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Article:

https://doi.org/10.1105/tpc.17.00537

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Figure 1. PSK signaling confer tomato plants immunity against *B. cinerea*.

(A–D) Tomato defense to *B. cinerea* is promoted by exogenous PSK. Four-week-old tomato plants were treated with 10 µM PSK, 10 µM dPSK, or water as control 12 h before *B. cinerea* inoculation. (A) Representative chlorophyll fluorescence imaging of the photochemical quantum yield of photosystem II (ΦPSII) at 2 days post *B. cinerea* inoculation (dpi). (B) Representative images of trypan blue staining for cell death in leaves at 2 dpi. Scale bar = 250 µm. (C) The quantification data of ΦPSII at 2 dpi. (D) Relative *B. cinerea* actin transcript abundance in infected leaves at 1 dpi.

(E) Effects of *B. cinerea* inoculation on the transcript abundance of PSK precursor genes and tyrosine sulfation processing gene *SlTPST* in leaves at 0.5 dpi. The transcript abundance of each gene under mock-inoculated condition were defined as 1. nd, not detected. The asterisk indicates a significant effect of *B. cinerea* inoculation with tomato plants.

(F–I) Effects of PSK-biosynthesis related genes silencing on tomato innate immunity against *B. cinerea*. (F) Representative chlorophyll fluorescence imaging of ΦPSII at 2 dpi. (G) Trypan blue staining for cell death in leaves at 2 dpi. Scale bar = 250 µm. (H) The quantification data of ΦPSII at 2 dpi. (I) Relative *B. cinerea* actin transcript abundance in infected leaves at 1 dpi.

The results in (C, D, E, H, and I) are presented as the mean values ± SD; n = 3. Different letters indicate significant differences between treatments (*P* < 0.05, Tukey’s test). The above experiments were repeated three times with similar results.
Figure 2. Characterization of tomato PSK receptors.

(A) Subcellular localization of SIPS KR1 and SIPS KR2. SIPS KR s-GFP and FLS 2-mCherry (marker for plasma membrane localization) plasmids were transiently co-expressed into *N. benthamiana* leaves. The GFP and mCherry signals were visualized under confocal microscopy after 48 h infiltration. Scale bar = 50 µm.

(B) Surface plasmon resonance analysis of the binding of PSK to potential tomato PSK receptors, SIPS KR1 and SIPS KR2. The curves represent the concentrations of the injected PSK and dPSK. From bottom to top, 0.39, 0.78, 3.125, 6.25, 12.5, and 25 µM were used for PSK. An additional 50 µM concentration was also used for dPSK. The recombinant extracellular portion of the SIPS KR1 and SIPS KR2 was immobilized onto the sensor chip. The obtained kinetic constants for specific binding are shown in each panel. RU, resonance units.

(C-F) Effects of *SIPS KR1-* and/or *SIPS KR2*-silencing on tomato innate immunity against *B. cinerea*. (C) Representative chlorophyll fluorescence imaging of the photochemical quantum yield of photosystem II (ΦPSII) at 2 days post *B. cinerea* inoculation (dpi). (D) Representative images of trypan blue staining for cell death in leaves at 2 dpi. Scale bar = 250 µm. (E) The quantification data of ΦPSII at 2 dpi. (F) Relative *B. cinerea actin* transcript abundance in infected leaves at 1 dpi.

The results in (E, F) are presented as the mean values ± SD; *n* = 3. Different letters indicate significant differences between treatments (*P* < 0.05, Tukey’s test). The above experiments were repeated two times with similar results.
Figure 3

(A) Effects of PSK application on endogenous leaf hormone contents (SA, JA, ET, and IAA). Four-week-old tomato plants were treated with 10 µM PSK, dPSK, or water as control 12 h before B. cinerea inoculation, and leaf samples were collected at 0.5 days post B. cinerea inoculation (dpi).

(B) Effects of PSK application on the transcript abundance of hormone signaling-related marker genes in leaves at 0.5 dpi. The elicitor application is as in (A). SA-related genes SlPR1b, JA-related genes SlCOI1, ET-related genes SlERF1, Auxin-related genes SlARF5.

(C) Effects of PSK application on trypan blue staining for cell death in hormone signaling-defective and control plants at 2 dpi. Tomato wild type (WT), mutants, or transgenic lines were treated with 10 µM PSK or water control 12 h before B. cinerea inoculation. The following tomato lines were used: SA accumulation-defective transgenic NahG and its WT line cv. Moneymaker (MM), JA-signaling mutant jai1-1 and its WT line cv. Castlemart (CM), ET-signaling mutant Nr and its WT line cv. Pearson, auxin signaling-insensitive mutant dgt and its WT line VFN8. Scale bar = 250 µm.

(D) Relative B. cinerea actin transcript abundance in infected hormone-related mutants in the presence or absence of PSK at 1 dpi, the elicitor application is as in (C).

The results in (A, B, and D) are presented as the mean values ± SD; n = 3. Different letters indicate significant differences between treatments (P < 0.05, Tukey’s test). The above experiments were repeated three times with similar results.
Figure 4. Auxin functions downstream of PSK-SIPS KR1 signaling in tomato immunity against *B. cinerea*.

(A) The changes of IAA content in PSK signaling component gene-silenced tomato plants. Leaf samples were collected at 0.5 days post *B. cinerea* inoculation (dpi).

(B) Effects of NAA application on leaf *B. cinerea* actin transcript abundance in target gene-silenced tomato plants at 1 dpi. PSK signaling component gene-silenced and TRV:0 control plants were treated with 10 nM NAA or water control 12 h before *B. cinerea* inoculation.

(C-D) SIPS KR1 silencing-compromised immunity was complemented by NAA but not by PSK. (C) Trypan blue staining for cell death as affected by SIPS KR1 silencing and application of PSK and NAA. Tomato SIPS KR1-silenced plants were treated with 10 µM PSK or 10 nM NAA 12 h before *B. cinerea* inoculation, and leaf samples were collected at 2 dpi. Scale bar = 250 µm. (D) Relative *B. cinerea* actin transcript abundance in infected leaves at 1 dpi.

(E) The effects of 10 nM NAA application on the transcript abundance of PSK signaling component genes in leaves under both mock- and *B. cinerea*-inoculated condition, samples were taken at 0.5 dpi.

The results in (A, B, D, and E) are presented as the mean values ± SD; *n* = 3. Different letters indicate significant differences between treatments (*P* < 0.05, Tukey’s test). The experiments in (A, B, and E) were repeated three times, and others were repeated two times with similar results.
Figure 5. Cytosolic Ca^{2+} elevation is induced and required for PSK-induced tomato immunity against *B. cinerea*.

(A-B) PSK-induced cytosolic Ca^{2+} elevation in leaves of aequorin-expressing tomato plants as affected by Ca^{2+} channel inhibitors (A) or *SlPSKR*s genes silencing (B). Tomato leaf discs were preincubated for 30 min with ruthenium red (RR) or verapamil (Ver) at 20 µM, and 10 µM PSK ligand was then added at time 0. The signals shown at 0.5-min intervals are the mean values ± SD, (n = 10–12). In (A), totally 50 leaf discs obtained from at least 5 plants were used for experiment and each treatment had 10 leaf discs; In (B), each 12 leaf discs obtained from independent 5 plants served as one treatment.

(C-F) Effects of Ca^{2+} channel inhibitor on tomato innate immunity against *B. cinerea*. The four-week-old tomato plants were treated with 10 µM PSK, 20 µM each Ca^{2+} channel inhibitor, or water as control 12 h before *B. cinerea* inoculation. (C) Representative chlorophyll fluorescence imaging of the photochemical quantum yield of photosystem II (ΦPSII) at 2 days post *B. cinerea* inoculation (dpi). (D) Representative images of trypan blue staining for cell death in leaves at 2 dpi. Scale bar = 250 µm. (E) The quantification data of ΦPSII at 2 dpi. (F) Relative *B. cinerea actin* transcript abundance in infected leaves at 1 dpi. The results in (E and F) are presented as the mean values ± SD; n = 3. Different letters indicate significant differences between treatments (P < 0.05, Tukey’s test).

The above experiments were repeated three times with similar results.
Figure 6

(A) BiFC analyses of the binding between SICaM2 and SIYUCs (left panel), and between SICaMs and SIYUC6 (right panel). Both spliced YFP constructs and FLS2-mCherry (marker for plasma membrane localization) plasmids were transiently co-expressed into N. benthamiana leaves. The YFP and mCherry signals were visualized under confocal microscopy after 48 h infiltration. Scale bar = 50 µm.

(B) The changes of BiFC fluorescence signal between p2YC-SlCaM2 and p2YN-SlYUC6 with or without 2 h

Figure 6. SICaMs binds to auxin biosynthetic protein SIYUCs.

(A) BiFC analyses of the binding between SICaM2 and SIYUCs (left panel), and between SICaMs and SIYUC6 (right panel). Both spliced YFP constructs and FLS2-mCherry (marker for plasma membrane localization) plasmids were transiently co-expressed into N. benthamiana leaves. The YFP and mCherry signals were visualized under confocal microscopy after 48 h infiltration. Scale bar = 50 µm.

(B) The changes of BiFC fluorescence signal between p2YC-SlCaM2 and p2YN-SlYUC6 with or without 2 h
of PSK (10 µM) application. Scale bar = 50 µm. The fluorescence signal intensity from three independent repeats was quantified and the data are shown as mean ± SD (n = 3). Asterisks indicates a significant effect of PSK application (P < 0.05, Tukey’s test).

(C) Co-IP analysis of association between HA-tagged SiCaM2 and FLAG-tagged SiYUC6 with or without application of 10 µM PSK, 20 µM CaCl2, and 20 µM Ca2+ channel inhibitor ruthenium red (RR) for 2 h. Total proteins were extracted from leaves transiently expressed with the SiCaM2-HA, SiYUC6-FLAG construct alone or their combinations after 48 h infiltration. The extracted proteins were immunoprecipitated with an anti-FLAG antibody and the presence of SiCaM2-HA and SiYUC6-FLAG in the immune complex was determined by immunoblot (IB) with the indicated antibody. The Co-IP band intensity (top) from three independent repeats was quantified by Image J software. The data are shown as mean ± SD (n = 3). Different letters indicate significant differences between treatments (P < 0.05, Tukey’s test).

The experiments in (A, B) were repeated three times, and experiments in (C) were repeated two times with similar results.
Figure 7. SlCaM2 silencing compromises the PSK-induced immunity and IAA accumulation.

(A) Representative chlorophyll fluorescence imaging of the photochemical quantum yield of photosystem II (ΦPSII) as affected by SlCaM2 silencing, and application of PSK and NAA. Tomato SlCaM2-silenced plants were treated with 10 µM PSK or 10 nM NAA 12 h before B. cinerea inoculation, and leaf samples were collected at 2 days post B. cinerea inoculation (dpi).

(B) The quantification data of ΦPSII at 2 dpi.

(C) Relative B. cinerea actin transcript abundance in infected leaves at 1 dpi.

(D) The changes of IAA content in SlCaM2-silenced tomato plants at 0.5 dpi, as affected by exogenous PSK and NAA application.

The results in (B to D) are presented as the mean values ± SD; n = 3. Different letters indicate significant differences between treatments (P < 0.05, Tukey’s test). The above experiments were repeated three times with similar results.
Figure 8. A working model of PSK-induced immunity against *B. cinerea* in tomato plants. PSK plays as a damage-associated molecular pattern (DAMP), its precursors and protein processing are activated upon *B. cinerea* inoculation. At the apoplast, PSK signaling peptide is mainly perceived by its receptor PSKR1, which transduce the signal into cytoplasm by initiating cytosolic Ca$^{2+}$ influx. The transient cytosolic Ca$^{2+}$ are further transduced to CaMs which binds to YUCs, promoting auxin biosynthesis and associated signaling to combat *B. cinerea* infection.