Supplementary Materials: The Cell Death Triggered by the Nuclear Localized RxLR Effector PITG_22798 from *Phytophthora infestans* Is Suppressed by the Effector AVR3b

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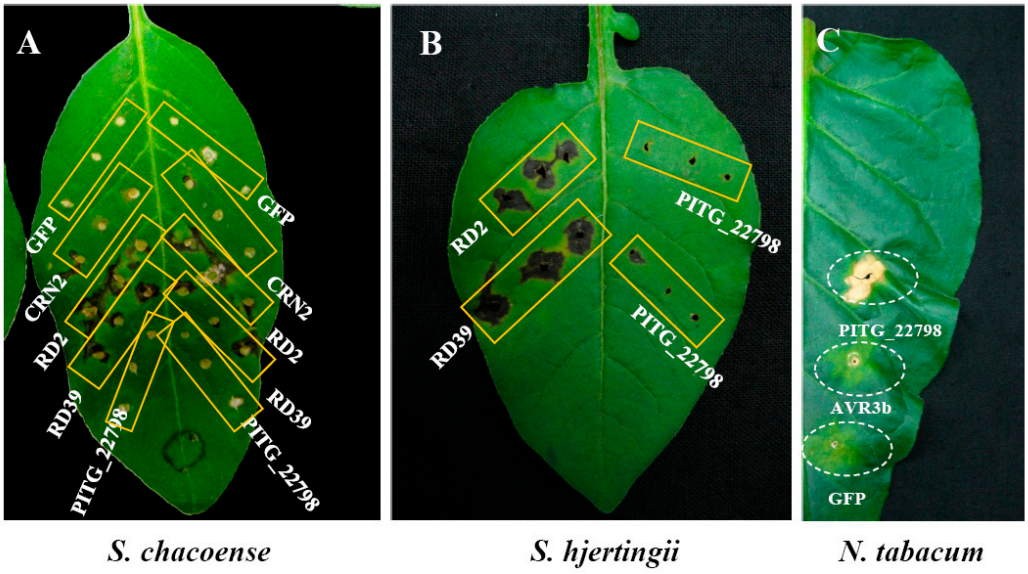

Figure S1. Functional screening of *P. infestans* effectors in wild potato species *S. chacoense*, *S. hjertingii*, and *N. tabacum*. *Agrobacterium* clones expressing *RD2*, *RD39*, and *PITG_22798* of *P. infestans* were tooth-pick inoculated in leaves of *S. chacoense* (**A**) and *S. hjertingii* (**B**). CRN2 (crinkling and necrosis induced protein gene 2) was used as a positive control and *GFP* (green fluorescent protein gene) was used as a negative control. Two weeks after inoculation, the expanding cell death was photographed. (**C**) Leaves of *N. tabacum* were infiltrated with *A. tumefaciens* carrying *PITG_22798*, *AVR3b*, and *GFP* (negative control). Photographs were taken 12 dpi.

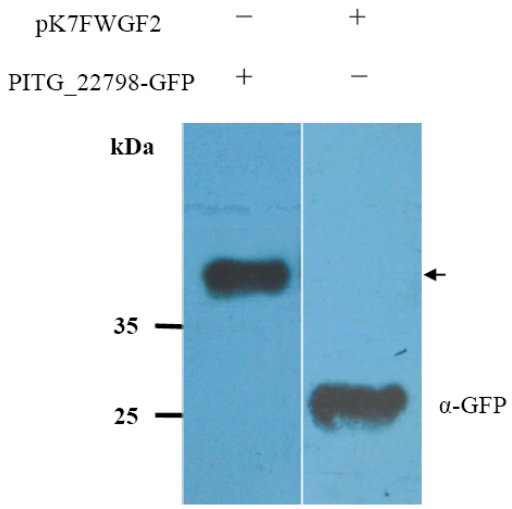

Figure S2. Western blot analysis of proteins PITG_22798-GFP and GFP-empty. Immunoblots show the stability of PITG_22798-GFP. Arrows indicated the expected size (43 kDa). Plus (+) and minus (−) signs indicate the presence or absence respectively.

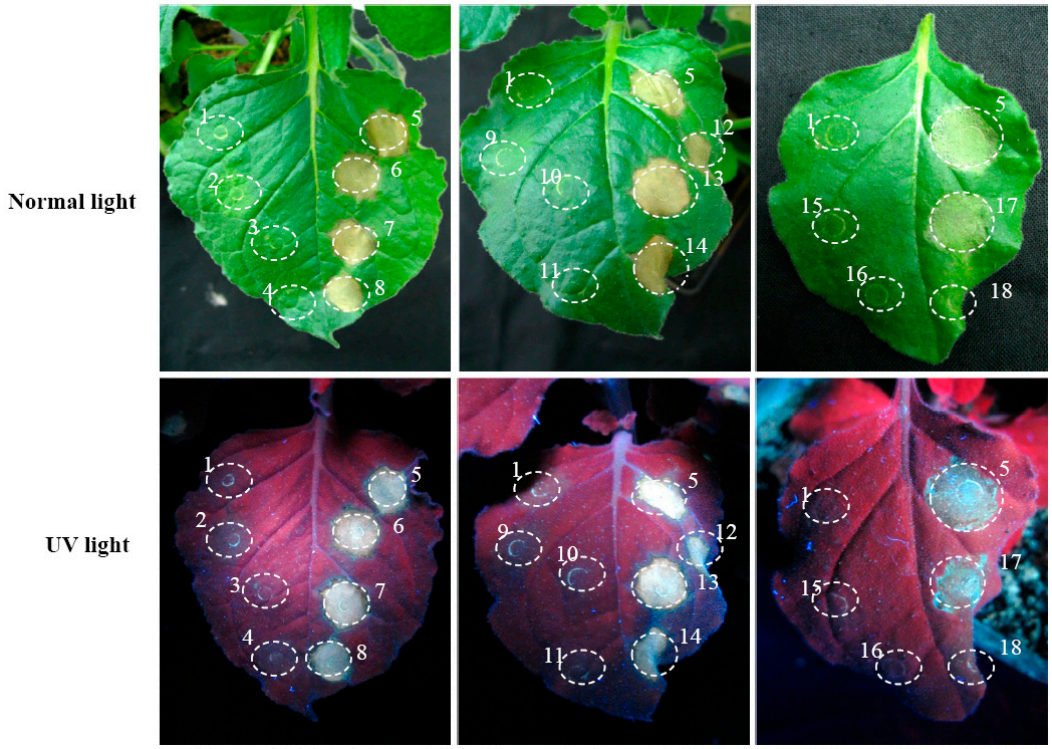

Figure S3. Test of effectors on the suppression of *PITG_22798*-induced cell death. *PITG_22798* was co-agroinfiltrated with the following effectors or controls in a 1:1 ratio with a final OD600 of about 0.4: (1) *GFP*; (2) *AVR2*; (3) *PITG_21388*; (4) *PITG_14783*; (5) *GFP+PITG_22798*; (6) *AVR2* + *PITG_22798*; (7) *PITG_21388* + *PITG_22798*; (8) *PITG_14783* + *PITG_22798*; (9) *PITG_20303*; (10) *PITG_13959*; (11) *PITG_23008*; (12) *PITG_20303* + *PITG_22798*; (13) *PITG_13959* + *PITG_22798*; (14) *PITG_23008* + *PITG_22798*; (15) *AVR3a*^{K1}; (16) *AVR3b*; (17) *AVR3a*^{KI} + *PITG_22798*; (18) *AVR3b* +*PITG_22798*. Pictures were taken at 7 dpi under normal light and UV light.

Table S1. Primers used in vectors construction, gene cloning, and PCR.

Primer Name	Gene Accession No.	Sequence (5′–3′)	Corresponding Plasmids
PITG_22798$^{1\text{-}170}$-F	XM_002998349	GCGATCGATATGAGATGCTACTACGTCCTTA	pGR106-*PITG_22798*$^{1\text{-}170}$
PITG_22798$^{1\text{-}170}$-R		GATGCGGCCGCTTAATCGTTGGTCCTCTTCCTTT	
PITG_22798$^{23\text{-}170}$-F		GCGATCGATATGGACTCAAGGCACAACAAGTTAGC	pGR106-*PITG_22798*$^{23\text{-}170}$
PITG_22798$^{23\text{-}170}$-R		GATGCGGCCGCTTAATCGTTGGTCCTCTTCCTTT	
PITG_22798$^{40\text{-}170}$-F		GCATCGATATGAGAGTGGAGCTATTTCTGCGTCGGA	pGR106-*PITG_22798*$^{40\text{-}170}$
PITG_22798$^{40\text{-}170}$-R		GATGCGGCCGCTTAATCGTTGGTCCTCTTCCTTT	
PITG_22798$^{40\text{-}156}$-F		GCATCGATATGAGAGTGGAGCTATTTCTGCGTCGGA	pGR106-*PITG_22798*$^{40\text{-}156}$
PITG_22798$^{40\text{-}156}$-R		GATGCGGCCGCATCAGCAGACTTTACGAAGAGCTCG	
PITG_22798$^{47\text{-}170}$-F		GCATCGATATGCGGAATGATGAATTGGACGCTG	pGR106-*PITG_22798*$^{47\text{-}170}$
PITG_22798$^{47\text{-}170}$-R		GATGCGGCCGCTTAATCGTTGGTCCTCTTCCTTT	
PITG_22798-GFP-F		AAAAAGCAGGCTTCACCATGGACTCAAGGCACAACAAGTT	pK7FWG2-*PITG_22798*
PITG_22798-GFP-R		AGAAAGCTGGGTCTTAATCGTTGGTCCTCTTCC	
ΔNLS-PITG_22798-GFP-F		AAAAAGCAGGCTTCACCATGGACTCAAGGCACAACAAGTT	pK7FWG2-ΔNLS-*PITG_22798*
ΔNLS-PITG_22798-GFP-R		AGAAAGCTGGGTCTTAATCGTTATCAGCAGACTT	
nls-PITG_22798-GFP-F		AAAAAGCAGGCTTCACCATGGACTCAAGGCACAACAAGTT	pB7WGF2-*PITG_22798*^{165A166A167A}
nls-PITG_22798-GFP-R		AGAAAGCTGGGTCTTAATCGTTGGTTGCTGCTGCTTTTAGCTT	
AVR3b-F	XM_002997802	ATCCCGGGATGACGTACTCGACTTCAAAGGGGGA	pGR106-*AVR3b*
AVR3b-R		GATGCGGCCGCTTAGAAATTGTTCTTTGCGGTCA	
PITG_23008-F	XM_002899560	GCATCGATATGAATTCTGCGGTTGCGGGCAAG	pGR106-*PITG_23008*
PITG_23008-R		GATGCGGCCGCTTATTTATAACCCAGTCTCATT	

Table S1. *Cont.*

Primer Name	Gene Accession No.	Sequence (5′–3′)	Corresponding Plasmids
PITG_21388-F	KF154438	CGCATCGATGGTTTCATCCAATCTCAACACCGCCG	pGR106-*PITG_21388*
PITG_21388-R		GATGCGGCCGCTATACGATGTCATAGCATGACA	
TRV-NbSGT1-F	AF494083	GCGAATTCCGAACAAGGCCATTGAGTTA	TRV-*NbSGT1*
TRV-NbSGT1-R		GATGGATCCCTCCTCTGGCTTCTGGTAAA	
TRV-NbHSP90-F	AY368904	GCGAATTCTGTCTGGGAATCTCAAGC	TRV-*NbHSP90*
TRV-NbHSP90-R		GATGGATCCTTCGTCAACCTCCTCTAC	
RT-PITG_22798-F		GACTCAAGGCACAACAAGTTAGC	
RT-PITG_22798-R		TTAATCGTTGGTCCTCTTCCTTT	
RT-NbSGT1-F	AF494083	TCGCCGTTGACCTGTACACTCA	
RT-NbSGT1-R		GCAGGTGTTATCTTGCCAAACA	
RT-NbHSP90-F	AY368904	ATGATTGGGCAATTTGGT	
RT-NbHSP90-R		ACACGACGCACATACAGC	
Nbef-1α-F	AY206004	CCAAGCTGACTGTGCTGTCC	
Nbef-1α-R		AAGCAAGCAATGCGTGCTC	
PiEF2-F	XM_002901697	TGACGCTATCGCCAAGGAATC	
PiEF2-R		TAACGCTGAGCCGTAATGGGGG	

Underline represent restriction sites, *Cla*I site (ATCGAT), *Sma*I site (CCCGGG), *Not*I site (GCGGCCGC), *BamH*I site (GGATCC), and *Eco*RI site (GAATTC).

Table S2. *Phytophthorainfestans* isolates used in this study.

Isolate	Virulence Spectra	Reference
EC1_DC2005	1.3.4.7.10.11	[1]
Ljx18	3.4.7.10.11	[2]
IPO-C	1.2.3.4.5.6.7.10.11	[3]
UK3928A	1.2.3.4.5.6.7.9.10.11	[4]
88069	1.3.4.7	[5]
PIC99183	1.3.4.5.7.8.10.11	[6]
HB09-41	1.2.3.4.5.6.7.8.9.10.11	Unpublished data
HB09-21	1.2.3.4.5.6.7.8.9.10.11	Unpublished data
HB09-23	1.2.3.4.5.6.7.8.9.10.11	Unpublished data
HB09-16-2	1.2.3.4.5.6.7.8.9.10.11	Unpublished data
HB09-14-2	1.2.3.4.5.6.7.8.9.10.11	[2]

Table S3. The RxLR effectors used in this study.

No.	RxLR Gene	*P. infestans* Isolate for Cloning Corresponding Gene
1	*AVR2*	88069
2	*AVR3a*^{KI}	88069
3	*AVR3b*	PIC99183
4	*PITG_21388*	HB09-14-2
5	*PITG_14783*	HB09-14-2
6	*PITG_20303*	HB09-14-2
7	*PITG_23008*	88069
8	*PITG_13959*	HB09-14-2
9	*PITG_22798*	88069
10	*PITG_22798*	Ljx18
11	*PITG_22798*	99183

The RxLR effectors were selected and cloned into the pGR106 vector according to previous studies [7,8].

Reference

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