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

TABLE S1 List of primers used in this study

^{*a*} PCR = PCR product length in basepair; ^{*b*} FP = forward primer; ^{*c*} RP = reverse primer

TABLE S1 Continued

Primer	Sequence 5' to 3'	Description	\mathbf{PCR}^{a}
Sol4OE5	GTTGTGTGTCCACCTGCCT	FP at +92 bp from ATG for	2,187 (with
	ATG	confirmation of 4OE strains	Sol4OE5 and
Sol4OE3	CGGGAAGCTGCGAGAAGA	4OE RP at +354 bp from stop	Sol4OE3
	TAAG	codon for confirmation of	primer pair)
		40E strains	
DW38	AGATGGTCAACGCTGCTT	pelA promoter primer for	\approx 2,200 (with
	AC	confirmation of 4OE strains,	DW38 and
		141 bp away from <i>Pvu</i> II site	Sol4OE3
			primer pair)
Sol1RT-F	TTGGTATTGGTTCGCTCGA	RT-PCR for <i>sol1</i>	530
	GGT		(615,
Sol1RT-R	TCAACAGCGGTTGACATC		for gDNA or
	CTCT		pre-mRNA)
Sol2RT-F	CACATCTCCATGGCTTTGG	RT-PCR for sol2	390
	CTC		(470,
Sol2RT-R	GTTCGCTGCTTAGCACCC		for gDNA or
	AGAA		pre-mRNA)
Sol3RT-F	GTTCGCCTTGATGGCAAG	RT-PCR for sol3	572
	ACTG		(622,
Sol3RT-R	CGCGCATCCAGAGGATGT		for gDNA or
	TCAA		pre-mRNA)
Sol4RT-F	TGGATCAACCAGATCGTC	RT-PCR for sol4	522
	CATC		(588,
Sol4RT-R	CTTACGGTGCAGTACGCAT		for gDNA or
	СТА		pre-mRNA)
Sol5RT-F	AGAACCCAGCGTGCATCT	RT-PCR for sol5	534
	ATAC		(631,
Sol5RT-R	GATCATGGAACCTCCCCAT		for gDNA or
	ATC		pre-mRNA)
Sol6RT-F	GCAAAGTGCTAACACCCG	RT-PCR for sol6	540
	СТСТ		(606,
Sol6RT-R	CGTTTAGCTGTTCTAGGCT		for gDNA or
	TGG		pre-mRNA)

^{*a*} PCR = PCR product length in basepair.

TABLE S1 Continued

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

^{*a*} PCR = PCR product length in basepair.

TABLE S1 Continued

Primer	Sequence 5' to 3'	Description	\mathbf{PCR}^{a}
ORF3realt-F	CCGCTCTAGCGATAAGAA	Real-time RT-PCR for ORF3	136
	GG		
ORF3realt-R	CGTAGCAACCTGATGCAA		
	С		
ORF10realt-F	CACTGCCATCCTTGAGAC	Real-time RT-PCR for	127
	AG	ORF10	
ORF10realt-R	GAGACTTCGCTGTTCTTGC		
	С		
Peudo6 realt-F	GCACTTTGACAGGCATAC	Real-time RT-PCR for	144
	AAAG	pseudogene 6	
Peudo6 realt-R	GAGTGTGGAGGCATGCAT		
	AG		
PKS1realt-F	CAACATGTCTCCACGTGA	Real-time RT-PCR for PKS1	130
	AG		
PKS1realt-R	AATGCGGTTCAGCTTTGTG		
	G		
PKS2realt-F	GAACCTTGCTGGTGCATC	Real-time RT-PCR for PKS2	123
	G		
PKS2realt-R	GCTTTGGACAGCGACTTG		
	AG		
Actin1realt-F	GTATCATGATCGGTATGGG	Real-time RT-PCR for Actin1	134
	ACAG		
Actin1realt-R	CCAGATCTTCTCCATGTCG		
	TCC		

^{*a*} PCR = PCR product length in basepair.

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

TABLE S2 The locations of putative binding motifs for a Zn(II)2Cys6 zinc cluster transcription factor in the promoter regions of biosynthetic genes for solanapyrone production

^a AR and AS prefixes indicate Ascochyta rabiei and Alternaria solani, respectively.

^{*b*} Perfect sites contain the 5'-GCC(N_{10})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.

^{*c*} Perfect sites contain the 5'-CCG(N_8)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.

^{*d*} 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 pHNU3PelA 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 = λ DNA digested with *HindIII*.

Α

Molly S.nodo Pseudogene_4 Pseudogene_5 Pseudogene_6	1 TANCOTACEJCACESCITESCCCOMEALACESTITESCCCOMCACTTTOCCAACECCCC 1 TANCOTACT ACCAACCACCCCCTTACCTAACCAACCCCCCACTTTOCDAACACCA 1 TANCOTACTE ACCESCESCE 20 AAAAACCCCCCAACTTTOCCAACECACCAC 1 TANCOTACTE ACCESCESCE 20 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	Molly S.modo 721 (3666766667477600000000000000000000000000	Nolly S.modo 1441 CAACTACTGTTEGECCACAGAGATTCTTAGCTTCTAACATACTACTACAACAACAACAACAACAACAACA
Molly S.nodo Pseudogene 4 Pseudogene_5 Pseudogene_6	61 ACCANETE A TRANSPORTER AND	Molly 3.modo 701 ABAAGAAGAAGAATATTACTAABAATAGGGTTATTACAGTCTCTAAGAACGGCTGGAAGA Pseudogene 4 700 AcaaGaAGCCGAGTATACCTAACAACTGGGTTATTACTGTCTCTAACAACAACTAGACTAGAATA Pseudogene_5 701 AcaaGaAGAAGTGTGCCGAGTATTAGAGTCTCTAACAA Pseudogene_6 700 ACAAGGAGCCGAGTATACCTAACAACTGGGTATTAGTATCTCTAACAA	Molly 5.modo 1801 CECENCGTANATERCXCANAAGAAAGGTAGAAGAAGGAAGGAAGGAAGGAAGGAAGG
Molly_8.modo Pseudogene 4 Pseudogene_5 Pseudogene_6	121 ALCOTCATATACAGACTOCCCTTCAGOCTETTLAS CALGACOCEAGACTORTETTEGAGOC 120 AAGOAGATATACAGOTTOCCCTACAGOCTATTTAS TALGACEGAAGACTOTCTTAGGAG 121 AAGOEGATATACAGOTTOCCCTECAGOCTATTTAS TALGACOCAAGACTOTCTTAGGAGO 122 AAGOEGATATACAGOTTOCCCTACAGOCTATTTAATAGACOCAAGACTOTCTTAGTAAC 120 AAGOAGATATACAGOTTOCCCTACAGOCTATTTAATAGACOCAAGACTA	Molly_S.modo 841 CAANTGAGCTCGGGTCTGCAGGGTAAAGCACTTTGATGAGCATAAAAGGGGGGGG	Nolly_S.modo 1961 NCGGICTICGIGGIACCACTCTIAAAGGAGTTTGGIGGGCGAGTAGAGGGAAGAAGAGGC Freudogene 4 1980 AGGGAGTGCGGCTEACTACTGITAAGGAGTTTAAGGCCGAAGTAATBGGAAGAAGAG Preudogene 5 1980 AGGGCCTINATACTACTCTIAAGTAGTTAAGGCCGCGTAGTAATBGGAAGAAGGAA Preudogene 6 1980 AGGGAGTGCAGCTAACTACTCTTAAGGAGTTTAAGGCCTGTAGTAATGGGAAGAAGGAAG
Molly_8.modo Pseudogene 4 Pseudogene_5 Pseudogene_6	191 осостосяс БатстяБалсо151стБайбалсястБаббатВабосст 180 йсостосясБатстяБалсо151стБайбалсястВаббатВабосст 181 осостосясБатВтэБалсбоссттаАлаластБаббабттаАлттистасбасВа 180 осостосясЛатстасАлсотоссттаАлаласттабталттаАсттастабаосст	Molly_3.mode 901 CAGOCECTATCEGOCTOCTTATERGACAGEACEACEACEACEACEACEACEACEACEACEACEACEACE	Molly_8.modo 1421 MGAMCEGGTGCGCGTGGTGGTGGCGGACCGTACCCAALGCCGTGTGGACGTGCAAG Preudogene 4 1420 MGAMCACGTGCCGTGTTAATACGACACTTAGTCTTTAAGCCGCTGTAGTACAA Preudogene 5 1420 MGAMCGAGGTGCCGTGTTAATACGACGACGTGCGTGAAGGCGTGCGT
Molly_S.modo Pseudogene 4 Pseudogene 5 Pseudogene_6	241 сттр <mark>абладсясаттост</mark> обосталадсал <u>д</u> сталс <mark>радс</mark> астваболоса, став 240 сттралосоваттосталосталатальносталовоса сталькосостал 241 дабле алботваттотабосталаталаносталовососталькосостал 240 сттралособаттоталосоталаталалосталомасса сталькососталановоса стал	Molly_S.mode 961 <mark>BGChätact#GAAGGATAAGAAGATTÄTC</mark> ACTCTGTGCATGCCTCCTCACTCACGACC Pseudogene 4 960 ACTAGTACTATAAGAAGATTATAGATTGTGACTCTGTGCATGCCCCTACACTTGTTÄCACC Pseudogene 5 961 A <u>B</u> TAÄTACTATAAGAM <u>GCAAAGATTGTATR</u> CTATGTATGCCTCTA <u>B</u> ACTTÄTGTTACC Pseudogene_6 960 ACTAGTACTATAAGAGTATAAGATTGTTATTCTTATGCACCCTACACTTGTTGCACC	Nolly S.nodo 1481 MGACHGGCATAAHGCCCCGTACGTAGAAAAHCCAGTGGAAGTAGAACTAGACTCTGDAFGATAT Preudogene 4 1480 MGACAGACATAACGCCCGAACATGTAGTAAGATGCCCAGAGTACTGTAGATTAGTGE Preudogene 5 1480 AHTTGGACATAACTGECCGAACATGTAGAAGAGCGTGGAAGTAGTGTGTAGAGTAGTATAGGCC Preudogene 6 1480 MGACAGACATAACGCCGAACATGTAGTAAAGATGCCTAGAGTACTATAGGACATTAGGCC
Molly_3.modo Pseudogene_4 Pseudogene_5 Pseudogene_6	801 TTGT <mark>R</mark> ŽAGANË TAËNËGËRAC TËGTINËGËËRGANTITCOT CIAGOTËGO NËGTOTËG 800 TTGTONËNTUTATANAGI TAGACINAGINAGINATITË COTAGOTËGI TAATOTAG 801 TTGTONËNTUTATANAGI TAGACINAGINAGINI TEGO OTTAGI TËGI TAATOTAG 800 TTGTO <mark>NGANGINI TAMAGI TAGACINAGINI GONINCTINO TA</mark> GOTIGOTINI TAMOT 800 TTGTO <mark>NGANGINI TAMAGI TAGACINAGINI GONINCTINO TA</mark> GOTIGOTINI TAMOT	Molly_S.modo 1021 TgCTGCLAGCTCTTHATGTGGGTGTTTTGCCTCTTTAAAAAAAGCCGTALAGAAGCCGTAAG Pseudogene_4 1020 TACTGCLAGCLACTTAACGTAGCTGTTTTGCCCGLATAAAAAAGCCTATGGCGCCTAAG Pseudogene_5 1021 TGTTAEAAGTACTAAGGTGTTTTGCCCCTGTAAAAAAAGCCTATAGCGCCTAAG Pseudogene_6 1020 TACTGCAGCTACTTAACGTAGGTGTTTTGCCCCTAAAAAAAA	Molly_S.modo 1741 [CATCTTCC]TTTTTCTTATTACTA_TACRAGECCBAAGTCGETTETTCCCCCABBATTAGSCTCB Pseudogene_4 1740 ATATTATTATTATTATTATTATTATTATTATTATTATTA
Molly_8.modo Pseudogene_4 Pseudogene 5 Pseudogene_6	941 CTGATATESCERAATTGECTSC22005GEGASCSC2005CE000000000000000000000000000000000	Molly_S.modo 1081 CCAAGGOCTGATGCCCAAGCGGATCACCCAGATGAAGAAACTTGAGTCCTAAGGOCT Pseudogene_4 1080 CCAAGGOCTAATGCCCAGGAAGATCACCCCGATTACTAAACTTAAGTTCTTACTAATGCCT Pseudogene 5 1081 CTAAGAGETEDATACGCAGGAGATAGACGEBEATTACTAAAGTTAGTCTTACTBAG Pseudogene_6 1080 CTAAGGOCTAATACGCAGPAGATCACCCCGATTACTAAACTTAAGTTCTACTGGCT	Molly_S.modo 1800 <mark>1757667676767676767676777767777677777777</mark>
Molly S.modo Pseudogene_4 Pseudogene_5 Pseudogene_6	422 Кабатлестті баталаласса басатасала саласттала база база база база база база база б	Molly S.modo 1141 TTAN <u>ECOCOCETTTGACCOLOCIANTACTECTAC</u> AN <mark>EATCEABGCACCETTTC</mark> GACEG Pseudogene_4 1140 TTANGCGCTTTTGACCCCTTAATTACTCACAACAACAACAACAACGETTTCGACAGGA Pseudogene_5 1141 TTABAC <u>ACCCTTTAACCCCTTAATTACTCACAACAACAACAACAAC</u>	Nolly S.modo 1855 GTACGTTA Pseudogene_4 1855 GTACGTTA Pseudogene_5 1855 GTACGTTA Pseudogene_6 1860 GTACGTTA
Molly S.modo Pseudogene_4 Pseudogene_5 Pseudogene_6	401 P. AMGAMMECCT <mark>E</mark> TGEBAGEATICTBAGET <mark>DATRIGAGALTGETTEE</mark> BETAANGA 400 P. AMGAMECCTTETAAGEATICTAAGEBETTAGEATTAGEATTAGTATTATTATAGAA 400 J. AMGAMECCTETGTAAGEATICTAAGEBETTAGEBETTAGEBETTETBEFEBETAGEA 400 J. AMGAMECCTETGTAAGEATICTAAGEATICTAAGAATGETTITATAGAA	Molly 5.modo 1201 CTGCAT BOTCS PETHIAC CAAL COORDATE TARGET CAGE COMPARED AND COORDATE TARGET CARD COORDATE CONTROL CO	
Molly S.modo Pseudogene_4 Pseudogene_5 Pseudogene_6	841 РЕЛЕФЛЯСКОМ И ВЛЕВОЛАТССТВАТСЯВОЛОНОТАКАЛАСТИ ПЛАВЛОГСТВОСТ 840 АЛАТАЛАКСТВАТАТАСТАТАСТКАЛВИЛИКАКССТАСАЛСТИТИВАСНОСТВОСТ 841 АЛЕВСАЛКОСТВАТАТАСЯЛСТВАТОВИТИВИСТВАТАСТИАЛВИТИВИ 840 АЛИСТАЛАНСТВАТАТАСТАСТИСТВАТОВЛОКАССТАСАЛСТИТАЛСАНСТСТВОСТ 840 АЛИСТАЛАНСТВАТАТАСТАССТАСАЛОНАНСКАССТАСАЛСТИТАЛСАНСТСТВОСТ	Molly 5.mode 1261 CCCENC STREET COLORS CALL COLORS CAL	
Nolly 3.nodo Pseudogene 4 Pseudogene_5 Pseudogene_6	601 TTATËATËGE <mark>CEAS</mark> ATËTC <mark>RAC</mark> HGANG <mark>YOTE</mark> GTTA <mark>SAGETCAR</mark> AGCEASËANGREGËC 600 TTATATAGETTTATATCTACAGAGCTGTTËTTATAGETTTAAGCGTTAGGAGAG 601 TTATATAGE <u>TTR</u> ACAGGAGAGCTGTTATAGGTTARAGCGTAAGGAGAG 800 TTATAATAGETGTTATATCTACAGGAGCTGTTGTTATAGGTCTAAGCGTAAGGGAGAC	Molly 3.modo 1931 TEGAÄTTÄCGÄTCÄCAATCENEGTTEGTÄÄÄKKEÄAGGÄAGAATTEAGAGÄCAENEÄÄTAGAT Pseudogene 4 1320 TAGAOTTTÄGATCTENATCEALAGCTATTTTÄÄGAAGAAGATTGAAGAACACCGÄAATAGCT Pseudogene 5 1320 TEGAÄTTÄGGATCECAATTÄÄEGCTGETTTÄÄAAGAAGAATTGAÄGAGACAE Pseudogene 6 1320 TAGAOTTGGGTCTCAATTGAAGCTGATTEGAAGAAGAATTGAAGAAAACAECCAAATAGCT	
Molly_S.nodo Pseudogene 4 Pseudogene_5 Pseudogene 6	661 CHANGECAGTGUAGCAGGUATGAGAGAGUAGGUGUGUAGCAGUGUGUGUGUGUGUGUGUGU	Molly_3.mode 1981 CACCAACETCIATEGTGERCCCTTTERARACETCICAAAACEGCCACCERTTEATEGCCC Pseudogene 4 1980 CACCACTTTAATAGTTAACCTCTTAATAGCTCACTEATAGCCCCAACCCTEATGCCC Pseudogene 5 1980 CACCACTTTAATAGTTAAEGCCTTTAATAACACTTEATAGCCCCACGCCTEATEGCCC Pseudogene 5 1980 CACCACTTTAATAGTTAAEGCCCACTTAATAACCTCCAAACCTCCAACCCTEATEGCCC	

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.

A ORF10_P450 001 MPYCPNWRNORKIYQSILNINAYGSLGALGAEAILTYQQLAHAPQNYYLTIRRYSTAVI XP_7784573 DI MPYCAEWRNORKIYQSILNITAYTSLCFLCBAEAULTIQQLMQTBEKYYLHIRRYSTAVI ORF10_P450 061 LSSVFGIRGPEBSHPNIQHLYKVQDQFTAILETGATPPVDIFFWLKKTPSFLAPWRNWAL XP_7784573 061 LSSVFGIRGPEBELPNIQKLYHVQDQFTAILETGATPPVDIFFWLKKTPSFLAPWRNWAL SSVFGIRGPEBELPNIQKLYHVQDQFTAILETGATPPVDIFFLKCTPTFMAPWRNWAL ORF10_P450 121 AIRCEQRSLYFELLIGAKERVAACAKRNCFMEXLLCESKFIKNGLDEHIAYIGGVLMEG XP_7784573 121 SIREEORLYFELLKGVKNRIGFVCVRCFMEXLLCESKFIKGLDEHIAYIGGVLMEG XP_7784573 121 SIREEORLYFELLKCVKNRIGFVCVRCFMEXLLCESKFIKGLDEHIAYIGGVLMEG XP_7784573 181 GSDTTSSTLLSFLLAVKYFNVLRKAGEEVERVCCVCRSFSFEDLAKMEYIKHCUTEVRT XF_7784573 181 GSDTTSSTLLSFLLAVKYFNVLRKAGEEVERVCCVCRSFSFEDLAKMEYIKHCUTEVRT

В	С	D
លក្ខវាល p450 001 នាក់ចុងសេចកម្មាន ទោះពីក្នុងទីហើយកែចុងក្លាំងពីកំពាំងទីកើនកំពុង ទោះពី xp_7784573 001	ORF10_P450_001_ATCCC_TT_CCTCG-CDFTCCCCIAN_CAGAGGAA_G_TTA_CAATCCAATCAACATC XP_7764573_001_ATCCCLTA_CCCAGAATCCCCAATCCAATCCAATCCAATCCAATCC	ORF10 P450 001 TOTOCTISCANATTIAAARTSAACAS TGANICGISCITATCANAGGIACHICGCGGGGG XP_7784573 001AGTTEANGACGCCGGGGAGTEITEACASTAAGGGCCCCAGGGICTHEASAGGGGG
ORFIG P450 055 Allow CIMBER AND CONTRACTOR CONTRACTOR AND CONTRACTOR CONT		ORF10 P450 055 0000700100 00007AT 5 OM OTTOTA NTO XP_7784573 055 00000000000000000000000000000000
ORFIO P450 115 AT A TOTAL TOTAL AND A CONTRACT AND	orfio P450 121 10-8 COLT 02 COLTANA TANTAN TANT	ORF10 9450 119 MARTOCATEATOCT TRAASACTAAATSICAAA TATUKA AATSI XP_7784573 114gattat taguta artottitata cotta baarto tagoog faratta
orfio P450 174 Bacctor Toctarean Crock Campa Construction and the Construction of the Construction of the Construction of the Construction Campa Construction Campa Campa Construction Campa Cam	ORF10 P450 181 DETECTION OF THE ATTENDED ATTENDE	ORF10_P450 179 CTTCC CAMER THE SECTO TAGGAMAGE HOSTATE OF SECTO CATOR OF XP_7784573 170 CTTCC AMAGE TO THE THE TAGGAMAGE TOSTA SALE AND SALE SECTO AND
ORFIO P450 234 OFTER ALTONIC TOTAL CARGONIC CARGONIC TOTAL TOTAL CARGONIC	ORF10 P450 241 200 2 30 20 20 20 20 20 20 20 20 20 20 20 20 20	ORF10 9450 239 COLTENTR CACEANTATING XP_7784573 230 ACCENTICONTITICATION TRACTAGEMENT ACCENTION ACCENTION
ORF10 P450 289 TROOM AN AGENT COARCE AN OTTAT COARAGE TO COARAGE AND C	ORF10 P450 301 ATATTCCCC SC GANA AN 5 5 5 1 5 5 1 5 5 1 5 5 1 5 5 1 5 5 1 5 5 1 5	ORF10 P450 290 CCT: TACAN CT 25 CA
ORFIO P450 349 CELETITE CONSIGNATION CONTRACTOR CONTRAC	ORF10 P450 361 COAN COCCAR CANADAGE AN CANTER STATUTED S	ORF10_P450_350_FLGAAACCACHCATEC
ORF10_P450_409_5470	ORF10 P450 421 (10 00 00 0 0 0 0 0 0 0 0 0 0 0 0 0 0	ORF10 P450 407 COMOCOL FREE CONTRACTOR CONCERNMENT OF CONTRACTOR FREE CONCERNMENT OF CONTRACTOR FREE CONTRACTOR FRE
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ORF10 P450 526 TT <mark>R</mark> 5 XP_7784573 526 AC <mark>B</mark> C	ORF10_P450 541 0000 000000000000000000000000000	ORF10 19450 527 FAACTICITICAR TOTAL TACATE OCHAAL TOTCHOOL TA TOACAAL CATTAI XE_7784573 522 FAATCAACATIM COMM CARCATE COMMAND CATCHOOL HE CTCOM OF COMMAND
		ORF10 P450 587 GANG AN CONTOCOLOGICA CITCLE A CITCLE AND AN AN XF_7794573 582 CANCINGTCANTER AND AN AND AN AND ANTER AND AN AND AND AND AND AND AND AND AND
		ORF10 P450 647 TREAMANDE THE BERT ACTICATING THE STATE AND CONCEPTE XP_7794573 642 TREAMANDE THE BOOM ACTICATING THE STATE OF CONCEPTE
		ORF10 P450 707 HALALACCACCACAGE AATOCATCAACATCACAACAACAACAACAACAACAACAACAACA

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.

KM 244525	001	
XP1397039	001	MHPTGRKRRVTICVPCYTRKQRCNRQYPCNHCTRRRRPEECVKQSIITGDPSNTFLLEP-
XP1905776	001	MQSSQRKRVVSSCIPCYTRKQKCNRQYPCNHCSRRRRPEQCA/NPSQATLPPSPPQTQKD
KM 244525	001	
XP1397039	060	-ARDPEPOEPPNVOLPNGSSKASPAEAONSASNSHSALAKSFGYFEHSDSNT
XP1905776	061	HLHDDEIQTDDSQQEAQAERRPSASSSVQDTINWGGLKEGREPTSLAEVFGYFENSKSNT
M 244525	0.01	
XD1397039	111	MALLKKWDLDSECDTDOSTOC-USTAWNETUKOWIEKI BEDET DELMOYEWDELNIKMKO
XP1905776	121	IALVRKLGADDDATGHSSEPAPVEEETAIIACRLFASMENRSILDFLVRYFIAEVSNMDO
KM 244525	039	TVHPERI AOVERWARMOT ISBNADLE FAVLMURI CAYASEFUPSOKYTVDT I KOBPLSD
XP1397039	170	I HEPESEL TOYOHWATKEWELSVEDUE FAALWLRIGAYSAOFLESPTHTUELTROSISD
KP1905776	181	INTYPEWELSHYOKWWEMERTSTAYGIEFAVLVLRICSYASOFLPSPTCTIDSIRGVPLAD
M 244525	099	IRSNCDRLAGELEGICNAAVLRGSLVRVOYMAFTAMCYECTSRIKLSMATTCCATPEACE
(P1397039	230	IRDICSHLEGELARACLSLNMKGSLVRVCHILEAAFRSSCEGRIDKEMEGHASACSAAOK
(P1905776	241	IRKSCDRUADALTFICSRLDARGSLIRVCHUAFACURSLCOGRTNAHMERUSCAURUAOR
CM 244525	159	VGLARDERKRE-DEGMDDLERELRRRMECHIRLAKAMDRVPEIVDAYCTVSLEOM
(P1397039	290	AGIHTVAPVPG-DDSSQVLEKEMRRRTLCGLYVLDSBLARCLDRVPFIPDNLVLETLER
(P1905776	301	IGLEVEATSGFYSPNMDBLEKEMRRRIFCNLYVWDSVLSKRLDCIPFLPD&LNPLTMPRM
M_244525	213	HLHETIANLCAPDLFTERVLQAQLVRFWKKLEAGNSAPGARPYDPVIAEERYCRFC
(P1397039	349	RLABFIEDLAASADAPEDFTERIMOVRLGREWRSFGSRRNLPYDPTHGEORYERFC
(P1905776	361	HIVLGUDDW-PQADAPDISTERVLEAQIANSWNTHGSGNWTEYDBURAEERYENSC
KM_244525	269	NEFLPELPAFFALEEN TEWEKHIPELERORVLEHVALFESVMHNFROLLRLOHHERS
KP1397039	405	ARTIFILEPARALNED - REWLIGQUERLEMORQULYTATISUSVCWNERFIDI LYKPGOVIS
121303776	410	Souther Be territed to ball and a ba
	0.07	
(P1397039	463	LEPYKOVLUOSCKOKLALAAVEELDAVTALETMECCSETTESATTENTEETAVVLSU-CI
(P1905776	476	LPAYKQILI SSHKRALAVIAINILEGVSALHIMMGGSHTEHASI TIPTEPAVVILCIOS
	0510.05	
M 244525	396	LOACDSNEMOTI FHINEFSLUSDISDNATDISCOCTORING DOCTORING CT
P1397039	523	OKDFPFDOGEDHPDII GLRWATIN RPRAVENALGRICMI AEVSEMA
P1905776	536	DGNFPPKAETTURRGSTTSMTKSSDPFGVGUTDVAKDECICAVE SALGRIOTIADMNHMA
M 244525	443	DICH HONGIMDHWOATLARSGTOTERMOSTETTTYNSSSSTSVONCHAHOUSSELDETE
(P1397039	572	ASCROVETCLFAKAARMEHSLEPYTPNESSRS-SSSPAILSN
P1905776	596	BVGARTHTREEGKEEKISDTEVSEA-NTLERL-DEDPIVVTD
M 244525	503	OROEGPTANAFRTDSNVFECLMMDLSTRALPMPSERCLEOLHEMSERCENEC
(P1397039	613	FINLEDCSCLWMSSETPTL#PD-MISTIAHCESYSMLQFTSPDLTSW
KP1905776	636	PALELSEAMNCLOTPDYAEVERLEENTPTTLESFSW
M_244525		
(P1397039	659	NGNFHS
KP1905776	673	F

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)2Cys6 (C₆) 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 C₆ 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 ≈ 10 kb of the flanking sequence. Horizontal solid line 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.

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