

Supplementary Figure 1

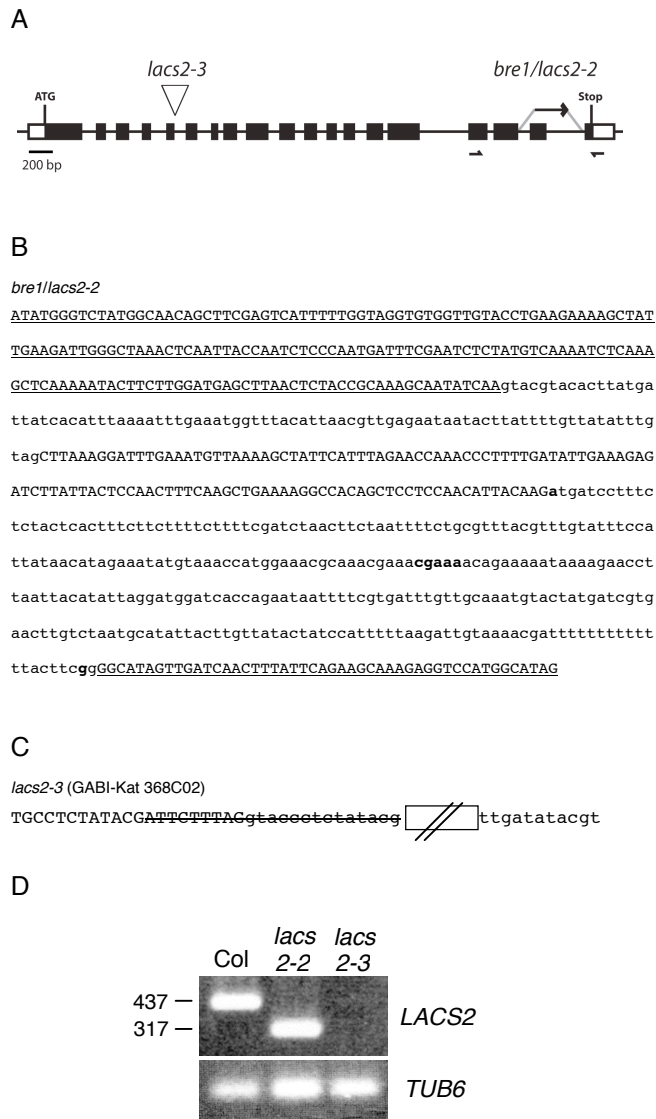
Complementation test between *bre1/lacs2-2* and *lacs2-3*



Calcofluor white-stained organs of F1 plants of a cross between *bre1/lacs2-2* and *lacs2-3*. Rosette leaf (left), young seedling (middle) and the tip of a flowering inflorescence (right) were analyzed.

Supplementary Figure 2

Characterization of the *lacs2-2* and *lacs2-3* mutations in the *LACS2* gene



(A) The *LACS2* gene structure and the mutation sites of the *lacs2* alleles. Black boxes represent exons, white boxes represent untranslated regions, and a black line represents introns. Aberrant splicing in the *lacs2-2* allele is indicated by a large arrow. T-DNA insertion in the *lacs2-3* allele is indicated by a triangle. Positions of primers used for RT-PCR are indicated by small arrows (not on scale).

(B) The genomic DNA sequence spanning the last 3 exons and 2 introns of the *LACS2* gene in Col-0 background. Capital letters represent nucleotides of exons, small letters represent nucleotides from introns. Polymorphisms found in the *lacs2-2* gene are indicated above the Col-0 sequence. Nucleotides present in the cDNA of *lacs2-2* gene are underlined.

(C) The genomic DNA sequence flanking the T-DNA insertion in the *lacs2-3* gene. Capital letters represent nucleotides from an exon, small letters represent nucleotides from an intron. The white box represents the T-DNA insertion site. A small deletion of 23 bp (strikethrough) genomic DNA was detected.

(D) Analysis of the *lacs2-2* and *lacs2-3* transcript. RT-PCR using primers flanking the 18th exon of *LACS2* (top) and of the β -tubulin gene *TUB6* (bottom).

For sequencing, genomic DNA was extracted using the Plant Mini Kit (Qiagen) and the PCR was performed with the Expand High Fidelity Kit (Roche). Genomic sequence of *lacs2-2* was identified by direct double-stranded sequencing of overlapping fragments from at least 2 independent PCR reactions.

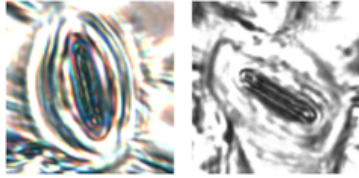
For determination of the cDNA sequences of *LACS2-2* and *LACS2-3*, RNA of young seedlings was extracted using the RNA Plant Mini kit (Qiagen). RNA was retrotranscribed using the Superscript III cDNA synthesis Kit (Invitrogen) followed by PCR using the Expand High Fidelity kit (Roche). The fragment flanking the 18th exon was amplified using the primers: 18-F: 5'-GGGTCTATGGCAACAGCTT-3' and 18-R: 5'-GATGAGAGGATCCGAACACAA-3'. The β -tubulin gene 6 (At5g12250) was amplified by using the primers: TUB-F 5'-ACCACTCCTAGCTTTGGTGATCTG-3' and TUB-R 5'-AGGTTCACTGCGAGCTTCCTCA-3'. RT-PCR using primers flanking the insertion site in *LACS2-3* led to the amplification of a fragment of the expected length in Col-0 and in *lacs2-2*, but not in *lacs2-3* (data not shown).

Sequence analysis of the *lacs2-2* gene detected polymorphisms in intron 18 at both the donor site (G to A) and the acceptor site (A to G) for splicing (Supplementary Figures 2A and 2B). Sequencing of the 3' end of the *LACS2-2* cDNA revealed that these differences in sequence lead to the removal of exon 18 from the cDNA (Figure 2D). The GG at the acceptor site might have created a stronger acceptor site or altered the secondary structure in a way that exon 18 was removed together with intron 17 and intron 18. Removal of exon 18 resulted in the loss of 40 amino acids from the protein, which is expected to reduce or eliminate LACS2 activity thus causing the mutant phenotype. In addition, a G to A transition was identified that caused an exchange from ARG₂₂₉ to HIS₂₂₉. The truncated transcript was present in the *lacs2-2* mutant in the same amounts as in Col-0, while no transcript was seen in *lacs2-3* (Figure 2D).

Sequence analysis of the *lacs2-3* allele showed that the T-DNA insertion in the *LACS2* gene at the junction of the 5th exon and intron was accompanied by the loss of the last nine nucleotides of exon 5 and the first 14 nucleotides of intron 5 (Figures 2A and 2B).

Supplementary Figure 3

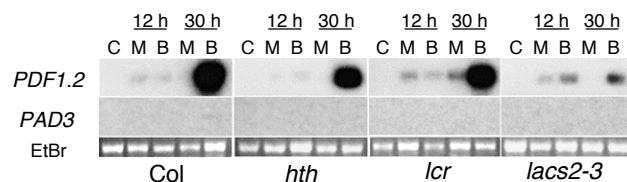
Stomata aperture under drought conditions



The aperture of stomata of leaves from 5 to 6-week-old deracinated rosettes were observed. 2 hours after excision expanded rosette leaves were pressed against fast drying nail polish (Manhattan) that had been applied to a microscopic slide. Pictures of the imprints were taken with a digital camera (Leica, DC 200) on a Diaplan microscope (500X, Leitz). Col-0 (left), *lacs2-3* (right).

Supplementary Figure 4

Expression of defence genes after inoculation with *B. cinerea*



Gel Blot analysis of 3 µg of total RNA of 5 to 6-week-old plants hybridized with a probe for *PDF1.2* and *PAD3*. An ethidium bromide-stained gel is presented as loading control. For each mutant, RNA of untreated control plants (C), mock-inoculated plants (M) and *Botrytis*-inoculated plants (B) are presented after 12 h and 30 h after inoculation, respectively.

RNA was prepared using the TRIzol® reagent (Molecular Research Center, Inc., Invitrogen). Gel Blot analyses were performed as described previously (Nawrath et al., 2002). ³²P-radioactive probes were synthesized using the Prime-a-gene kit (Invitrogen). Probes for *PDF1.2* and *PAD3* were prepared as described previously (Penninckx et al., 1998; Zhou et al., 1999).

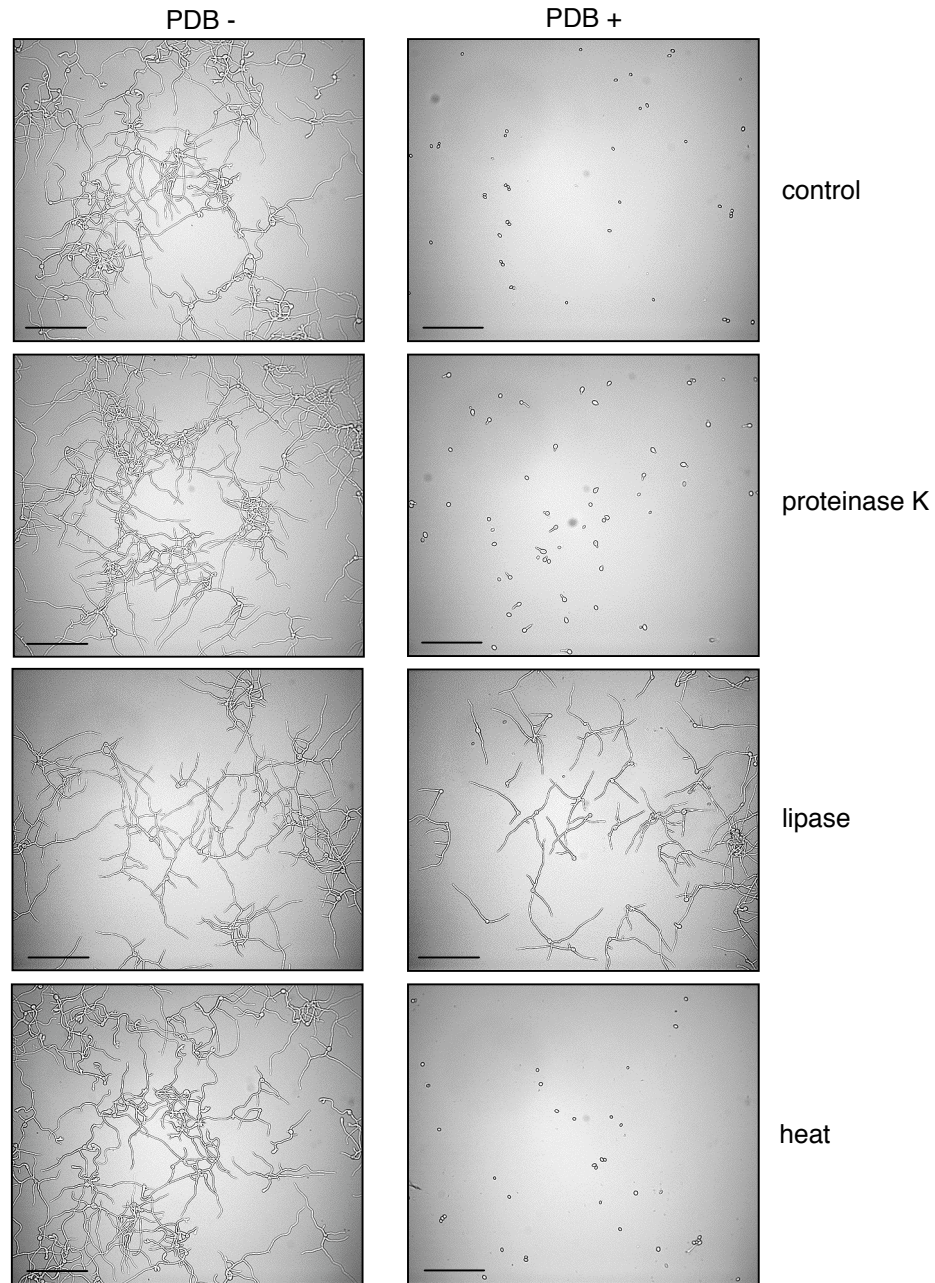
Nawrath, C., Heck, S., Parinthewong, N. and Metraux, J.P. (2002) EDS5, an essential component of salicylic acid-dependent signaling for disease resistance in Arabidopsis, is a member of the MATE transporter family. *Plant Cell* **14**: 275-286.

Penninckx, I.A., Thomma, B.P., Buchala, A., Métraux, J.-P. and Broekaert, W.F. (1998) Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in Arabidopsis. *Plant Cell* **10**: 2103-2113.

Zhou, N., Tootle, T.L. and Glazebrook, J. (1999) Arabidopsis PAD3, a gene required for camalexin biosynthesis, encodes a putative cytochrome P450 monooxygenase. *Plant Cell* **11**: 2419-2428.

Supplementary Figure 5

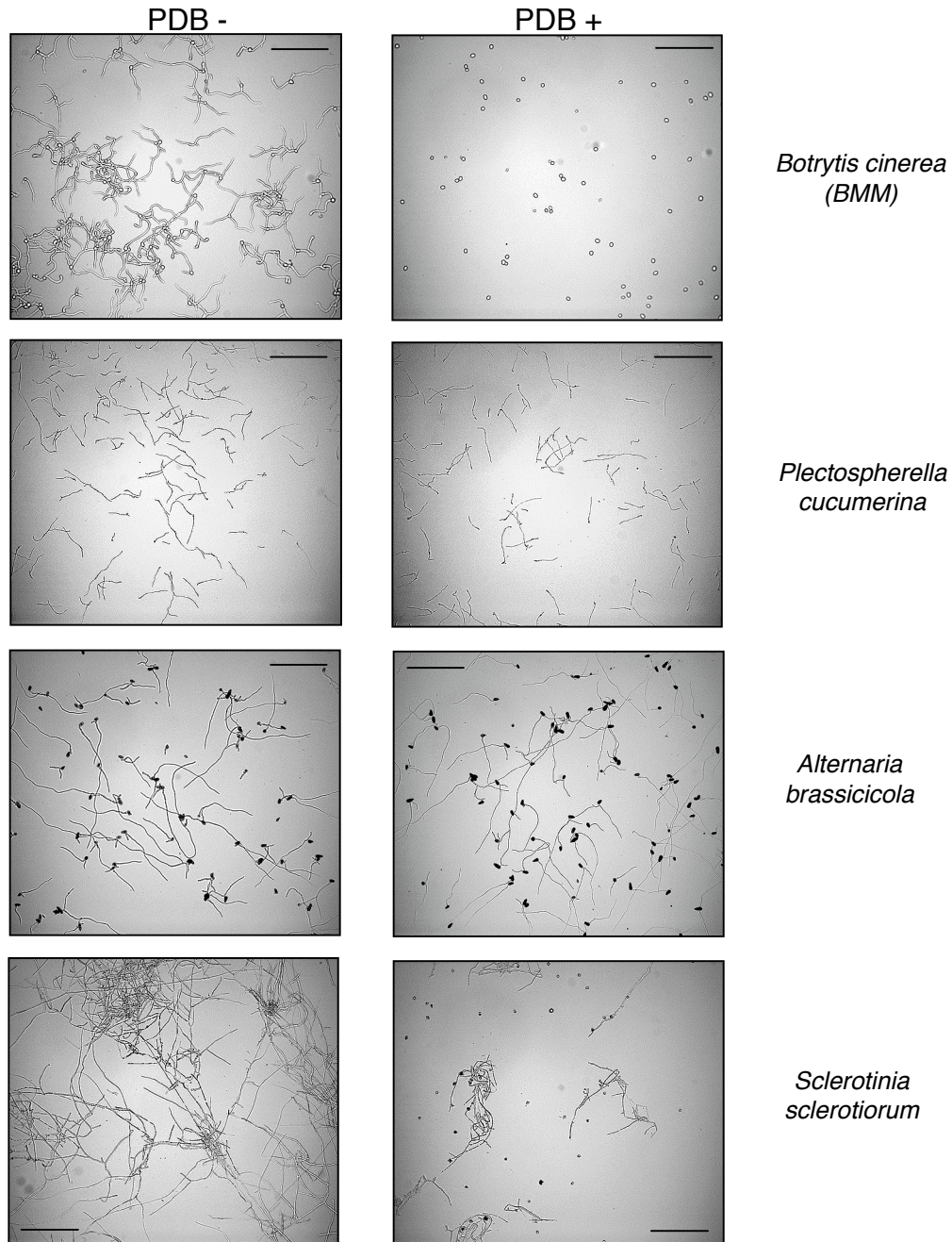
In vitro growth of *Botrytis* hyphae in the presence of differently treated PDB-diffusates from *lacs2*



Microscopy pictures of germination and growth of *Botrytis in vitro* in the presence of 1/4 PDB (left) or 1/4 PDB that was incubated on *lacs2* plants for 42 h (right) that had both undergone different treatments. No treatment (control), proteinase K at 0.035 u/ μ l (protease), treatment with the lipase from *Mucor miehei* at 32 u/ μ l (lipase), and 95°C for 1 h (heat) are presented. Bar = 0.2 μ m.

Supplementary Figure 6

Effect of PDB-diffusate from *lacs2* on different necrotrophic fungi



Microscopy pictures of hyphae of different necrotrophic fungi grown in 1/4 PDB medium (left) or in 1/4 PBD-medium that was incubated for 40 h on *lacs2* plants (right). 3 μ l of a spore suspension (6×10^4 spores/ml) of *B. cinerea*, *A. brassicicola* and *P. cucumerina*, and macerated mycelium for *S. sclerotiorum* (0.1 ml mycelium/ml medium) were mixed with 9 μ l of the different PDB media and incubated for 14 h under 100% humidity at 20°C on a glass slide. Pictures were taken with a digital camera (Leica, DC 300) on a Diaplan microscope (Leitz). Bar = 0.2 μ m.

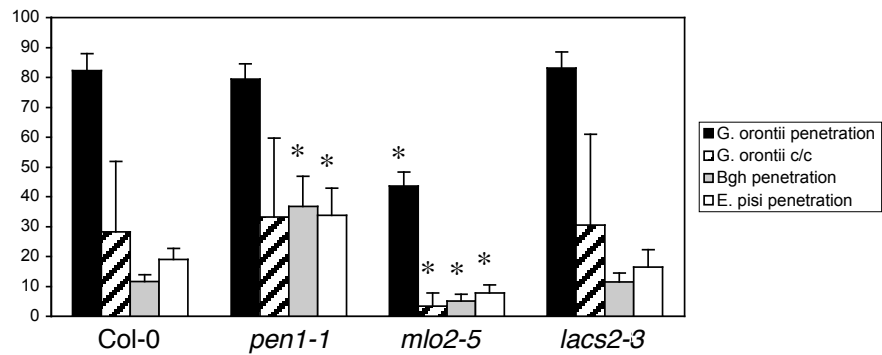
Supplementary Figure 7

Analysis of responses to adapted and non-adapted powdery mildew fungi

A



B

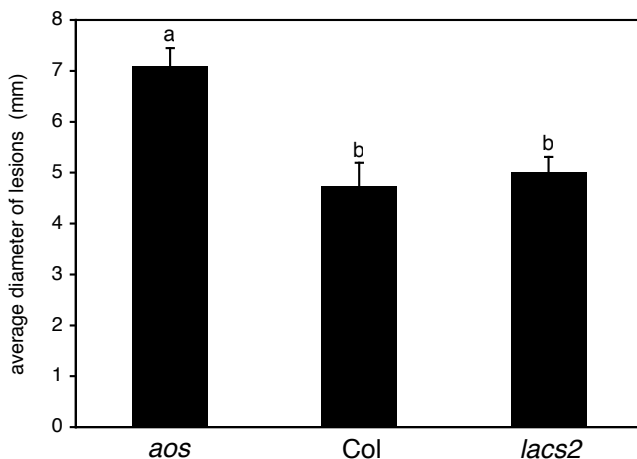


(A) Macroscopic phenotypes 10 days post inoculation (dpi) with the adapted powdery mildew *Golovinomyces orontii*

(B) Percentage host cell entry after 48 hours post inoculation for *G. orontii* and *Blumeria graminis* f. sp. *hordei* (*Bgh*) and 6 dpi for *Erysiphe pisi* and counts of conidiophores per colony for *G. orontii* 7 dpi.

Supplementary Figure 8

Analysis of the symptom development after inoculation with *Plectosphaerella cucumerina*



Plectosphaerella cucumerina was grown on 1x PDA medium at 20-22°C. Spores were harvested in water after 2 weeks of growth. 5 μ l droplets of a suspension of 2×10^6 spores/ml in 1/4 PDB were placed on leaves of 5 week-old plants and incubated under a transparent lid at 100% humidity. Diameter of lesions that developed 4 days after inoculation was measured. The mean of the lesion diameter was taken as a measure for susceptibility of the plant (n=6; \pm SE). Values not connected by the same letter are significantly different at $p < 0.005$ using ANOVA performing comparison for all pairs with Turkey Kramer HSD test. The experiment was repeated with equivalent results.