

**TABLE S1** List of primers used in this study

<b>Primer</b>	<b>Sequence 5' to 3'</b>	<b>Description</b>	<b>PCR<sup>a</sup></b>
primer-1	GCGTCGATGCAGATTCTTA CTTA	flanking region upstream of <i>sol4</i> FP <sup>b</sup>	838
primer-2	TTGACCTCCACTAGCTCCA GCCAAGCCCATCGTGTGT ACTCATGTGGTT	flanking region upstream of <i>sol4</i> RP <sup>c</sup> with overhang sequence of 5' end of <i>hph</i>	
primer-3	GCAAAGGAATAGAGTAGA TGCCGACCGTTAGGCGAT GGAGTAGAGAGAAG	flanking region downstream of <i>sol4</i> RP with overhang sequence of 3' end of <i>hph</i>	1,094
primer-4	CATCCAAGCGAGCGAGAA TGGAG	flanking region downstream of <i>sol4</i> FP	
HYG-F	GGCTTGGCTGGAGCTAGT GGAG	FP to amplify <i>hph</i> from pDWJ5	1,372
HYG-R	CGGTCGGCATCTACTCTAT TCCTT	RP to amplify <i>hph</i> from pDWJ5	
primer-7	AGAGTAAGCGGAGCGGAT CAAAG	flanking region upstream of <i>sol4</i> FP nested primer	
primer-8	CCAGAAGTGGACCTCTGA TGCGA	flanking region downstream of <i>sol4</i> RP nested primer	
YG-F	CGATGTAGGAGGGCGTGG ATATGTCC	FP coding region of <i>hph</i> for overlapping extension, pair with primer-8	
HY-R	GTATTGACCGATTCTTGC GGTCCGAA	RP coding region of <i>hph</i> for overlapping extension, pair with primer-7	
primer-11	GTGTGTCCACCTGCCTATG TATC	FP upstream of <i>sol4</i> , verification primer	1,950 (in wild type)
primer-12	CACGCTTCTTCCCGTGCAT CTG	RP downstream of <i>sol4</i> , verification primer	1,581 (in <i>sol4</i> )
Sol4BamH5	GGATCCATGATGCCGTCCA CCCTCATC	FP at ATG with <i>Bam</i> HI cut site for 4OE construction	2,029
Sol4Hind3	AAGCTTTCGCAAGGTCTG GGAAATTC	RP at +276 bp from stop codon with <i>Hind</i> III cut site for 4OE construction	

<sup>a</sup> PCR = PCR product length in basepair; <sup>b</sup> FP = forward primer; <sup>c</sup> RP = reverse primer

**TABLE S1** Continued

<b>Primer</b>	<b>Sequence 5' to 3'</b>	<b>Description</b>	<b>PCR<sup>a</sup></b>
Sol4OE5	GTTGTGTGTCCACCTGCCT ATG	FP at +92 bp from ATG for confirmation of 4OE strains	2,187 (with Sol4OE5 and Sol4OE3 primer pair)
Sol4OE3	CGGGAAGCTGCGAGAAGA TAAG	4OE RP at +354 bp from stop codon for confirmation of 4OE strains	
DW38	AGATGGTCAACGCTGCTT AC	<i>pelA</i> promoter primer for confirmation of 4OE strains, 141 bp away from <i>PvuII</i> site	≈ 2,200 (with DW38 and Sol4OE3 primer pair)
Sol1RT-F	TTGGTATTGGTTCGCTCGA GGT	RT-PCR for <i>sol1</i>	530 (615, for gDNA or pre-mRNA)
Sol1RT-R	TCAACAGCGGTTGACATC CTCT		
Sol2RT-F	CACATCTCCATGGCTTTGG CTC	RT-PCR for <i>sol2</i>	390 (470, for gDNA or pre-mRNA)
Sol2RT-R	GTTCGCTGCTTAGCACCC AGAA		
Sol3RT-F	GTTCGCCTTGATGGCAAG ACTG	RT-PCR for <i>sol3</i>	572 (622, for gDNA or pre-mRNA)
Sol3RT-R	CGCGCATCCAGAGGATGT TCAA		
Sol4RT-F	TGGATCAACCAGATCGTC CATC	RT-PCR for <i>sol4</i>	522 (588, for gDNA or pre-mRNA)
Sol4RT-R	CTTACGGTGCAGTACGCAT CTA		
Sol5RT-F	AGAACCCAGCGTGCATCT ATAC	RT-PCR for <i>sol5</i>	534 (631, for gDNA or pre-mRNA)
Sol5RT-R	GATCATGGAACCTCCCAT ATC		
Sol6RT-F	GCAAAGTGCTAACACCCG CTCT	RT-PCR for <i>sol6</i>	540 (606, for gDNA or pre-mRNA)
Sol6RT-R	CGTTTAGCTGTTCTAGGCT TGG		

<sup>a</sup> PCR = PCR product length in basepair.

**TABLE S1** Continued

<b>Primer</b>	<b>Sequence 5' to 3'</b>	<b>Description</b>	<b>PCR<sup>a</sup></b>
Actin1RT-F	CAATGGTTCGGGTATGTGC AAG	RT-PCR for <i>Actin1</i>	482 (633, for gDNA or pre-mRNA)
Actin1RT-R	GAAGAGCGAAACCCTCGT AGAT		
Sol1realt-F	GTTGGCATGGGCTGTAGAT G	Real-time RT-PCR for <i>sol1</i>	129
Sol1realt-R	ATGGTGGAAATCCCTTGCG AG		
Sol2realt-F	CACTACATGCTCGATGAAT GC	Real-time RT-PCR for <i>sol2</i>	123
Sol2realt-R	GAACATTGGCACACCGAA G		
Sol3realt-F	CGTTCAGGTTAATCACCTG G	Real-time RT-PCR for <i>sol3</i>	126
Sol3realt-R	CAGAAGTGGACCTCTGAT G		
Sol4realt-F	CAACCTTCGCCTTGCAAA AG	Real-time RT-PCR for <i>sol4</i>	140
Sol4realt-R	GAAGTACTCTCTCGGTGA AC		
Sol5realt-F	CGCGAACAATTTTGGCATT G	Real-time RT-PCR for <i>sol5</i>	162
Sol5realt-R	ATGGCAGACTTCTTGTC CA G		
Sol6realt-F	CAAGCAATACGGACCGGT G	Real-time RT-PCR for <i>sol6</i>	135
Sol6realt-R	GGAAACGAGATGCATCGA TAG		
ORF2realt-F	ATTGGACCCGCACCAAAT AC	Real-time RT-PCR for ORF2	140
ORF2realt-R	GTCTTCATGGGATCTCCAA GG		

<sup>a</sup> PCR = PCR product length in basepair.

**TABLE S1** Continued

<b>Primer</b>	<b>Sequence 5' to 3'</b>	<b>Description</b>	<b>PCR<sup>a</sup></b>
ORF3real-F	CCGCTCTAGCGATAAGAA GG	Real-time RT-PCR for ORF3	136
ORF3real-R	CGTAGCAACCTGATGCAA C		
ORF10real-F	CACTGCCATCCTTGAGAC AG	Real-time RT-PCR for ORF10	127
ORF10real-R	GAGACTTCGCTGTTCTTGC C		
Pseudo6 real-F	GCACTTTGACAGGCATAC AAAG	Real-time RT-PCR for pseudogene 6	144
Pseudo6 real-R	GAGTGTGGAGGCATGCAT AG		
PKS1real-F	CAACATGTCTCCACGTGA AG	Real-time RT-PCR for PKS1	130
PKS1real-R	AATGCGGTTTCAGCTTTGTG G		
PKS2real-F	GAACCTTGCTGGTGCATC G	Real-time RT-PCR for PKS2	123
PKS2real-R	GCTTTGGACAGCGACTTG AG		
Actin1real-F	GTATCATGATCGGTATGGG ACAG	Real-time RT-PCR for <i>Actin1</i>	134
Actin1real-R	CCAGATCTTCTCCATGTCG TCC		

<sup>a</sup> PCR = PCR product length in basepair.

**TABLE S2** The locations of putative binding motifs for a Zn(II)<sub>2</sub>Cys<sub>6</sub> zinc cluster transcription factor in the promoter regions of biosynthetic genes for solanapyrone production

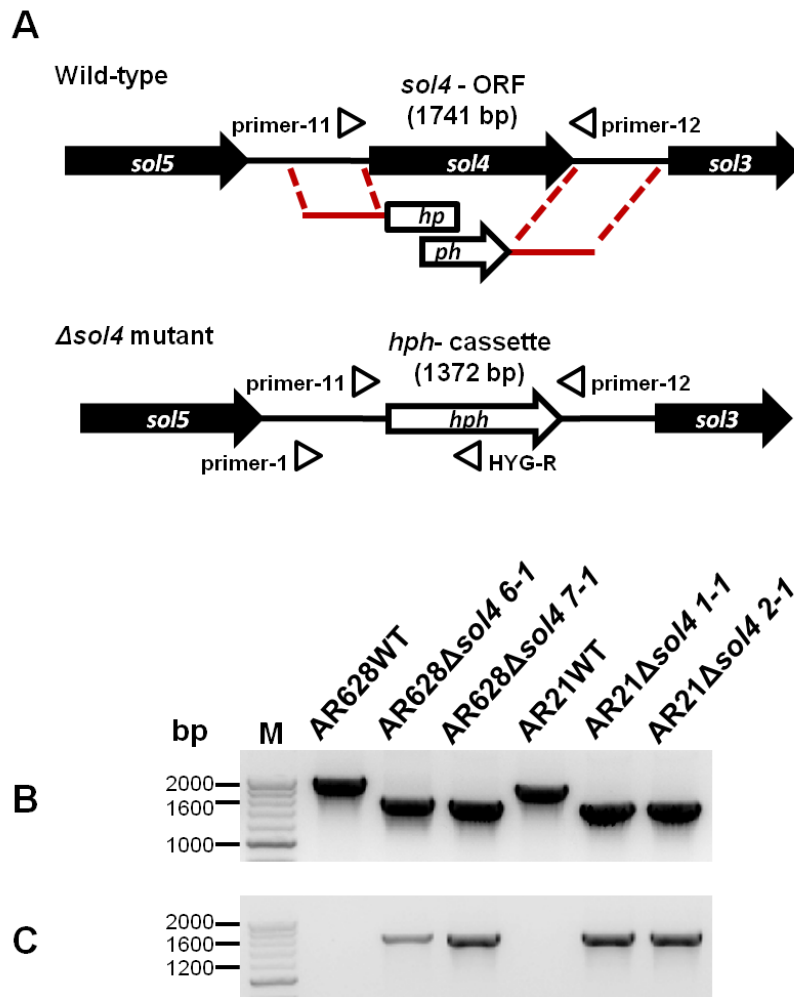
Gene <sup>a</sup>	Binding site I <sup>b</sup>	Binding site II <sup>c</sup>
AR <i>sol1</i>	285 <sup>d</sup> , [G <b>CA</b> (N <sub>10</sub> )GGC]	None
AR <i>sol2</i>	320, [ <b>ACC</b> (N <sub>10</sub> )GGC]	321, [CCG(N <sub>8</sub> )CGG]
AR <i>sol3</i>	252, [GCC(N <sub>10</sub> )GGC]	253, [CCG(N <sub>8</sub> )CGG]
AR <i>sol5</i>	355, [GCC(N <sub>10</sub> )GGC]	356, [CCG(N <sub>8</sub> )CGG]
AR <i>sol6</i>	224, [GCC(N <sub>10</sub> )GGC]	225, [CCG(N <sub>8</sub> )CGG]
AS <i>sol1</i>	438, [G <b>AC</b> (N <sub>10</sub> )GGC]	439, [ <b>ACG</b> (N <sub>8</sub> )CGG]
AS <i>sol2</i>	320, [ <b>ACC</b> (N <sub>10</sub> )GGC]	321, [CCG(N <sub>8</sub> )CGG]
AS <i>sol3</i>	259, [GCC(N <sub>10</sub> )GGC]	260, [CCG(N <sub>8</sub> )CGG]
AS <i>sol5</i>	357, [GCC(N <sub>10</sub> )GGC]	358, [CCG(N <sub>8</sub> )CGG]
AS <i>sol6</i>	310, [GCC(N <sub>10</sub> )GGC]	311, [CCG(N <sub>8</sub> )CGG]

<sup>a</sup> AR and AS prefixes indicate *Ascochyta rabiei* and *Alternaria solani*, respectively.

<sup>b</sup> Perfect sites contain the 5'-GCC(N<sub>10</sub>)GGC-3' consensus motif. Imperfect sites contain a single base pair mismatch in one of the two triplet half sites (GCC or GGC), highlighted in bold.

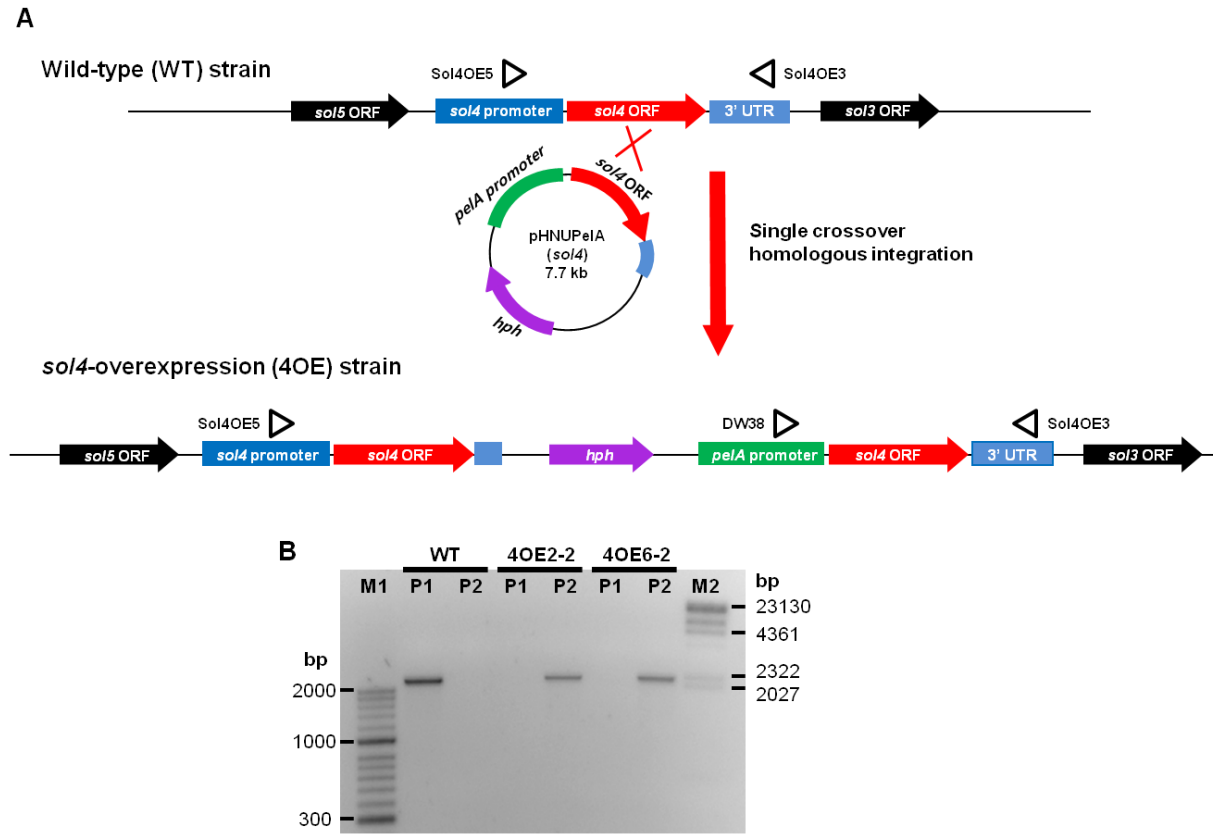
<sup>c</sup> Perfect sites contain the 5'-CCG(N<sub>8</sub>)CGG-3' consensus motif. Imperfect sites contain a single base pair mismatch in one of the two triplet half sites (CCG or CGG), highlighted in bold.

<sup>d</sup> The sites represent those found within 500 bp upstream of the ATG start codon. The numbers indicate relative position of the binding site from ATG start codon.



**FIG S1** Gene replacement via split-marker strategy and PCR analysis of *sol4*-deletion. (A)

Schematic diagram of targeted gene replacement strategy. (B) PCR analysis of *A. rabiei* wild-type isolates and their  $\Delta$ *sol4* mutants. Replacement of the *sol4* gene by the *hph* cassette was verified in two independent mutants from each isolate using a primer pair, primer-11 and primer-12 (wild-types, 1,950 bp;  $\Delta$ *sol4* mutants, 1,581 bp). (C) PCR verification for homologous integration of the replacement fragment to the correct genomic site with a primer pair, primer-1 and HY-R (1,604 bp).



**FIG S2** Verification of integration of pHNUPelA plasmid carrying *sol4* open reading frame in *sol4*-overexpression (4OE) strains. (A) Strategy to overexpress the *sol4* gene and positioning of the primers used for a diagnostic PCR. (B) PCR analysis of AR628 wild-type (WT) strain and two independent 4OE strains confirmed a single crossover event at the *sol4* genomic locus in 4OE strains, using two primer pairs, Sol4OE5 and Sol4OE3 (P1) and DW38 and Sol4OE3 (P2). M1 = 100 bp DNA ladder, M2 =  $\lambda$  DNA digested with *HindIII*.





**FIG S3** Multiple DNA sequence alignment of *Molly* transposon from *Stagonospora nodorum* and degenerated transposons (pseudogene 4–6) located proximal to the solanapyrone gene cluster in *A. rabiei*. Identical nucleotides are shaded in black. The regions marked with purple line indicate terminal inverted repeat. The coding regions of putative transposase are indicated by arrows. The first RIP mutation (CpA to TpA) which introduced premature stop codon in the transposase domain of pseudogene 4, 5, or 6 are indicated by red boxes in comparison to the functional *Molly* transposon. Note that the four elements are of nearly identical length and include 5' and 3' TA insertion sites.



**FIG S4** Pairwise sequence alignment of *ORF10* and a *P450* homologous gene found in *Coniosporium apollinis*. (A) Pairwise comparison of deduced amino acid sequence of *ORF10* and the homologous *P450* gene from *Coniosporium apollinis* (GenBank accession: XP\_007784573). Only a region conserved with *ORF10* (240 out of 535 residues) was shown for the *P450*. Alignment shaded to indicate similarity with black corresponding to blocks of identical residues, and with grey corresponding to conservative substitutions. Pairwise DNA sequence comparison between upstream of *ORF10* and the corresponding region of the hypothetical protein (B), between the coding region of *ORF10* and the corresponding region of the hypothetical protein (C) and between downstream of *ORF10* and the corresponding region of the hypothetical protein (D). Note that the coding region of *ORF10* has no indels and is more conserved with the hypothetical protein compared with the upstream or downstream regions.

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KM_244525 001 -----
XP1397039 001 MHPTGRKRRVITCVPCYTRKQRCNRQYPCNHCTRRRRPEECVYQSIITGDPSNTFLLEP-
XP1905776 001 MQSSQRKRVSVCIPCYTRKQRCNRQYPCNHCSRRRRPEQCAVNPSEQATLPPSPPTQKD

KM_244525 001 -----
XP1397039 060 -ARDPE-----PQEPNVQLPNGSSKASP--AEAQNSASNSHSALAKSFGYFEHSDSNT
XP1905776 061 HLHDEIQTDDSQEQAQAERRPASSSSVQDTINWGGLKEGREPTSLAEVFGYFENSKSNT

KM_244525 001 -----MPSTLINDVQCELKLLHARGIVDFLIQYFEDINWINQ
XP1397039 111 MALLKKWDLDEGDTDQSTQG-LSTAVLETVKQVLEKTPFRFILDFLMQYFVDEINWVKQ
XP1905776 121 IALVRKLGADDDATGHSSEPAFVPEETAIIAQRLFASMPNRSILDFLWRYEIAEVSWMQD

KM_244525 039 IVHPPRLLAQYEDWKKMDTISRVADEFAVLMRLICAYASHFLPSQKYTVDTIKGRPLSD
XP1397039 170 ITHPPSELTQYQHWTKKWPLSVDDIEFALVLRICAYSAQFLPSPTHLLDRIRGQSLSD
XP1905776 181 LIYPPWFLSHYQKWWEMERTSTAYGIEFAVLVLRICSYASQFLPSPTCTLDSIRGVLAD

KM_244525 099 IRSNCDRLAGLEGICNAAVLRGSLVRVCHMAFTAMCYECSRIKLSWATLCCAIREAQE
XP1397039 230 IRDTCSEHLGSLARRACLNLNWRGSLVRVCHLFAAFRSSCEGRDCKPWEHTASACSAAOK
XP1905776 241 IRKSCDRVADALTFICSRLDARGLSLRVCHVAFAGTRSLCQGRTNAHWEALSCAVRVAQR

KM_244525 159 VGLHREPPKRC-DDGMDDLERELRRRRCFNLR-----LAKALDRVPFLVDAYCTVSLPQM
XP1397039 290 AGHTVAVPVC-EDSSQVLEKEMRRRITCGLYVLDSELARQLDRVPFLFDNLVLETLPRRL
XP1905776 301 IGLEVDTATSGFYSPNMDELEKEMRRRRCFNLYVWDSVLSKRLDCRPFIPDALNPDTPRM

KM_244525 213 HLEETIANL----CAPDLFTErvLOAQLVRFWKKLEAGNSAPGAFPYDPVIAEERYQRFC
XP1397039 349 RLAEFTICDLAASADAPEFTErLMOVRLGRFWRSFGSRR----NIPYDPTGEGQRYERFC
XP1905776 361 HLVLGLDDV-PQADAPDLFTErvLEAQLANFWRTHGSGN----ATEYDPTAAEERYEKFC

KM_244525 269 NEFLPELPAFALEFN--TDWDKHIPDLERQRVLFHVALFESVMHNFROLRLDQHHLRS
XP1397039 405 AEYIPILPPAVALNPD--RRWDGQLPELPMORCLLYIAFFSVQGNFRFLLIKPGQVTS
XP1905776 416 SEFLPEIPVFALYEDTCDRWDARLPLELQRCQMFMAILESLCHNFRPALFQEPNSIQQ

KM_244525 327 LPNSRMLVTCHRHTLATAAMGLFQSVTSLHAMMSFNQTKLSLVIEYHFETAVVLSL-CI
XP1397039 463 LPPYKQVLLQSQKQLALAAEELDAVTPLHIMFGGSHTRFSAIISNTFERAVVLLILCT
XP1905776 476 LPAYKQILLSEKRALAVIAANILEGVSLHLMMGGSHTRHASLIIPTFERAVVLLCLCS

KM_244525 386 LQACDSNGMQII---EHINFFSLHSPLSPNMIDISCQCLRAIKRARRCLEMMSLISVMA
XP1397039 523 QKDFPFQGE-----DHPDILGLRVATLTPRRMRAVERALGRLCMIAEVSEMA
XP1905776 536 DGNFPKAEITLARGSTTSMTKSSDPEFGVWTDVAKDECIQAVRSALGRLOTADMNHMA

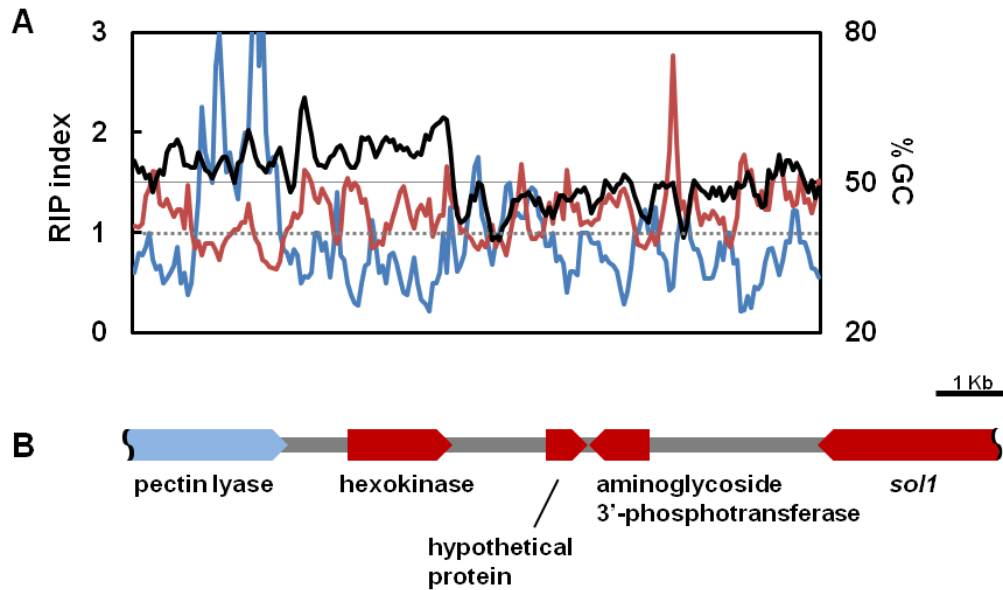
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XP1397039 572 ASCAQVVTQLFAKAARMEH----SLEPYTPNESRS-SSSPAILSN-----
XP1905776 596 EVGARTLTRLGKTEKISD----TTVSEA-NTLRL-EDPIVVD-----

KM_244525 503 QRQEGPTANAFRTDSNVFEGMLWDLSTIALPLDPSFLGLEQLBMSL--QRMVGL---
XP1397039 613 -----FLNLEDGSGLWMSSEIPLMPD-MSTIAHGQSYSLQFPSHILTSW
XP1905776 636 -----PALQ---LSEANCLQTPDYAEVFLMENT-----PTTMSVFSW

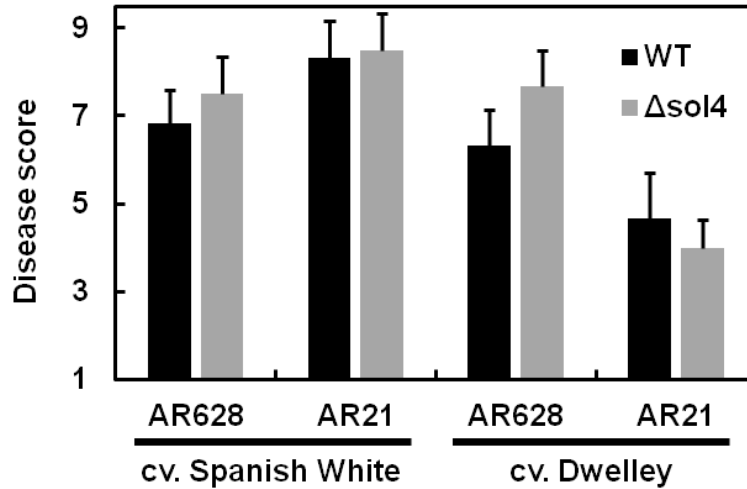
KM_244525 -----
XP1397039 659 NGNFHS
XP1905776 673 F-----

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**FIG S5** Multiple amino acid sequence alignment of *A. rabiei* Sol4 (KM244525) and two hypothetical proteins from *Aspergillus niger* (XP\_001397039) and *Podospora anserina* (XP\_001905776). Alignment was shaded to indicate similarity with black corresponding to blocks of identical residues, and with grey corresponding to conservative substitutions. Zn(II)<sub>2</sub>Cys<sub>6</sub> (C<sub>6</sub>) zinc cluster DNA-binding domain is boxed in purple and middle homology region (MHR) is marked with blue line. The coiled coil region in Sol4 amino acid sequence detected by COILS program (1) is boxed in green (a region with prediction values greater than 0.8 in scanning windows of 14). No coiled coil region was predicted for the two hypothetical proteins. Note that Sol4 lacks the C<sub>6</sub> zinc cluster DNA-binding domain.



**FIG S6** Gene content of the flanking region of *sol1* gene in *Alternaria solani*. (A) GC content (%), black line), RIP index I (TpA/ApT, blue line) and RIP index II (CpA+TpG/ApC+ GpT, red line) were calculated in a 200-bp window, which was slid in 50-bp increments across  $\approx 10$  kb of the flanking sequence. Horizontal solid lines are provided to show the 50% GC mark, while dashed line shows a RIP index value of 1. (B) Schematic diagram of arrangement and orientation of three open reading frames (red boxes) and one pseudogene (blue box). Genes found were pectin lyase (similar to XM\_001796354, 67% identity,  $E$ -value =  $4e-155$ ), hexokinase (similar to XP\_008029266, 94% identity,  $E$ -value = 0), hypothetical protein (similar to XP\_007692661, 88% identity,  $E$ -value =  $1e-119$ ), and aminoglycoside 3'-phosphotransferase (similar to XP\_007692660, 85% identity,  $E$ -value = 0). Note that the partial coding region of pectin lyase gene showed strong RIP response.



**FIG S7** Comparison of virulence between  $\Delta sol4$  mutants and their wild-type progenitors. Mean disease severity of two chickpea cultivars Spanish White and Dwelley caused by wild-type strains AR628 (pathotype II), AR21 (pathotype I), and their corresponding  $\Delta sol4$  mutants. Disease score was assessed 2 weeks after inoculation, based on the 1–9 rating scale (1 = no symptom, 9 = dead plants) as described previously (2). Student’s *t*-test showed that the disease severity caused by the  $\Delta sol4$  mutants was not different from that caused by their respective wild-type progenitors.

## REFERENCES

1. **Lupas A, Van Dyke M, Stock J.** 1991. Predicting coiled coils from protein sequences. *Science* **252**:1162-1164.
2. **Chen W, Coyne CJ, Peever TL, Muehlbauer FJ.** 2004. Characterization of chickpea differentials for pathogenicity assay of ascochyta blight and identification of chickpea accessions resistant to *Didymella rabiei*. *Plant Pathol.* **53**:759-769.