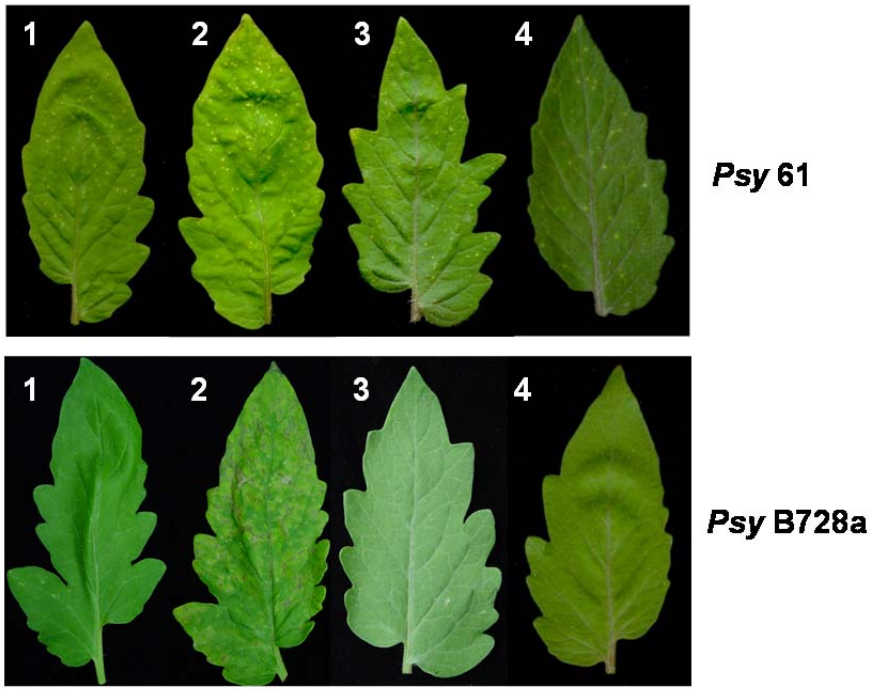
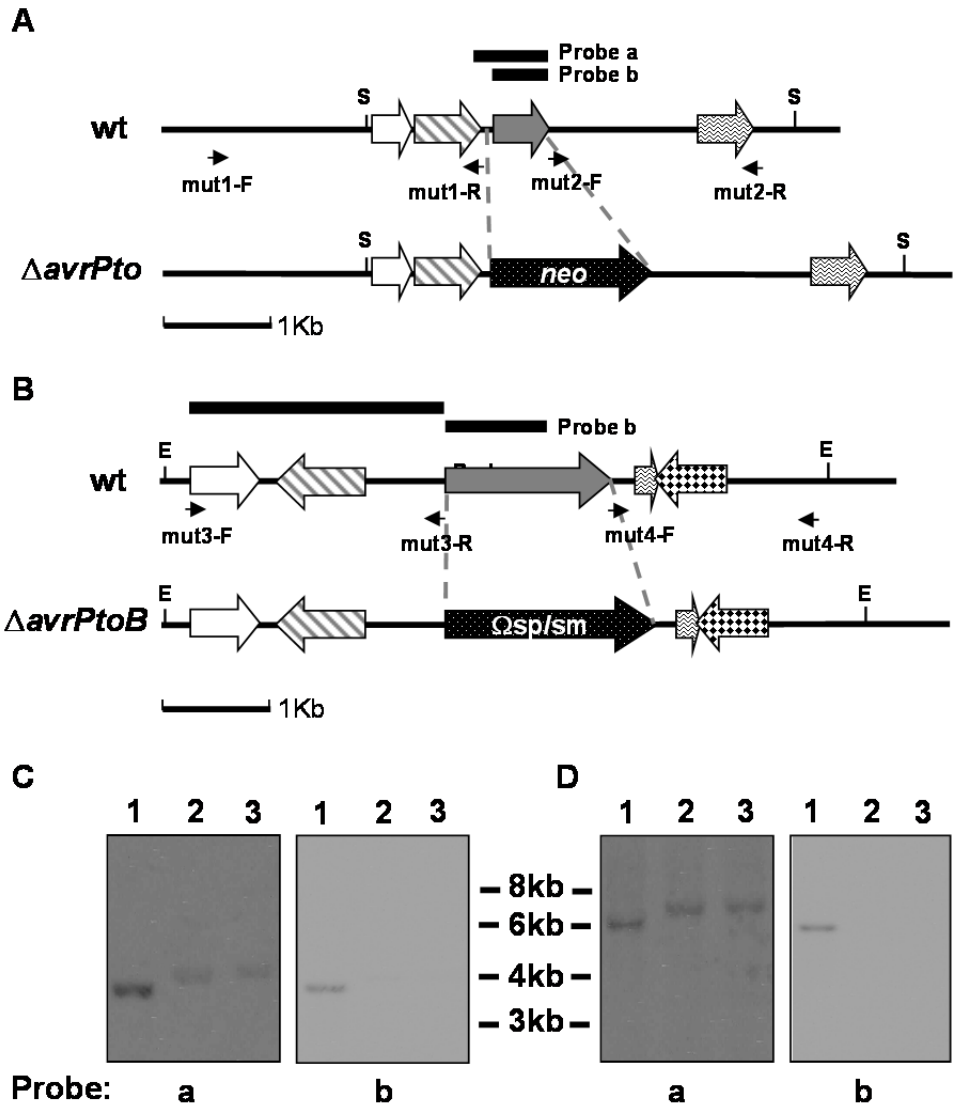
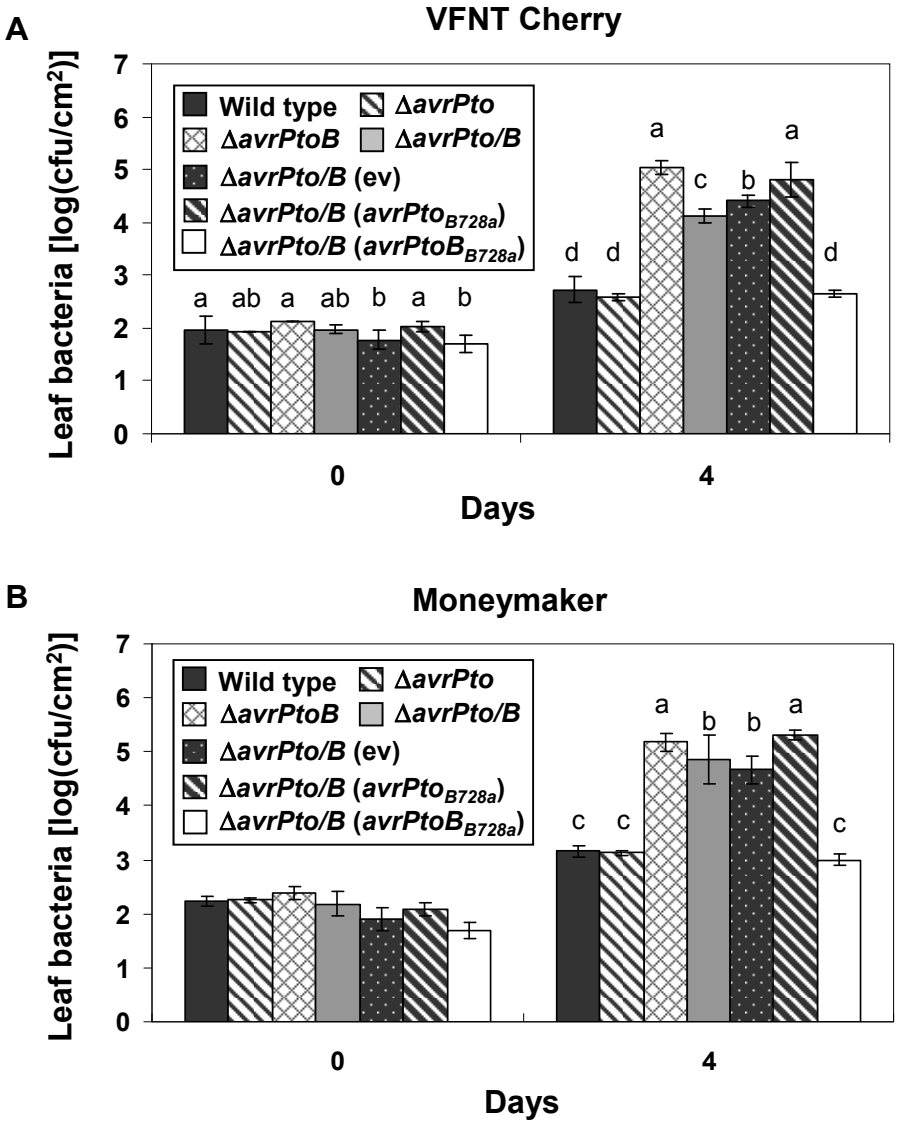


	(1) RG-PtoR	(2) RG-prf3	(3) Moneymaker	(4) VFNT Cherry
Psy 61	C	C	C	C
Psy B728a	I	C	I	I

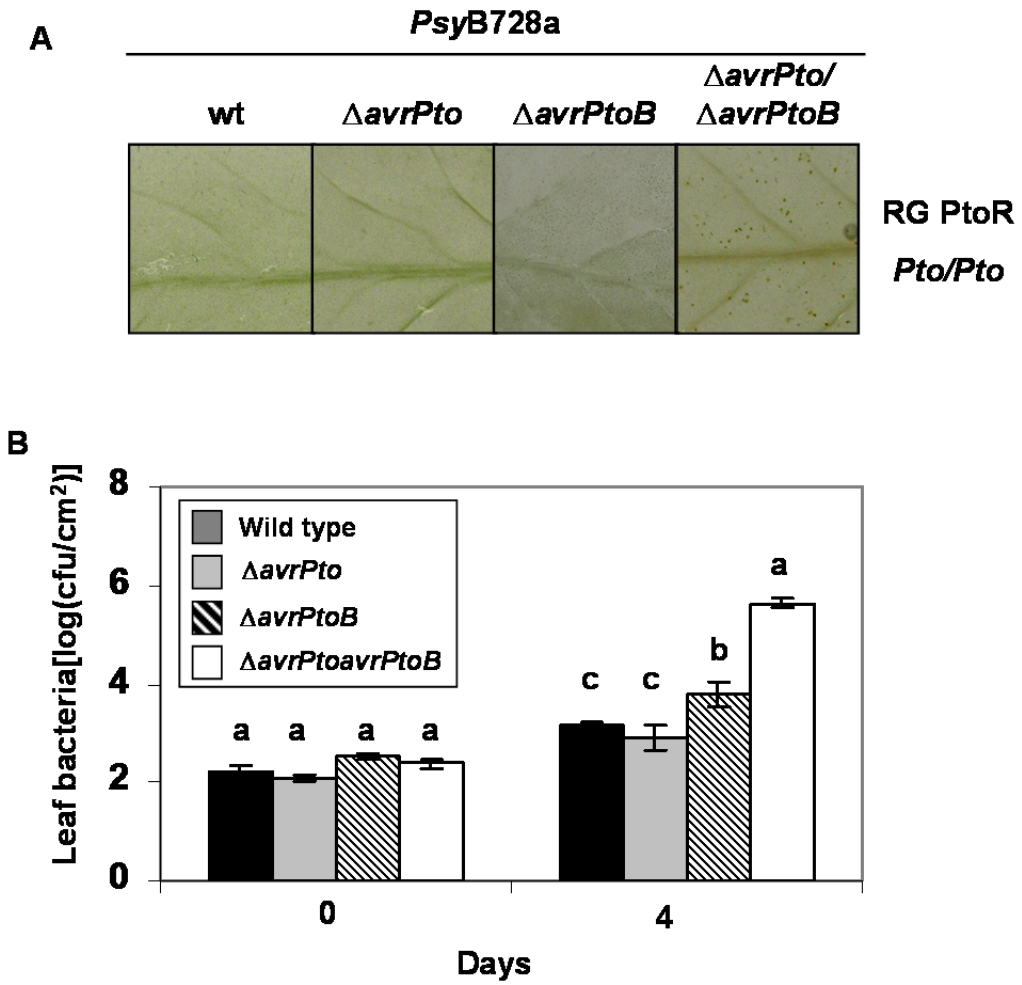
Supplementary Fig. S1. Responses of tomato leaves to two bean pathogens, *Pseudomonas syringae* pv. *syringae* strains 61 and B728a. Disease symptoms on leaves of tomato cultivars Rio Grande-PtoR (1), Rio Grande-prf3 (2), Moneymaker (3) or VFNT Cherry (4) were monitored after vacuum infiltration with 2 x 10^4 CFU/ml of *Psy*61 (upper panel) or *Psy*B728a (lower panel). C, compatible interaction (disease); I, incompatible interaction (no disease).



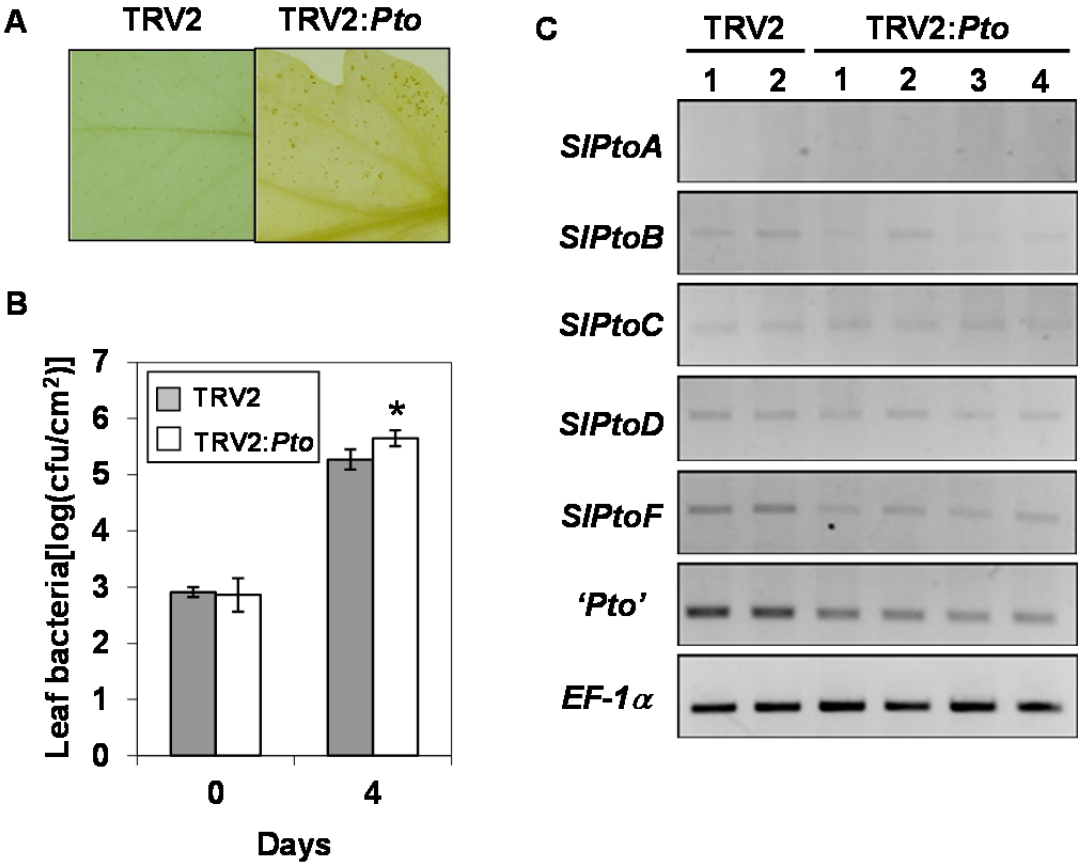
Supplementary Fig. S2. Generation of *avrPto*- and *avrPtoB*-deletion mutants of *Psy*B728a. **A,** The gene structures of *avrPto* and **B,** *avrPtoB* of *Psy*B728a and the location of the kanamycin resistance gene or the omega fragment in the derived mutants. Probes used in Southern blot analysis are indicated as bold lines. E: *Eco*RI site; S: *Stu*I site. Numbers above the gene structure are the locations in the *Psy*B278a genome. **C,** Southern blot analysis for deletion of *avrPto* in *Psy*B728a. 1, wild-type *Psy*B728a; 2, *Psy*B728aΔ*avrPto*; and 3, *Psy*B728aΔ*avrPto*Δ*avrPtoB*. **D,** Southern blot analysis for deletion of *avrPtoB* in *Psy*B728a. 1, *Psy*B728a wild-type; 2, *Psy*B728aΔ*avrPtoB*; and 3, *Psy*B728aΔ*avrPto*Δ*avrPtoB*.



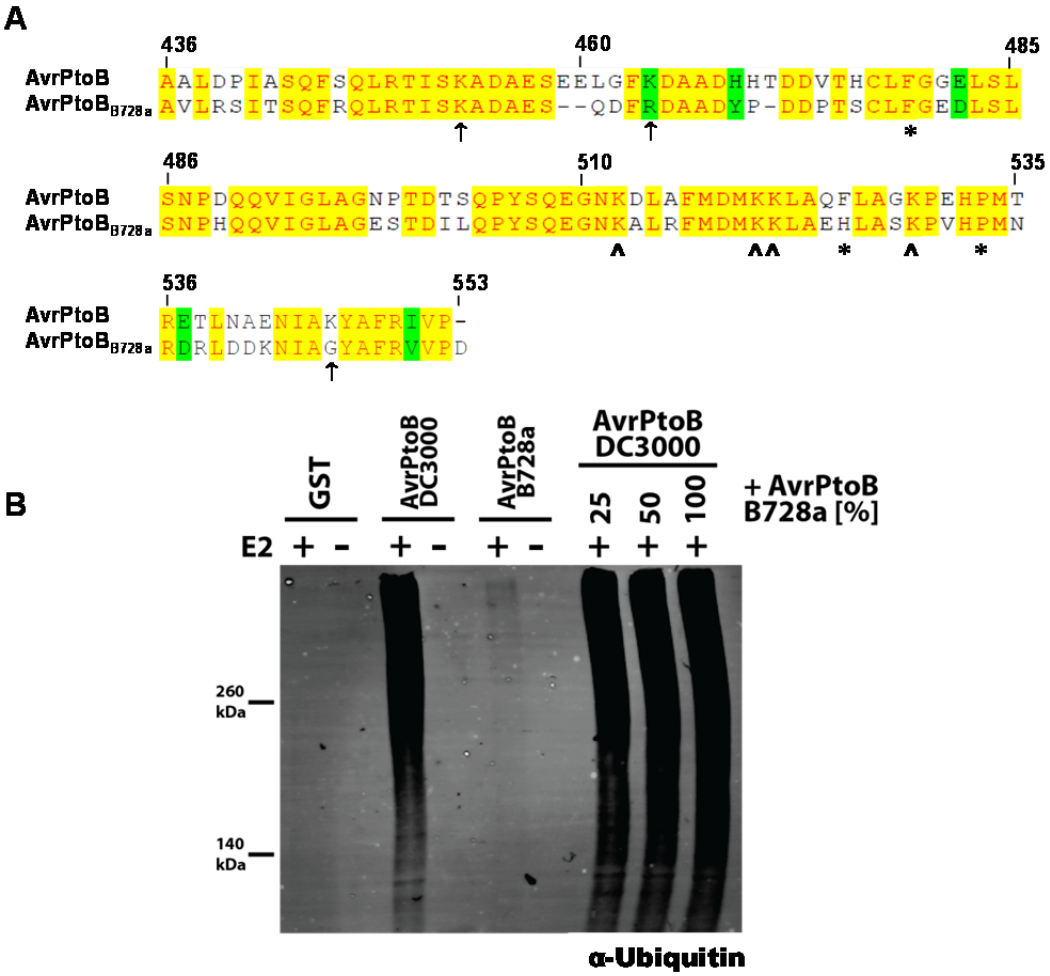
Supplementary Fig. S3. A, Responses of leaves of VFNT Cherry and **B,** Moneymaker to PsyB728a, its derivative mutants, and each cognate complementary strain. Bacterial populations were measured in tomato leaves at 0 and 4 days postinoculation (dpi) after vacuum infiltration with 2×10^4 CFU/ml of *Psy*B728a wild type, Δ*avrPto*, Δ*avrPtoB*, Δ*avrPto*Δ*avrPtoB* (Δ*avrPto/B*), and Δ*avrPto*Δ*avrPtoB* carrying pCPP45 [Δ*avrPto/B*(ev)], pCPP45::*avrPto*$_{B728a}$ [Δ*avrPto/B*(*avrPto*$_{B728a}$)], and pCPP45::*avrPtoB*$_{B728a}$ [Δ*avrPto/B*(*avrPtoB*$_{B728a}$)]. The error bars indicate the standard deviations for three replicates. Data analysis was performed using Duncan's multiple-range test. Means with the same letter above the bars are not different at a significance level of 5%. Each experiment was performed three times with similar results.



Supplementary Fig. S4. Responses of leaves of Rio Grande-PtoR to *Psy*B728a and its derivative mutants. **A,** Disease symptoms were observed only when tomato plants were inoculated with 2×10^4 CFU/ml *Psy*B728aΔ*avrPto*Δ*avrPtoB*. Photographs were taken 7 days after vacuum infiltration. **B,** Bacterial populations in tomato leaves at 0 and 4 days postinoculation. The error bars indicate the standard deviations for three replicates. Data analysis was performed using Duncan's multiple-range test. Means with the same letter above the bars are not different at a significance level of 5%. Each experiment was performed three times with similar results.



Supplementary Fig. S5. Resistance elicited by *avrPtoB*$_{B728a}$ is dependent on *Pto* family members. **A,** *Psy*B728a did not trigger resistance on VFNT Cherry that was silenced by tobacco rattle virus (TRV2) VIGS for *Pto* orthologs, and specks were observed 4 days after bacterial inoculation. At 7 days post-inoculation (dpi), chlorophyll was removed from the leaves using ethanol before photographs were taken. **B,** Bacterial populations in VFNT Cherry leaves silenced for *Pto* orthologs and infiltrated with *Psy*B728a. The error bars indicate the standard deviations for three replicates. Data analysis was performed using Student's *t*-test, and an asterisk (*) indicates significant difference at a level of 5%. Each experiment was performed three times with similar results. **C,** Silencing of *Pto* orthologs in VFNT Cherry was evaluated using semi-quantitative RT-PCR. Primers specific to the *Pto* ortholog shown or that would amplify all *Pto*-like sequences (designated as '*Pto*') were used. Note that VFNT Cherry does not have the *Pto* gene itself. Expression level of *EF-1α* was used as a control.



Supplementary Fig. S6. AvrPtoB$_{B728a}$ protein preparations do not contain a detectable E3-ligase inhibitory activity. **A,** Amino acid sequence alignment of the E3 ligase domains of AvrPtoB and AvrPtoB$_{B728a}$. Alignment was performed using ClustalW program in Vector NTI. Black arrows (↑) indicate lysine residues that do not have a known role in AvrPtoB ubiquitination activity, the carets (^) indicate that substitutions at these lysines cause loss in ubiquitination activity, and stars (*) represent putative E2-binding sites. **B,** AvrPtoB from *Pst*DC3000 or AvrPtoB$_{B728a}$ from *Psy*B728a were purified from *E. coli* as GST fusions and subjected to *in vitro* ubiquitination assays in the presence and absence of E2 conjugating enzyme as described in Fig. 5. To test for the presence of an E3-inhibitory activity in the AvrPtoB$_{B728a}$ preparations, increasing amounts of AvrPtoB$_{B728a}$ were included in AvrPtoB assay. No inhibitory effect of the AvrPtoB$_{B728a}$ preparation on the E3 ligase activity of AvrPtoB samples was observed.

Table S1. Primers used in this study

Primer	Sequence (5′→3′)	Note
AvrPtoB728a-1	GGGGTACCTTACTGTCCGCCAAACTCAA	Anneal to the upstream of *hrp* box
AvrPtoB728a-2	GGTCTAGAGCTACAATCCAGTTCCTCTACAAA	Anneal to downstream of av*rPto*$_{B728a}$ ORF
B728amut1-F	AAGGTACCAGCTCGACATTGCCTTTCTCA	To generate pΔavrPto$_{B728a}$
B728amut1-R	AACTCGAGCCTGGATCCGCCGACACAT	To generate pΔavrPto$_{B728a}$
B728amut2-F	AACTCGAGCTCCTAGCCCGCACCGTAAC	To generate pΔavrPto$_{B728a}$
B728amut2-R	AATCTAGACTATATGAAGGACCCAGCAAAACC	To generate pΔavrPto$_{B728a}$
B728a-mt1	AAGGTACCTTGGCGCACTGACCCTGGTGAT	To generate pΔavrPtoB$_{B728a}$
B728a-mt2	AAGATATCGATGGCCCTGCTCCGTTGAT	To generate pΔavrPtoB$_{B728a}$
B728a-mt3	AAGATATCGCATCCCATGAACAGAGACA	To generate pΔavrPtoB$_{B728a}$
B728a-mt4	AAGGATCCTCGACATGGCGAATTGTTG	To generate pΔavrPtoB$_{B728a}$
SlPtoA-1	AGATGCTCTCTCACTTCCGC	For RT-PCR
SlPtoA-2	CACCATCCCCTTCGAAATAT	For RT-PCR
SlPtoB-F	CACAAGGTATTGAAGAGTTC	For RT-PCR
SlPtoB-R	TCTTCTGCGTCTCATCATCA	For RT-PCR
SlPtoC-1	CTCGAGATATGGCGTTCCTT	For RT-PCR
SlPtoC-2	TGTGCTAAGATGGGTTTGAT	For RT-PCR
SlPtoD-F	TGTCTTCCCACTATGAGCTG	For RT-PCR
SlPtoD-R	AAGTGCATACTCCAGTTTCC	For RT-PCR
SlPtoF-1	GCTTCAAACTCTTTTGAAAG	For RT-PCR
SlPtoF-2	TCACGTCTGTGGTTTGATAA	For RT-PCR

*The recognition sequence of each restriction endonuclease is underlined and as follows: *Eco*RI, GAATTC; *Eco*RV, GATATC; *Kpn*I, GGTACC; *Hin*dIII, AAGCTT; *Xba*I, TCTAGA; *Xho*I, CTCGAG

SUPPLEMENTARY MATERIALS AND METHODS

Southern blot analysis.

Genomic DNA (5 μg) extracted from *Psy*B728a, *Psy*B728a Δ*avrPto*$_{B728a}$, *Psy*B728aΔ*avrPtoB*$_{B728a}$, or *Psy*B728aΔ*avrPto*$_{B728a}$ Δ*avrPtoB*$_{B728a}$ was digested with *Eco*RI or *Stu*I at 37°C overnight before separation in a 1% agarose gel. DNA was then transferred onto a Hybond N+ nylon membrane (Amersham Biosciences, Buckinghamshire, U.K.) with 10× SSC (1.5 M NaCl and 0.15 M sodium acetate, pH7.0) by the capillary method. Southern blot analysis was performed using a digoxigenin (DIG) system followed by procedures recommended by the manufacturer (Roche Applied Science, Indianapolis, IN, U.S.A.). Probes used to check the *avrPto* deletions were DNA fragments containing *hrp* promoter and ORF (probe a) and ORF (probe b) of *avrPto*$_{B728a}$. Probes used to confirm the *avrPtoB* deletion are upstream sequence (probe a) and partial ORF (1 to 630 bp; probe b) of *avrPtoB*$_{B728a}$. Prehybridization and hybridization were carried out at 65°C for 4 hours and overnight, respectively, in a standard hybridization buffer (5× SSC, 0.1% (wt/vol) lauroylsarcosine, 0.02% (wt/vol) SDS, and 1% blocking reagent). The membrane was washed twice in 2× SSC with 0.1% SDS at room temperature for 5 minutes, and twice in 0.5× SSC with 0.1% SDS at 65°C for 15 minutes before incubation of the membrane in the blocking solution (1% blocking reagent in maleic acid buffer, which contains 100 mM maleic acid, 150 mM NaCl, and is adjusted pH7.5) for 30 minutes followed by incubation with a horseradish peroxidase (HRP)-conjugated anti-DIG antibody (1:10,000 dilution) for 30 minutes. After washing twice with the washing buffer (1× malic acid buffer and 0.1% Tween 20) for 15 minutes each, CSPD was applied to the membrane, incubated at 37°C for 10 minutes, and then the probe-target hybrids were detected using a G-Box systems (Syngene, Cambridge, U.K.).