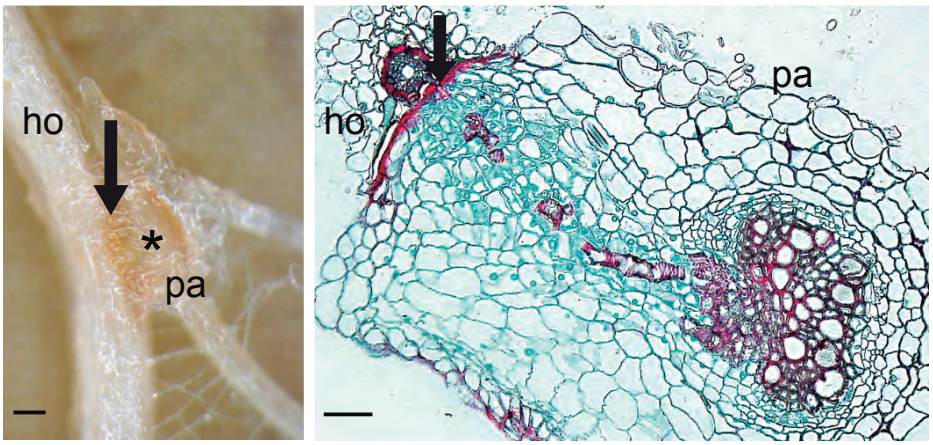
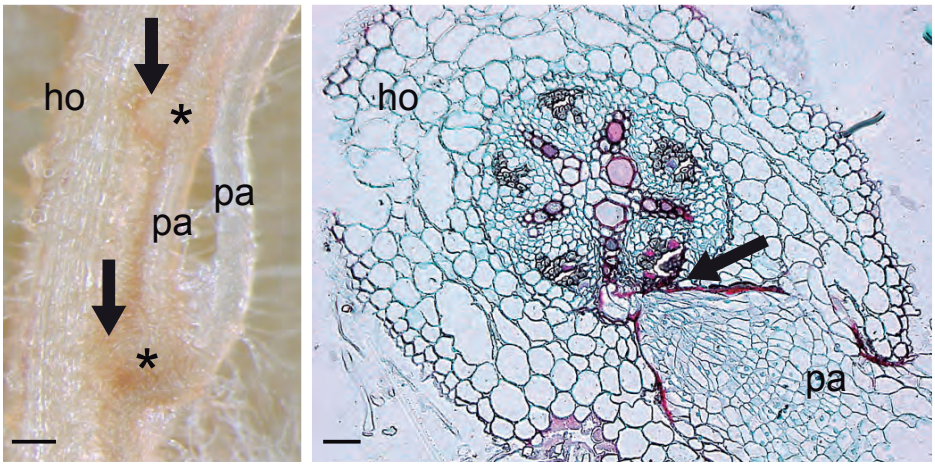
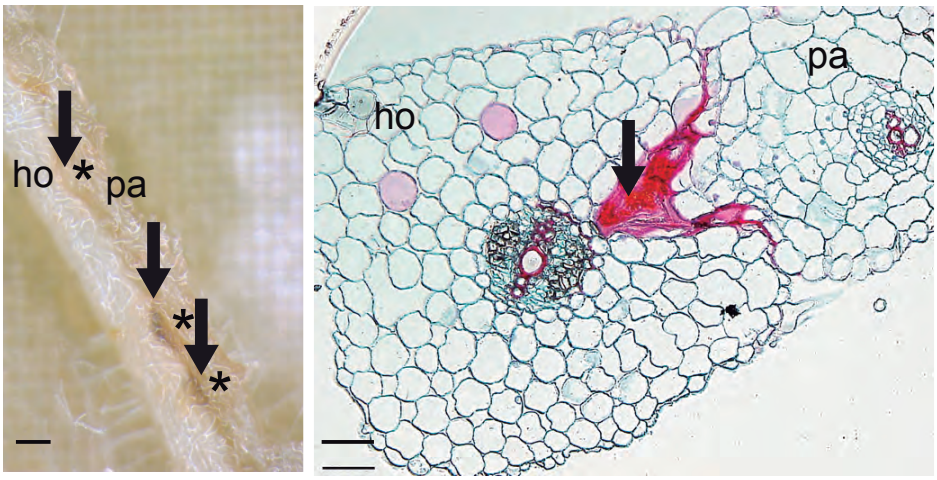
A Host: maize



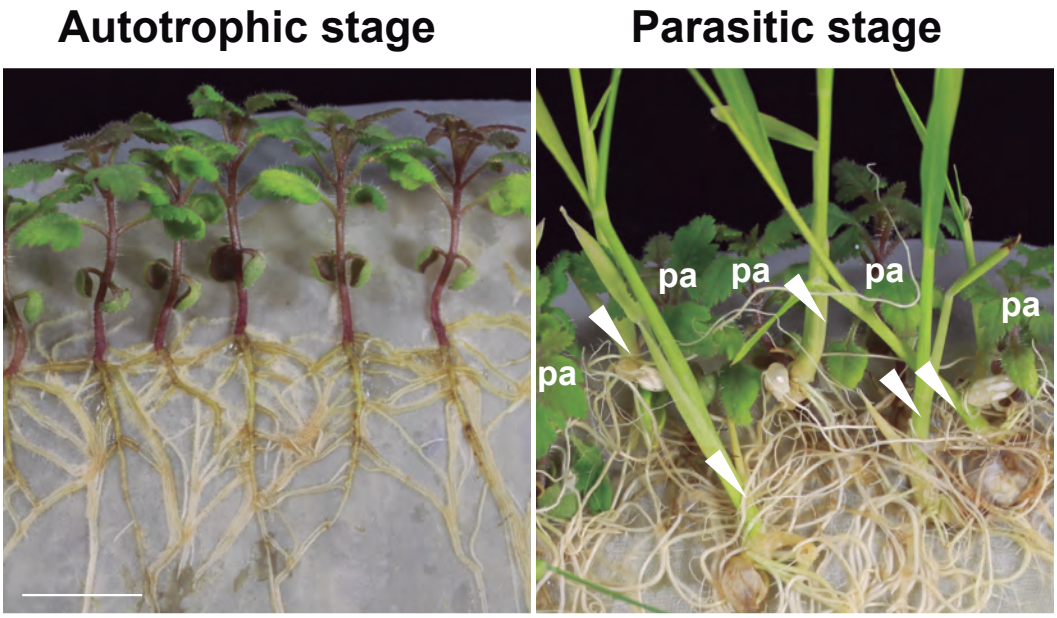
B Host: cowpea



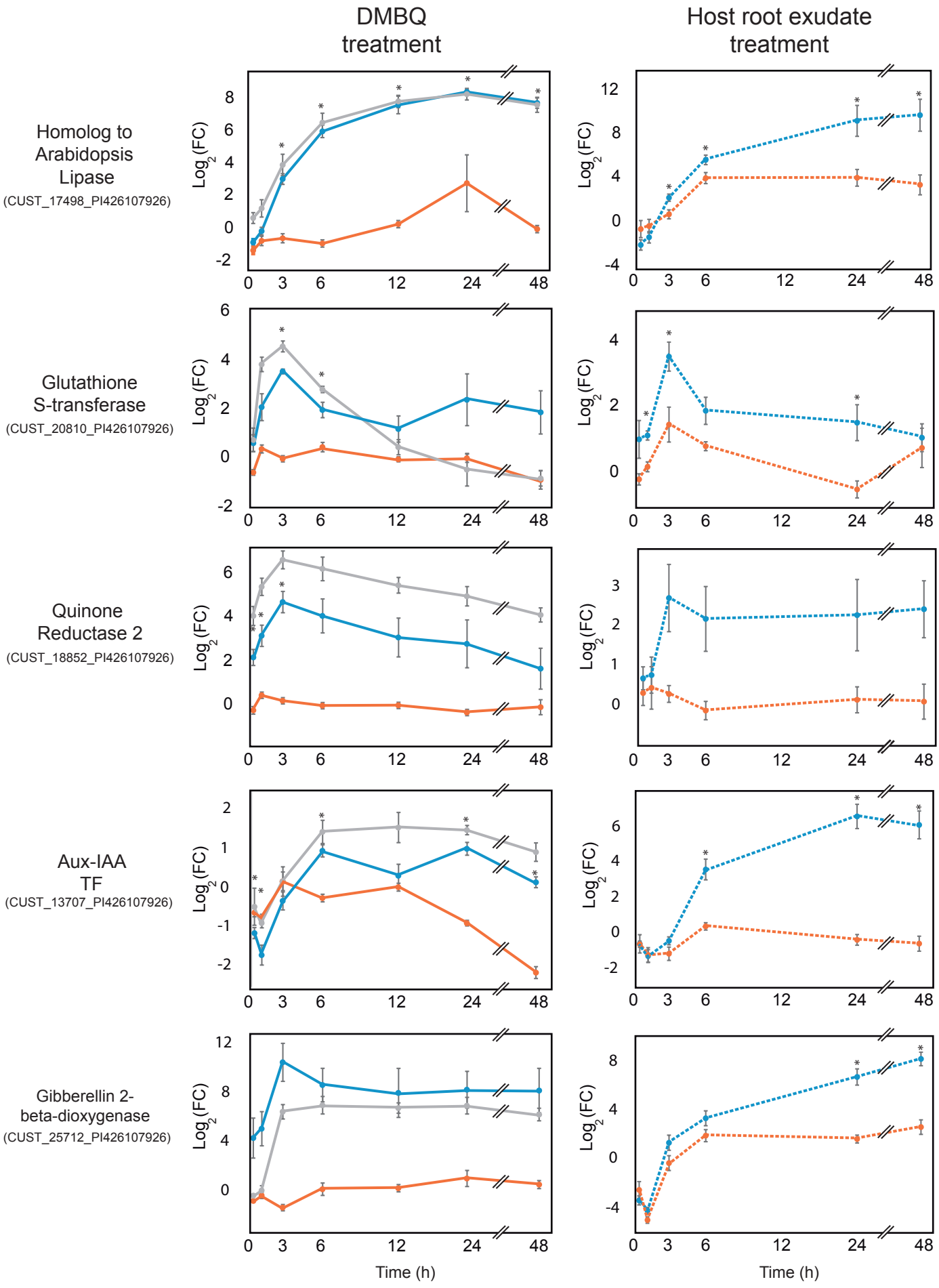
C Host: *Lotus japonicus*

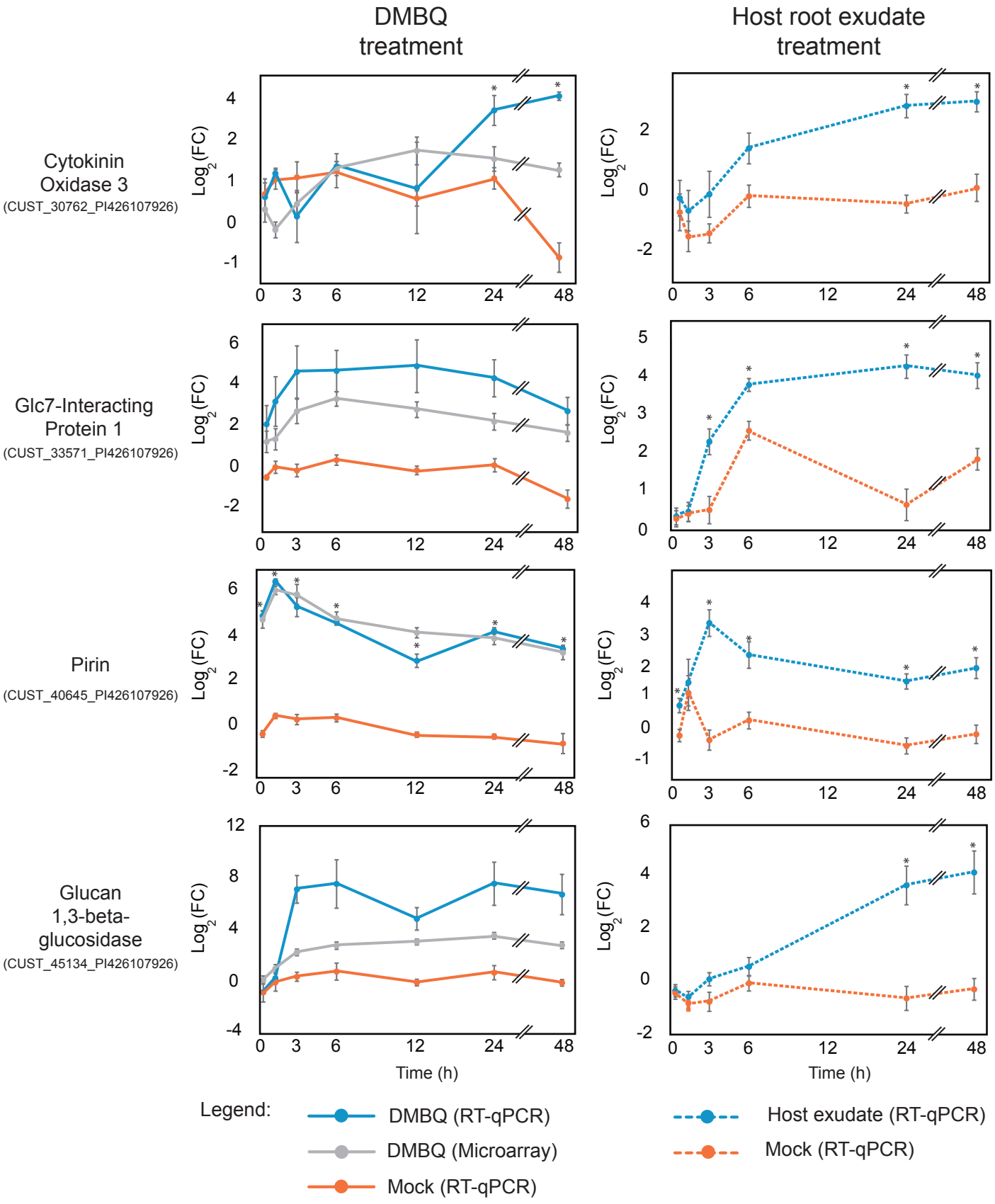


Supplemental Figure 1. Host specificity of the parasitic plant *P. japonicum*. (A - C) Interaction of *P. japonicum* with maize (A), cowpea (B) and *Lotus japonicus* (C) roots. On the right are shown the respective double-stained cross sections of haustoria embedded with Tecnovit 7100 solution stained with Safranin O and Fast Green dyes. Note that *L. japonicus* is a nonhost for *P. japonicum*. Black arrows point to the interface between the host and parasite. Asterisks mark the haustoria, pa: parasite root and ho: putative host root. Bars on the left photographs correspond to 200 µm and bars on the right cross sections correspond to 50 µm.

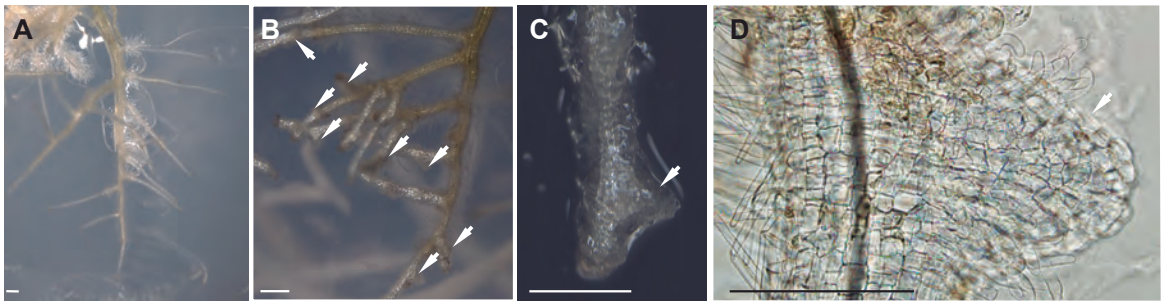


Supplemental Figure 2. *P. japonicum* grown in rhizotron with or without rice hosts. *P. japonicum* plants were grown in rhizotron chamber without (left) or with (right) rice hosts. Parasites are labeled with pa and hosts are labeled with white arrowheads in the right panel. Bar: 1 cm.

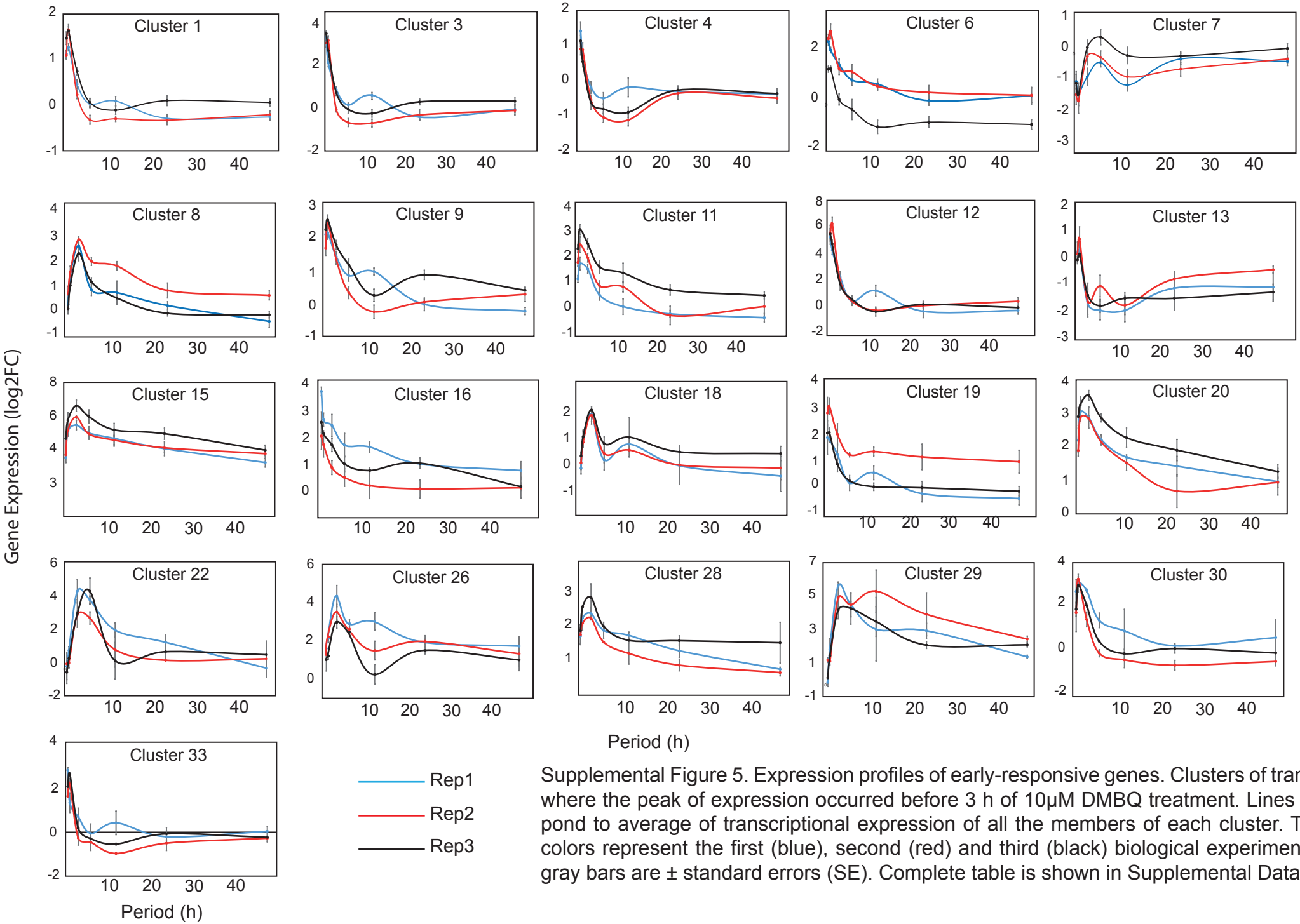

DMBQ treatment
Host root exudate treatment
Homolog to Arabidopsis Lipase
(CUST_17498_PI426107926)
Glutathione S-transferase
(CUST_20810_PI426107926)
Quinone Reductase 2
(CUST_18852_PI426107926)
Aux-IAA TF
(CUST_13707_PI426107926)
Gibberellin 2-beta-dioxygenase
(CUST_25712_PI426107926)
Log2 (FC)
Time (h)


DMBQ treatment
Host root exudate treatment
Cytokinin Oxidase 3
(CUST_30762_PI426107926)
Glc7-Interacting Protein 1
(CUST_33571_PI426107926)
Pirin
(CUST_40645_PI426107926)
Glucan 1,3-beta-glucosidase
(CUST_45134_PI426107926)
Log2 (FC)
Time (h)
Legend:
DMBQ (RT-qPCR)
DMBQ (Microarray)
Mock (RT-qPCR)
Host exudate (RT-qPCR)
Mock (RT-qPCR)

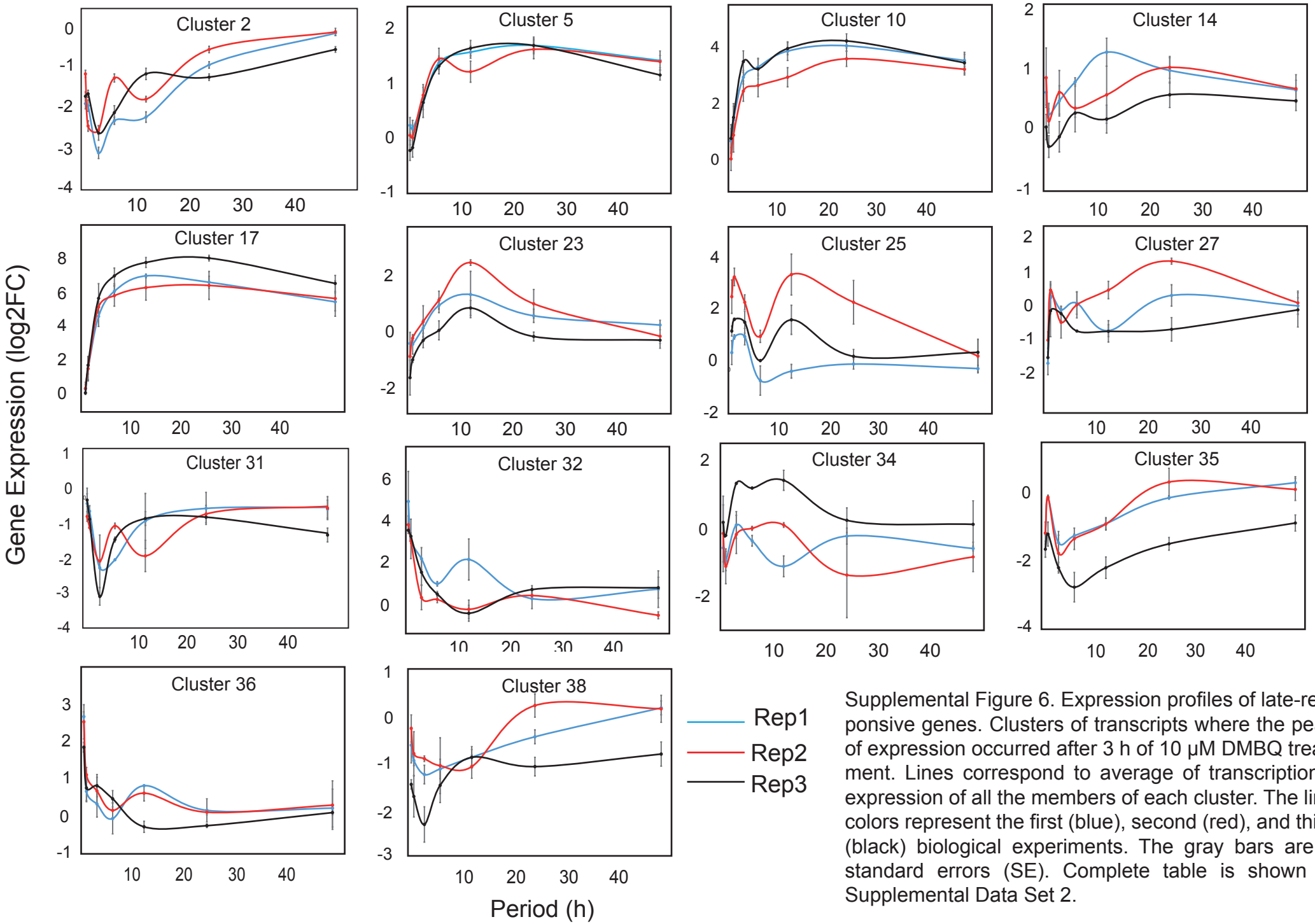
Supplemental Figure 3. RT-qPCR validation of the expression profiles of selected genes. Fold--induction of gene expression after DMBQ (solid orange lines) or mock (solid blue lines on the left column) treatment. For comparison, microarray data are shown in the graphs of DMBQ-treated roots (solid gray lines). Host rice root exudate (dashed orange lines) or mock (dashed blue lines) treatments. Data are shown as log2 fold-induction of treated roots compared to non-treated roots at 30 min, 1 h, 3 h, 6 h, 24 h and 48 h of treatment period. Two-week-old *P. japonicum* plants were grown vertically to prevent the roots from entering the agar media, thereby forcing the roots to be in contact with 10 µM DMBQ solution (on the left) or with rice root exudate (on the right). The values are the average of at least two biological replicates with at least two technical replicates. Error bars indicate ± standard errors (SE). Each experiment used pooled RNA extracted from the roots of total 20-30 plants. Asterisks indicate significant differences (p value<0.05) compared to mock-treatment in RT-qPCR experiments. Primers specific for the reference *Pj-PTB* gene were used for normalization.



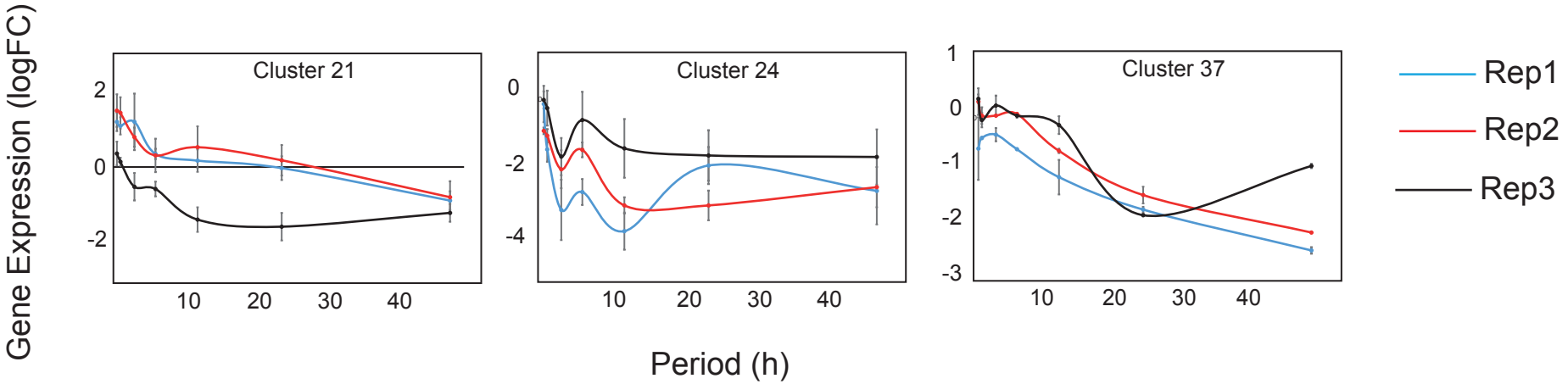
Supplemental Figure 4. *P. japonicum* roots at 48 h after rice root exudate treatment. (A) Non-transformed roots in normal growth conditions and (B) non-transformed roots treated 48 h with rice root exudate. (C-D) Magnification of host-induced haustoria in non-transformed roots. Root tissues were cleared with chloral hydrate and observed under bright-field microscope (D). The black bar corresponds to 500 µm and white bars correspond 250 µm. White arrows indicate haustoria.



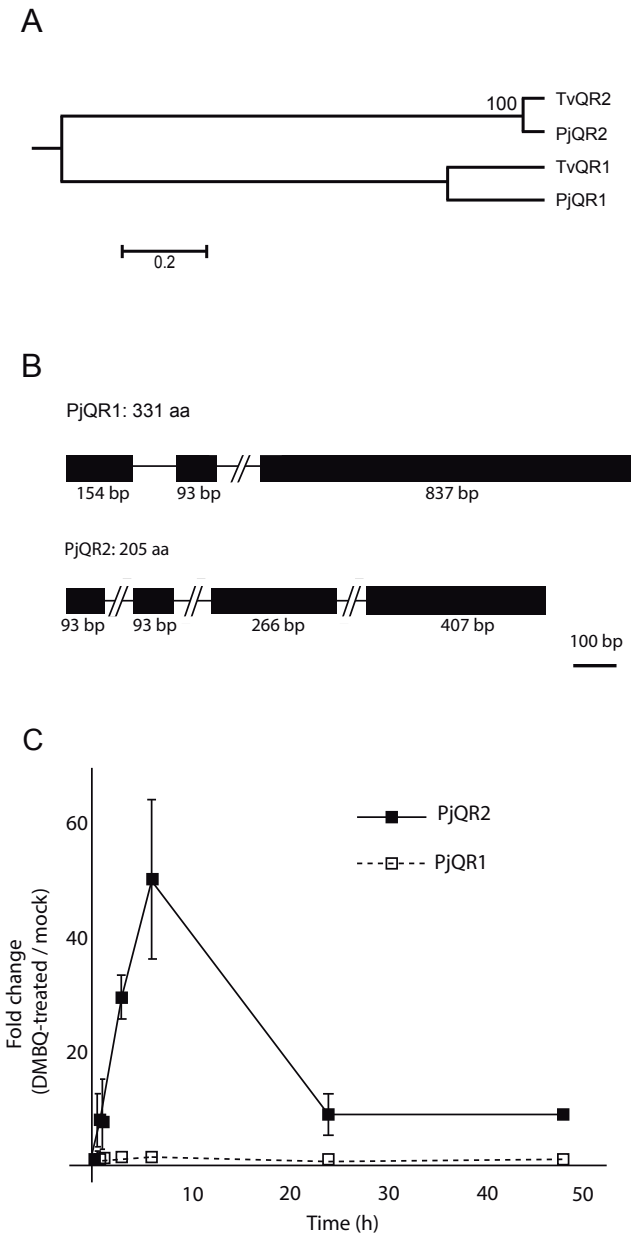
Supplemental Figure 5. Expression profiles of early-responsive genes. Clusters of transcripts where the peak of expression occurred before 3 h of 10μM DMBQ treatment. Lines correspond to average of transcriptional expression of all the members of each cluster. The line colors represent the first (blue), second (red) and third (black) biological experiments. The gray bars are ± standard errors (SE). Complete table is shown in Supplemental Data Set 2.



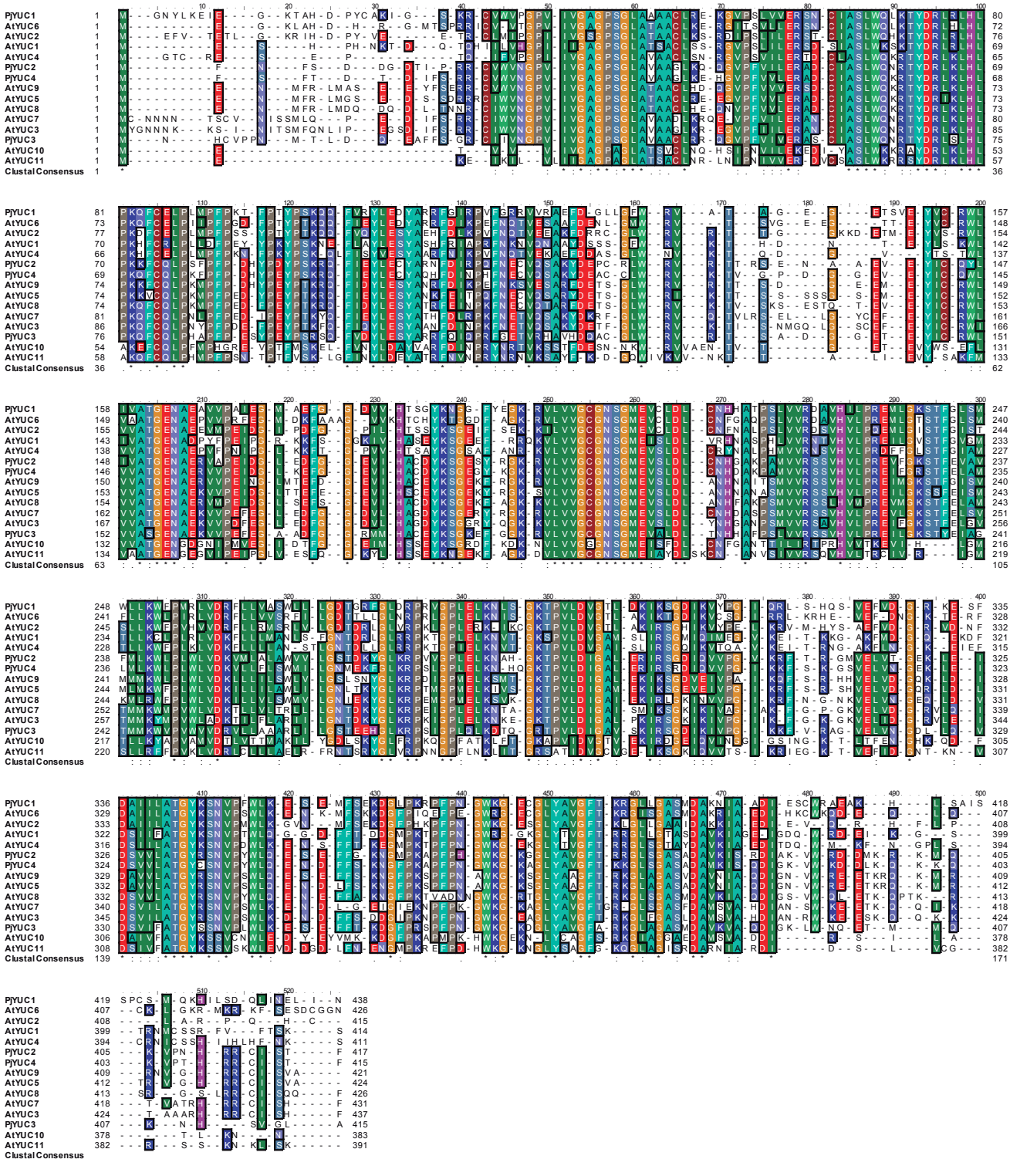
Supplemental Figure 6. Expression profiles of late-responsive genes. Clusters of transcripts where the peak of expression occurred after 3 h of 10 µM DMBQ treatment. Lines correspond to average of transcriptional expression of all the members of each cluster. The line colors represent the first (blue), second (red), and third (black) biological experiments. The gray bars are ± standard errors (SE). Complete table is shown in Supplemental Data Set 2.



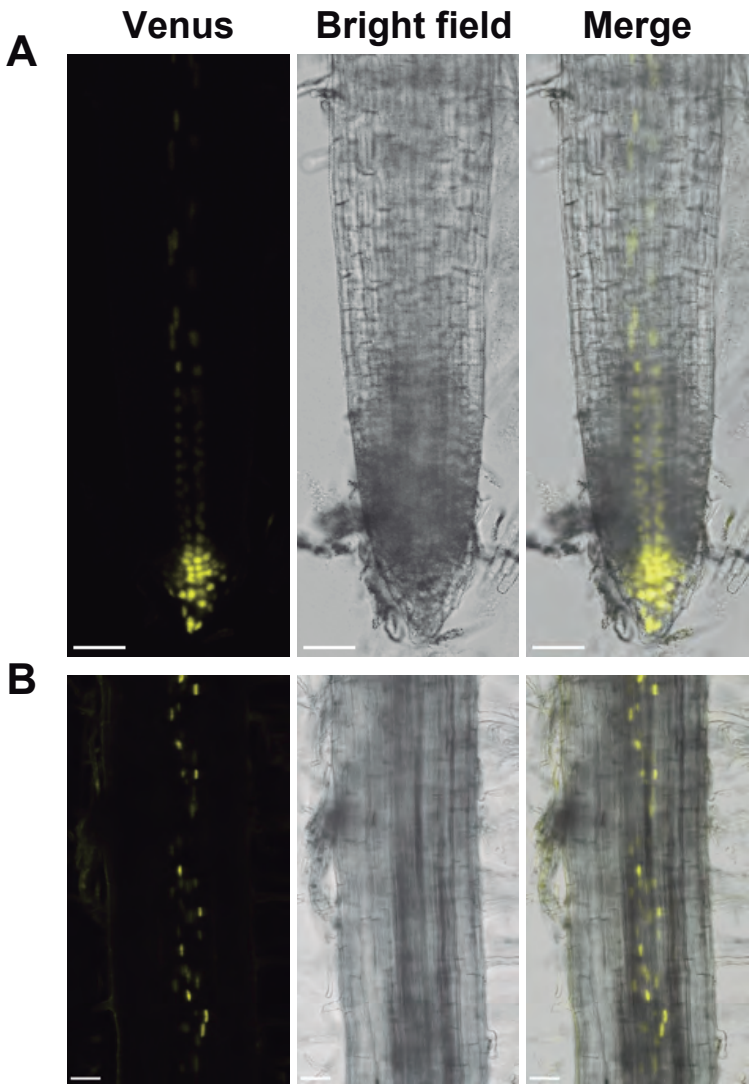
Supplemental Figure 7. Expression profiles of down-regulated genes. Lines correspond to average of transcriptional expression of all the members of each cluster. The line colors represent the first (blue), second (red), and third (black) biological experiments. The gray bars are ± standard errors (SE). Complete table is shown in Supplemental Data Set 2.



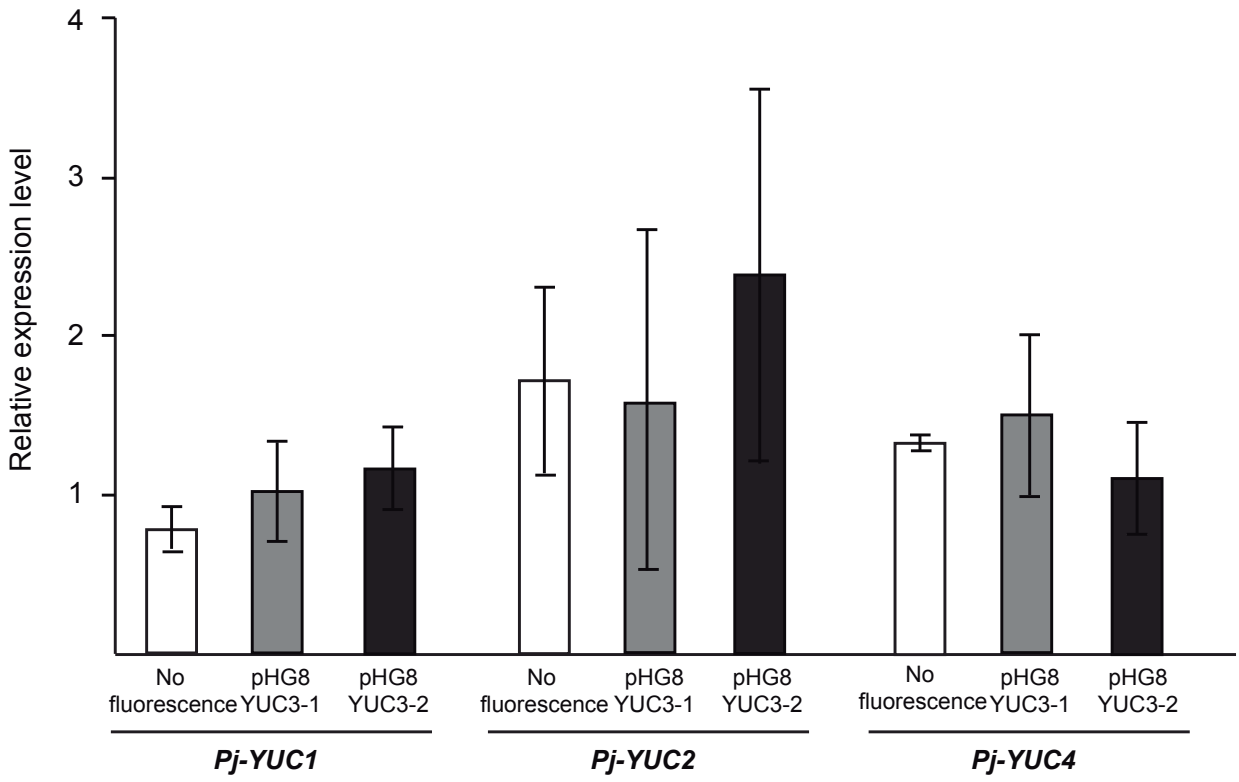
Supplemental Figure 8. *QR2* rather than *QR1* is differentially regulated during parasitism. (A) The UPGMA phylogenetic tree based on full-length protein alignment of Pj-QR1, Pj-QR2 and their homologs in *T. vesicolor*, Tv-QR1 and Tv-QR2. Bootstrap values are shown in percentage at the internal nodes. (B) Schematic view of the *Pj-QR1* and *Pj-QR2* genes, black boxes represent exons and lines are introns. The bar corresponds to 100 bp. (C) Relative expression levels of *QR1* and *QR2* measured by RT-qPCR. Values correspond to the ratio between the expression of 10 µM DMBQ-treated roots compared to mock-treated roots (0.1%(v/v) DMSO) sampled at 0.5 h, 1 h, 3 h, 6 h, 24 h and 48 h after treatment. Data represent the average ± standard errors (SE) of three independent biological replicates .



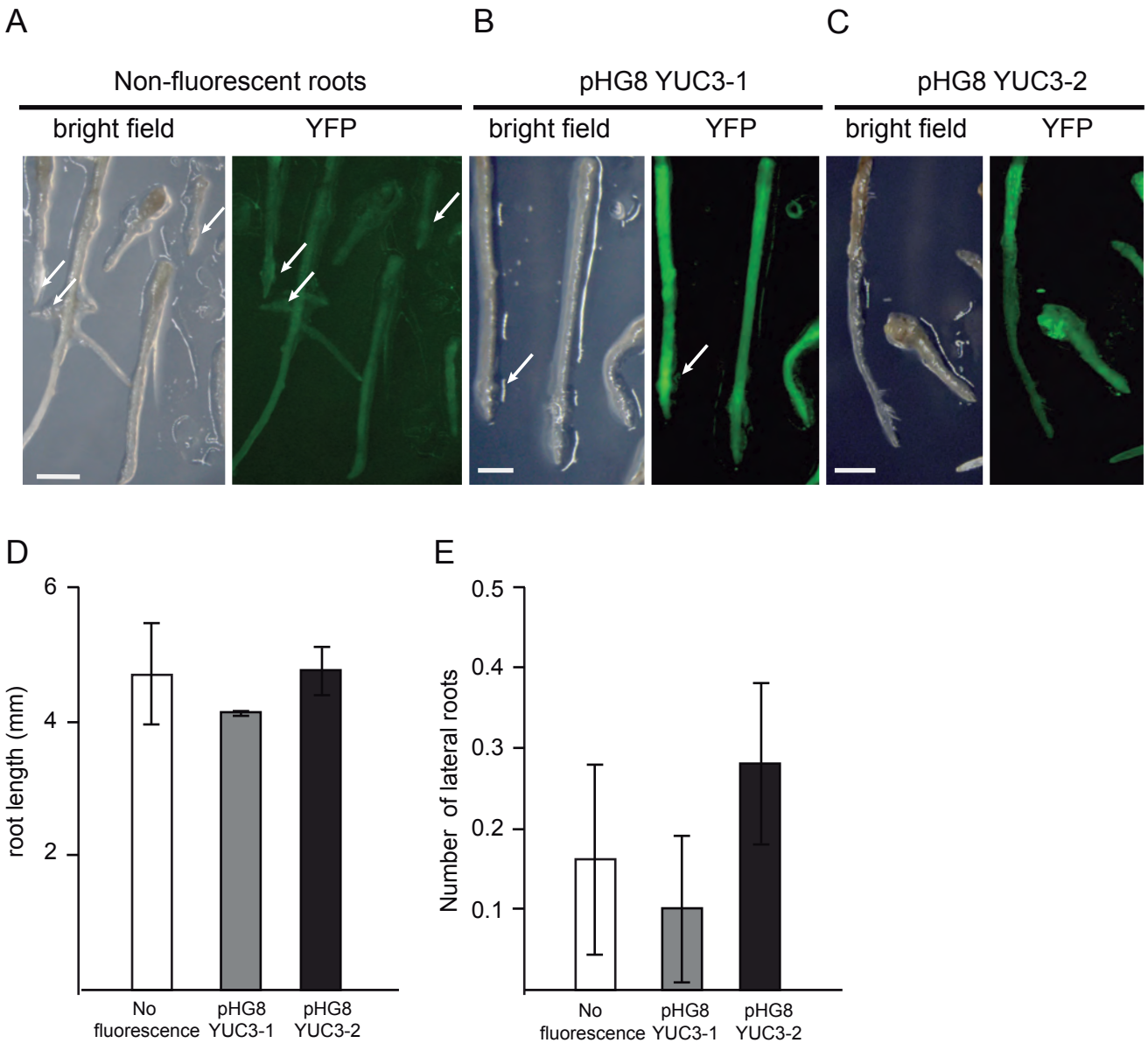
Supplemental Figure 9. Alignment of Pj-YUCs and At-YUCs amino acid sequences. The sequences were aligned using CLC Genomics Workbench (v4.8) and the figure was generated by BioEdit. Residues are background-shaded according to their identity or similarity scores calculated from protein similarity matrix BLOSUM62 with the default cut-off value in BioEdit software. Amino acids with similar property are shaded with the same color.



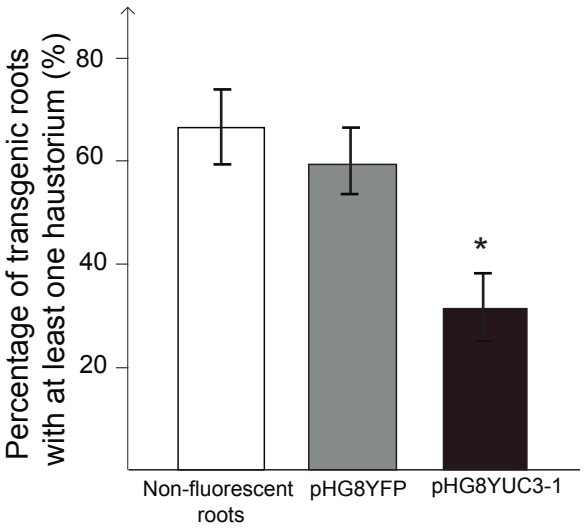
Supplemental Figure 10. Expression pattern of DR5 promoter in *P. japonicum* roots. Confocal images of transgenic hairy roots carrying *pDR5rev:3xVenus-N7*. (A) at root apical meristem. (B) mature zone. Bars correspond to 50 μm.



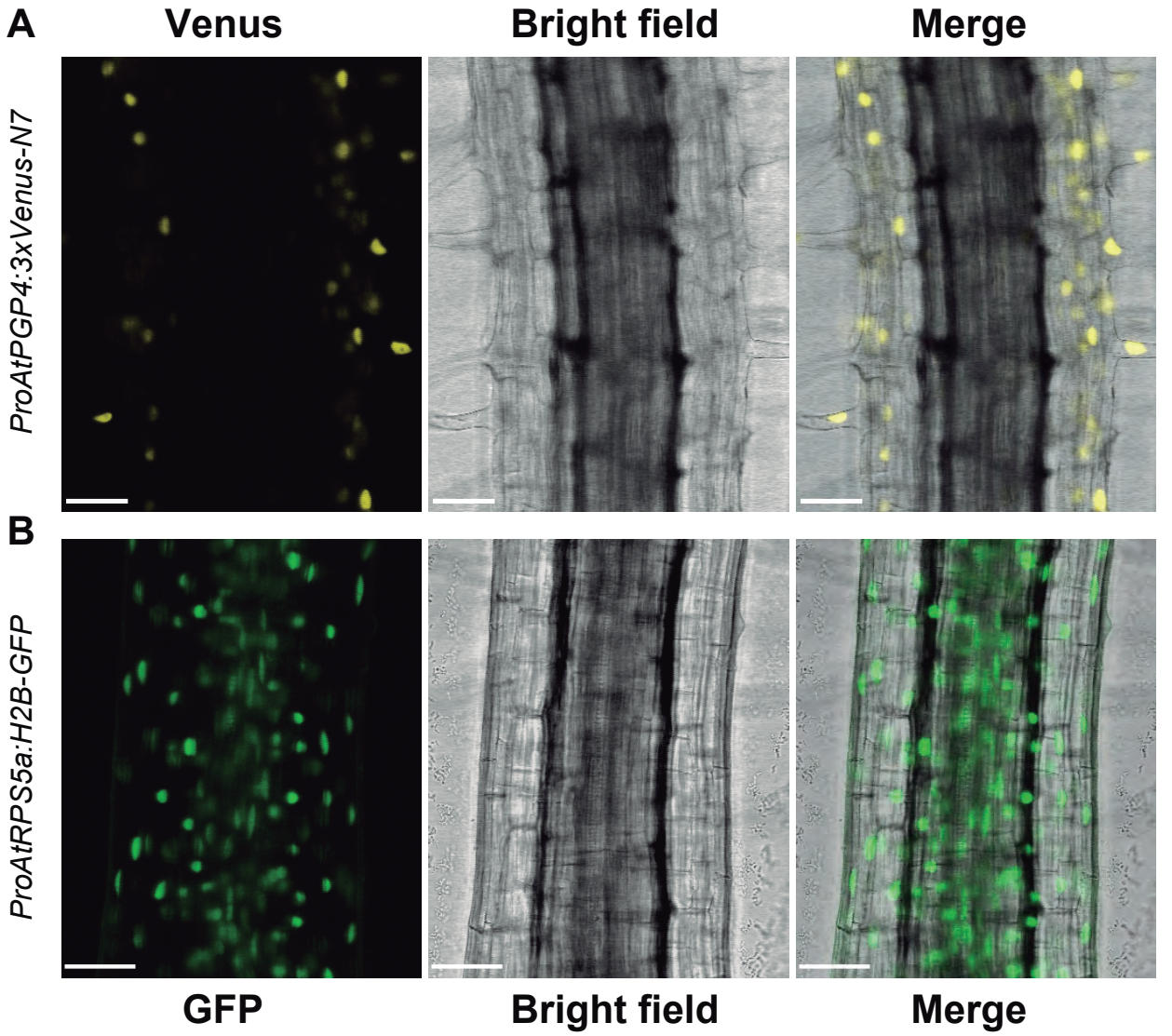
Supplemental Figure 11. Transcript levels of *YUC* homologs in transgenic roots. Transcript levels were measured by RT-qPCR in transgenic roots transformed with non-fluorescent hairy roots, pHG8-YUC3-1 and pHG8YUC3-2 after 48 h in contact with host *Arabidopsis* roots using primer pairs specific for the *Pj-YUC1*, *Pj-YUC2* and *Pj-YUC4* genes. Data correspond to the average ± standard error of three to five biological replicates with 5-15 independent transgenic roots per experiment.

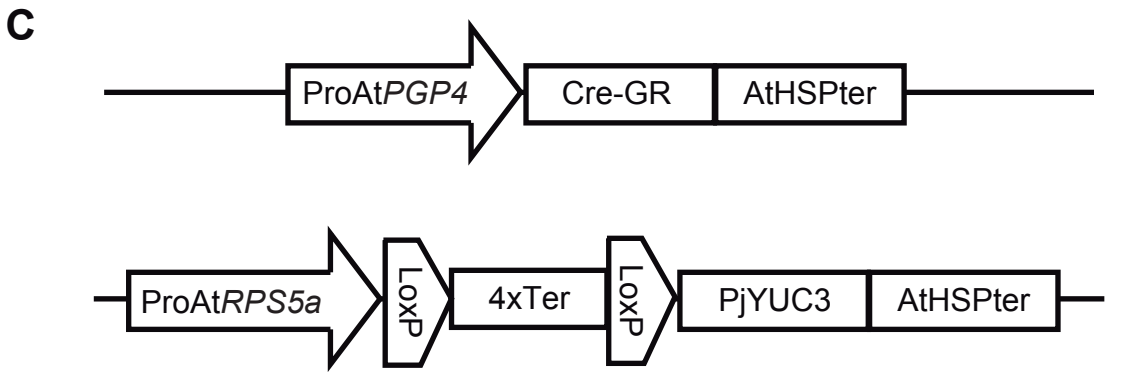


Supplemental Figure 12. Morphology of transformed hairy roots. (A - C) Photographs of transgenic roots transformed with the control (A) or silencing constructs (B, C) after 48 h of DMBQ treatment, under bright field (left) and fluorescence (right). Arrows point to haustoria. Bars correspond to 1 cm. (D) Root length of transgenic roots. (E) Number of lateral roots per transgenic root. Data correspond to the average ± standard errorof three to five biological replicates with 5-15 independent transgenic roots per experiment.



Supplemental Figure 13. RNAi lines of *P. japonicum* roots targeted to *YUC3* show reduced haustorium formation induced by rice root exudates. Graph shows the percentage of transgenetic roots with at least one haustorium. Roots transformed with empty vector pHG8-YFP and no-fluorescent hairy roots were used as control. Non-fluorescent roots represent those transgenetic hairy roots without the T-DNA containing the fluorescent protein gene. Root silenced for Pj-*YUC3* gene carries the construct pHG8YUC3-1. Asterisk (*) represent $\alpha = 0.01$ by t-Test assuming unequal variances. Values represent average ± SD of three to six independent experiments with 10 to 40 transformed roots per experiment. Total n = 99, 90 and 89 in no fluorescent roots, pHG8-YFP and pHG8YUC3-1, respectively.





Supplemental Figure 14. Expression patterns of *At-PGP4* promoter and *At-RPS5a* promoter in *P. japonicum*. (A) At-*PGP4* promoter expression in *P. japonicum* root transformed with *AtPGP4:3x-Venus-N7*. (B) At-*RPS5a* promoter expression in *P. japonicum* root transformed with *AtRPS5a: :H2B-GFP*. Bars correspond to 50 μm. (C) Schematic view of AtPGP4>>PjYUC3 construct.

Supplemental Table 1 - Total number of reads obtained in each RNA-seq library

	Sequencer	Material	Read	Total sequence
Library 1	Illumina Hi-seq2000	Autotrophic Stage	24,537,964 reads (90 bp reads)	2,208,416,760 bp
Library 2	Illumina Hi-seq2000	Haustoria	25,841,404 reads (90 bp reads)	2,325,726,360 bp
Library 3	Roche 454 FLX	Mixed	888,638 reads (average 333.8 bp)	296,626,260 bp

Supplemental Table 2. Investigation on the rice contamination in *P. japonicum* unigenes

e-value threshold	Number of contigs matched with rice sequences*	Minimum alignment length
1e-10	238	37 bp
1e-20	198	54 bp
1e-30	176	73 bp
1e-40	160	92 bp

*Number of *P. japonicum* unigenes that show local alignment in the indicated e-value and 100% identity with rice cDNAs (ver 6.1)

Supplemental Table 3: Summary of *de novo* assembly of *P. japonicum* transcriptome

	Values
Number of contigs	57,939
Total residues (bp)	47,011,167
Minimum legth (bp)	300
Maximum length (bp)	12,710
Average length (bp)	812
N50 length (bp)	1,090
Putative protein coding[a]	47,817 (82.5%)
Putative proteins with start codon	17,044 (35.6 % of predicted proteins)
Blast hits to *Arabidopsis*[b]	34,897 (60.23%)
Blast hits RefSeq-prot[b]	38,372 (66.23%)
Blast hits to nr[b]	37,259 (64,3%)

[a] Number of ESTScan-predicted protein sequences more than 50 aa
[b] Blastx with threshold e-value less than 1e-10.