SUPPLEMENTARY TABLES

Table S1. Genes expressed in the guard cell detected by RNA-seq in two biological replicates (sample 1 and 2). Genes highlighted in yellow were selected for RT-qPCR experiments. FPKM values indicate the normalized expression of each gene in each sample. The link at the right column will lead to the description of each gene available at TAIR.

http://www.scientificsocieties.org/MPMIXtras/2013/MPMI-03-13-0081-TA_TableS1.xlsx

Table S2 Part A. All GO terms with a minimum of five entries identified in the guard cell transcriptome. Out of the 18,994 transcripts, 510 could not be grouped into any GO term, thus the query total is 18,484 transcripts. The letters P, F, and C refer to the GO terms Biological Process, Molecular Function, and Cellular Component, respectively. **Part B.** All GO terms with a minimum of five entries identified in the guard cell transcriptome in comparison to the Arabidopsis reference gene model (TAIR10 with 28,352 annotated genes). Singular Enrichment Analysis (SEA) was performed using AgriGO (Du et al. 2010). Statistical significance was detected with the Fisher's exact test (*P* value) with Benjamini-Hochberg-FDR correction. The letters P, F, and C refer to the GO terms Biological Process, Molecular Function, and Cellular Component, respectively. **Part C.** GO terms significantly enriched (FDR < 0.01) in the guard cell transcriptome (GCT; 18,484 transcripts) in comparison to the Arabidopsis reference gene model (TAIR10; 28,352 transcripts). Singular Enrichment Analysis (SEA) was performed using AgriGO (Du et al. 2010). Statistical significance was detected with the Fisher's exact test (*P* value) with Benjamini-Hochberg correction (FDR < 0.01). The letters P, F, and C refer to the GO terms Biological Process, Molecular Function, and Cellular Component, respectively.

http://www.scientificsocieties.org/MPMIXtras/2013/MPMI-03-13-0081-TA_TableS2.xlsx

Table S3. Gene-specific primers used in qPCR reactions and the expected amplicon sizes.

Gene	Primer	Sequence (5'-3')	Size(bp)
JAZ1 (At1g19180)	Forward	CGTGTAGTCGATTGAGTCAGTATCTAAAAGAGAACG	180
	Reverse	CGGTTTAACATCTTGAACCATGGAATCCATGTTAG	
JAZ8 (AT1G30135)	Forward	CAGCAAAATTGTGACTTGGAACTTCGTC	230
	Reverse	GTTATTCTTTGAGATTCTTCATTTGGTTGTGG	
DND1 (At5g15410)	Forward	GCAACACGCTGTATTGCGAGAACA	133
	Reverse	AGAAGGATGCAGAAGGTCACTGGT	
S6K1 (At3g08730)	Forward	CTTCCAAGTCGCCTTTCTG	84
	Reverse	CAAGCTTCCGCAGTTTCT	
LHY (AT1G01060)	Forward	GAGAGCCTGAAACGCTATAC	84
	Reverse	GAGACAACAACAGCAACAAC	
NINJA (At4g28910)	Forward	CAACAGGTTGTTTGCCTTCGCCTT	91
	Reverse	AGGAGGGATTGTCGCACTTTCTCA	
SKIP (At1g77180)	Forward	ACAGTACCCAAGTCTCCCTCGTTT	145
	Reverse	ACTCTCCCTGTTACTGTCGATGCT	
JAZ2 (At1g74950)	Forward	CTTCTTCCTCTTCCTCTGGGACCAAAG	125
	Reverse	CATCAAACACCATAACTCGACCACCG	
PPC2 (At2g42600)	Forward	CTTCAGGAGTTACTCGCGGGTTTC	176
	Reverse	GGATGAGCTACTTCCATGAGACAATCTGG	
TUB4 (At5g44340)	Forward	GCAGAGATGAGATGGTTAAGA	110
	Reverse	AACGCTGACGAGTGTATG	
ACT2 (At3g18780)	Forward	CACTTGCACCAAGCAGCATGAAGA	80
	Reverse	AATGGAACCACCGATCCAGACACT	

SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. Flow chart of the GCP preparation procedure. The left column represents the short protocol (2 h) and the right column indicates the steps with longer incubation times taking >6 h to complete the procedure. The two procedures (short and long) were performed in the presence or absence of the transcription inhibitors cordycepin (0.01%) and actinomycin D (0.0033%).

Fig. S2. Quality of RNA extracted from GCPs using Qiagen columns. **A,** RIN values of RNA samples obtained from GCPs isolated following the short (2 h) or long (>6 h) methods with or without antibiotics. Results are shown as mean (n=4) ± standard error. **B,** Representative electronic gel derived from the BioAnalyzer profiles of RNA samples. Lanes were loaded as follows: 1 = RNA ladder, 2-5 = RNA extracted from GCPs isolated using short (2 and 4) or long incubations (3 and 5) in the absence (2 and 3) or presence (4 and 5) of antibiotics. The numbers on the left corresponds to the fragment size in bp. RIN values of these samples ranged from 4.7 to 6.6.

Fig. S3. Agarose gel showing cDNA smears synthesized through reverse transcriptase reactions. Reactions were carried out with RNA samples extracted from GCP preparations in the presence or absence of antibiotics (+ and – symbols on top of the gel lanes) using long or short procedure.

Fig. S4. qPCR efficiency calculated based on the linear regression between the amount of cDNA template in the reaction and the cycle threshold (C_T). Results are shown as mean (n=3) ± standard error. *ACT2* and *TUB4* were used as reference gene for qPCR analysis.

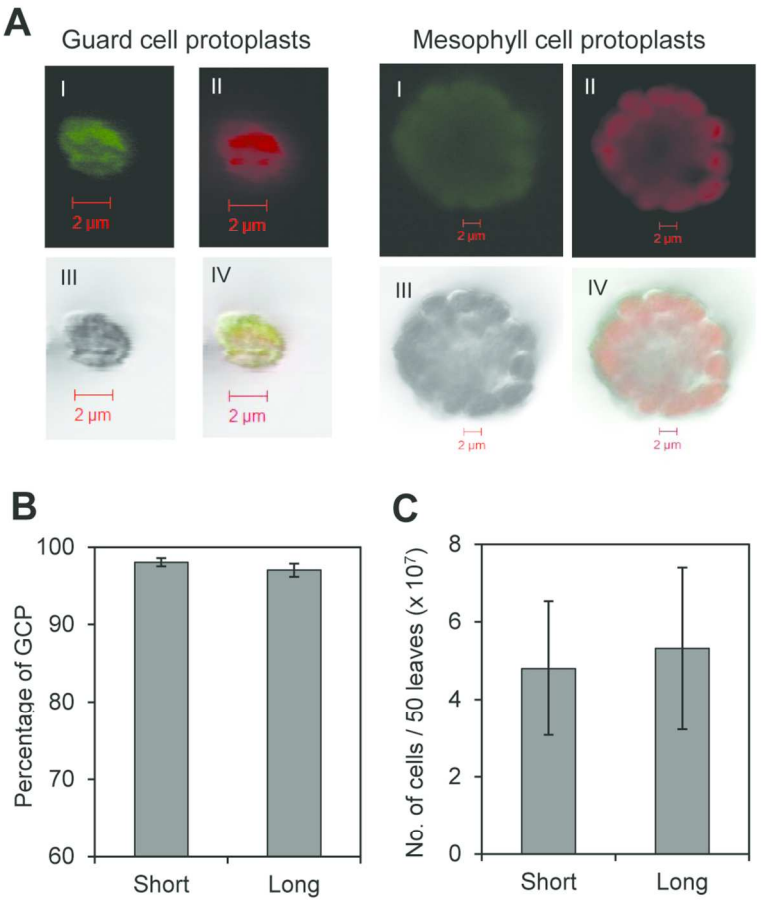

Fig. 1. Assessing the yield and purity of GCP preparations. **A,** Laser scanning confocal micrographs of guard cell and mesophyll cell protoplasts (I=green channel, II=red channel, III=DIC, IV=merged channels). Note the size difference. **B,** Purity of GCPs extracted using long and short incubation protocols calculated as percentages of total protoplast extracted (MCP and GCP). **C,** Number of GCPs isolated in long and short methods. Results are shown as means (n=3) ± standard error.

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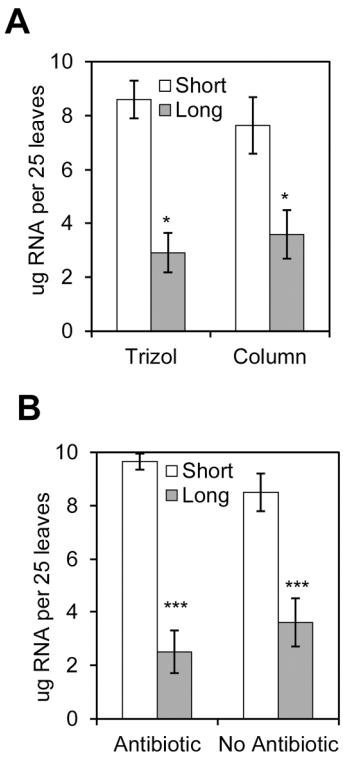

Fig. 2. Amount of RNA extracted from long and short protocols. **A,** GCPs were isolated from 50 leaves and GCP suspension was equally divided for total RNA extraction using either the Qiagen column or Trizol reagent, thus yield is expressed in µg per 25 leaves. Transcription inhibitors were not added during guard cell protoplasting. **B,** Total RNA extracted from GCPs using Qiagen column in presence or absence of the transcription inhibitor antibiotics cordycepin (0.01%) and actinomycin D (0.0033%). Results are shown as means (n=3) ± standard error. Statistical significance between the means (short versus long) was detected with two-tailed Student's *t*-test (*** refers to $P<0.001$, * refers to $P<0.05$).

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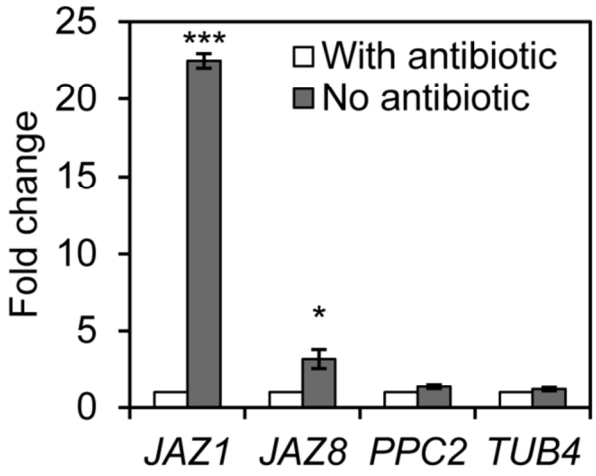

Fig. 3. Effect of transcription inhibitor antibiotics (actinomycin and cordycepin) in wound-responsive gene transcription during GCP preparation (2-h procedure). Transcript abundance of the indicated genes relative to the procedure with antibiotics was determined by RT-qPCR analysis. Results are shown as mean (n=6) ± standard error. Statistical significance of the difference between means (with antibiotics versus without antibiotics) was detected with two-tailed Student's *t*-test (*** = $P<0.001$, * = $P<0.05$).

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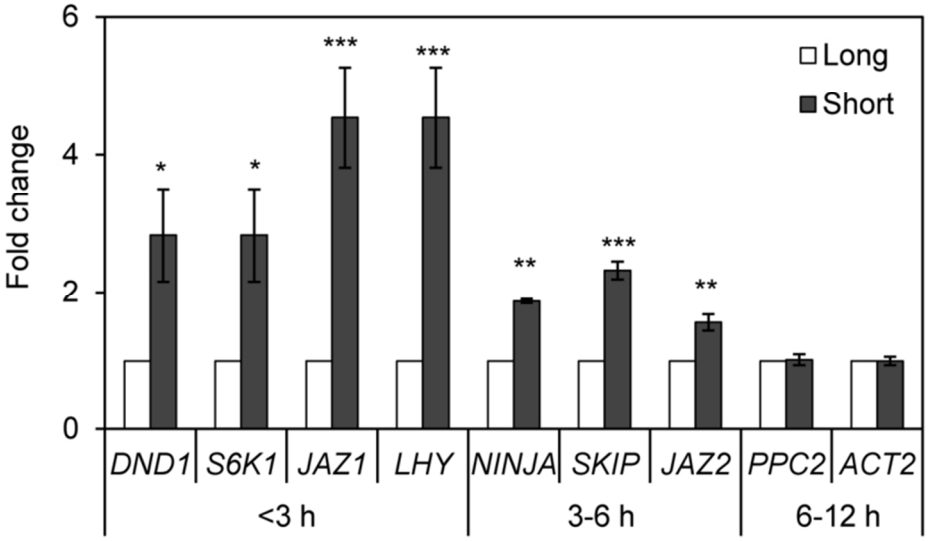

Fig. 4. Effect of GCP preparation time on transcript abundance. Long procedure takes >6 h whereas the short procedure can be finished in about 2 h. Transcript abundance of the indicated genes relative to the >6 h procedure was determined by RT-qPCR analysis. Results are shown as mean (n=6) ± standard error. Statistical significance of the difference between means (short versus long procedure) was detected with two-tailed Student's *t*-test (*** = $P<0.001$, ** = $P<0.01$, * = $P<0.05$).

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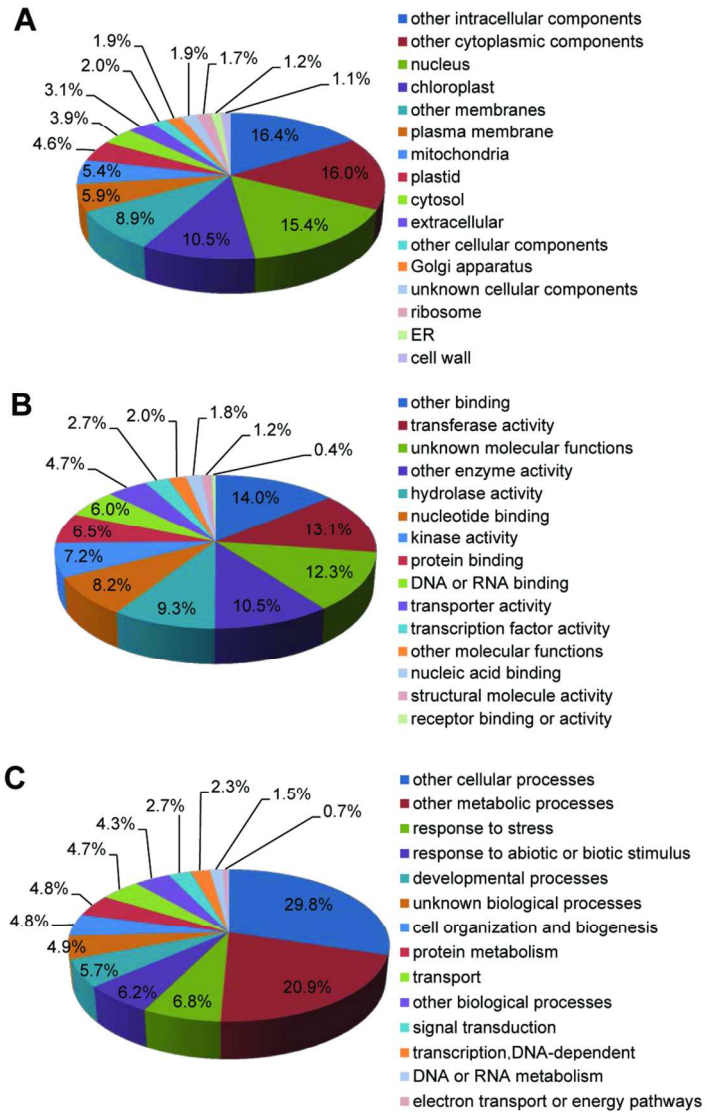

Fig. 5. Functional categorization of guard cell expressed genes (18,994) according to the three broad Gene Ontology categories cellular component (A), molecular function (B), and biological process (C) using the GO slim tool available at TAIR.

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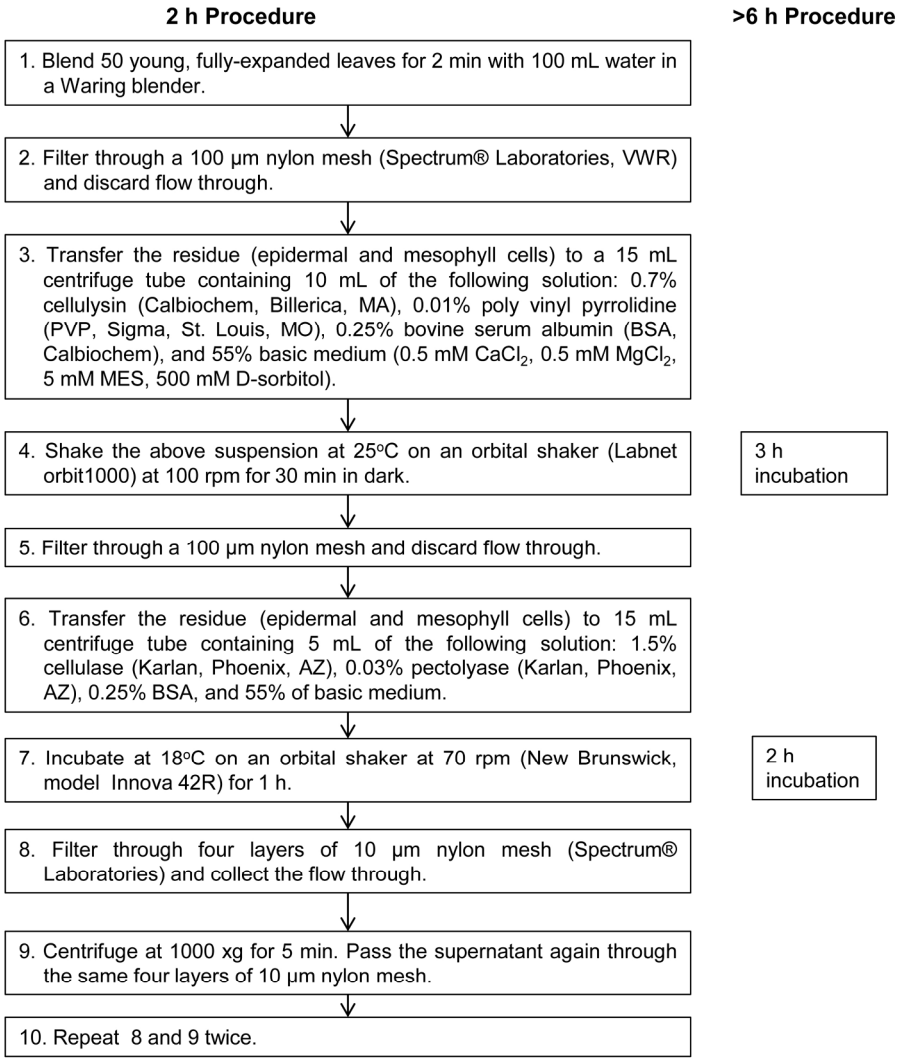

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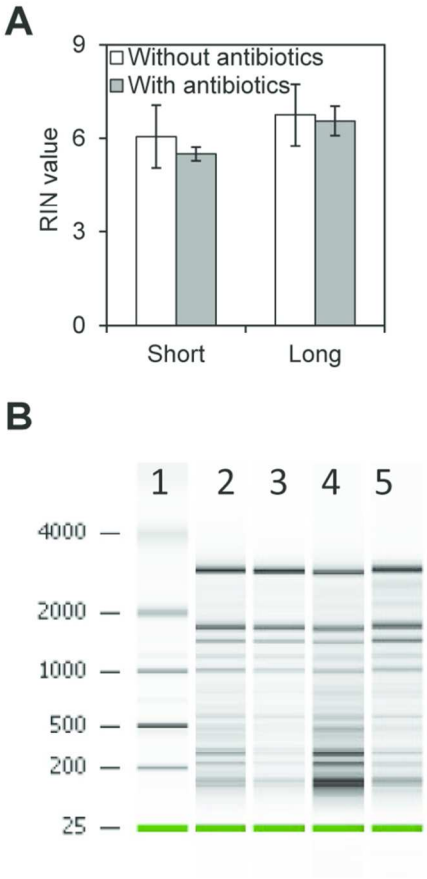

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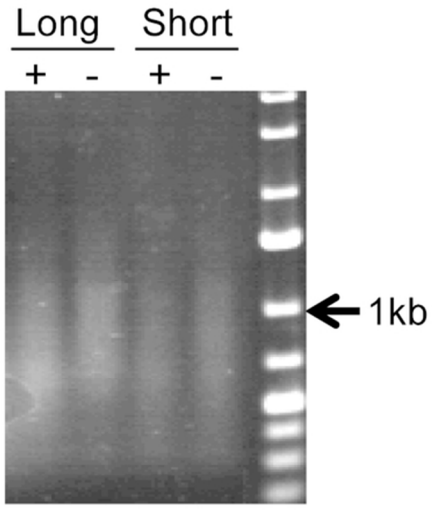

Fig. S3. Agarose gel showing cDNA smears synthesized through reverse transcriptase reactions. Reactions were carried out with RNA samples extracted from GCP preparations in the presence or absence of antibiotics (+ and – symbols on top of the gel lanes) using long or short procedure.

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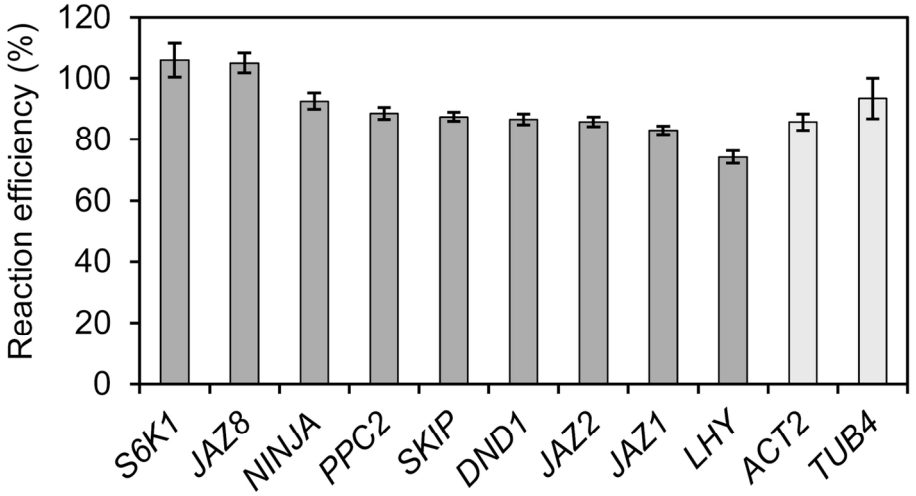

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138x108mm (300 x 300 DPI)