Supporting Information

Hacquard et al. 10.1073/pnas.1306807110

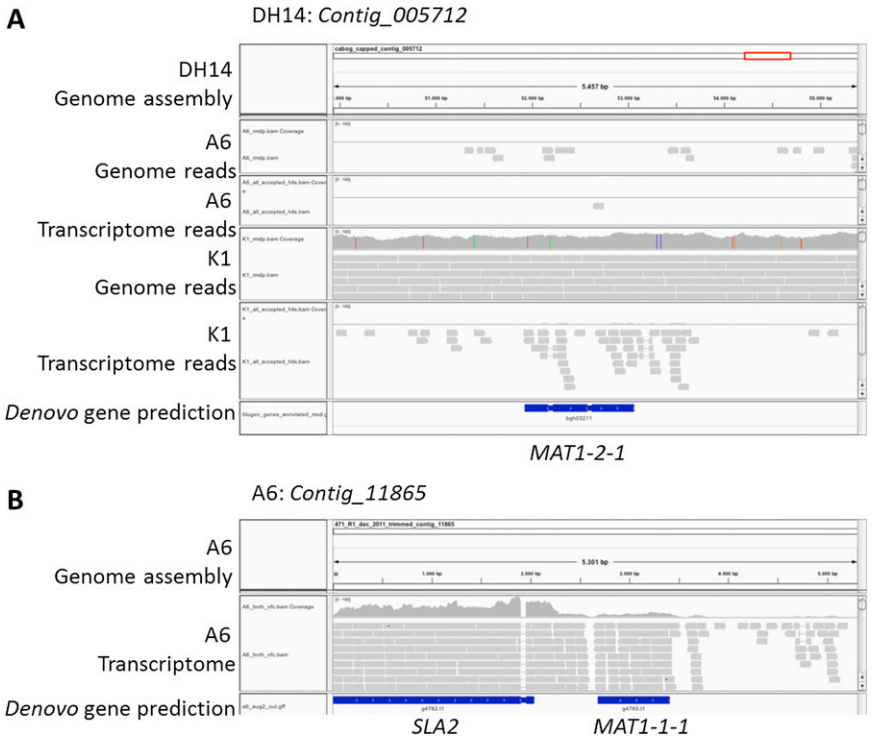
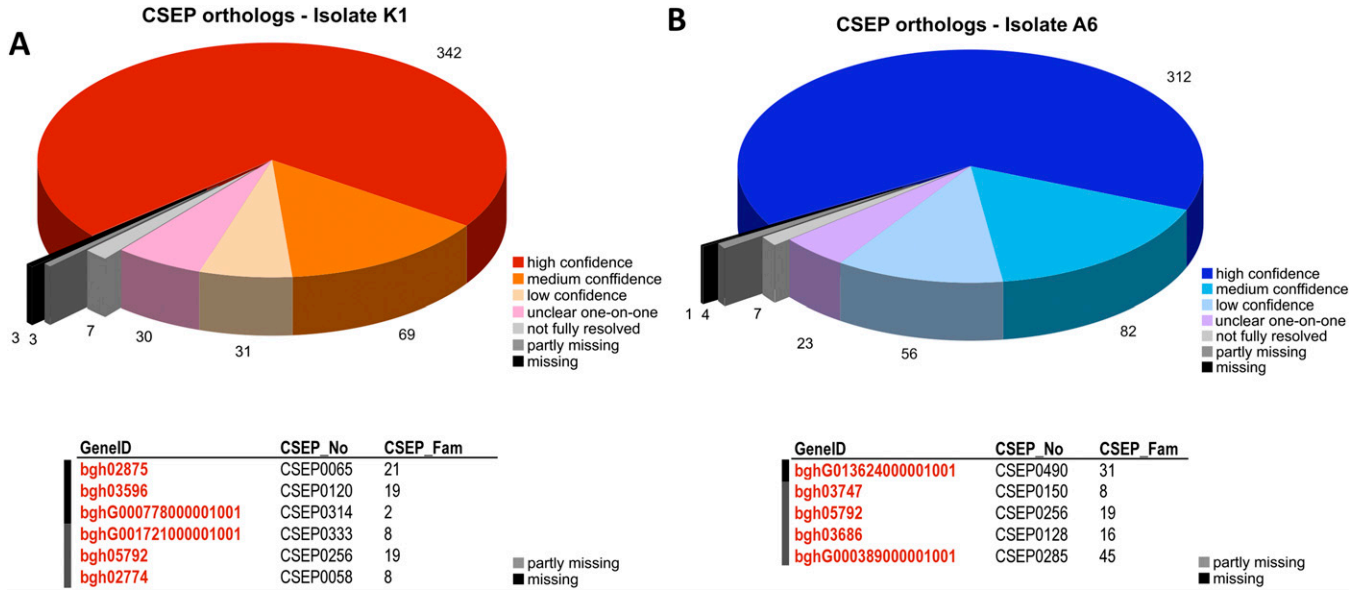


Fig. S1. Visualization of genome and transcriptome sequencing read coverage of the DH14 reference isolate contig that contains the *MAT-1–2-1* mating-type gene. (*A*) Genome and transcriptome reads of isolate A6 and K1 mapped to the contig of the reference genome *Bgh* DH14 that contains *MAT1-2–1*. (*B*) Genome and transcriptome reads of isolate A6 mapped to the genome contig of isolate A6 which contains *MAT1-1–1*. Visualization was done via the Integrative Genomics Viewer (www.broadinstitute.org/igv).



GeneID	CSEP_No	CSEP_Fam
bgh02875	CSEP0065	21
bgh03596	CSEP0120	19
bghG000778000001001	CSEP0314	2
bghG001721000001001	CSEP0333	8
bgh05792	CSEP0256	19
bgh02774	CSEP0058	8

partly missing
missing

GeneID	CSEP_No	CSEP_Fam
bghG013624000001001	CSEP0490	31
bgh03747	CSEP0150	8
bgh05792	CSEP0256	19
bgh03686	CSEP0128	16
bghG000389000001001	CSEP0285	45

partly missing
missing

Fig. S2. Identification of orthologous candidate-secreted effector proteins (CSEP) genes between *Blumeria graminis* f. sp. *hordei* (*Bgh*) isolates. (*A*) Summary of DH14 *CSEP* orthologs present or absent in *Bgh* isolate K1. (*B*) Summary of DH14 *CSEP* orthologs present or absent in *Bgh* isolate A6. The pie charts show the amount of *CSEPs* present in the *Bgh* isolate DH14 for which an ortholog could or could not be identified in the respective isolate. The tables below give the IDs of those *CSEPs* that are (partly) missing in the respective isolate. "High confidence" indicates that an ortholog was found with at least two of the used tools (gmap threshold: ≥99% similarity and coverage); "medium confidence" that an ortholog was found with one of the three tools (gmap threshold: ≥99% similarity and coverage); "low confidence" that an ortholog was found only with gmap and at a lower threshold (between 90% and 99% coverage/similarity) and/or verified by manual inspection of the A6/K1 genome-sequencing read alignment to the DH14 genome; "unclear one-on-one" that, due to the presence of close paralogs in DH14, the exact ortholog relation is not fully resolvable; "not fully resolved" that no ortholog was found with any tool (also for lower gmap threshold) and that alignment of A6/K1 genome-sequencing reads shows gaps; "partly missing" that no ortholog was found with any tool and only a small part of the DH14 gene model is covered by A6/K1 genome-sequencing reads; "missing" that no ortholog was found with any tool and that the DH14 gene model is not covered by any A6/K1 genome-sequencing reads.

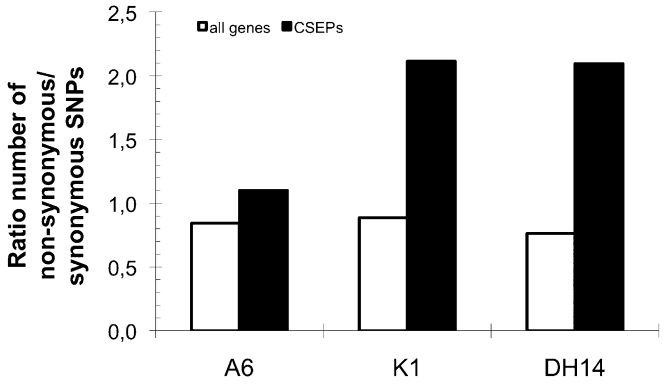


Fig. S3. Total ratio of nonsynonymous substitutions vs. synonymous substitutions for genes encoding CSEPs compared with the complete gene repertoire in each of the three isolates.

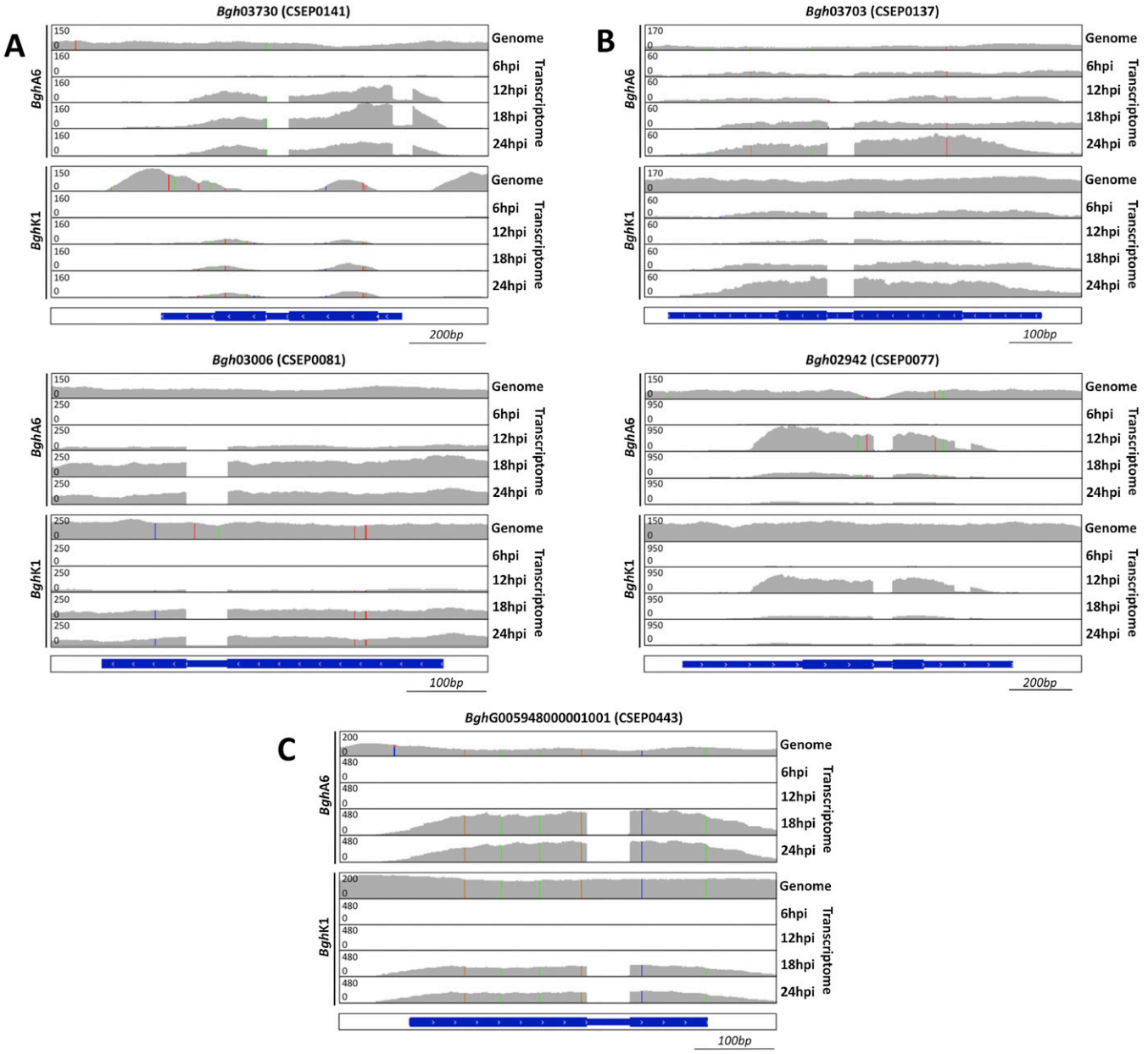


Fig. S4. Visualization of genome and transcriptome sequencing read coverage and SNP locations for five plant induced powdery mildew genes encoding fast-evolving candidate secreted effectors. The alignment of the sequenced reads of *B. graminis* f. sp. *hordei* isolates A6 and K1 to the DH14 isolate reference genome and the positions of observed SNPs were visualized using the Integrative Genomics Viewer (www.broadinstitute.org/igv). For each isolate, the first lane corresponds to the genome sequencing coverage. The four lanes below reflect transcript accumulation during powdery mildew infection on *Arabidopsis* at intervals corresponding to conidiospore germination [6 h postinoculation (hpi)], penetration of the plant epidermis (12 hpi), haustorial initials (18 hpi), and mature haustoria (24 hpi). In the last lane, the predicted DH14 gene model (with or without UTR extremities) is indicated by blue boxes. *CSEP* genes were selected based on Fig. 2*B*. (*A*) *CSEP0141* and *CSEP0081* accumulate SNPs only in *Bgh* isolate K1. (*B*) *CSEP0137* and *CSEP0077* accumulate SNPs only in *Bgh* isolate A6. (*C*) *CSEP0441* accumulates SNPs in both isolates A6 and K1, which means that the respective positions are specifically different in DH14 from the other two isolates.

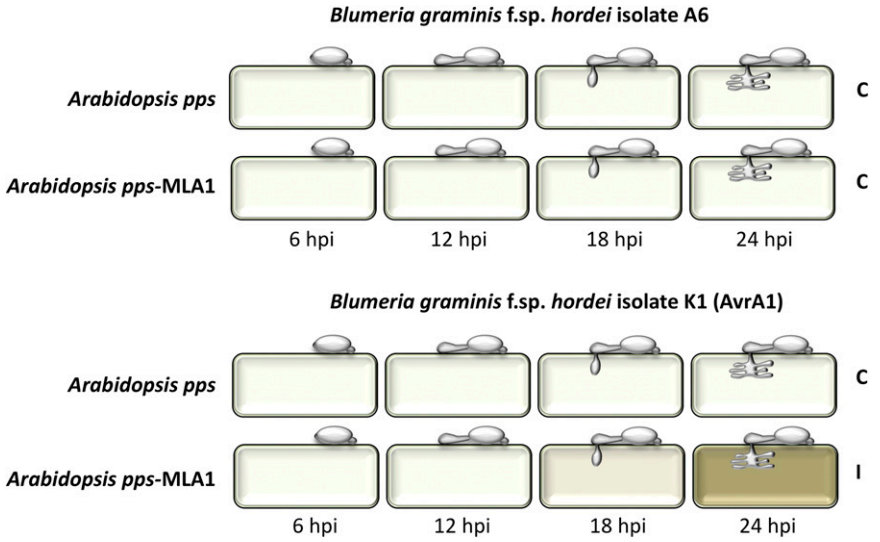


Fig. S5. Schematic diagram representing the four powdery mildew infection stages used for RNA sequencing during *Arabidopsis* infection. Partially immunocompromised *Arabidopsis* (*pps*: *pen2*, *pad4*, *sag101* background), on which *B. graminis* f. sp. *hordei* isolates A6 and K1 are able to grow and reproduce, was used as susceptible host (compatible interaction). *Arabidopsis pps*-expressing barley MLA1, which specifically recognizes *Bgh* isolate K1 (expressing the cognate AvrA1 effector), was used to monitor fungal gene expression during the incompatible interaction (1). Samples were harvested at intervals corresponding to conidiospore germination (6 hpi), penetration of the plant epidermis (12 hpi), haustorial initials (18 hpi), and mature haustoria (24 hpi). The brown cell indicates the hypersensitive response detected at 24 hpi in the incompatible interaction (1).

1. Maekawa T, Kracher B, Vernaldi S, Ver Loren van Themaat E, Schulze-Lefert P (2012) Conservation of NLR-triggered immunity across plant lineages. *Proc Natl Acad Sci USA* 109(49): 20119–20123.

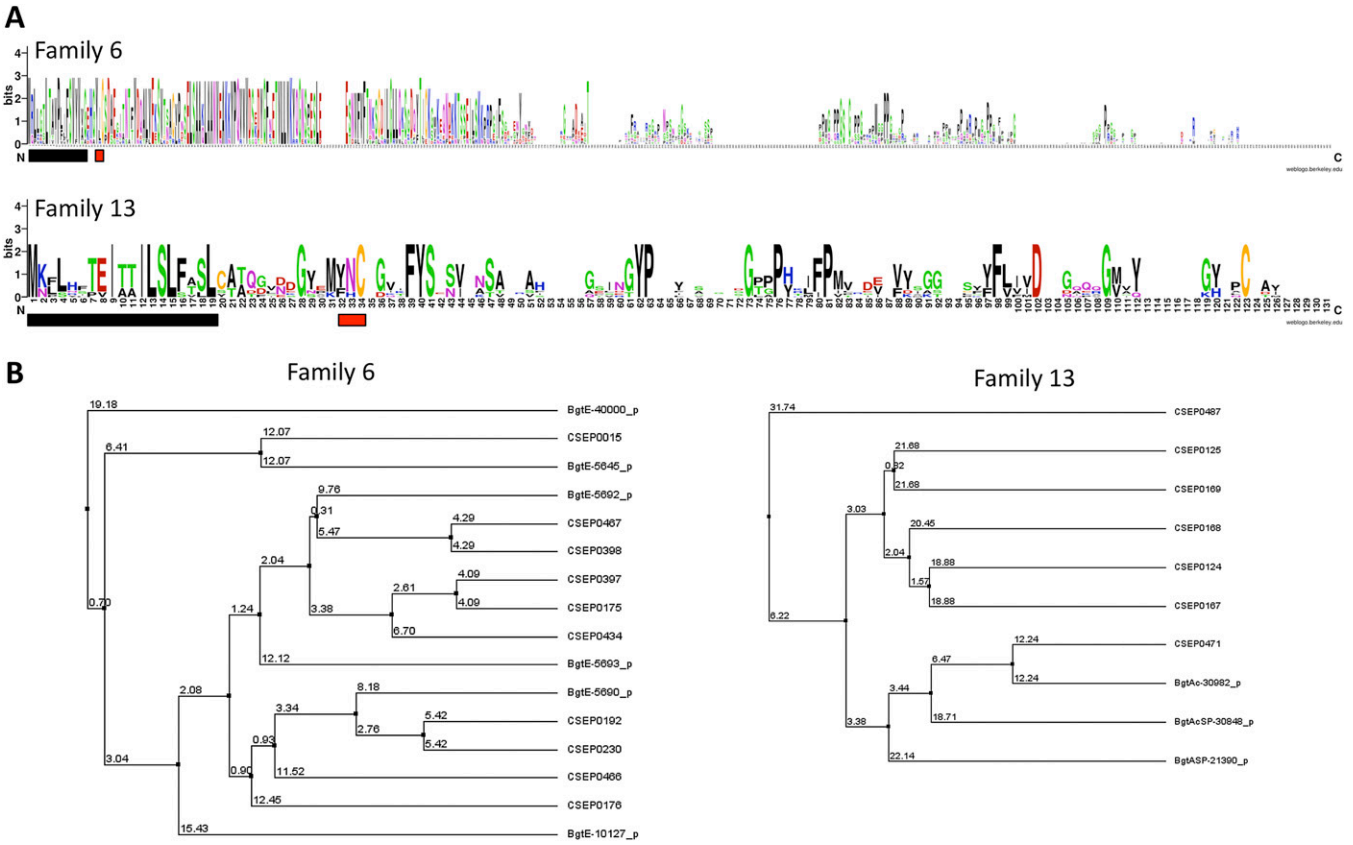


Fig. S6. Conservation profiles and phylogenetic analysis of CSEP families 6 and 13. (*A*) Conservation profiles of CSEP families 6 and 13. Secretion signals and YxC motifs (1) are highlighted with black and red bars, respectively. (*B*) Phylogenetic tree of *Bgh* and *Bgt* CSEP families 6 and 13 as identified with the Markov cluster (MCL) algorithm. The tree was generated via Jalview using clustalw for multiple alignment and percentage identity as average distance.

1. Godfrey D, et al. (2010) Powdery mildew fungal effector candidates share N-terminal Y/F/WxC-motif. *BMC Genomics* 11:317.

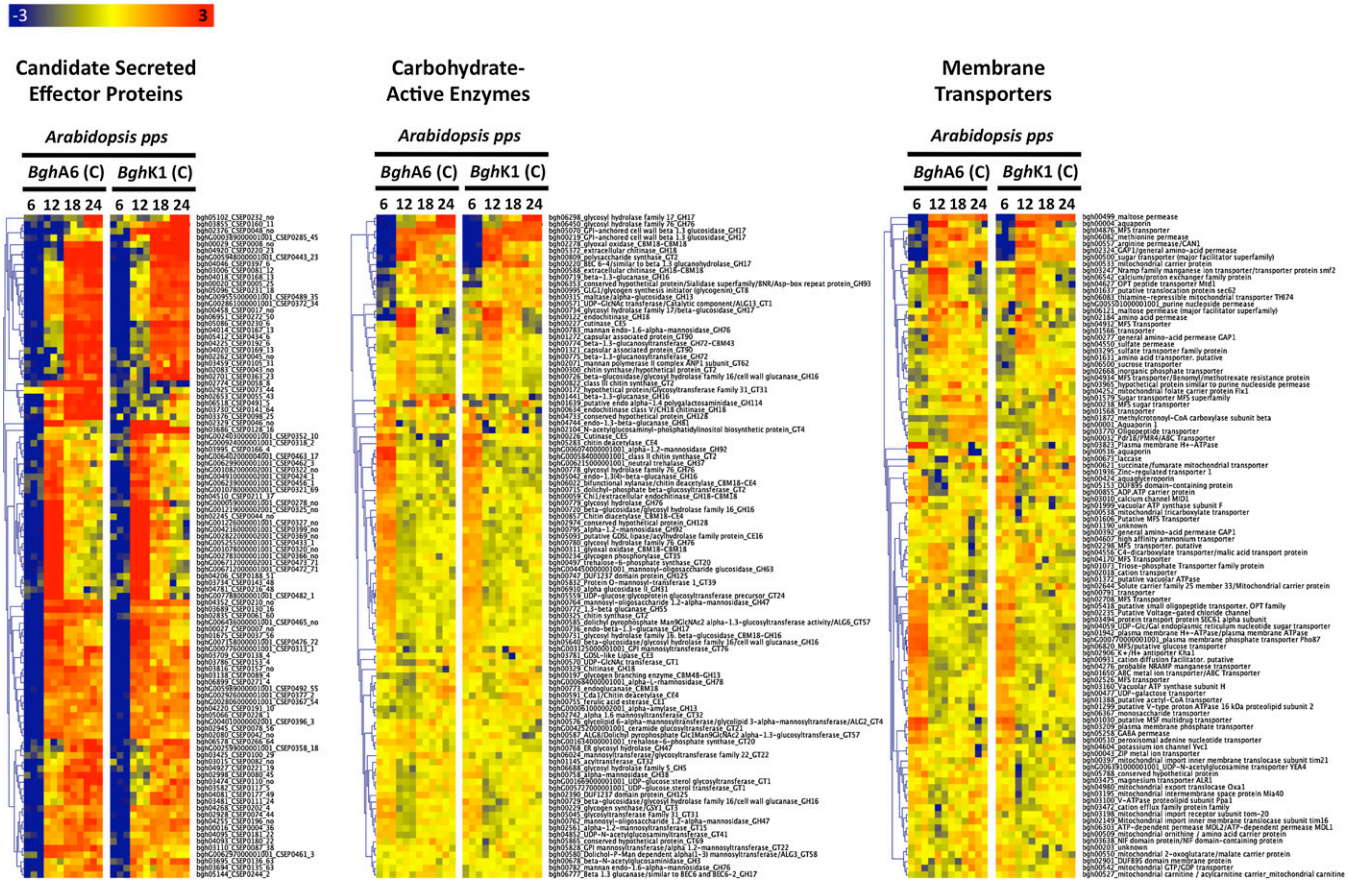


Fig. S7. Heatmaps of three pathogenicity-related gene categories in *B. graminis* f. sp. *hordei* isolates A6 and K1 during infection on *Arabidopsis*. For each category (CSEPs, carbohydrate active-enzymes, and membrane transporters), the 100 genes showing the highest Δ expression ratio levels during the compatible time-course infection on *Arabidopsis* leaves (*pps*: *pen2*, *pad4*, *sag101* background) are presented. Overrepresented (dark red) and underrepresented transcripts (dark blue) are depicted as log2-fold changes relative to the mean expression measured across all samples. Six hours postinoculation, conidiospore germination; 12 hpi, penetration of the plant epidermis; 18 hpi, haustorial initials; and 24 hpi, mature haustoria. For each gene, the identification number (www.blugen.org/) and the corresponding description are presented. CSEP identification numbers were retrieved from ref. 1, and CAZymes modules are described in the CAZy database (www.cazy.org).

1. Pedersen C, et al. (2012) Structure and evolution of barley powdery mildew effector candidates. *BMC Genomics* 13:694.

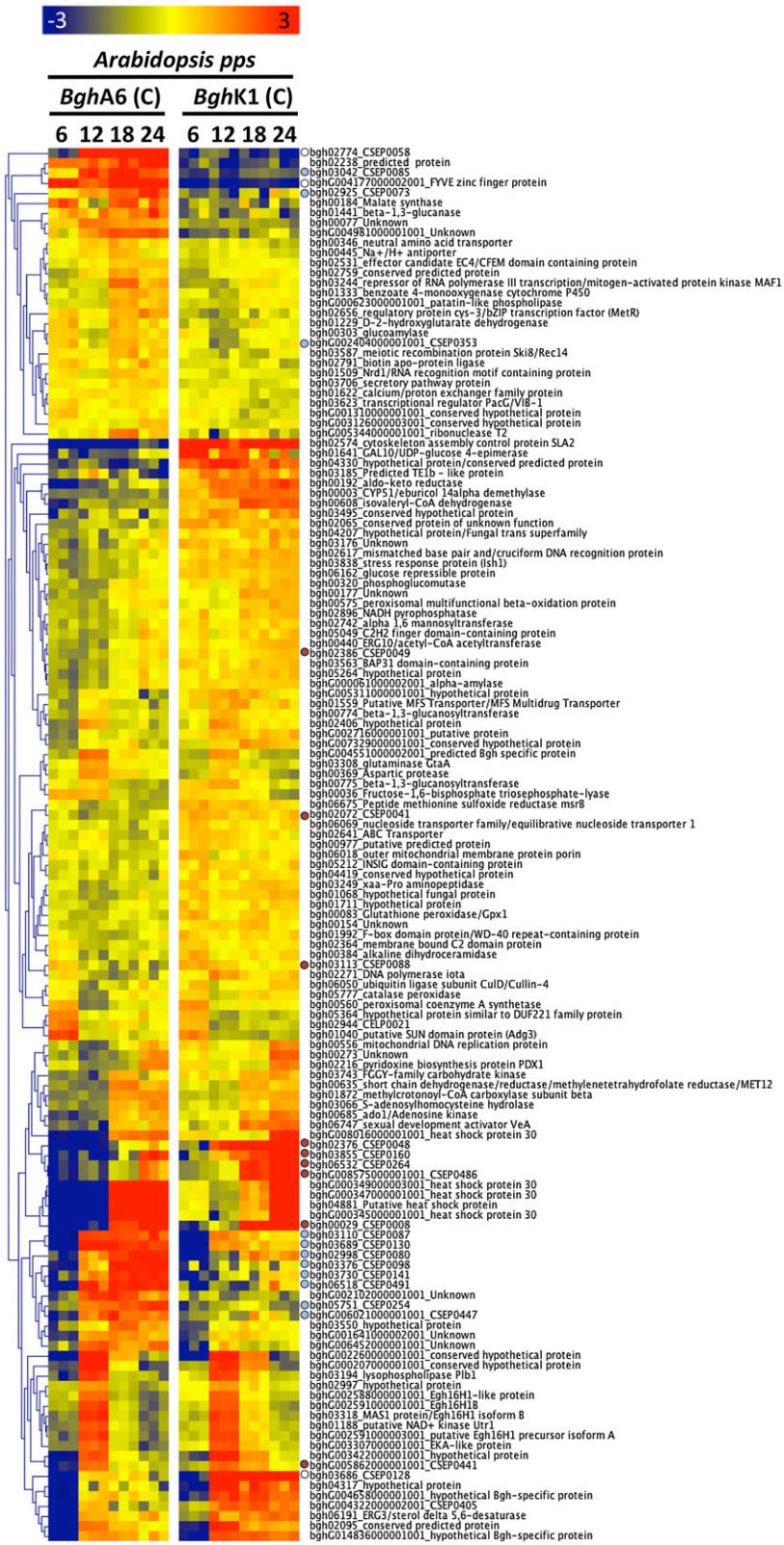


Fig. S8. Heatmap of *B. graminis* f. sp. *hordei* (*Bgh*) genes differentially expressed for at least three of the four analyzed time points between the two isolates A6 and K1 (fdr < 0.01). Partially immunocompromised *Arabidopsis* (*pps*: *pen2*, *pad4*, *sag101* background), on which *Bgh* isolates A6 and K1 are able to grow and reproduce, was used as susceptible host. *Arabidopsis pps* plants were inoculated with either *Bgh* isolate A6 or K1, and samples were harvested at time points corresponding to conidiospore germination (6 hpi), penetration of the plant epidermis (12 hpi), haustorial initials (18 hpi), and mature haustoria (24 hpi). White circles, genes with isolate-specific expression that were previously identified as missing (Table 1 and Fig. S2). Blue circles, *CSEPs* expressed in *Bgh* isolate A6 but not or weakly expressed in isolate K1. Red circles, *CSEPs* expressed in *Bgh* isolate K1 but not or weakly expressed in isolate A6.

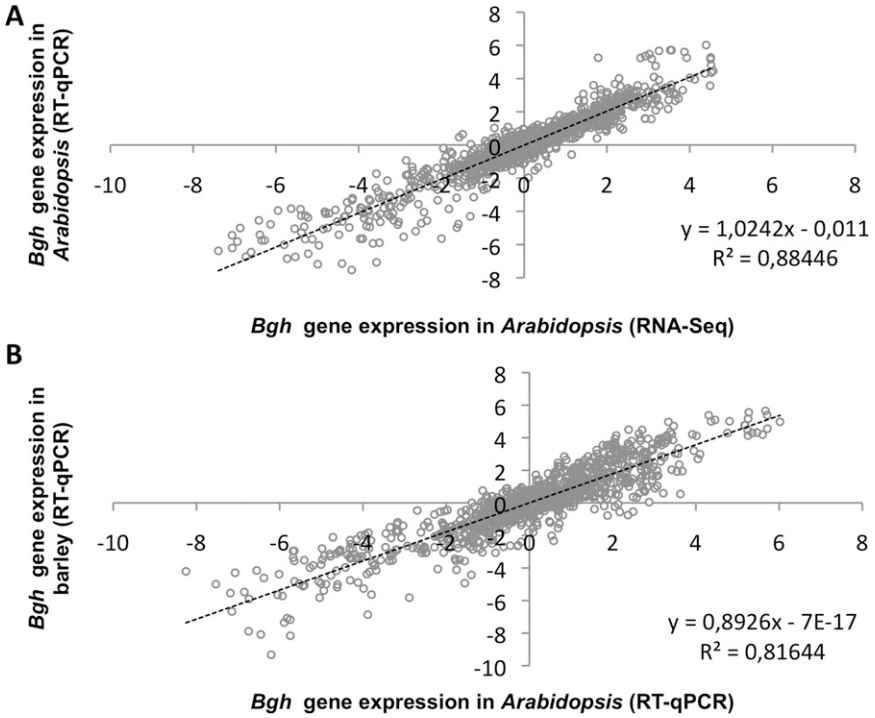


Fig. S9. Validation of RNA-seq data using RT-quantitative PCR (RT-qPCR) and analysis of the correlation between powdery mildew gene expression profiles measured during infection on *Arabidopsis* and barley host plants. A subset of 45 genes showing remarkable expression patterns based on RNA sequencing during powdery mildew infection on *Arabidopsis* was selected for RT-qPCR analysis. Gene expression levels were normalized using the two *B. graminis* f. sp. *hordei* (*Bgh*) reference genes actin and α-tubulin. (*A*) Correlation between *Bgh* gene expression levels measured by RT-qPCR and RNA sequencing during infection on the non host partially immunocompromised *Arabidopsis* (*pps*: *pen2*, *pad4*, *sag101* background). (*B*) Correlation between *Bgh* gene expression levels measured by RT-qPCR during infection on the non-host partially immunocompromised *Arabidopsis* and the natural host barley.