See also S000308; Overrepresented in the promoters of nuclear genes encoding components of the oxidative phosphorylation (OxPhos) machinery from both Arabidopsis and rice (Welchen and Gonzalez, 2006);) PLACE
ACCCTNAT	4.7	39	8.25E-15	ACCCTNAT NCS-motif

Supplementary Table 16:

Patterns of autophagy related transcripts

AGI	Pattern	Symbol	Description
AT5G05150	0	ATG18E	homolog of yeast autophagy 18 E
AT3G13970	E-EN	APG12B	Autophagy 12B
AT3G07525	E-EN	ATG10	Autophagy 10
AT4G30510	E-EN	ATG18B	homolog of yeast autophagy 18 B
AT3G56440	E-EN	ATG18D	homolog of yeast autophagy 18 D
AT5G45900	E-EN	ATG7	Autophagy 7
AT3G18770	E-E-U-E		Autophagy-related protein 13
AT2G31260	E-E-U-EN	APG9	Autophagy 9
AT2G40810	E-E-U-EN	ATG18C	homolog of yeast autophagy 18 C
AT5G61500	E-E-U-EN	ATG3	Authophagy 3
AT3G61710	E-E-U-EN	ATG6	Autophagy 6
AT4G21980	E-E-U-EN	ATG8A	Autophagy 8A
AT4G04620	E-E-U-EN	ATG8B	Autophagy 8B
AT2G45170	E-E-U-EN	ATG8E	Autophagy 8E
AT3G06420	E-E-U-EN	ATG8H	Autophagy 8H
AT3G49590	E-E-U-EN		Autophagy-related protein 13
AT3G62770	E-U-U-EN	ATG18A	Autophagy 18A
AT3G60640	E-U-U-EN	ATG8G	Autophagy 8G
AT3G19190	U-E-E-EN	ATG2	Autophagy 2
AT5G17290	U-E-E-EN	ATG5	Autophagy 5
AT5G54730	U-EN	ATG18F	homolog of yeast autophagy 18 F
AT1G54710	U-EN	ATG18H	homolog of yeast autophagy 18 H
AT1G62040	U-EN	ATG8C	Autophagy 8C
AT2G05630	U-E-U-E	ATG8D	ATG8D
AT1G54210	U-E-U-EN	ATG12A	Autophagy 12A
AT4G16520	U-E-U-EN	ATG8F	Autophagy 8F
AT1G03380	U-U-U-EN	ATG18G	homolog of yeast autophagy 18 G
AT3G15580	U-U-U-EN	ATG8H/I	Autophagy 8H/I

Supplementary Table 17:

Cell wall. Transcripts associated with cell wall organisation, biogenesis and loosening, as well as cell wall thickening.

Cell wall biogenesis, organization and loosening			
AGI	**Pattern**	**Symbols**	**Description**
AT1G43790	E-E-D-EN	TED6	tracheary element differentiation-related 6
AT1G69530	E-E-D-EN	ATEXPA1, EXP1, AT-EXP1, ATEXP1, ATHEXP ALPHA 1.2, EXPA1	expansin A1
AT1G78580	E-E-D-ED	ATTPS1, TPS1	trehalose-6-phosphate synthase
AT2G28950	E-E-D-ED	ATEXPA6, ATEXP6, ATHEXP ALPHA 1.8, EXPA6	expansin A6
AT2G35620	E-E-D-ED	FEI2	Leucine-rich repeat protein kinase family protein
AT2G38080	E-D-E-EN	IRX12, LAC4, ATLMCO4, LMCO4	Laccase/Diphenol oxidase family protein
AT2G40610	E-E-D-EN	ATEXPA8, EXP8, ATEXP8, ATHEXP ALPHA 1.11, EXPA8	expansin A8
AT3G29030	E-E-D-ED	ATEXPA5, ATEXP5, ATHEXP ALPHA 1.4, EXP5, EXPA5	expansin A5
AT3G55500	E-D-E-EN	ATEXPA16, EXP16, ATEXP16, ATHEXP ALPHA 1.7, EXPA16	expansin A16
AT4G01630	E-E-D-EN	ATEXPA17, ATEXP17, ATHEXP ALPHA 1.13, EXPA17	expansin A17
AT4G14940	E-D-E-E	ATAO1, AO1	amine oxidase 1
AT4G26690	E-E-D-EN	SHV3, MRH5, GPDL2	PLC-like phosphodiesterase family protein
AT4G38210	E-E-D-EN	ATEXPA20, EXP20, ATEXP20, ATHEXP ALPHA 1.23, EXPA20	expansin A20
AT4G39350	E-E-D-ED	CESA2, ATH-A, ATCESA2	cellulose synthase A2
AT5G02260	E-E-D-ED	ATEXPA9, EXP9, ATEXP9, ATHEXP ALPHA 1.10, EXPA9	expansin A9
AT5G05170	E-E-D-ED	CESA3, IXR1, ATCESA3, ATH-B, CEV1	Cellulose synthase family protein
AT5G09870	E-E-D-ED	CESA5	cellulose synthase 5
AT5G16490	E-D-E-EN	RIC4	ROP-interactive CRIB motif-containing protein 4
AT5G17420	E-D-E-EN	IRX3, CESA7, ATCESA7, MUR10	Cellulose synthase family protein
AT5G48920	E-D-E-E	TED7	tracheary element differentiation-related 7
AT5G56320	E-E-D-EN	ATEXPA14, EXP14, ATEXP14, ATHEXP ALPHA 1.5, EXPA14	expansin A14
AT5G60920	E-E-D-ED	COB	COBRA-like extracellular glycosyl-phosphatidyl inositol-
AT5G64740	E-E-D-ED	CESA6, IXR2, E112, PRC1	cellulose synthase 6

Cell wall thickening			
AGI	**Pattern**	**Symbols**	**Description**
AT1G24100	U-U-E-ED	UGT74B1	UDP-glucosyl transferase 74B1
AT2G22330	U-E-E-E	CYP79B3	cytochrome P450, family 79, subfamily B, polypeptide 3
AT4G31500	U-U-E-ED	CYP83B1, SUR2, RNT1, RED1, ATR4	cytochrome P450, family 83, subfamily B, polypeptide 1
AT5G44070	U-E-E-E	CAD1, ARA8, ATPCS1, PCS1	phytochelatin synthase 1 (PCS1)
AT5G57220	U-E-E-E	CYP81F2	cytochrome P450, family 81, subfamily F, polypeptide 2

Supplementary Table 18:

Plastid gene expression. Transcript and protein expression patterns of plastid encoded genes and the sigma factors regulating plastid gene expression. In blue, patterns with a downward trend across stages, in yellow patterns with an upward trend, and in green an intermediate pattern. Pattern '0' indicates that the transcript or protein was detected, but was not considered to change significantly.

Chlorplast genes

AGI	pattern transcript	pattern protein	Symbols	Description
ATCG00020	U-E-U-E	0	PSBA	photosystem II reaction center protein A
ATCG00040	D-E		MATK	maturase K
ATCG00065	D-E-U-E	0	RPS12A,RPS12	ribosomal protein S12A
ATCG00070	0		PSBK	photosystem II reaction center protein K precursor
ATCG00080	0		PSBI	photosystem II reaction center protein I
ATCG00120	D-E	U-U-U-E	ATPA	ATP synthase subunit alpha
ATCG00130	D-E	U-E-E-E	ATPF	ATPase, F0 complex, subunit B/B', bacterial/chloroplast
ATCG00140	D-E		ATPH	ATP synthase subunit C family protein
ATCG00150	D-E		ATPI	ATPase, F0 complex, subunit A protein
ATCG00160	D-E-E-E	0	RPS2	ribosomal protein S2
ATCG00170	D-E-E-E		RPOC2	DNA-directed RNA polymerase family protein
ATCG00180	D-E-E-E	E-E	RPOC1	DNA-directed RNA polymerase family protein
ATCG00190	D-E-E-E	D-E	RPOB	RNA polymerase subunit beta
ATCG00210	0		YCF6	electron transporter, transferring electrons within cytochrome b6/f complex of photos
ATCG00220	0		PSBM	photosystem II reaction center protein M
ATCG00270	U-E	0	PSBD	photosystem II reaction center protein D
ATCG00280	U-E-U-E		PSBC	photosystem II reaction center protein C
ATCG00300	0		YCF9	YCF9
ATCG00330	0		RPS14	chloroplast ribosomal protein S14
ATCG00340	0		PSAB	Photosystem I, PsaA/PsaB protein
ATCG00350	0		PSAA	Photosystem I, PsaA/PsaB protein
ATCG00360	D-E-E-E		YCF3	Tetratricopeptide repeat (TPR)-like superfamily protein
ATCG00380	D-E-E-E	D-E	RPS4	chloroplast ribosomal protein S4
ATCG00420	0	0	NDHJ	NADH dehydrogenase subunit J
ATCG00430	0	0	PSBG	photosystem II reaction center protein G
ATCG00440	0		NDHC	NADH:ubiquinone/plastoquinone oxidoreductase, chain 3 protein
ATCG00470	D-E	U-E-E-E	ATPE	ATP synthase epsilon chain
ATCG00480	D-E-D-E	0	ATPB,PB	ATP synthase subunit beta
ATCG00490	E-E-D-E	0	RBCL	ribulose-bisphosphate carboxylases
ATCG00500	0	E-D-D-E	ACCD	acetyl-CoA carboxylase carboxyl transferase subunit beta
ATCG00510	0		PSAI	photsystem I subunit I
ATCG00520	0		YCF4	unfolded protein binding
ATCG00530	0		YCF10	CemA-like proton extrusion protein-related
ATCG00540	D-E-D-EN	0	PETA	photosynthetic electron transfer A
ATCG00550	0		PSBJ	photosystem II reaction center protein J
ATCG00560	0		PSBL	photosystem II reaction center protein L
ATCG00570	E-E	0	PSBF	photosystem II reaction center protein F
ATCG00580	E-E		PSBE	photosystem II reaction center protein E
ATCG00590	U-E-E-E		ORF31	electron carriers
ATCG00600	U-E-E-E		PETG	Cytochrome b6-f complex, subunit V.
ATCG00630	0		PSAJ	PSAJ
ATCG00640	0	D-E	RPL33	ribosomal protein L33
ATCG00650	D-E-E-E	0	RPS18	ribosomal protein S18
ATCG00660	D-E-D-EN		RPL20	ribosomal protein L20
ATCG00670	D-E-D-EN	0	CLPP1,PCLPP	plastid-encoded CLP P
ATCG00680	0	0	PSBB	photosystem II reaction center protein B
ATCG00690	U-E		PSBT,PSBTC	photosystem II reaction center protein T
ATCG00700	0		PSBN	photosystem II reaction center protein N
ATCG00710	0	0	PSBH	photosystem II reaction center protein H

ATCG00720	U-E-E-E		PETB	photosynthetic electron transfer B
ATCG00730	0		PETD	photosynthetic electron transfer D
ATCG00740	D-E-D-E	D-E	RPOA	RNA polymerase subunit alpha
ATCG00750	D-E-D-EN		RPS11	ribosomal protein S11
ATCG00760	D-D-E-E		RPL36	ribosomal protein L36
ATCG00770	D-E	E-E-D-E	RPS8	ribosomal protein S8
ATCG00780	D-D-D-EN	0	RPL14	ribosomal protein L14
ATCG00790	D-D-D-EN	0	RPL16	ribosomal protein L16
ATCG00800	D-E-D-E	0	NULL	structural constituent of ribosome
ATCG00810	D-E		RPL22	ribosomal protein L22
ATCG00820	D-E	E-E	RPS19	ribosomal protein S19
ATCG00830	D-E-E-EN	0	RPL2.1	ribosomal protein L2
ATCG00840	D-E	0	RPL23.1,RPL23	ribosomal protein L23.1
ATCG00860	D-E-D-E		YCF2.1	Chloroplast Ycf2;ATPase, AAA type, core
ATCG00870	D-E-D-E		ORF77.1	Protein precursor Ycf15, putative, chloroplast
ATCG00890	D-E-E-E		NDHB.1	NADH-Ubiquinone/plastoquinone (complex I) protein
ATCG00900	D-E-E-E	0	RPS7.1,RPS7	Ribosomal protein S7p/S5e family protein
ATCG00905		0	RPS12C,RPS12	ribosomal protein S12C
ATCG00920	0		ribosomal_rna	chloroplast-encoded 16S ribosomal RNA
ATCG00950	D-E-E-E		ribosomal_rna	chloroplast-encoded 23S ribosomal RNA
ATCG01000	0		YCF1.1	Ycf1 protein
ATCG01010	E-E-D-E		NDHF	NADH-Ubiquinone oxidoreductase (complex I), chain 5 protein
ATCG01020	E-E-D-EN		RPL32	ribosomal protein L32
ATCG01040	0		YCF5	Cytochrome C assembly protein
ATCG01050	0		NDHD	NADH-Ubiquinone/plastoquinone (complex I) protein
ATCG01060	E-E-D-E	0	PSAC	iron-sulfur cluster binding;electron carriers;4 iron, 4 sulfur cluster binding
ATCG01070	E-EN		NDHE	NADH-ubiquinone/plastoquinone oxidoreductase chain 4L
ATCG01080	D-E		NDHG	NADH:ubiquinone/plastoquinone oxidoreductase, chain 6
ATCG01090	E-E-D-E	0	NDHI	NADPH dehydrogenases
ATCG01100	E-E-D-EN		NDHA	NADH dehydrogenase family protein
ATCG01110	D-E	U-E	NDHH	NAD(P)H dehydrogenase subunit H
ATCG01120	D-E-E-E	E-E	RPS15	chloroplast ribosomal protein S15
ATCG01230		0	RPS12B,RPS12	ribosomal protein S12B
ATCG01240		0	RPS7.2	ribosomal protein S7
ATCG01300		0	RPL23.2	ribosomal protein L23
ATCG01310		0	RPL2.2	ribosomal protein L2

Sigma factors

AGI	pattern transcript	pattern protein	Symbols	Description
AT1G08540	E-E-D-ED		SIG2	RNApolymerase sigma subunit 2
AT1G64860	E-EN		SIG1	sigma factor A
AT2G36990	E-E-D-ED		SIG6	RNApolymerase sigma-subunit F
AT3G53920	0		SIG3	RNApolymerase sigma-subunit C
AT5G13730	E-ED		SIG4	sigma factor 4
AT5G24120	U-E-E-EN		SIG5	sigma factor E

Supplementary Table 20:

Defence response to fungus. Transcripts that account for the overrepresentation of the GO category *defence response to fungus* in the transcripts that are more abundant in SOW than in SWD leaves. Pattern '0' indicates that the transcript levels were not changing significantly and were therefore not subjected to the clustering. maxDiff referes to the maximum difference between EOD and EON in the four stages and meanDiff the average difference.

AGI	pvalue	maxDiff	meanDiff	pattern SOW	pattern SWD	distinct_symbols	Description
AT5G11270	0.002	0.87	0.61	D-D-D-ED	D-E-D-ED	OCP3	overexpressor of cationic peroxidase 3
AT4G20970	0.003	1.31	0.82	D-ED	0		basic helix-loop-helix (bHLH) DNA-binding superfamily protein
AT3G49120	0.003	1.67	0.93	E-U-U-ED	U-E-U-ED	ATPERX34, PERX34, PRXCB, ATPCB, PRX34	peroxidase CB
AT2G43510	0.004	1.17	0.71	E-U-U-E	U-U-U-ED	ATTI1, TI1	trypsin inhibitor protein 1
AT5G64120	0.008	2.38	1.25	U-U-U-E	E-E-U-E	NULL	Peroxidase superfamily protein
AT2G39200	0.009	1.05	0.61	E-E-U-E	E-E-U-E	MLO12, ATMLO12	Seven transmembrane MLO family protein
AT2G30770	0.012	1.34	0.64	E-E-U-E	E-E-U-E	CYP71A13	cytochrome P450, family 71, subfamily A, polypeptide 13
AT3G52400	0.013	1.85	0.77	U-U-U-EN	U-U-U-E	SYP122, ATSYP122	syntaxin of plants 122
AT3G04720	0.015	2.06	1.20	U-U-E-E	U-U-U-ED	PR4, HEL, PR-4	pathogenesis-related 4
AT5G61600	0.016	1.43	0.66	U-E-E-E	U-E	ERF104	ethylene response factor 104
AT5G57220	0.017	1.18	0.65	U-E-E-E	U-E-E-E	CYP81F2	cytochrome P450, family 81, subfamily F, polypeptide 2
AT4G31800	0.019	1.13	0.63	U-ED	E-E-U-ED	WRKY18, ATWRKY18	WRKY DNA-binding protein 18
AT3G15356	0.034	2.34	1.06	U-E-E-EN	U-EN	NULL	Legume lectin family protein
AT4G37150	0.043	2.18	0.71	E-E-U-E	E-E-U-E	ATMES9, MES9	methyl esterase 9
AT1G15010	0.046	2.59	0.85	U-EN	E-E-U-EN	NULL	unknown protein

Supplementary Table 23:

Proteins with significant differences between SOW and SWD.

agi	pvalue	maxDiff	meanDiff	correlation	higher in	pattern SOW	pattern SWD	Name	Description
AT2G42540	0.0002	1.76	1.48	0.98	SWD	E-E-U-E	E-E-U-E	COR15A, COR15	cold-regulated 15a
AT5G15970	0.0016	0.82	0.65	0.95	SWD	D-E-U-E		KIN2, COR6.6	stress-responsive protein (KIN2) / stress-induced protein (KIN2) / cold-responsive protein (COR6.6) /
AT4G11380	0.0016	1.20	0.77	0.60	SWD				Adaptin family protein
AT2G42530	0.0020	0.98	0.69	0.19	SWD	D-E-U-E		COR15B	cold regulated 15b
AT3G08940	0.0020	1.45	0.99	0.71	SWD		E-E	LHCB4.2	light harvesting complex photosystem II
AT4G25100	0.0041	1.07	0.71	0.93	SWD	U-E	U-E-E-E	FSD1, ATFSD1	Fe superoxide dismutase 1
AT5G54270	0.0049	0.99	0.62	0.76	SWD	E-E		LHCB3, LHCB3*1	light-harvesting chlorophyll B-binding protein 3
AT1G79600	0.0060	1.17	0.65	0.69	SWD				Protein kinase superfamily protein
AT4G39960	0.0062	0.96	0.59	0.62	SWD				Molecular chaperone Hsp40/DnaJ family protein
AT5G64350	0.0171	4.02	1.90	0.74	SWD	D-E-E-E		FKBP12, ATFKBP12	FK506-binding protein 12
AT5G43060	0.0192	2.06	1.08	-0.64	SWD				Granulin repeat cysteine protease family protein
AT5G43330	0.0205	1.48	0.77	0.02	SWD				Lactate/malate dehydrogenase family protein
AT2G26930	0.0278	1.38	0.63	0.07	SWD			ATCDPMEK, PDE277, ISPE, CDPMEK	4-(cytidine 5'-phospho)-2-C-methyl-D-erithritol kinase
AT5G10920	0.0294	1.60	0.78	0.48	SWD				L-Aspartase-like family protein
AT3G28220	0.0386	1.98	0.91	0.02	SWD		E-E-U-E		TRAF-like family protein
AT5G49030	0.0463	1.23	0.65	-0.64	SWD			OVA2	tRNA synthetase class I (I, L, M and V) family protein
AT4G27090	0.0011	1.71	1.27	0.74	SOW	E-E	D-E		Ribosomal protein L14
ATCG01120	0.0015	1.77	1.13	0.76	SOW	E-E		RPS15	chloroplast ribosomal protein S15
AT5G54600	0.0018	1.14	0.67	0.60	SOW	E-E			Translation protein SH3-like family protein
AT1G04270	0.0035	2.93	1.98	0.81	SOW			RPS15	cytosolic ribosomal protein S15
AT2G41100	0.0054	1.31	0.78	0.67	SOW	U-E-E-E		TCH3, ATCAL4	Calcium-binding EF hand family protein
AT2G43030	0.0061	0.94	0.60	0.69	SOW	E-E			Ribosomal protein L3 family protein
AT3G44890	0.0066	1.04	0.64	0.83	SOW	E-E		RPL9	ribosomal protein L9
AT1G05190	0.0074	1.03	0.63	0.55	SOW			emb2394	Ribosomal protein L6 family
AT2G41950	0.0074	1.12	0.69	0.62	SOW				unknown protein
AT3G15190	0.0097	2.85	1.71	0.45	SOW	E-E	D-E		chloroplast 30S ribosomal protein S20, putative
AT1G75350	0.0107	1.17	0.68	0.52	SOW	E-E		emb2184	Ribosomal protein L31
AT1G56500	0.0129	1.31	0.65	0.71	SOW		D-E		haloacid dehalogenase-like hydrolase family protein
ATCG00650	0.0192	1.14	0.67	0.40	SOW			RPS18	ribosomal protein S18
AT2G18020	0.0249	1.43	0.59	-0.10	SOW			EMB2296	Ribosomal protein L2 family
AT2G05380	0.0258	2.10	1.00	0.74	SOW	E-E-U-E		GRP3S	glycine-rich protein 3 short isoform
AT5G40950	0.0291	2.83	1.52	-0.60	SOW	E-E		RPL27	ribosomal protein large subunit 27
AT1G50250	0.0311	1.15	0.64	0.12	SOW			FTSH1	FTSH protease 1
AT5G38410	0.0420	2.43	1.23	0.31	SOW	E-E			Ribulose bisphosphate carboxylase (small chain) family protein

Supplementary Table 24:

SOW iTRAQ labelling scheme. 8plex iTRAQ labelling scheme for the short day optimal watering experiment; Reference = mixed rosette sample, EN = end-of-night, ED= end-of-day. In each field the mass of the reporter ion of the 8-plex iTRAQ reagent is given at the top, and the sample aliquot at the bottom.

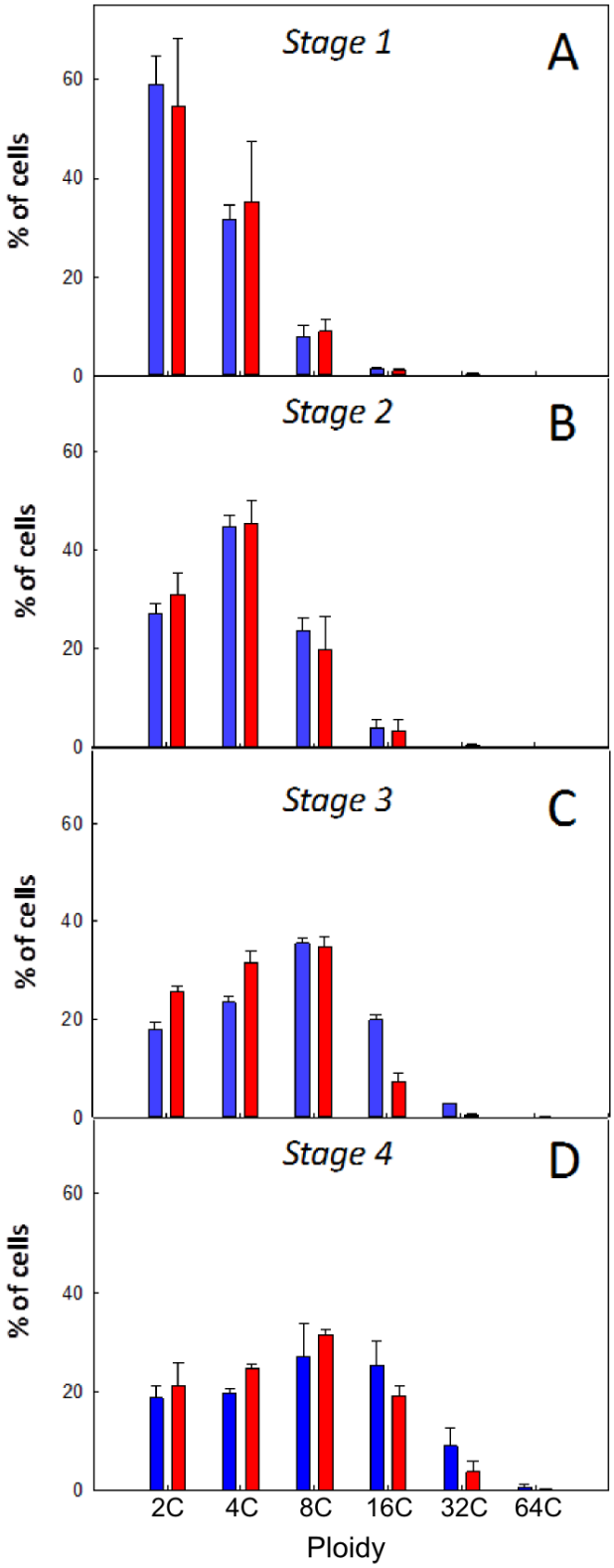
SOW	Sample/ Reaction	stage 1 EN	stage 1 ED	stage 2 EN	stage 2 ED	stage 3 EN	stage 3 ED	stage 4 EN	stage 4 ED	Reference 1	Reference 2
Biol. Replicate 1	ae1_1	-	-	113 PE3_1	114 PE4_1	115 PE5_1	116 PE6_1	117 PE7_1	118 PE8_1	119 c_1	121 c_2
	ae1_5	121 PE47_1	113 PE48_1	-	-	114 PE5_4	115 PE6_4	116 PE7_4	117 PE8_4	118 c_25	119 c_26
	ae1_6	117 PE47_2	118 PE48_2	119 PE3_4	121 PE4_4	-	-	113 PE7_5	114 PE8_5	115 c_27	116 c_28
	ae1_7	116 PE47_2	117 PE48_2	118 PE3_5	119 PE4_5	121 PE5_5	113 PE6_5	-	-	114 c_29	115 c_30
Biol. Replicate 2	ae2_1	-	-	114 PE14_1	115 PE15_1	116 PE16_1	117 PE17_1	118 PE18_1	119 PE19_1	121 c_3	113 c_4
	ae2_2	119 PE51_1	121 PE52_1	-	-	113 PE16_2	114 PE17_2	115 PE18_2	116 PE19_2	117 c_13	118 c_14
	ae2_3	118 PE51_2	119 PE52_2	121 PE14_2	113 PE15_2	-	-	114 PE18_3	115 PE19_3	116 c_15	117 c_16
	ae2_4	115 PE51_2	116 PE52_2	117 PE14_3	118 PE15_3	119 PE16_3	121 PE17_3	-	-	113 c_17	114 c_18
Biol. Replicate 3	ae3_1	-	-	115 PE25_1	116 PE26_1	117 PE27_1	118 PE28_1	119 PE29_1	121 PE30_1	114 c_5	113 c_6
	ae3_2	113 PE55_1	114 PE56_1	-	-	118 PE27_2	119 PE28_2	121 PE29_2	116 PE30_2	117 c_19	115 c_20
	ae3_3	114 PE55_2	115 PE56_2	116 PE25_2	117 PE26_2	-	-	118 PE29_3	113 PE30_3	121 c_21	119 c_22
	ae3_4	119 PE55_2	121 PE56_2	113 PE25_3	114 PE26_3	115 PE27_3	116 PE28_3	-	-	118 c_23	117 c_24

Supplementary Table 25:

SWD iTRAQ labelling scheme 8plex iTRAQ labelling scheme for the short day water deficit experiment; Reference = mixed rosette sample, EN = end-of-night, ED= end-of-day. In each field the mass of the reporter ion of the 8-plex iTRAQ reagent is given at the top, and the sample aliquot at the bottom.

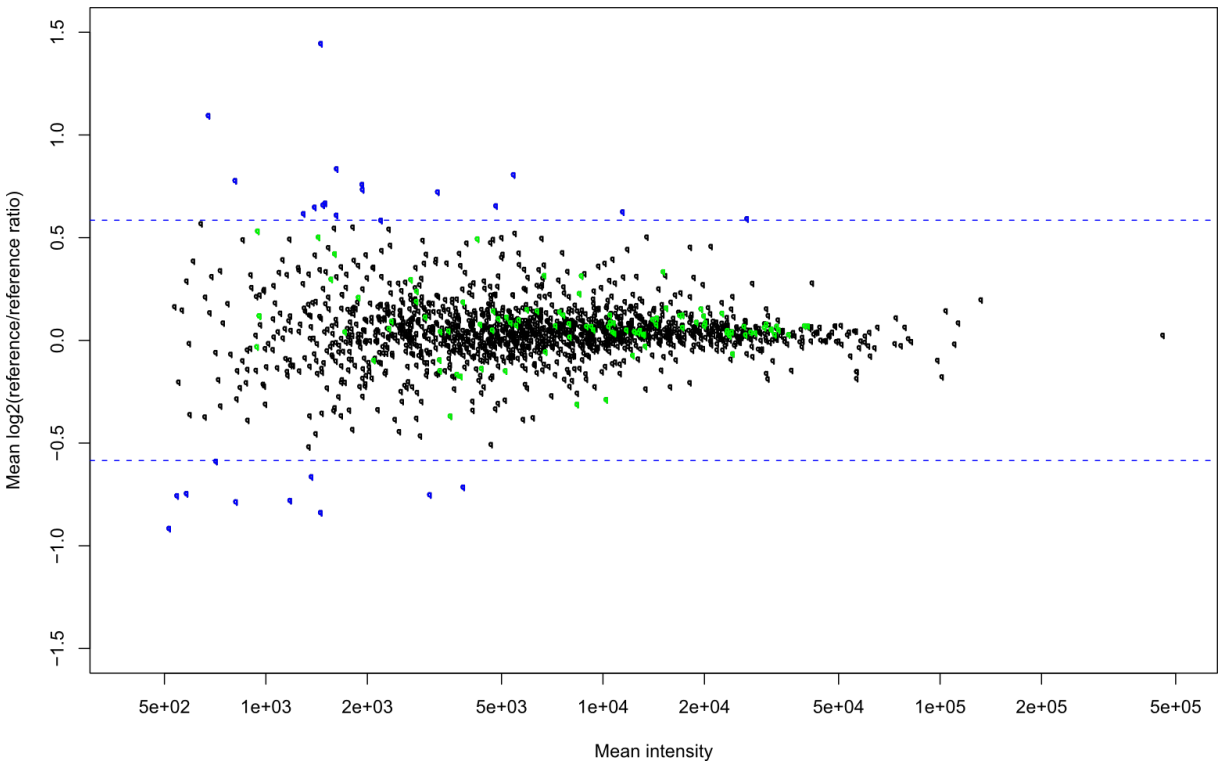
SWD	Sample/ Reaction	stage 1 EN	stage 1 ED	stage 2 EN	stage 2 ED	stage 3 EN	stage 3 ED	stage 4 EN	stage 4 ED	Reference 1	Reference 2
Biol. Replicate 1	ae4_1			113 PE38.1	114 PE39.1	115 PE40.1	116 PE41.1	117 PE42.1	118 PE43.1	119 c_1	121 c_2
	ae4_2	121 PE36.1	113 PE37.1			114 PE40.2	115 PE41.2	116 PE42.2	117 PE43.2	118 c_3	119 c_4
	ae4_3	117 PE36.2	118 PE37.2	119 PE38.2	121 PE39.2			113 PE42.3	114 PE43.3	115 c_5	116 c_6
	ae4_4	116 PE36.3	117 PE37.3	118 PE38.3	119 PE39.3	121 PE40.3	113 PE41.3			114 c_7	115 c_8
Biol. Replicate 2	ae5_1			114 PE59.1	115 PE60.1	116 PE61.1	117 PE62.1	118 PE63.1	119 PE64.1	121 c_9	113 c_10
	ae5_2	119 PE49_1	121 PE50_1			113 PE61.2	114 PE62.2	115 PE63.2	116 PE64.2	117 c_11	118 c_12
	ae5_3	118 PE49_2	119 PE50_2	121 PE59.2	113 PE60.2			114 PE63.3	115 PE64.3	116 c_13	117 c_14
	ae5_4	115 PE49_3	116 PE50_3	117 PE59.3	118 PE60.3	119 PE61.3	121 PE62.3			113 c_15	114 c_16
Biol. Replicate 3	ae6_1			115 PE70.1	116 PE71.1	117 PE72.1	118 PE73.1	119 PE74.1	121 PE75.1	113 c_17	114 c_18
	ae6_2	113 PE68.1	114 PE69.1			115 PE72.2	116 PE73.2	117 PE74.2	118 PE75.2	119 c_19	121 c_20
	ae6_3	114 PE68.2	115 PE69.2	116 PE70.2	117 PE71.2			118 PE74.3	119 PE75.3	121 c_21	113 c_22
	ae6_4	119 PE68.3	121 PE69.3	113 PE70.3	114 PE71.3	115 PE72.3	116 PE73.3			117 c_23	118 c_24

Supplementary Figure 1:



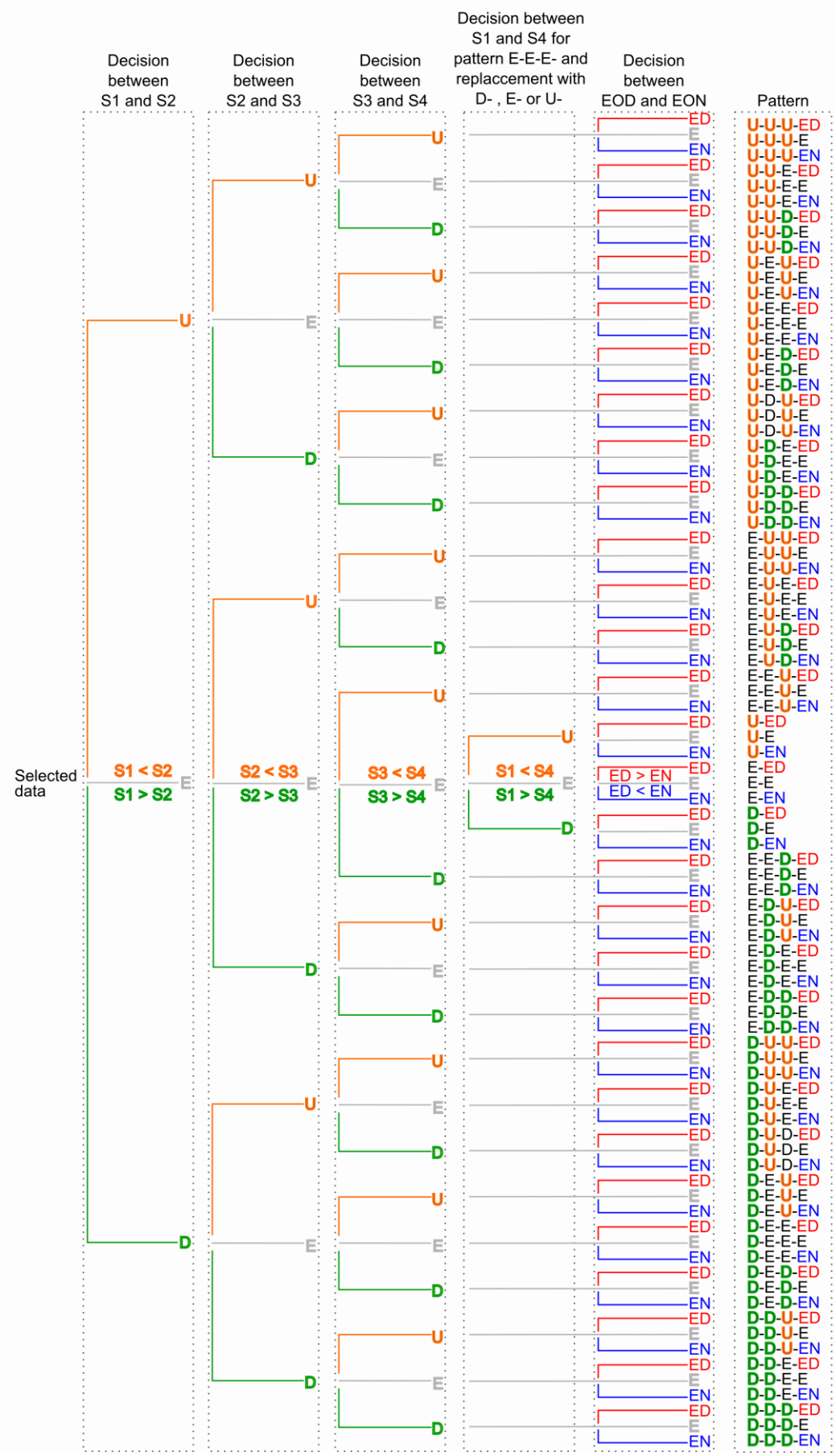
Stage-specific ploidy levels. Percentage of leaf cells at each ploidy level for the four stages as defined in Figure 3 in SOW (blue bars) and SWD (red bars) conditions. The four stages (1, 2, 3, 4) are represented in A, B, C and D, respectively. Data are mean and SD values with $n \geq 5$.

Supplementary Figure 2:

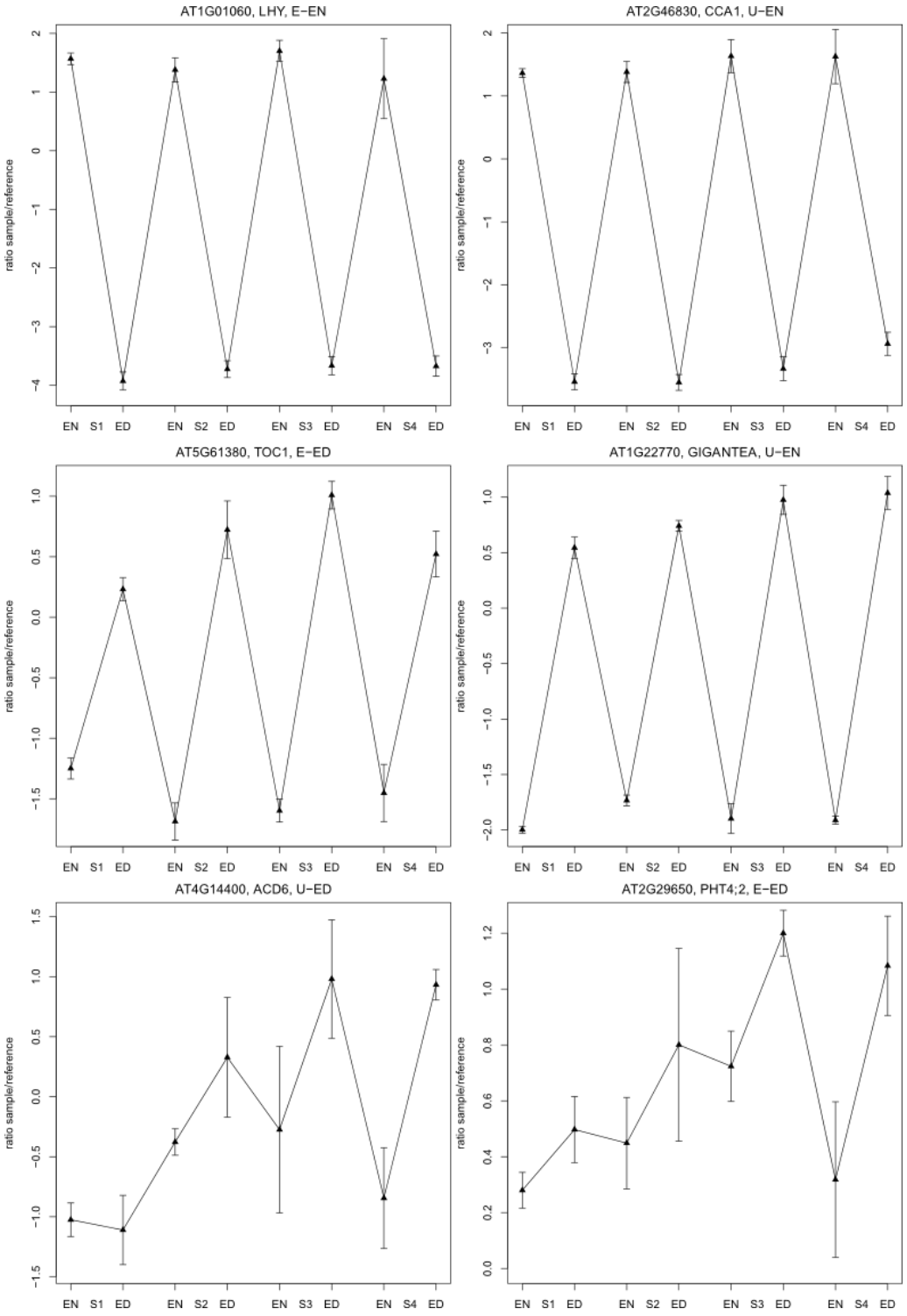


Technical variability of protein quantification by iTRAQ. As two reference samples were included in each of the 12 iTRAQ labelling experiments (see Supplementary Table 24), the reference/reference log-transformed ratios, which should ideally be 0, were used to assess the technical variability in the data and the validity of the cut-off criteria to find significantly changing proteins. The cut-off criteria are a combination of a significance test (p-value < 0.05) and a fold-change threshold (fold-change > 1.5), and when applying these to the reference/reference protein ratios no protein passed both criteria. For visualisation we plotted the mean log-transformed reference/reference protein ratios versus the mean intensity of the reference peaks. The data is presented for the 1639 proteins with a reference/reference ratio in at least two of the three biological replicates in the SOW experiment. In blue are the proteins with fold-change > 1.5 and in green those with p-value < 0.05 in a one-sample two-sided Student's t-Test. The dashed blue lines indicate the fold-change cut-off.

Supplementary Figure 3:

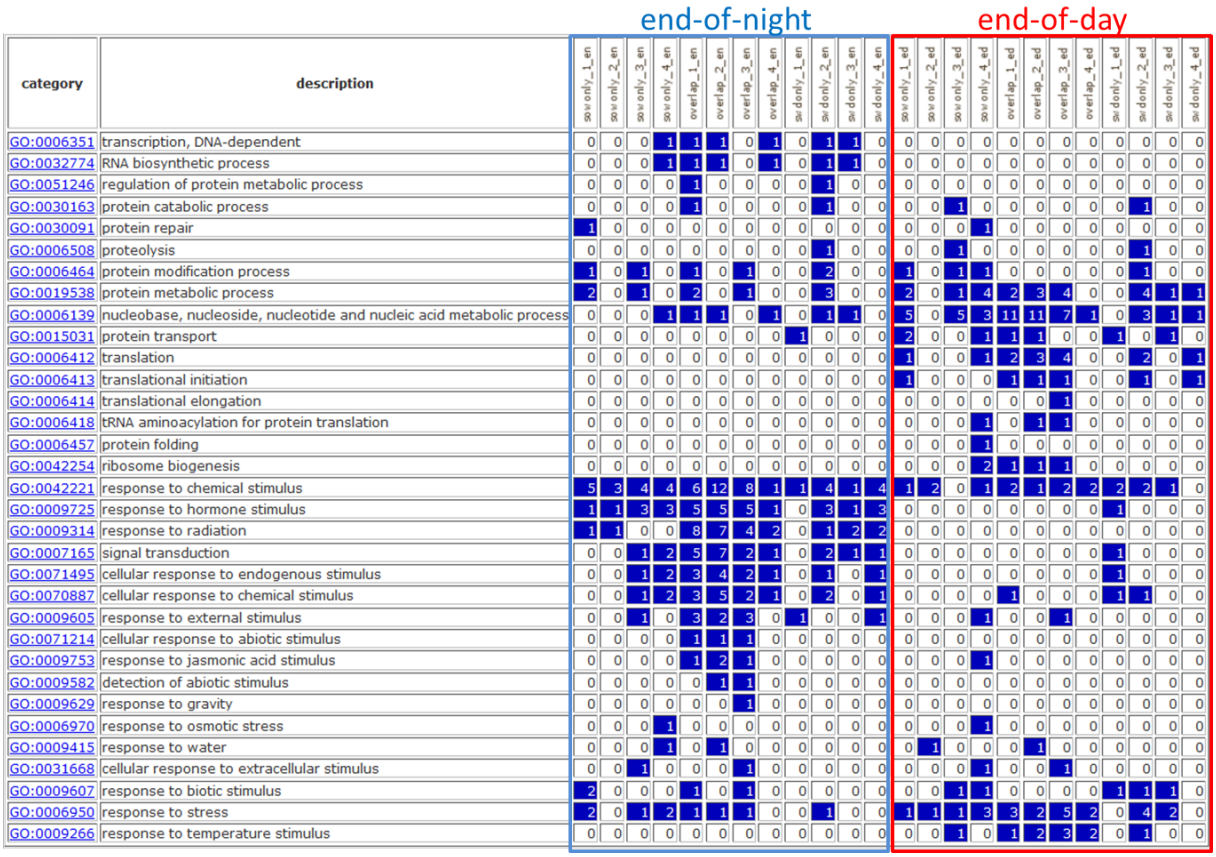


Supplementary Figure 8:



Example transcripts with EON and EOD changes. Plots of the mean transcript sample/reference ratios and their standard deviations in the 8 time points for the central clock components LHY, CCA1 and TOC1, GIGANTEA, ACD6 and PHT4;2. For each transcript, the pattern into which it was clustered is given in the header line.

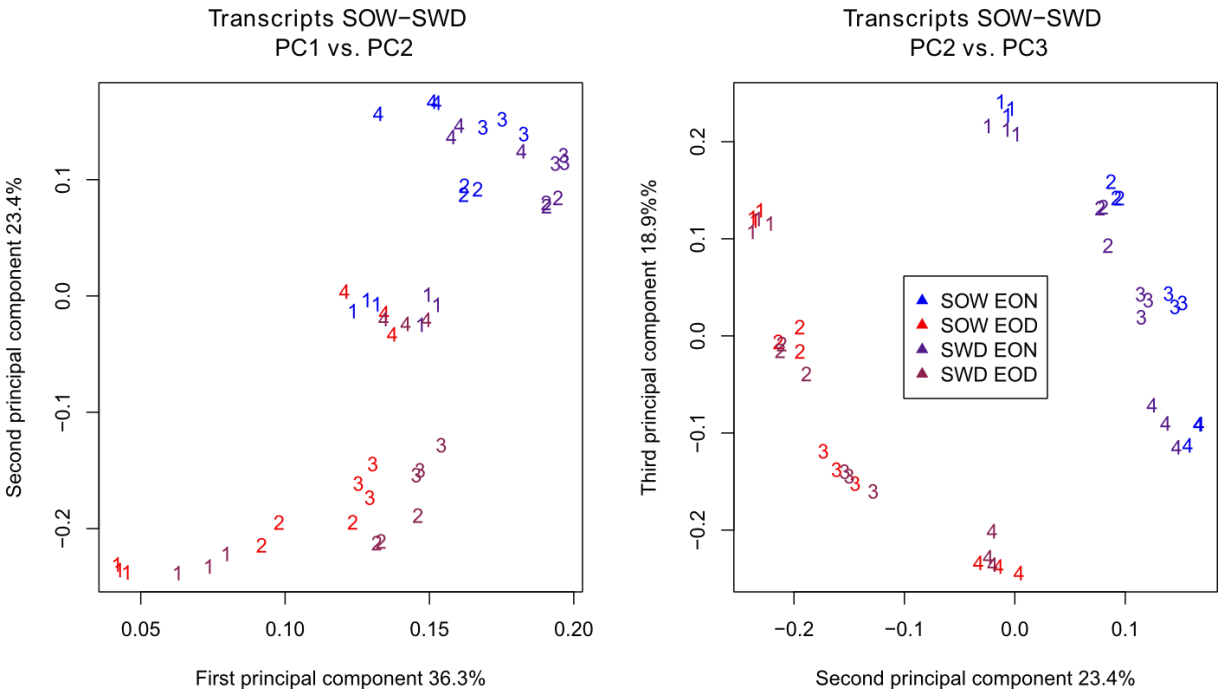
Supplementary Figure 9:



category	description	end-of-night												end-of-day											
		sov only_1_en	sov only_2_en	sov only_3_en	sov only_4_en	overlap_1_en	overlap_2_en	overlap_3_en	overlap_4_en	su donly_1_en	su donly_2_en	su donly_3_en	su donly_4_en	sov only_1_ed	sov only_2_ed	sov only_3_ed	sov only_4_ed	overlap_1_ed	overlap_2_ed	overlap_3_ed	overlap_4_ed	su donly_1_ed	su donly_2_ed	su donly_3_ed	su donly_4_ed
GO:0006351	transcription, DNA-dependent	0	0	0	1	1	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
GO:0032774	RNA biosynthetic process	0	0	0	1	1	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
GO:0051246	regulation of protein metabolic process	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GO:0030163	protein catabolic process	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0
GO:0030091	protein repair	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
GO:0006508	proteolysis	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0	0
GO:0006464	protein modification process	1	0	1	0	1	0	1	0	0	2	0	0	1	0	1	1	0	0	0	0	0	1	0	0
GO:0019538	protein metabolic process	2	0	1	0	2	0	1	0	0	3	0	0	2	0	1	4	2	3	4	0	0	4	1	1
GO:0006139	nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	0	0	0	1	1	1	0	1	0	1	1	0	5	0	5	3	11	11	7	1	0	3	1	1
GO:0015031	protein transport	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	1	1	1	0	0	1	0	1	0
GO:0006412	translation	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	2	3	4	0	0	2	0	1
GO:0006413	translational initiation	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	1	0	0	1	0	1
GO:0006414	translational elongation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
GO:0006418	tRNA aminoacylation for protein translation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0
GO:0006457	protein folding	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
GO:0042254	ribosome biogenesis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	1	1	0	0	0	0	0
GO:0042221	response to chemical stimulus	5	3	4	4	6	12	8	1	1	4	1	4	1	2	0	1	2	1	2	2	2	2	1	0
GO:0009725	response to hormone stimulus	1	1	3	3	5	5	5	1	0	3	1	3	0	0	0	0	0	0	0	0	1	0	0	0
GO:0009314	response to radiation	1	1	0	0	8	7	4	2	0	1	2	2	0	0	0	0	0	0	0	0	0	0	0	0
GO:0007165	signal transduction	0	0	1	2	5	7	2	1	0	2	1	1	0	0	0	0	0	0	0	0	1	0	0	0
GO:0071495	cellular response to endogenous stimulus	0	0	1	2	3	4	2	1	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0
GO:0070887	cellular response to chemical stimulus	0	0	1	2	3	5	2	1	0	2	0	1	0	0	0	0	1	0	0	0	1	1	0	0
GO:0009605	response to external stimulus	0	0	1	0	3	2	3	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0
GO:0071214	cellular response to abiotic stimulus	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GO:0009753	response to jasmonic acid stimulus	0	0	0	0	1	2	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
GO:0009582	detection of abiotic stimulus	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GO:0009629	response to gravity	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
GO:0006970	response to osmotic stress	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
GO:0009415	response to water	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
GO:0031668	cellular response to extracellular stimulus	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0
GO:0009607	response to biotic stimulus	2	0	0	0	1	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	1	0
GO:0006950	response to stress	2	0	1	2	1	1	1	0	0	1	0	0	1	1	1	3	3	2	5	2	0	4	2	0
GO:0009266	response to temperature stimulus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	2	3	2	0	1	0	0

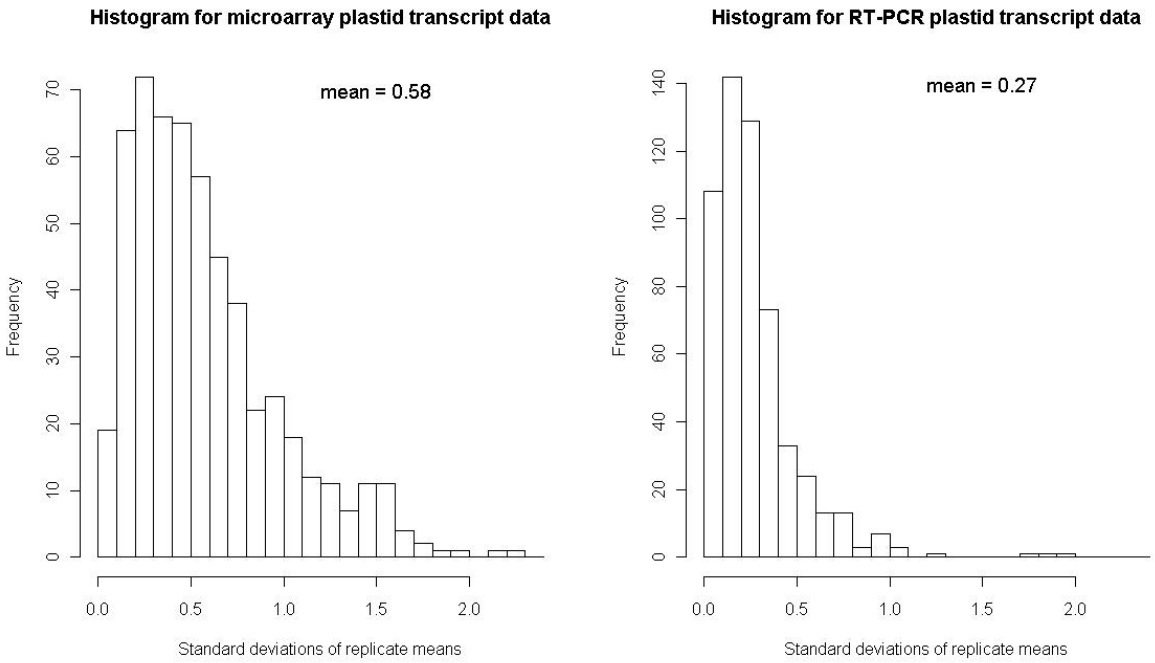
Functional categories over-represented in transcripts higher at EOD or EON. The lists with transcripts for each group were subjected to an assessment of over-representation of GO categories. The categories with p-value < 0.01 were assigned to higher-order GO categories in protein and nucleic acid metabolism and response to stimulus. The numbers inside the cells indicate the number of over-represented GO categories for each group that fall into the higher-order GO categories.

Supplementary Figure 10:



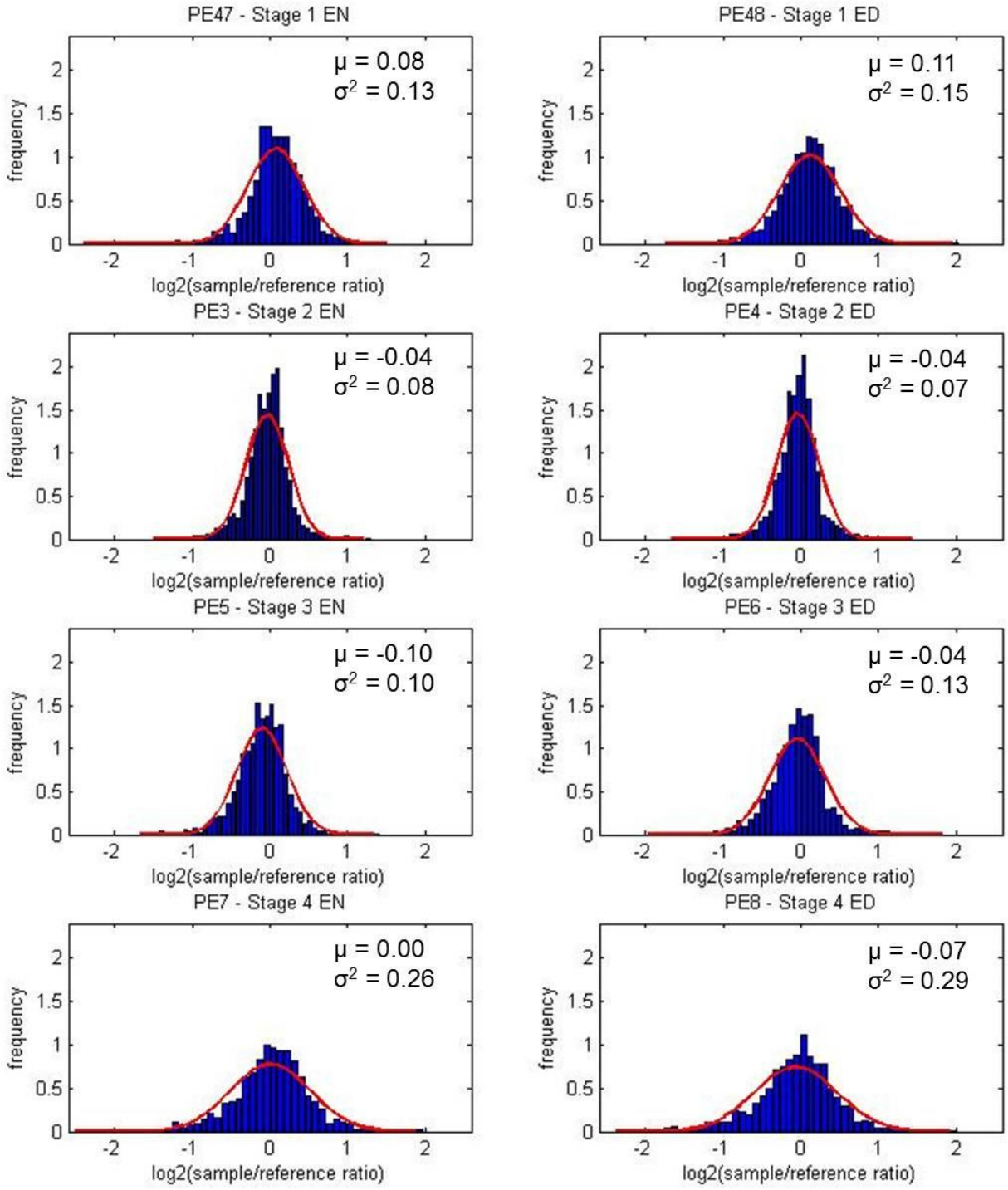
Results of the PCA analysis after combination of the transcript data from the SOW and SWD experiments.

Supplementary Figure 11



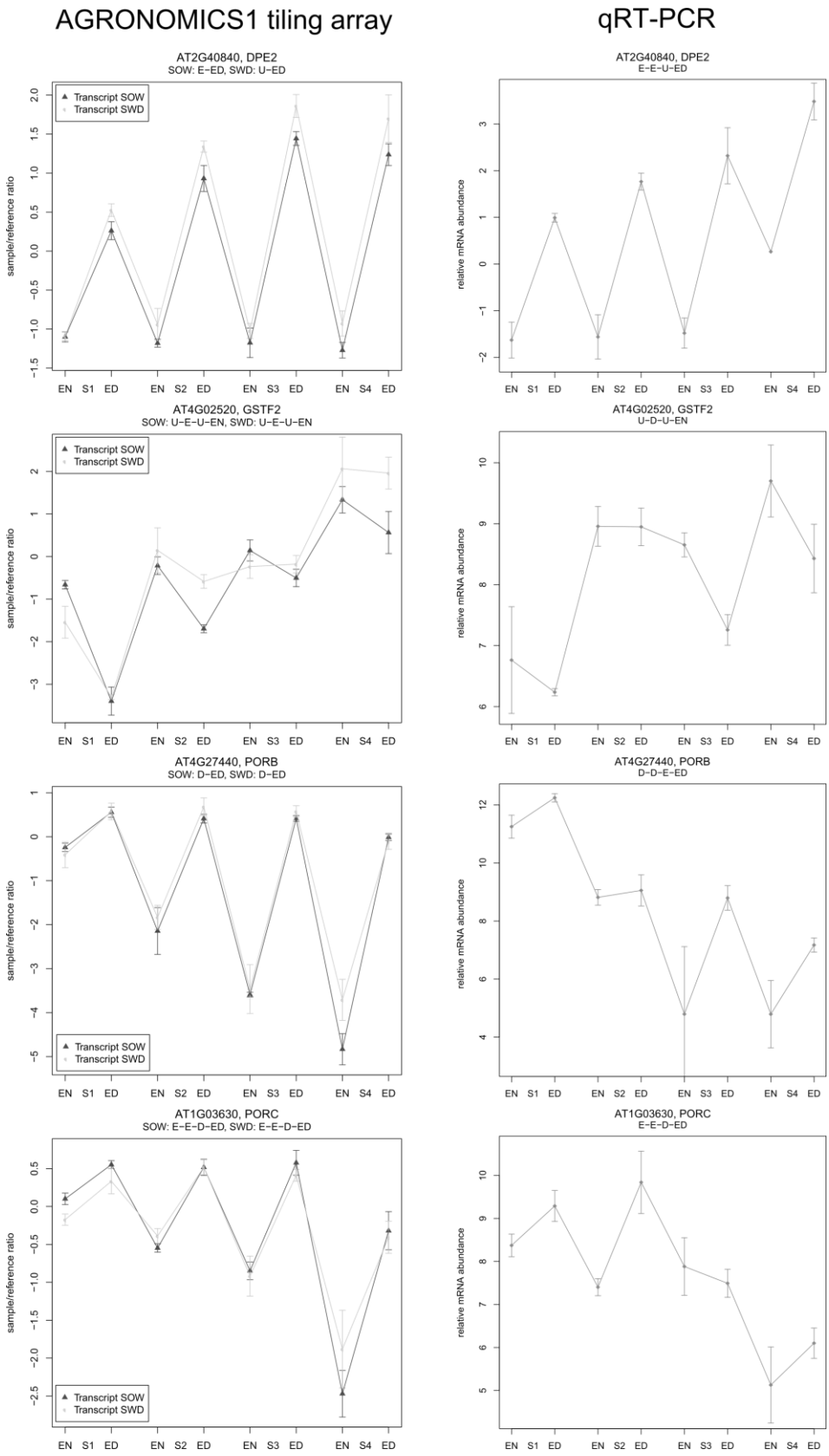
Histograms of the standard deviations of the replicate means for the microarray (left) and RT-PCR (right) plastid transcript data. The mean values indicated at the upper right of each panel indicate that the RT-PCR data have a smaller mean standard deviation.

Supplementary Figure 12:



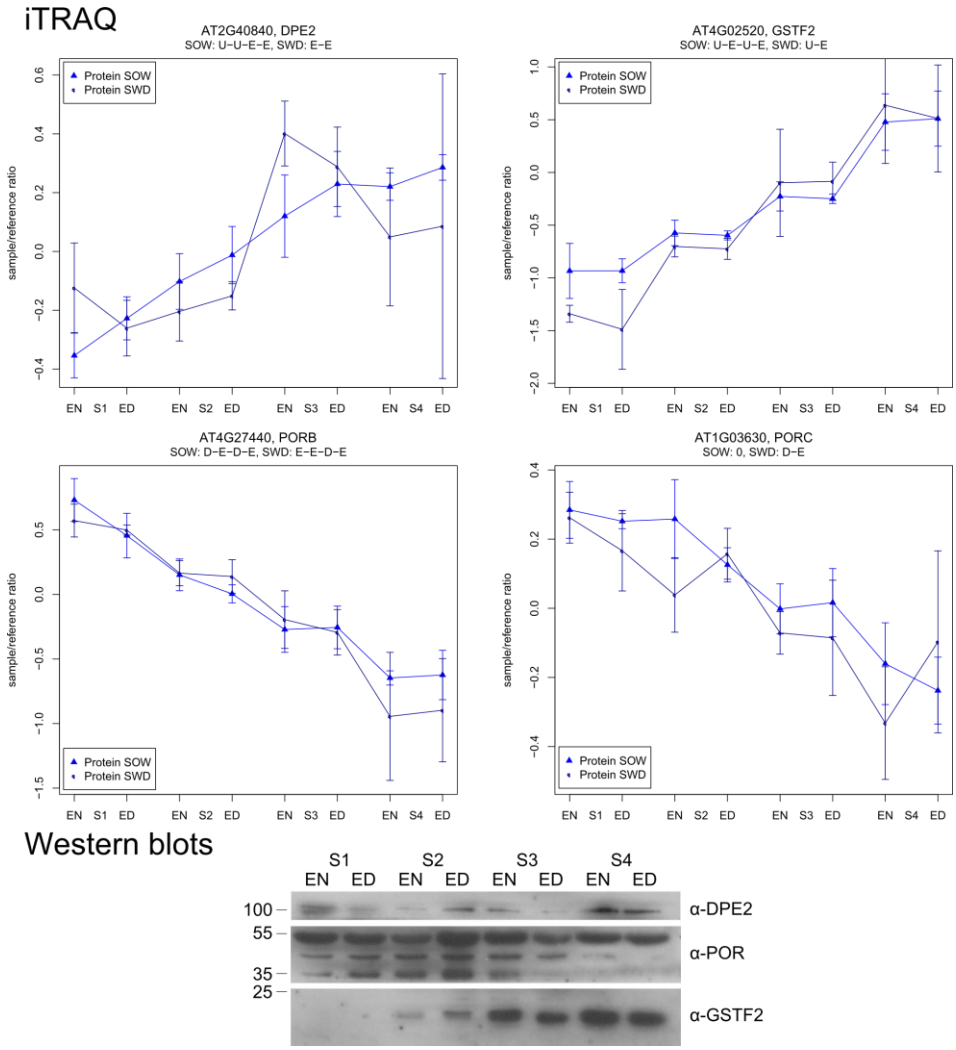
Histograms of the log2-transformed sample/reference protein ratios in biological replicate 1 with the mean and the variance of the ratios. The theoretical normal distribution is plotted in red.

Supplementary Figure 13:



Comparison of the transcript variation patterns in the AGRONOMICS1 tiling array data for SOW and SWD (left panels) and the qRT-PCR data for SWD (right panels).

Supplementary Figure 14:



Comparison of the protein variation patterns in the iTRAQ data for SOW and SWD (upper panels) and a qualitative Western blot for DPE2, POR and GSTF2 in SWD (lowest panel).The membrane sections probed with the α-POR and α-GSTF2 antibodies were generated from the same gel, the signals therefore serve as mutual loading controls. At the left, the molecular weight is indicated.