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Supplementary Material

Expression of a *Nicotiana tabacum* pathogen-induced gene is involved in the susceptibility to black shank

Roxana Portieles^A, Eduardo Canales^A, Osmaní Chacon^B, Yussuan Silva^B, Ingrid Hernández^A, Yunior López^A, Mayra Rodríguez^A, Ryohei Terauchi^C, Hideo Matsumura^D, Carlos Borroto^E, Jonathan D. Walton^F, Ramon Santos^G and Orlando Borrás-Hidalgo^{A,H}

^ACentre for Genetic Engineering and Biotechnology, PO Box 6162, Havana, 10 600, Cuba.

^BTobacco Research Institute, Carretera de Tumbadero 8, PO Box 6063, Havana, Cuba.

^CIwate Biotechnology Research Centre, Kitakami, Iwate, 024-0003, Japan.

^DGene Research Center, Shinshu University, Ueda 386-8567, Japan.

^ECentro de Investigación Científica de Yucatán, Calle 43 No. 130, Colonia Chuburná de Hidalgo, 97200 Mérida, Yucatán, México.

^FDepartment of Energy Plant Research Laboratory, Michigan State University, East Lansing, USA 48824.

^GCentro de Bioplantas, Carretera de Morón Km 9, Ciego de Ávila, C. P. 69450, Cuba.

^HCorresponding author. Email: orlando.borras@cigb.edu.cu

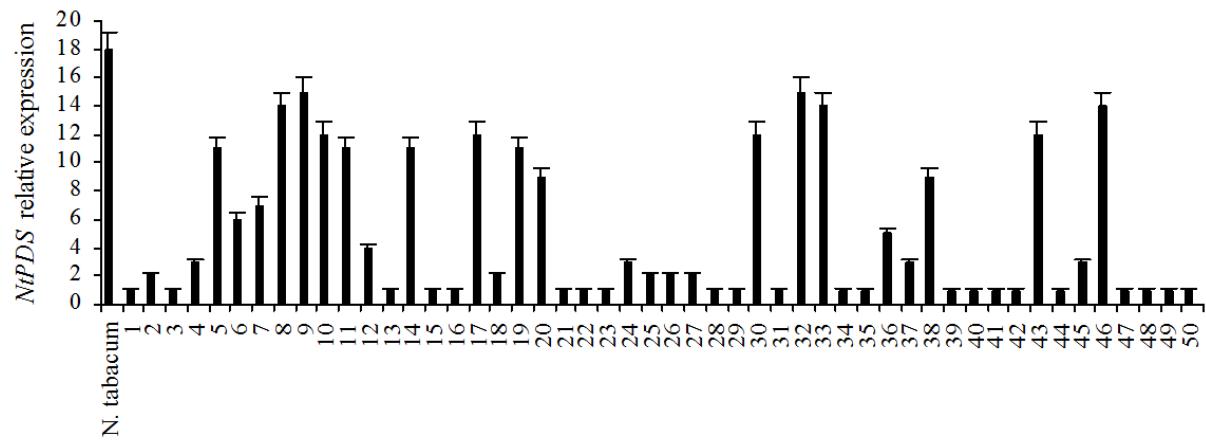
NtPIP	MVLETQLGLTNHQSYAHERNYYSDSSHGGSMQMTRPSGYSTMYPQGQSTHIHMLMGHG C QH
Q9FXT0	MVLQTQLGLNKHQSYAHEQNYYSDSSHGGSMQMTRPSGYSTMYPQGQSTHNHMMMGHGGQH
F2VJ77	MVLQTQLGLNKHQSYAHEQNYYSDSSHGGSMQMTRPSGYSTLPYDQSTHNHMMMGHGGQH ***** ***** ***** ***** ***** ***** ***** ***** ***** ***** ***** *****
NtPIP	HGGHYGGHDHGHHGGYYGSHGHGAHM P HDSTNISS C TSMIHSDGGYGSGM Q SAHM T AM
Q9FXT0	HGGHYGGHDHGHHGGYYGSHGHGAHM P HDSTNFSSSTSMI I HS D GGYGSGM Q SAHM S AM
F2VJ77	HGGPFGGHGHGHGGQYGS H GHHGAHM P HHSANFSSSTNMVHS D GGYGSGM Q STHM P AM ***** ***** ***** ***** ***** ***** ***** ***** ***** ***** ***** *****
NtPIP	GMASTNYHG H GYGGSHPSHYSQS Q KFNWALKDLEE
Q9FXT0	GMG S TNYHG H GYGGSHPSHYSQS Q KFNWALKDLEE
F2VJ77	GMGLTNYHG H GYGGSHPS Q YSQS Q KFNWALKDLEE ** ***** ***** ***** ***** ***** *****

Fig. S1. Comparison of the putative sequence of *NtPIP* protein and those of other *N. tabacum* species. The sequence alignment was performed with CLUSTAL X software (Thompson *et al.* 1997). The yellow shadow represent the substitution of amino acid in *NtPIP* regard the others members.

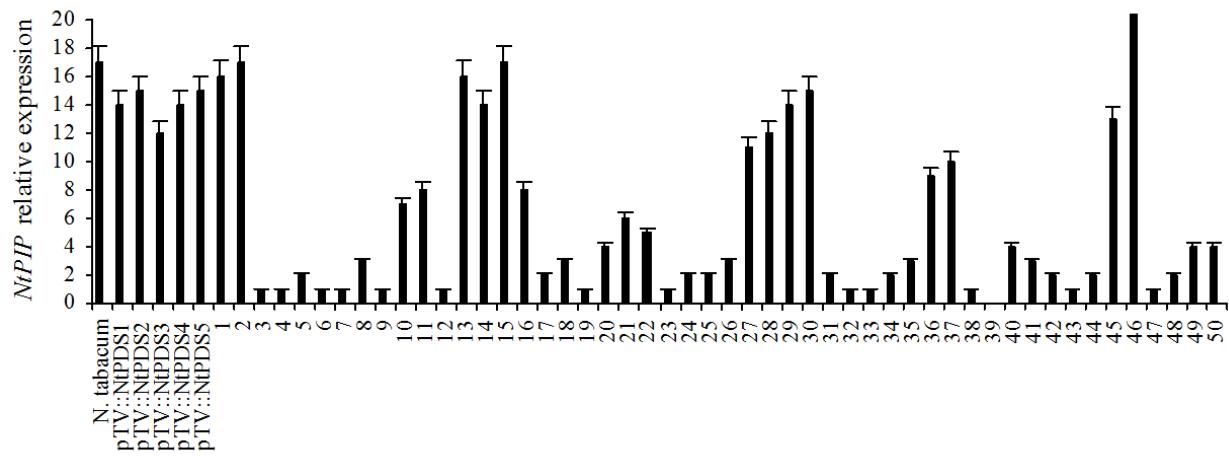
Reference

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**, 4876–4882. doi:10.1093/nar/25.24.4876

(a)



(b)



(c)



Fig. S2. Greenhouse evaluation of reaction of *N. tabacum* cv. 'Sumatra' plants with a silenced *NtPIP* gene to *P. parasitica* var. *nicotianae*. Relative expression of *NtPDS* (a) and *NtPIP* (b) genes in *N. tabacum* cv. 'Sumatra' plants at 20 days post infiltration. Bars represent mean values and standard error of the results obtained from three replicates. Real-time PCR was used to measure the relative expression of transcript level of *NtPDS* and *NtPIP* as compared to the constitutively expressed 26S rRNA gene used as an endogenous control. A total of 15 plants with the lowest *NtPIP* relative expression were used to calculate the stem disease rating after disease susceptibility testing. (c) Disease phenotype of silenced (center) and non-silenced *N. tabacum* cv. 'Sumatra' (left and right) plants at 5 days post inoculation.

Table S1. Primers used in this study

Primers used for 3'-RACE PCR and real time PCR	
Tag_4774	5'-TATGGTGAAAGTATGTGTGTTT-3'
Tag_7582	5'-GCTGCTGTCGGAATAGCAGTTT-3'
Tag_25308	5'-GGGCATATGCCGCCCTGATGT-3'
Tag_22906	5'-GCTGCTGTCGGAATAACAGTTT-3'
Tag_18123	5'-GCTGACGACATATGGGTAGATT-3'
Tag_11525	5'-GCCATGGTTATGGTGGCAGCCA-3'
Tag_42	5'-GTTATGGTGGCAGCCACCCTAG-3'
Primers used for construction of VIGS vectors.	
<i>NtPIP</i> Fw	5'-CATGGCGGCTCTATGCAAATGAC-3'
<i>NtPIP</i> Rv	5'-CAGTATGATCCATAGTGTGATGGTG-3'
PDS Fw	5'-TTCTTCAGGAGAACATGGTTCAA-3'
PDS Rv	5'-TCCACAATCGGCATGCAA-3'
Primers used to evaluate gene silencing efficiency VIGS-silenced plants	
<i>NtPIP</i> Fw	5'-CATGGCGGCTCTATGCAAATGAC-3'
<i>NtPIP</i> Rv	5'-CAGTATGATCCATAGTGTGATGGTG-3'
PDS Fw	5'-TTCTTCAGGAGAACATGGTTCAA-3'
PDS Rv	5'-TCCACAATCGGCATGCAA-3'
Primers used to transient expression of <i>NtPIP</i> gene	
<i>NtPIP</i> Fw	5'-ATCGATATGGTTCTCCAAACTCAAC-3'
<i>NtPIP</i> Rv	5'-GTCGACTTATTCCCTCCAAATCCTTAAGAGC-3'
Reference gene primers	
26S rRNA Fw	5'-CACGGACCAAGGAGTCTGACAT-3'
26S rRNA Rv	5'-TCCCACCAATCAGCTTCCTTAC-3'