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(54) **COMPOSITIONS AND METHODS TO PROTECT CELLS BY BLOCKING ENTRY OF PATHOGEN PROTEINS**

**Publication Classification**

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*C12N 5/077* (2010.01)  
*C12N 5/078* (2010.01)  
*C12N 5/079* (2010.01)

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(52) **U.S. Cl.** ..... **436/501**; 435/410; 435/412; 435/414; 435/415; 435/417; 435/416; 435/325; 435/350; 435/351; 435/366; 435/368; 435/369; 435/370; 435/371; 435/372

(21) Appl. No.: **12/944,345**

(57) **ABSTRACT**

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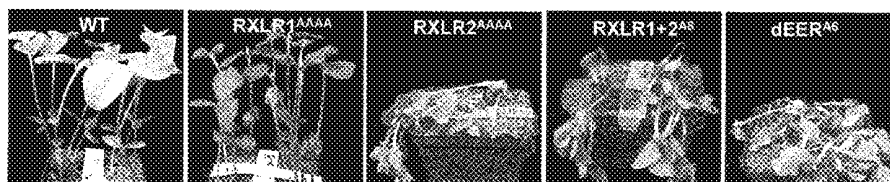
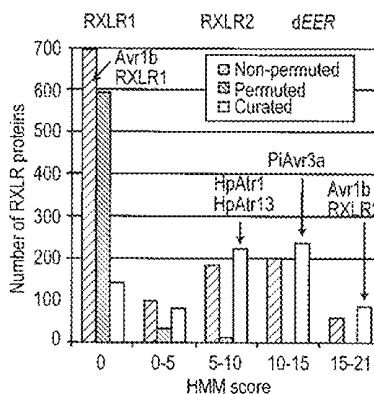
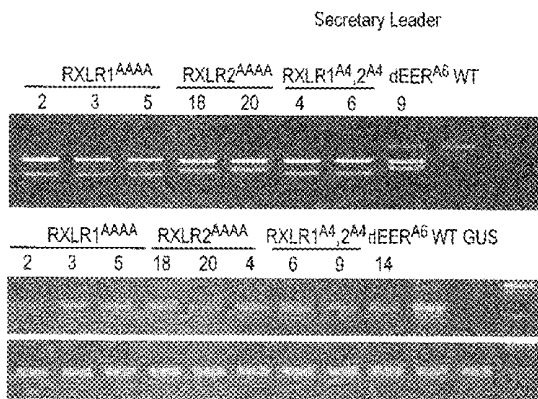
**Related U.S. Application Data**

(63) Continuation-in-part of application No. 12/468,470, filed on May 19, 2009, Continuation-in-part of application No. PCT/US2009/044489, filed on May 19, 2009.

Pathogenic effector proteins include one or more virulence motifs of amino acid consensus sequence BXZ, where B=RK or H; X=any amino acid or is absent; Z=L, M, I, W, Y or F) which bind to target polar lipids on a host (plant or animal) cell as a prerequisite for translocation of the pathogenic effector proteins into the cell. Translocation is prevented by binding blocking compounds to one or more motifs of the effector protein or to the lipid ligands of the host cell. The blocking compounds include synthetic or naturally occurring polypeptides which bind the polar lipids or the motifs, various polar lipids, the hydrophilic head-groups of polar lipids, etc. Suitable blocking compounds can be identified by assays demonstrating binding to the motifs or to the target polar lipids.

(60) Provisional application No. 61/260,227, filed on Nov. 11, 2009, provisional application No. 61/128,080, filed on May 19, 2008, provisional application No. 61/160,059, filed on Mar. 13, 2009.

	10      20      30      40      50      60
SEQ ID NO: 2 sAvr1b WT	MRLSFVLSLVVAIGYVVTCTNATEYSDETNIAMVESPDLVRRSLRNGDIAGGRFLRAHEEDDAGERT.....
SEQ ID NO: 3 sAvr1b RXLR1 <sup>AAAA</sup>	MRLSFVLSLVVAIGYVVTCTNATEYSDETNIAMVESPDLVRAAAAANGDIAGGRFLRAHEEDDAGERT.....
SEQ ID NO: 4 sAvr1b RXLR2 <sup>AAAA</sup>	MRLSFVLSLVVAIGYVVTCTNATEYSDETNIAMVESPDLVRRSLRNGDIAGGAAAAAHEEDDAGERT.....
SEQ ID NO: 5 sAvr1b RXLR1 <sup>AAAA</sup> 2 <sup>AAAA</sup>	MRLSFVLSLVVAIGYVVTCTNATEYSDETNIAMVESPDLVRAAAAANGDIAGGAAAAAHEEDDAGERT.....
SEQ ID NO: 6 sAvr1b dEER <sup>A6</sup>	MRLSFVLSLVVAIGYVVTCTNATEYSDETNIAMVESPDLVRRSLRNGDIAGGRFLRAHAAAAAGAAT.....



SEQ ID NO: 2 sAvr1b WT  
 SEQ ID NO: 3 sAvr1b RXLR1<sup>AAAA</sup>  
 SEQ ID NO: 4 sAvr1b RXLR2<sup>AAAA</sup>  
 SEQ ID NO: 5 sAvr1b RXLR1<sup>AAAA</sup> 2<sup>AAAA</sup>  
 SEQ ID NO: 6 sAvr1b dEER<sup>A6</sup>

1 10 20 30 40 50 60

MRLSFVLSLVAIGYVYVTCNATEYSDETNIAMVSPDLVRRSLRNGDIAGGRFLRAHEEDDAGERT.....  
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Secretary Leader

Figure 1A

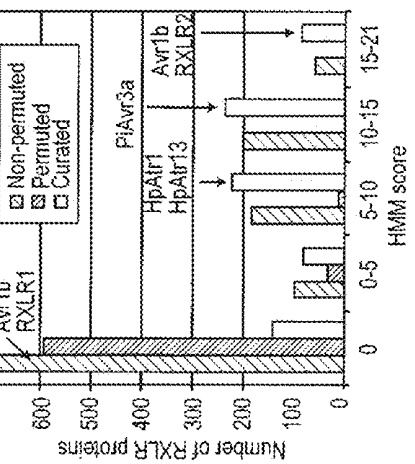


Figure 1D

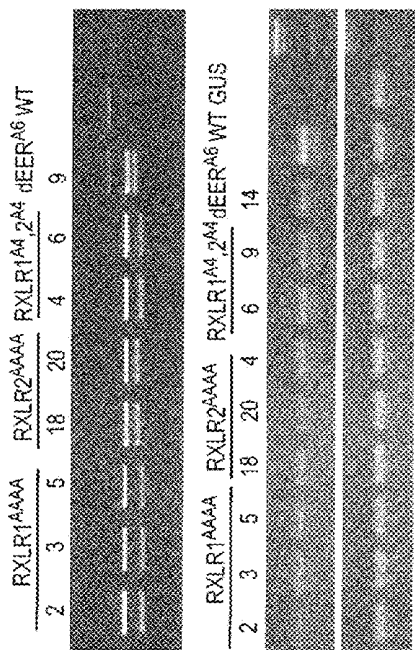


Figure 1B

Figure 1C

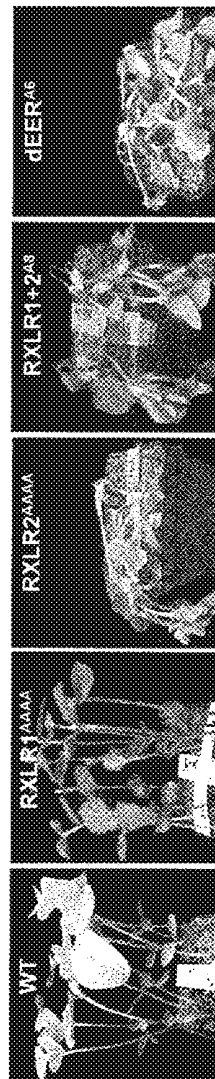


Figure 1E

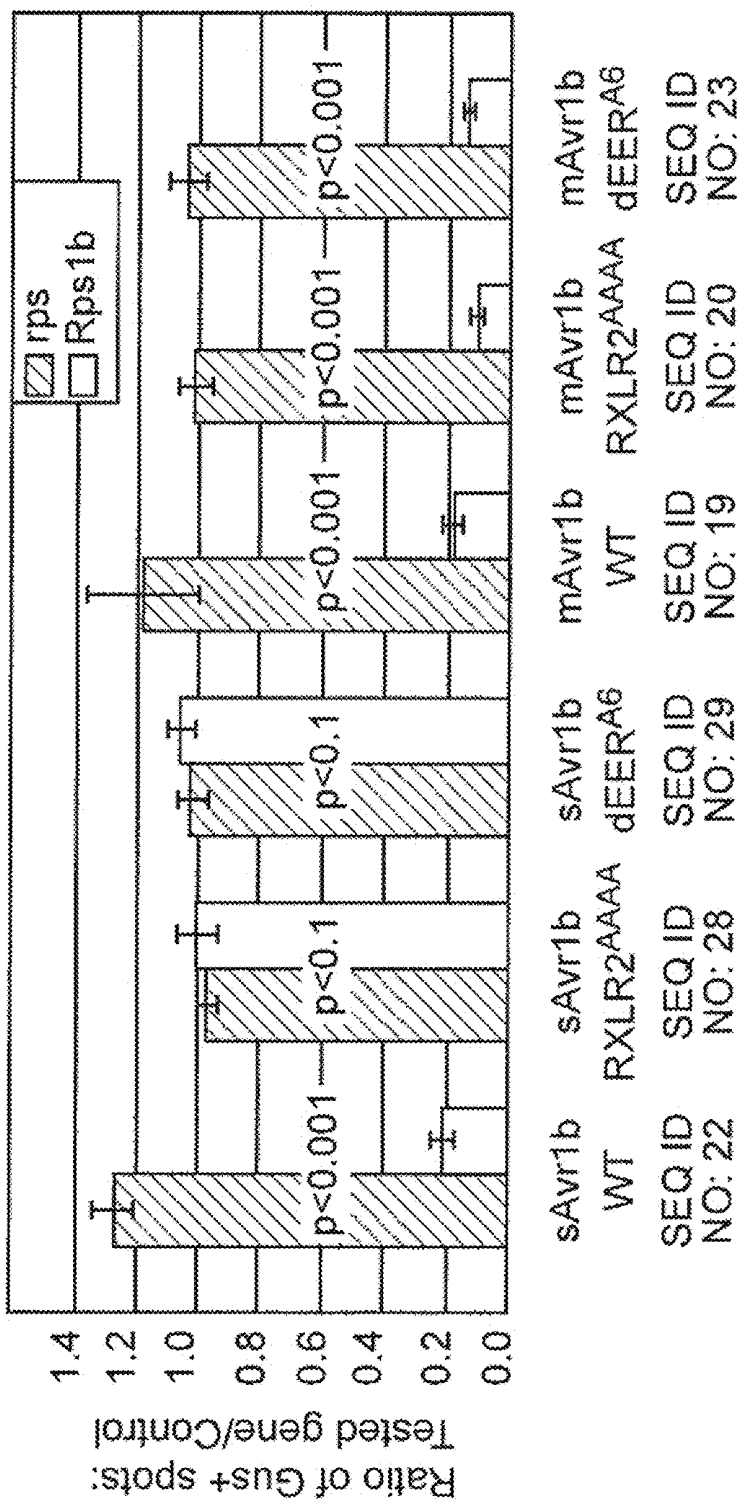


Figure 2

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 SEQ ID NO: 4 sAvr1bRXLR2<sup>AAAA</sup>  
 MRLSFVLSLVAIGYVWTCNATEYSDETNIAMVESPDIVRRSLRNGDIAGGAAAAAHEEDDAGERfsvt.....  
 SEQ ID NO: 8 PsAvr4/6::Avr1bC1  
 MGLHKGFFVAVALLLIIVAAPADAITDESOPRDATIVDAPLTGRGANARYLRTSTSIKAPDAQLP-  
 STKAAIASSVTKEEEERKISTGLSKLRQKLSKRFHDIPDWLLQLQAFLSVGLHHLfsvt.....  
 SEQ ID NO: 7 HpAvh341::Avr1bC1  
 MRLHYVGPVVAALAAASAHLQWSDSASDLORTDEARQOPYINDKTKRFLTSEDKDLPPLVTS-  
 DGYSALLPQGGNDRVLRSDIGDGDYEEERSKIKRHKRKSHTGSHGVLDfsvt.....  
 SEQ ID NO: 9 mAvr1bC1 MK

Figure 3A

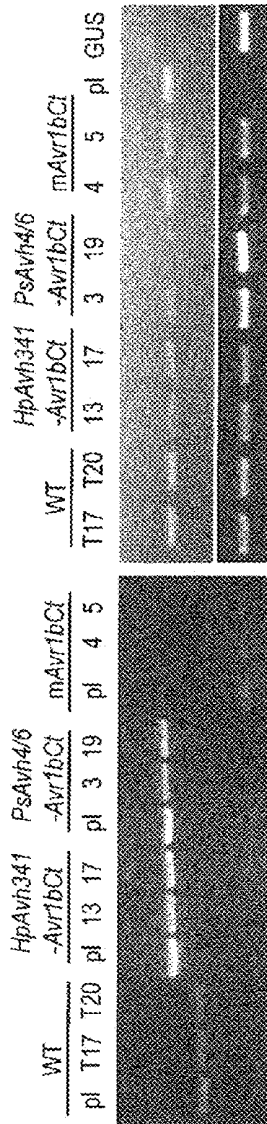
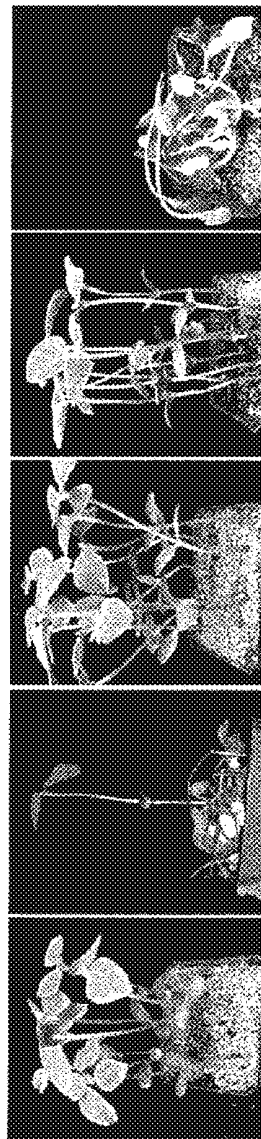


Figure 3B



T17 (Avr1b WT) sAvr1b RXLR2<sup>AAAA</sup>-18  
 HpAvh341-Avr1bC1-13  
 PsAvr4/6-Avr1bC1-19  
 mAvr1bC1-4

Figure 3D

SEQ ID NO: 2 2 sAvr1b  
 SEQ ID NO: 41 sAvr1b RXLR2<sup>Arg8</sup>  
 SEQ ID NO: 42 sAvr1b RXLR2<sup>TAT</sup>  
 SEQ ID NO: 43 sAvr1b<sup>PfGBP</sup>  
 SEQ ID NO: 44 sAvr1b<sup>PfHRP</sup>  
 SEQ ID NO: 45 sAvr1b<sup>Pf1615c</sup>

MRLSFVLSLVAIGYVWTCNATEYSDETNIAMVESPDVRRSLRNGDIAGG-RFLR-----AHEEDDAGERTFSV...  
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Figure 4A

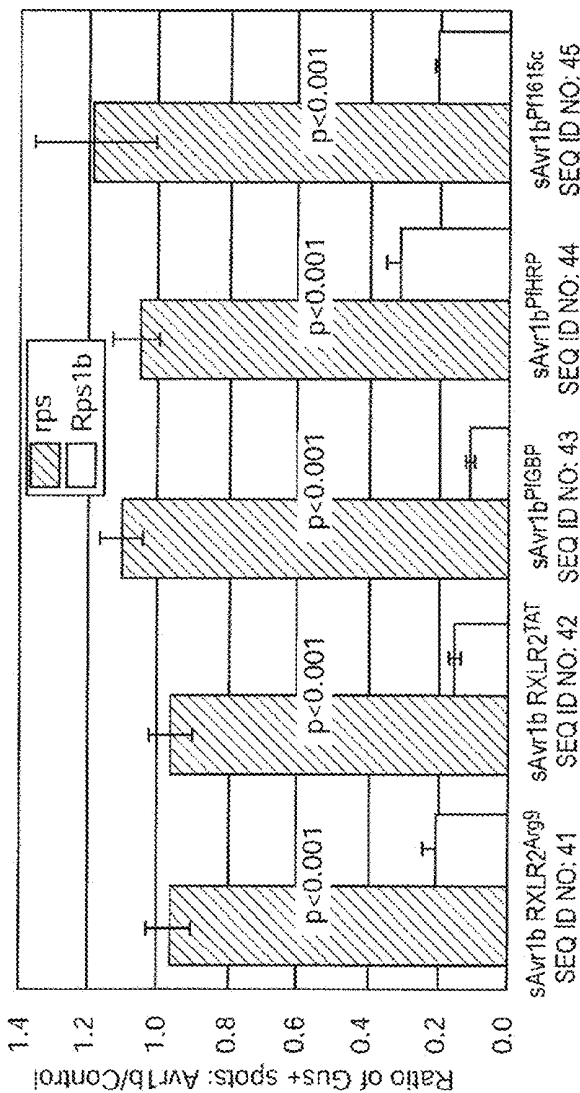


Figure 4B

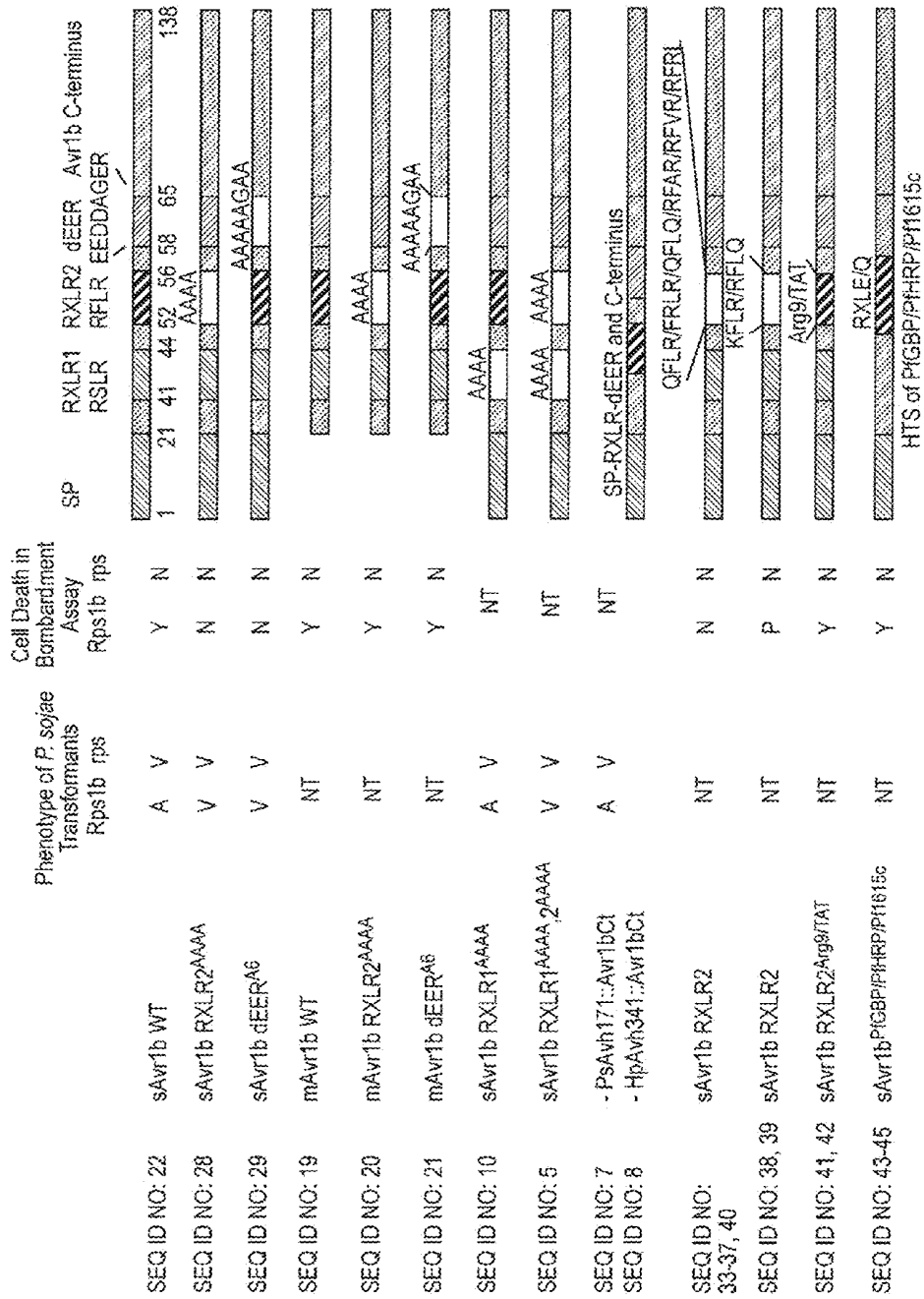


Figure 5

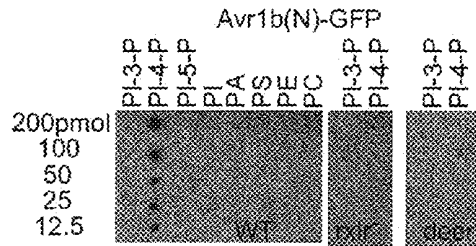


Figure 6A

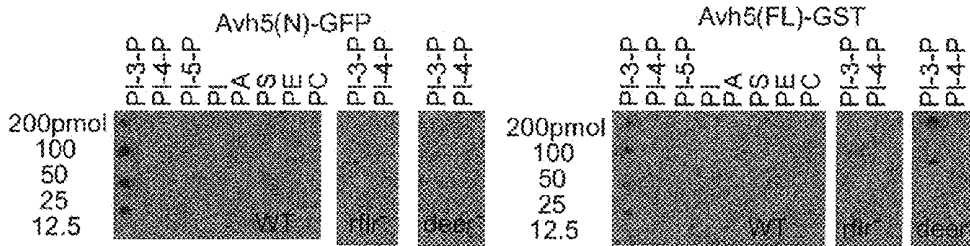


Figure 6B

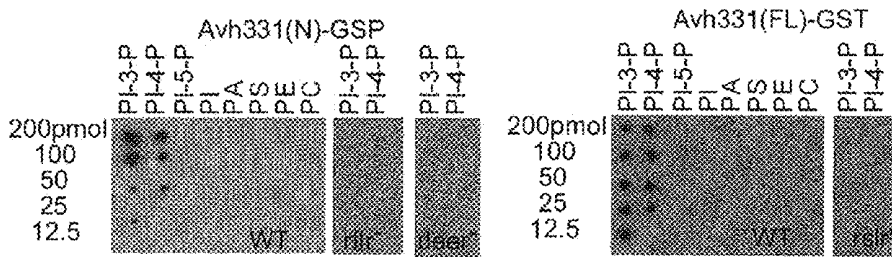


Figure 6C

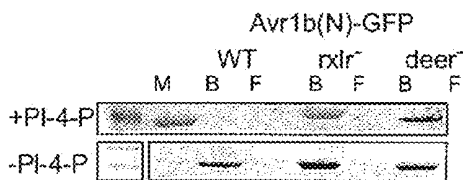


Figure 6D

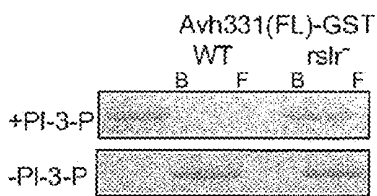
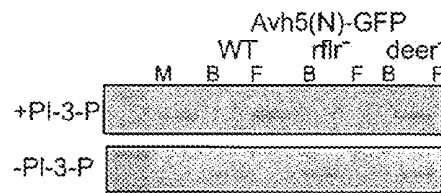


Figure 6F

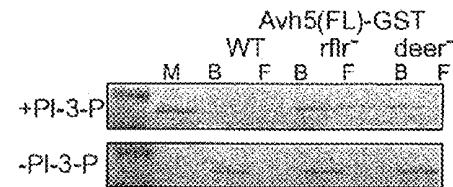


Figure 6E

Protein	Sequence	Binding PI-3-P PI-4-P	Relative Ablation	Root Cell Entry	Human Cell Entry	Inhibition by IP2
SEQ ID NO: 46 Avr1b	TEYSDETNIAMVESPDLVRRSLRNGDIAGGRFLRAHEEDDAGERITFSVTD	-	1.00	Y	Y	Y
SEQ ID NO: 47 Avr1b rxlr	TEYSDETNIAMVESPDLVRAAAANGDIAGGAAAHAHEEDDAGERITFSVTD	-	0.00	N	N	nt
SEQ ID NO: 48 Avr1b deer	TEYSDETNIAMVESPDLVRRSLRNGDIAGGRFLRAHAAAAGERTFSVTD	-	0.00	N	N	nt
SEQ ID NO: 116 Avh331	LTCAISEQQTREPELCEFFSVRSWSPSTISDGACIALVSAEQGATAGRNT LSLRSMMA TEDMATSTRSLRSQATNVDDANVSIENRGM	+	1.07	Y	Y	Y
SEQ ID NO: 117 Avh331 rslr	LTCAISEQQTREPELCEFFSVRSWSPSTISDGACIALVSAEQGATAGRNT LSLRSMMA TEDMATSTAAAASQATNVDDANVSIENRGM	-	0.00	N	N	nt
SEQ ID NO: 118 Avh331 deer	LTCAISEQQTREPELCEFFSVRSWSPSTISDGACIALVSAEQGATAGRNT LSLRSMMA TEDMATSTRSLRSQATNVAAAANVSIANRGM	-	0.00	N	N	N
SEQ ID NO: 113 Avh5	TRYPDDANLQSVNAPVQTVTRSRRELRTADIDVYEPVYVHNPKG	+	nt	Y	Y	Y
SEQ ID NO: 114 Avh5 rslr	TRYPDDANLQSVNAPVQTVTRSRRAAATAADIDVYEPVYVHNPKG	-	nt	N	N	nt
SEQ ID NO: 115 Avh5 deer	TRYPDDANLQSVNAPVQTVTRSRRELRTAATAIYVAPVYVHNPKG	-	nt	N	N	nt
SEQ ID NO: 119 AvrL567	MEHVPAELTRYSEGTYREYRPTASVLSGLVKYKNDMEQMTMP	+	0.70	Y	Y	Y
SEQ ID NO: 120 AvrL567/flyr-de	MEHVPAELTRYSEGTYRABAAASPTASVLSGLVKYKVAQAQMTMP	-	0.00	N	N	nt
SEQ ID NO: 123 PfHRP11	FNNNLCCKNAKGLNLKRLLEHQAHVDDAHHHHVAD	+	1.15	Y	Y	Y
SEQ ID NO: 124 PfHRP11 pexel	FNNNLCCKNAKGLNLKAAAATQAHVDDAHHHHVAD	-	nt	N	N	nt
SEQ ID NO: 121 PfGBP	DKYEKAVDYGFRESKILAEGETICARKEKTLTKRSKQK	+	0.97	Y	nt	nt
SEQ ID NO: 122 PfGBP pexel	DKYEKAVDYGFRESAAAAGEDTCARKEKTLTKRSKQK	-	nt	N	nt	nt
SEQ ID NO: 125 Pf1615c	SYNKINSSTYTHSRLLKLEFITLEEKTYNALQEMLDSDV	+	1.11	Y	nt	nt
SEQ ID NO: 126 Pf1615c pexel	SYNKINSSTYTHSAAAAALEFITLEEKTYNALQEMLDSDV	-	nt	N	nt	nt
SEQ ID NO: 112 Arg9	RRRRRRRR	+	1.13	Y	Y	N

Figure 6G

secretion signal peptide

Avr1b- MRLSFVLSLVVAIGYVVTQNAITEYSDETNIAMVESPDLVRRSLRNGDIAGGRFLRAHEEDDAGERITFSVTDLWNVKVAKKLAKA

6x-His-tag

EK Site

HA-tag

Arg-GFP- MRGSHHHHHHGMASMTGGQOMGRNLVDDDDKDRWGSRSIRRRRRRRRRRFEFRSTMSGYPDYDPDYAGSMGSGIGRPTST  
SSLVAAAAATMSKEELFTGWPIVELDGDVNGHKFVSVEGEGDATYKLTLD

Figure 6H

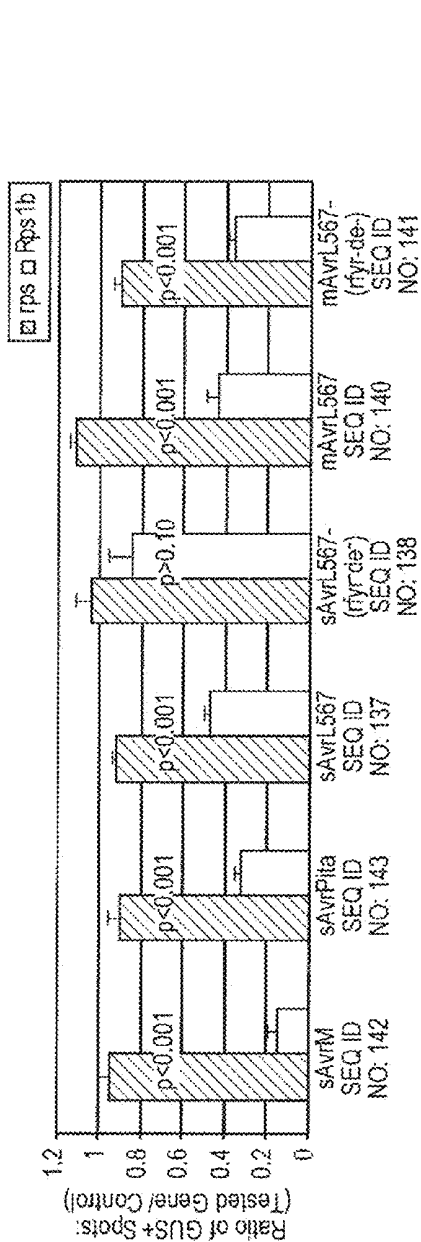


Figure 7A

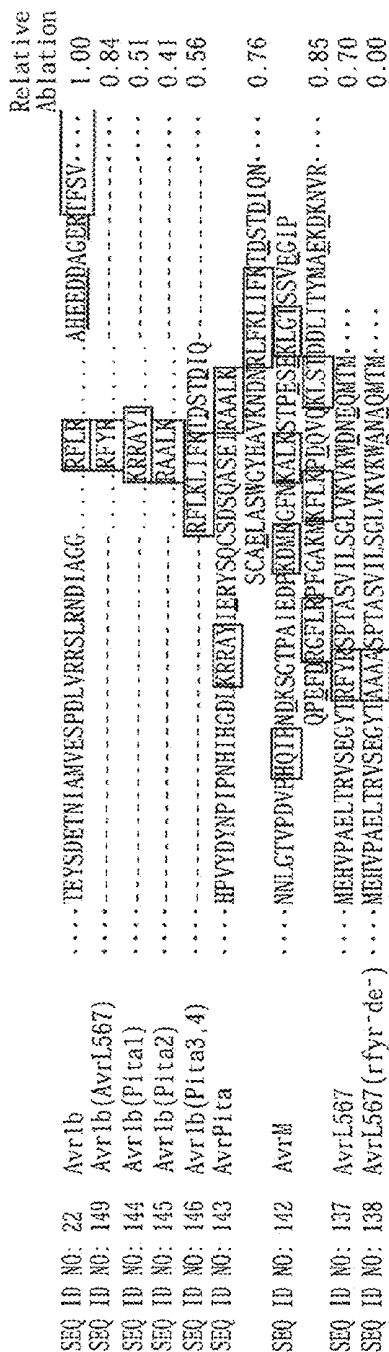
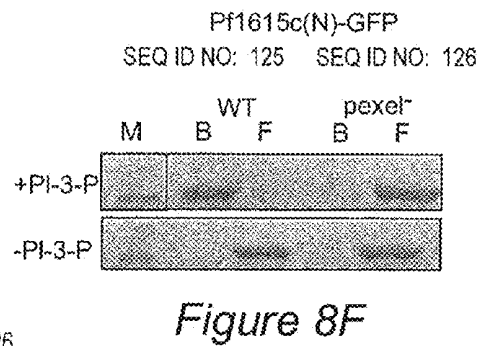
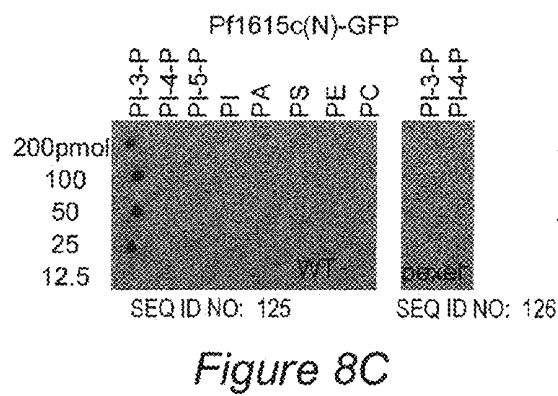
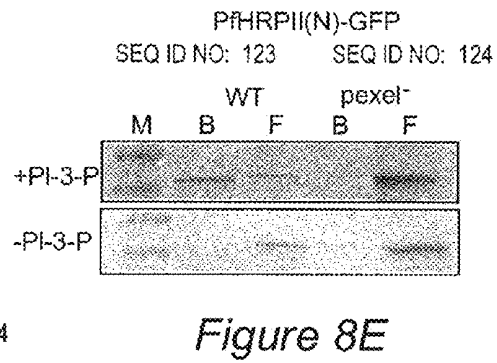
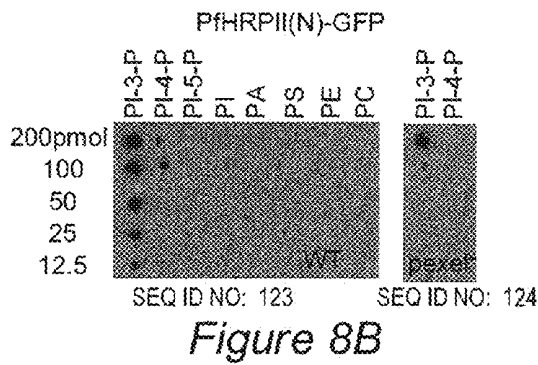
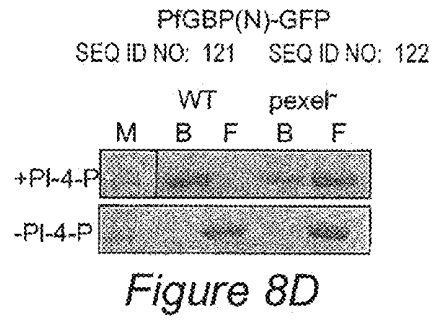
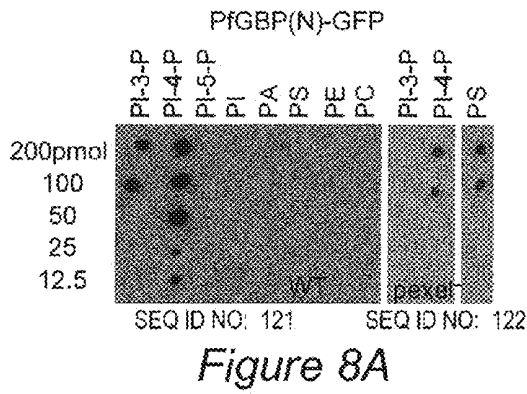


Figure 7B

SEQ ID NO:	AVr1b	TEYSDETNIAMVESPDIVRRSLRNDIAGGRELRAHEEDDAGERTFSV	Relative Ablation
1	AVr1b	TEYSDETNIAMVESPDIVRRSLRNDIAGGRELRAHEEDDAGERTFSV	1.00
3	AVr1b(AAAA)	-----AAAA-----	0.00
38	AVr1b(1K)	-----K-----	0.42
147	AVr1b(1H)	-----H-----	0.89
37	AVr1b(1Q)	-----Q-----	0.00
148	AVr1b(3F)	-----F-----	1.01
149	AVr1b(3Y)	-----Y-----	0.84
150	AVr1b(3I)	-----I-----	0.52
151	AVr1b(3M)	-----M-----	0.89
40	AVr1b(3V)	-----V-----	0.00
35	AVr1b(3A)	-----A-----	0.00
39	AVr1b(40)	-----Q-----	0.74
152	AVr1b(4G)	-----G-----	0.88
153	AVr1b(2M3V)	-----MV-----	0.71
154	AVr1b(2L3T4G)	-----LTG-----	0.56

Figure 7C



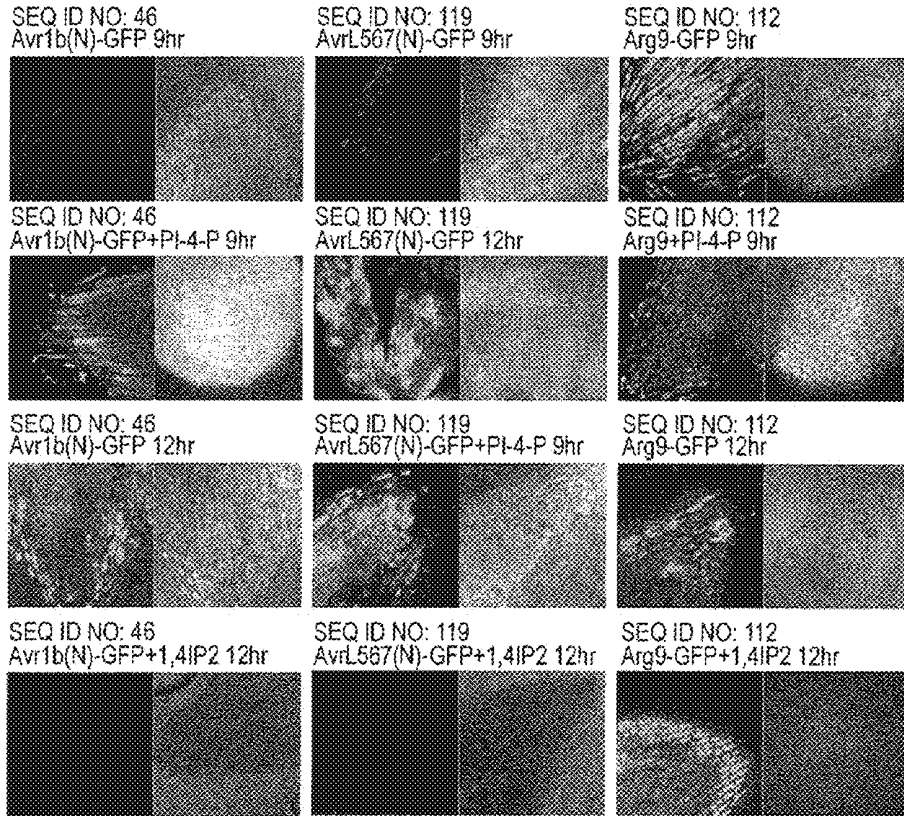


Figure 9A

Figure 9B

Figure 9C

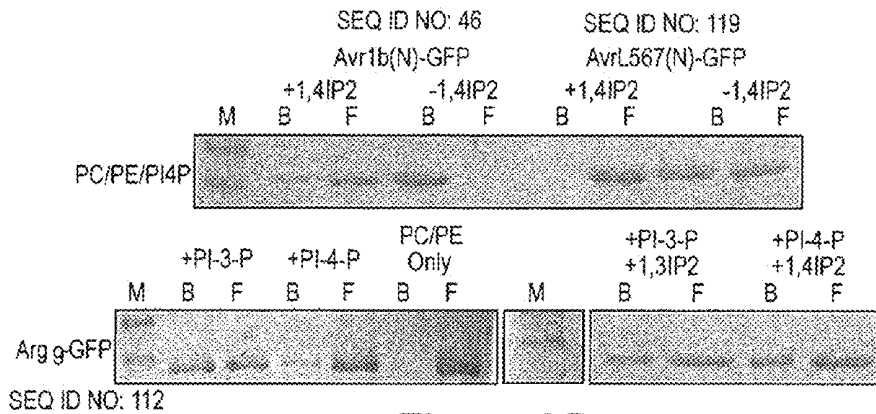
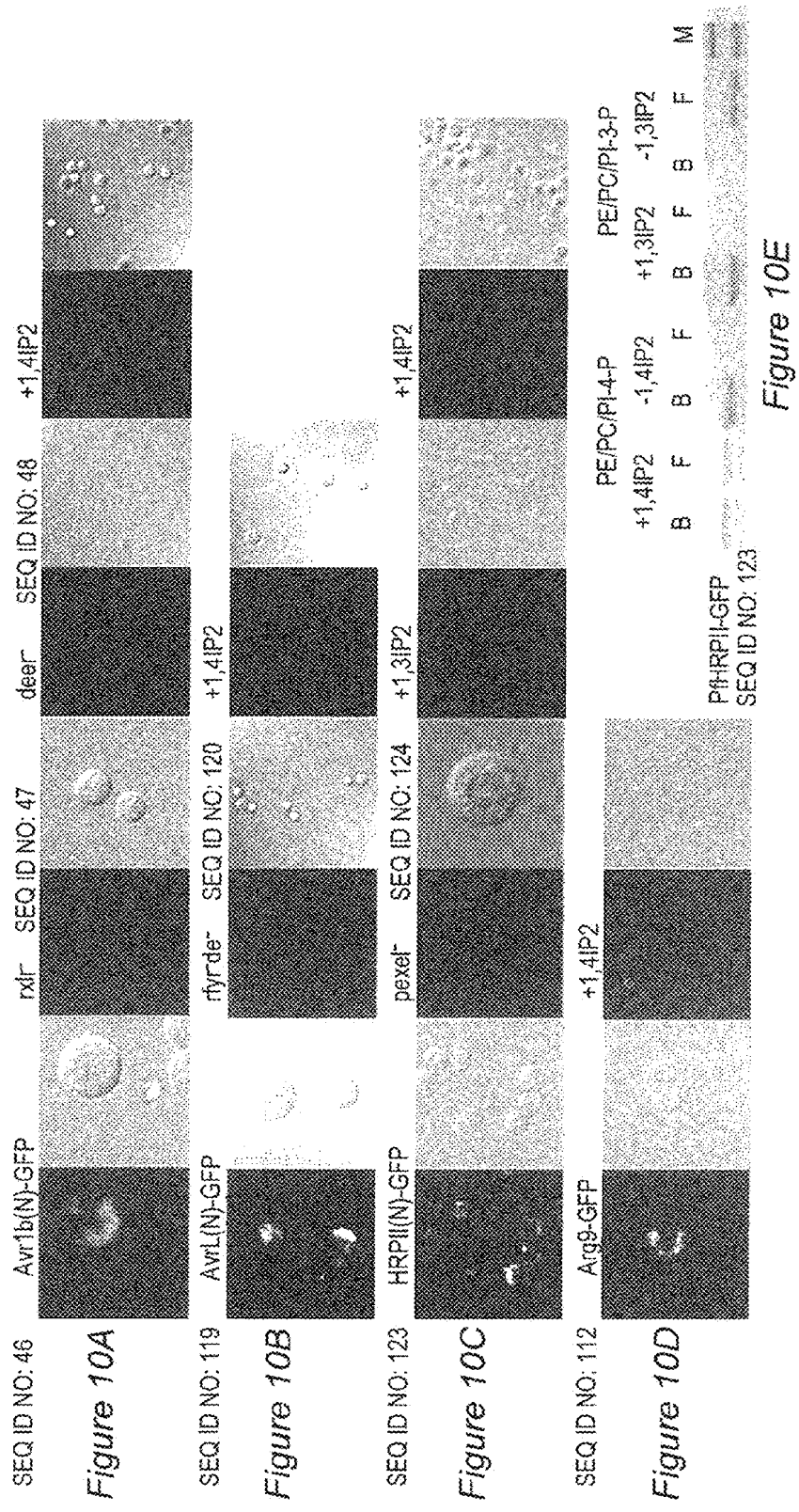


Figure 9D



SEQ ID NO:	Plasmid Name	Sources	Construct	Construction strategy
1	pUN	pHMT35N (5)	<i>Npt II</i> gene for G418 resistance fused to <i>P. sojae</i> rPL41 promoter in pUC19	Ham34 promoter of pHMT35N replaced by <i>P. sojae</i> rPL41 promoter using primers UF and UR
2	pHamAvr1b	pHMT35N	<i>Avr1b-1</i> gene fused to Ham34 promoter and terminator for <i>P. sojae</i> transformation in pUC19	<i>NptII</i> gene of pHMT35N replaced with <i>P. sojae Avr1b-1</i> using PrimerC and PrimerD
3	M1	pHMT35N	<i>Avr1b-1</i> (RXLR1 <sup>AAAA</sup> ) mutant fused to Ham34 promoter and terminator	Same as pHHamAvr1b with mutation introduced by primers motif1F and motif1R
4	M2	pHMT35N	<i>Avr1b-1</i> (RXLR2 <sup>AAAA</sup> ) mutant fused to Ham34 promoter and terminator	Same as pHHamAvr1b with mutation introduced by primers motif2F and motif2R
5	M1+2	pHMT35N	<i>Avr1b-1</i> (RXLR1 <sup>AAAA</sup> ,2 <sup>AAAA</sup> ) mutant fused to Ham34 promoter and terminator	Same as pHHamAvr1b with mutation introduced by primers motif1+2F and motif1+2R
6	M3	pHMT35N	<i>Avr1b-1</i> (dEER <sup>46</sup> ) mutant fused to Ham34 promoter and terminator	Same as pHHamAvr1b with mutation introduced by primers motif3F and motif3R
7	pHamAvh341	pHMT35N	<i>HpAvh341</i> fused with C-terminal domain of <i>Avr1b-1</i> , driven by Ham34 promoter and terminator	<i>NptII</i> gene replaced with <i>HpAvh341-Avr1b</i> fusion using primers <i>HpAvh341F</i> , <i>HpAvh341R</i> and PrimerD
8	pHamAvh171	pHMT35N	<i>Ps Avh171</i> (=Avr4/6) fused with C-terminal of <i>Avr1b-1</i> driven by Ham34 promoter and terminator	<i>NptII</i> gene replaced with <i>Ps Avh171-Avr1b</i> fusion using primers <i>PsAvh171F</i> , <i>PsAvh171R</i> and PrimerD
9	pHamAvr1bCt	pHMT35N	C-terminus of <i>Avr1b-1</i> gene fused to Ham34 promoter and terminator for <i>P. sojae</i> transformation in pUC19	<i>NptII</i> gene of pHMT35N replaced with <i>P. sojae Avr1b-1</i> C terminus using primers 1bRXLR(-)F and PrimerD
10	UNM1	pUN and M1	Ham34::Avr1b (RXLR1 <sup>AAAA</sup> ) inserted together with rPL41::NptII for <i>P. sojae</i> transformation	Selection and Gus expression cassettes in pCambia1305.2 replaced by inserts from pUN and M1, respectively
11	UNM2	pUN and M2	Ham34::Avr1b (RXLR2 <sup>AAAA</sup> ) inserted together with rPL41::NptII for <i>P. sojae</i> transformation	Selection and Gus expression cassettes in pCambia1305.2 replaced by inserts from pUN and M2, respectively
12	UNM1+2	pUN and M1+2	Ham34::Avr1b (RXLR1 <sup>AAAA</sup> ,2 <sup>AAAA</sup> ) inserted together with rPL41::NptII for <i>P. sojae</i> transformation	Selection and Gus expression cassettes in pCambia1305.2 replaced by inserts from pUN and M1+2, respectively

Figure 11A

SEQ ID NO:	Plasmid Name	Sources	Construct	Construction strategy
13	UNM3	pUN and M3	Ham34::Avr1b(dEER <sup>AB</sup> ) inserted together with rpl41::NptII for <i>P. sojae</i> transformation	Selection and Gus expression cassettes in pCambia1305.2 replaced by inserts from pUN and M3, respectively
14	pCambiaAvr1b	pCambia1305.2	CaMV 35S promoter fused to full length <i>Avr1b-1</i> in GUS-containing vector	HMT gene in pCambia1305.2 replaced by <i>Avr1b-1</i> using primers Avr1bfull_F and Avr1bR
15	pCambiaM2	pCambia1305.2	CaMV 35S promoter fused to <i>Avr1b-1</i> (RXLR2 <sup>AAAA</sup> ) in GUS-containing vector	HMT gene in pCambia1305.2 replaced by Avr1b(RXLR2) using primers Avr1bfull_F and Avr1bR
14	pCambia-mAvr1b	pCambia1305.2	CaMV 35S promoter fused to leaderless <i>Avr1b-1</i> in GUS-containing vector	HMT gene in pCambia1305.2 replaced by mature <i>Avr1b-1</i> using primers Avr1bF and Avr1bR
15	pCambia-mM2	pCambia1305.2	CaMV 35S promoter fused to leaderless <i>Avr1b-1</i> (RXLR2 <sup>AAAA</sup> ) in GUS-containing vector	HMT gene in pCambia1305.2 replaced by mAvr1b(RXLR2) using primers Avr1bF and Avr1bR
16	pCa-GUS(-)	pCambia1305.2	Empty vector as the control	GUS expression cassette was removed by <i>Sph</i> I restriction and re-ligation for co-transformation experiments
17	pCaAvr1b	pCambiaAvr1b	CaMV 35S promoter fused to <i>Avr1b-1</i> in GUS-free vector	
18	pCaM2	pCambiaM2	CaMV 35S promoter fused to <i>Avr1b-1</i> (RXLR2 <sup>AAAA</sup> ) in GUS-free vector	
19	pCa-mAvr1b	pCambia-mAvr1b	CaMV 35S promoter fused to leaderless <i>Avr1b-1</i> in GUS-free vector	
20	pCa-mM2	pCambia-mM2	CaMV 35S promoter fused to leaderless <i>Avr1b-1</i> (RXLR2 <sup>AAAA</sup> ) in GUS-free vector	
21	pUCAvr1b	pCambiaAvr1b	CaMV 35S promoter fused to <i>Avr1b-1</i> with <i>Xma</i> I site	Avr1b expression cassette was inserted into pUC19 by PCR with primers Avr1b_EcoRI and Avr1b_HindIII
22	pUCAvr1bXK	pUCAvr1b	<i>Kpn</i> I site added to 3' end pUCAvr1b to facilitate later constructions	Amplification with primers Avr1b_EcoRI, Avr1b_HindIII and Avr1b_genegun_KpnI
23	pUCmAvr1b(dEER)	pUCAvr1bXK and M3	CaMV 35S promoter fused to mature <i>Avr1b-1</i> (dEER <sup>AB</sup> ) using <i>Xma</i> I and <i>Kpn</i> I sites	Ligation of <i>Xma</i> I- <i>Kpn</i> I fragment from M3 into pUCAvr1bXK

Figure 11B

SEQ ID NO:	Plasmid Name	Sources	Construct	Construction strategy
24	pUCmAvr1bGFP	pAcGFP1-N	Fusion of leaderless <i>Avr1b-1</i> with <i>AcGFP</i> from pAcGFP1-N (Clontech, # 632485) placed under control of CaMV 35S promoter	AcGFP fused to relevant <i>Avr1b</i> sequence by PCR with primers <i>Avr1bF</i> or <i>Avr1bfull_F</i> plus <i>GFPF</i> and <i>GFPR</i> . PCR product inserted into pUCAvr1bXK using <i>Xma</i> I and <i>Kpn</i> I
25	pUCsAvr1bGFP	pUCAvr1bXK	Fusion of normal <i>Avr1b-1</i> with <i>AcGFP</i> placed under control of CaMV 35S promoter	
26	pUCGFP	pUCsAvr1bGFP	CaMV 35S promoter fused to <i>AcGFP</i>	Deletion of <i>Avr1b</i> sequences by self-ligation of <i>Xma</i> I/ <i>Age</i> I restricted pUCsAvr1bGFP
27	pUCsGFP		CaMV 35S promoter fusion of <i>Avr1b</i> secretory leader to <i>AcGFP</i> placed under control of CaMV 35S promoter	Deletion of <i>Avr1b</i> sequences encoding mature protein by self-ligation of <i>Nco</i> I restricted pUCsAvr1bGFP
28	pUCM2GFP	pUCAvr1bXK	<i>Avr1b-1</i> (RXLR2 <sup>AAAA</sup> )- <i>AcGFP</i> fusion placed under control of CaMV 35S promoter	AcGFP fused to relevant <i>Avr1b</i> sequence by PCR with primers <i>Avr1bfull_F</i> , <i>GFPF</i> and <i>GFPR</i> . PCR product inserted into pUCAvr1bXK using <i>Xma</i> I and <i>Kpn</i> I
29	pUCM3GFP		<i>Avr1b-1</i> (dEER <sup>AD</sup> )- <i>AcGFP</i> fusion placed under control of CaMV 35S promoter	
30	pUCAvr1b(R9)GFP		<i>Avr1b-1</i> (RXLR2 <sup>Ag9</sup> )- <i>AcGFP</i> fusion placed under control of CaMV 35S promoter	
31	pUCAvr1b(TAT)GFP		<i>Avr1b-1</i> (RXLR2 <sup>TAT</sup> )- <i>AcGFP</i> fusion placed under control of CaMV 35S promoter	
32	pUCNgo	pUCAvr1bXK	Addition of <i>NgoMIV</i> site upstream of RFLR in N-terminus of <i>Avr1b</i> (T150C) in pUCAvr1bXK to facilitate later manipulations	GCCGGT in was mutated to GCCGGc using primer <i>Avr1bSac</i>
33	pUCRFRL	pUCAvr1bXK	CaMV 35S promoter fused to <i>Avr1b-1</i> genes carrying indicated mutations	Wild type <i>Avr1b</i> in pUCAvr1bXK was replaced with the corresponding mutants using <i>Xma</i> I and <i>Kpn</i> I
34	pUCFRLR			
35	pUCRFAR			
36	pUCQFLQ			
37	pUCQFLR			
38	pUCKFLR	pUCNgo		<i>Avr1b</i> in pUCNgo was replaced with the corresponding mutants by <i>NgoMIV</i> and <i>Kpn</i> I
39	pUCRFLQ			

Figure 11C

SEQ ID NO:	Plasmid Name	Sources	Construct	Construction strategy
40	pUCRFVR			
41	pUCAvr9R			
42	pUCAvrTAT			
43	pUCAvrPfGBP	pUCAvr1bXK		Wild type Avr1b in pUCAvr1bXK was replaced with the corresponding mutants using <i>Xma</i> I and <i>Kpn</i> I
44	pUCAvrPfHRP			
45	pUCAvrPf1815			
46	pAvr1bGFP	pR9GFP	Replacement of codons encoding nine arginine motif in pR9GFP with Avr1b residues 33 to 71 from wild type Avr1b or from Avr1b RXLR or dEER mutants	Amplification with primers AvrGFPF, AvrGFPR1 and AvrGFPR2
47	pAvr1b(M1+2)GFP			
48	pAvr1b(M3)GFP			

Figure 11D

SEQ ID NO.	Name	Applications	Sequence (from 5' to 3')*
49	UF	<i>Hind</i> III and <i>Xma</i> I sites added to rpL41 promoter to drive G418 resistance gene	ataagccttgaatTCTGGCGTTCATCTCCGACG
50	UR		ttcccccggTGGATGCTCAGATGctagcGTC
51	HamF	<i>Bremia</i> Ham34 promoter internal primer	TTCTCCTTTTCACTCTCACG
52	HamR	<i>Bremia</i> Ham34 Terminator internal primer	AGACACAAAATCTGCAACTTC
53	Avr1bReF	<i>Avr1b-1</i> internal primers for PCR	ACCTTCAGCGTGACTGACCT
54	Avr1bReR		GCGATTGCCAACCAGTTCT
55	ActinF	<i>P. sojae</i> Actin gene internal primers for the reference in RT-PCR	CGACATCCGTAAGGACCTGT
56	ActinR		TTCGAGATCCACATCTGCTG
57	PrimerC	<i>Kpn</i> I sites added flanking <i>Avr1b-1</i> for insertion downstream of HAM34 promoter	gggggtaccgacaacaATGCGTCTATCTTTTGTGCT
58	PrimerD		gggggtaccTCAGCTCTGATACCGGTGAA
59	Avr1bF	<i>Xho</i> I site and initiation codon added to 5' end of mature <i>Avr1b-1</i> for insertion downstream of CaMV 35S promoter	atgcactcagcgtttgcagatcccggggggaatgagatgACTGAGTACTCCGACGAA
60	Avr1bR	<i>Xho</i> I site to 3' end of <i>Avr1b-1</i> for insertion downstream of CaMV 35S promoter	atgcactcagcgtttgcagatcccggggggaatgagatgACTGAGTACTCCGACGAA
61	Avr1bfull_F	Same as Avr1bF but for secretory <i>Avr1b-1</i>	atgcactcagcgtttgcagatcccggggggaatgagatgACTGAGTACTCCGACGAA
62	Motif1F	Introduction of RXLR1 <sup>AAAA</sup> mutation, creating a <i>Pst</i> I site	TCGTCGGTgctcagcgtctAACGGCGACATTGCCGGTGG
63	Motif1R		TCGTCGGTtagcagcgtcagcACGGACGAGATCTGGAGATT
64	Motif2F	Introduction of RXLR2 <sup>AAAA</sup> mutation, creating a <i>Pst</i> I site	CCGGTGGAgctcagcgtctGCTCATGAAGAGGACGATGC
65	Motif2R		TCATGAGCagcagcgtcagcTCCACCGGCAATGTCGCCGT
66	Motif1+2F	Introduction of RXLR1 <sup>AAAA</sup> & RXLR2 <sup>AAAA</sup> mutations, creating 2 <i>Pst</i> I sites	gctcagcgtctAACGGCGACATTGCCGGTGGAgctcagcgtctGCTCATGAAGAGGACGATGC
67	Motif1+2R		agcagcgtcagcTCCACCGGCAATGTCGCCGTtagcagcgtcagcACGGACGAGATCTGGAGATT
68	Motif3F	Introduction of dEER <sup>80</sup> mutation, creating a <i>Pst</i> I site	gctcagcagcctGCGGGGgctgctACCTTCAGCGTGACTGACCT
69	Motif3R		agcagcCCCCGcagcgtcagcATGAGCTCGAAGAAATCTTC
70	HpAvh341F	5' flanking <i>Xma</i> I site added to Hp Avh341 for insertion downstream of HAM34 promoter	attcccccgggacaacaATGCGACTCCACTACGTG
71	HpAvh341R	For fusion with <i>Avr1b-1</i>	GTCAGTCACGCTGAAGGTATCGAGAACGCCATGCCAT

Figure 12A

SEQ ID NO.	Name	Applications	Sequence (from 5' to 3')*
72	PsAvh171F	5' flanking <i>Xma</i> I site added to PsAvh171 for insertion downstream of HAM34 promoter	attcccccgggacaacaATGGGCTCCACAAGGGCT
73	PsAvh171R	For fusion with <i>Avr1b-1</i>	GTCAGTCACGCTGAAGGT TAGGTGGTGTAGTCCGAC
74	1bRXLR(-)F	For deletion of N-terminus of <i>Avr1b-1</i>	aacccccggacaacaafgACCTTCAGCGTGACTGAC
75	<i>Avr1b</i> _EcoRI	Primers for making moving <i>Avr1b</i> expression cassette into pUC19 vector with <i>Xma</i> I and <i>Kpn</i> I sites flanking <i>Avr1b-1</i> gene	GGAGgaaTTcGCTGGCTGGTGGCAGGAT
76	<i>Avr1b</i> _HindIII		GTATTGGCTAGAGaAGCTTGCCA
77	<i>Avr1b</i> _gene_gun_KpnI		AGAAACTCGAGCTTGTCCGATCGACAGATCCGGTCCGCAggtACcTCAGCTCTGATAC
78	M2_F1	Replacement of RFLR with mutation encoding RFRL	GAAGATTTcgaCtTGCTCATGAAG
79	M2_R1		CTTCATGAGCaaGtcGAAATCTTC
80	M2_F2	Replacement of RFLR with the mutation encoding FRLR	TGCCGGTGGAtttagaCTTCGAGCTC
81	M2_R2		GAGCTCGAAGtctaaTCCACCGGCA
82	M2_F3	Replacement of RFLR with the mutation encoding RFAR	GAAGATTTgcaCGAGCTCAT
83	M2_R3		ATGAGCTCGtgcAAATCTTC
84	M2_F4	Replacement of RFLR with the mutation encoding QFLQ	GGTGGAcagTTTCTTCaaGCTCATGAAG
85	M2_R4		AGCtGAAGAAActgTCCACCGGCAATG
86	GFPP	For fusion with <i>Avr1b-1</i> to make <i>Avr1b-1-AcGFP</i>	CTTTCACCGGTATCAGAGC ggtaccgcccacclatgGTGAGCAAGGGCGCCGAG
87	GFPR	Addition of <i>Kpn</i> I after stop codon of GFP	AAggtACCtcaCTTGTACAGCTCATCCAT
88	<i>Avr1b</i> Sac	Addition of NgoMIV site upstream of RFLR	TCATGAGCTCGAAGAAATCTTCcgCCGGCAATG
89	<i>Avr</i> RFLQ	Replacement of RFLR with the mutation encoding RFLQ	CATTGCCGGcGGAAGATTTCTTCaAGC
90	<i>Avr</i> QFLR	Replacement of RFLR with the mutation encoding QFLR	CATTGCCGGcGGAcAATTC
91	<i>Avr</i> RFVR	Replacement of RFLR with the mutation encoding RFVR	CATTGCCGGcGGAAGATTTgTTC
92	<i>Avr</i> KFLR	Replacement of RFLR with the mutation encoding KFLR	CATTGCCGGcGGAAagTTTCTTC
93	<i>Avr</i> 9R1	Replacement of RFLR with the sequence encoding the 9 arginine motif	cgtcgacgtcggcgacgcGCTCATGAAGAGGACGATG
94	<i>Avr</i> 9R2		CATTGCCGGcGGAcgacggcgacgtcgcgtcggcgacg
95	<i>Avr</i> TAT1	Replacement of RFLR with the sequence encoding the TAT motif	taagaaacgccgtcagcagcgtcgaGCTCATGAAGAGGACGATG
96	<i>Avir</i> TAT2		CATTGCCGGcGGAtatggacgtaagaaacgccgtcagc
97	GBPF1	Replacement of RFLR-dEER with the sequence from Pf	gaaggagaagactacactccggaaaagcaagcaaaag ACCTTCAGCGTGACTGACC

Figure 12B

SEQ ID NO:	Name	Applications	Sequence (from 5' to 3')*
98	GBPF2	GBP130	ctcgtatactggcagagggcgaagatacctgcgcaaggaggagaaga ctacact
99	GBPF3		gtatgagaagcggtagattacggcttfcgagagtcctgtatactggcagag
100	GBPR		ctaccgctttctcatacttaic TGC GTTGCAGGTCACGAC
101	HRPF1	Replacement of RFLR-dEER with the sequence from Pf HRPII	tgtcgacgatgcgcaaccatgcacaccatgtgcagat ACCTTCAGC GTGACTGACC
102	HRPF2		aacctcaacaagagactgttgcacgagacacaagcacatgctgacgatg cgcacc
103	HRPF3		acaataaccctgtgtagtaagaatgctaaggcttgaacctcaacaagaga ctg
104	HRPR1		actacacaggfiattgftaaa TGC GTTGCAGGTCACGAC
105	1615F1	Replacement of RFLR-dEER with the sequence from Pf 1615c	cagtcaatgcattacaagaaatgtttagatgatagtg ACCTTCAGC GTGACTGACC
106	1615F2		ctcaagcagttggagttcatcacattggaagagaagacagtcaatgcatta caag
107	1615F3		aagatcaactcgtcatctactatatacacacagtagaatacicaagcagttgg agttc
108	1615R1		agatgacgagttgatcttgttgaact TGC GTTGCAGGTCACGA C
109	AvrGFPF	Replacement of codons encoding nine arginine motif in pR9GFP with Avr1b residues 33 to 71 from wild type Avr1b or from Avr1b RXLR or dEER mutants	gatctagatctGTGGAATCTCCAGATCTC
110	AvrGFPR1		GTCATATGGATAGCCGGACAT GTCAGTCACGCTGAA GGT
111	AvrGFPR2		GATCCCATGGAGCCAGCATAGTCTGGGACGTCATAT GGATAGCCGGA

Figure 12C

SEQ ID NO:	Plasmid	Recipient	Expression	Description*	Cloning Strategy†
112	Arg9-GFP		E. coli	9Arg fused to the N-terminus of GFP.	Received from H.J.Lee (Chang, M., Chou, J. C. & Lee, H. J. Cellular internalization of fluorescent proteins via arginine-rich intracellular delivery peptide in plant cells. Plant Cell Physiol 46, 482-8 (2005)) Described in Figure 11
46	Avr1b(N)-GFP	Arg9-GFP	E. coli	Amino acids 33-71 of Avr1b fused to the N-terminus of GFP.	Described in Figure 11
47	Avr1b(N)(xlr)-GFP	Arg9-GFP	E. coli	Amino acids 33-71 of Avr1b fused to the N-terminus of GFP. RSLR and RFLR both mutated to AAAA.	Described in Figure 11
48	Avr1b(N)(deer)-GFP	Arg9-GFP	E. coli	Amino acids 33-71 of Avr1b fused to the N-terminus of GFP. EEDDAGER mutated to AAAAAGAA.	Described in Figure 11
113	Avh5(N)-GFP	Arg9-GFP	E. coli	Amino acids 19-63 of Avh5 fused to the N-terminus of GFP.	2-Step PCR of Avh5 using Avh5-ExpGFP-BglII and Avh5R2 then Avh5-ExpGFP-BglII and LinkerR2. BglII and NcoI cloning.
114	Avh5(N)(rfr)-GFP	Arg9-GFP	E. coli	Amino acids 19-63 of Avh5 fused to the N-terminus of GFP. RFLR mutated to AAAA.	2-Step PCR of Avh5RXL mutant from pGEX4T1 using Avh5-ExpGFP-BglII and Avh5R2 then Avh5-ExpGFP-BglII and LinkerR2. BglII and NcoI cloning.
115	Avh5(N)(deer)-GFP	Arg9-GFP	E. coli	Amino acids 19-63 of Avh5 fused to the N-terminus of GFP. DTDVVEPK mutated to ATAIVYAPA	2-Step PCR of Avh5dEER mutant from pGEX4T1 using Avh5-ExpGFP-BglII and Avh5R2 then Avh5-ExpGFP-BglII and LinkerR2. BglII and NcoI cloning.
116	Avr1k(N)-GFP	Arg9-GFP	E. coli	Amino acids 21-108 of Avr1k fused to the N-terminus of GFP.	2-Step PCR of Avr1k using Avr1k-ExpGFP-BglII and Avr1kR2 then Avr1k-ExpGFP-BglII and LinkerR2. BglII and NcoI cloning.

Figure 13A

SEQ ID NO:	Plasmid	Recipient	Expression	Description*	Cloning Strategy†
117	Avr1k(N)(s/r)-GFP	Arg9-GFP	<i>E. coli</i>	Amino acids 21-108 of Avr1k fused to the N-terminus of GFP. RSLR mutated to AAAAA	2-Step PCR of Avr1kRXL mutant from pGEX4T1 using Avr1k-ExpGFP-BglII and Avr1kR2 then Avr1k-ExpGFP-BglII and LinkerR2. BglII and NcoI cloning.
118	Avr1k(N)(dear)-GFP	Arg9-GFP	<i>E. coli</i>	Amino acids 21-108 of Avr1k fused to the N-terminus of GFP. DDDANVSIENR mutated to AAAANVSIANA	2-Step PCR of Avr1kdEER mutant from pGEX4T1 using Avr1k-ExpGFP-BglII and Avr1kR2 then Avr1k-ExpGFP-BglII and LinkerR2. BglII and NcoI cloning.
119	AvrL567(N)-GFP	Arg9-GFP	<i>E. coli</i>	Amino acids 24-67 of AvrL fused to the N-terminus of GFP	2-Step PCR of AvrL567 from pUCmAvrL567 using AvrL-ExpGFP-BglII and AvrL5R2 then AvrL-ExpGFP-BglII and LinkerR2. BglII and NcoI cloning.
120	AvrL567(N)(rfy-de)-GFP	Arg9-GFP	<i>E. coli</i>	Amino acids 24-67 of AvrL fused to the N-terminus of GFP. RFYRSP TASVILSGLVKVKWONE mutated to AAAASPTASVILSGLVKVKWANA	2-Step PCR of AvrL567m from pUCmAvrL567(rfy-de) using AvrL-ExpGFP-BglII and AvrL5R2 then AvrL-ExpGFP-BglII and LinkerR2. BglII and NcoI cloning.
121	PIGBP(N)-GFP	Arg9-GFP	<i>E. coli</i>	Amino acids 70-107 of PIGBP fused to the N-terminus of GFP	2-Step PCR of PIGBP using PIGBP-ExpGFP-BglII and PIGBPR2 then PIGBP-ExpGFP-BglII and LinkerR2. BglII and NcoI cloning.
122	PIGBP(N)(pexel)-GFP	Arg9-GFP	<i>E. coli</i>	Amino acids 70-107 of PIGBP fused to the N-terminus of GFP. RILAE mutated to AAAAA	3-Step PCR of PIGBP mutating RILAE to AAAAA using PIGBPF1 & PIGBPR2, then PIGBPF2 and PIGBPR2, then PIGBPEXP-BglII and LinkerR2. BglII and NcoI cloning.

Figure 13B

SEQ ID NO:	Plasmid	Recipient	Expression	Description*	Cloning Strategy
123	PfHRPII(N)-GFP	Arg9-GFP	<i>E. coli</i>	Amino acids 27-63 of PfHRPII fused to the N-terminus of GFP	2-Step PCR of PfHRPII using PfHRPII-ExpGFP-BglII and PfHRPIIR2 then PfHRPII-ExpGFP-BglII and LinkerR2. BglII and NcoI cloning.
124	PfHRPII(N)(pexet)-GFP	Arg9-GFP	<i>E. coli</i>	Amino acids 27-63 of PfHRPII fused to the N-terminus of GFP. RLLHE mutated to AAAAA	4-Step PCR of PfHRPII mutating RLLHE to AAAAA using PfHRPIIF1 and PfHRPIIR2, then PfHRPIIF2 and PfHRPIIR2, then PfHRPIIF3 and PfHRPIIR2, then PfHRPII-ExpGFP-BglII and LinkerR2. BglII and NcoI cloning.
125	Pf1615c(N)-GFP	Arg9-GFP	<i>E. coli</i>	Amino acids 25-65 Pf1615c fused to the N-terminus of GFP	2-Step PCR of Pf1615c using Pf1615c-ExpGFP-BglII and Pf1615cR2 then Pf1615c-ExpGFP-BglII and LinkerR2. BglII and NcoI cloning.
126	Pf1615c(N)(pexet)-GFP	Arg9-GFP	<i>E. coli</i>	Amino acids 25-65 Pf1615c fused to the N-terminus of GFP. RILKQ mutated to AAAAA	3-Step PCR of Pf1615c mutating RILKQ to AAAAA using Pf1615cF1 and Pf1615cR2, then Pf1615cF2 and Pf1615cR2, then Pf1615c-ExpGFP and LinkerR2. BglII and NcoI cloning.
127	GST-Avr1k	pGEX4T1	<i>E. coli</i>	mature Avr1k fused to the C-terminus of GST	PCR of Avr1k using Avr1kF-GST-BamHI and Avr1kR-GST-SalI. BamHI and SalI.
128	GST-Avr1k(rslr)	pGEX4T1	<i>E. coli</i>	mature Avr1k fused to the C-terminus of GST. RSLR mutated to AAAAA	2-Step PCR of Avr1kRXLRL using Avr1kF-GST-BamHI and Avr1kR2-RXLRL, and Avr1kF2-RXLRL and Avr1kR-GST-SalI. Products were fused using Avr1kF-GST-BamHI and Avr1kR-GST-SalI.

Figure 13C

SEQ ID NO:	Plasmid	Recipient	Expression	Description*	Cloning Strategy
129	GST-Avh5	pGEX4T1	<i>E. coli</i>	mature Avh5 fused to the C-terminus of GST	PCR of Avh5 using Avh5F-GST-BamHI and Avh5R-GST-Sall. BamHI and Sall
130	GST-Avh5(rflr)	pGEX4T1	<i>E. coli</i>	mature Avh5 fused to the C-terminus of GST.RFLR mutated to A4AA	2-Step PCR of Avh5 using Avh5F-GST-BamHI and Avh5-RXLRF, and Avh5-RXLRR and Avh5R-GST-Sall. Products were fused using Avh5F-GST-BamHI and Avh5R-GST-Sall.
131	GST-Avh5(deer)	pGEX4T1	<i>E. coli</i>	mature Avh5 fused to the C-terminus of GST. DTDIVYEPK mutated to ATAIVYAPA	2-Step PCR of Avh5 using Avh5F-GST-BamHI and Avh5-dEERF, and Avh5-dEER and Avh5R-GST-Sall. Products were fused using Avh5F-GST-BamHI and Avh5R-GST-Sall.
132	pUCsAvr1k	pUCAvr1bXK	Transient soybean expression	mature Avr1k fused to the secreted leader of Avr1b in the transient assay vector	PCR of Avr1k using Avr1kF-NcoI and Avr1kR-KpnI. NcoI and KpnI.
133	pUCsAvr1k(rsir)	pUCAvr1bXK	Transient soybean expression	mature Avr1k fused to the secreted leader of Avr1b in the transient assay vector. RSLR mutated to A4AA	PCR of Avr1k(rflr) using Avr1kF-NcoI and Avr1kR-KpnI. NcoI and KpnI.
134	pUCsAvr1k(deer)	pUCAvr1bXK	Transient soybean expression	mature Avr1k fused to the secreted leader of Avr1b in the transient assay vector. AAAANVSIANA	PCR of Avr1k(deer) using Avr1kF-NcoI and Avr1kR-KpnI. NcoI and KpnI.
135	pUCmAvr1k	pUCAvr1bXK	Transient soybean expression	mature Avr1k cloned into transient bombardment vector with Avr1b signal peptide	PCR of Avr1k using Avr1kF-XmaI and Avr1kR-Kpn.I XmaI and KpnI.

Figure 13D

SEQ ID NO:	Plasmid	Recipient	Expression	Description*	Cloning Strategy†
136	pUCmAvr1k(rsir)	pJCAvr1bXK	Transient soybean expression	mature Avr1k cloned transient bombardment vector with Avr1b signal peptide. RSLR mutated to AAAA	PCR of Avr1k(rsir) using Avr1kF-XmaI and Avr1kR-KpnI. XmaI and KpnI.
137	pUCmAvr1k(deer)	pJCAvr1bXK	Transient soybean expression	mature Avr1k cloned into transient bombardment vector with Avr1b signal peptide.	PCR of Avr1k(deer) using Avr1kF-XmaI and Avr1kR-KpnI. XmaI and KpnI.
138	pUCsAvrL567-Avr1b	pJCNgo	Transient soybean expression	Amino acids 24-67 of AvrL replaced the RXLR region of sAvr1b in the transient assay vector	4-Step PCR Avr1b. AvrLF1 and Avr1b-KpnI, then AvrLF2 Avr1b-KpnI, then AvrLF3 and Avr1b-KpnI, then AvrLF4 and Avr1b-KpnI.
139	pUCsAvrL567(rfyrd)-Avr1b	pJCNgo	Transient soybean expression	Amino acids 24-67 of AvrL replaced the RXLR region of sAvr1b in the transient assay vector. RFYR-DE of AvrL mutated to AAAA-AA	2-Step PCR AvrL567-Avr1b. AvrLF and AvrLmR. AvrLmF and Avr1b-KpnI. Fuse the two pieces using AvrLF and Avr1b-KpnI.
140	pUCmAvrL567-Avr1b	pJCAvr1bXK	Transient soybean expression	Amino acids 24-67 of AvrL replaced the RXLR region of mAvr1b in the transient assay vector	PCR pUCsAvrL567. mAvrLF and Avr1b-KpnI of XmaI and KpnI.
141	pUCmAvrL567(rfyrd)-Avr1b	pJCAvr1bXK	Transient soybean expression	Amino acids 24-67 of AvrL replaced the RXLR region of mAvr1b in the transient assay vector. RFYR-DE of AvrL mutated to AAAA-AA	PCR pUCsAvrL567(rfyrd). mAvrLF and Avr1b-KpnI. XmaI and KpnI.
142	pUCsAvrM-Avr1b	pJCAvr1bXK	Transient soybean expression	N-terminus of AvrM replaced the RXLR region of sAvr1b in the transient assay vector.	3-Step PCR-AvrM1 and AvrM2 and AvrM3, AvrM3 and Avr1b-KpnI. NcoI and KpnI.

Figure 13E

SEQ ID NO:	Plasmid	Recipient	Expression	Description*	Cloning Strategy†
143	pUCsAvrPita-Avr1b	pUCAvr1bXX	Transient soybean expression	N-terminus AvrPita replaced the RXLR region of sAvr1b in the transient assay vector.	4-Step PCR. AvrPita, AvrPitaF and AvrPitaR1, AvrPitaF and AvrPitaR2, Avr1b, AvrPitaF1mid and Avr1b-Kpnl of Avr1b, AvrPitaF2mid and Avr1b-Kpnl. Fusion using both products. AvrPitaF and Avr1b-Kpnl. NcoI and Kpnl.
144	pUCsAvr1b(KRRAYIER)	pUCAvr1bXX	Transient soybean expression	RFLR motif of Avr1b was replaced with KRRAYIER by PCR and fused to pUCAvr1bXX. XmaI/Kpnl	PCR of Avr1b using Avr1b(KRRAYIER)F and Avr1b-Kpnl. NgoMIV and Kpnl.
145	pUCsAvr1b(RAALK)	pUCAvr1bXX	Transient soybean expression	RFLR motif of Avr1b was replaced with by RAALK PCR and fused to pUCAvr1bXX. XmaI/Kpnl	PCR of Avr1b using Avr1b(RAALK)F and Avr1b-Kpnl. NgoMIV and Kpnl.
146	pUCsAvr1b (RLFKLIFKTDSTDIQ)	pUCAvr1bXX	Transient soybean expression	RFLR motif of Avr1b was replaced with RFLKLFKTDSTDIQ by PCR and fused to pUCAvr1bXX. XmaI/Kpnl	3-Step PCR. PCR of Avr1b Avr1bFulF and Avr1b(RFLKLFKTDSTDIQ)R. PCR of Avr1b Avr1b(RFLKLFKTDSTDIQ)F and Avr1b-Kpnl. PCR fusion of products. Avr1bFulF and Avr1b-Kpnl. XmaI & Kpnl.
38	pUCsAvr1b(KFLR)	pUCAvr1bNgo	Transient soybean expression	RFLR motif of Avr1b was replaced with KFLR by PCR and fused to pUCAvr1bXX. NgoMIV/Kpnl	Described in Figure 11
147	pUCsAvr1b(HFLR)	pUCAvr1bNgo	Transient soybean expression	RFLR motif of Avr1b was replaced with HFLR by PCR and fused to pUCAvr1bXX. NgoMIV/Kpnl	PCR of Avr1b using Avr1b(HFLR)F and Avr1b-Kpnl. NgoMIV and Kpnl.
37	pUCsAvr1b(QFLR)	pUCAvr1bNgo	Transient soybean expression	RFLR motif of Avr1b was replaced with QFLR by PCR and fused to pUCAvr1bXX. NgoMIV/Kpnl	Described in Figure 11

Figure 13F

SEQ ID NO:	Plasmid	Recipient	Expression	Description*	Cloning Strategy†
148	pUCsAvr1b(RFFR)	pUCAvr1bNgo	Transient soybean expression	RFLR motif of Avr1b was replaced with RFFR by PCR and fused to pUCAvr1bXK. NgoMIV/KpnI	PCR of Avr1b using Avr1b(RFFR)F and Avr1b-KpnI. NgoMIV and KpnI.
149	pUCsAvr1b(RFYR)	pUCAvr1bNgo	Transient soybean expression	RFLR motif of Avr1b was replaced with RFYR by PCR and fused to pUCAvr1bXK. NgoMIV/KpnI	PCR of Avr1b using Avr1b(RFYR)F and Avr1b-KpnI. NgoMIV and KpnI.
150	pUCsAvr1b(RFIR)	pUCAvr1bNgo	Transient soybean expression	RFLR motif of Avr1b was replaced with RFIR by PCR and fused to pUCAvr1bXK. NgoMIV/KpnI	PCR of Avr1b using Avr1b(RFIR)F and Avr1b-KpnI. NgoMIV and KpnI.
151	pUCsAvr1b(RFMR)	pUCAvr1bNgo	Transient soybean expression	RFLR motif of Avr1b was replaced with RFMR by PCR and fused to pUCAvr1bXK. NgoMIV/KpnI	PCR of Avr1b using Avr1b(RFMR)F and Avr1b-KpnI. NgoMIV and KpnI.
40	pUCsAvr1b(RFVR)	pUCAvr1bNgo	Transient soybean expression	RFLR motif of Avr1b was replaced with RFVR by PCR and fused to pUCAvr1bXK. NgoMIV/KpnI	Described in Figure 11
35	pUCsAvr1b(RFAR)	pUCAvr1bNgo	Transient soybean expression	RFLR motif of Avr1b was replaced with RFAR by PCR and fused to pUCAvr1bXK. NgoMIV/KpnI	Described in Figure 11
39	pUCsAvr1b(RFLQ)	pUCAvr1bNgo	Transient soybean expression	RFLR motif of Avr1b was replaced with RFLQ by PCR and fused to pUCAvr1bXK. NgoMIV/KpnI	Described in Figure 11
152	pUCsAvr1b(RFLG)	pUCAvr1bNgo	Transient soybean expression	RFLR motif of Avr1b was replaced with RFLG by PCR and fused to pUCAvr1bXK. NgoMIV/KpnI	PCR of Avr1b using Avr1b(RFLG)F and Avr1b-KpnI. NgoMIV and KpnI.
153	pUCsAvr1b(RMVR)	pUCAvr1bNgo	Transient soybean expression	RFLR motif of Avr1b was replaced with RMVR by PCR and fused to pUCAvr1bXK. NgoMIV/KpnI	PCR of Avr1b using Avr1b(RMVR)F and Avr1b-KpnI. NgoMIV and KpnI.

Figure 13G

SEQ ID NO:	Plasmid	Recipient	Expression	Description*	Cloning Strategy
154	pUCsAvr1b(RLGT)	pUCAvr1bNgo	Transient soybean expression	RFLR motif of Avr1b was replaced with RLGT by PCR and fused to pUCAvr1bXK NgoMIV/KpnI	PCR of Avr1b using Avr1b(RLGT)F and Avr1b-KpnI. NgoMIV and KpnI
155	pUCsAvr1b(RLTQ)	pUCAvr1bNgo	Transient soybean expression	RFLR motif of Avr1b was replaced with RLTQ by PCR and fused to pUCAvr1bXK NgoMIV/KpnI	PCR of Avr1b using Avr1b(RLTQ)F and Avr1b-KpnI. NgoMIV and KpnI
41	pUCAvr1b(Arg9)	pUCAvr1bNgo	Transient soybean expression	RFLR motif of Avr1b was replaced with 9 Arg residues by PCR and fused to pUCAvr1bXK NgoMIV/KpnI	Described in Figure 11
224	Pita(N)-GFP	Arg9-GFP	E. coli	Amino acids 25-100 of Avr Pita fused to the N-terminus of GFP	2-Step PCR using PitaF and PitaR and LinkerR2. BgIII and NcoI.
225	Pitam(N)-GFP	Arg9-GFP	E. coli	Amino acids 25-100 of Avr Pita fused to the N-terminus of GFP. Mutation of putative FXLR motifs KRRAY, RYSQ, RAALK, HAVK, RLFR to SASAS, AASA, ASSAA, ASAA, AASA.	2-Step PCR using PitaF and PitaR and LinkerR2. BgIII and NcoI.
226	Ps87(N)-GFP	Arg9-GFP	E. coli	Amino acids 23-69 of Ps87 fused to the N-terminus of GFP.	2 Step PCR using 87F and 87R and LinkerR2. BgIII and NcoI
227	Ps87m(N)-GFP	Arg9-GFP	E. coli	Amino acids 23-69 of Ps87 fused to the N-terminus of GFP. KRLTG mutated to AAAAA	2 Step PCR using 87F and 87R and LinkerR2. BgIII and NcoI
228	AvrM(N)-GFP	Arg9-GFP	E. coli	Amino acids 54-151 of AvrM fused to the N-terminus of GFP.	2 Step PCR using AvrMF and AvrMR and LinkerR2. BgIII and NcoI

Figure 13H

SEQ ID NO.	Plasmid	Recipient	Expression	Description*	Cloning Strategy
229	AvrMm(N)-GFP	Arg9-GFP	E. coli	Amino acids 54-151 of AvrM fused to the N-terminus of GFP. HPIQ, KSGT, KDMK, KALK, KLG, RGFLR, KFLK, KLST, KNVR mutated to AAAA,AAAA,AAAA,AAAA,AAAA,AAA AA,AAAA,AAAA,AAAA	2 Step PCR using AvrMF and AvrMR and LinkerR2. BgIII and NcoI
230	P123(N)-GFP	Arg9-GFP	E. coli	Amino acids 23- 59 of AvrP123 were fused to the N-terminus of GFP.	2 Step PCR using P123F and P123R and LinkerR2. BgIII and NcoI.
231	P123m(N)-GFP	Arg9-GFP	E. coli	Amino acids 23- 59 of AvrP123 were fused to the N-terminus of GFP. RL TQ was mutated to AAAA	2 Step PCR using P123F and P123R and LinkerR2. BgIII and NcoI.
232	P4(N)-GFP	Arg9-GFP	E. coli	Amino acids 29-61 of AvrP4 were fused to the N-terminus of GFP.	2 Step PCR using P4F and P4R and LinkerR2. BgIII and NcoI.
233	P4m(N)-GFP	Arg9-GFP	E. coli	Amino acids 29-61 of AvrP4 were fused to the N-terminus of GFP. RDIQ was mutated to AAAA.	2 Step PCR using P4F and P4R and LinkerR2. BgIII and NcoI.

Figure 13I

SEQ ID NO:	Primer Name	Function	Sequence (5' to 3')
156	LinkerR2	Recreates the linker region between GFP and Arg9 for RXLR regions for all GFP expression constructs. NcoI	GCATCCCATGGAGCCAGCATAGTCTGGGACGT CATATGGATAGCCGGACATGG
157	Avr1k-GFPExp-BgIII	Forward primer for Avr1k RXLR Region. BgIII site for cloning into GFP expression vector	ATGCAGATCTCTCACTTGGCCACCCTCCGAGC
158	Avr1kR2	Reverse primer for Avr1k RXLR region. Adds nucleotides to facilitate space between GFP and RXLR	CATATGGATAGCCGGACATGGTGGATCTGTC GACTCTGTTTCAATCG
159	Avr1kΔEERR2	Reverse primer for Avr1k RXLR region ΔEER mutant. Adds nucleotides to facilitate space between GFP and RXLR	CATATGGATAGCCGGACATGGTGGATCTGTCG ACTGCGTTTCCAAT
160	Avr5-GFPExp-BgIII	Forward primer for Avr5 RXLR Region. BgIII site for cloning into GFP expression vector	ACTCAGATCTACAAGAGTCCCGGACGA
161	Avr5R2	Reverse primer for Avr5 RXLR region. Adds nucleotides to facilitate space between GFP and RXLR	CATATGGATAGCCGGACATGGTGGATCTG TCGACTTGGCCAGGATTATG
162	AvrL-GFPExp-BgIII	Forward primer for AvrL567 RXLR Region. BgIII site for cloning into GFP expression vector	ATCGAGATCTATGGGAATGGAACATG
163	AvrLRevMod1	Reverse primer for AvrL567 RXLR region. Adds nucleotides to facilitate space between GFP and RXLR	GATAGCCGGACATGGTGGATCTGTCCGCCACA GGTCAGTCAACCG
164	AvrLRevMod2	Adds remaining nucleotides for linker sequence between GFP Expression vector and RXLR region	TCCCATGGAGCCAGCATAGTCTGGGACGTCATA TGGATAGCCGGACATGGTGGATCT
165	PIGBP-GFPExp-BgIII	Forward primer for PIGBP RXLR Region. BgIII site for cloning into GFP expression vector	ATCGAGATCTGATAAGTATGAGAAAGCG
166	PIGBPR2	Reverse primer for PIGBP7 RXLR region. Adds nucleotides to facilitate space between GFP and RXLR	CATATGGATAGCCGGACATGGTGGATCTGTCGAC CGTTTGGCTTTCCTTTCCGG
167	PIGFPm-GFPExp-F1	Forward primer 1 for PIGBP RXLR Region. RILAE to AAAAA	GATTACGGCTTCGAGAGTCTGcagcagcagcagca GGCGAAGATACCTGGG
168	PIGFPm-GFPExp-F2	Forward primer 2 for PIGBP RXLR Region. RILAE to AAAAA	ATGCAGATCTGATAAGTATGAGAAAGCGGTAGATT ACGGCTTCGAGAGTCT
169	PfHRP1I-GFPExp-BgIII	Forward primer for PfHRP1I RXLR Region. BgIII site for cloning into GFP expression vector	ATGCAGATCTTTTAACAATAACC
170	PfHRP1IIR2	Reverse primer for PfHRP1I RXLR region. Adds nucleotides to facilitate space between GFP and RXLR	CATATGGATAGCCGGACATGGTGGATCTGTCGAC ATCTGCAACATGG

Figure 14A

SEQ ID NO.	Primer Name	Function	Sequence (5' to 3')
171	PfHRP1lm-GFPExp-F1	Forward primer 1 for PIGBP RXLR Region. RLLHE to AAAAA	GGCTTGAACCTCAACAAAGgcagcagcagcagca ACACAAGCCACATG
172	PfHRP1lm-GFPExp-F2	Forward primer 2 for PIGBP RXLR Region. RLLHE to AAAAA	GTACTAAGAAATGCTAAAGGCTTGAACCTCAACAAAG
173	PfHRP1lm-GFPExp-F3	Forward primer 3 for PIGBP RXLR Region. RLLHE to AAAAA	ATGCAGATCTTTTAACAATAACCTGTGTAGTAAGA ATGCTAAAGGC
174	Pf1615cGFPExp-BgIII	Forward primer for Pf1615c RXLR Region. BgIII site for cloning into GFP expression vector	ATGCAGATCTAGTTACAACAAGATCAAC
175	Pf1615cR2	Reverse primer for Pf1615c RXLR region. Adds nucleotides to facilitate space between GFP and RXLR	CATATGGATAGCCGGACATGGTGGATCTGTCGAC ACTATCATCTAAC
176	Pf1615cm-GFPExp-F1	Forward primer 1 for Pf1615c RXLR Region. RILKQ to AAAAA	CATGTACTTATACACACAGTgcagcagcagcagca TTGGAGTTCATCACATTGG
177	Pf1615cm-GFPExp-F2	Forward primer 2 for Pf1615c RXLR Region. RILKQ to AAAAA	ATGCAGATCTAGTTACAACAAGATCAACCTGTCATC TACTTATACACACAGTgcagca
178	Avr1kF-GST-BamHI	Forward primer for mature Avr1k. BamHI site for cloning into PGEX4T1 vector	CCGTGGATCCCCTCAGTTGCCGCCACCTCCG
179	Avr1kR-GST-SalI	Reverse primer for mature Avr1k. SalI site for cloning into PGEX4T1 vector	TCCGAGTCCGACTCAGATAATCATGATGCTGT
180	Avr5F-GST-BamHI	Forward primer for mature Avr5. BamHI site for cloning into PGEX4T1 vector	GGGTGGATCCACAAGAGTCCCGGACGA
181	Avr5R-GST-SalI	Reverse primer for mature Avr5. SalI site for cloning into PGEX4T1 vector	TCCGAGTCCGACTCAGCTTGGCCCTCTTCCGAT
182	Avr1kF2-RXLR	Forward primer for producing RXLR mutation in Avr1k. RSLR to AAAAA	CAgcgggggggggTCTCAAGGTACGGAACG
183	Avr1kR2-RXLR	Reverse primer for producing RXLR mutation in Avr1k. RSLR to AAAAA	GTTCTGTAGCTTGAGAGcggccggccggcTGTCTGGAG GTTGCCATGTC
184	Avr1kF2-dEER	Forward primer for producing dEER mutation in Avr1k. DDDANVSIENR to AAAANVSIANA	GGCTAACGTTTCGATTgcgAACgcggGGATGAACCCCTTCA G
185	Avr1kR2-dEER	Reverse primer for producing dEER mutation in Avr1k. DDDANVSIENR to AAAANVSIANA	CGCAATCGAAACGTTAGCggcggccggcCACC TTCGTAG

Figure 14B

SEQ ID NO:	Primer Name	Function	Sequence (5' to 3')
186	Avr1kF-NcoI	Forward primer for mature Avr1k. NcoI site for cloning into transient assay vector with Avr1b signal peptide	GCGTCCATGGGAGCTCACITGGCCACCCTCCG
187	Avr1kR-KpnI	Reverse primer for mature Avr1k. KpnI site for cloning into transient assay vector	TCGAGGTACCTCAGATAATCATGATGCTGT
188	Avr1kF-XmaI	Forward primer for mature Avr1k. XmaI site for cloning into transient assay vector	AGATCCGGGGGGCAGTGAGATAIGCTCACTTGGCGC CACCTCCGAGCAACAG
189	AvrL567-F4	Forward primer for AvrL567 RXLR region NcoI site for cloning into transient assay vector with Avr1b signal peptide	ATCGCCATGGGAATGGAACTGGAACTGTACCAGCAGAGTT GACCAGAGTCAGCGAAGGG
190	AvrL567-F3	Forward primer for AvrL567 RXLR region	ACCAGAGTCAGCGAAGGGGTATACACGATTTTA CCGGTCCCAACGGCTAGTGTAAATAC
191	AvrL567-F2	Forward primer for AvrL567 RXLR region	CAACGGCTAGTGTAACTACTGTGAGGATGGTA AAGGTTAAATGGGATAATGAACAAATG
192	AvrL567-F1	Forward primer for AvrL567 RXLR region	TGGATAATGAACAAATGACGATGCCGACCTT CAGCGTGACTGACCTGTGG
193	AvrL567-F	Forward primer for AvrL567 RXLR region XmaI site for cloning into transient assay vector	AGATCCCGGGGGCAATGAGATATGATGGAACATGTA CCAGCAG
194	AvrL567MR	Reverse primer for AvrL567 mutation. RFYR-DxE to AAAA-AxA	TCCTGACAGATTACACTAGCCGTTGG GGAgcagggggcgcTGTATACCCCTTCGGTG
195	AvrL567MF	Forward primer for AvrL567 mutation. RFYR-DxE to AAAA-AxA	GTGTAATACTGTGAGGATGGTAAAGGTT AAATGGgcTAA TgcCAAAATGACGATGCCG
77	Avr1b-KpnI Dou et al <sup>8</sup>	Reverse Primer for Avr1b in the transient assay vector	AGAAACTCGAGCTTGTGATCGACAGATCCGGTCCGGC AggTACcTCAGCTCTGATAC
196	Avr1bF-ijk-F Dou et al <sup>8</sup>	Forward primer for Avr1b with signal peptide. XmaI site	atcgcactgagctttgcagatcccggggggcaatgagataTGGGTCT ATCTTTTGTG
197	AvrPitaF	Forward primer for fusing N-terminus of AvrPita wild type into Avr1b	ATCGCCATGGGACACCCAGTTACGATTACAATC
198	AvrPitaF2mid	Forward primer for fusing N-terminus of AvrPita wild type into Avr1b	CACGCCGTTAAAAATGACAAATCCGGTTATTTAGAT TAATCTTTAAAACTGACAGCACAG

Figure 14C

SEQ ID NO:	Primer Name	Function	Sequence (5' to 3')
199	AvrPitaF1mid	Forward primer for fusing N-terminus of AvrPita wild type into Avr1b	CTTTAAAACTGACAGCACAGATATTCAAAACACC TTCAGCGTGACTGACCTG
200	AvrPitaR1	Reverse primer for fusing N-terminus of AvrPita wild type into Avr1b	CCACGAGGGGAGCTCGGCACAACCTTTTAGCGC GGCACGAAATTC
201	AvrPitaR2	Reverse primer for fusing N-terminus of AvrPita wild type into Avr1b	CCGATTGTCAATTTTAACGGCGGTGATGCCCCAC GAGGGAGCTCGGCAC
202	AvrM-F3	Forward primer for fusing N-terminus of AvrM wild type into Avr1b	ATCGCCATGGGAAACAACCTTGGAAACAGTACCCGA TGTGCCACATC
203	AvrM-F2	Forward primer for fusing N-terminus of AvrM wild type into Avr1b	CAGTACGGGATGTGCCACATCAAAATTCCAAATGA CAAAAGTGGTACTCTCGCCATTG
204	AvrM-F1	Forward primer for fusing N-terminus of AvrM wild type into Avr1b	CAAAAGTGGTACTCTCGCCATTGAAGACCCCAAA GATATGAAAGGATTCAATAAGGCTC
205	AvrM-R	Reverse Primer for fusing N-terminus of AvrM wild type into Avr1b	CCACAGTCAAGTACGCTGAAGGTTTCGTACATTT TTATCTTTTTCGGATGATG
206	Avr1b(KRRAYIER)F	Forward primer for replacing RFLR motif of Avr1b with KRRAYIER	GACATTCGCCGGGGGAAAAGGGGGGchattatt gaagcSCTCATGAAGAGGACGATGC
207	Avr1b(RAALK)F	Forward primer for replacing RFLR motif of Avr1b with RAALK	GACATTCGCCGGGGGAcgtgcggcgaataaaaAG TGCTCATGAAGAGGACGATGC
208	Avr1b (RLFKLIFKTDSDI Q)F	Forward primer for replacing RFLR motif of Avr1b with RFLKIFKTDSDI	ctttaaacaigacagcacagattt:aaGCTCATGA AGAGGACCGATGCC
209	Avr1b (RLFKLIFKTDSDI Q)R	Reverse primer for replacing RFLR motif of Avr1b with RFLKIFKTDSDI	cigfgctgcagtttaagataatftaaataacgTCC GCCGGCAATGTCCGGCTTC
210	Avr1b(KFLR)F Dou et al <sup>8</sup>	Forward primer for replacing RFLR motif of Avr1b with KFLR	CATTGCCGGcGGAAagTTTCTTTC
211	Avr1b(HFLR)F	Forward primer for replacing RFLR motif of Avr1b with HFLR	CGACATTCGCCGGGGGacacTTTCTTCGAGCTCATGAAG AGGACCGATG
212	Avr1b(QFLR)F Dou et al <sup>8</sup>	Forward primer for replacing RFLR motif of Avr1b with QFLR	CATTGCCGGcGGcAcaATTTC

Figure 14D

SEQ ID NO:	Primer Name	Function	Sequence (5' to 3')
213	Avr1b(RFFR)F	Forward primer for replacing RFLR motif of Avr1b with RFFR	CGACATTGCCGGCGGAAGATTTTTCGAGGCTCATGAAGAGGACGATG
214	Avr1b(RFYR)F	Forward primer for replacing RFLR motif of Avr1b with RFYR	CGACATTGCCGGCGGAAGATTTTTCGAGGCTCATGAAGAGGACGATG
215	Avr1b(RFIR)F	Forward primer for replacing RFLR motif of Avr1b with RFIR	CGACATTGCCGGCGGAAGATTTTTCGAGGCTCATGAAGAGGACGATG
216	Avr1b(RFMR)F	Forward primer for replacing RFLR motif of Avr1b with RFMR	CGACATTGCCGGCGGAAGATTTTTCGAGGCTCATGAAGAGGACGATG
217	Avr1b(RFVR)F Dou et al's	Forward primer for replacing RFLR motif of Avr1b with RFVR	CATTGCCGGCGGAAGATTTTgcTGG
218	Avr1b(RFAR)F Dou et al's	Forward primer for replacing RFLR motif of Avr1b with RFAR	CATTGCCGGCGGAAGATTTCTTCaAGC
219	Avr1b(RFLQ)F Dou et al's	Forward primer for replacing RFLR motif of Avr1b with RFLQ	CATTGCCGGCGGAAGATTTCTTCaAGC
220	Avr1b(RFLG)F	Forward primer for replacing RFLR motif of Avr1b with RFLG	CGACATTGCCGGCGGAAGATTTCTTgGAGGCTCATGAAGAGGACGATG
221	Avr1b(RMVR)F	Forward primer for replacing RFLR motif of Avr1b with RMVR	CGACATTGCCGGCGGAAGATTTggTTCGAGGCTCA
222	Avr1b(RLGT)F	Forward primer for replacing RFLR motif of Avr1b with RLGT	CGACATTGCCGGCGGAAGAcTTggaacAGCTCA
223	Avr1b(RLTQ)F	Forward primer for replacing RFLR motif of Avr1b with RLTQ	CGACATTGCCGGCGGAAGAcTTacTcaAGCTCA
234	P4ForGFPExp-BglII	Forward primer to PCR AvrP4 or AvrP4 mutant	atgcagatcGAAATTTCTTAGAGGATGCACGAGATATCC
235	P4RevGFPPr2	Reverse primer to PCR AvrP4 or AvrP4 mutant	CTGACAGCTCGCGCGATAGGCAAGAAAAGTCGACACGATCCACCATGCGCGCTATCCATATG
236	P123F or GFPExp-BglII	Forward primer to PCR AvrP123 or AvrP123 mutant	atgcagatcCAGTATGTTGTTGATCCAGG
237	P123RevGFPPr2	Reverse primer to PCR AvrP123 or AvrP123 mutant	CATATGGATAGCCGGACATGGTGGATCTGTCGACACA
238	Ps87ForGFP-BglII	Forward primer to PCR Ps87 or Ps87 mutant	ACTTGGGGTGGCTTCACATCCAC ATCCAGATCTACTCTGTTGAACGTTGACT

Figure 14E

SEQ ID NO:	Primer Name	Function	Sequence (5' to 3')
239	Ps87RevGFP2	Reverse primer to PCR Ps87 or Ps87 mutant	CATATGGATAGCCCGGACATGGTGGATCTGTGGACGTTTGAATATCTGTGC
240	AvrMForGFP-EgIII	Forward primer to PCR AvrM or AvrM mutant	atgcagatcATGGGAACAACCCITGGAACAGTACCG
241	AvrMRevGFP2	Reverse primer to PCR AvrM or AvrM mutant	CATATGGATAGCCCGGACATGGTGGATCTGTGGACTCGTACATTTTATCTTTTCTGCCATG
242	AvrPitaForGFP-BglII	Forward primer to PCR AvrPita or AvrPita mutant	AGTCAGATCTCACCCAGTTACGATTACAATCC
243	AvrPitaRevGFP2	Forward primer to PCR AvrPita or AvrPita mutant	CATATGGATAGCCCGGACATGGTGGATCTGTGGACGTTTGAATATCTGTGC
244	Ps87m8R	For replacing KRLTG with AAAAA	GAAACgagcagcgggcaAACCTCGAGGGCAATGTGTC
245	Ps87m8F	For replacing KRLTG with AAAAA	GTTTgacgcgctgcgcGTTTCTCTCTTTCATCATTTCC
246	AvrMimR1	Reverse Primers for replacing HPIQ, KDMK, KALK, KLGT, RGFLR, KFLK, KLST motifs in AvrM with ASSA, SASA, SAAA, AAAAA, SAASA, SAAS, ASSA	CATTgcatgaagcTGGCACATCCGGTACTGTTC
247	AvrMimR2		cagcgcATTGAAATCCgctgagggcactTGGGTCTTCAATGGCAGGAG
248	AvrMimR3		GACGAagcgcagcggcTTCGGATTCTGGAGTAGAag
249	AvrMimR4		CAAAAGGcagcagcagcagcactGTCAAATCTGGTTGAGGGATC
250	AvrMimR5		gatgcCTGAACCTTGGTCCGGactagcggctgaCATTTTGGCTCCAAAAGGgagcag
251	AvrMimF1	Reverse Primers for replacing HPIQ, KDMK, KALK, KLGT, RGFLR, KFLK, KLST motifs in AvrM with ASSA, SASA, SAAA, AAAAA, SAASA, SAAS, ASSA	GTGCCAgatcactgcaAATGACAAAAGTGGTACTCCTG
252	AvrMimF2		cagcaGGATTCAAATagcctgpcgcaTCTACTCCAGAATCCGAAAAAC

Figure 14F

SEQ ID NO:	Primer Name	Function	Sequence (5' to 3')
253	AvrMmF3		GAAgccgctggggctTCGTCAGTTGAAGGGATCCCTC
254	AvrMmF4		CagtgagcctctgctgCCTTTTGGAGCAAAAATG
255	AvrMmF5		gICCGGACCCAAAGTTCAGgcatctcagccGATGATCTCATCA CATAATG
256	AvrP4mR	Reverse primer used to replace RDIQ in AvrP4 with AAAA	
257	AvrP4mF	Forward primer used to replace RDIQ in AvrP4 with AAAA	
258	AvrP123mF	Forward primer used to replace RLIQ in AvrP123 with AAAA	
259	AvrP123mR	Reverse primer used to replace RLIQ in AvrP123 with AAAA	

Figure 14G

SEQ ID NO:	Protein		RXLN Motifs of each fungal or mutations therein	PA Binding	Root Cell Entry
224	AvrPita-GFP	wild-type	KRRAY, RYSQ, RAALK, HAVK, RLFR	Y	Y
225	AvrPita <sub>m</sub> -GFP	mutant	SASAS, AASA, ASSAA, ASAA, AASA	N	N
226	Ps87-GFP	wild-type	KRLTG	Y	NT
227	Ps87 <sub>m</sub> -GFP	mutant	AAAAA	N	NT
228	AvrM-GFP	wild-type	HPIQ, KSGT, KDMK, KALK, KLGT, RGFLR, KFLK, KLST, KNVR	Y	NT
229	AvrM <sub>m</sub> -GFP	mutant	AAAA,AAAA,AAAA,AAAA,AAAA,AAA AA,AAAA,AAAA,AAAA	N	NT
230	AvrP123-GFP	wild-type	RLTQ	Y	NT
231	AvrP123 <sub>m</sub> -GFP	mutant	AAAA	N	NT
232	AvrP4-GFP	wild-type	RDIQ	Y	NT
233	AvrP4 <sub>m</sub> -GFP	mutant	AAAA	N	NT

Figure 15

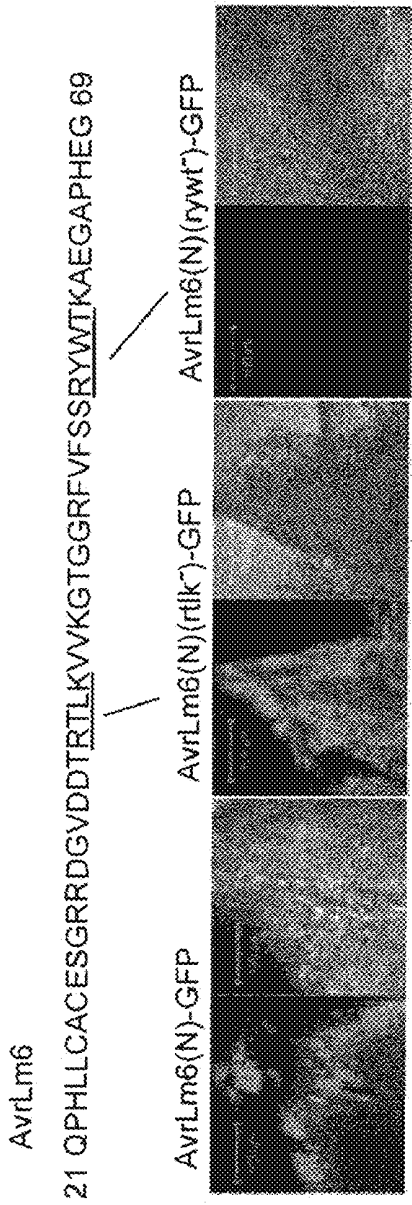


Figure 16A



Figure 16B

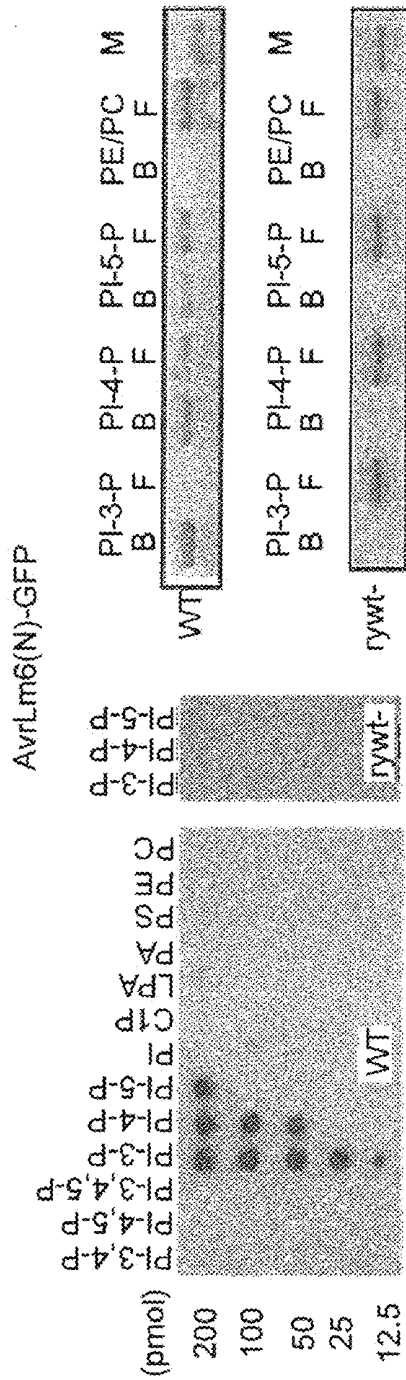


Figure 16C

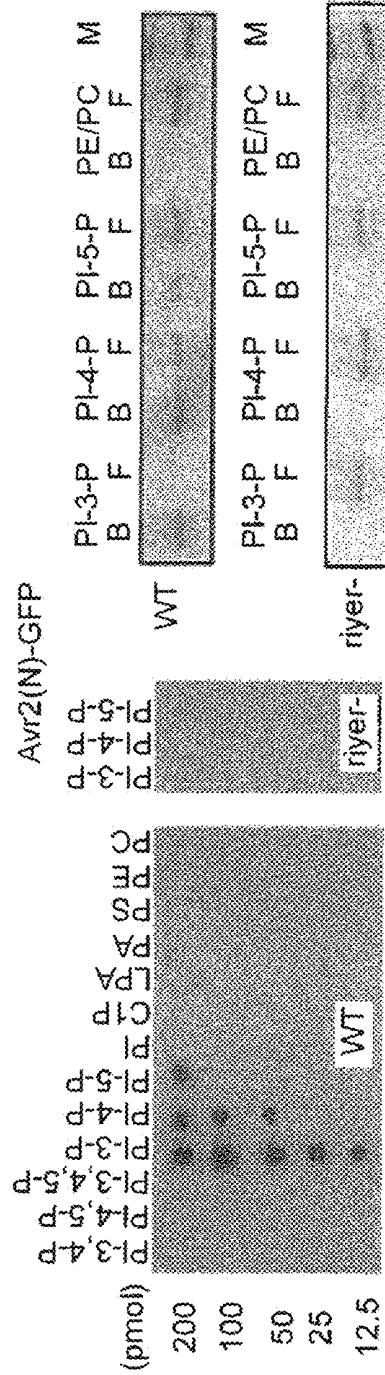


Figure 16D

Af2 VAMGVSEQRKANERKMDARRMARNFNIDTSGETQEEDERKRVLRDNKVVLDLPLPANRKHPSHTAESFYIDYP

Cng2 QQGQKANAREQHRGRKTNLTIKLPGAHSHYKAKFEGCMVVLQDKKLYVEHAGCESLAYAHP

AviLm4/7 CREASISGEIRYPQGTCTKTEALNDCNKVTKGLIDFSQSHQRRAWGIDMTAKVQ

Figure 17A

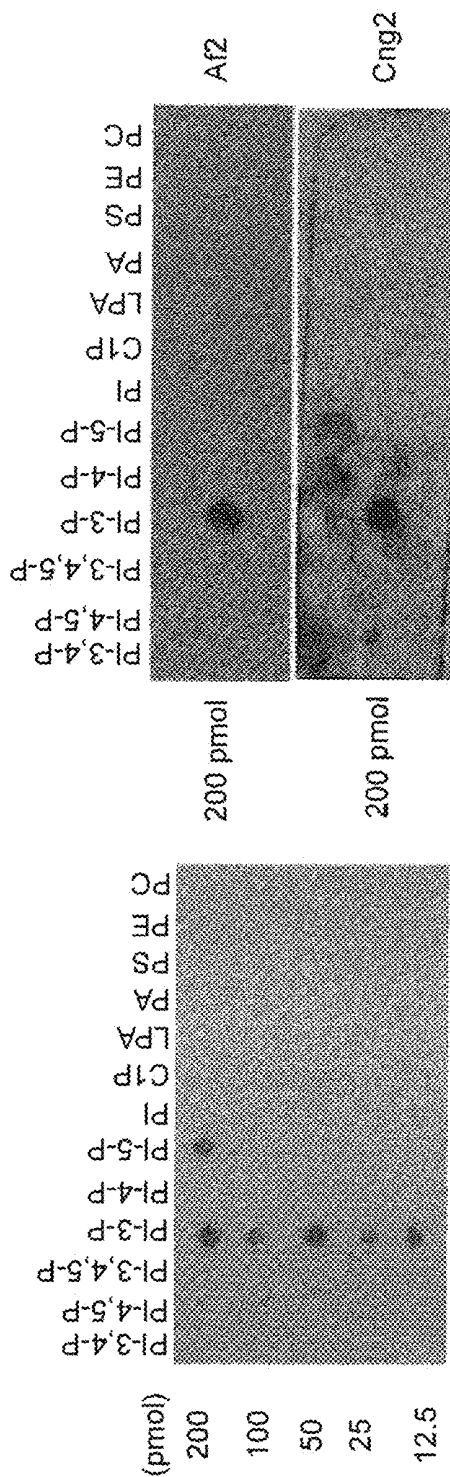


Figure 17B

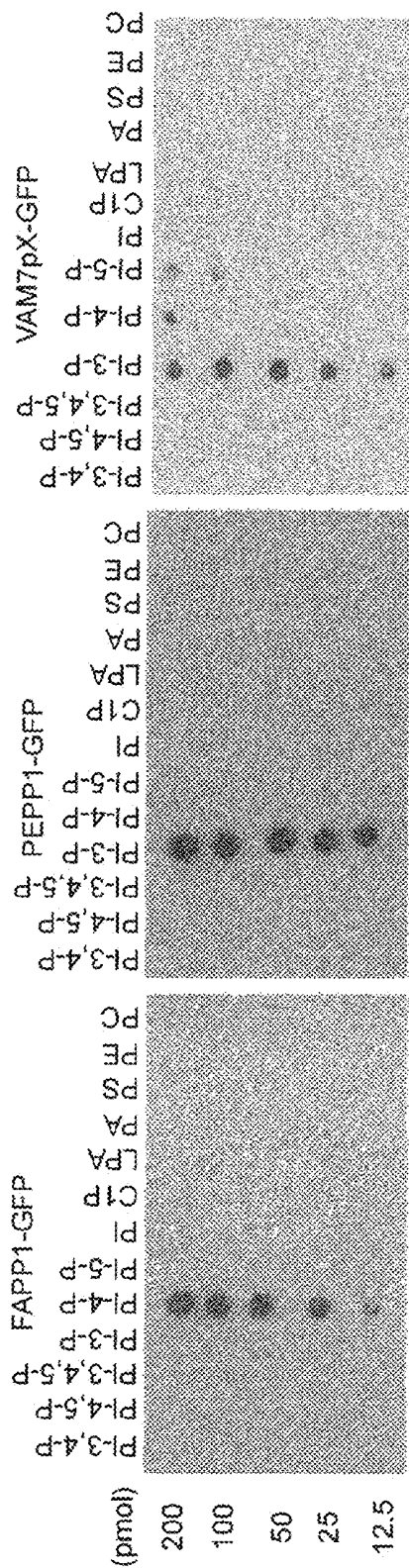


Figure 18A

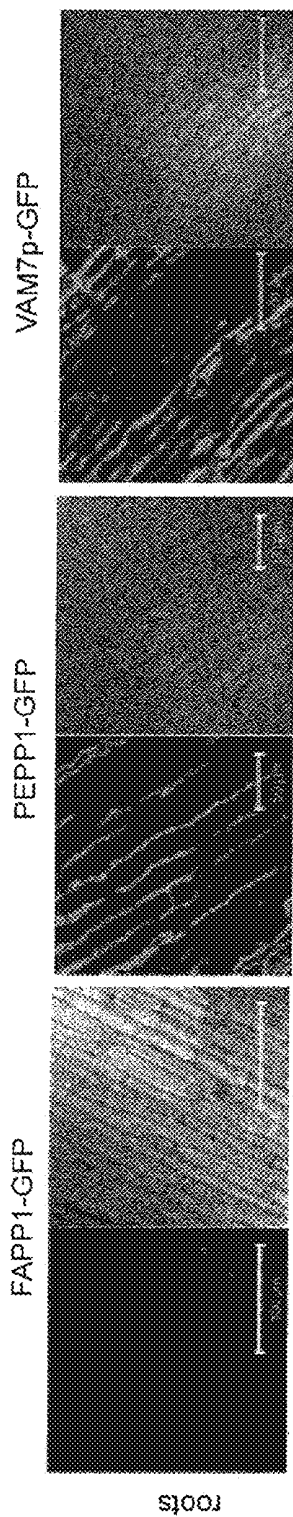


Figure 18B

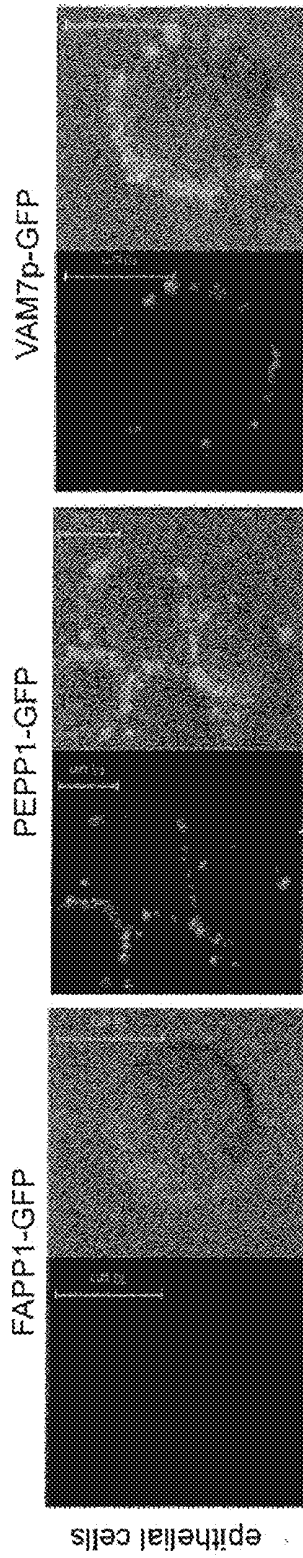


Figure 18C

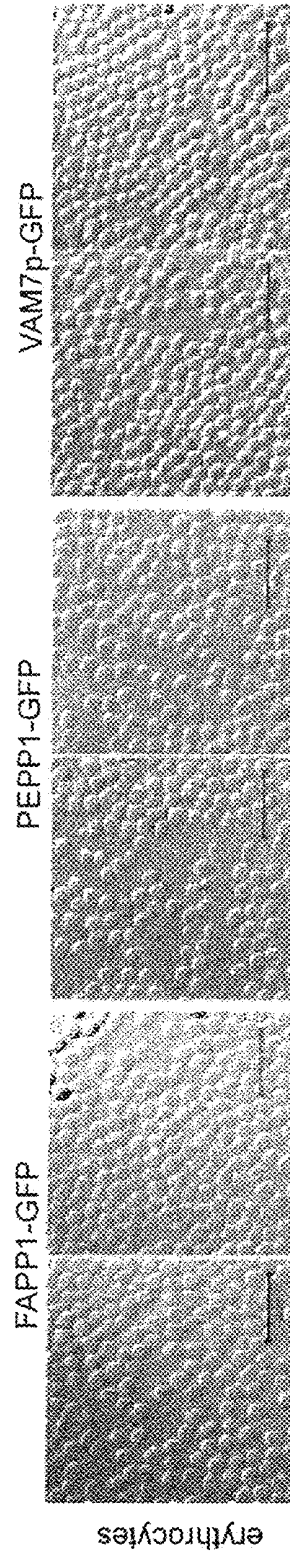


Figure 18D

	Entry
Avr1b	1.00
Avr1b(AAAA)	0.00
Avr1b(RFRL)	0.00
Avr1b(FRLR)	0.00
Avr1b(1K)	0.42
Avr1b(1H)	0.89
Avr1b(1Q)	0.00
Avr1b(3F)	1.01
Avr1b(3Y)	0.84
Avr1b(3I)	0.52
Avr1b(3M)	0.89
Avr1b(3V)	0.00
Avr1b(3A)	0.00
Avr1b(4Q)	0.74
Avr1b(4G)	0.88
Avr1b(2M3V)	0.71
Avr1b(2L3G4T)	0.56
Avr1b(2L3T4Q)	0.66

```

TEYSDETNIAMVESPDLVRRSLRNGDIAGGRFLRAHEEDDAGERTFSV
-----AAAA-----RL-----FR-----K-----H-----Q-----F-----Y-----I-----M-----V-----A-----Q-----G-----MV-----LGT-----LTQ-----
    
```

Figure 19

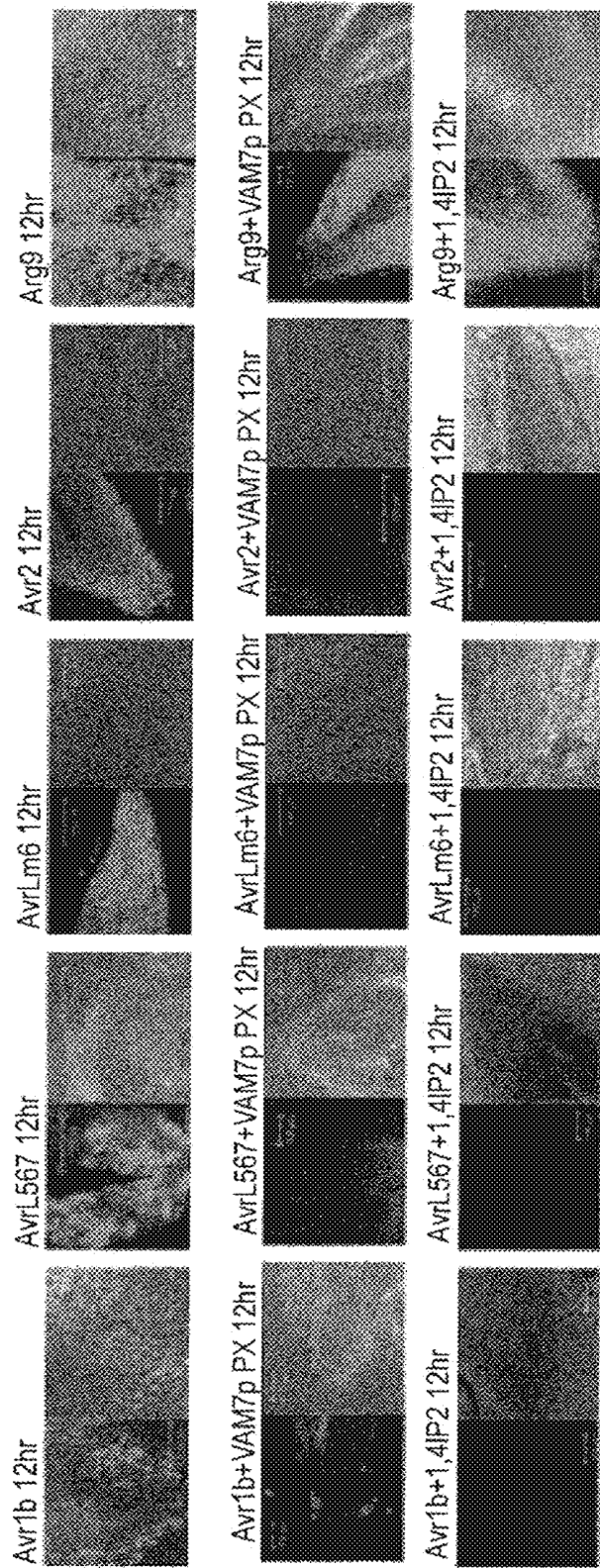


Figure 20A

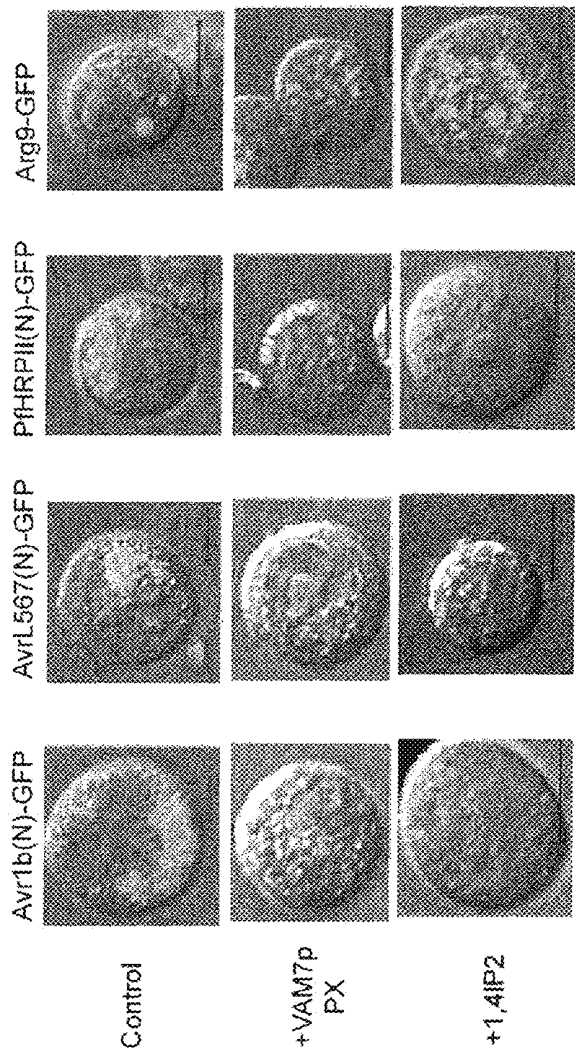


Figure 20B

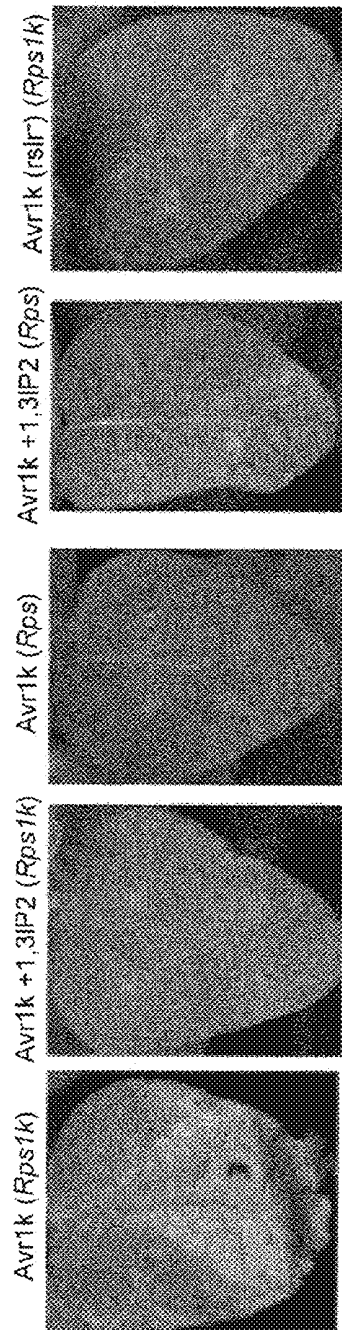


Figure 20C

## COMPOSITIONS AND METHODS TO PROTECT CELLS BY BLOCKING ENTRY OF PATHOGEN PROTEINS

### CROSS-REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims priority to U.S. Provisional Patent Application Ser. No. 61/128,080, filed May 19, 2008; Ser. No. 61/160,059, filed Mar. 13, 2009; and Ser. No. 61/260,227, filed Nov. 11, 2009. This application is a continuation-in-part of U.S. patent application Ser. No. 12/468,470 filed May 19, 2009; and of International patent application PCT/US09/044,489, filed May 19, 2009. The complete contents of each of these applications are herein incorporated by reference.

### STATEMENT OF GOVERNMENT INTEREST

**[0002]** This invention was made with funding under USDA/CSREES/NRICGP Grant No. 2007-35319-18100, and the United States government has certain rights in the invention.

### SEQUENCE LISTING

**[0003]** This application includes as the Sequence Listing the complete contents of the accompanying text file "Sequence.txt", created Nov. 10, 2010, containing 307,236 bytes, hereby incorporated by reference.

### BACKGROUND OF THE INVENTION

**[0004]** 1. Field of the Invention

**[0005]** The present invention generally relates to prevention of microbial, especially oomycete or fungal, disease and, more particularly, to cellular targets for blocking entry of pathogen effector proteins into plant or animal cells. The invention also provides compositions and methods for identifying compounds that block entry of pathogen effector proteins into cells, and treatments using such compounds.

**[0006]** 2. Background Description

**[0007]** Fungi and parasites such as *Plasmodium* are eukaryotes, which are organisms that have complex internal cell structures (bacteria and viruses have simpler structures and are excluded). Infections by parasites and fungi are especially difficult to develop drugs for because humans are also eukaryotes, so many drugs toxic to these organisms are also toxic to humans. In addition to the malaria parasite, *Plasmodium*, other eukaryotic pathogens of humans include the parasites *Schistosoma*, *Onchocerca*, *Trypanosoma*, and *Leishmania*, fungi that afflict AIDS patients such as *Candida*, *Histoplasma*, *Cryptococcus* and *Aspergillus*, and the Valley Fever fungus, *Coccidioides*, that affects healthy people in the Southwest. Fungal spores are also responsible for allergies, asthma and mold-related illnesses.

**[0008]** Eukaryotic pathogens of plants are also a major problem in agriculture, horticulture and forestry, and include fungi and fungal-like organisms related to marine algae called oomycetes. These diseases cause billions of dollars in losses each year. Some fungal plant pathogens include rust fungi, such as the new virulent wheat rust fungus, Ug99, that is sweeping through Africa and the middle east, and the rice blast fungus which causes major losses to the US and Asian rice crop each year. Oomycete pathogens include the late blight pathogen of potato (*Phytophthora infestans*) that causes the Irish potato famine and still causes \$5 billion in

losses worldwide annually, *Phytophthora ramorum* that causes Sudden Oak Death in California, and *Phytophthora sojae* that caused \$1-2b damage to the US soybean crop. Worldwide transport of plants and plant products across diverse ecosystems has hastened the spread of many plant pathogens. With the increased pressure on agricultural production systems due to competing needs for food and biofuels, there is an urgent need to explore new highly efficacious strategies for biotechnology-based approaches to disease control.

**[0009]** Eukaryotic pathogens of both humans and plants release protein toxins called effectors that have the ability to infiltrate inside host cells, across the membrane barrier that normally surrounds the host cells. Once the effectors enter the host cell, they reprogram the cells to suppress or block the immune responses of the host and to make the host tissue more congenial for reproduction and spread of the pathogen. Therefore, drugs that could block the entry of effector proteins into host cells would potentially suppress infection by a broad range of eukaryotic pathogens important to medicine and agriculture.

**[0010]** Like animals, plants have evolved defense mechanisms that afford some protection from pathogens. Constitutive defenses include structures such as the cuticle and preformed anti-microbial chemicals. Plants have also evolved an active defense response that is induced by detection of an attacking pathogen. The response includes rapid synthesis of anti-microbial chemicals and proteins, and a programmed cell death (PCD) response, called the hypersensitive response (HR). The ability of plants to detect and respond to pathogens is mediated by various receptors and signal transduction pathways that have close similarities to the innate immunity mechanisms of animals. Unfortunately, however, pathogens of both plants and animals have evolved mechanisms to avoid or suppress host defenses, thereby retaining the ability to cause many destructive diseases affecting crops and forests.

**[0011]** Oomycetes are fungus-like organisms many of which are pathogens. For example, most of the more than 80 species of the oomycete genus *Phytophthora* are destructive pathogens, including the potato late blight pathogen, *Phytophthora infestans*, which caused the Irish potato famine in the 18th century, the soybean root and stem rot pathogen *P. sojae*, and *Phytophthora ramorum*, the causative agent of Sudden Oak Death that is currently ravishing oak forests in California. The closely related oomycete genus *Pythium* contains more than 100 species, most of which are also pathogens. The oomycetes also include a number of commercially important and diverse downy mildew pathogens that are obligate parasites, often with narrow host ranges.

**[0012]** The sequencing of Expressed Sequence Tags (ESTs) and genomes from several oomycete pathogens has been completed or is under way. Draft genome sequences for the soybean pathogen *Phytophthora sojae* and for *P. ramorum* have been completed; those of *P. infestans* and the *Arabidopsis* downy mildew pathogen *Hyaloperonospora arabidopsidis* are nearing completion; and genome sequencing of the broad host range plant pathogens *Phytophthora capsici* and *Pythium ultimum* and the fish pathogen *Saprolegnia parasitica* is also underway. In addition, substantial libraries of EST sequences are available for most of these species, as well as for *Phytophthora parasitica* and the Saprolegniomycete plant pathogens *Aphanomyces euteiches* and *Aphanomyces cochlidioides*. The mining of these pathogen sequences by comparative genomics and the prediction of which proteins

are secreted by the pathogens has resulted in identification of large numbers of candidate genes that potentially encode proteins involved in plant infection.

**[0013]** Among these are the so-called “effector proteins” or “effectors”. Effectors, which are secreted by plant pathogens and have the ability to enter plant cells, have been documented for many classes of plant pathogens, including bacteria, fungi, oomycetes and nematodes. Once inside a host cell, the major function of an effector protein is to suppress the signal transduction pathways that mediate plants defense responses, and many effector proteins also suppress host programmed cell death. The activities of fungal effector proteins are known to include chitin-binding, cytotoxicity, metalloprotease activity, and protease inhibition. Pathogen effectors may also reprogram the plant cell to promote nutrition of the pathogen.

**[0014]** In response to pathogen attacks mediated by effectors, plants have evolved certain resistance (“R”) genes that encode receptor proteins having the ability to bind and sequester, and thereby inactivate, the pathogen effector proteins. (In fact, effectors were initially discovered based on their ability to trigger responses mediated by R gene-encoded host receptors). Pathogen genes encoding effectors are referred to as avirulence (Avr) genes, because, in practice, they actually prevent infection of host plants which contain cognate receptor proteins by binding to the receptor, thereby alerting the plant to their presence, and initiating an anti-pathogen response. In contrast, genes encoding plant effectors for which cognate plant receptors do not exist are referred to as virulence genes.

**[0015]** Genetic mapping of oomycete Avr genes led to the cloning of the first four effector genes: Avr1b-1 from *P. sojae* (Shan, W., Cao, M., Leung, D. & Tyler, B. M. The Avr1b locus of *Phytophthora sojae* encodes an elicitor and a regulator required for avirulence on soybean plants carrying resistance gene Rps1b. *Mol. Plant. Microbe Interact* 17, 394-403 (2004); Avr3a from *P. infestans* (Armstrong, M. R. et al. An ancestral oomycete locus contains late blight avirulence gene Avr3a, encoding a protein that is recognized in the host cytoplasm. *Proc Natl Acad Sci USA* 102, 7766-71 (2005); and ATR1 (Rehmany, A. P., Gordon, A., Rose, L. E., Allen, R. L., Armstrong, M. R., Whisson, S. C., Kamoun, S., Tyler, B. M., Birch, P. R., and Beynon, J. L. (2005). Differential recognition of highly divergent downy mildew avirulence gene alleles by RPP1 resistance genes from two *Arabidopsis* lines. *Plant Cell* 17, 1839-1850), and ATR13 (Allen, R. L., Bittner-Eddy, P. D., Grenville-Briggs, L. J., Meitz, J. C., Rehmany, A. P., Rose, L. E., and Beynon, J. L. (2004). Host-parasite coevolutionary conflict between *Arabidopsis* and downy mildew. *Science* 306, 1957-1960), both from *H. arabidopsidis*. Many other effector genes have subsequently been identified and analyses have shown that all effector genes encode small secreted hydrophilic proteins that lack disulfide bonds. Significantly, effector proteins have the ability to enter plant cells unaided by any other pathogen encoded molecules. Thus, the mechanism of entry must lie in the effector proteins themselves. Sequence comparisons have led to the identification of two common motifs in the N-terminus region of effector proteins: 1) RxLR or RXLR (which stands for “arginine, any amino acid, leucine, arginine”); and 2) dEER (which stands for “aspartate which is not highly conserved, glutamate, glutamate, arginine”) (Birch, P. R., Rehmany, A. P., Pritchard, L., Kamoun, S., and Beynon, J. L. 2006. *Trends Microbiol* 14, 8-11; Tyler, 2006. *Science* 313, 1261-1266; Rehmany, et al.

2005. *Plant Cell* 17, 1839-1850). These motifs have been suspected of being responsible for the ability of effector proteins to enter plant cells. This speculation has been encouraged by the observation that a similar N-terminal “Pexel” motif (RxLxE/Q, i.e. “arginine, any amino acid, leucine, any amino acid, then aspartate or glutamate or glutamine”) is required for effectors of the malaria parasite, *Plasmodium*, to cross the host parasitophorous vacuolar membrane into the cytoplasm of red blood cells. In addition, experimental evidence has shown that mutations in either the RxLR or dEER motifs can alter an effector’s ability to translocate into host cells.

**[0016]** Effectors of fungal plant pathogens have also been predicted to translocate into host cells because many plants (e.g. flax and rice) possess intracellular receptors, encoded by major resistance (R) genes, which mediate a rapid defense response when fungal effectors are present. However, prior to the present invention, fungal effectors had not been well characterized and the presence of amino acid sequence motifs that mediated entry into host cells had not been demonstrated.

**[0017]** In spite of previous suspicions concerning putative involvement of the RxLR and dEER motifs in effector translocation, the precise mode of and requirements for translocation of effector proteins were not known. And, in fact, it was previously not known whether fungal pathogens even possessed these or analogous motifs. This lack of knowledge had hindered the development of effective methods to combat the infection of both plant and animal host cells by oomycete, fungal and *Plasmodium* pathogens. Further, the lack of detailed characterization of the RxLR and dEER motifs and their flanking sequences has prevented the selection, from an enormous pool of genomic sequence data, of genes that likely encode additional effector proteins, the identification of which could lead to strategies for inhibiting their pathogenic action in cells.

#### SUMMARY OF THE INVENTION

**[0018]** The present invention provides methods to block the entry of pathogen effector proteins into host cells (e.g., “translocation”), thereby preventing host cell infection. The methods are based on the discovery that binding of polar lipids such as phosphatidyl-inositol-3-phosphate (PI-3-P) and/or phosphatidyl-inositol-4-phosphate (PI-4-P) and/or phosphatidic acid to effector molecules via a virulence motif is a prerequisite to translocation of the effector into a host cell, and that when binding is blocked, translocation, and hence infection of the cell, does not occur. The motifs have the sequence “BXZ” where B=arginine, lysine or histidine; X=any amino acid or no amino acid (i.e. X may be absent); and Z=leucine, methionine, isoleucine, tryptophan, tyrosine or phenylalanine. The BXZ motif encompasses a family of motifs, which frequently occur at or near the N-terminus of an effector protein, examples of which include but are not limited to RxLR (which may function in concert with a dEER motif), Pexel, RYWT, RIYER, RSLR, RRLLR, RRFLR, and RFYR, and others, all of which may collectively be referred to herein as “virulence motifs.”

**[0019]** Binding may be prevented by any of several strategies including but not limited to i) blocking the effector motif itself, and ii) blocking the lipids of the cell to which the motif binds. The motif itself may be blocked by e.g. inositol 1,4-diphosphate, or by other inositol containing phosphatidic acids, phospholipids and sphingolipids, or any other compound which binds to one or the virulence motif. Blocking of

the lipid (generally at or on the cell surface) can be accomplished using, for example, proteins or peptides or other molecules that bind the lipids (e.g. mimetics of the motifs, or proteins or other compounds that destroy, remove or block access to phosphatidyl-inositol-3-phosphate (PI-3-P) and/or phosphatidyl-inositol-4-phosphate (PI-4-P) and/or phosphatidic acid and/or any other polar lipid that is bound by an effector) thereby preventing effector binding to a motif. According to the invention, the entry of effector proteins from oomycetes, fungi, and other types of pathogens, including human pathogens (e.g. *Plasmodium*) may be blocked. For example, *Plasmodium* effector proteins include a Pexel motif which is selectively bound as a prerequisite for translocation. Blocking of effector entry prevents the pathogen from inhibiting host cell defense mechanisms and allows the host to mount an effective response to the pathogen.

**[0020]** The invention also provides elucidation of the structural requirements of the virulence motifs in oomycetes and fungi, and of the sequences which flank the motifs, leading to the ability to predict which genes in the genome of a pathogen are likely to encode effector molecules.

**[0021]** According to an embodiment the invention, translocation of an effector protein from a pathogen, such as a bacteria, fungus, oomycete, protozoa or nematode, into a host including animals (including humans) and plants is prevented by selectively binding a blocking compound to one or more BXZ virulence motifs of the effector protein. According to another embodiment, translocation of an effector protein from a pathogen is prevented by selectively binding a blocking compound to one or more polar lipids which would otherwise bind an effector motif. By preventing entry of the effector protein into the cell, the host cell defense mechanisms are permitted to mount an effective defense against the pathogen (it being recognized that after entry, the effector protein would compromise the host cell defense mechanisms). Thus, the invention provides a mechanism to avoid the adverse outcomes attributed to pathogenic effector proteins, and it is applicable in promoting the health and viability of both plants and animals.

**[0022]** Another embodiment of the invention pertains to identifying compounds which are suitable for use in protecting cells (animal and plant) from pathogenic effector proteins. In operation, an assay is used to determine whether or not a compound binds to one or more motifs of an effector protein which are bound by phosphoinositides or another polar lipid as a prerequisite for translocation. The assay may include pathogenic effector proteins which include a BXZ motif (e.g. RxLRPexel, RYWT, RIYER, RSLR, RRLLR, RRFLR, and RFYR or similar motifs), and/or may include protein substrates which present one or more BXZ motifs in a manner which can be bound by a candidate compound. Alternatively, an assay may include one or more polar lipids which bind to the motifs, in order to identify compounds which bind and thus would block motif binding. Such assays may include competition assays between candidate compounds and compounds (e.g. peptides or proteins) which contain the motif(s). The assay may be in the solid or liquid phase and may employ fluorescent, phosphorescent, chemiluminescent, colorimetric, or other suitable labels to indicate binding of a candidate compound to one or more motifs which are required to be bound by phosphoinositides or another polar lipid as a prerequisite for translocation.

**[0023]** Yet another embodiment of the invention pertains to a methodology of identifying whether an amino acid

sequence of a protein in a pathogen is part of an effector protein. In this embodiment, hidden Markov modeling (HMM) is used to compare flanking sequences of a BXZ sequence (e.g. of an RxLR sequence) to determine whether the structural features for the motif are present. Through identification of effector proteins, effective strategies for preventing entry of the effector proteins into a cell (animal or plant) can be pursued.

#### BRIEF DESCRIPTION OF THE DRAWINGS

**[0024]** FIG. 1A-E. RxLR and dEER motifs are required for Avr1b function in *P. sojae* transformants. (A) Sequences of mutations in the RxLR1, RxLR2 and dEER motifs. Bold indicates amino acids of the RxLR motifs and the alanines used to replace them in the mutations. Italics indicate the dEER motif and the alanines used to replace it in the mutant. (B) Pst I restriction analysis of PCR products amplified from Avr1b-1 transformants using primers specific for the HAM34 promoter and terminator regions. Pst I restriction profiles of Avr1b(RxLR1<sup>AAAA</sup>), Avr1b(RxLR2<sup>AAAA</sup>), Avr1b(RxLR1<sup>AAAA</sup>, 2<sup>AAAA</sup>), Avr1b(dEER<sup>A6</sup>) and wild type (WT) Avr1b are distinguished from each other because the mutations introduce a Pst I site. Avr1b(dEER<sup>A6</sup>)-9 was confirmed by sequencing the PCR product. C, Detection of Avr1b mRNA in *P. sojae* stable transformants by RT-PCR. Upper panel shows amplification with primers internal to the Avr1b C-terminus. Lower panel shows amplification with *P. sojae* actin primers. *P. sojae* stable transformants were the same as for (B) except that an amplification reaction is also shown from RNA from a *P. sojae* transformant containing a  $\beta$ -glucuronidase gene (GUS). No amplification was observed when reverse transcriptase was omitted from the reactions. (D) Distributions of HMM scores of RxLR flanking regions for all RxLR-containing secreted proteins from *P. sojae* and *P. ramorum* (non-permuted), for all secreted proteins retaining an RxLR string after sequence permutation (permuted), and for all high quality RxLR-effector candidates identified by Jiang et al (2008) (curated). The locations on the distribution of the HMM scores of the RxLR strings of known avirulence proteins and HpAvh341 are shown by the arrows. (E) Phenotype of L77-1863 (Rps1b) seedlings inoculated on the hypocotyls with transformants carrying the indicated wild type or mutant Avr1b-1 genes and photographed 4 days later.

**[0025]** FIG. 2. RxLR and dEER functions confirmed by particle bombardment assay. Soybean leaves were bombarded using a double-barreled device that delivered Avr1b-1 DNA-bearing particles to one side of the leaf and control (empty vector) DNA to the other; both sides received GUS DNA. Ratio of blue spots in the presence of Avr1b-1 compared to the control. sAvr1b indicates a gene encoding secretory Avr1b and mAvr1b indicates one encoding mature Avr1b (lacking the secretory leader). WT indicates wild-type RxLR motif, RxLR2<sup>AAAA</sup> indicates the four alanine replacement of the RxLR2 motif, dEERA6 indicates the six alanine replacement of the dEER motif. Averages and standard errors are from 16 pairs of shots. p values comparing results from cultivars with Rps1b (L77-1863) or without (rps; Williams) were calculated using the Wilcoxon rank sum test.

**[0026]** FIG. 3A-D. *P. sojae* stable transformants show that two other Avh proteins can replace the RxLR and dEER region of Avr1b. (A) Sequences of the N-termini of wild type and mutant Avr1b proteins, and of fusions with two other Avh proteins. Underlined, secretory leader; bold, RxLR motifs; italics, dEER motifs. The C-terminal sequence of Avr1b is

shown in lowercase. (B) PCR analysis of DNA from *P. sojae* stable transformants. WT: p1=pHamAvr1b plasmid DNA, T17 and T20=two transformants with wild type Avr1b-1 transgenes. HpAvh341-Avr1bCt: p1=pHamAvh341 plasmid DNA (encoding Hp Avh341-Avr1bCt), 13 and 17=two transformants containing pHamAvh341. PsAvr4/6-Avr1bCt: pHamAvh171 plasmid DNA (encoding Ps Avr4/6-Avr1bCt), 3 and 19=two transformants containing pHamAvh171. mAvr1bCt: p1=pHamAvr1bCt plasmid DNA (encoding mAvr1bCt protein), 4 and 5=two transformants containing pHamAvr1bCt. The sizes of the PCR products for Avr1b-1, pHamAvh341, pHamAvh171 and pHamAvr1bCt are 577 bp, 721 bp, 748 bp and 385 bp respectively. C, Detection of Avr1b mRNA in *P. sojae* stable transformants by RT-PCR. Upper panel shows amplification with primers internal to the Avr1b C-terminus. Lower panel shows amplification with *P. sojae* actin primers. *P. sojae* stable transformants were the same as for (B) except that an amplification reaction is also shown from RNA from a *P. sojae* transformant containing a  $\beta$ -glucuronidase gene (GUS). p1=pHamAvr1b plasmid DNA as template. No amplification was observed when reverse transcriptase was omitted from the reactions (D) Phenotype of L77-1863 (Rps1b) seedlings inoculated on the hypocotyls with the indicated transformants carrying wild type or mutant Avr1b-1 genes and photographed 4 days later. HpAvh341-Avr1b-17, PsAvr4/6-Avr1b-3 and mAvr1bCt-5 gave similar results to HpAvh341-Avr1b-13, PsAvr4/6-Avr1b-19 and mAvr1bCt-4 (Table 1).

**[0027]** FIGS. 4A and B. Functional replacement of Avr1b host targeting signal with protein transduction motifs and *Plasmodium* host targeting signals. (A) Sequences of modified Avr1b proteins. PfGBP, PfHRP and Pf1615c refer to the *Plasmodium* Pf GBP-130, Pf HRPII and Pf PFE1615c proteins. All non-native Avr1b sequences are underlined, Avr1b RxLR2 and *Plasmodium* RxLXE/Q motifs are in bold, and acidic residues in the dEER region are in italics. The Avr1b secretory leader was used in all constructs. (B) Ratio of blue spots in the presence of Avr1b-1 compared to the control, assayed as described in FIG. 2. Constructs are as in (A). Averages and standard errors are from 8 pairs of shots.

**[0028]** FIG. 5. Summary of Avr1b-1 mutations and their phenotypes in *P. sojae* stable transformants and soybean transient expression assays. A, Avirulent; V, Virulent; NT, Not Tested; Y, significantly fewer blue (GUS-positive) tissue patches from GUS expression resulting from Avr1b-induced cell death; N, not significantly fewer blue tissue patches; P, partial reduction in blue tissue patches; SP, signal peptide.

**[0029]** FIGS. 6A-H. Binding of oomycete effector proteins to phosphoinositides. a-c, Filter-binding assays. d-f, Liposome binding assays. RxLR and dEER mutations are described in FIGS. 6G and H. (N)-GFP indicates a fusion of the N-terminal domain to GFP. (FL)-GST indicates a fusion of the full length effector proteins (without signal peptide) to GST. In d-f, B and F indicate liposome-bound and -free proteins respectively; M=size markers. PI-3-P=phosphatidylinositol-3-phosphate; PI-4-P=phosphatidyl inositol-4-phosphate; PI-5-P=phosphatidyl inositol-5-phosphate; PI=phosphatidyl inositol; PA=phosphatidic acid; PS=phosphatidyl serine; PE=phosphatidyl ethanolamine; PC=phosphatidyl choline. No mutant proteins bound to PI-5-P, PT, PA, PS, PE or PC (not shown).

**[0030]** FIGS. 7A-C. Identification of host-targeting signals in fungal effectors a, Particle bombardment cell re-entry assays of fungal effectors fused to Avr1b. N-terminal

sequences of AvrL567, AvrM and AvrPi-ta (shown in b) were fused to the secretory leader (s) and C-terminal domain of Avr1b. AvrL567-Avr1b fusions lacking the secretory leader (m) or with mutations in the putative RxLR and dEER motif (rfyr-de-) were also assayed. Effector re-entry resulting in cell killing was measured by double-barreled particle bombardment in which parallel bombardments with a beta-glucuronidase (GUS) reporter gene, with and without the Avr1b fusion, were compared in the presence of resistance gene Rps1b (cultivar L77-1863) or in its absence (rps; cultivar Williams). Averages and standard errors shown are from 14-16 pairs of bombardments. P values were calculated using the Wilcoxon rank sum test. b, N-terminal sequences of effectors tested in a, with RXLR-like motifs shaded and dEER-like motifs underlined. The start of the Avr1b C-terminal domain used for all fusions is boxed. Three sequences containing potential motifs from AvrPi-ta and one from AvrL567 were inserted into Avr1b in place of the RFLR motif. Cell entry activity of each sequence is given as relative ablation. Ablation=[1-(GUS+spots on Rps1b)/(GUS+spots on rps)]. Relative ablation=ablation of construct/ablation of wild-type Avr1b. In the sequence alignment, dashes indicate identical residues, periods indicate gaps in the alignment and \* \* \* \* indicates Avr1b sequences.

**[0031]** FIGS. 8A-F. Binding of *P. falciparum* effector fusion proteins to phosphoinositides a-c, Filter binding assays Mutant proteins did not bind to PI-5-P, PI, PA, PS, PE or PC (not shown) except PfGBP(N)-GFP(pexel-) which bound weakly to PS. d-f, Binding of wild-type and mutant fusion proteins to PT-3-P or PI-4-P in liposomes; B and F indicate liposome-bound and -free proteins respectively; M=size markers.

**[0032]** FIGS. 9A-D. Modulation of effector entry into root cells by phosphoinositides a, Stimulation of Avr1b(N)-GFP entry by PI-4-P and inhibition by IP2. b, Stimulation of AvrL567(N)-GFP entry by PI-4-P and inhibition by IP2. c, Entry of Arg9-GFP is not stimulated by PI-4-P nor inhibited by IP2. In each case, 1 mg/ml protein was incubated with soybean root tips for 9 hr or 12 hr then washed and photographed. Either 250  $\mu$ M di-octanoyl-PI-4-P or 500  $\mu$ M IP2 was preincubated with the proteins for 30 min prior to exposure to the roots. Paired light micrographs and fluorescence optical sections are from the same root tips in each case. Lighting and photographic exposure were identical for all photographs. d, Inhibition of effector-binding to liposomes by IP2. Binding of Avr1b(N)-GFP, AvrL567(N)-GFP and Arg9-GFP to liposomes containing PI-4-P was measured in the presence or absence of 300  $\mu$ M inositol 1,4 diphosphate (1,4IP2). Also, binding of Arg9-GFP to liposomes containing PI-3-P was measured in the presence or absence of 300  $\mu$ M inositol 1,3 diphosphate (1,3IP2).

**[0033]** FIGS. 10A-E. Effector entry into human cells and inhibition by inositol diphosphates a-d, Cells of the human lung epithelial cell line A549 were incubated with the indicated fusion proteins (1 mg/ml) for 15 hr, in the presence or absence of 430  $\mu$ M inositol 1,3 diphosphate (1,3IP2), 440  $\mu$ M inositol 1,4 diphosphate (1,4IP2) or 240  $\mu$ M dioctanoyl-PI-4-P, then washed and photographed. Paired light micrographs and fluorescence optical sections are from the same cells in each case. Lighting and photographic exposure were identical for all photographs.

**[0034]** FIGS. 11A-B. Description of plasmids used in Example 1.

**[0035]** FIGS. 12A-B. Oligonucleotides used for plasmid construction. Uppercase letters indicate bases that match the initial template. Lower case letters indicate mutations or 5' extensions that do not match the initial template. Restriction sites introduced into the amplicon are underlined. A pipe (|) indicates the boundary between Avr1b-1 sequences and fused sequences (Avh, GFP or *Plasmodium* RXLX motif) in the fusion oligonucleotides.

**[0036]** FIGS. 13 A-F. Description of plasmids used in Example 2.

**[0037]** FIG. 14A-F. Oligonucleotides used. Restriction sites are in bold. Mutations created by the primers are in lower case.

**[0038]** FIG. 15. Binding of fungal effector proteins to phosphatidic acid shown in tabular form. Filter-binding assay were used to test which polar lipids were bound by the indicated fungal effector proteins. The N-terminus of each fungal effector (documented in the sequence list) was fused to GFP, the fusion proteins were purified from *E. coli*, and then tested for binding to the same polar lipids as documented in FIG. 6. Mutant proteins contained amino acid substitutions in the motifs listed in the row above (A=alanine; S=serine). All the wild-type proteins listed bound phosphatidic acid, but none of the mutants did.

**[0039]** FIG. 16A-D. Effectors from non-haustorial fungal pathogens enter via RXLR-mediated PI-3-P binding. A. N-terminus of AvrLm6 (SEQ ID NO: 319) showing functional and non-functional RXLR motifs. 1 mg/mL GFP fusion proteins were incubated with soybean root cells for 12 hr then washed for 2 hr. B. N-terminus of Avr2 (SEQ ID NO: 326) showing functional and non-functional RXLR motifs. 1 mg/mL GFP fusion proteins were incubated with soybean root cells for 12 hr then washed for 2 hr. C. Binding of AvrLm6-GFP to lipids in filter-(left) and liposome-(right) binding assays. D. Binding of Avr2-GFP to lipids in filter-(left) and liposome-(right) binding assays. In both C and D lipids are: PI phosphatidyl (ptd)-inositol; C1P=ceramide-1 phosphate; LPA=lysophosphatidic acid; PA=phosphatidic acid; PS=ptd-serine; PE=ptd-ethanolamine; PC=ptd-choline. In the liposome assays (right panels), B=bound; F=free; M=markers.

**[0040]** FIGS. 17A and B. Binding of three fungal effectors to phosphoinositides. A. N-terminal sequences of effector-like proteins Af2 from *Aspergillus fumigatus* (Af2; XP 752996.1), CNg2 from *Cryptococcus neoformans* (Cng2; AAW43853.1) and AvrLm4/7 from *Leptosphaeria macularis*. Candidate RXLR-like motifs are boxed. B. Sequences shown in A were fused to GFP and the expressed proteins tested for lipid binding using filter-binding assays as described in FIG. 16D.

**[0041]** FIG. 18A-D. PI-3-P is located on the surface of root cells and epithelial cells, but not erythrocytes. A. Binding of biosensors to phosphoinositides in filter assays, as described for FIG. 16. B. Binding of biosensors to root cells. Fusion proteins were incubated with soybean root cells for 12 hr then washed for 2 hr. Pairs of fluorescence and light micrographs are shown. Bars=50  $\mu$ M or 100  $\mu$ M. C. Binding of biosensors to epithelial cells. Fusion proteins were incubated with cells for 2 hr then washed for 30 min. Pairs of fluorescence micrographs and fluorescence/light overlays are shown. Bars=10  $\mu$ M. D. Binding of biosensors to human erythrocytes. Fusion proteins were incubated with cells for 2 hr then washed for 30 min. Two independent fluorescence/light overlays are shown. Bars=20  $\mu$ M.

**[0042]** FIG. 19. Mutations of the Avr1b peptide (SEQ ID NO: 305) containing the RXLR motif assayed using the double barrel particle bombardment assay. Cell entry activity measured as cell death in the presence of Rps1b relative to wild-type Avr1b. Dashes indicate identical residues; ●●●● indicates Avr1b sequences.

**[0043]** FIG. 20A-C. PI-3-P binding proteins and inositol diphosphate block effector entry. A. Blocking binding of effector-GFP fusions into root cells. Fusion proteins were incubated with soybean root cells for 12 hr then washed for 2 hr. Inositol 1,4 diphosphate (500  $\mu$ M) was preincubated with the fusion proteins for 30 min. 5 mg/ml VAMP7 PX proteins was preincubated with the roots for 2 hr. Pairs of fluorescence and light micrographs are shown. Bars=100  $\mu$ M. B. Binding of biosensors to epithelial cells. Fusion proteins were incubated with cells for 2 hr then washed for 30 min. Inositol 1,4 diphosphate (500  $\mu$ M) was preincubated with the fusion proteins for 30 min. 5 mg/ml VAMP7 PX proteins was preincubated with the cells for 2 hr. Overlays of fluorescence and light micrographs are shown. Bars=10  $\mu$ M. C. Full-length Avr1k protein, with or without the RXLR mutation, was produced in *E. coli*. 0.25 mg/mL protein was infiltrated into the primary unifoliate leaves of 13 day old seedlings of cultivars Williams (no rps gene) or Williams 82 (Rps1k). Where indicated, the protein was co-infiltrated with 500  $\mu$ M 1,3IP2. The plants leaves were photographed 5 days after infiltration.

#### DETAILED DESCRIPTION

**[0044]** Since many resistance genes against oomycetes encode intracellular proteins, and since several cognate oomycete avirulence genes encode secreted proteins, it has been inferred that there must be a mechanism for translocating the avirulence proteins into the plant cells. Since the RxLR and dEER motifs were first identified during the *Phytophthora* genome sequence annotation, there has been extensive speculation that these motifs are involved in transporting avirulence proteins into host cells. Importantly, proteins in this family use the N-terminal motifs RxLR and dEER to cross the host plasma cell membrane autonomously, i.e. no other proteins are necessary to effect this translocation. Once inside the host cell, the proteins suppress host defense signaling. The importance of this effector family is underlined by the fact that plants have evolved intracellular defense receptors to detect the effectors and trigger a rapid counter-attack.

**[0045]** The present invention establishes that effectors of fungal plant pathogens contain virulence motifs with consensus sequence BXZ, where B is R, K or H; X is any amino acid and may be absent; and Z is a hydrophobic amino acid, generally L, M, I, W, Y or F. The sequence BXZ represents three contiguous amino acids, or two contiguous amino acids if X is absent. The BXZ family of motifs includes exemplary motifs such as RxLR (which may function with a dEER motif, an exemplary RxLR motif being RSLR) and related functional variants thereof (e.g. RRFLR, RRFLR), as well as exemplary motifs RFYR, RYWT, RIYER, etc). The virulence motifs are responsible for binding of the pathogen effector proteins to polar lipids (e.g. phosphatidyl-inositol-3-phosphate (PI-3-P) and/or phosphatidyl-inositol-4-phosphate (PI-4-P) and/or phosphatidic acid) at or near the surface of a host cell. Stimulation of host cell entry by, for example, PI-4-P, and inhibition by inositol 1, 4 diphosphate suggests that the binding of effectors to polar lipids (such as phosphoinositides, phospholipids and sphingolipids) mediates cell entry of the effectors. All effectors that were tested could also enter

human cells, suggesting that this mode of effector entry may be very widespread in plant and animal (including human) pathogenesis, including that which utilizes the *Plasmodium* Pexel ("P") motif. Identification of this broad spectrum of effectors containing virulence motifs thereof constitutes a novel target class, and raises the possibility of targeted blockade of pathogen effector proteins. This knowledge can be exploited to develop new classes of antibiotic treatments to prevent a wide variety of pathogenic fungal, Plasmodial and oomycete infections in plants and animals.

**[0046]** The invention also identifies the sequence requirements for the function of the virulence motifs and/or domains. With respect to the BXZ motif, it has been determined that generally only the presence of arginine, lysine or histidine at the first position and the presence of leucine, isoleucine, methionine, tyrosine, phenylalanine or tryptophan at the third position are required to enable function. However, in some embodiments, methionine or leucine at the second position may allow function if none of leucine, isoleucine, methionine, tyrosine, phenylalanine or tryptophan are present at the third position. In some embodiments, the sequences flanking the motifs are required for function. As used herein the term "domain", in some embodiments, refers to a region or regions of the primary sequence of an effector protein containing more than one virulence motif, e.g. both the RxLR and dEER motifs, or one or more analogous virulence motifs as described herein. The sequence requirements can be defined by a hidden markov model. For example, mutational analysis of the RxLR motif shows that, in some embodiments, the requirement for the first and third positions are quite strict. Furthermore, reversing the order of residues 1 and 2 or of 3 and 4 also abolishes activity, indicating that the mere presence of positive charge and hydrophobicity within the motif are insufficient. The arginine at position 4 is more flexible and can be replaced by lysine or glutamine. Naturally occurring functional variants of RxLR include lysine, histidine, threonine, glycine and alanine at the fourth position.

**[0047]** As a result of these findings, the invention provides methods to inhibit the entry, into a host cell, of effector proteins expressed by pathogens and containing the virulence motifs. The method is carried out by blocking the interaction, for example, by the binding of the motifs to a natural ligand such as a polar lipid, exemplified by phospholipids (e.g. phosphoinositides) and/or sphingolipids. Blocking may be accomplished by any of several means, for example, by exposing one or more virulence motifs to one or more molecules or molecular species which are capable of binding to or otherwise interacting with the virulence motifs, thus preventing the polar lipid (e.g. phosphoinositide, phospholipid or sphingolipid) from binding to the virulence motif. Herein, molecular species such as phosphoinositides, phospholipids and/or sphingolipids which, in nature, bind to one or more motifs of an effector molecule as described herein, causing the effector protein to translocate into the targeted host cell, may be referred to as "natural molecules" or "natural ligands" of the motif. Conversely, the motifs disclosed herein may be considered "natural ligands" of the polar lipids to which they bind. These designations distinguish them from the blocking molecules of the invention, which are added exogenously to cells and used to prevent binding of the natural ligands to the motifs, thereby preventing translocation of the effector protein into the cell. The blocking molecules may or may not be molecules that occur in nature, but if they are, then when used

in the present invention, they are isolated or substantially purified, or chemically synthesized.

**[0048]** Blocking molecules of choice include but are not limited to lipid-derived molecules which bind to the motif but not in a manner that results in entry of the effector protein into the cell, e.g. molecules that are sterically related to natural ligands but which do not comprise all requisite properties for enabling translocation of the effector. In other embodiments, the blocking molecules are inositol or inositol derivatives (e.g. various phosphorylated inositols such as inositol mono-phosphate, various inositol diphosphates such as inositol 1, 4 diphosphate, and other similar molecules); or peptides that bind to the motif and block access to the motif by natural ligands; or peptides that bind to the motif and target the effector for protease degradation; or peptides that bind to the motif and anchor the effector to an external structure such as a cell wall or cell matrix such that the effector cannot enter the cell; or molecules that bind to the motif and cause chemical modification of the effector so that it can no longer enter cells; or other "small molecule" compounds that possess the geometric and charge requisites for binding to one or more of the motifs, thereby blocking the binding of the natural ligand that is responsible for effector translocation.

**[0049]** In one embodiment of the invention, the blocking molecule is a peptide, in particular a peptide with an amino acid primary sequence that is designed to include amino acid residues with charges suitable for interacting with and/or binding to the charged residues of the motif. In such a peptide, the amino acid sequence is designed so that charged atoms or groups (especially of the side chains) are spatially arranged in a manner that allows, for example, negatively charged side chains to be within bonding distance of positively charged side chains of e.g. R residues of the motif, or for aliphatic side chains of the peptide to interact with aliphatic side chains of the motif, etc. Approaches to synthetic peptide design are described, for example, by Devlin et al. (Devlin, H., Pangniban, L. C. and Devlin, P. E. (1990) Random peptide libraries: a source of specific protein binding molecules. *Science*, 249, 404-406) and Scott and Smith (Scott, J. K. and Smith, G. P. (1990) Searching for peptide ligands with an epitope library. *Science*, 249, 386-390). Such peptides may be designed to be stable by e.g. by avoiding the use of known protease cleavage sites in the sequence; by introducing various non-natural amino acids; or by various modifications to amino acids (e.g. amidation, sulfonation, etc.) that increase the stability of the molecule, so long as such modifications do not interfere with binding to the effector motif.

**[0050]** The binding or interaction of the blocking molecule (s) may be of any suitable type, and will depend on the nature of the blocking molecule. For example, the binding may be covalent and hence essentially irreversible. Thus, in some embodiments of the invention, the blocking molecule is one that, upon contact with one or more chemically reactive functional groups of the motif or polar lipid, forms a covalent bond with the one or more functional groups that participate in binding, or with functional groups of adjacent portions of the molecule (e.g. adjacent residues of an effector protein) in a manner that blocks access to the motif (e.g. by phospholipids and/or sphingolipids that are natural ligands of the motif) and/or to the polar lipid, which would otherwise permit translocation of the effector into the host cell that the pathogen is trying to infect. Usually, however, the binding is non-covalent and comprises, for example, electrostatic and/or charge interactions, hydrophobic interactions, van der Waals interactions,

etc. For a blocking molecule to be effective, a  $K_d$  better than ten-fold less than the concentration of the competing natural ligand (polar lipid or motif) in the region of the host membrane is preferred (a lower  $K_d$  indicates tighter binding). In this manner, binding of the natural ligand is prevented or at least attenuated or slowed so as to render the natural ligands ineffective in enabling the effector molecule to enter the targeted host cell, and infection of the host by the pathogen which manufactured the effector molecule is prevented, or attenuated or slowed. Those of skill in the art will recognize that much benefit can accrue from a treatment that inhibits a pathogen, even if inhibition is not absolute, but merely attenuates or slows the symptoms of infection. "Inhibition" or "prevention of infection" as used herein is intended to encompass all such degrees of inhibition, blocking, etc. In addition, in the case of plants, the overall biomass of harvested plants or plant products, and therefore the usefulness of the crop, may be advantageously increased even if some plants remain affected by the pathogen after treatment as described herein.

**[0051]** "Motifs" to which natural ligand binding is blocked include BXZ motifs as described herein, as well as RxLR motifs, dEER motifs, the Pexel motif, and the RYWT, RIYER, RSLR, RRLR, RRFLR, and RFYR motifs. Those of skill in the art will recognize that many effector proteins contain both an RxLR motif and a dEER motif, or one or more virulence motifs as described herein. According to the invention, in one embodiment, the binding of a blocking molecule (which in some embodiments may be a natural ligand such as a phospholipid or sphingolipid) to one or more (e.g. either one or the other, or both, of the RxLR and dEER motifs) is blocked, and blocking occurs in a manner that prevents the effector protein that bears the motif(s) from entering the host cell. In another embodiment, the binding of a natural ligand to a Pexel motif is blocked, and blocking occurs in a manner that prevents the effector protein that bears the motif from entering a host cell. In another embodiment, the binding of a natural ligand to a RYWT, RIYER, RSLR, RRLR, RRFLR, and/or RFYR motif is blocked, and blocking occurs in a manner that prevents the effector protein that bears the motif from entering a host cell. In some embodiments, the blocking molecule binds to or interacts directly with residues of one or more of the virulence motifs, if two or more virulence motifs are present in an effector. However, this need not always be the case, as binding to a single motif may be sufficient. In other embodiments, the blocking molecule (or molecules) binds to or interacts with adjacent residues. "Adjacent residues" may, but need not necessarily be, adjacent in primary sequence to the motif. They may also be in proximity due to the secondary or tertiary structure of the effector molecule. For example, in one embodiment, the effector protein comprises an RxLR motif followed by at least one aspartate or one glutamate residue within a 60 amino acid carboxy terminal flanking sequence. In other words, within the effector protein, the sequence which is attached directly to the carboxy terminus of the RxLR motif (which, in primary sequence, follows immediately after the carboxyl terminal R of the motif) contains at least one aspartate residue and/or at least one glutamate residue within the first 60 amino acids of the sequence.

**[0052]** In addition, the RxLR motifs that are targeted for blocking by the methods of the invention include but are not limited to those which comprise at least one of a two or three amino acid sequence selected from the group consisting of: arginine, any amino acid, leucine; histidine, any amino acid,

leucine; lysine, any amino acid, leucine; arginine, any amino acid, isoleucine; histidine, any amino acid, isoleucine; lysine, any amino acid, isoleucine; arginine, any amino acid, methionine; histidine, any amino acid, methionine; lysine, any amino acid, methionine; arginine, any amino acid, tyrosine; histidine, any amino acid, tyrosine; lysine, any amino acid, tyrosine; arginine, any amino acid, phenylalanine; histidine, any amino acid, phenylalanine; lysine, any amino acid, phenylalanine; arginine, any amino acid, tryptophan; histidine, any amino acid, tryptophan; lysine, any amino acid, tryptophan; arginine, any amino acid, valine; histidine, any amino acid, valine; lysine, any amino acid, valine; arginine, leucine; histidine, leucine; lysine, leucine; arginine, isoleucine; histidine, isoleucine; lysine, isoleucine; arginine, methionine; histidine, methionine; lysine, methionine; arginine, tyrosine; histidine, tyrosine; lysine, tyrosine; arginine, phenylalanine; histidine, phenylalanine; lysine, phenylalanine; arginine, tryptophan; histidine, tryptophan; lysine, tryptophan; arginine, valine; histidine, valine; and lysine, valine. In some embodiments, the R of the RxLR motif is preceded by R, i.e. the motif is RRxLR, with "x" being any amino acid, or in particular L or F. These particular sequence may be represented by standard conventions using the single letter abbreviation for the amino acid and an "x" for the variable residue, e.g. as RXL for "arginine, any amino acid, leucine".

**[0053]** In addition, blocking may be accomplished by exposing one or more of the polar lipids to which the motifs bind (i.e. "target lipids") to one or more molecules or molecular species which are capable of binding to or otherwise interacting with the targeted polar lipid, thus preventing the motif (and hence the effector molecule) from binding to the polar lipid. In this embodiment, blocking molecules include but are not limited to: peptides, proteins, and other molecules which bind the polar lipid(s), for example, peptide mimetics of one or more effector motifs, small molecules or drugs which bind the polar lipids, various charged species which bind to the polar lipids, etc. The blocking molecule may bind to the natural target lipid (e.g. phosphoinositide, phospholipid, sphingolipid or other polar lipid) in order to block the binding of the effector to its target. In one embodiment the blocking molecule may be a naturally occurring protein that binds to the target lipid, such as a protein containing, for example, a C1, C2, PH, FYVE, PX, ENTH, ANTH, BAR, FERM, PDZ, and tubby domains (Stahelin, R. V. (2009). Lipid binding domains: more than simple lipid effectors. Lipid Res 50 Suppl, S299-304). In another embodiment, the blocking molecule may be a peptide with an amino acid primary sequence that is designed to include amino acid residues with charges suitable for interacting with and/or binding to the target phosphoinositide, phospholipid or sphingolipid. In yet another embodiment, the blocking molecule may be a polypeptide (e.g. a peptide, polypeptide, etc.) which includes one or more motif sequences and/or is a mimetic of one or more motif sequences. Blocking molecules that target the polar lipid ligands of the motif may bind to any portion of the lipid that prevents effector binding, or even to adjacent molecules or cellular components that sterically interfere with motif-lipid binding.

**[0054]** The host cells that are protected from effector protein invasion include many species of plant and animal cells, including human cells. Examples of plant cells that can benefit from the practice of the invention include but are not limited to: wheat, maize, rice, sorghum, barley, oats, millet,

soybean, common bean (e.g. *Phaseolus* species), green pea (*Pisum* species), cowpea, chickpea, alfalfa, clover, tomato, potato, tobacco, pepper, egg plant, grape, strawberry, raspberry, cranberry, blueberry, blackberry, hops, walnut, apple, peach, plum, pistachio, apricot, almond, pear, avocado, cacao, coffee, tea, pineapple, passionfruit, coconut, date and oil palm, citrus, safflower, carrot, sesame, common bean, banana, citrus (e.g. orange, lemon, grapefruit), papaya, macadamia, guava, pomegranate, pecan, *Brassica* species (canola, cabbage, cauliflower, mustard etc), cucurbits (pumpkin, cantaloupe, squash, zucchini, melons etc), cotton, sugar cane, sugar beets, sunflower, lettuce, onion, garlic, ornamental cut flowers, grasses used in lawns, athletic fields, golf courses and pastures (e.g. *Festuca*, *Lolium*, *Zoysia*, *Agrostis*, *Cynodon*, *Dactylis*, *Phleum*, *Phalaris*, *Poa*, *Bromua* and *Agropyron* species), etc.

**[0055]** Examples of animal cells that may benefit from the practice of the invention include but are not limited to: humans, cattle, sheep, pigs, goats, horses, cats, dogs, chickens, turkeys, bees, salmon, trout, bass, catfish, shellfish, crayfish, lobsters, shrimp, crabs, etc.

**[0056]** Many types of invasive pathogens may be targeted and their effector proteins prevented from entering host cells by the methods of the invention. Examples of such pathogens include but are not limited to: any *Phytophthora* species, e.g. *Phytophthora infestans*, *Phytophthora sojae*, *Phytophthora ramorum*, *Phytophthora parasitica*, *Phytophthora capsici*, *Phytophthora nicotianae*, *Phytophthora cinnamomi*, *Phytophthora cryptogea*, *Phytophthora drechsleri*, *Phytophthora cactorum*, *Phytophthora cambivora*, *Phytophthora citrophthera*, *Phytophthora citricola*, *Phytophthora megasperma*, *Phytophthora palmivora*, *Phytophthora Megakarya*, *Phytophthora boehmeriae*, *Phytophthora kernoviae*, *Phytophthora erythroseptica*, *Phytophthora fragariae*, *Phytophthora heveae*, *Phytophthora lateralis*, *Phytophthora syringae*; any *Pythium* species, e.g. *Pythium ultimum*, *Pythium aphanidermatum*, *Pythium irregulare*, *Pythium graminicola*, *Pythium arrhenomanes*, *Pythium insidiosum*; any downy mildew species; any *Peronospora* species, e.g. *Peronospora tabacina*, *Peronospora destructor*, *Peronospora sparsa*, *Peronospora viciae*; any *Bremia* species, e.g. *Bremia lactucae*; any *Plasmopora* species, e.g. *Plasmopora viticola*, *Plasmopara halstedii*; any *Pseudoperonospora* species, e.g. *Pseudoperonospora cuhensis*, *Pseudoperonospora humuli*; any *Sclerospora* species e.g. *Sclerospora graminicola*; any *Peronosclerospora* species, e.g. *Peronosclerospora philippinensis*, *Peronosclerospora sorghi*, *Peronosclerospora sacchari*; any *Sclerophthora* species, e.g. *Sclerophthora rayssiae*, *Sclerophthora macrospora*; any *Albugo* species, e.g. *Albugo candida*; any *Aphanomyces* species, e.g. *Aphanomyces cochlioides*, *Aphanomyces euteiches*, *Aphanomyces invadans*; any *Saprolegnia* species, e.g. *Saprolegnia parasitica*; any *Achlya* species; any rust fungi; any smut fungi; any bunt fungi; any powdery mildew fungi; any *Puccinia* species, *Puccinia striiformis*, *Puccinia graminis*, *Puccinia triticina* (syn. *Puccinia recondita*), *Puccinia sorghi*, *Puccinia schedonnardii*, *Puccinia cacabata*; any *Phakopsora* species, e.g. *Phakopsora pachyrhizi*, *Phakopsora gossypii*; any *Phoma* species, e.g. *Phoma glycincicola*; any *Ascochyta* species, e.g. *Ascochyta gossypii*; any *Cryphonectria* species, e.g. *Cryphonectria parasitica*; any *Magnaporthe* species, e.g. *Magnaporthe oryzae*; any *Gaeumannomyces* species, e.g. *Gaeumannomyces graminis*; any *Synchytrium* species, e.g. *Synchytrium endobioticum*; any *Ustilago* species, e.g. *Ustilago maydis*,

*Ustilago tritici*, *Ustilagoidea virens*; any *Tilletia* species, e.g. *Tilletia indica*, *Tilletia caries*, *Tilletia foetida*, *Tilletia barclayana*; any *Erysiphe* species, e.g. *Erysiphe necator* (formerly *Uncinula necator*); any *Blumeria* species, e.g. *Blumeria graminis*; *Podosphaera oxyacanthae*; any *Alternaria* species, e.g. *Alternaria alternata*; any *Botrytis* species, e.g. *Botrytis cinerea*; any *Diaporthe* species, e.g. *Diaporthe phaseolorum*; any *Fusarium* species, e.g. *Fusarium graminearum*, *Fusarium oxysporum* (e.g. f.sp. *lycopersici*), *Fusarium moniliforme*, *Fusarium solani*; any *Leptosphaeria* species, e.g. *Leptosphaeria maculans*, *Leptosphaeria maydis*; any *Macrophomina* species, e.g. *Macrophomina phaseolina*; any *Monilinia* species, e.g. *Monilinia fructicola*; any *Mycosphaerella* species, e.g. *Mycosphaerella graminicola*, *Mycosphaerella fijiensis*, *Mycosphaerella tassiana*, *Mycosphaerella zae-maydis*; any *Phialophora* species, e.g. *Phialophora gregata*; any *Phymatotrichopsis* species, e.g. *Phymatotrichopsis omnivora*; any *Taphrina* species, e.g. *Taphrina deformans*; any *Aspergillus* species, e.g. *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus famigatus*; any *Verticillium* species, e.g. *Verticillium dahliae*, *Verticillium albo-atrum*, *Rhizoctonia solani*, *Ophiostoma ulmi* (syn. *Ceratocystis ulmi*), *Ophiostoma novo-ulmi*; any *Septoria* species, e.g. *Septoria avenae*; any *Pyrenophora* species, e.g. *Pyrenophora tritici-repentis*; any *Colletotrichum* species, e.g. *Colletotrichum graminicola*; any *Sclerotinia* species, e.g. *Sclerotinia sclerotiorum*; any *Sclerotium* species, e.g. *Sclerotium rolfsii*; any *Thielaviopsis* species, e.g. *Thielaviopsis basicola*; any *Coccidioides* species, e.g. *Coccidioides immitis*; any *Paracoccidioides* species, e.g. *Paracoccidioides brasiliensis*; any *Pneumocystis* species, e.g. *Pneumocystis carinii*; any *Histoplasma* species, e.g. *Histoplasma capsulatum*; any *Cryptococcus* species, e.g. *Cryptococcus neoformans*; any *Candida* species, e.g. *Candida albicans*; any apicomplexan parasite species such as: any *Plasmodium* species, e.g. *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*; any *Babesia* species, e.g. *Babesia bovis*, *Babesia bigemina*; any *Cryptosporidium* species, e.g. *Cryptosporidium parvum*; any *Toxoplasma* species, e.g. *Toxoplasma gondii*; any *Trypanosomatid* species such as: any *Trypanosoma* species, e.g. *Trypanosoma brucei*, *Trypanosoma cruzi*, *Trypanosoma congolense*, *Trypanosoma vivax*; any *Leishmania* species, e.g. *Leishmania donovani*. Any amoeboid parasites; any *Entamoeba* species, e.g. *Entamoeba histolytica*; any *Mastigamoeba* species; any *Schistosoma* species; any *Onchocerca* species; any *Giardia* species; any microsporidial species; any *Enterocytozoon* species; any *Encephalitozoon* species, e.g. *Encephalitozoon cuniculi*, etc.

**[0057]** As a result of the practice of the methods of the invention, entry of a pathogen effector protein into the host cell is prevented, inhibited, slowed, or otherwise decreased or lessened. As a result, the host cell can mount a robust or normal immune response to the pathogen, and infection of the host organism is averted (prevented), or the degree of infection (i.e. the deleterious symptoms that typically accompany the presence of an infection by the pathogen) are eliminated or decreased. Thus, some aspects of the invention also include methods of preventing or attenuating the symptoms of infection usually caused in a host organism by a pathogen which employs effector proteins comprising one or more virulence motifs as described herein to enter host cells. In some embodiments, the methods are used to prevent infection and/or symptoms of infection. In other embodiments, infection may have already started but the methods of the invention can

be used to curtail the spread of the infection to other organisms, or to lessen the symptoms in an organism that is already afflicted. This is the case, in particular, with *Plasmodium* infections, where the methods of the invention are especially useful in preventing the subsequent rounds of parasite multiplication after initial infection, or with other pathogens that multiply logarithmically. In addition, the invention provides methods of maintaining a host cell's ability to mount an immune response to a pathogen, the pathogen being one that produces effector proteins that comprise one or more of the motifs described herein, and the method involving blocking the effector protein from entering the host cell by preventing the binding of its natural ligand.

**[0058]** The mode of administration of the blocking molecules of the invention will depend on several factors, including the nature of the molecule and the host. Generally, the blocking molecule will be in a composition or formulation suitable for administration. If the host organism is a plant, application is generally in the form of a foliar spray or watering solution of e.g. an aqueous or oil solution that includes the blocking molecule in a concentration sufficient to block effector molecules of pathogens which are likely to attack the plant. For administration to an animal, which may be a human, any suitable composition, many of which are known in the art, may be employed, e.g. various pills, powders, liquids, injectable formulations, etc. Likewise, any suitable means may be used, including but not limited to by injection (e.g. subcutaneous or intramuscular), inhalation, orally, intranasally, by ingestion of a food product containing the protein, etc. In addition, the compositions may include one or more than one blocking molecule. For example, a preparation for application to plants may include molecules that block the effector proteins of one or of several different types of pathogen. In addition, the blocking molecules may be administered to plants in conjunction with other beneficial substances, such as fertilizers, various pesticides, growth factors, etc. The same is true for administration to animals, where one or more than one type of blocking molecule may be administered, and may be administered in conjunction with other beneficial substances such as chemotherapeutic agents that also have activity against the pathogen.

**[0059]** In some embodiments, a combination of blocking agents is utilized, e.g. two or more agents that act on or effect the effector protein, or two or more agents that act on or effect the lipid, or a mixture of blocking agents, one or more of which acts on the effector protein, and one or more of which acts on the lipid.

**[0060]** In another aspect, the invention provides elucidation of the structural requirements of the virulence motifs (e.g. BXZ motifs, such as RXLR, RYWT, RIYER and related or similarly functioning motifs) in oomycetes, fungi, and other pathogens, and of the sequences which flank the motifs, thereby allowing, for example, the design of molecules to bind the motif. As a result, the invention also provides methods to predict which genes in the genome of a pathogen are likely to encode effector molecules. This is significant because, as demonstrated herein, the mere presence of a sequence conforming to the RXLR and dEER motif in a protein may not be sufficient to insure that the protein is an effector, i.e. that the protein is able to traverse the cell wall and enter the cell upon binding to phospholipids or sphingolipids. Functional virulence motifs (or functional virulence domains) may have additional requirements, particularly in the flanking sequences, as described herein. In one embodi-

ment, the invention describes a non-random distribution of amino acid residues in the regions flanking the RXLR motif, represented by a position-weight matrix. One method of predicting whether or not a gene encodes a true effector protein involves the use of a hidden markov model (HMM) based on the position-weight matrix. This method may also be applied to the analysis of other BXZ motifs.

**[0061]** The development of this aspect of the invention was based in the observation of differences in activity between two putative RXLR motifs, RXLR1 and RXLR2, as described in detail in Example 1 below. The differences suggested that surrounding sequences are also important to the activity of an RXLR motif. To define the differences in the surrounding sequences a hidden markov model (HMM) was created using the 10 amino acid residues to the left and right of the RXLR motifs of all of known *P. sojae* and *P. ramorum* Avh genes. Using this HMM, the sequences surrounding RXLR2 (which was an authentic, functional motif) had a high score of 18.5, representing an excellent match to the consensus flanking sequence, and very unlikely to have been found in a random sequence. In contrast, sequences surrounding RXLR1 (a non-functional motif) had a low, non-significant score of 0.0. Using the same HMM, the sequences surrounding the RXLR motif of *P. infestans* Avr3a scored 10.9. Using a similar HMM derived from *Hyaloperonospora parasitica* Avh genes, the sequences surrounding the RXLR motifs of the *H. parasitica* Atr1 and Atr13 proteins had scores of 9.8 and 6.3 respectively. These and other comparisons described in Example 1, showed that HMM scores of zero, such as that of Avr1b RXLR1, are characteristic of RXLR strings found at random, i.e. such sequences are not likely to represent true RXLR motifs. On the other hand, HMM scores over 5.0 are characteristic of non-random occurrences of RXLR strings, and the proteins in which such non-random strings occur are likely to be authentic functional RXLR sequences. In other words, such sequences are likely, upon binding a phospholipid or sphingolipid, to promote or allow the translocation, into a host cell, of effector (avirulence) proteins in which they are located or of which they form a part. HMM scores between 0 and 5 are equivocal and cannot be assigned to either category of protein (random RXLR strings vs authentic RXLR motifs). This methodology may also be applied to other virulence motifs, e.g. RYWT, RIYER, etc.

**[0062]** For further discussions of the use of a hidden markov model, see U.S. Pat. No. 6,128,587 to Sjolander (Oct. 3, 2000), the complete contents of which are hereby incorporated by reference.

**[0063]** The invention also provides a method for screening compounds to identify those that inhibit binding of phosphoinositides, phospholipids or sphingolipids (or other natural lipid ligands) to a BXZ motif (e.g. to an RXLR motif, and optionally also to a dEER motif) in an effector protein, and which thus can inhibit translocation of effector proteins that have these motifs into cells. The method involves exposing a candidate or putative blocking compound to one or more BXZ virulence motifs (e.g. one or both of the RXLR and dEER motifs, and/or one or both of RYWT and RIYER) under conditions suitable for binding of either the natural phospholipid/sphingolipid ligands or phosphoinositides to the motifs (phosphoinositides are known to be capable of such binding). The screening is evaluated in that if the candidate compound is able to bind to one or more of the virulence motifs, and especially to two motifs when they are found to be present in a single effector (e.g. RXLR and dEER)

then the compound is selected as a compound that will inhibit binding of the natural ligands to the motifs in an effector protein, and prevent entry of the effector protein into a cell. This is especially the case if the blocking compound is able to competitively bind to the motif in the presence of a natural ligand.

**[0064]** Alternatively, such screening methods may be adapted and used to identify blocking compounds which bind to or otherwise interfere with the polar lipid to which the motif binds.

**[0065]** Blocking of effector-lipid binding may also be prevented or attenuated using genetic engineering/molecular biology techniques. Such techniques may target one or both of the effector (e.g. the motif sequence) and the lipid to which a motif binds. For example, host cells (e.g. in plants) may be genetically engineered to express inhibitory RNA (e.g. siRNA) that inhibits one or more enzymes involved in synthesis of a target lipid, or in transport of the lipid to the cell surface where it is accessible to the motif. Alternatively, host cells may be genetically engineered to produce peptides or proteins which contain one or more motifs, in a manner that promotes expression of the peptides/proteins and their binding to at least one target lipid. Other strategies will occur to those of skill in the art, and all such methods are encompassed by the invention.

**[0066]** The invention also provides methods for preventing pathogens from invading or infecting cells, by prophylactically applying one or more of the blocking compounds described herein to a substrate with which a pathogen and a cell the pathogen might infect may come in contact. The method inhibits entry, into the cell, of a pathogenic effector protein (entry of the pathogenic effector protein requiring binding of at least one motif of the effector protein as described herein to at least one polar lipid of the cell) and comprises the step of contacting a substrate which contains or is likely to contain a pathogen comprising the pathogenic effector protein with a blocking compound as described herein. By "contacting" is meant applying, permeating, coating or otherwise placing the blocking compound on the substrate. The blocking compound is capable of i) binding to at least one motif of the pathogenic effector protein; or ii) binding to at least one polar lipid of said cell (the polar lipid being the cellular ligand of the effector protein motif). Binding of the blocking compound prevents entry of the pathogenic effector protein into said cell (and hence prevents infection of the cell by the pathogen), if the pathogen comes into contact with the substrate. The blocking agent may be applied to the substrate by any suitable means, e.g. by spraying, painting, coating, etc., or even by manufacturing the substrate to contain the blocking agent (e.g. fabric), or by permeating or soaking the substrate with the blocking agent, etc. The substrate may be any suitable substrate that may be contacted by the pathogen, and which usually will be or may also come into contact with a cell which might be infected by the pathogen, or in some cases may be the cell or a collection of cells which may encounter or be exposed to the pathogen. Exemplary substrates include but are not limited to: plants (e.g. to leaves, fruit, roots, etc.) for example, by spraying or otherwise placing the blocking agent, sometimes, though not always, on an exterior surface of the plant (e.g. mature plants, plants "in the field", plants in green houses, seedlings, seeds, sprouts, etc.); fabrics (e.g. fabrics used for tents, mosquito netting, clothing, etc.), water (e.g. bodies of water, swamps, pools of standing water, wells, drinking water, etc.); skin, hair and/or fur, eyes,

ear and nasal passages, the mouth, etc., e.g. of an animal (e.g. mammals such as humans, or other mammals, and also reptiles, fish, birds, etc. i.e. veterinary applications are also contemplated). For animals, application may be external by the application of e.g. lotions, sprays, rinses, mists, drops, washes, (e.g. mouthwash), etc; (internal administration is also contemplated, as described above). The substrate may also be an insect, e.g. an insect that is known or suspected of carrying the pathogen which comprises the effector protein, or is capable of synthesizing the effector protein.

**[0067]** The invention is further illustrated by the following Examples, which should not be interpreted as limiting the invention in any way.

## EXAMPLES

### Example 1

RXLR-mediated entry of *Phytophthora sojae* effector Avr1b (SEQ ID NO: 2) into soybean cells does not require pathogen encoded machinery

**[0068]** Effector proteins secreted by oomycete and fungal pathogens have been inferred to enter host cells, where they interact with host resistance gene products. Using the effector protein Avr1b of *Phytophthora sojae*, an oomycete pathogen of soybean, we show that a pair of sequence motifs, RXLR and dEER, plus surrounding sequences, (SEQ ID NO: 46) are both necessary and sufficient to deliver the protein into plant cells. Particle bombardment experiments demonstrate that these motifs function in the absence of the pathogen, indicating that no additional pathogen encoded machinery is required for effector protein entry into host cells. Furthermore, fusion of the Avr1b RXLR and dEER domain to green fluorescent protein (GFP) allows GFP to enter soybean root cells autonomously. The conclusion that RXLR and dEER serve to transduce oomycete effectors into host cells indicates that the more than 370 RXLR and dEER containing proteins encoded in the genome sequence of *P. sojae* are candidate effectors. We further show that the RXLR and dEER motifs can be replaced by the closely related erythrocyte targeting signals found in effector proteins of *Plasmodium*, the protozoan that causes malaria in humans. Mutational analysis of the RXLR motif shows that the required residues are very similar in the motifs of *Plasmodium* and *Phytophthora*. Thus the machinery of the hosts (soybean and human) targeted by the effectors may be very ancient.

RXLR2 and dEER Motifs of Avr1b are Required for its Avirulence Function in Transgenic *P. sojae* Lines

**[0069]** To test the function of the RXLR and dEER motifs of Avr1b, transgenic *P. sojae* strains that expressed either wild-type or mutant Avr1b-1 genes were created. Wild-type Avr1b contains two RXLR motifs, RXLR1 and RXLR2 (FIG. 1A). Mutations in either or both of the RXLR motifs (SEQ ID NO: 3,4,5), in addition to a mutation in the dEER motif (FIG. 1A) (SEQ ID NO: 6), were created. The Avr1b-1 gene constructs were fused to a strong constitutive promoter, HAM34 (Judelson, H., Tyler, B. M., and Michelmore, R. W. 1991. Mol. Plant-Microbe Interact. 4, 602-607.), and introduced into a strain, P7076, that expresses a variant Avr1b protein that does not confer avirulence against Rps1b-containing soybeans (Shan, W., Cao, M., Leung, D., and Tyler, B. M. 2004. Mol. Plant-Microbe Interact. 17, 394-403). Two independent transformants (T17 and T20) expressing wild type Avr1b-1 (FIG. 1B, C) lost the ability to infect soybean plants carrying Rps1b, but were unaffected in their ability to infect

plants lacking Rps1b (FIG. 1E, Table 1). This demonstrated that they had acquired avirulence against Rps1b as a result of a functional Avr1b gene product. This result was confirmed using two different pairs of isolines of soybean that differed only in the presence of Rps1b, namely Williams (no Rps gene) with L77-1863 (Rps1b; Williams background) and HARO(1-7)1 (No Rps; Harosoy background) with HARO13 (Rps1b; Harosoy background) (FIG. 1E; Table 1).

**[0070]** In contrast, in five independent transformants expressing the RXLR2<sup>AAAA</sup> (SEQ ID NO 4) mutant, there was no gain of avirulence against Rps1b cultivars, despite the

the RXLR<sup>1</sup> motif was intact in the RXLR2<sup>AAAA</sup> mutant, the motif appeared to be non-functional. Consistent with this inference, the RXLR1<sup>AAAA</sup> mutation (SEQ ID NO 3) did not abolish avirulence in three independent transformants (FIG. 1E, Table 1). As expected, avirulence was lost in the RXLR1<sup>AAAA</sup>, RXLR2<sup>AAAA</sup> double mutants (FIG. 1E, Table 1) (SEQ ID NO: 5). A mutation in the dEER motif (SEQ ID NO: 6) also abolished avirulence (in two independent transformants) indicating that this motif is also required for the function of the protein (FIG. 1E, Table 1).

TABLE 1

Molecular characterization and avirulence testing of <i>P. sojae</i> stable transformants						
Strains	Transgene (PCR) <sup>a</sup>	Expression (RT-PCR) <sup>b</sup>	Transformant Validation <sup>c</sup>	Avirulence (Surviving seedlings) <sup>d</sup>		
				Rps1 <sup>b</sup>	rps	p <sup>e</sup>
P7076 (Gus)						
GUS	no	no	P7076 (sAvr1b WT)	1/23	1/22	0.77
T17	yes	yes	Pst I	20/30	0/21	3.9E-07
T20	yes	yes	Sequence	25/44	2/28	1.2E-05
P7076 (sAvr1b RXLR1 <sup>AAAA</sup> )						
RXLR1-2	yes	yes	Pst I	32/36	3/20	5.0E-08
RXLR1-3	yes	yes	Pst I	31/54	3/21	6.4E-04
RXLR1-5	yes	yes	Pst I	37/57	7/23	5.1E-03
P7076 (sAvr1b RXLR2 <sup>AAAA</sup> )						
RXLR2-18	yes	yes	Pst I	6/44	3/17	0.48
RXLR2-20	yes	yes	Pst I	4/46	3/19	0.33
P7076 (sAvr1b RXLR1 <sup>AAAA</sup> ; RXLR2 <sup>AAAA</sup> )						
RXLR1+2-4	yes	yes	Pst	5/31	2/16	0.55
RXLR1+2-6	yes	yes	Pst I	4/43	1/21	0.47
P7076 (sAvr1b dEER <sup>46</sup> )						
dEER-9	yes	yes	Sequence	4/59	2/23	0.79
dEER-14	yes	yes	Pst I	4/40	3/15	0.28
Ps Avr4/6-Avr1bCt						
4/6-1b-3	yes	yes	size	15/24	3/11	0.057
4/6-1b-19	yes	yes	size	14/26	3/16	0.025
Hp 341-Avr1bCt						
341-1b-13	yes	yes	size	16/19	0/11	6.6E-06
341-1b-17	yes	yes	size	23/24	0/7	3.0E-06
mAvr1bCt						
mAvr1bCt-4	yes	yes	size	3/20	3/16	0.77
mAvr1bCt-5	yes	yes	size	2/21	1/15	0.63

<sup>a</sup>The presence of transgenes was verified by PCR as described in the Materials and Methods. + = transgene present; - = transgene not detected.

<sup>b</sup>Transgene expression was determined by qualitative RT-PCR (RT-PCR) and by quantitative RT-PCR (q-PCR) as described in the Materials and Methods. Yes = transgene transcripts present; no = transgene transcripts not detected; ND = not determined.

<sup>c</sup>The presence of the relevant mutation in the transforming plasmid was verified by sequencing in every case. The presence of the correct mutation within the transgenes of each transformed strain was verified after PCR amplification of the Avr1b-1 transgene by Pst I digestion or by sequencing in the case of the mutants (e.g. FIG. 1) or by size in the case of the Avh gene fusions and N-terminal deletion (e.g. FIG. 4).

<sup>d</sup>The avirulence of each transgenic strain was tested by inoculation of seedlings containing Rps1b (L77-1863) or no rps gene (Williams), as described in the Materials and Methods. The number of surviving seedlings/total inoculated seedlings is shown, summed from all replicates.

<sup>e</sup>Fisher's exact test (one tailed) was used to compare the frequency of seedling survival between rps and Rps1b plants. A significant p value (0.05) indicates that the transformant's phenotype is avirulent.

presence of abundant mRNA from the transgene (FIG. 1E, Table 1). Thus, the RXLR<sup>2</sup> motif is necessary for Avr1b activity when the protein is delivered by the pathogen. Since

**[0071]** The difference in activity between RXLR1 (SEQ ID NO: 3) and RXLR2 (SEQ ID NO: 4) suggests that surrounding sequences are important to the activity of an RXLR motif.

To define the differences in the surrounding sequences a hidden markov model (HMM) was created using the 10 amino acid residues to the left and right of the RXLR motifs of all of the *P. sojae* and *P. ramorum* Avh genes (Tyler et al., 2006; Jiang et al., 2008). Using this HMM, the sequences surrounding RXLR2 (SEQ ID NO: 5) had a high score of 18.5, representing an excellent match to the consensus flanking sequence, very unlikely to have been found in a random sequence. In contrast, sequences surrounding RXLR1 (SEQ ID NO: 3) had a low, non-significant score of 0.0. Using the same HMM, the sequences surrounding the RXLR motif of *P. infestans* Avr3a scored 10.9. Using a similar HMM derived from *Hyaloperonospora parasitica* Avh genes, the sequences surrounding the RXLR motifs of the *H. parasitica* Atr1 and Atr13 proteins had scores of 9.8 and 6.3 respectively.

**[0072]** To establish the significance of these HMM scores, the *Phytophthora* HMM was used to score the RXLR motifs of 1240 RXLR-containing sequences identified from a pool of all putative secreted *P. sojae* and *P. ramorum* proteins by Jiang et al. (2008). As a control, 639 RXLR-containing sequences were scored found after permuting the sequences of all the putative secreted *P. sojae* and *P. ramorum* proteins (Jiang et al., 2008). As shown in FIG. 1D, the RXLR strings of 698 (56%) of the 1240 real proteins had an HMM score of zero, while the RXLR strings of 595 (93%) of the permuted proteins had a zero score, and only 13 (1.8%) scored above 5.0. In contrast, of 765 proteins that Jiang et al. (2008) identified as high quality candidate effectors, only 18% had an HMM score of zero, and 543 (72%) had a score over 5.0. From this comparison we conclude that HMM scores of zero, such as that of Avr1b RXLR1 (SEQ ID NO: 3), are characteristic of RXLR strings found at random, while scores over 5.0 are characteristic of non-random occurrences of RXLR strings and of the RXLR strings of functional avirulence proteins. HMM scores between 0 and 5 are equivocal. The curated Avh genes with a score of zero may represent pseudogenes as many of them were identified principally by C-terminal sequence similarity.

The Interaction Between Avr1b and the Rps1b Gene Product Occurs Within Host Cells and Does Not Require the RXLR and dEER Motifs

**[0073]** To confirm that the site of interaction of Avr1b with the Rps1b gene product is within the plant cell, particle bombardment was used to introduce DNA encoding Avr1b proteins lacking a secretory leader into soybean cells together with DNA encoding  $\beta$ -glucuronidase (GUS). This assay measures the functional interaction of the Avr1b protein with the intracellular product of the soybean Rps1b gene; when the two proteins interact, programmed cell death is triggered in the transformed cells ablating the development of tissue patches expressing GUS. Since the Avr1b protein lacks its normal secretory leader, the protein should be synthesized in the plant cytoplasm. To facilitate the comparison of test and control bombardments, a novel double-barreled attachment for the Bio-Rad Gene Gun was utilized (Dou et al., 2008). The gun shooting two different DNA samples side-by-side into a leaf in the same shot, which greatly improves the reproducibility of the results (Dou et al., 2008). FIG. 2 shows that delivery of DNA encoding leader-less Avr1b protein (SEQ ID NO: 19) into soybean cells significantly reduced the number of blue GUS-positive patches when the Rps1b gene was present, but not when Rps1b was absent (FIG. 2A). This is consistent with a cytoplasmic location for the Avr1b-Rps1b interaction. When RXLR2 or dEER motifs were replaced by

four or six alanine residues, respectively [FIG. 2A, mAvr1b (RXLR2<sup>AAAA</sup>) (SEQ ID NO: 20) and mAvr1b(dEER<sup>d6</sup>) (SEQ ID NO: 23)], the interaction of the cytoplasmic, leader-less Avr1b with Rps1b was unaffected (FIG. 2A), indicating that the RXLR2 and dEER motifs were not required for the interaction.

RXLR-Mediated Transit into Soybean Cells Does Not Require the Pathogen

**[0074]** To test whether RXLR function requires the presence of the pathogen, the bombardment assay was used to determine the effect of the RXLR2<sup>AAAA</sup> mutation on secreted Avr1b protein (SEQ ID NO: 18). When soybean cells were bombarded with DNA encoding wild type Avr1b (SEQ ID NO: 22), including its normal secretory leader, a reduction in GUS-positive blue spots was observed comparable to that observed for the non-secreted protein [FIG. 2, sAvr1b(WT)]. However when the RXLR2<sup>AAAA</sup> (SEQ ID NO: 28) or dEERA6 (SEQ ID NO: 29) mutations were present in the bombarded DNA, there was no reduction in the number of blue spots [FIG. 2, sAvr1b(RXLR2<sup>AAAA</sup>) (SEQ ID NO: 11) and sAvr1b(dEER<sup>d6</sup>) (SEQ ID NO: 13)]. From these results it may be inferred first that the secretory leader is functional in soybean and targets Avr1b protein to the outside of the cell; second, that the RXLR2 (but not RXLR1) and dEER motifs are required for Avr1b protein to re-enter the cell, which confirms the conclusion from the *P. sojae* transformation experiments. Importantly, the results also show that RXLR-dEER-mediated entry does not require the presence of the pathogen.

**[0075]** To support the inference that the secretory leader of Avr1b was correctly exporting the protein from the plant cells in the bombardment assay, a gene encoding *Aequorea coerulescens* green fluorescent protein (acGFP; "GFP" herein) fused either to the Avr1b leader (SEQ ID NO: 27) or to full-length Avr1b (SEQ ID NO: 25) was constructed. These fusions enabled tracking of the proteins and checking their stability. To aid in visualization onion bulb epidermal cells were used rather than soybean cells. GFP was exported from the cells and accumulated in the apoplast when the secretory leader was attached to GFP but accumulated in the cytoplasm and nucleus when the leader was not attached (not shown). When full length Avr1b was fused to GFP (SEQ ID NO: 25), the proteins also accumulated in the apoplast if a mutation was present in either the RXLR2 motif (SEQ ID No: 28) or in the dEER motif (SEQ ID No: 29). This observation confirmed that the protein encoded by these mutants was stable and correctly targeted outside of the cells. When cells expressing Avr1b-GFP fusion proteins with RXLR mutations were plasmolyzed by treatment with 0.8M mannitol for 15 min, the GFP was associated with the cell wall and not with the plasma cell membrane (not shown). Furthermore, GFP protein could be seen diffusing into the apoplast between pairs of neighboring cells (not shown). Similar observations were made when cells expressing secreted GFP or Avr1b-GFP fusion proteins with a dEER mutation were plasmolyzed (not shown). If the RXLR2 and dEER motifs were intact however, the sAvr1b-GFP protein fusion accumulated in the cytoplasm and nucleus of the cells, similar to the mAvr1b-GFP (SEQ ID NO: 24) fusion lacking the leader. When cells expressing sAvr1b-GFP fusion proteins were plasmolyzed by treatment with 0.8M mannitol for 15 min, the GFP could be observed to have either fully or partially returned to the inside of the cells.

These results supported our conclusion that the RXLR2 and dEER motifs act together to enable Avr1b protein to re-enter the plant cells.

The Avr1b RXLR and dEER Motifs are Sufficient to Target GFP to Soybean Cells

**[0076]** The RXLR and dEER region of Avr1b was fused to GFP (SEQ ID NO: 46), and the fusion protein was synthesized in *E. coli* and partially purified. Root tips of soybean seedlings were incubated with the isolated fusion protein for 12 hours, washed for four hours in water, then observed under light and UV microscopy to localize the GFP. GFP accumulated inside many of the root cells, whereas buffer alone did not produce any fluorescence. The optical sections produced by the confocal microscope revealed that the protein penetrated approximately 10 cell layers deep during the 12 hour incubation. The characteristic accumulation of GFP in the nuclei of the treated cells is comparable to the pattern observed when GFP is expressed in planta, and verifies that the GFP is located inside the cells. The nuclear localization of the protein also indicates that the cells are alive. If mutations were present in the RXLR or dEER motifs of the fusion protein, GFP did not accumulate inside the soybean root cells. When the RXLR and dEER region was replaced by the artificial protein transduction motif Arg9 (SEQ ID NO: 112), GFP once again entered the soybean root cells and accumulated in the nuclei.

Avr1b RXLR and dEER Motifs can be Replaced by RXLR and dEER-Containing Protein Sequences Encoded by Bioinformatically Identified Avh Genes

**[0077]** To determine if the RXLR and dEER motifs of bioinformatically identified Avh genes could functionally replace the RXLR2 and dEER motifs of Avr1b-1, full length Avh genes from *P. sojae* and *H. parasitica* were fused to an Avr1b-1 N-terminal deletion mutant lacking the RXLR and dEER motifs. The fusion genes were then introduced into *P. sojae* and the transformants were tested for avirulence on Rps1b-containing soybean cultivars. Both Avh genes, *P. sojae* Avh171 (since identified as Avr4/6; Dau et al., 2008) (SEQ ID NO: 8) and *H. parasitica* Avh341 (SEQ ID NO: 7), could replace the requirement for the RXLR2 and dEER motifs as judged by the avirulence of the transformants on Rps1b-containing cultivars, whereas transformants containing only the C-terminus of Avr1b fused to an initiator methionine remained virulent (FIG. 3 and Table 1). This result indicates that the RXLR and dEER motifs form a distinct transferable functional domain of Avr1b and other Avh proteins. The HMM scores of the RXLR-dEER motifs of Ps Avr4/6 and Hp Avh341 are both well within the functional range (6.9 and 14.2, respectively).

The Avr1b Host Targeting Signal can be Functionally Replaced by Autonomous Protein Transduction Motifs

**[0078]** Protein transduction domains (PTDs) capable of autonomously carrying proteins across plasma cell membranes have been described and characterized in the HIV-1 Tat protein. Arginine-rich peptides such as Arg9 can also carry out this function. To compare RXLR and dEER mediated effector delivery with the function of PTDs, the RXLR2 motif of Avr1b was replaced with the TAT PTD (SEQ ID NO: 42) or with Arg9 (FIG. 4A) (SEQ ID NO: 41). The resultant proteins were treated using the particle bombardment assay, and both PTDs could functionally replace the RXLR2 motif of Avr1b, restoring the avirulence reaction of Avr1b with Rps1b (FIG. 4B). Furthermore, when the version of secreted

Avr1b that contained the Arg9 sequence in place of the RXLR2 motif was fused to GFP (SEQ ID NO: 30), the fusion protein accumulated in the cytoplasm and the nucleus of bombarded onion bulb cells rather than the apoplast, confirming that Arg9 could functionally replace RXLR2 (not shown). Similar results were obtained when the version of secreted Avr1b that contained the TAT PTD was fused in place of the RXLR2 motif to GFP (SEQ ID NO: 31). Finally, when Arg9 was fused to GFP (SEQ ID NO: 112), the isolated proteins could enter soybean root cells directly (not shown).

The Avr1b Host Targeting Signal is Interchangeable with Host Targeting Signals from *Plasmodium* Effectors

**[0079]** To test if the erythrocyte targeting signals of *Plasmodium* effector proteins could functionally replace the RXLR and dEER region of Avr1b, the residues of Avr1b from the end of the secretory leader to the end of the dEER motif were replaced with the mature N-termini of three different *Plasmodium* effector proteins that are targeted to the erythrocyte cytoplasm, namely PfGBP-130 (SEQ ID NO: 121), PfHRP2 (SEQ ID NO: 123) and PfPFE1615c (SEQ ID NO: 125) (Bhattacharjee, S., Hiller, N. L., Liolios, K., Win, J., Kanneganti, T. D., Young, C., Kamoun, S., and Haldar, K. 2006. *PLoS Pathog* 2, e50.). The entire 37-41 amino acid region of each *Plasmodium* effector required for transduction was used (FIG. 4A). As shown in FIG. 4B, all three *Plasmodium* host targeting domains could functionally replace the Avr1b N-terminus in targeting Avr1b to the soybean cytoplasm, assuming that they do not simply interfere with secretion.

Functional Characterization of the RXLR Motif

**[0080]** To experimentally characterize the sequence requirements of the RXLR motif, a series of mutations were introduced into the motif in a version of the Avr1b-1 gene that retained the secretory leader, and assayed the mutants using the bombardment assay (Table 2). Mutations which targeted the arginine at position 1 or the leucine at position 3 has the strongest effect on the ability of Avr1b to ablate GUS-positive tissue patches. Replacement of R1 with lysine reduced function significantly (33% ablation compared to 78%;  $p < 0.001$ ) while glutamine replacement completely abolished it. Replacement of L3 with alanine or even the relatively conservative valine also completely abolished function. Replacement of the arginine at position 4 with a glutamine slightly but significantly reduced function (58% ablation compared to 72%;  $p < 0.001$ ). Reversing the order within the first and second two pairs of positively-charged and hydrophobic residues (RFLR->RFLR; RFLR->RFRL) completely abolished avirulence activity, indicating that positions of R1 and L3 were critical, not just their presence.

TABLE 2

Function of RXLR2 mutants of Avr1b assayed by particle bombardment						
SEQ	RXLR2	Ratio of GUS-positive spots <sup>a</sup>		ablation <sup>c</sup>	p value <sup>d</sup>	Activity
ID NO	sequence <sup>a</sup>	rps	Rps1b	tion <sup>c</sup>	p value <sup>d</sup>	ity
22	RFLR	1.26 ± 0.07	0.28 ± 0.03	0.78	<0.001	Yes
20	AAAA	0.93 ± 0.04	0.96 ± 0.05	0	>0.1	No
38	KFLR	1.04 ± 0.04	0.70 ± 0.04	0.33 <sup>e</sup>	<0.001	Partial
37	QFLR	0.95 ± 0.03	0.99 ± 0.03	0	>0.1	No

TABLE 2-continued

Function of RXLR2 mutants of Avr1b assayed by particle bombardment						
SEQ	RXLR2	Ratio of GUS-positive spots <sup>a</sup>		ablation <sup>c</sup>	p value <sup>d</sup>	Activity
		rps	Rps1b			
ID NO	sequence <sup>e</sup>					
31	<u>F</u> R <u>L</u> R	1.00 ± 0.04	0.98 ± 0.05	0	>0.1	No
34	R <u>F</u> L <u>Q</u>	0.98 ± 0.07	0.41 ± 0.08	0.58 <sup>e</sup>	<0.001	Partial
36	<u>Q</u> F <u>L</u> Q	1.03 ± 0.05	1.05 ± 0.05	0	>0.1	No
35	R <u>F</u> <u>A</u> R	0.94 ± 0.03	0.91 ± 0.05	0	>0.1	No
40	R <u>F</u> <u>Y</u> R	0.95 ± 0.05	1.03 ± 0.07	0	>0.1	No
33	R <u>F</u> R <u>L</u>	1.02 ± 0.04	0.96 ± 0.04	0	>0.1	No

<sup>a</sup>Amino acid sequence of RXLR2 in wild-type and mutants. RFLR is the wild-type. Altered residues are underlined.

<sup>b</sup>Ratio of blue spots in the presence of various RXLR2 mutants of Avr1b-1, compared to the control empty vector when bombarded onto leaves from rps plants (Williams) or Rps1b plants (L77-1863). Averages and standard errors are from 16 pairs of shots.

<sup>c</sup>Ablation calculated as 1 - (Rps1b ratio)/(rps ratio) for ratios significantly different between rps and Rps1b.

<sup>d</sup>p values comparing results from rps and Rps1b cultivars were calculated using the Wilcoxon rank sum test.

<sup>e</sup>Ablations for KFLR and RFLQ were significantly different than wildtype (RFLR) with p < 0.001.

**[0081]** This example demonstrates that

1) both the RXLR2 and dEER motifs of Avr1b are required for this protein to confer avirulence on *P. sojae* transformants (summarized in FIG. 5);

2) the RXLR2 and dEER motifs are not required to trigger an interaction with the Rps1b gene product when the Avr1b protein is synthesized in the soybean cytoplasm;

3) when Avr1b protein is directed to be secreted out of the soybean cell, the RXLR2 and dEER motifs are once more required for the protein to trigger an interaction with Rps1b, which is consistent with the motifs being required for the Avr1b protein to re-enter the soybean cell across the plasma cell membrane; 4) fusion of the RXLR and dEER region to GFP (SEQ ID NO: 46) enabled the isolated fusion protein to enter soybean root cells in the absence of the pathogen, but only if the RXLR and dEER motifs were both intact; and 5) RXLR-dependent entry of Avr1b does not require the presence of the pathogen. These observations lead to the conclusion that the RXLR and dEER motifs do indeed have the function of transporting avirulence proteins into host cells.

**[0082]** In addition, the data presented in this Example characterizes the RXLR2 and dEER motifs as follows: 1) arginine at position 1 and leucine at position 3 are essential for function of the RXLR motif. However, there is not a strong requirement for the arginine at position 4. Therefore by functional assays, the oomycete RXLR motif resembles the *Plasmodium* motif (RXLxD/E/Q) even more closely than previously noted; and 2) the amino acid sequences flanking the RXLR2 and dEER motifs are required in addition to the motifs themselves for the transit of Avr1b into soybean. Further, the region from residues 33 to 71 (19aa to the left of RXLR2 and baa to the right of dEER) were sufficient for protein translocation.

**[0083]** The Avr1b protein requires not only the RXLR motif itself, but also non-random surrounding sequences including the dEER motif. These surrounding sequences are not enriched in positive and hydrophobic residues, but instead are enriched in acidic and hydrophilic residues. Furthermore, our RXLR mutagenesis results show that the presence of basic and hydrophobic residues is not sufficient for RXLR function; instead the order of the amino acid residues is very

important, and very subtle mutations such as RFLR→RFVR or QFLR abolish function. Therefore, oomycete effectors may utilize a novel mechanism for translocation across the membrane, possibly involving host cell surface machinery (such as a receptor) that is more complex than just the phospholipid bilayer. The *Plasmodium* Pexel/VTF motif also requires surrounding sequences that are enriched in acidic and hydrophilic residues and is functionally interchangeable with the oomycete RXLR domain in both erythrocytes and in soybean tissue (this study). Thus oomycetes and *Plasmodium* both may target host cell surface machinery that is common to plants and vertebrate animals but different than that targeted by animal PTDs. The targeted machinery, if common, must not only be very ancient, but also must serve an irreplaceable function in the host organisms since it must have been preserved against strong negative selection pressure resulting from exploitation by the pathogens.

**[0084]** These results do not indicate which specific flanking sequences are required. However, HMMs constructed from the 10 amino acid residues flanking the upstream and downstream sides of all *P. sojae* and *P. ramorum* Avh RXLR motifs, clearly separated the RXLR motifs of functional avirulence proteins from RXLR motifs obtained by chance from real or permuted proteins sequences. These findings indicate that reliable bioinformatic searches for RXLR effector candidates should include the use of HMMs to evaluate the sequences flanking putative RXLR and dEER motifs. METHODS: Plasmids and oligonucleotides used in the study are depicted in tabular form as FIGS. 11A and B and FIGS. 12A and B, respectively.

*P. sojae* isolates and transformation: *P. sojae* isolate P7076 (Race 19) was routinely grown and maintained on V8 agar. The *P. sojae* transformation procedure was described by Dou et al (Dou, D., Kale, S. D., Wang, X., Chen, Y., Wang, Q., Wang, X., Jiang, R. H. Y., Arredondo, F. D., Anderson, R., Thakur, P., McDowell, J., Wang, Y., and Tyler, B. M. (2008) Plant Cell 20(4), 1118-1133).

Characterization, of *P. sojae* transformants: *P. sojae* transformants were selected that grew well on V8 medium with 50 µg/ml G418, and were cultured in V8 liquid medium for 3 days. The mycelia were harvested, frozen in liquid nitrogen and ground to a powder for DNA or RNA extraction. Genomic DNA was isolated from mycelium using known techniques. DNA samples were quantified using a Nanodrop ND-1000 spectrophotometer (Thermo Scientific). The presence of Avr1b-1 transgenes was verified by PCR amplification from 100 ng genomic DNA using a program of 94° C. for 2 min, 30 cycles of 94° C. for 30 s, 56° C. for 30 s, 72° C. for 30 s, and 72° C. for 5 min with primers of HamF and HamR (TS1). All the transformed *P. sojae* were double-checked by Pst I restriction and/or sequence. RNA was extracted from each sample using RNeasy Plant Mini Kit (QIAGEN, cat #74904) with β-mercaptoethanol added buffer RLT and genomic DNA was removed using RNase-Free DNase (QIAGEN, cat #79254) according to the manufacturer's recommendations. RNA was quantified using a Nanodrop ND-1000 spectrophotometer. Avr1b-1 transgene transcription was verified by RT-PCR using the internal primers, Avr1bReF and Avr1bReR (TS1) and *P. sojae* actin was used as the reference.

Phenotypic assays for avirulence: Avr1b phenotypic expression was assayed using soybean cultivars HARO(1-7) (rps), Haro13 (Harosoy background, Rps1b), Williams (rps) and L77-1863 (Williams background, Rps1b). Seedlings were

grown in the greenhouse or in a growth chamber (Percival AR-36L) with a program of 24° C. at daytime and 22° C. at night with a 14 hr day length under fluorescent light (250  $\mu\text{mol photons s}^{-1}\text{m}^{-2}$ ). The virulence of each transformant was evaluated using hypocotyl inoculation. 1-2 days after the first primary leaf appeared, the hypocotyl of the soybean was wounded with a short incision and the incision was inoculated with a small piece of V8 agar cut from the edge of a 3 day old colony. Thereafter, the plants were incubated in a growth chamber under the conditions described above. The numbers of dead and surviving plants were counted 4 days after inoculation, and summed over 2-5 replicates. The differences between the numbers of surviving plants from rps and Rps1b cultivars were compared using Fisher's exact test. Only the transformants producing a significant difference between rps and Rps1b cultivars were judged as avirulent.

**Particle bombardment assays:** Particle bombardment assays were carried out using a double-barreled extension of the Bio-Rad He/1000 Particle Delivery System ((Dou, D., Kale, S. D., Wang, X., Chen, Y., Wang, Q., Wang, X., Jiang, R. H. Y., Arredondo, F. D., Anderson, R., Thakur, P., McDowell, J., Wang, Y., and Tyler, B. M. (2008) *Plant Cell* 20(4), 1118-1133). Analyzing the bombardment data as a ratio between the test and control shots improves the reproducibility of the measurements greatly. The avirulence activity of the Avr1b-1 constructs was measured as the reduction in the number of blue spots comparing the Avr1b-1+GUS bombardment with the GUS+control bombardment. For each paired shot the logarithm of the ratio of the spot numbers of Avr1b-1 to that of the control was calculated, then the log-ratios obtained from the Rps1b and non-Rps1b leaves were compared using the Wilcoxon rank sum test.

**Bombardment assays of onion bulb cells with GFP constructs:** Preparation of DNA-particle mixtures was as described above. 5 mm hemispherical layers of yellow and white onion bulbs were bombarded without the double barrel attachment under a 26 psi vacuum, using a rupture pressure of 1100 psi. The onion layers were incubated between 24-48 hr at 25° C., then viewed with a Zeiss Axioskop2 Plus microscope using a 480 nm filter for GFP fluorescence. Images were captured using a Qimaging Retiga 1300 Camera. To further confirm the GFP had been secreted out of the onion cells, plasmolysis was performed for 15 min in 0.8 M mannitol and cells were observed in a Zeiss LSM510 laser scanning confocal microscope (Jena, Germany) with an argon laser excitation wavelength of 488 nm.

**RXLR-GFP Fusion Protein Expression and Purification:** Residues 33 to 71 of Avr1b (VESPDLVRRSLRNGDIAGGRFLRAHEEDDAGERTFSVTD (SEQ ID NO: 46) including the RXLR1, RXLR2 and dEER motifs were fused to GFP, replacing the Arg9 encoding sequences in vector pR9GFP (SEQ ID NO: 112), called pR9 by Chang et al., (Chang, M., Chou, J. C. and Lee, H. J. (2005) *Plant and Cell Physiology* 46, 482-488). pR9GFP, which also adds an N-terminal His6 tag, was derived by Chang et al (2005) from Ptat-HA. C43 (DE3) *E. coli* cells containing RXLR-GFP fusion constructs or pR9 were grown in 200 mL of LB containing ampicillin 100  $\mu\text{g}/\text{mL}$  in a 1 L baffled flask shaken at 240 rpm at 37° C. until reaching an OD of 0.4, at which point the cells were induced by addition of 1 mL of 1M IPTG (final [5 mM]). After 4 hours further growth at the same conditions, the cells were harvested by centrifugation at 4° C. and then stored at -20° C. Visual confirmation of GFP expression was noted by the green color of the bacterial cell pellet. To extract the GFP

fusion proteins, cells were thawed on ice for 20 min then 4 mL of lysis buffer (50 mM  $\text{NaH}_2\text{PO}_4$ , 300 mM NaCl, 10 mM imidazole, pH 8.0) were added per 1 g of wet cell weight. Lysozyme (Sigma-Aldrich, cat# L6876) was added to a final concentration of 1 mg/mL then the suspension was incubated for 20 min on ice. Sonication (Branson sonifier 150D, with Double stepped micro tip, 3 mm) was done at 300 W at 15 sec bursts four times with 15 sec cooling periods between each burst. The lysate was centrifuged at 10,000 $\times$ g for 30 minutes at 4° C., then the supernatant was transferred to a fresh tube and kept on ice until use. 5  $\mu\text{L}$  of each sample was stored for SDS-PAGE analysis. Protein purification using Ni-NTA affinity chromatography was performed using the QiaExpressionist protocol. 2 mL of 50% Ni-NTA super flow slurry (Qiagen) was loaded on a column. The column was washed twice with 5 mL of wash buffer (50 mM  $\text{NaH}_2\text{PO}_4$ , 300 mM NaCl, 20 mM imidazole, pH 8.0). The protein sample was loaded onto the column and then the column was washed twice with 10 vol (10 mL) of wash buffer. The protein was eluted with 4 mL of elution buffer (50 mM  $\text{NaH}_2\text{PO}_4$ , 300 mM NaCl, 200 mM imidazole, pH 8.0) into 1 mL fractions. These fractions were pooled and concentrated to 3001 using a centrifugal protein concentrator (Amicon Centriplus Centrifugal Filter Device MWCO-3 kDa) at 13,500 $\times$ g. The sample was then mixed with an equal volume of 50 mM MES buffer pH 5.8. The protein concentration was measured at 280 nm using a nanodrop spectrophotometer (ND-1000) and adjusted to 8 mg/mL. All purified GFP preparations fluoresced normally under UV illumination.

**RXLR-GFP Fusion Protein Root Cell Transduction Assay:** Root tips were cut into lengths of between 0.5 cm and 1 cm, and then were washed with water. Each root tip was completely submerged in 20  $\mu\text{L}$  of the protein solution (8 mg/ml in 25 mM MES pH 5.8) in a eppendorf tube. The samples were incubated overnight at 28° C. (~12 hours). The roots were then washed in 200 mL of water for 4 hours while shaken at 100 rpm on a rotary shaker. The roots were then viewed using a Zeiss LSM510 laser scanning confocal microscope with an argon laser excitation wavelength of 488 nm. For nuclear staining, the roots were stained with DAPI (4',6-diamidino-2-phenylindole) (Sigma-Aldrich cat# D8417) and viewed with a 405 nm filter.

**Hidden Markov Model Analysis:** By using the program HMMER 2.3.2 (Eddy, S. R. (1998). Profile hidden Markov models. *Bioinformatics* 14, 755-763; and website located at [hmm.janelia.org](http://hmm.janelia.org)), an HMM was built from the full set of 765 high quality candidate effectors identified from the *P. sojae* and *P. ramorum* genomes by Jiang et al (Jiang, R. H. Y., Tripathy, S., Govers, F. & Tyler, B. M. *Proc. Natl. Acad. Sci. USA* 105, 4874-4879 (2008), using the 10 amino acids on the left side of each RXLR motif together with the 10 amino acids on the right side each RXLR motif. The same procedure was used to build an HMM from a curated list of 191 high quality candidate effectors from *Hyaloperonospora parasitica* developed at the *H. parasitica* genome annotation jamboree in August 2007 and available at [pmgn.vbi.vt.edu](http://pmgn.vbi.vt.edu). To estimate the significance of HMM scores, all proteins (1240) with a predicted N-terminal signal peptide (SP) and the string RXLR located between 30 and 60 amino acids after the SP cleavage site were obtained by translating the genome sequences of *P. sojae* and *P. ramorum* in all reading frames. The sequences of all the putative secreted proteins were permuted (other than the signal peptide) and RXLR-containing sequences were again identified; 639 of the permuted proteins

had RXLR strings, indicating that about 639 of the 1240 detected RXLR motifs could be expected by chance. The distributions of HMM scores the set of 1240 real proteins, the 639 permuted proteins and the 765 curated proteins were then calculated. The frequency that a permuted protein received a score between 0 and 5.0 was 0.044. The frequency that a permuted protein received a score better than 5.0 was 0.018. Accession Numbers The sequences reported herein have been deposited in the GenBank database, namely Hp Avh341 (EF681127). Accession numbers for sequences already in GenBank are Ps Avr1b-1 (AAM20936), Ps Avr4/6 (ABS50087), Pi Avr3a (CA172345); Hp Atr1 (AY842877), Hp Atr13 (AY785301).

#### Example 2

##### Effector Host-Targeting Signals of Eukaryotic Pathogens Bind Phosphoinositides or Phosphatidic Acid

**[0085]** Pathogens of both plants and animals produce effectors and/or toxins that act within the cytoplasm of host cells to suppress host defenses and cause disease. Effector proteins of oomycete plant pathogens utilize N-terminal motifs, RXLR and dEER, to enter host cells, and a similar motif, Pexel (RXLxE/D/Q), is used by *Plasmodium* effectors to enter erythrocytes. This Example shows that effectors of fungal plant pathogens contain functional variants of the RXLR and dEER motifs, and that the oomycete and fungal RXLR and dEER motifs, as well as the *Plasmodium* Pexel motifs, are responsible for binding of the effectors to phosphatidyl-inositol-3-phosphate (PI-3-P) and/or phosphatidyl-inositol-4-phosphate (PI-4-P). Stimulation of host cell entry by PI-4-P, and inhibition by inositol 1,4 diphosphate suggest that phosphoinositide binding mediates cell entry. All the effectors could also enter human cells, suggesting that phosphoinositide-mediated effector entry may be very widespread in plant, animal and human pathogenesis.

Oomycete RXLR and dEER Domain Binds Phosphoinositides

**[0086]** The RXLR and dEER domain of *P. sojae* Avr1b enables translocation of green fluorescent protein (GFP) into plant cells without any pathogen-encoded machinery (see Example 1), and the same is true for two additional bioinformatically predicted effectors, Avh5 (SEQ ID NO: 129) and Avh331 (SEQ ID NO: 127). In these experiments, accumulation of the GFP fusion proteins inside the cells was confirmed by the accumulation of GFP within the nuclei of the cells (a natural property of GFP), and by plasmolysis experiments. In principle, a cell entry domain could bind either a (glyco)protein or (glyco)lipid receptor. After noting that beta-type phosphatidylinositol-4-phosphate kinases from rice and *Arabidopsis* contained a PI-4-P binding domain consisting of 14 and 11 tandem RXLR and dEER motifs respectively, experiments were conducted to test whether oomycete the RXLR and dEER domain could bind phosphoinositides. An array of 8 different lipids found in plant cell membranes were spotted in decreasing amounts onto a Hybond-C extra membrane. Then the membrane was probed with GFP fused to the N-terminal RXLR and dEER domains of Avr1b (SEQ ID NO: 46), Avh331 (SEQ ID NO: 116) or Avh5 (SEQ ID NO: 113). FIG. 6 shows that the Avr1b- and Avh331-GFP fusions bound to PI-4-P while Avh331- and Avh5-GFP fusions bound to PI-3-P. Alanine substitutions mutations in either the RXLR or the dEER motif of any of the three fusions abolished binding, just as they abolished entry into soybean root cells. Fusions of

full-length Avh5 (SEQ ID NO: 129) or Avh331 (SEQ ID NO: 127) proteins at their N-termini to glutathione-S-transferase (GST) could also bind the same phosphoinositides as just their N-terminal domains fused at their C-termini to GFP and binding by the full length proteins also required intact RXLR and dEER motifs (FIG. 6) (full-length Avr1b could not be produced in *E. coli*).

**[0087]** To independently confirm binding of the effector RXLR and dEER domains to the phosphoinositides, the binding of the fusion proteins to liposomes composed of phosphatidyl-choline (PC) and phosphatidyl-ethanolamine (PE) was tested. In the absence of phosphoinositides, neither the effector N terminus-GFP fusion proteins nor the full length GST-effector fusion proteins bound the liposomes (FIG. 6). However, when either PI-3-P or PI-4-P were included, all the fusion proteins bound to the liposomes. In every case, when any of the RXLR or the dEER motifs were mutated by alanine substitutions, the mutant fusion proteins lost their ability to bind the liposomes (FIG. 6).

##### Identification of Fungal Effector Translocation Domains

**[0088]** To test whether fungal effectors contain N-terminal cell entry domains, N-terminal segments from the fungal effectors AvrL567 (SEQ ID NO: 138) and AvrM (SEQ ID NO: 142) of *M. lini* and from AvrPi-ta (SEQ ID NO: 143) of *M. oryzae* were fused to the C-terminus of Avr1b, in the presence of the Avr1b secretory leader, then tested the fusions in a particle bombardment cell re-entry assay that measures the ability of a motif to carry an Avr1b reporter protein back into soybean leaf cells after secretion. FIG. 7A shows that all three fungal N-terminal segments had significant ability to deliver Avr1b back into soybean leaf cells.

**[0089]** Since the fungal effectors contained no obvious RXLR or dEER motifs, we decided to define experimentally the range of residues within the RXLR motif of Avr1b that could permit cell entry, using the particle bombardment cell re-entry assay. The results revealed that lysine (K) or histidine (H) but not glutamine (Q) could replace the arginine at position 1 in the motif, that any large hydrophobic residue (isoleucine, I; methionine, M; phenylalanine, F; tyrosine, Y) could replace the leucine at position 3, albeit with varying efficiencies, but valine (V) and alanine (A) could not. At position 4, all residues tested (lysine, K; glutamine, Q; glycine, G) allowed function. Furthermore, the presence of either an L or M residue at position 2 could substitute for a large hydrophobic residue at position 3.

**[0090]** Using this information, one, seven and four potential cell entry motifs were identified in N-terminal regions of AvrL567 (SEQ ID NO: 138), AvrM (SEQ ID NO: 142) and AvrPi-ta (SEQ ID NO: 143), respectively (FIG. 7B). The single motif in AvrL567, RFYR, had a particularly good match to the oomycete RXLR motif, and RFYR had already been shown to be functional (FIGS. 7B and 7C). Four of the candidate motifs in the AvrPi-ta N-terminus, including two that overlapped at one residue (RFLK and KLIFK (SEQ ID NO: 146)), were tested for cell entry activity by substituting them for the RXLR motif of Avr1b. The two single motifs and the overlapping pair were all active in the cell re-entry assay (FIG. 7B).

**[0091]** The N-terminus of AvrL567 was subjected to further analysis by mutagenesis and root cell entry assays. Alanine substitutions in the RFYR motif and in two downstream acidic residues that might act as a dEER motif (FIG. 7B) (SEQ ID NO: 139), abolished the activity of the AvrL567

N-terminal domain in the particle bombardment cell re-entry assay (FIGS. 7A and B). To confirm the cell entry activity of the AvrL567 N-terminal domain, it was fused to GFP (creating AvrL567(N)-GFP (SEQ ID NO: 119)), with and without (SEQ ID NO: 120) the alanine substitutions, and then the fusion proteins were tested for cell entry in the soybean root uptake assay. The GFP-fusion with the intact AvrL567 N-terminus (SEQ ID NO: 119) efficiently accumulated in the root cells, including the nuclei, whereas the fusion with the mutated RFYR and acidic residues (rfyr-de-) (SEQ ID NO: 120) did not (not shown). Thus the RFYR motif and the two downstream acidic residues appear to act as a RXLR and dEER motif in *M. lini* AvrL567.

#### Fungal and Apicomplexan Effectors Bind Phosphoinositides

**[0092]** Both filter binding and liposome binding were used to test whether the N-terminal domain of AvrL567 bound phosphoinositides. AvrL567(N)-GFP (SEQ ID NO: 119) bound PI-3-P in both assays. Binding of AvrL567(N)-GFP to PI-4-P was also detected in the liposome assay though it is not as strong as to PI-3-P. Mutation of the RXLR and dEER-like motif to alanines (rfyr-de-mutant) (SEQ ID NO: 120) resulted in a loss of binding to the phosphoinositides in both assays.

**[0093]** Filter binding assays were used to determine if five additional fungal effectors could bind phospholipids. The N-terminal sequences of the following effectors were fused to GFP: *Magnaporthe grisea* AvrPita (SEQ ID NO: 224), *Puccinia graminis* Ps87 (SEQ ID NO: 226); *Melampsora lini* AvrM (SEQ ID NO: 228); *Melampsora lini* AvrP123 (SEQ ID NO: 230); *Melampsora lini* AvrP4 (SEQ ID NO: 232). The results, summarized in tabular form in FIG. 15, showed that all five effectors bound phosphatidic acid. Mutations in RXLR-like motifs found in AvrPita (SEQ ID NO: 225), Ps87 (SEQ ID NO: 227), AvrM (SEQ ID NO: 228), AvrP123 (SEQ ID NO: 230) and AvrP4 (SEQ ID NO: 232) all abolished binding to phosphatidic acid. In the case of AvrPita, the mutant protein (SEQ ID NO: 225) was unable to enter soybean root cells, whereas the wildtype protein (SEQ ID NO: 224) could enter root cells, suggesting that binding of AvrPita to phosphatidic acid was required to enter plant cells.

**[0094]** The host targeting signals (HTS) of three *Plasmodium falciparum* effectors, PfGBP (SEQ ID NO: 121), PfHRP2 (SEQ ID NO: 123), and Pf1615c (SEQ ID NO: 125) can carry Avr1b into soybean leaf cells and onion bulb epidermal cells<sup>8</sup>. The three signals can also carry purified GFP into soybean root cells and this activity requires intact Pexel motifs. To test whether the three signals also could bind phosphoinositides, the HTS-GFP fusion proteins were tested using filter binding and liposome binding assays. The PfGBP HTS fusion (SEQ ID NO: 121) could bind PI-4-P and also, more weakly, PI-3-P (FIG. 8A). The PfHRP2 HTS fusion (SEQ ID NO: 123) could bind PI-3-P, and also rather weakly, PI-4-P (FIG. 8B). The Pf1615c HTS fusion (SEQ ID NO: 125) could bind specifically to PI-3-P (FIG. 8C). Liposome binding assays confirmed binding of all the fusion proteins to PI-3-P or PI-4-P (FIGS. 8D-F). In both assays, alanine substitutions in the Pexel motifs of each effector abolished phosphoinositide binding (FIG. 8A-F) (SEQ ID NO: 122, 124, 126).

#### Modulation of Effector Entry by Exogenous Phosphoinositides

**[0095]** The binding of phosphoinositides to the effector cell entry domains suggested that these phospholipids might

serve as a cell entry receptor in each case. Tomato cells secreted PI-4-P when stimulated by fungal xylanase, suggesting that free PI-4-P might exist in the plant apoplast<sup>24</sup>. Therefore, increasing the concentration of free phosphoinositide by exogenous addition might stimulate RXLR and dEER-mediated uptake. To test this hypothesis, a soluble form of PI-4-P, di-octanoyl-PI-4-P (250  $\mu$ M), was mixed with the Avr1b GFP fusion, Avr1b(N)-GFP, for 30 min prior to exposure to soybean roots. After 9 hr, strong stimulation of Avr1b(N)-GFP uptake by PI-4-P was evident (FIG. 9A). The phospholipids, PI, PC and PE all did not stimulate uptake. Uptake of the fungal AvrL567(N)-GFP fusion (SEQ ID NO: 119) was also strongly stimulated by PI-4-P (FIG. 9B), even though it binds most strongly to PI-3-P.

**[0096]** A synthetic cell entry motif composed of nine-arginine residues (Arg<sup>9</sup>) (SEQ ID NO: 112) was previously shown to deliver Avr1b into soybean leaf cells and into onion epidermal leaf cells in particle bombardment cell re-entry assays. The motif could also enable uptake of purified GFP into soybean root cells<sup>8</sup> and into maize and onion cells. The mechanism of uptake has been proposed to be a plant form of macropinocytosis. The Arg<sup>9</sup>-GFP fusion protein binds PI-3-P, PI-4-P and phosphatidyl serine, albeit weakly (FIG. 9D). FIG. 9C shows that di-octanoyl-PI-4-P does not stimulate uptake of the Arg<sup>9</sup>-GFP fusion protein in soybean root cells, suggesting that the stimulation by PI-4-P is specific to RXLR and dEER-mediated uptake. This conclusion is supported by the observation that exogenous PI-4-P did not promote the uptake of Avr1b(N)-GFP (SEQ ID NO: 46) and AvrL567(N)-GFP (SEQ ID NO: 119) proteins containing alanine substitutions in the RXLR and dEER motifs.

**[0097]** Inositol-1,4-diphosphate (IP2) represents the hydrophilic head-group of PI-4-P. Preincubation with 100  $\mu$ M IP2 inhibited binding of Avr1b(N)-GFP (SEQ ID NO: 46) to PI-4-P-containing liposomes and could completely block binding of AvrL567(N)-GFP (SEQ ID NO: 119) to PI-4-P-containing liposomes, presumably via competitive inhibition. To test whether IP2 could block effector uptake in planta, which would imply that a PI-4-P-like molecule mediated uptake in planta, Avr1b(N)-GFP (SEQ ID NO: 46) or AvrL567(N)-GFP (SEQ ID NO: 119) was preincubated with 500  $\mu$ M IP2 for 30 min prior to exposure to soybean roots. IP2 almost completely blocked uptake of both Avr1b(N)-GFP (FIG. 9A) (SEQ ID NO: 46) or AvrL567(N)-GFP (FIG. 9B) (SEQ ID NO: 119) into soybean cells. IP2 could not inhibit the binding of Arg<sup>9</sup>-GFP to liposomes (FIG. 9D) and uptake of Arg<sup>9</sup>-GFP (SEQ ID NO: 112) was completely unaffected by preincubation with IP2 (FIG. 9C), supporting the conclusion that IP2 specifically blocks RXLR and dEER motif-mediated protein uptake.

#### Effector Entry into Human Cells

**[0098]** Phosphatidyl-inositol-phosphates are universally found in eukaryotic cells. Since a number of human and animal diseases are caused by fungi and oomycetes, as well as by apicomplexan parasites, we tested the possibility that RXLR and dEER motifs might mediate protein entry into human cells, using the human lung epithelial cell line A549 as a model. Avr1b(N)-GFP (FIG. 10A) (SEQ ID NO: 46), AvrL567(N)-GFP (FIG. 10B) (SEQ ID NO: 119) and PfHRP2(N)-GFP (FIG. 10C) (SEQ ID NO: 123) (SEQ ID NO: 60) could all enter the A549 cells, but entry did not occur if alanine substitutions were present in the RXLR or Pexel motifs of the proteins (SEQ ID NO: 47,48,120,124). In these experiments, accumulation of the GFP fusion proteins inside

the cells was confirmed by the accumulation of GFP within vesicle-like structures within the cells, and by the fact that the cells were treated with protease (trypsin) prior to photographing. Protein accumulation was strongly inhibited in each case by 1,4-IP<sub>2</sub>, supporting the hypothesis that entry was mediated by phosphoinositide binding. Exogenous di-octanoyl-PI-4-P did not stimulate accumulation, suggesting that the availability of PI-4-P or other phosphatidylinositides in the growth medium was not limiting. Inositol 1,3 diphosphate (1,3-IP<sub>2</sub>), the headgroup of PI-3-P, could also inhibit entry of PfHRP11 (N)-GFP (FIG. 10C) (SEQ ID NO: 123), consistent with the observation that the protein bound PI-3-P more strongly than PI-4-P in the filter-binding assay.

**Discussion:** Two independent assays, namely filter-binding and liposome-binding, demonstrated that the N-terminus of all seven effectors tested could bind to either PI-3-P or PI-4-P, but not to PI-5-P nor to any other phospholipids tested. The primary structures of the RXLR and dEER effector domains do not resemble any known phosphoinositide binding domains. However, the binding of the pathogen RXLR and dEER domains to phosphoinositides is concordant with the binding of the RXLR and dEER domains of rice and *Arabidopsis* (3-type PI-4-kinases. In the oomycete effector proteins, the dEER motif is variably spaced from the RXLR motif, so if residues from both motifs contact the phosphoinositide head group, the protein must fold so as to bring the two motifs into proximity. The three dimensional structure of the RXLR and dEER or Pexel domain is not yet available for any oomycete or apicomplexan effector proteins, respectively. However, the crystal structure of AvrL567 has been determined. In this structure, the RFYR motif adopts a beta-stranded conformation on the surface of the protein. It will be interesting to determine if the structure of AvrL567 changes in solution in the presence of a phosphoinositide.

**[0099]** The stimulation of protein entry into soybean root cells by PI-4-P and the inhibition of entry by IP<sub>2</sub> together support the hypothesis that binding to phosphoinositides mediates entry of these pathogen effectors into plant cells. Similar findings with a human lung epithelial cell line suggest the possibility that effectors of oomycetes and fungi that infect humans and other animals might enter host cells via a similar mechanism. This mechanism appears to be different than reported for other peptides with cell entry activity because entry of those other peptides is not dependent on phosphoinositides.

**[0100]** These data demonstrate that several fungal effectors contain N-terminal domains that are capable of carrying Avr1b into soybean leaf cells. Within these domains are RXLR-like motifs that can replace the Avr1b RXLR motif in carrying reporter proteins into host cells. In the one case tested in detail so far, AvrL567, the motif RFYR was identified as necessary for the activity of the N-terminal cell entry domain, and for the binding of the domain to PI-3-P. The mutagenesis survey of the Avr1b RXLR motif and the diverse functional motifs found in the fungal effectors together suggest that a wide diversity of RXLR-like sequences support binding of phosphoinositides. Bioinformatic screens with the highly redundant motif suggested by the data identifies huge numbers of matches, most of which are likely spurious as judged by searches of permuted protein sequences. Thus, it is very likely that there are additional requirements for phosphoinositide binding. Both the RXLR and dEER motifs of Avr1b and the Pexel motifs of *Plasmodium* effectors are

insufficient by themselves to facilitate cell entry; in both cases flanking sequences are required.

**[0101]** Other than in apicomplexan parasites, eukaryotic pathogens of humans and other animals have not been reported to produce effector proteins that can cross host membranes into the cytoplasm of host cells. The finding that phosphoinositide-binding effectors from oomycete and fungal plant pathogens can cross the membranes of human cells predicts that oomycete and fungal pathogens of humans and other animals may also utilize this mechanism to debilitate their hosts. Possible examples include oomycete pathogens of marine animals from the genera *Saprolegnia* and *Aphanomyces*, extracellular fungal pathogens such as *Pneumocystis carinii*, *Coccidioides immitis* and *Aspergillus fumigatus*, and intra-phagosomal fungal pathogens of humans such as *Cryptococcus neoformans* and *Histoplasma capsulatum*.

**[0102]** The binding of phosphoinositides or other polar lipids to effector cell entry domains from diverse kingdoms will provide a powerful biochemical tool for screening or directly isolating new candidate effector proteins from all classes of microbes. It may also enable detection of phosphoinositide-binding plant proteins (or other polar-lipid-binding proteins) that can traffic through the apoplast and enter into target cells to transduce signals. Some precedents for such proteins already exist, such as the *Drosophila* antennapedia transcription factor that can move from cell to cell via an arginine-rich cell entry motif.

**[0103]** Understanding the role of phosphoinositides and other polar lipids in pathogen effector entry also opens the possibility of targeting cell entry domains for preventative or therapeutic intervention in both agriculture and medicine. The finding that IP<sub>2</sub> can block effector entry into both plant and human cells provides a proof-of-concept for this approach.

#### Methods Summary

**[0104]** Cloning was performed according to known molecular biology techniques. Proteins were expressed in *E. coli* BL21DE3 and purified using affinity chromatography. Plasmids and oligonucleotides used in the study are depicted in tabular form as FIGS. 13A-F and FIGS. 14A-D, respectively.

**[0105]** Soybean seeds were germinated in vermiculite for 3-5 days. Roots were washed with water thoroughly to remove any debris. Approximately 1.5 cm root tips were cut and placed into the protein solution (50  $\mu$ l-25 mM MES pH 5.8, 50  $\mu$ g protein) and incubated for 12-15 hr at 28° C. Then the root tips were rinsed with water and washed in 75 mL of water for 2 hr on an orbital shaker at 90 rpm. Roots were examined using a Zeiss LSM510 laser scanning confocal microscope with an argon laser excitation wavelength of 488 nm.

**[0106]** Lipid filter arrays were prepared by pipetting 1  $\mu$ L PI-3-P, PI-5-P (Cayman Chemical), PS, PC, PE, PA, or PI-4-P (Avanti Polar Lipids, Cayman Chemical) at various concentrations on Hybond-C extra membranes.

**[0107]** Liposomes were prepared from a suspension of 0.71  $\mu$ g/ml phosphatidyl-choline, 0.29  $\mu$ g/ml phosphatidyl-ethanolamine (PC/PE) or 0.64  $\mu$ g/ml phosphatidyl-choline, 0.26  $\mu$ g/ml phosphatidyl-ethanolamine, 0.1  $\mu$ g/ml phosphatidylinositol-phosphate (PC/PE/PI-x-P). The lipid mixtures were dried under vacuum overnight, then the resultant lipid films were rehydrated at 1 mg/mL (total lipid) in 20 mM Tris-HCl (pH 6.8) 100 mM NaCl, 2 mM dithiothreitol by three cycles

of freeze-thawing. Large unilamellar vesicles were formed by extruding the lipid suspension through a 0.1- $\mu$ M filter (nucleopore track-etch membrane, Whatman) 20 times and were used immediately. Effector fusion proteins were centrifuged at 100,000 g for 20 min at 25° C. prior to assay to remove protein aggregates. 10  $\mu$ g of protein was added to 50  $\mu$ g of liposomes and incubated for 1 hr at room temperature. Protein-liposome mixtures were centrifuged at 100,000 g for 15 min at 25° C. Pellets containing liposome-bound proteins and supernatants containing free proteins, were then analyzed by SDS-PAGE.

### Example 3

#### Assay for Screening Compound Libraries to Identify Novel Compounds that Interfere with the RXLR and dEER-Mediated Uptake of Effector Proteins into Plant or Human Cells

**[0108]** The binding of phosphoinositides (PI-3-P or PI-4-P) and phosphatidic acid to effector cell entry domains indicates that these phospholipids may serve as a cell entry receptors. Increasing the concentration of free phosphoinositide such as di-octanoyl-PI-4-P by exogenous addition stimulated RXLR and dEER-mediated uptake of the Avr1b GFP fusion, Avr1b (N)-GFP, into soybean roots and human cells. Furthermore, preincubation with inositol 1,4 diphosphate (IP2) inhibited binding of Avr1b(N)-GFP to PI-4-P-containing liposomes presumably via competitive inhibition. In addition, IP2 almost completely blocked uptake of both Avr1b(N)-GFP into soybean root cells and human cells in cell culture. Therefore, an assay is devised for screening compound libraries to identify novel compounds that interfere with the RXLR and dEER-mediated uptake of effector proteins into plant or human cells, through inhibition of the binding of, or interaction between phospholipids PI-3-P or PI-4-P and RXLR and dEER motif containing proteins. Plasmids encoding the Avr1b sequence are expressed in BL21 *E. coli* cells and the protein are purified and diluted into appropriate binding buffer at an appropriate concentration, and thirty microliters are dispensed into each well coated 96 or 384 well plates using an automated dispenser. Through a robotized transfer mechanism involving steel pins, each of the Avr1b protein-containing wells (in a 96 or 384 well plate) receive 300 nanoliter of a compound from the compound libraries, followed by incubating the plate at room temperature for 60 minutes. An equal volume of 2 $\times$  stock solution of fluorescently labeled soluble PI-4-P (Echelon Inc. BODIPY FL Phosphatidylinositol(4) Phosphate catalog #C-04F6a; BF-PI-4-P) is prepared in suitable buffer and 30 microliter of this solution is dispensed into each well of Avr1b coated preincubated 384 well plates using an automated dispenser. After BF-PI-4-P addition, the plate is incubated in dark for 60 minutes, followed by the measurement of fluorescence, utilizing a Synergy plate reader integrated with a biostack. The reactions are performed in duplicates and with negative controls, where the interactions are measured in the absence of the protein or fluorescently labeled BF-PI-4-P, and positive controls where the interaction is measured in the presence of a range of concentrations of inositol 1,4 diphosphate (IP2). The readouts are stored and analyzed for the identification of potential inhibitors of the reaction. Statistical analysis are performed utilizing a combination of parameters and compounds that showed statistically significant inhibition are selected. Briefly, the background absorbance is subtracted

from the test reads. Subsequently, the net absorbance is compared to controls wells, that did not receive the test compounds and the percent decrease in absorbance is measured by the following formula: Percent inhibition=[(Fluorescence in test well/Fluorescence in control wells) $\times$ 100]. In excess of one hundred thousand drug-like, diverse heterocyclic chemical compounds are screened during this process for their potential to inhibit the interactions between BF-PI-4-P and Avr1b. These compounds are obtained from several sources including established chemical vendors like Asinex, Analyticon, Biomol, Bionet, ChemDiv, Enamine, Maybridge, Spectrum, TimTec as well as a range of diversity oriented synthesis compounds that have been generated by academic research laboratories from around the world. Typical screening identifies several hundred compounds that inhibit the reaction at a statistically significant >40% levels. Successful events in this initial screen lead to the consolidation of select wells from the original library stock to generate a new second generation of plate for screening the activity of these compounds at three compound concentrations to allow the calculation of a preliminary 1050 value. A select group of compounds is then selected that showed >50% inhibition. Larger quantities of select compounds are ordered from the specific vendors (above) for rescreening in the soybean root or human cell uptake assays for their potential to inhibit the uptake of Avr1b (N)-GFP into soybean roots cells or human cells in culture.

### Example 4

#### Screening Assays for Novel Compounds that Inhibit Plant Oomycete or Fungal Infection Through Blocking of RXLR and dEER Containing Effector Protein Action

**[0109]** To further characterize candidate protective compounds obtained from the RXLR and dEER protein screen, the ability of the compounds to protect against oomycete or fungal pathogen infection are tested in detached leaf assays. The following detached leaf assays are used for soybean, potato, tomato, tobacco, grape, rice, and wheat. The assays are used to test for infection by *Phytophthora* oomycete pathogens (soybean, potato, tomato, tobacco), downy mildew oomycete pathogens (tobacco and grape), rust fungi (soybean and wheat), *Magnaporthe* blast fungi (rice and wheat), and powdery mildew fungi (soybean, potato, tomato, tobacco, grape, wheat).

**[0110]** Expanded leaves are removed from young growth chamber-grown plants with the petioles intact (soybean, potato, tomato, tobacco, grape), or are clipped from the mother plant with sterile scissors (wheat and rice). The petioles or cut ends of the leaves are placed into plastic test tubes containing an aqueous solution of a suitable range of concentrations of each compound (determined from the biochemical IC<sub>50</sub>). The leaves are then fastened into a horizontal position, but with the petioles or cut ends bent down into the tubes. The plants are then placed in a lighted growth chamber at 30% humidity for 6 hr to enable the compounds to be drawn into the leaves by transpiration.

**[0111]** The plants are then inoculated with pathogen spores. *Phytophthora* infections (*P. sojae* on soybean; *P. infestans* on tomato and potato; *P. parasitica* on tobacco) are initiated by spraying the leaves with an aqueous suspension of zoospores at a suitable concentration. Infections with rust fungi (*Phakopsora pachyrizi* for soybean; *Puccinia striiformis* f. sp. *tritici* (stripe rust) *Puccinia triticina* (leaf rust),

*Puccinia graminis* f. sp. *tritici* (stem rust) for wheat) are initiated by spraying the leaves with an aqueous suspension of urediniospores at a suitable concentration. Infections with downy mildew oomycetes (*Peronospora tabacina* on tobacco; *Plasmopora viticola* on grape) and infections of *Magnaporthe* blast fungi (*Magnaporthe oryzae* for rice; *Magnaporthe grisea* on wheat) are initiated by spraying the leaves with an aqueous suspension of conidia at a suitable concentration. In each case, after spraying with the pathogen spore suspension, the plants are replaced into the growth chamber at high (90%) humidity at a suitable temperature (15° C. for *Phytophthora infestans* and downy mildew oomycetes; 25° C. for *P. sojae*; 20° C. for all others) until symptoms develop (3-7 days). Infections with powdery mildew (*Microsphaera diffusa* on soybean; *Erysiphe cichoracearum* on potato and tobacco; *Leveillula taurica* on tomato or potato; *Erysiphe necator* on grape; *Blumeria graminis* f. sp. *tritici* on wheat) are done dry. The arrays of plant leaves are placed into a dusting tower and heavily infected leaves are introduced into the top of the tower and shaken vigorously for one minute, then the spores are allowed to settle for 20 min. The plants are replaced into growth chambers maintained at 70% humidity (except *Microsphaera diffusa* which is favored by low humidity of 30%) and 25° C.

[0112] To assay disease development in each case, the leaves are photographed, and from the photographs the numbers of lesions on each leaf are counted (total lesions and sporulating lesion) and the areas of the lesions are determined digitally. The results are assessed statistically by reference to negative controls (water), positive controls (benomyl for the fungi and metalaxyl for the oomycetes).

#### Example 5

##### Protecting Plants Against Oomycete Infection by Blocking Effector Entry Using Peptide Receptor Mimics

[0113] Eukaryotic pathogens such as oomycetes, fungi and apicomplexan parasites deliver hundreds of effector proteins into the cytoplasm of their host cells. Delivery of these proteins is key to the pathogenic success of these organisms. The similarity between oomycete and apicomplexan effector delivery systems has been noted for some time. The discovery that inositol 1,4 diphosphate can inhibit oomycete and fungal effector uptake (Example 2) shows that effector entry can be blocked by externally applied small molecular weight compounds. This Example describes experiments that test whether infection by oomycetes, and possibly by fungi, can be mitigated by inhibiting effector entry using host-synthesized peptides that mimic inositol 1,4 diphosphate. Biotrophic and hemi-biotrophic oomycete pathogens that are likely to use RXLR and dEER effectors include more than 80 species of *Phytophthora* and more than 500 species of downy mildews that together attack almost every crop species and horticultural species of economic importance. Peptides that could inhibit RXLR and dEER effector entry could thus provide broad-spectrum protection against many of these pathogens. Even if protection is narrow, and multiple peptides must be selected for each species of pathogen, this approach offers an important new weapon against these highly adaptable pathogens. The fact that one fungal effector from a rust pathogen also may use phosphoinositides to enter host cells suggests that the protection provided by anti-RXLR and dEER

peptides may extend to many biotrophic and hemi-biotrophic fungal pathogens such as rusts, smuts, powdery mildews and the rice blast fungus.

[0114] At least three commercially available phage display libraries are screened against a panel of effectors that have been well-characterized and/or are strongly expressed at the outset of *P. sojae* or *P. infestans* infection. The phage are eluted from the effectors using a rising concentration gradient of IP2 or soluble PI-P in order to identify those phage that have the greatest affinity for the PI-P binding sites of the effectors. The candidates obtained are evaluated for their binding to all panel members, and to RXLR and dEER mutants of the panel members. In addition, their affinity for both soluble and liposome-bound PI-4-P and PI-3-P is measured. Synthetic peptides corresponding to candidates with the highest affinities are prepared commercially and tested for their ability to inhibit uptake into plant and human cells. The most promising peptides at this point (broadest specificity, highest affinity and/or strongest inhibition of effector entry) are targeted for optimization of their breadth and affinity of binding. Two optimization strategies are used. Firstly, PCR-directed random mutagenesis of selected peptides is carried out and high affinity, broad-spectrum mutants are selected by phage display. Loss-of-activity mutants are also characterized to help identify important residues. As a parallel alternative approach, targeted mutagenesis of selected peptides is carried out based on bioinformatic analysis of all the phage peptide sequences obtained (both high quality and low quality peptides). Surface Plasmon resonance and NMR characterization of the binding of the most promising peptides to their target effector(s) also yields important information. The cycle of selecting promising peptides and optimizing them is repeated as needed, or until little further improvement is obtained. At this point the most promising peptides are fused to selected secreted plant proteins, and the chimeric proteins are expressed in plant tissues by transient expression. The expression levels and stabilities of the chimeric proteins are assessed, as well as the ability of the expressed proteins to reduce entry of effector proteins and reduce infection by *P. sojae* or *P. infestans*. The resistance of the plant tissue to additional pathogens is also evaluated. Stable transgenic plants expressing the chimeric proteins are produced, and are evaluated systematically for disease resistance against diverse pathogens.

[0115] Initially the Ph.D.-C7C random peptide phage display library available from New England Biolabs, Inc. is screened. In this library, a loop of 7 random amino acid residues is constrained by a disulfide bond at the base of the loop where it is fused to the N-terminus of the pill coat protein. This configuration was chosen because the loop will eventually be transferred to a secreted plant protein, and the disulfide bond will ensure that the loop has a similar structure in that context as on the phage. The library contains  $1.2 \times 10^9$  independent phage, providing about 60% statistical coverage of the total theoretical complexity of a heptapeptide library ( $207 = 1.3 \times 10^9$ ). Other possible libraries for screening include Ph.D.-7 and the Ph.D.-12 libraries that contain 7 or 12 random residues respectively, but without a disulfide bond; both have a complexity of around  $2.8 \times 10^9$ .

[0116] Two approaches are used to select RXLR and dEER-specific phage. In the first, individual effectors are screened. Since this allows interactions with residues outside the immediate RXLR and dEER region, inhibitory peptides with a narrow specificity are likely to be obtained. In a

complementary approach, the phage are selected on several different effectors successively, in order to only obtain peptides with broad specificity. Both strategies are adjusted as needed.

**[0117]** The one-effector-at-a-time strategy targets Avr1b, Avh331 (Avr1k), Avh5, Avh6 and Avh172. Because the first two effectors are avirulence gene products that trigger plant defense responses mediated by resistance (R) genes, the efficacy of candidate inhibitory peptides is also tested in planta by their ability to inhibit the R gene mediated response to the effectors. Avh6 and Avh172 are major early-expressed effectors, so targeting them singly also has a measurable effect on pathogen virulence. Avh5 is included because its NMR characterization is well advanced. In each case, two different fusions are produced: GFP and GST (glutathione-S-transferase) to reduce the chance of selecting phage that bind to an irrelevant part of the protein.

**[0118]** In the second strategy, three pools of effectors are created, and the phage are successively selected on the different pools. One example of such a set of pools is: pool 1=Avr1b+Avh331+Avh6; pool 2=Avh5+Avh172+Avh152; pool 3=Avh38+Avh260+AvrL567. Each effector listed is either an avirulence protein or a strongly-early-expressed *P. sojae* effector, or is otherwise well-characterized. By using pools, the risk that a single chosen effector may be problematic is reduced, and by using three different pools for the successive selection steps, the likelihood of finding broad specificity peptides is increased. The order of the pools used for selection is varied. The composition of the pools may be varied once data on the specificity of each effector for PI-3-P or PI-4-P is available, and/or if production of some chosen effector proteins in *E. coli* proves problematic.

**[0119]** Panning is carried out in microtiter tray wells; if sufficient enrichment of peptides is not seen in the wells, then the proteins are bound onto beads and the beads are used for panning. The phage are step eluted with different concentrations of inositol diphosphates (IP2) or soluble (e.g. di-hexanoyl) phosphatidyl phosphates. The choice of 1,3 IP2, 1,4 IP2, PI-3-P or PI-4-P, and the choice of concentrations is finalized once more precise data on the binding constant of the effectors for the phosphoinositides is available.

Characterization of Discovered Peptides for Binding to Multiple Effectors, and for Ability to Block Entry of Key Oomycete and Fungal Effectors into Plant Cells.

**[0120]** Each selected peptide is screened against a panel of all the effectors mentioned listed above, plus a selection of 10 *P. infestans* infection-induced effectors and several fungal effectors. RXLR and dEER region mutants are included to identify peptides that interact with those motifs. Phage with the broad specificity and a set of peptides with complementary sets of targets are identified. Screening is done in a western dot blot format in which effectors bound to a filter are probed with the phage and then with an anti-M13 antibody. Alternatively, the phage are panned against effectors arrayed in microtiter wells, and then detected by spotting onto an *E. coli* lawn with a replicator. The affinity of the phage for the effector is initially estimated by doing binding experiments in the presence of different concentrations of PI-Ps or IP2s. An oomycete effector protein microarray containing all 1440 effectors from *P. sojae*, *P. infestans*, *P. ramorum* and *H. arabidopsidis* is ideal for comprehensive screening of the most promising phage. The most promising peptides are tested for the ability to block effector-GFP entry into root cells. To obtain sufficient peptides for these experiments, synthetic

peptides are ordered from a commercial supplier. A quantitative cell entry assay using luciferase and suspension culture cells may also be employed. Binding of the most promising peptides to key effectors is further characterized by NMR and surface plasmon resonance (SPR).

Concatenation and Mutagenesis of the Most Promising Peptides to Further Optimize Broad-Spectrum Binding and Ability to Block Effector Entry into Plant Cells.

**[0121]** Bioinformatic comparisons of peptide sequences having different affinities and ranges of specificity provide important starting clues about the potential for using targeted mutations to improve affinity and specificity of the identified peptides. Alternatively, phage display technology is a proven platform for improving binding via random mutagenesis. A single randomized oligonucleotide is used to mutagenize the 21 nucleotides encoding each peptide loop. Selection of phage on a range of different effectors is used to improve the breadth of specificity. Selection of phage in the presence of free peptide having the original sequence is used to select for improved affinity. An alternative approach to improving the breadth of specificity is to concatenate several peptides, with a spacer or linker sequence in between. This is an acceptable construction for in planta expression. The concatenated peptides are tested to ensure that they retain their original affinities and breadth of specificity.

Fuse Peptides to Small Secreted Plant Proteins, and Test the Effects of their Expression in Planta on Effector Entry and on Disease Resistance.

**[0122]** For evaluation in planta, the peptide mimics are fused to larger proteins normally produced during infection to promote the peptides' stability and reduce their potential susceptibility to endogenous plant proteases. The fusions are evaluated in three steps: (i) exogenous application of purified proteins to plant tissues; (ii) transient expression in plants; and (iii) expression in stable transgenic plants. A variety of candidate proteins are evaluated for fusions with the peptide mimics, including highly stable plant proteins such as PR1a, lipid transfer proteins, protease inhibitors and proteases. Fusion to a protease inhibitor promotes stability, while conversely, fusion to a protease more effectively targets pathogen effectors for proteolysis. Generally, the mimic is attached to the C-terminus of the "carrier" protein via a suitable spacer so that the native N-terminal secretory leader can be used. Initially a single peptide mimic is attached to each carrier. Once attachment of single peptides has been validated, multiple peptides are attached in tandem to improve the breadth of binding and/or for better efficacy against effectors with multiple phosphoinositide binding sites. C-terminal green fluorescent protein (GFP) fusions are used to evaluate the stability and localization of the proteins in planta.

Expression in *E. coli* or *Pichia pastoris* and Evaluation of Purified Proteins.

**[0123]** Fusion proteins are expressed in *E. coli* or, due to the necessity to correctly form disulfide bonds, in eukaryotic expression system based on *Pichia pastoris*. The purified peptide-fusion proteins are tested for effector binding in vitro to ensure they retain binding activity as fusions. They are then introduced into leaf and root tissues (by infiltration and direct uptake, respectively) from soybean and *N. benthamiana* to test their stability in planta (via western blots) and to test their ability to inhibit the uptake of exogenously applied effector-reporter fusions into the plant cells. Uptake assays based on

suspension cultures cells and on protoplasts may also be used to distinguish between stability and effectiveness in effector uptake inhibition.

#### Transient Expression in Planta.

**[0124]** Excellent virus-based transient expression systems now exist for soybean and *Arabidopsis*. Infiltration of *Agrobacterium tumefaciens* strains harboring vectors designed to deliver gene expression constructs into plant cells locally (Agroinfiltration) and particle bombardment have also been used extensively. Initially a quantitative “double-barrel” particle bombardment assay is used to measure the ability of plant-expressed peptide fusion proteins to interfere with effector entry into soybean cells, using either the native effectors, Avr1b or Avh331 (i.e. Avr1k), or fusions of other effectors to an Avr1b reporter. By using GFP-fusions in conjunction with onion epidermal cell bombardment direct visualization of the localization of the peptide fusions or the targeted effector (or both together if one carries a red fluorescent protein, e.g. mCherry) is possible. Bi-molecular fluorescence complementation (BiFC; “split-YFP”) is used to verify effector-peptide interaction in planta in the onion system.

**[0125]** In order to evaluate the potential effect of peptide-fusion expression in planta on pathogen infection, the BPMV system is used to transiently express the peptide fusion proteins in soybean and Agroinfiltration to transiently express the proteins in *N. benthamiana*. Versions that include GFP to facilitate evaluation of stability and localization are used. Transcription of the constructs is confirmed using RT-PCR or northern analysis. Protein levels are evaluated by western blots, and confocal microscopy is used to verify that the proteins are being delivered to the apoplast.

**[0126]** Areas of plant tissue transiently expressing the peptide fusions are inoculated with *P. sojae* (soybean) or *P. infestans* (*N. benthamiana*) and disease development is evaluated. Empty vectors and vectors with the entire construct minus the peptide mimic are used as negative controls in these experiments. *N. benthamiana* tissue is tested for its response to the blue mold downy mildew pathogen *Peronospora tabacina*. Soybean leaf tissue is tested for resistance to the soybean rust fungus, *Phakopsora pachyrhizi*.

**[0127]** Hairy root cultures of soybean expressing the peptides are created, and assayed them for *P. sojae* resistance, to show that expression of Avr1b and Avh331 confers increased susceptibility to *P. sojae*. Stably transformed soybean, *N. benthamiana* and *Arabidopsis* plants expressing the fusion proteins are tested and display resistance to a variety of oomycete and fungal pathogens.

#### Example 6

##### Identification of Additional Virulence Motifs

**[0128]** The previous Examples present results showing that a fungal effector protein, AvrL567, from a rust fungus that forms haustoria (specialized feeding structures) enters plant cells via RXLR sequence motif-mediated binding to a phosphatidylinositol within the plant cell wall. In this Example, results are presented which show that effector protein Avr2 from the tomato pathogen *Fusarium oxysporum* Esp. *lycopersici* and effector protein AvrLm6 from the *Brassica* pathogen *Leptosphaeria maculans* also enter plants via the same mechanism. *Fusarium oxysporum* f.sp. *lycopersici* is a xylem dwelling pathogen and *Leptosphaeria maculans* is an apoplastic pathogen.

**[0129]** AvrLm6 contains two RXLR-like sequences, RYWT and RTLK. Mutations in the second motif (RYWT) but not the first (RTLK) abolish entry of AvrLm6-GFP fusions into root cells (FIG. 16A). Avr2 also contains two RXLR-like sequences, RMLH and RIYER. Mutations in the second motif (RIYER) but not the first (RMLH) abolish entry of Avr2-green fluorescent protein (GFP) fusion proteins into root cells (FIG. 16B). Both effector-GFP fusions bind PI-3-P strongly, PI-4-P moderately and PI-5-P weakly, but there was no binding to any other lipids (FIGS. 16C and D). In each case, the PI-P binding is dependent on the functional RXLR-like sequences, RYWT (in AvrLm6) and RIYER (in Avr2). Entry of AvrLm6 (FIG. 16E) and Avr2 (FIG. 16F) is inhibited in both cases by inositol 1,4 diphosphate (FIG. 16E), inositol 1,3 diphosphate (not shown) and by the PI-3-P binding proteins VAMP7 PX, indicating that entry into the soybean root cells is dependent on the presence of PI-3-P.

**[0130]** It has been found that GFP fusions to the N-termini of three more fungal effectors or effector-like proteins bind PI-3-P. The proteins are *Leptosphaeria maculans* effector AvrLm4/7 and two bioinformatically-predicted effector-like proteins from the human pathogens *Cryptococcus neoformans* (Cng2; AAW43853.1) and *Aspergillus fumigatus* (Af2; XP\_752996.1). Each N-terminal domain contains potential RXLR-like motifs (FIG. 17A).

**[0131]** These findings extend previously findings that several effectors from oomycete plant pathogens and apicomplexan pathogens of vertebrates (e.g. *Plasmodium falciparum*) bind phosphoinositides, particularly PI-3-P, which enables them to enter plant and animal cells.

#### Example 7

##### Occurrence of RXLR-Like Motifs in Effector Like Proteins from a Wide Diversity of Oomycetes, Fungi and Insects

**[0132]** Using a bioinformatic approach informed by detailed mutagenesis of the Avr1b RXLR motif, we have identified candidate RXLR-like motifs in 20 experimentally validated fungal effectors, as well as in 13 experimentally validated oomycete effectors (Table 1). The fungal effectors include an effector (MiSSP7) from a mutualistic ectomycorrhizal fungus, *Laccaria bicolor* (Martin et al., 2008).

**[0133]** Some sucking and chewing insects produce effector-like proteins, including hessian flies (*Mayetiola destructor*) (Behura et al., 2004) and pea aphids (*Acyrtosiphon pisum*) (Mutti et al., 2008). We have identified candidate RXLR-like motifs in the N-terminus of effectors vH9 and vH13 from *Mayetiola destructor*, and in the effector C002 from *Acyrtosiphon pisum* (Table 3).

**[0134]** Among oomycetes, RXLR-containing effectors have so far been documented in pathogens from the order Peronosporales. We have identified RXLR-like motifs in bioinformatically predicted effectors from *Pythium ultimum* (Pythiales) and *Albugo candida* (Albuginales), suggesting that RXLR-like effectors may be common to the entire oomycete Phylum (Table 4).

**[0135]** We have also identified RXLR-like motifs in bioinformatically predicted effectors from the necrotrophic plant pathogens *Pyrenophora tritici-repentis* and *Alternaria brassicicola*, and from the human pathogens *Cryptococcus neoformans*, *Aspergillus fumigatus* and *Coccidioides immitis* (Table 4).

[0136] Thus, phosphoinositide binding, particularly to PI-3-P, is a common property of most if not all eukaryotic effectors that can autonomously enter host plant or animal cells across their plasma membranes, including effectors produced by host-associated oomycetes, fungi and animals (e.g. insects and nematodes), and including pathogens, mutualists,

commensals, ectosymbionts and endosymbionts. A further corollary is that chemical or transgenic control measures that target the interaction of RXLR-like sequences with phosphoinositides will potentially be effective against a wide range of oomycete, fungal and animal pathogens.

TABLE 3

RXLR-like sequences in experimentally verified effectors.		King-	N-terminal
Effector Species		dom	Amino Acid Sequence
Avr1a	<i>Phytophthora sojae</i>	O	SENAFSAATDADQATVSKLAAAEFDLVDV LTESKRSLRATVDDGEER (SEQ ID NO: 304)
Avr1b	<i>Phytophthora sojae</i>	O	TEYSDETNIAMVESPDVLRSLRNGDIAGGR FLRAHEEDDAGERTFSV (SEQ ID NO: 305)
Avr1k	<i>Phytophthora sojae</i>	O	LTCATSEQQTRPELCFFFSVRSWPSTISDGA CLALVSAEQGATAGRNTLSLRSMATEDM ATST <b>ESLR</b> SQATNVDDANVSIENR (SEQ ID NO: 306)
Avr3a	<i>Phytophthora sojae</i>	O	LSTTNANQAKI IKGTSPGGHSP <b>RL</b> LRAYQPD DEGDSPEDR (SEQ ID NO: 307)
Avr3c	<i>Phytophthora sojae</i>	O	VEPSATSTVEVAEVQARGADK <b>RELR</b> SLQTE EE QGDSVDNEAEDGSEER (SEQ ID NO: 308)
Avr46	<i>Phytophthora sojae</i>	O	ITDESQPRDATIVDAPLTGRGANARY <b>LRT</b> ST SIIKAPDAQLPSTKAAIAS (SEQ ID NO: 309)
Avh172	<i>Phytophthora sojae</i>	O	TAEVDSKTALAAEVPAAIR <b>SL</b> ESDTPAS <b>RL</b> L RTGTVTSADNEDR (SEQ ID NO: 310)
Avr3a	<i>Phytophthora infestans</i>	O	IDQTKVLVYGTPAHYI <b>HS</b> AG <b>RL</b> LRKNEE NEETSEER (SEQ ID NO: 311)
Avr4	<i>Phytophthora infestans</i>	O	KADSLARTVSVVDNVK <b>SR</b> FLRAQ <b>TD</b> EK NEER (SEQ ID NO: 312)
AvrB1b1	<i>Phytophthora infestans</i>	O	AVSSNLNTAVNYASTSKIRFLSTEYNADEKR SLRGDYNNEVTKEPNTSDE (SEQ ID NO: 313)
AvrB1b2	<i>Phytophthora infestans</i>	O	VAAFPIPDESRLSKTSPDTPAP <b>SL</b> REIAEQE VIQSGR (SEQ ID NO: 314)
Atr1	<i>Hyaloperonospora arabidopsidis</i>	O	TESSETSGTIVHVFPLRDVADHRNDA LIN <b>RAL</b> RAQTALDDDEER (SEQ ID NO: 315)
Atr13	<i>Hyaloperonospora arabidopsidis</i>	O	LLHAHALHEDETGVTAG <b>RL</b> RAAASEVFGL SRASFGLGKAQDPLDKFF (SEQ ID NO: 316)
AvrLm1	<i>Leptosphaeria maculans</i>	F	SPATKNNVNQPLDNI <b>SR</b> SEWKS <b>VQ</b> IS PVKEHSAKTADNTENNHN <b>LEKRV</b> TSF HMKRTFTLALENTFYAMAWLIDFSFS EEGEPHFSYKLQ (SEQ ID NO: 317)
AvrLm4/7	<i>Leptosphaeria maculans</i>	F	CREASISGEIRYPQGTCP <b>TK</b> TEALNDC NKVT <b>KGL</b> IDFSQ <b>SH</b> RAWGIDMT (SEQ ID NO: 318)
AvrLm6	<i>Leptosphaeria maculans</i>	F	QHLLCACESGRRDGVDD <b>TR</b> TLKVVKG <b>TGG</b> RFV <b>FSSR</b> Y <b>WT</b> KAEGAPHE (SEQ ID NO: 319)
Avr-Pita	<i>Magnaporthe oryzae</i>	F	HPVVDYNPINHIHGDLK <b>RR</b> AY <b>LE</b> RY <b>SQ</b> CS DSQASEI <b>RA</b> ALKSCAELASWGYH <b>AVK</b> SD N <b>LEK</b> L <b>LE</b> FKTD <b>ST</b> DIQ <b>N</b> (SEQ ID NO: 320)
Avr-Pii	<i>Magnaporthe oryzae</i>	F	LPTPASLNGNTEVATISDV <b>KLE</b> ARS <b>DT</b> TY <b>HK</b> CSKCGYGSDDSDAYFN <b>HK</b> C (SEQ ID NO: 321)

TABLE 3 -continued

RXLR-like sequences in experimentally verified effectors.		
Effector Species	King- dom	N-terminal Amino Acid Sequence
Avr-Pia	<i>Magnaporthe oryzae</i>	F RFCVYYDGHLPATREVLMLMYVRIGTTATITA RGHEFEVEAKDQCKVILITNG (SEQ ID NO: 322)
Avr-Pizt	<i>Magnaporthe oryzae</i>	F SFVQCNIHLLLYNGRHHGTIRKKAGWAV RFYEEKPGQPRLVAICKNA (SEQ ID NO: 323)
Avr-PikD	<i>Magnaporthe oryzae</i>	F ETGNKYIEKRAIDLRSRERDPNFFDHPGIPVPE CFWFMFKNNVRQ (SEQ ID NO: 324)
Avr1 (Six4)	<i>Fusarium oxysporum f. sp. lycopersici</i>	F LPKGEEGDIIGTFNFSSSDSQPLKIHVVDTPTD SSGSNLVKRSA (SEQ ID NO: 325)
Avr2 (Six3)	<i>Fusarium oxysporum f. sp. lycopersici</i>	F LPVEDADSSVQQLQGRGNPYCVFPGRTSS TSFTTSFSTEPLGYARMLHRDPPYERAGNSG LNHR <b>IYER</b> SRVGGRLRTVIDV (SEQ ID NO: 326)
Avr3 (Six1)	<i>Fusarium oxysporum f. sp. lycopersici</i>	F QEAAVREPQIFPNLTYTEYLDKVAASHGSP DKSDLPWNDTMGSFPGNETDDGVQTETGSS LSRRGHIVNLRKREPFGESRNDRVTD (SEQ ID NO: 327)
Six2	<i>Fusarium oxysporum f. sp. lycopersici</i>	F NPAGDSLPPDDAHLDPDRRLSPSEVQALKKAQ IYPPGYIHKRVTFEGEKDAV (SEQ ID NO: 328)
Six5	<i>Fusarium oxysporum f. sp. lycopersici</i>	F RDHQYCACQSGSGDSIDIDATTQLQNDNS KSYLWAQTSPAYWFADRHK (SEQ ID NO: 329)
Six6	<i>Fusarium oxysporum f. sp. lycopersici</i>	F GPLAQTESADVAEHTINYIDIAPEEFEPK ANLSSLVSRDTLPVST (SEQ ID NO: 330)
AvrL567	<i>Melampsora lini</i>	F MEHVPAELTRVSEGYT <b>REYR</b> SPTASVILSG LVKVKWDNEQMTMPLFKWIG (SEQ ID NO: 331)
AvrM	<i>Melampsora lini</i>	F SLSNNLGTVPDVP <b>HQIP</b> NDKSGT <b>PAIED</b> PKA AIEDPK <b>DMKGENKAL</b> KSTPESEK <b>LTSS</b> VE GIPQPEFDR <b>GFLE</b> PPGAKMK <b>FLKPD</b> QVQ KLSTDDLITYM (SEQ ID NO: 332)
AvrP123	<i>Melampsora lini</i>	F QYVVDPGFGEIECMCG <b>QIARLTOR</b> PPDVE CEAT (SEQ ID NO: 333)
AvrP4	<i>Melampsora lini</i>	F EFLEDAR <b>DIQ</b> GFSRKSGSKLEESDSSRDRO (SEQ ID NO: 334)
STP1	<i>Ustilago maydis</i>	F NGSISNASHHHQ <b>ERMV</b> RQ <b>RHI</b> EAR <b>SAM</b> SWL TK <b>ISSK</b> ASDW <b>MFG</b> SVHAP <b>NLDK</b> DL <b>KPLV</b> GGVAVMPKMPY (SEQ ID NO: 335)
MISSP7	<i>Laccaria bicolor</i>	F SPVPGEVGLVERGPIPNVAVFRVPEPNFFKD LLRALQASQGGDLHR (SEQ ID NO: 336)
C007	<i>Acyrtosiphon pisum</i>	I SAAEPYDEQEASVELPMEHRQCDEYK <b>SKI</b> WDKAFSNQ <b>EAMQ</b> LMELTFNT <b>GKEL</b> GSHEV (SEQ ID NO: 337)
vH13	<i>Mayetiola destructor</i>	I SPLPLAYT <b>DQVYDACD</b> R <b>QFDE</b> TVRNSQPL (SEQ ID NO: 338)

TABLE 3 -continued

RXLR-like sequences in experimentally verified effectors.		
Effector Species	King-	N-terminal dom Amino Acid Sequence
v19 <i>Mayetiola destructor</i>	I	LVLDTRAMPETDFEKALKEWNRVQTLVLIA PEQRTMVLIAEHLTNLKKMNVDSPPGGSFL YLKDGDPVIKLPVSEHFEITFRGPYGVDKNE <u>SFYMPKLKLLIVEDADANDKLIKLFVSQHS</u> <u>RTLKTLDLVAANYRTLRLTLGAMKHIEEFVT</u> SPP (SEQ ID NO: 339)

Effectors named AvrXXX are avirulence gene products.  
 Motifs experimentally verified as functional are in bold and underlined.  
 Motifs experimentally determined to be non-functional are marked in italics.  
 Putative motifs are underlined.  
 Sequences shown are from the N-terminus of the respective proteins.  
 Note that *Laccaria bicolor* is a mutualistic fungus.  
 Kingdoms:  
 O = oomycetes;  
 F = fungi;  
 I = insects

TABLE 4

RXLR-like sequences in bioinformatically predicted effectors.		
Predicted Effector	Species	King- dom N-terminal Amino Acid Sequence
Avh5	<i>Phytophthora sojae</i>	O TRVPDDANLQSVNAPVQTVTRS <b>RRLLR</b> TADT DIVVEPKVHNPGKKQVFIE (SEQ ID NO: 340)
PYU1_T005878	<i>Pythium ultimum</i>	O MMPSTDAHGYIAPPPAQYKDPATATNYNAIIT ASINTAPACKK <b>WDDNP</b> TANT <b>KTE</b> TAAPKKS YTS <b>LKQMLDKK</b> VPGCONSRDFTPIK <b>TKTYK</b> TMEWQND (SEQ ID NO: 341)
PYU1_T001475	<i>Pythium ultimum</i>	O HSQMTVPNPKFSDVSKANSPLGTIDGPTVMPP PAGQSYAMGTD <b>TNIKAYVE</b> AFKQ <b>TKW</b> TL KDLIMDKY <b>VEDGNI</b> PDRACGLTD <b>KTYM</b> QLP DKYVVW (SEQ ID NO: 342)
AcEff1	<i>Albugo candida</i>	O STMGLSN <b>SRHLED</b> AVE <b>VLGDL</b> KL <b>NQ</b> DEKQ NENEVDKNN <b>SKG</b> DR <b>ESS</b> (SEQ ID NO: 343)
Af13	<i>Aspergillus flavus</i>	F AGLPVFPNQAVLRPSLALPGD <b>NSHRY</b> SLPMFD LQPWERVDEI <b>RLARKG</b> YLYGSP (SEQ ID NO: 344)
PTRG_02320	<i>Pyrenophora triticirepentis</i>	F QVKGNAIRCGDDKSDQDT <b>ENE</b> CREMETS D <b>RTLKINGE</b> FRGNA (SEQ ID NO: 345)
AB09791.1	<i>Alternaria brassicicola</i>	F APASYPASALGKRWVDTTGGQKMPAHFVST VRKL <b>STEK</b> LK <b>TKRQ</b> L <b>DQLL</b> (SEQ ID NO: 346)
Cng1	<i>Cryptococcus neoformans</i>	F LTV <b>PQ</b> AHRETLE <b>EAG</b> KLTTIA <b>INTK</b> LIK <b>DV</b> TVGTMSSVFPDGT <b>DNG</b> GRP (SEQ ID NO: 347)
Cng2	<i>Cryptococcus neoformans</i>	F IQQGQKANARE <b>QHR</b> GRK <b>NLT</b> IKLPG <b>AHSYK</b> AKFEGCMV <b>VLQDKKLY</b> VEHAGCESLAYAHP (SEQ ID NO: 348)
Af1	<i>Aspergillus fumigatus</i>	F IPSAFPODNAVNQVLLSDSYQDQSVSSISAED DAQNSAVIHIG <b>ES</b> ETMRAPS <b>WFT</b> STLMAR <b>LL</b> ALSTTG <b>VST</b> IFPDPLPGNSHAPPSVAGLP (SEQ ID NO: 349)
Af2	<i>Aspergillus fumigatus</i>	F VAMGVSEQRKANERKMD <b>ARRM</b> ARFNIDIETS GETQ <b>E</b> DEIRG <b>KR</b> IVLEDNKVYLLDDPLPANRK HPSHTAESFYIDYP (SEQ ID NO: 350)

TABLE 4 -continued

RXLR-like sequences in bioinformatically predicted effectors.			
Predicted Effector	Species	King- dom	N-terminal Amino Acid Sequence
Af3	<i>Aspergillus fumigatus</i>	F	PVVPGQTVMEPSAALPDDGDHLYTLPMFDIR PWERVSEVRLAREGYLYG (SEQ ID NO: 351)
Ci1	<i>Coccidioides immitus</i>	F	SPVFPGGDKRDALYQKPIAPAGEFPFDSSPPEA RMTIPYADNEPDSLSIPSWPTTHLLARLLGL STTGVLSTVFPRTNRDPALVGV (SEQ ID NO: 352)
Ci2	<i>Coccidioides immitus</i>	F	IRSSQRSQRQEHRSRKMNLIVSCSDPSRKS DVDGCFVVLNRHKLWLASRPSDGEANEPSDD ATRFKASLHQCHH (SEQ ID NO: 353)

Motifs experimentally verified as functional are in bold and underlined.

Putative motifs are underlined.

Sequences shown are from the N-terminus of the respective proteins.

Note that *Cryptococcus neoformans*, *Aspergillus fumigatus* and *Coccidioides immitus* are human pathogens. *Pyrenophora tritici-repentis* and *Alternaria brassicicola* are necrotrophic plant pathogens.

Kingdoms:

O = oomycetes;

F = fungi.

#### Example 8

##### Phosphatidylinositol-3-phosphate is the Natural Target of RXLR(-Like) Effectors

**[0137]** Of the eight oomycete and fungal effectors tested to date, seven have a preference for binding to PI-3-P, and one (Avr1b) has a preference for PI-4-P. Neither of PI-3-P nor PI-4-P has been reported to occur in the outer leaflet of the plasma membrane of plant or animal cells (Boon et al., 2002). Two papers have reported secretion of PI-4-P by plant cells (Regente et al., 2008; Gonorazsky et al., 2008).

**[0138]** In order to test directly for the presence of PI-3-P and PI-4-P, we fused the PX domain of VAM7p and the PH domains of the human proteins FAPP1 and PEPP1 to green fluorescent protein (GFP). PEPP1 and FAPP1 bind very specifically to PI-3-P and PI-4-P, respectively (FIG. 18A) (Dowler et al., 2000). VAM7p has a preference for PI-3-P but can bind weakly to PI-4-P and PI-5-P (FIG. 18A) (Lee et al., 2006). The GFP fusion proteins were used to stain the surface of cells of human lung epithelial cell line A549 and soybean root cells. The results reveal very clearly that PI-3-P is present uniformly on the outer surface of roots cells (FIG. 18B), and at specific sites on the surface of the epithelial cells (FIG. 18C).

**[0139]** As can be seen, PI-4-P could not be detected in either case. Neither PI-3-P nor PI-4-P could be detected on the surface of erythrocytes. The lack of PI-3-P or PI-4-P on the surface of erythrocytes is significant because nearly all published studies documenting the absence of PI-3-P or PI-4-P from the outside of eukaryotic cells utilized erythrocytes. Our results suggest that erythrocytes may be an exception in this regard.

**[0140]** The finding of PI-3-P on the outside of plant and animal cells, combined with the preference for most effectors for PI-3-P is consistent with PI-3-P being the principal receptor mediating entry of the effectors into plant and animal cells. The absence of PI-4-P from the membranes suggests PI-4-P is unlikely to be the principal route of cell entry. However, PI-4-P has been reported to be secreted from plant

cells under certain conditions (Regente et al., 2008; Gonorazky et al., 2008), and it is possible that some effectors such as Avr1b have evolved to respond to PI-4-P.

#### Example 9

**[0141]** Since several of the fungal effectors that were tested contained no obvious RXLR or dEER motifs, we used the leaf bombardment assay to define the range of residues within the RXLR motif of Avr1b that could permit cell entry. The results (FIG. 19) revealed that lysine or histidine but not glutamine could replace the arginine at position 1 in the motif, that any large hydrophobic residue (isoleucine, methionine, phenylalanine, or tyrosine) could replace the leucine at position 3, but valine and alanine could not. At position 4, all residues tested allowed function. Furthermore, the presence of either a leucine or methionine residue at position 2 could substitute for leucine at position 3. The effector binding motif was thus refined to BXZ, where B=R, K or H; X is any amino acid and may be absent; and z=L, M, I, W, Y, or F.

#### Example 10

##### A PI-3-P-Binding Protein Blocks Entry of Effectors into Plant and Human Cells

**[0142]** To determine more directly if PI-3-P mediates host cell entry, we pre-incubated soybean roots and epithelial cells with unlabeled VAM7p PX protein, which binds PI-3-P, prior to exposing the cells to effector-GFP fusions. Pre-incubation with VAM7p PX protein strongly inhibited entry by GFP fusions of the oomycete effector Avr1b, and the fungal effectors AvrL567, Avr2 and AvrLm6 into soybean root cells (FIG. 20A) but did not inhibit entry of a synthetic cell permeable protein Arg9-GFP that does not bind phosphoinositides. Similarly, VAM7p PX protein strongly inhibited the entry by GFP fusions of Avr1b, AvrL567 and the *Plasmodium* effector PfHRP2 into human epithelial cells, but did not inhibit entry of Arg9-GFP.

**[0143]** These results strongly support the hypothesis that PI-3-P binding is necessary for the effector GFP fusions to enter plant and animal cells.

**[0144]** The previous examples showed that the head group mimic 1,4 inositol diphosphate (1,4IP2) could inhibit entry of effector-GFP fusions into soybean root cells and human epithelial cells (FIGS. 20A and B). Presumably the binding of 1,4IP2 to the effectors is strong enough to compete with binding for cellular PI-3-P.

**[0145]** To test the ability of inositol diphosphate to inhibit entry of a native effector (not a GFP fusion) into plant cells, we produced full length protein of the oomycete effector Avr1k (from *Phytophthora sojae*) in *E. coli*. We then infiltrated the purified protein into soybean leaves which did or did not carry the resistance gene Rps1k. In the presence of Rps1k, Avr1k triggers a programmed cell death response called the hypersensitive response (HR) (FIG. 20C, panel 1). In the presence of 1,3IP2, however, no HR was observed, consistent with 1,3IP2 blocking the entry of the effector into the leaf cells. When the RXLR motif of Avr1k (RSLR) was mutated, no HR was observed, even in the absence of 1,3IP2, confirming the RXLR motif was essential for cell entry. When the Rps1k gene was absent, no HR was observed, as expected. These results show that entry of RXLR effectors into plant cells requires binding to PI-3-P.

#### Example 11

##### Methods to Block Effector Entry Using Small-Molecule Drugs

**[0146]** The ability to block effector entry using 1,3IP2 or 1,4IP2 provides a proof-of-concept for treating oomycete or fungal infections of plants or animals, including humans, with drugs that block the PI-3-P-binding sites of the effectors. Such drugs may not need to fully block all effectors in order to be effective. Since a principal function of effectors is to suppress the host defense responses, even partial inhibition of effector entry may be sufficient to obtain protein. This point may be important because some forms of genetic resistance in plants (major gene resistance) rely upon entry of effectors into the plant cells (the resistance gene product encodes a receptor that detects the presence of an intracellular effector).

**[0147]** Drugs are also used to interfere with biosynthesis or export of PI-3-P to the outer leaflet. Inside the cell, PI-3-P can be formed by the action of phosphatidylinositol-3-kinases on phosphatidylinositol, and by the action of phosphatidylinositol 4,5 phosphatases on phosphatidylinositol 3,4 diphosphate, phosphatidylinositol 3,5 diphosphate and phosphatidylinositol 3,4,5 diphosphate. Any drug which inhibited these enzymes could lower the levels of external PI-3-P. Currently it is not known how PI-3-P reaches the outer leaflet. All known PI-3-P forming enzymes are located on the cytoplasmic face of membranes. PI-3-P could reach the external leaflet of the plasma membrane or the luminal face of secretory vesicles by the action of floppases or a scramblases. Alternatively PI-3-P might be transported to the outer leaflet by an ABC transporter or by a secreted lipid transfer protein. Any of the proteins involved in this process could be targeted with drugs, provided that they did not disrupt normal cell physiology. In addition to drugs that directly target the proteins described above, and drug that targets biosynthesis of the proteins, for example siRNAs, are also effective.

**[0148]** Some specific examples of drugs that may be used in the practice of the invention include but are not limited to

membrane-permeant derivatives of inositol diphosphates (Li et al., 1992) and bis(hydroxymethyl)-inositol (Flu et al., 2000).

**[0149]** Alternatively, drugs that bind directly to PI-3-P making it unavailable to effectors are effective. For example, neomycin binds PI-4,5-P2 very effectively; thus neomycin, neomycin derivatives or other aminoglycosides that bind PI-3-P may be used.

**[0150]** If effective drugs cause toxicity, forms of the drugs which are activated only when in close proximity to the pathogen are used. For example, infection in both plant and animal cell systems results in local high concentrations of hydrogen peroxide. A pro-drug that is activated by oxidation or peroxidation mitigates toxicity.

#### Example 12

##### Methods to Block Effector Entry Using Polypeptides

**[0151]** Polypeptides with the properties described in Example 10 may also be utilized. Polypeptides may have some advantage over chemicals, in plant and animal systems, in that the host organism can be genetically engineered to produce the polypeptide, simplifying delivery and reducing cost. The ability to produce and select large numbers of variant polypeptides via phage display technologies provides additional power to improve specificity, if needed. Random peptides or single chain antibodies selected by phage display are used to block the PI-3-P binding sites of effectors. Additionally such effector-binding proteins could be fused to proteases to facilitate degradation of the effectors.

**[0152]** The ability to block effector entry by pre-incubation with PI-3-P-binding proteins provides a strong indication that secretion of PI-3-P-binding proteins could provide protection against infection, especially if the secreted protein could be targeted to the infection site. Additionally, secretion of enzymes which can hydrolyze PI-3-P or modify it in other ways may be effective in reducing the level of PI-3-P available to transport effectors into cells. Examples of such enzymes include but are not limited to PI-3-P 4,5 kinases, PI-3-phosphatases, or phospholipases, etc. Examples of these enzymes have been described in the literature (Falasca et al., 2006).

**[0153]** Additionally, enzymes (e.g. from microbes) that cause novel modifications of PI-3-P such as methylases, acetylases or glycosylases may be used. A particularly useful enzyme is a phospholipase C that can cleave PI-3-P into diacylglycerol and 1,3-inositol diphosphate; not only is the level of PI-3-P reduced but the inhibitor 1,3-inositol diphosphate would be produced as a result. Currently known phosphatidylinositol-specific phospholipase C's are specific for phosphatidylinositol, glycosyl phosphatidylinositol-protein anchors, or for phosphatidylinositol-4-phosphate and phosphatidylinositol-4,5-diphosphate. In some embodiments, systematic mutagenesis is used to modify the specificity of a phosphatidylinositol specific phospholipase C so that it could cleave PI-3-P.

**[0154]** Further, in cases where polypeptides that manipulate PI-3-P levels cause deleterious physiological effects on the host, transgenic hosts are produced in which the polypeptide gene is transcribed only during infection. Alternatively, or jointly with this strategy, the polypeptide is targeted to the site of infection. For example, the *Arabidopsis* protein. RPW8 is specifically targeted to haustoria of certain

oomycetes and fungi (Wang et al., 2009). RPW8 is used to target anti-effector polypeptides to the haustorial space.

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Gln Leu Pro Ser Thr Lys Ala Ala Ile Ala Ser Ser Val Thr Lys Glu
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85 90 95  
Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp  
100 105 110  
Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr  
115 120 125  
Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser  
130 135

<210> SEQ ID NO 10  
<211> LENGTH: 138  
<212> TYPE: PRT  
<213> ORGANISM: *Phytophthora sojae*

<400> SEQUENCE: 10

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val  
1 5 10 15  
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met  
20 25 30  
Val Glu Ser Pro Asp Leu Val Arg Ala Ala Ala Ala Asn Gly Asp Ile  
35 40 45  
Ala Gly Gly Arg Phe Leu Arg Ala His Glu Glu Asp Asp Ala Gly Glu  
50 55 60  
Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys  
65 70 75 80  
Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr  
85 90 95  
Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp  
100 105 110  
Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr  
115 120 125  
Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser  
130 135

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<210> SEQ ID NO 11
<211> LENGTH: 138
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

<400> SEQUENCE: 11
Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1          5          10          15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
20          25          30
Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile
35          40          45
Ala Gly Gly Ala Ala Ala Ala Ala His Glu Glu Asp Asp Ala Gly Glu
50          55          60
Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys
65          70          75          80
Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr
85          90          95
Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp
100         105         110
Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr
115         120         125
Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser
130          135

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<210> SEQ ID NO 12
<211> LENGTH: 138
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

<400> SEQUENCE: 12
Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1          5          10          15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
20          25          30
Val Glu Ser Pro Asp Leu Val Arg Ala Ala Ala Ala Asn Gly Asp Ile
35          40          45
Ala Gly Gly Ala Ala Ala Ala Ala His Glu Glu Asp Asp Ala Gly Glu
50          55          60
Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys
65          70          75          80
Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr
85          90          95
Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp
100         105         110
Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr
115         120         125
Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser
130          135

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<210> SEQ ID NO 13
<211> LENGTH: 138
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

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&lt;400&gt; SEQUENCE: 13

```

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1           5           10           15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
          20           25           30
Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile
          35           40           45
Ala Gly Gly Arg Phe Leu Arg Ala His Ala Ala Ala Ala Gly Ala
          50           55           60
Ala Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys
65           70           75           80
Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr
          85           90           95
Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp
          100          105          110
Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr
          115          120          125
Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser
          130          135

```

&lt;210&gt; SEQ ID NO 14

&lt;211&gt; LENGTH: 138

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

&lt;400&gt; SEQUENCE: 14

```

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1           5           10           15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
          20           25           30
Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile
          35           40           45
Ala Gly Gly Arg Phe Leu Arg Ala His Glu Glu Asp Asp Ala Gly Glu
          50           55           60
Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys
65           70           75           80
Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr
          85           90           95
Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp
          100          105          110
Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr
          115          120          125
Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser
          130          135

```

&lt;210&gt; SEQ ID NO 15

&lt;211&gt; LENGTH: 138

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

&lt;400&gt; SEQUENCE: 15

```

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1           5           10           15

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Asp Phe Gln Gly Lys Ala Glu Thr Val Lys Val Ser Val Val Asp Glu  
 245 250 255  
 Glu Gly Lys Val Val Ala Ser Thr Glu Gly Leu Ser Gly Asn Val Glu  
 260 265 270  
 Ile Pro Asn Val Ile Leu Trp Glu Pro Leu Asn Thr Tyr Leu Tyr Gln  
 275 280 285  
 Ile Lys Val Glu Leu Val Asn Asp Gly Leu Thr Ile Asp Val Tyr Glu  
 290 295 300  
 Glu Pro Phe Gly Val Arg Thr Val Glu Val Asn Asp Gly Lys Phe Leu  
 305 310 315 320  
 Ile Asn Asn Lys Pro Phe Tyr Phe Lys Gly Phe Gly Lys His Glu Asp  
 325 330 335  
 Thr Pro Ile Asn Gly Arg Gly Phe Asn Glu Ala Ser Asn Val Met Asp  
 340 345 350  
 Phe Asn Ile Leu Lys Trp Ile Gly Ala Asn Ser Phe Arg Thr Ala His  
 355 360 365  
 Tyr Pro Tyr Ser Glu Glu Leu Met Arg Leu Ala Asp Arg Glu Gly Leu  
 370 375 380  
 Val Val Ile Asp Glu Thr Pro Ala Val Gly Val His Leu Asn Phe Met  
 385 390 395 400  
 Ala Thr Thr Gly Leu Gly Glu Gly Ser Glu Arg Val Ser Thr Trp Glu  
 405 410 415  
 Lys Ile Arg Thr Phe Glu His His Gln Asp Val Leu Arg Glu Leu Val  
 420 425 430  
 Ser Arg Asp Lys Asn His Pro Ser Val Val Met Trp Ser Ile Ala Asn  
 435 440 445  
 Glu Ala Ala Thr Glu Glu Glu Gly Ala Tyr Glu Tyr Phe Lys Pro Leu  
 450 455 460  
 Val Glu Leu Thr Lys Glu Leu Asp Pro Gln Lys Arg Pro Val Thr Ile  
 465 470 475 480  
 Val Leu Phe Val Met Ala Thr Pro Glu Thr Asp Lys Val Ala Glu Leu  
 485 490 495  
 Ile Asp Val Ile Ala Leu Asn Arg Tyr Asn Gly Trp Tyr Phe Asp Gly  
 500 505 510  
 Gly Asp Leu Glu Ala Ala Lys Val His Leu Arg Gln Glu Phe His Ala  
 515 520 525  
 Trp Asn Lys Arg Cys Pro Gly Lys Pro Ile Met Ile Thr Glu Tyr Gly  
 530 535 540  
 Ala Asp Thr Val Ala Gly Phe His Asp Ile Asp Pro Val Met Phe Thr  
 545 550 555 560  
 Glu Glu Tyr Gln Val Glu Tyr Tyr Gln Ala Asn His Val Val Phe Asp  
 565 570 575  
 Glu Phe Glu Asn Phe Val Gly Glu Gln Ala Trp Asn Phe Ala Asp Phe  
 580 585 590  
 Ala Thr Ser Gln Gly Val Met Arg Val Gln Gly Asn Lys Lys Gly Val  
 595 600 605  
 Phe Thr Arg Asp Arg Lys Pro Lys Leu Ala Ala His Val Phe Arg Glu  
 610 615 620  
 Arg Trp Thr Asn Ile Pro Asp Phe Gly Tyr Lys Asn Ala Ser His His  
 625 630 635 640

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His His His His Val  
645

<210> SEQ ID NO 17  
 <211> LENGTH: 138  
 <212> TYPE: PRT  
 <213> ORGANISM: *Phytophthora sojae*

<400> SEQUENCE: 17

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val  
 1 5 10 15  
 Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met  
 20 25 30  
 Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile  
 35 40 45  
 Ala Gly Gly Arg Phe Leu Arg Ala His Glu Glu Asp Asp Ala Gly Glu  
 50 55 60  
 Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys  
 65 70 75 80  
 Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr  
 85 90 95  
 Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp  
 100 105 110  
 Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr  
 115 120 125  
 Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser  
 130 135

<210> SEQ ID NO 18  
 <211> LENGTH: 138  
 <212> TYPE: PRT  
 <213> ORGANISM: *Phytophthora sojae*

<400> SEQUENCE: 18

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val  
 1 5 10 15  
 Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met  
 20 25 30  
 Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile  
 35 40 45  
 Ala Gly Gly Ala Ala Ala Ala Ala His Glu Glu Asp Asp Ala Gly Glu  
 50 55 60  
 Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys  
 65 70 75 80  
 Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr  
 85 90 95  
 Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp  
 100 105 110  
 Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr  
 115 120 125  
 Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser  
 130 135

<210> SEQ ID NO 19  
 <211> LENGTH: 117

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<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

<400> SEQUENCE: 19

Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met Val Glu Ser Pro Asp
1           5           10           15
Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile Ala Gly Gly Arg Phe
20          25          30
Leu Arg Ala His Glu Glu Asp Asp Ala Gly Glu Arg Thr Phe Ser Val
35          40          45
Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys Leu Ala Lys Ala Met
50          55          60
Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr Glu Lys Trp Ala Lys
65          70          75          80
Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp Leu Ala Ile Ala Asp
85          90          95
Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr Asn Gly Tyr Thr Phe
100         105         110
His Arg Tyr Gln Ser
115

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<210> SEQ ID NO 20
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

<400> SEQUENCE: 20

Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met Val Glu Ser Pro Asp
1           5           10           15
Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile Ala Gly Gly Ala Ala
20          25          30
Ala Ala Ala His Glu Glu Asp Asp Ala Gly Glu Arg Thr Phe Ser Val
35          40          45
Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys Leu Ala Lys Ala Met
50          55          60
Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr Glu Lys Trp Ala Lys
65          70          75          80
Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp Leu Ala Ile Ala Asp
85          90          95
Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr Asn Gly Tyr Thr Phe
100         105         110
His Arg Tyr Gln Ser
115

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<210> SEQ ID NO 21
<211> LENGTH: 138
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

<400> SEQUENCE: 21

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1           5           10           15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
20          25          30
Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile

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      35          40          45
Ala Gly Gly Arg Phe Leu Arg Ala His Glu Glu Asp Asp Ala Gly Glu
  50          55          60
Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys
  65          70          75          80
Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr
  85          90          95
Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp
 100          105          110
Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr
 115          120          125
Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser
 130          135

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<210> SEQ ID NO 22
<211> LENGTH: 138
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

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<400> SEQUENCE: 22

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```

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
  1          5          10          15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
  20          25          30
Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile
  35          40          45
Ala Gly Gly Arg Phe Leu Arg Ala His Glu Glu Asp Asp Ala Gly Glu
  50          55          60
Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys
  65          70          75          80
Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr
  85          90          95
Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp
 100          105          110
Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr
 115          120          125
Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser
 130          135

```

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<210> SEQ ID NO 23
<211> LENGTH: 117
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

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<400> SEQUENCE: 23

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```

Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met Val Glu Ser Pro Asp
  1          5          10          15
Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile Ala Gly Gly Arg Phe
  20          25          30
Leu Arg Ala His Ala Ala Ala Ala Ala Gly Ala Ala Thr Phe Ser Val
  35          40          45
Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys Leu Ala Lys Ala Met
  50          55          60
Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr Glu Lys Trp Ala Lys

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65              70              75              80
Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp Leu Ala Ile Ala Asp
      85              90              95
Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr Asn Gly Tyr Thr Phe
      100             105             110
His Arg Tyr Gln Ser
      115

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&lt;210&gt; SEQ ID NO 24

&lt;211&gt; LENGTH: 360

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

&lt;400&gt; SEQUENCE: 24

```

Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met Val Glu Ser Pro Asp
1          5          10          15
Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile Ala Gly Gly Arg Phe
20         25         30
Leu Arg Ala His Glu Glu Asp Asp Ala Gly Glu Arg Thr Phe Ser Val
35         40         45
Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys Leu Ala Lys Ala Met
50         55         60
Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr Glu Lys Trp Ala Lys
65         70         75         80
Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp Leu Ala Ile Ala Asp
85         90         95
Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr Asn Gly Tyr Thr Phe
100        105        110
His Arg Tyr Gln Ser Gly Thr Ala Thr Met Val Ser Lys Gly Ala Glu
115        120        125
Leu Phe Thr Gly Ile Val Pro Ile Leu Ile Glu Leu Asn Gly Asp Val
130        135        140
Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr
145        150        155        160
Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro
165        170        175
Val Pro Trp Pro Thr Leu Val Thr Thr Leu Ser Tyr Gly Val Gln Cys
180        185        190
Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser
195        200        205
Ala Met Pro Glu Gly Tyr Ile Gln Glu Arg Thr Ile Phe Phe Glu Asp
210        215        220
Asp Gly Asn Tyr Lys Ser Arg Ala Glu Val Lys Phe Glu Gly Asp Thr
225        230        235        240
Leu Val Asn Arg Ile Glu Leu Thr Gly Thr Asp Phe Lys Glu Asp Gly
245        250        255
Asn Ile Leu Gly Asn Lys Met Glu Tyr Asn Tyr Asn Ala His Asn Val
260        265        270
Tyr Ile Met Thr Asp Lys Ala Lys Asn Gly Ile Lys Val Asn Phe Lys
275        280        285
Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr
290        295        300

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Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn  
305 310 315 320

His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys  
325 330 335

Arg Asp His Met Ile Tyr Phe Gly Phe Val Thr Ala Ala Ala Ile Thr  
340 345 350

His Gly Met Asp Glu Leu Tyr Lys  
355 360

<210> SEQ ID NO 25  
 <211> LENGTH: 381  
 <212> TYPE: PRT  
 <213> ORGANISM: *Phytophthora sojae*

<400> SEQUENCE: 25

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val  
1 5 10 15

Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met  
20 25 30

Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile  
35 40 45

Ala Gly Gly Arg Phe Leu Arg Ala His Glu Glu Asp Asp Ala Gly Glu  
50 55 60

Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys  
65 70 75 80

Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr  
85 90 95

Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp  
100 105 110

Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr  
115 120 125

Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser Gly Thr Ala Thr Met Val  
130 135 140

Ser Lys Gly Ala Glu Leu Phe Thr Gly Ile Val Pro Ile Leu Ile Glu  
145 150 155 160

Leu Asn Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly  
165 170 175

Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr  
180 185 190

Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Ser  
195 200 205

Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His  
210 215 220

Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln Glu Arg Thr  
225 230 235 240

Ile Phe Phe Glu Asp Asp Gly Asn Tyr Lys Ser Arg Ala Glu Val Lys  
245 250 255

Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Thr Gly Thr Asp  
260 265 270

Phe Lys Glu Asp Gly Asn Ile Leu Gly Asn Lys Met Glu Tyr Asn Tyr  
275 280 285

Asn Ala His Asn Val Tyr Ile Met Thr Asp Lys Ala Lys Asn Gly Ile  
290 295 300

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Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln  
305 310 315 320

Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val  
325 330 335

Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys  
340 345 350

Asp Pro Asn Glu Lys Arg Asp His Met Ile Tyr Phe Gly Phe Val Thr  
355 360 365

Ala Ala Ala Ile Thr His Gly Met Asp Glu Leu Tyr Lys  
370 375 380

<210> SEQ ID NO 26

<211> LENGTH: 239

<212> TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

<400> SEQUENCE: 26

Met Val Ser Lys Gly Ala Glu Leu Phe Thr Gly Ile Val Pro Ile Leu  
1 5 10 15

Ile Glu Leu Asn Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly  
20 25 30

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile  
35 40 45

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr  
50 55 60

Leu Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys  
65 70 75 80

Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln Glu  
85 90 95

Arg Thr Ile Phe Phe Glu Asp Asp Gly Asn Tyr Lys Ser Arg Ala Glu  
100 105 110

Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Thr Gly  
115 120 125

Thr Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly Asn Lys Met Glu Tyr  
130 135 140

Asn Tyr Asn Ala His Asn Val Tyr Ile Met Thr Asp Lys Ala Lys Asn  
145 150 155 160

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser  
165 170 175

Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly  
180 185 190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu  
195 200 205

Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Tyr Phe Gly Phe  
210 215 220

Val Thr Ala Ala Ala Ile Thr His Gly Met Asp Glu Leu Tyr Lys  
225 230 235

<210> SEQ ID NO 27

<211> LENGTH: 270

<212> TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

<400> SEQUENCE: 27

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Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1           5           10           15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
           20           25           30
Val Ser Lys Gly Ala Glu Leu Phe Thr Gly Ile Val Pro Ile Leu Ile
           35           40           45
Glu Leu Asn Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
           50           55           60
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
65           70           75           80
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu
           85           90           95
Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
           100          105          110
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln Glu Arg
115          120          125
Thr Ile Phe Phe Glu Asp Asp Gly Asn Tyr Lys Ser Arg Ala Glu Val
130          135          140
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Thr Gly Thr
145          150          155
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly Asn Lys Met Glu Tyr Asn
165          170          175
Tyr Asn Ala His Asn Val Tyr Ile Met Thr Asp Lys Ala Lys Asn Gly
180          185          190
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
195          200          205
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
210          215          220
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
225          230          235          240
Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Tyr Phe Gly Phe Val
245          250          255
Thr Ala Ala Ala Ile Thr His Gly Met Asp Glu Leu Tyr Lys
260          265          270

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&lt;210&gt; SEQ ID NO 28

&lt;211&gt; LENGTH: 381

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

&lt;400&gt; SEQUENCE: 28

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Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1           5           10           15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
           20           25           30
Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile
           35           40           45
Ala Gly Gly Ala Ala Ala Ala Ala His Glu Glu Asp Asp Ala Gly Glu
50           55           60
Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys
65           70           75           80
Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr

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85				90				95							
Glu	Lys	Trp	Ala	Lys	Lys	Gly	Tyr	Ser	Leu	Asp	Lys	Ile	Lys	Asn	Trp
			100					105						110	
Leu	Ala	Ile	Ala	Asp	Pro	Lys	Gln	Lys	Gly	Lys	Tyr	Asp	Arg	Ile	Tyr
		115					120					125			
Asn	Gly	Tyr	Thr	Phe	His	Arg	Tyr	Gln	Ser	Gly	Thr	Ala	Thr	Met	Val
		130					135					140			
Ser	Lys	Gly	Ala	Glu	Leu	Phe	Thr	Gly	Ile	Val	Pro	Ile	Leu	Ile	Glu
		145			150					155					160
Leu	Asn	Gly	Asp	Val	Asn	Gly	His	Lys	Phe	Ser	Val	Ser	Gly	Glu	Gly
				165					170					175	
Glu	Gly	Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr	Leu	Lys	Phe	Ile	Cys	Thr
			180					185						190	
Thr	Gly	Lys	Leu	Pro	Val	Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr	Leu	Ser
			195				200					205			
Tyr	Gly	Val	Gln	Cys	Phe	Ser	Arg	Tyr	Pro	Asp	His	Met	Lys	Gln	His
		210					215				220				
Asp	Phe	Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly	Tyr	Ile	Gln	Glu	Arg	Thr
					230					235					240
Ile	Phe	Phe	Glu	Asp	Asp	Gly	Asn	Tyr	Lys	Ser	Arg	Ala	Glu	Val	Lys
				245					250					255	
Phe	Glu	Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile	Glu	Leu	Thr	Gly	Thr	Asp
			260					265						270	
Phe	Lys	Glu	Asp	Gly	Asn	Ile	Leu	Gly	Asn	Lys	Met	Glu	Tyr	Asn	Tyr
			275				280					285			
Asn	Ala	His	Asn	Val	Tyr	Ile	Met	Thr	Asp	Lys	Ala	Lys	Asn	Gly	Ile
			290				295				300				
Lys	Val	Asn	Phe	Lys	Ile	Arg	His	Asn	Ile	Glu	Asp	Gly	Ser	Val	Gln
					310					315					320
Leu	Ala	Asp	His	Tyr	Gln	Gln	Asn	Thr	Pro	Ile	Gly	Asp	Gly	Pro	Val
				325					330					335	
Leu	Leu	Pro	Asp	Asn	His	Tyr	Leu	Ser	Thr	Gln	Ser	Ala	Leu	Ser	Lys
			340						345					350	
Asp	Pro	Asn	Glu	Lys	Arg	Asp	His	Met	Ile	Tyr	Phe	Gly	Phe	Val	Thr
			355				360					365			
Ala	Ala	Ala	Ile	Thr	His	Gly	Met	Asp	Glu	Leu	Tyr	Lys			
			370				375					380			

&lt;210&gt; SEQ ID NO 29

&lt;211&gt; LENGTH: 381

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Phytophthora sojae

&lt;400&gt; SEQUENCE: 29

Met	Arg	Leu	Ser	Phe	Val	Leu	Ser	Leu	Val	Val	Ala	Ile	Gly	Tyr	Val
1				5					10					15	
Val	Thr	Cys	Asn	Ala	Thr	Glu	Tyr	Ser	Asp	Glu	Thr	Asn	Ile	Ala	Met
			20					25					30		
Val	Glu	Ser	Pro	Asp	Leu	Val	Arg	Arg	Ser	Leu	Arg	Asn	Gly	Asp	Ile
			35				40					45			
Ala	Gly	Gly	Arg	Phe	Leu	Arg	Ala	His	Ala	Ala	Ala	Ala	Ala	Gly	Ala
			50				55					60			

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Ala Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys  
65 70 75 80

Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr  
85 90 95

Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp  
100 105 110

Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr  
115 120 125

Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser Gly Thr Ala Thr Met Val  
130 135 140

Ser Lys Gly Ala Glu Leu Phe Thr Gly Ile Val Pro Ile Leu Ile Glu  
145 150 155 160

Leu Asn Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly  
165 170 175

Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr  
180 185 190

Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Ser  
195 200 205

Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His  
210 215 220

Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln Glu Arg Thr  
225 230 235 240

Ile Phe Phe Glu Asp Asp Gly Asn Tyr Lys Ser Arg Ala Glu Val Lys  
245 250 255

Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Thr Gly Thr Asp  
260 265 270

Phe Lys Glu Asp Gly Asn Ile Leu Gly Asn Lys Met Glu Tyr Asn Tyr  
275 280 285

Asn Ala His Asn Val Tyr Ile Met Thr Asp Lys Ala Lys Asn Gly Ile  
290 295 300

Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln  
305 310 315 320

Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val  
325 330 335

Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys  
340 345 350

Asp Pro Asn Glu Lys Arg Asp His Met Ile Tyr Phe Gly Phe Val Thr  
355 360 365

Ala Ala Ala Ile Thr His Gly Met Asp Glu Leu Tyr Lys  
370 375 380

&lt;210&gt; SEQ ID NO 30

&lt;211&gt; LENGTH: 386

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Synthetic Sequence of 9 arginines replacing the RXLR region of secreted AvriB fused to GFP

&lt;400&gt; SEQUENCE: 30

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val  
1 5 10 15

Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met  
20 25 30

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Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile  
           35                                  40                                  45  
 Ala Gly Gly Arg Arg Arg Arg Arg Arg Arg Arg Arg Ala His Glu Glu  
           50                                  55                                  60  
 Asp Asp Ala Gly Glu Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys  
   65                                  70                                  75                                  80  
 Val Ala Ala Lys Lys Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys  
                                   85                                  90  
 Glu Gln Lys Ala Tyr Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp  
                                   100                                  105                                  110  
 Lys Ile Lys Asn Trp Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys  
           115                                  120                                  125  
 Tyr Asp Arg Ile Tyr Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser Gly  
   130                                  135                                  140  
 Thr Ala Thr Met Val Ser Lys Gly Ala Glu Leu Phe Thr Gly Ile Val  
   145                                  150                                  155                                  160  
 Pro Ile Leu Ile Glu Leu Asn Gly Asp Val Asn Gly His Lys Phe Ser  
           165                                  170                                  175  
 Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu  
           180                                  185                                  190  
 Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu  
           195                                  200  
 Val Thr Thr Leu Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp  
   210                                  215                                  220  
 His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr  
   225                                  230                                  235                                  240  
 Ile Gln Glu Arg Thr Ile Phe Phe Glu Asp Asp Gly Asn Tyr Lys Ser  
           245                                  250                                  255  
 Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu  
           260                                  265                                  270  
 Leu Thr Gly Thr Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly Asn Lys  
           275                                  280                                  285  
 Met Glu Tyr Asn Tyr Asn Ala His Asn Val Tyr Ile Met Thr Asp Lys  
   290                                  295                                  300  
 Ala Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu  
   305                                  310                                  315                                  320  
 Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile  
           325                                  330                                  335  
 Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln  
           340                                  345                                  350  
 Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Tyr  
           355                                  360                                  365  
 Phe Gly Phe Val Thr Ala Ala Ala Ile Thr His Gly Met Asp Glu Leu  
   370                                  375                                  380  
 Tyr Lys  
 385

&lt;210&gt; SEQ ID NO 31

&lt;211&gt; LENGTH: 388

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

-continued

<223> OTHER INFORMATION: Synthetic sequence consisting of P. sojae Avrb1 with a known TAT motif from Lentivirus HIV inserted in the position of the second RXLR sequence fused to GFP

<400> SEQUENCE: 31

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val  
1 5 10 15

Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met  
20 25 30

Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile  
35 40 45

Ala Gly Gly Tyr Gly Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala His  
50 55 60

Glu Glu Asp Asp Ala Gly Glu Arg Thr Phe Ser Val Thr Asp Leu Trp  
65 70 75 80

Asn Lys Val Ala Ala Lys Lys Leu Ala Lys Ala Met Leu Ala Asp Pro  
85 90 95

Ser Lys Glu Gln Lys Ala Tyr Glu Lys Trp Ala Lys Lys Gly Tyr Ser  
100 105 110

Leu Asp Lys Ile Lys Asn Trp Leu Ala Ile Ala Asp Pro Lys Gln Lys  
115 120 125

Gly Lys Tyr Asp Arg Ile Tyr Asn Gly Tyr Thr Phe His Arg Tyr Gln  
130 135 140

Ser Gly Thr Ala Thr Met Val Ser Lys Gly Ala Glu Leu Phe Thr Gly  
145 150 155 160

Ile Val Pro Ile Leu Ile Glu Leu Asn Gly Asp Val Asn Gly His Lys  
165 170 175

Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu  
180 185 190

Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro  
195 200 205

Thr Leu Val Thr Thr Leu Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr  
210 215 220

Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu  
225 230 235 240

Gly Tyr Ile Gln Glu Arg Thr Ile Phe Phe Glu Asp Asp Gly Asn Tyr  
245 250 255

Lys Ser Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg  
260 265 270

Ile Glu Leu Thr Gly Thr Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly  
275 280 285

Asn Lys Met Glu Tyr Asn Tyr Asn Ala His Asn Val Tyr Ile Met Thr  
290 295 300

Asp Lys Ala Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn  
305 310 315 320

Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr  
325 330 335

Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser  
340 345 350

Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met  
355 360 365

Ile Tyr Phe Gly Phe Val Thr Ala Ala Ala Ile Thr His Gly Met Asp

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370          375          380
Glu Leu Tyr Lys
385

<210> SEQ ID NO 32
<211> LENGTH: 138
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

<400> SEQUENCE: 32
Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1          5          10          15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
20         25         30
Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile
35         40         45
Ala Gly Gly Arg Phe Leu Arg Ala His Glu Glu Asp Asp Ala Gly Glu
50         55         60
Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys
65         70         75         80
Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr
85         90         95
Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp
100        105        110
Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr
115        120        125
Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser
130        135

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<210> SEQ ID NO 33
<211> LENGTH: 138
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

<400> SEQUENCE: 33
Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1          5          10          15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
20         25         30
Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile
35         40         45
Ala Gly Gly Arg Phe Arg Leu Ala His Glu Glu Asp Asp Ala Gly Glu
50         55         60
Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys
65         70         75         80
Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr
85         90         95
Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp
100        105        110
Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr
115        120        125
Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser
130        135

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<210> SEQ ID NO 34
<211> LENGTH: 138
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

<400> SEQUENCE: 34
Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1           5           10           15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
           20           25           30
Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile
           35           40           45
Ala Gly Gly Phe Arg Leu Arg Ala His Glu Glu Asp Asp Ala Gly Glu
           50           55           60
Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys
65           70           75           80
Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr
           85           90           95
Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp
           100          105          110
Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr
           115          120          125
Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser
           130          135

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<210> SEQ ID NO 35
<211> LENGTH: 138
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

<400> SEQUENCE: 35
Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1           5           10           15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
           20           25           30
Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile
           35           40           45
Ala Gly Gly Arg Phe Ala Arg Ala His Glu Glu Asp Asp Ala Gly Glu
           50           55           60
Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys
65           70           75           80
Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr
           85           90           95
Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp
           100          105          110
Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr
           115          120          125
Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser
           130          135

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<210> SEQ ID NO 36
<211> LENGTH: 138
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

<400> SEQUENCE: 36

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Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1           5           10           15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
                20           25           30
Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile
                35           40           45
Ala Gly Gly Gln Phe Leu Gln Ala His Glu Glu Asp Asp Ala Gly Glu
                50           55           60
Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys
65           70           75           80
Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr
                85           90           95
Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp
                100           105           110
Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr
                115           120           125
Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser
130           135

```

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<210> SEQ ID NO 37
<211> LENGTH: 138
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

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<400> SEQUENCE: 37

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```

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1           5           10           15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
                20           25           30
Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile
                35           40           45
Ala Gly Gly Gln Phe Leu Arg Ala His Glu Glu Asp Asp Ala Gly Glu
                50           55           60
Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys
65           70           75           80
Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr
                85           90           95
Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp
                100           105           110
Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr
                115           120           125
Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser
130           135

```

```

<210> SEQ ID NO 38
<211> LENGTH: 138
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

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<400> SEQUENCE: 38

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```

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1           5           10           15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
                20           25           30

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Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile
    35                                40                                45
Ala Gly Gly Lys Phe Leu Arg Ala His Glu Glu Asp Asp Ala Gly Glu
    50                                55                                60
Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys
    65                                70                                75                                80
Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr
    85                                90                                95
Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp
    100                               105                               110
Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr
    115                               120                               125
Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser
    130                               135

```

```

<210> SEQ ID NO 39
<211> LENGTH: 138
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

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<400> SEQUENCE: 39

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```

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
 1      5      10      15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
 20     25     30
Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile
 35     40     45
Ala Gly Gly Arg Phe Leu Gln Ala His Glu Glu Asp Asp Ala Gly Glu
 50     55     60
Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys
 65     70     75     80
Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr
 85     90     95
Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp
 100    105    110
Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr
 115    120    125
Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser
 130    135

```

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<210> SEQ ID NO 40
<211> LENGTH: 138
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

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<400> SEQUENCE: 40

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Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
 1      5      10      15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
 20     25     30
Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile
 35     40     45
Ala Gly Gly Arg Phe Val Arg Ala His Glu Glu Asp Asp Ala Gly Glu
 50     55     60

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Ala Gly Gly Tyr Gly Arg Lys Lys Lys Arg Arg Gln Arg Arg Arg Ala  
 50 55 60

His Glu Glu Asp Asp Ala Gly Glu Arg Thr Phe Ser Val Thr Asp Leu  
 65 70 75 80

Trp Asn Lys Val Ala Ala Lys Lys Leu Ala Lys Ala Met Leu Ala Asp  
 85 90 95

Pro Ser Lys Glu Gln Lys Ala Tyr Glu Lys Trp Ala Lys Lys Gly Tyr  
 100 105 110

Ser Leu Asp Lys Ile Lys Asn Trp Leu Ala Ile Ala Asp Pro Lys Gln  
 115 120 125

Lys Gly Lys Tyr Asp Arg Ile Tyr Asn Gly Tyr Thr Phe His Arg Tyr  
 130 135 140

Gln Ser  
 145

<210> SEQ ID NO 43  
 <211> LENGTH: 138  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetic sequence consisting of P. sojæ  
 Avr1b with a RXLR motif from Plasmodium falciparum GBP protein  
 inserted where the second RXLR sequence is found.

<400> SEQUENCE: 43

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val  
 1 5 10 15

Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met  
 20 25 30

Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile  
 35 40 45

Ala Gly Gly Arg Phe Leu Arg Ala His Glu Glu Asp Asp Ala Gly Glu  
 50 55 60

Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys  
 65 70 75 80

Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr  
 85 90 95

Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp  
 100 105 110

Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr  
 115 120 125

Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser  
 130 135

<210> SEQ ID NO 44  
 <211> LENGTH: 132  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetic sequence consisting of P. sojæ  
 Avr1b with a RXLRsequence of Plasmodium falciparum HRPII protein  
 inserted where the second RXLR sequence is found.

<400> SEQUENCE: 44

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val  
 1 5 10 15

Val Thr Cys Asn Ala Phe Asn Asn Asn Leu Cys Ser Lys Asn Ala Lys

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                20                25                30
Gly Leu Asn Leu Asn Lys Arg Leu Leu His Glu Thr Gln Ala His Val
   35                40                45
Asp Asp Ala His His Ala His His Val Ala Asp Thr Phe Ser Val Thr
   50                55                60
Asp Leu Trp Asn Lys Val Ala Ala Lys Lys Leu Ala Lys Ala Met Leu
   65                70                75                80
Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr Glu Lys Trp Ala Lys Lys
   85                90                95
Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp Leu Ala Ile Ala Asp Pro
  100                105                110
Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr Asn Gly Tyr Thr Phe His
  115                120                125
Arg Tyr Gln Ser
  130

```

```

<210> SEQ ID NO 45
<211> LENGTH: 135
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic sequence consisting of P. sojae
    Avr1b with a RXLRsequence from Plasmodium falciparum 1615c
    protein inserted where the second RXLR sequence is found.

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<400> SEQUENCE: 45

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```

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
  1                5                10                15
Val Thr Cys Asn Ala Ser Tyr Asn Lys Ile Asn Ser Ser Ser Thr Tyr
  20                25                30
Thr His Ser Arg Ile Leu Lys Gln Leu Glu Phe Ile Thr Leu Glu Glu
  35                40                45
Lys Thr Val Asn Ala Leu Gln Glu Met Leu Asp Asp Ser Val Thr Phe
  50                55                60
Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys Leu Ala Lys
  65                70                75                80
Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr Glu Lys Trp
  85                90                95
Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp Leu Ala Ile
  100               105               110
Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr Asn Gly Tyr
  115               120               125
Thr Phe His Arg Tyr Gln Ser
  130               135

```

```

<210> SEQ ID NO 46
<211> LENGTH: 365
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

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```

<400> SEQUENCE: 46

```

```

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
  1                5                10                15
Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
  20                25                30

```

-continued

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Arg Trp Gly Ser Arg Ser Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala
 35          40          45

Met Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp
 50          55          60

Ile Ala Gly Gly Arg Phe Leu Arg Ala His Glu Glu Asp Asp Ala Gly
 65          70          75          80

Glu Arg Thr Phe Ser Val Thr Asp Glu Phe Arg Ser Thr Met Ser Gly
 85          90          95

Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Ser Met Gly Ser Gly Ile
 100         105         110

Gln Arg Pro Thr Ser Thr Ser Ser Leu Val Ala Ala Ala Ala Thr Met
 115         120         125

Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu
 130         135         140

Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly
 145         150         155         160

Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr
 165         170         175

Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Ser
 180         185         190

Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His
 195         200         205

Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr
 210         215         220

Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys
 225         230         235         240

Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp
 245         250         255

Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr
 260         265         270

Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile
 275         280         285

Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln
 290         295         300

Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val
 305         310         315

Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys
 325         330         335

Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr
 340         345         350

Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
 355         360         365

```

&lt;210&gt; SEQ ID NO 47

&lt;211&gt; LENGTH: 365

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

&lt;400&gt; SEQUENCE: 47

```

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
 1          5          10          15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
 20         25         30

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Arg Trp Gly Ser Arg Ser Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala  
 35 40 45  
 Met Val Glu Ser Pro Asp Leu Val Arg Ala Ala Ala Asn Gly Asp  
 50 55 60  
 Ile Ala Gly Gly Ala Ala Ala Ala Ala His Glu Glu Asp Asp Ala Gly  
 65 70 75 80  
 Glu Arg Thr Phe Ser Val Thr Asp Glu Phe Arg Ser Thr Met Ser Gly  
 85 90 95  
 Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Ser Met Gly Ser Gly Ile  
 100 105 110  
 Gln Arg Pro Thr Ser Thr Ser Ser Leu Val Ala Ala Ala Ala Thr Met  
 115 120 125  
 Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu  
 130 135 140  
 Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly  
 145 150 155 160  
 Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr  
 165 170 175  
 Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Ser  
 180 185 190  
 Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His  
 195 200 205  
 Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr  
 210 215 220  
 Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys  
 225 230 235 240  
 Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp  
 245 250 255  
 Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr  
 260 265 270  
 Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile  
 275 280 285  
 Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln  
 290 295 300  
 Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val  
 305 310 315 320  
 Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys  
 325 330 335  
 Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr  
 340 345 350  
 Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys  
 355 360 365

&lt;210&gt; SEQ ID NO 48

&lt;211&gt; LENGTH: 365

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Phytophthora sojae

&lt;400&gt; SEQUENCE: 48

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
 1 5 10 15  
 Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp

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20				25				30							
Arg	Trp	Gly	Ser	Arg	Ser	Thr	Glu	Tyr	Ser	Asp	Glu	Thr	Asn	Ile	Ala
		35					40					45			
Met	Val	Glu	Ser	Pro	Asp	Leu	Val	Arg	Arg	Ser	Leu	Arg	Asn	Gly	Asp
	50					55					60				
Ile	Ala	Gly	Gly	Arg	Phe	Leu	Arg	Ala	His	Ala	Ala	Ala	Ala	Ala	Gly
65					70					75					80
Ala	Ala	Thr	Phe	Ser	Val	Thr	Asp	Glu	Phe	Arg	Ser	Thr	Met	Ser	Gly
			85							90					95
Tyr	Pro	Tyr	Asp	Val	Pro	Asp	Tyr	Ala	Gly	Ser	Met	Gly	Ser	Gly	Ile
			100						105					110	
Gln	Arg	Pro	Thr	Ser	Thr	Ser	Ser	Leu	Val	Ala	Ala	Ala	Ala	Thr	Met
		115					120					125			
Ser	Lys	Gly	Glu	Glu	Leu	Phe	Thr	Gly	Val	Val	Pro	Ile	Leu	Val	Glu
	130					135					140				
Leu	Asp	Gly	Asp	Val	Asn	Gly	His	Lys	Phe	Ser	Val	Ser	Gly	Glu	Gly
145					150					155					160
Glu	Gly	Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr	Leu	Lys	Phe	Ile	Cys	Thr
				165					170					175	
Thr	Gly	Lys	Leu	Pro	Val	Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr	Phe	Ser
			180					185						190	
Tyr	Gly	Val	Gln	Cys	Phe	Ser	Arg	Tyr	Pro	Asp	His	Met	Lys	Gln	His
		195					200					205			
Asp	Phe	Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly	Tyr	Val	Gln	Glu	Arg	Thr
	210					215					220				
Ile	Phe	Phe	Lys	Asp	Asp	Gly	Asn	Tyr	Lys	Thr	Arg	Ala	Glu	Val	Lys
225					230					235					240
Phe	Glu	Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile	Glu	Leu	Lys	Gly	Ile	Asp
			245					250						255	
Phe	Lys	Glu	Asp	Gly	Asn	Ile	Leu	Gly	His	Lys	Leu	Glu	Tyr	Asn	Tyr
			260					265						270	
Asn	Ser	His	Asn	Val	Tyr	Ile	Met	Ala	Asp	Lys	Gln	Lys	Asn	Gly	Ile
		275					280					285			
Lys	Val	Asn	Phe	Lys	Ile	Arg	His	Asn	Ile	Glu	Asp	Gly	Ser	Val	Gln
	290					295					300				
Leu	Ala	Asp	His	Tyr	Gln	Gln	Asn	Thr	Pro	Ile	Gly	Asp	Gly	Pro	Val
305					310					315				320	
Leu	Leu	Pro	Asp	Asn	His	Tyr	Leu	Ser	Thr	Gln	Ser	Ala	Leu	Ser	Lys
			325					330						335	
Asp	Pro	Asn	Glu	Lys	Arg	Asp	His	Met	Val	Leu	Leu	Glu	Phe	Val	Thr
		340						345						350	
Ala	Ala	Gly	Ile	Thr	His	Gly	Met	Asp	Glu	Leu	Tyr	Lys			
		355					360					365			

&lt;210&gt; SEQ ID NO 49

&lt;211&gt; LENGTH: 31

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Artificial Sequence

&lt;220&gt; FEATURE:

&lt;223&gt; OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

&lt;400&gt; SEQUENCE: 49

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ataagcttga atctggcgtt catctccgac g 31

<210> SEQ ID NO 50  
<211> LENGTH: 30  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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sites to facilitate cloning

<400> SEQUENCE: 50

ttcccgggtg gatgctcaga tgctagcgtc 30

<210> SEQ ID NO 51  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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sites to facilitate cloning

<400> SEQUENCE: 51

ttctcctttt cactctcaeg 20

<210> SEQ ID NO 52  
<211> LENGTH: 21  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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<400> SEQUENCE: 52

agacacaaaa tctgcaactt c 21

<210> SEQ ID NO 53  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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sites to facilitate cloning

<400> SEQUENCE: 53

accttcagcg tgactgacct 20

<210> SEQ ID NO 54  
<211> LENGTH: 19  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 54

gcgattgccca accagttct 19

<210> SEQ ID NO 55  
<211> LENGTH: 20

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<212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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 <400> SEQUENCE: 55  
  
 cgacatccgt aaggacctgt 20  
  
 <210> SEQ ID NO 56  
 <211> LENGTH: 20  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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 sites to facilitate cloning  
  
 <400> SEQUENCE: 56  
  
 ttcgagatcc acatctgctg 20  
  
 <210> SEQ ID NO 57  
 <211> LENGTH: 35  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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 sites to facilitate cloning  
  
 <400> SEQUENCE: 57  
  
 ggggtaccga caacaatgcg tctatctttt gtgct 35  
  
 <210> SEQ ID NO 58  
 <211> LENGTH: 28  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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 sites to facilitate cloning  
  
 <400> SEQUENCE: 58  
  
 ggggtacctc agctctgata ccggtgaa 28  
  
 <210> SEQ ID NO 59  
 <211> LENGTH: 62  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
 amplify genes from genomes or plasmids with various restriction  
 sites to facilitate cloning  
  
 <400> SEQUENCE: 59  
  
 atcgcaactcg agcttttcgca gatcccgggg ggcaatgaga tatgactgag tactccgacg 60  
  
 aa 62  
  
 <210> SEQ ID NO 60  
 <211> LENGTH: 63  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to

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amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 60

atcgcaactcg agcttgcga tcgacagatc cggtcggcat ctacttcagc tctgataccg 60

gtg 63

<210> SEQ ID NO 61

<211> LENGTH: 59

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 61

atcgcaactcg agctttgcga gatcccgagg ggcaatgaga tatgcgtcta tctttgtg 59

<210> SEQ ID NO 62

<211> LENGTH: 40

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 62

tcgtccgtgc tgcagctgct aacggcgaca ttgccggtgg 40

<210> SEQ ID NO 63

<211> LENGTH: 40

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 63

tcgccgttag cagctgcagc acggacgaga tctggagatt 40

<210> SEQ ID NO 64

<211> LENGTH: 40

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 64

ccggtggagc tgcagctgct gctcatgaag aggacgatgc 40

<210> SEQ ID NO 65

<211> LENGTH: 40

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 65

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tcatgagcag cagctgcagc tccaccggca atgtcgccgt 40

<210> SEQ ID NO 66  
 <211> LENGTH: 65  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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 sites to facilitate cloning

<400> SEQUENCE: 66

gctgcagctg ctaacggcga cattgccggt ggagctgcag ctgctgctca tgaagaggac 60

gatgc 65

<210> SEQ ID NO 67  
 <211> LENGTH: 65  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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 sites to facilitate cloning

<400> SEQUENCE: 67

agcagctgca gctccaccgg caatgtcgcc gttagcagct gcagcacgga cgagatctgg 60

agatt 65

<210> SEQ ID NO 68  
 <211> LENGTH: 44  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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 sites to facilitate cloning

<400> SEQUENCE: 68

gctgcagcag ctgcgggggc tgctaccttc agcgtgactg acct 44

<210> SEQ ID NO 69  
 <211> LENGTH: 44  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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 sites to facilitate cloning

<400> SEQUENCE: 69

agcagcccc gcagctgctg cagcatgagc tcgaagaaat cttc 44

<210> SEQ ID NO 70  
 <211> LENGTH: 34  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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 sites to facilitate cloning

<400> SEQUENCE: 70

attccccggg acaacaatgc gactccacta cgtg 34

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<210> SEQ ID NO 71  
<211> LENGTH: 38  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 71  
gtcagtcacg ctgaaggtat cgagaacgcc atgcccat 38

<210> SEQ ID NO 72  
<211> LENGTH: 35  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 72  
attccccgggg acaacaatgg gcctccacaa gggct 35

<210> SEQ ID NO 73  
<211> LENGTH: 36  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 73  
gtcagtcacg ctgaagggtta ggtggtgtag tccgac 36

<210> SEQ ID NO 74  
<211> LENGTH: 35  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 74  
aaccggggac aacaatgacc ttcagcgtga ctgac 35

<210> SEQ ID NO 75  
<211> LENGTH: 28  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 75  
ggaggaattc gctggctggt ggcaggat 28

<210> SEQ ID NO 76  
<211> LENGTH: 23  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 76  
 gtattggcta gagaagcttg cca 23

<210> SEQ ID NO 77  
 <211> LENGTH: 57  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 77  
 agaaactoga gcttgtcgat cgacagatcc ggtcggcagg tacctcagct ctgatac 57

<210> SEQ ID NO 78  
 <211> LENGTH: 24  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 78  
 gaagatttcg acttgctcat gaag 24

<210> SEQ ID NO 79  
 <211> LENGTH: 24  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 79  
 cttcatgagc aagtcgaaat cttc 24

<210> SEQ ID NO 80  
 <211> LENGTH: 26  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 80  
 tgccgggtgga tttagacttc gagctc 26

<210> SEQ ID NO 81  
 <211> LENGTH: 26  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 81

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gagctcgaag tctaaatcca cggca 26

<210> SEQ ID NO 82  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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sites to facilitate cloning

<400> SEQUENCE: 82

gaagatttgc acgagctcat 20

<210> SEQ ID NO 83  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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<400> SEQUENCE: 83

atgagctcgt gcaaattcttc 20

<210> SEQ ID NO 84  
<211> LENGTH: 28  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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<400> SEQUENCE: 84

ggtagacagt ttcttcaagc tcatgaag 28

<210> SEQ ID NO 85  
<211> LENGTH: 28  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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sites to facilitate cloning

<400> SEQUENCE: 85

agcttgaaga aactgtccac cggcaatg 28

<210> SEQ ID NO 86  
<211> LENGTH: 52  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 86

cttccaccgg taccagagcg gtaccgccac catggtgagc aagggcgccg ag 52

<210> SEQ ID NO 87  
<211> LENGTH: 29

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<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 87

aaggtacctc acttgtagacag ctcatccat 29

<210> SEQ ID NO 88  
<211> LENGTH: 33  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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sites to facilitate cloning

<400> SEQUENCE: 88

tcatgagctc gaagaaatct tccgccggca atg 33

<210> SEQ ID NO 89  
<211> LENGTH: 27  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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sites to facilitate cloning

<400> SEQUENCE: 89

cattgccggc ggaagatttc ttcaagc 27

<210> SEQ ID NO 90  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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<400> SEQUENCE: 90

cattgccggc ggacaatttc 20

<210> SEQ ID NO 91  
<211> LENGTH: 23  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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<400> SEQUENCE: 91

cattgccggc ggaagatttg ttc 23

<210> SEQ ID NO 92  
<211> LENGTH: 23  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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sites to facilitate cloning

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<400> SEQUENCE: 92

cattgccggc ggaaagtttc ttc 23

<210> SEQ ID NO 93

<211> LENGTH: 37

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 93

cgtcgacgtc ggcgacggc tcatgaagag gacgatg 37

<210> SEQ ID NO 94

<211> LENGTH: 39

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 94

cattgccggc ggacgacggc gacgtcgacg tcggcgacg 39

<210> SEQ ID NO 95

<211> LENGTH: 44

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 95

taagaaaacg cgtcagcgac gtcgagctca tgaagaggac gatg 44

<210> SEQ ID NO 96

<211> LENGTH: 38

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 96

cattgccggc ggatatggac gtaagaaacg ccgtcagc 38

<210> SEQ ID NO 97

<211> LENGTH: 56

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 97

gaaggagaag actacactcc ggaaaagcaa gcaaaagacc ttcagcgtga ctgacc 56

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<210> SEQ ID NO 98  
<211> LENGTH: 55  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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sites to facilitate cloning

<400> SEQUENCE: 98

ctcgtatact ggcagagggc gaagatacct gcgcaaggaa ggagaagact acact 55

<210> SEQ ID NO 99  
<211> LENGTH: 52  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
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sites to facilitate cloning

<400> SEQUENCE: 99

gtatgagaaa gcggtagatt acggctttcg agagtctcgt atactggcag ag 52

<210> SEQ ID NO 100  
<211> LENGTH: 40  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 100

ctaccgcttt ctcatactta tctgcgttgc aggtcaccgac 40

<210> SEQ ID NO 101  
<211> LENGTH: 56  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 101

tgtcgacgat ggcaccatg cacaccatgt tgcagatacc ttcagcgtga ctgacc 56

<210> SEQ ID NO 102  
<211> LENGTH: 55  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 102

aacctcaaca agagactggt gcacgagaca caagcacatg tcgacgatgc gcacc 55

<210> SEQ ID NO 103  
<211> LENGTH: 53  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to

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amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 103

acaataacct gtgtagtaag aatgctaaag gcttgaacct caacaagaga ctg 53

<210> SEQ ID NO 104

<211> LENGTH: 39

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 104

actacacagg ttattgtaa atgcgttgca ggtcacgac 39

<210> SEQ ID NO 105

<211> LENGTH: 57

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 105

cagtcaatgc attacaagaa atgtagatg atagtgtcac ctcagcgtg actgacc 57

<210> SEQ ID NO 106

<211> LENGTH: 55

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 106

ctcaagcagt tggagtcat cacattggaa gagaagacag tcaatgcatt acaag 55

<210> SEQ ID NO 107

<211> LENGTH: 57

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 107

aagatcaact cgtcatctac ttatacacac agtagaatac tcaagcagtt ggagttc 57

<210> SEQ ID NO 108

<211> LENGTH: 45

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 108

agatgacgag ttgatcttgt tgtaacttgc gttgcagtc acgac 45

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<210> SEQ ID NO 109  
 <211> LENGTH: 29  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 109

gatctagatc tgtggaatct ccagatctc 29

<210> SEQ ID NO 110  
 <211> LENGTH: 39  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 110

gtcatatgga tagccggaca tgtcagtcac gctgaaggt 39

<210> SEQ ID NO 111  
 <211> LENGTH: 47  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 111

gatcccatgg agccagcata gtctgggacg tcatatggat agccgga 47

<210> SEQ ID NO 112  
 <211> LENGTH: 324  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetic construct fusing 9 arginine residues to a linker sequence to humanized GFP

<400> SEQUENCE: 112

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
 1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
 20 25 30

Arg Trp Gly Ser Arg Ser Arg Arg Arg Arg Arg Arg Arg Arg Glu  
 35 40 45

Phe Arg Ser Thr Met Ser Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala  
 50 55 60

Gly Ser Met Gly Ser Gly Ile Gln Arg Pro Thr Ser Thr Ser Ser Leu  
 65 70 75 80

Val Ala Ala Ala Ala Thr Met Ser Lys Gly Glu Glu Leu Phe Thr Gly  
 85 90 95

Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys  
 100 105 110

Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu



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145		150		155		160
Gly	Lys	Leu	Thr	Leu	Lys	Val
		165		170		175
Pro	Trp	Pro	Thr	Leu	Val	Thr
		180		185		190
Ser	Arg	Tyr	Pro	Asp	His	Met
		195		200		205
Met	Pro	Glu	Gly	Tyr	Val	Gln
		210		215		220
Gly	Asn	Tyr	Lys	Thr	Arg	Ala
		225		230		235
Val	Asn	Arg	Ile	Glu	Leu	Lys
		245		250		255
Ile	Leu	Gly	His	Lys	Leu	Glu
		260		265		270
Ile	Met	Ala	Asp	Lys	Gln	Lys
		275		280		285
Arg	His	Asn	Ile	Glu	Asp	Gly
		290		295		300
Gln	Asn	Thr	Pro	Ile	Gly	Asp
		305		310		315
Tyr	Leu	Ser	Thr	Gln	Ser	Ala
		325		330		335
Asp	His	Met	Val	Leu	Leu	Glu
		340		345		350
Gly	Met	Asp	Glu	Leu	Tyr	Lys
		355				

&lt;210&gt; SEQ ID NO 114

&lt;211&gt; LENGTH: 359

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

&lt;400&gt; SEQUENCE: 114

Met	Arg	Gly	Ser	His	His	His	His	His	His	Gly	Met	Ala	Ser	Met	Thr
1				5					10					15	
Gly	Gly	Gln	Gln	Met	Gly	Arg	Asp	Leu	Tyr	Asp	Asp	Asp	Asp	Lys	Asp
		20						25						30	
Arg	Trp	Gly	Ser	Arg	Ser	Thr	Arg	Val	Pro	Asp	Asp	Ala	Asn	Leu	Gln
		35				40						45			
Ser	Val	Asn	Ala	Pro	Val	Gln	Thr	Val	Thr	Arg	Ser	Arg	Ala	Ala	Ala
		50				55					60				
Ala	Thr	Ala	Asp	Thr	Asp	Ile	Val	Tyr	Glu	Pro	Lys	Val	His	Asn	Pro
		65			70				75					80	
Gly	Lys	Glu	Phe	Arg	Ser	Thr	Met	Ser	Gly	Tyr	Pro	Tyr	Asp	Val	Pro
		85							90					95	
Asp	Tyr	Ala	Gly	Ser	Met	Gly	Ser	Gly	Ile	Gln	Arg	Pro	Thr	Ser	Thr
		100						105						110	
Ser	Ser	Leu	Val	Ala	Ala	Ala	Ala	Thr	Met	Ser	Lys	Gly	Glu	Glu	Leu
		115					120					125			
Phe	Thr	Gly	Val	Val	Pro	Ile	Leu	Val	Glu	Leu	Asp	Gly	Asp	Val	Asn
		130				135					140				

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Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr  
 145 150 155 160  
 Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val  
 165 170 175  
 Pro Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe  
 180 185 190  
 Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala  
 195 200 205  
 Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp  
 210 215 220  
 Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu  
 225 230 235 240  
 Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn  
 245 250 255  
 Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr  
 260 265 270  
 Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile  
 275 280 285  
 Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln  
 290 295 300  
 Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His  
 305 310 315 320  
 Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg  
 325 330 335  
 Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His  
 340 345 350  
 Gly Met Asp Glu Leu Tyr Lys  
 355

&lt;210&gt; SEQ ID NO 115

&lt;211&gt; LENGTH: 359

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

&lt;400&gt; SEQUENCE: 115

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
 1 5 10 15  
 Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
 20 25 30  
 Arg Trp Gly Ser Arg Ser Thr Arg Val Pro Asp Asp Ala Asn Leu Gln  
 35 40 45  
 Ser Val Asn Ala Pro Val Gln Thr Val Thr Arg Ser Arg Arg Phe Leu  
 50 55 60  
 Arg Thr Ala Ala Thr Ala Ile Val Tyr Ala Pro Ala Val His Asn Pro  
 65 70 75 80  
 Gly Lys Glu Phe Arg Ser Thr Met Ser Gly Tyr Pro Tyr Asp Val Pro  
 85 90 95  
 Asp Tyr Ala Gly Ser Met Gly Ser Gly Ile Gln Arg Pro Thr Ser Thr  
 100 105 110  
 Ser Ser Leu Val Ala Ala Ala Ala Thr Met Ser Lys Gly Glu Glu Leu  
 115 120 125  
 Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn  
 130 135 140

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Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr  
 145 150 155 160  
 Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val  
 165 170 175  
 Pro Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe  
 180 185 190  
 Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala  
 195 200 205  
 Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp  
 210 215 220  
 Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu  
 225 230 235 240  
 Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn  
 245 250 255  
 Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr  
 260 265 270  
 Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile  
 275 280 285  
 Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln  
 290 295 300  
 Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His  
 305 310 315 320  
 Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg  
 325 330 335  
 Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His  
 340 345 350  
 Gly Met Asp Glu Leu Tyr Lys  
 355

&lt;210&gt; SEQ ID NO 116

&lt;211&gt; LENGTH: 403

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

&lt;400&gt; SEQUENCE: 116

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
 1 5 10 15  
 Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
 20 25 30  
 Arg Trp Gly Ser Arg Ser Leu Thr Cys Ala Thr Ser Glu Gln Gln Thr  
 35 40 45  
 Arg Pro Glu Leu Cys Phe Phe Phe Ser Val Arg Ser Ser Trp Pro Ser  
 50 55 60  
 Thr Ile Ser Asp Gly Ala Cys Leu Ala Leu Val Ser Ala Glu Gln Gly  
 65 70 75 80  
 Ala Thr Ala Gly Arg Asn Thr Leu Ser Leu Arg Ser Met Met Ala Thr  
 85 90 95  
 Glu Asp Met Ala Thr Ser Thr Arg Ser Leu Arg Ser Gln Ala Thr Asn  
 100 105 110  
 Val Asp Asp Asp Ala Asn Val Ser Ile Glu Asn Arg Gly Met Glu Phe  
 115 120 125  
 Arg Ser Thr Met Ser Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly





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35					40					45					
Arg	Pro	Glu	Leu	Cys	Phe	Phe	Phe	Ser	Val	Arg	Ser	Ser	Trp	Pro	Ser
50					55					60					
Thr	Ile	Ser	Asp	Gly	Ala	Cys	Leu	Ala	Leu	Val	Ser	Ala	Glu	Gln	Gly
65					70					75					80
Ala	Thr	Ala	Gly	Arg	Asn	Thr	Leu	Ser	Leu	Arg	Ser	Met	Met	Ala	Thr
				85					90					95	
Glu	Asp	Met	Ala	Thr	Ser	Thr	Arg	Ser	Leu	Arg	Ser	Gln	Ala	Thr	Asn
			100					105					110		
Val	Ala	Ala	Ala	Ala	Asn	Val	Ser	Ile	Ala	Asn	Ala	Gly	Met	Glu	Phe
			115				120					125			
Arg	Ser	Thr	Met	Ser	Gly	Tyr	Pro	Tyr	Asp	Val	Pro	Asp	Tyr	Ala	Gly
			130			135					140				
Ser	Met	Gly	Ser	Gly	Ile	Gln	Arg	Pro	Thr	Ser	Thr	Ser	Ser	Leu	Val
				150						155					160
Ala	Ala	Ala	Ala	Thr	Met	Ser	Lys	Gly	Glu	Glu	Leu	Phe	Thr	Gly	Val
				165					170					175	
Val	Pro	Ile	Leu	Val	Glu	Leu	Asp	Gly	Asp	Val	Asn	Gly	His	Lys	Phe
			180					185					190		
Ser	Val	Ser	Gly	Glu	Gly	Glu	Gly	Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr
			195				200					205			
Leu	Lys	Phe	Ile	Cys	Thr	Thr	Gly	Lys	Leu	Pro	Val	Pro	Trp	Pro	Thr
					210		215				220				
Leu	Val	Thr	Thr	Phe	Ser	Tyr	Gly	Val	Gln	Cys	Phe	Ser	Arg	Tyr	Pro
				230					235					240	
Asp	His	Met	Lys	Gln	His	Asp	Phe	Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly
				245					250					255	
Tyr	Val	Gln	Glu	Arg	Thr	Ile	Phe	Phe	Lys	Asp	Asp	Gly	Asn	Tyr	Lys
			260						265				270		
Thr	Arg	Ala	Glu	Val	Lys	Phe	Glu	Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile
			275				280					285			
Glu	Leu	Lys	Gly	Ile	Asp	Phe	Lys	Glu	Asp	Gly	Asn	Ile	Leu	Gly	His
				290		295					300				
Lys	Leu	Glu	Tyr	Asn	Tyr	Asn	Ser	His	Asn	Val	Tyr	Ile	Met	Ala	Asp
				310						315				320	
Lys	Gln	Lys	Asn	Gly	Ile	Lys	Val	Asn	Phe	Lys	Ile	Arg	His	Asn	Ile
				325					330					335	
Glu	Asp	Gly	Ser	Val	Gln	Leu	Ala	Asp	His	Tyr	Gln	Gln	Asn	Thr	Pro
				340				345					350		
Ile	Gly	Asp	Gly	Pro	Val	Leu	Leu	Pro	Asp	Asn	His	Tyr	Leu	Ser	Thr
			355				360					365			
Gln	Ser	Ala	Leu	Ser	Lys	Asp	Pro	Asn	Glu	Lys	Arg	Asp	His	Met	Val
				370		375					380				
Leu	Leu	Glu	Phe	Val	Thr	Ala	Ala	Gly	Ile	Thr	His	Gly	Met	Asp	Glu
				385		390				395					400
Leu	Tyr	Lys													

&lt;210&gt; SEQ ID NO 119

&lt;211&gt; LENGTH: 359

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Melampsora lini

-continued

&lt;400&gt; SEQUENCE: 119

```

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1      5      10      15
Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
20      25      30
Arg Trp Gly Ser Arg Ser Met Glu His Val Pro Ala Glu Leu Thr Arg
35      40      45
Val Ser Glu Gly Tyr Thr Arg Phe Tyr Arg Ser Pro Thr Ala Ser Val
50      55
Ile Leu Ser Gly Leu Val Lys Val Lys Trp Asp Asn Glu Gln Met Thr
65      70      75      80
Met Pro Glu Phe Arg Ser Thr Met Ser Gly Tyr Pro Tyr Asp Val Pro
85      90      95
Asp Tyr Ala Gly Ser Met Gly Ser Gly Ile Gln Arg Pro Thr Ser Thr
100     105     110
Ser Ser Leu Val Ala Ala Ala Ala Thr Met Ser Lys Gly Glu Glu Leu
115     120     125
Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn
130     135     140
Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr
145     150     155     160
Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val
165     170     175
Pro Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe
180     185     190
Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala
195     200     205
Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp
210     215     220
Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu
225     230     235     240
Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn
245     250     255
Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr
260     265     270
Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile
275     280     285
Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln
290     295     300
Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His
305     310     315     320
Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg
325     330     335
Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His
340     345     350
Gly Met Asp Glu Leu Tyr Lys
355

```

&lt;210&gt; SEQ ID NO 120

&lt;211&gt; LENGTH: 359

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Melampsora lini

-continued

&lt;400&gt; SEQUENCE: 120

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
 1 5 10 15  
 Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Lys Asp  
 20 25 30  
 Arg Trp Gly Ser Arg Ser Met Glu His Val Pro Ala Glu Leu Thr Arg  
 35 40 45  
 Val Ser Glu Gly Tyr Thr Ala Ala Ala Ala Ser Pro Thr Ala Ser Val  
 50 55 60  
 Ile Leu Ser Gly Leu Val Lys Val Lys Trp Ala Asn Ala Gln Met Thr  
 65 70 75 80  
 Met Pro Glu Phe Arg Ser Thr Met Ser Gly Tyr Pro Tyr Asp Val Pro  
 85 90 95  
 Asp Tyr Ala Gly Ser Met Gly Ser Gly Ile Gln Arg Pro Thr Ser Thr  
 100 105 110  
 Ser Ser Leu Val Ala Ala Ala Ala Thr Met Ser Lys Gly Glu Glu Leu  
 115 120 125  
 Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn  
 130 135 140  
 Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr  
 145 150 155 160  
 Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val  
 165 170 175  
 Pro Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe  
 180 185 190  
 Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala  
 195 200 205  
 Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp  
 210 215 220  
 Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu  
 225 230 235 240  
 Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn  
 245 250 255  
 Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr  
 260 265 270  
 Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile  
 275 280 285  
 Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln  
 290 295 300  
 Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His  
 305 310 315 320  
 Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg  
 325 330 335  
 Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His  
 340 345 350  
 Gly Met Asp Glu Leu Tyr Lys  
 355

&lt;210&gt; SEQ ID NO 121

&lt;211&gt; LENGTH: 353

&lt;212&gt; TYPE: PRT

-continued

&lt;213&gt; ORGANISM: Plasmodium falciparum

&lt;400&gt; SEQUENCE: 121

```

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1          5          10
Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
20          25          30
Arg Trp Gly Ser Arg Ser Asp Lys Tyr Glu Lys Ala Val Asp Tyr Gly
35          40          45
Phe Arg Glu Ser Arg Ile Leu Ala Glu Gly Glu Asp Thr Cys Ala Arg
50          55          60
Lys Glu Lys Thr Thr Leu Arg Lys Ser Lys Gln Lys Glu Phe Arg Ser
65          70          75
Thr Met Ser Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Ser Met
85          90          95
Gly Ser Gly Ile Gln Arg Pro Thr Ser Thr Ser Ser Leu Val Ala Ala
100         105         110
Ala Ala Thr Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
115         120         125
Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
130         135         140
Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
145         150         155
Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
165         170         175
Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
180         185         190
Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
195         200         205
Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg
210         215         220
Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
225         230         235
Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu
245         250         255
Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln
260         265         270
Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp
275         280         285
Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
290         295         300
Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
305         310         315
Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
325         330         335
Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr
340         345         350

```

Lys

&lt;210&gt; SEQ ID NO 122

&lt;211&gt; LENGTH: 353

&lt;212&gt; TYPE: PRT

-continued

<213> ORGANISM: *Phytophthora sojae*

&lt;400&gt; SEQUENCE: 122

```

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1          5          10
Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
20          25          30
Arg Trp Gly Ser Arg Ser Asp Lys Tyr Glu Lys Ala Val Asp Tyr Gly
35          40          45
Phe Arg Glu Ser Ala Ala Ala Ala Ala Gly Glu Asp Thr Cys Ala Arg
50          55          60
Lys Glu Lys Thr Thr Leu Arg Lys Ser Lys Gln Lys Glu Phe Arg Ser
65          70          75          80
Thr Met Ser Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Ser Met
85          90          95
Gly Ser Gly Ile Gln Arg Pro Thr Ser Thr Ser Ser Leu Val Ala Ala
100         105         110
Ala Ala Thr Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
115         120         125
Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
130         135         140
Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
145         150         155         160
Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
165         170         175
Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
180         185         190
Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
195         200         205
Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg
210         215         220
Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
225         230         235         240
Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu
245         250         255
Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln
260         265         270
Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp
275         280         285
Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
290         295         300
Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
305         310         315         320
Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
325         330         335
Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr
340         345         350

```

Lys

&lt;210&gt; SEQ ID NO 123

&lt;211&gt; LENGTH: 353

&lt;212&gt; TYPE: PRT

-continued

&lt;213&gt; ORGANISM: Plasmodium falciparum

&lt;400&gt; SEQUENCE: 123

```

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1          5          10
Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
20          25          30
Arg Trp Gly Ser Arg Ser Phe Asn Asn Asn Leu Cys Ser Lys Asn Ala
35          40          45
Lys Gly Leu Asn Leu Asn Lys Arg Leu Leu His Glu Thr Gln Ala His
50          55          60
Val Asp Asp Ala His His Ala His His Val Ala Asp Glu Phe Arg Ser
65          70          75          80
Thr Met Ser Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Ser Met
85          90          95
Gly Ser Gly Ile Gln Arg Pro Thr Ser Thr Ser Ser Leu Val Ala Ala
100         105         110
Ala Ala Thr Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
115         120         125
Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
130         135         140
Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
145         150         155         160
Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
165         170         175
Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
180         185         190
Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
195         200         205
Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg
210         215         220
Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
225         230         235         240
Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu
245         250         255
Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln
260         265         270
Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp
275         280         285
Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
290         295         300
Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
305         310         315         320
Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
325         330         335
Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr
340         345         350

```

Lys

&lt;210&gt; SEQ ID NO 124

&lt;211&gt; LENGTH: 353

&lt;212&gt; TYPE: PRT

-continued

&lt;213&gt; ORGANISM: Plasmodium falciparum

&lt;400&gt; SEQUENCE: 124

```

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1          5          10
Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
20          25          30
Arg Trp Gly Ser Arg Ser Phe Asn Asn Asn Leu Cys Ser Lys Asn Ala
35          40          45
Lys Gly Leu Asn Leu Asn Lys Ala Ala Ala Ala Ala Thr Gln Ala His
50          55          60
Val Asp Asp Ala His His Ala His His Val Ala Asp Glu Phe Arg Ser
65          70          75
Thr Met Ser Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Ser Met
85          90          95
Gly Ser Gly Ile Gln Arg Pro Thr Ser Thr Ser Ser Leu Val Ala Ala
100         105         110
Ala Ala Thr Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
115         120         125
Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
130         135         140
Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
145         150         155
Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
165         170         175
Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
180         185         190
Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
195         200         205
Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg
210         215         220
Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
225         230         235
Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu
245         250         255
Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln
260         265         270
Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp
275         280         285
Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
290         295         300
Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
305         310         315
Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
325         330         335
Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr
340         345         350

```

Lys

&lt;210&gt; SEQ ID NO 125

&lt;211&gt; LENGTH: 356

&lt;212&gt; TYPE: PRT

-continued

&lt;213&gt; ORGANISM: Plasmodium falciparum

&lt;400&gt; SEQUENCE: 125

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
 1 5 10 15  
 Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
 20 25 30  
 Arg Trp Gly Ser Arg Ser Ser Tyr Asn Lys Ile Asn Ser Ser Ser Thr  
 35 40 45  
 Tyr Thr His Ser Arg Ile Leu Lys Gln Leu Glu Phe Ile Thr Leu Glu  
 50 55 60  
 Glu Lys Thr Val Asn Ala Leu Gln Glu Met Leu Asp Asp Ser Val Glu  
 65 70 75 80  
 Phe Arg Ser Thr Met Ser Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala  
 85 90 95  
 Gly Ser Met Gly Ser Gly Ile Gln Arg Pro Thr Ser Thr Ser Ser Leu  
 100 105 110  
 Val Ala Ala Ala Ala Thr Met Ser Lys Gly Glu Glu Leu Phe Thr Gly  
 115 120 125  
 Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys  
 130 135 140  
 Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu  
 145 150 155 160  
 Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro  
 165 170 175  
 Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr  
 180 185 190  
 Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu  
 195 200 205  
 Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr  
 210 215 220  
 Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg  
 225 230 235 240  
 Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly  
 245 250 255  
 His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala  
 260 265 270  
 Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn  
 275 280 285  
 Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr  
 290 295 300  
 Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser  
 305 310 315 320  
 Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met  
 325 330 335  
 Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp  
 340 345 350  
 Glu Leu Tyr Lys  
 355

&lt;210&gt; SEQ ID NO 126

&lt;211&gt; LENGTH: 356

-continued

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Plasmodium falciparum

&lt;400&gt; SEQUENCE: 126

```

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1      5      10      15
Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Lys Asp
20      25      30
Arg Trp Gly Ser Arg Ser Ser Tyr Asn Lys Ile Asn Ser Ser Ser Thr
35      40      45
Tyr Thr His Ser Ala Ala Ala Ala Leu Glu Phe Ile Thr Leu Glu
50      55      60
Glu Lys Thr Val Asn Ala Leu Gln Glu Met Leu Asp Asp Ser Val Glu
65      70      75      80
Phe Arg Ser Thr Met Ser Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala
85      90      95
Gly Ser Met Gly Ser Gly Ile Gln Arg Pro Thr Ser Thr Ser Ser Leu
100     105     110
Val Ala Ala Ala Ala Thr Met Ser Lys Gly Glu Glu Leu Phe Thr Gly
115     120     125
Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys
130     135     140
Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu
145     150     155     160
Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro
165     170     175
Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr
180     185     190
Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu
195     200     205
Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr
210     215     220
Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg
225     230     235     240
Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly
245     250     255
His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala
260     265     270
Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn
275     280     285
Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr
290     295     300
Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser
305     310     315     320
Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met
325     330     335
Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp
340     345     350
Glu Leu Tyr Lys
355

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&lt;210&gt; SEQ ID NO 127

-continued

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<211> LENGTH: 485
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

<400> SEQUENCE: 127

Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro
1          5          10          15
Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
20          25          30
Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
35          40          45
Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
50          55          60
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65          70          75          80
Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
85          90          95
Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
100         105         110
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
115         120         125
Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
130         135         140
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145         150         155         160
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
165         170         175
Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
180         185         190
Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
195         200         205
Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
210         215         220
Gly Ser Leu Thr Cys Ala Thr Ser Glu Gln Gln Thr Arg Pro Glu Leu
225         230         235         240
Cys Phe Phe Phe Ser Val Arg Ser Ser Trp Pro Ser Thr Ile Ser Asp
245         250         255
Gly Ala Cys Leu Ala Leu Val Ser Ala Glu Gln Gly Ala Thr Ala Gly
260         265         270
Arg Asn Thr Leu Ser Leu Arg Ser Met Met Ala Thr Glu Asp Met Ala
275         280         285
Thr Ser Thr Arg Ser Leu Arg Ser Gln Ala Thr Asn Val Asp Asp Asp
290         295         300
Ala Asn Val Ser Ile Glu Asn Arg Gly Met Asn Pro Ser Val Leu Thr
305         310         315         320
Lys Leu Gly Glu Phe Ala Ser Thr Leu Thr Ala Gly Asn Thr Ala Asn
325         330         335
Lys Leu Trp Leu Met Ala Asp Val Asp Pro Lys Ser Ala Phe Lys Leu
340         345         350
Leu Gly Leu Asp Met Pro Gly Val Arg Phe Ile Asp Asn Pro Lys Met
355         360         365
Leu Gln Trp Leu Lys Phe Thr Lys Ala Tyr Leu Asp Met Lys Lys Ser

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370          375          380
Gly Phe Gly Glu Thr Ser Ala His Ala Leu Leu Tyr Glu Lys Ile Gly
385          390          395          400
Gly Pro Asp Leu Ser Leu Leu Leu Leu Ser Leu Lys Asp Ala Pro Asp
          405          410          415
Ala Asn Ser Leu Val Gln Lys Leu Thr Asn Ser Gln Phe Gly Met Trp
          420          425          430
His Asp Ala Arg Ile Glu Pro Glu Gln Leu Ala Gln Thr Val Phe Lys
          435          440          445
Ile Gln Asp Val Arg Lys Leu Pro Lys Asn Asp Pro Lys Leu Gln Val
          450          455          460
Ile Asp Asp Tyr Ala Lys Tyr His Arg Lys His Arg Lys Phe Leu Asn
          465          470          475          480
Ser Ile Met Ile Ile
          485

<210> SEQ ID NO 128
<211> LENGTH: 485
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

<400> SEQUENCE: 128
Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro
1          5          10          15
Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
          20          25          30
Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
          35          40          45
Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
          50          55          60
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
          65          70          75          80
Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
          85          90          95
Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
          100          105          110
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
          115          120          125
Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
          130          135          140
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
          145          150          155          160
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
          165          170          175
Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
          180          185          190
Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
          195          200          205
Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
          210          215          220
Gly Ser Leu Thr Cys Ala Thr Ser Glu Gln Gln Thr Arg Pro Glu Leu
          225          230          235          240

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Cys Phe Phe Phe Ser Val Arg Ser Ser Trp Pro Ser Thr Ile Ser Asp  
 245 250 255  
 Gly Ala Cys Leu Ala Leu Val Ser Ala Glu Gln Gly Ala Thr Ala Gly  
 260 265 270  
 Arg Asn Thr Leu Ser Leu Arg Ser Met Met Ala Thr Glu Asp Met Ala  
 275 280 285  
 Thr Ser Thr Ala Ala Ala Ala Ser Gln Ala Thr Asn Val Asp Asp Asp  
 290 295 300  
 Ala Asn Val Ser Ile Glu Asn Arg Gly Met Asn Pro Ser Val Leu Thr  
 305 310 315 320  
 Lys Leu Gly Glu Phe Ala Ser Thr Leu Thr Ala Gly Asn Thr Ala Asn  
 325 330 335  
 Lys Leu Trp Leu Met Ala Asp Val Asp Pro Lys Ser Ala Phe Lys Leu  
 340 345 350  
 Leu Gly Leu Asp Met Pro Gly Val Arg Phe Ile Asp Asn Pro Lys Met  
 355 360 365  
 Leu Gln Trp Leu Lys Phe Thr Lys Ala Tyr Leu Asp Met Lys Lys Ser  
 370 375 380  
 Gly Phe Gly Glu Thr Ser Ala His Ala Leu Leu Tyr Glu Lys Ile Gly  
 385 390 395 400  
 Gly Pro Asp Leu Ser Leu Leu Leu Leu Ser Leu Lys Asp Ala Pro Asp  
 405 410 415  
 Ala Asn Ser Leu Val Gln Lys Leu Thr Asn Ser Gln Phe Gly Met Trp  
 420 425 430  
 His Asp Ala Arg Ile Glu Pro Glu Gln Leu Ala Gln Thr Val Phe Lys  
 435 440 445  
 Ile Gln Asp Val Arg Lys Leu Pro Lys Asn Asp Pro Lys Leu Gln Val  
 450 455 460  
 Ile Asp Asp Tyr Ala Lys Tyr His Arg Lys His Arg Lys Phe Leu Asn  
 465 470 475 480  
 Ser Ile Met Ile Ile  
 485

&lt;210&gt; SEQ ID NO 129

&lt;211&gt; LENGTH: 342

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

&lt;400&gt; SEQUENCE: 129

Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro  
 1 5 10 15  
 Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu  
 20 25 30  
 Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu  
 35 40 45  
 Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys  
 50 55 60  
 Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn  
 65 70 75 80  
 Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu  
 85 90 95  
 Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser  
 100 105 110



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115				120				125							
Met	Leu	Lys	Met	Phe	Glu	Asp	Arg	Leu	Cys	His	Lys	Thr	Tyr	Leu	Asn
130						135					140				
Gly	Asp	His	Val	Thr	His	Pro	Asp	Phe	Met	Leu	Tyr	Asp	Ala	Leu	Asp
145					150					155					160
Val	Val	Leu	Tyr	Met	Asp	Pro	Met	Cys	Leu	Asp	Ala	Phe	Pro	Lys	Leu
				165					170						175
Val	Cys	Phe	Lys	Lys	Arg	Ile	Glu	Ala	Ile	Pro	Gln	Ile	Asp	Lys	Tyr
			180					185					190		
Leu	Lys	Ser	Ser	Lys	Tyr	Ile	Ala	Trp	Pro	Leu	Gln	Gly	Trp	Gln	Ala
			195				200					205			
Thr	Phe	Gly	Gly	Gly	Asp	His	Pro	Pro	Lys	Ser	Asp	Leu	Val	Pro	Arg
			210			215					220				
Gly	Ser	Thr	Arg	Val	Pro	Asp	Asp	Ala	Asn	Leu	Gln	Ser	Val	Asn	Ala
225					230					235					240
Pro	Val	Gln	Thr	Val	Thr	Arg	Ser	Arg	Ala	Ala	Ala	Ala	Thr	Ala	Asp
			245					250						255	
Thr	Asp	Ile	Val	Tyr	Glu	Pro	Lys	Val	His	Asn	Pro	Gly	Lys	Lys	Gln
			260					265					270		
Val	Phe	Ile	Glu	Asp	Lys	Leu	Gln	Lys	Ala	Leu	Thr	Asp	Pro	Lys	Lys
			275				280					285			
Asn	Lys	Lys	Leu	Tyr	Ala	Arg	Trp	Tyr	Asn	Ser	Gly	Phe	Thr	Val	Lys
			290			295					300				
Gln	Val	Glu	Gly	Gly	Leu	Asp	Gln	Asn	Glu	Asn	Arg	Glu	Leu	Glu	Leu
305					310					315					320
Thr	Tyr	Lys	Asn	Leu	Ala	Leu	Gly	Tyr	Ala	Lys	Tyr	Tyr	Gln	Ala	Arg
			325					330						335	
Arg	Ser	Gln	Glu	Ala	Lys										
			340												

&lt;210&gt; SEQ ID NO 131

&lt;211&gt; LENGTH: 342

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Phytophthora sojae

&lt;400&gt; SEQUENCE: 131

Met	Ser	Pro	Ile	Leu	Gly	Tyr	Trp	Lys	Ile	Lys	Gly	Leu	Val	Gln	Pro
1				5					10					15	
Thr	Arg	Leu	Leu	Leu	Glu	Tyr	Leu	Glu	Glu	Lys	Tyr	Glu	Glu	His	Leu
			20					25					30		
Tyr	Glu	Arg	Asp	Glu	Gly	Asp	Lys	Trp	Arg	Asn	Lys	Lys	Phe	Glu	Leu
		35					40					45			
Gly	Leu	Glu	Phe	Pro	Asn	Leu	Pro	Tyr	Tyr	Ile	Asp	Gly	Asp	Val	Lys
		50				55					60				
Leu	Thr	Gln	Ser	Met	Ala	Ile	Ile	Arg	Tyr	Ile	Ala	Asp	Lys	His	Asn
65					70					75					80
Met	Leu	Gly	Gly	Cys	Pro	Lys	Glu	Arg	Ala	Glu	Ile	Ser	Met	Leu	Glu
				85					90					95	
Gly	Ala	Val	Leu	Asp	Ile	Arg	Tyr	Gly	Val	Ser	Arg	Ile	Ala	Tyr	Ser
			100					105					110		
Lys	Asp	Phe	Glu	Thr	Leu	Lys	Val	Asp	Phe	Leu	Ser	Lys	Leu	Pro	Glu
			115				120					125			

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Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
 130                               135                140

Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145                               150                155                160

Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
165                               170                175

Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
180                               185                190

Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
195                               200                205

Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
210                               215                220

Gly Ser Thr Arg Val Pro Asp Asp Ala Asn Leu Gln Ser Val Asn Ala
225                               230                235                240

Pro Val Gln Thr Val Thr Arg Ser Arg Arg Phe Leu Arg Thr Ala Ala
245                               250                255

Thr Ala Ile Val Tyr Ala Pro Ala Val His Asn Pro Gly Lys Lys Gln
260                               265                270

Val Phe Ile Glu Asp Lys Leu Gln Lys Ala Leu Thr Asp Pro Lys Lys
275                               280                285

Asn Lys Lys Leu Tyr Ala Arg Trp Tyr Asn Ser Gly Phe Thr Val Lys
290                               295                300

Gln Val Glu Gly Gly Leu Asp Gln Asn Glu Asn Arg Glu Leu Glu Leu
305                               310                315                320

Thr Tyr Lys Asn Leu Ala Leu Gly Tyr Ala Lys Tyr Tyr Gln Ala Arg
325                               330                335

Arg Ser Gln Glu Ala Lys
340

```

&lt;210&gt; SEQ ID NO 132

&lt;211&gt; LENGTH: 279

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

&lt;400&gt; SEQUENCE: 132

```

Met Met Gln Trp Ser Ala Ile Leu Ile Arg Thr Cys Phe Ser Gly Ser
 1      5      10      15

Gly Gly Glu Ala Leu Thr Cys Ala Thr Ser Glu Gln Gln Thr Arg Pro
 20      25      30

Glu Leu Cys Phe Phe Phe Ser Val Arg Ser Ser Trp Pro Ser Thr Ile
 35      40      45

Ser Asp Gly Ala Cys Leu Ala Leu Val Ser Ala Glu Gln Gly Ala Thr
 50      55      60

Ala Gly Arg Asn Thr Leu Ser Leu Arg Ser Met Met Ala Thr Glu Asp
 65      70      75      80

Met Ala Thr Ser Thr Arg Ser Leu Arg Ser Gln Ala Thr Asn Val Asp
 85      90      95

Asp Asp Ala Asn Val Ser Ile Glu Asn Arg Gly Met Asn Pro Ser Val
100      105      110

Leu Thr Lys Leu Gly Glu Phe Ala Ser Thr Leu Thr Ala Gly Asn Thr
115      120      125

Ala Asn Lys Leu Trp Leu Met Ala Asp Val Asp Pro Lys Ser Ala Phe
130      135      140

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Lys Leu Leu Gly Leu Asp Met Pro Gly Val Arg Phe Ile Asp Asn Pro  
 145 150 155 160  
 Lys Met Leu Gln Trp Leu Lys Phe Thr Lys Ala Tyr Leu Asp Met Lys  
 165 170 175  
 Lys Ser Gly Phe Gly Glu Thr Ser Ala His Ala Leu Leu Tyr Glu Lys  
 180 185 190  
 Ile Gly Gly Pro Asp Leu Ser Leu Leu Leu Leu Ser Leu Lys Asp Ala  
 195 200 205  
 Pro Asp Ala Asn Ser Leu Val Gln Lys Leu Thr Asn Ser Gln Phe Gly  
 210 215 220  
 Met Trp His Asp Ala Arg Ile Glu Pro Glu Gln Leu Ala Gln Thr Val  
 225 230 235 240  
 Phe Lys Ile Gln Asp Val Arg Lys Leu Pro Lys Asn Asp Pro Lys Leu  
 245 250 255  
 Gln Val Ile Asp Asp Tyr Ala Lys Tyr His Arg Lys His Arg Lys Phe  
 260 265 270  
 Leu Asn Ser Ile Met Ile Ile  
 275

&lt;210&gt; SEQ ID NO 133

&lt;211&gt; LENGTH: 279

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

&lt;400&gt; SEQUENCE: 133

Met Met Gln Trp Ser Ala Ile Leu Ile Arg Thr Cys Phe Ser Gly Ser  
 1 5 10 15  
 Gly Gly Glu Ala Leu Thr Cys Ala Thr Ser Glu Gln Gln Thr Arg Pro  
 20 25 30  
 Glu Leu Cys Phe Phe Phe Ser Val Arg Ser Ser Trp Pro Ser Thr Ile  
 35 40 45  
 Ser Asp Gly Ala Cys Leu Ala Leu Val Ser Ala Glu Gln Gly Ala Thr  
 50 55 60  
 Ala Gly Arg Asn Thr Leu Ser Leu Arg Ser Met Met Ala Thr Glu Asp  
 65 70 75 80  
 Met Ala Thr Ser Thr Ala Ala Ala Ala Ser Gln Ala Thr Asn Val Asp  
 85 90 95  
 Asp Asp Ala Asn Val Ser Ile Glu Asn Arg Gly Met Asn Pro Ser Val  
 100 105 110  
 Leu Thr Lys Leu Gly Glu Phe Ala Ser Thr Leu Thr Ala Gly Asn Thr  
 115 120 125  
 Ala Asn Lys Leu Trp Leu Met Ala Asp Val Asp Pro Lys Ser Ala Phe  
 130 135 140  
 Lys Leu Leu Gly Leu Asp Met Pro Gly Val Arg Phe Ile Asp Asn Pro  
 145 150 155 160  
 Lys Met Leu Gln Trp Leu Lys Phe Thr Lys Ala Tyr Leu Asp Met Lys  
 165 170 175  
 Lys Ser Gly Phe Gly Glu Thr Ser Ala His Ala Leu Leu Tyr Glu Lys  
 180 185 190  
 Ile Gly Gly Pro Asp Leu Ser Leu Leu Leu Leu Ser Leu Lys Asp Ala  
 195 200 205  
 Pro Asp Ala Asn Ser Leu Val Gln Lys Leu Thr Asn Ser Gln Phe Gly

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210          215          220
Met Trp His Asp Ala Arg Ile Glu Pro Glu Gln Leu Ala Gln Thr Val
225          230          235          240
Phe Lys Ile Gln Asp Val Arg Lys Leu Pro Lys Asn Asp Pro Lys Leu
245          250          255
Gln Val Ile Asp Asp Tyr Ala Lys Tyr His Arg Lys His Arg Lys Phe
260          265          270
Leu Asn Ser Ile Met Ile Ile
275

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<210> SEQ ID NO 134
<211> LENGTH: 279
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae
<400> SEQUENCE: 134

```

```

Met Met Gln Trp Ser Ala Ile Leu Ile Arg Thr Cys Phe Ser Gly Ser
1          5          10          15
Gly Gly Glu Ala Leu Thr Cys Ala Thr Ser Glu Gln Gln Thr Arg Pro
20          25          30
Glu Leu Cys Phe Phe Phe Ser Val Arg Ser Ser Trp Pro Ser Thr Ile
35          40          45
Ser Asp Gly Ala Cys Leu Ala Leu Val Ser Ala Glu Gln Gly Ala Thr
50          55          60
Ala Gly Arg Asn Thr Leu Ser Leu Arg Ser Met Met Ala Thr Glu Asp
65          70          75          80
Met Ala Thr Ser Thr Arg Ser Leu Arg Ser Gln Ala Thr Asn Val Ala
85          90          95
Ala Ala Ala Asn Val Ser Ile Ala Asn Ala Gly Met Asn Pro Ser Val
100          105          110
Leu Thr Lys Leu Gly Glu Phe Ala Ser Thr Leu Thr Ala Gly Asn Thr
115          120          125
Ala Asn Lys Leu Trp Leu Met Ala Asp Val Asp Pro Lys Ser Ala Phe
130          135          140
Lys Leu Leu Gly Leu Asp Met Pro Gly Val Arg Phe Ile Asp Asn Pro
145          150          155          160
Lys Met Leu Gln Trp Leu Lys Phe Thr Lys Ala Tyr Leu Asp Met Lys
165          170          175
Lys Ser Gly Phe Gly Glu Thr Ser Ala His Ala Leu Leu Tyr Glu Lys
180          185          190
Ile Gly Gly Pro Asp Leu Ser Leu Leu Leu Leu Ser Leu Lys Asp Ala
195          200          205
Pro Asp Ala Asn Ser Leu Val Gln Lys Leu Thr Asn Ser Gln Phe Gly
210          215          220
Met Trp His Asp Ala Arg Ile Glu Pro Glu Gln Leu Ala Gln Thr Val
225          230          235          240
Phe Lys Ile Gln Asp Val Arg Lys Leu Pro Lys Asn Asp Pro Lys Leu
245          250          255
Gln Val Ile Asp Asp Tyr Ala Lys Tyr His Arg Lys His Arg Lys Phe
260          265          270
Leu Asn Ser Ile Met Ile Ile
275

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<210> SEQ ID NO 135
<211> LENGTH: 259
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

<400> SEQUENCE: 135
Leu Thr Cys Ala Thr Ser Glu Gln Gln Thr Arg Pro Glu Leu Cys Phe
1      5      10      15
Phe Phe Ser Val Arg Ser Ser Trp Pro Ser Thr Ile Ser Asp Gly Ala
20     25     30
Cys Leu Ala Leu Val Ser Ala Glu Gln Gly Ala Thr Ala Gly Arg Asn
35     40     45
Thr Leu Ser Leu Arg Ser Met Met Ala Thr Glu Asp Met Ala Thr Ser
50     55     60
Thr Arg Ser Leu Arg Ser Gln Ala Thr Asn Val Asp Asp Asp Ala Asn
65     70     75     80
Val Ser Ile Glu Asn Arg Gly Met Asn Pro Ser Val Leu Thr Lys Leu
85     90     95
Gly Glu Phe Ala Ser Thr Leu Thr Ala Gly Asn Thr Ala Asn Lys Leu
100    105    110
Trp Leu Met Ala Asp Val Asp Pro Lys Ser Ala Phe Lys Leu Leu Gly
115    120    125
Leu Asp Met Pro Gly Val Arg Phe Ile Asp Asn Pro Lys Met Leu Gln
130    135    140
Trp Leu Lys Phe Thr Lys Ala Tyr Leu Asp Met Lys Lys Ser Gly Phe
145    150    155    160
Gly Glu Thr Ser Ala His Ala Leu Leu Tyr Glu Lys Ile Gly Gly Pro
165    170    175
Asp Leu Ser Leu Leu Leu Leu Ser Leu Lys Asp Ala Pro Asp Ala Asn
180    185    190
Ser Leu Val Gln Lys Leu Thr Asn Ser Gln Phe Gly Met Trp His Asp
195    200    205
Ala Arg Ile Glu Pro Glu Gln Leu Ala Gln Thr Val Phe Lys Ile Gln
210    215    220
Asp Val Arg Lys Leu Pro Lys Asn Asp Pro Lys Leu Gln Val Ile Asp
225    230    235    240
Asp Tyr Ala Lys Tyr His Arg Lys His Arg Lys Phe Leu Asn Ser Ile
245    250    255

Met Ile Ile

<210> SEQ ID NO 136
<211> LENGTH: 259
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

<400> SEQUENCE: 136
Leu Thr Cys Ala Thr Ser Glu Gln Gln Thr Arg Pro Glu Leu Cys Phe
1      5      10      15
Phe Phe Ser Val Arg Ser Ser Trp Pro Ser Thr Ile Ser Asp Gly Ala
20     25     30
Cys Leu Ala Leu Val Ser Ala Glu Gln Gly Ala Thr Ala Gly Arg Asn
35     40     45
Thr Leu Ser Leu Arg Ser Met Met Ala Thr Glu Asp Met Ala Thr Ser

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50					55					60					
Thr	Ala	Ala	Ala	Ala	Ser	Gln	Ala	Thr	Asn	Val	Asp	Asp	Asp	Ala	Asn
65					70					75					80
Val	Ser	Ile	Glu	Asn	Arg	Gly	Met	Asn	Pro	Ser	Val	Leu	Thr	Lys	Leu
				85					90					95	
Gly	Glu	Phe	Ala	Ser	Thr	Leu	Thr	Ala	Gly	Asn	Thr	Ala	Asn	Lys	Leu
			100					105					110		
Trp	Leu	Met	Ala	Asp	Val	Asp	Pro	Lys	Ser	Ala	Phe	Lys	Leu	Leu	Gly
		115					120					125			
Leu	Asp	Met	Pro	Gly	Val	Arg	Phe	Ile	Asp	Asn	Pro	Lys	Met	Leu	Gln
		130				135					140				
Trp	Leu	Lys	Phe	Thr	Lys	Ala	Tyr	Leu	Asp	Met	Lys	Lys	Ser	Gly	Phe
				150							155				160
Gly	Glu	Thr	Ser	Ala	His	Ala	Leu	Leu	Tyr	Glu	Lys	Ile	Gly	Gly	Pro
				165					170					175	
Asp	Leu	Ser	Leu	Leu	Leu	Ser	Leu	Lys	Asp	Ala	Pro	Asp	Ala	Asn	
			180					185				190			
Ser	Leu	Val	Gln	Lys	Leu	Thr	Asn	Ser	Gln	Phe	Gly	Met	Trp	His	Asp
		195					200					205			
Ala	Arg	Ile	Glu	Pro	Glu	Gln	Leu	Ala	Gln	Thr	Val	Phe	Lys	Ile	Gln
		210				215					220				
Asp	Val	Arg	Lys	Leu	Pro	Lys	Asn	Asp	Pro	Lys	Leu	Gln	Val	Ile	Asp
				230					235					240	
Asp	Tyr	Ala	Lys	Tyr	His	Arg	Lys	His	Arg	Lys	Phe	Leu	Asn	Ser	Ile
				245					250					255	

Met Ile Ile

<210> SEQ ID NO 137  
 <211> LENGTH: 259  
 <212> TYPE: PRT  
 <213> ORGANISM: Phytophthora sojae

<400> SEQUENCE: 137

Leu	Thr	Cys	Ala	Thr	Ser	Glu	Gln	Gln	Thr	Arg	Pro	Glu	Leu	Cys	Phe
1				5					10					15	
Phe	Phe	Ser	Val	Arg	Ser	Ser	Trp	Pro	Ser	Thr	Ile	Ser	Asp	Gly	Ala
			20					25					30		
Cys	Leu	Ala	Leu	Val	Ser	Ala	Glu	Gln	Gly	Ala	Thr	Ala	Gly	Arg	Asn
		35					40					45			
Thr	Leu	Ser	Leu	Arg	Ser	Met	Met	Ala	Thr	Glu	Asp	Met	Ala	Thr	Ser
		50				55					60				
Thr	Arg	Ser	Leu	Arg	Ser	Gln	Ala	Thr	Asn	Val	Ala	Ala	Ala	Ala	Asn
				70					75					80	
Val	Ser	Ile	Ala	Asn	Ala	Gly	Met	Asn	Pro	Ser	Val	Leu	Thr	Lys	Leu
				85					90					95	
Gly	Glu	Phe	Ala	Ser	Thr	Leu	Thr	Ala	Gly	Asn	Thr	Ala	Asn	Lys	Leu
			100					105					110		
Trp	Leu	Met	Ala	Asp	Val	Asp	Pro	Lys	Ser	Ala	Phe	Lys	Leu	Leu	Gly
		115					120					125			
Leu	Asp	Met	Pro	Gly	Val	Arg	Phe	Ile	Asp	Asn	Pro	Lys	Met	Leu	Gln
		130				135					140				
Trp	Leu	Lys	Phe	Thr	Lys	Ala	Tyr	Leu	Asp	Met	Lys	Lys	Ser	Gly	Phe

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145              150              155              160
Gly Glu Thr Ser Ala His Ala Leu Leu Tyr Glu Lys Ile Gly Gly Pro
      165              170              175

Asp Leu Ser Leu Leu Leu Leu Ser Leu Lys Asp Ala Pro Asp Ala Asn
      180              185              190

Ser Leu Val Gln Lys Leu Thr Asn Ser Gln Phe Gly Met Trp His Asp
      195              200              205

Ala Arg Ile Glu Pro Glu Gln Leu Ala Gln Thr Val Phe Lys Ile Gln
      210              215              220

Asp Val Arg Lys Leu Pro Lys Asn Asp Pro Lys Leu Gln Val Ile Asp
      225              230              235              240

Asp Tyr Ala Lys Tyr His Arg Lys His Arg Lys Phe Leu Asn Ser Ile
      245              250              255

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Met Ile Ile

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<210> SEQ ID NO 138
<211> LENGTH: 137
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Sequence of amino acids 24-67 of
      avrL567 replacing theRXLR region of secreted AvrIb

```

&lt;400&gt; SEQUENCE: 138

```

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1      5      10      15

Val Thr Cys Asn Ala Met Glu His Val Pro Ala Glu Leu Thr Arg Val
      20      25      30

Ser Glu Gly Tyr Thr Arg Phe Tyr Arg Ser Pro Thr Ala Ser Val Ile
      35      40      45

Leu Ser Gly Leu Val Lys Val Lys Trp Asp Asn Glu Gln Met Thr Met
      50      55      60

Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys Leu
      65      70      75      80

Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr Glu
      85      90      95

Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp Leu
      100     105     110

Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr Asn
      115     120     125

Gly Tyr Thr Phe His Arg Tyr Gln Ser
      130     135

```

```

<210> SEQ ID NO 139
<211> LENGTH: 137
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Sequence of amino acids 24-67 of
      avrL567 replacing theRXLR region of secreted AvrIb. RFYR-DE
      mutated to AAAA-AA

```

&lt;400&gt; SEQUENCE: 139

```

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1      5      10      15

Val Thr Cys Asn Ala Met Glu His Val Pro Ala Glu Leu Thr Arg Val

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                20                25                30
Ser  Glu  Gly  Tyr  Thr  Ala  Ala  Ala  Ala  Ser  Pro  Thr  Ala  Ser  Val  Ile
   35                40                45
Leu  Ser  Gly  Leu  Val  Lys  Val  Lys  Trp  Ala  Asn  Ala  Gln  Met  Thr  Met
   50                55                60
Thr  Phe  Ser  Val  Thr  Asp  Leu  Trp  Asn  Lys  Val  Ala  Ala  Lys  Lys  Leu
   65                70                75                80
Ala  Lys  Ala  Met  Leu  Ala  Asp  Pro  Ser  Lys  Glu  Gln  Lys  Ala  Tyr  Glu
   85                90                95
Lys  Trp  Ala  Lys  Lys  Gly  Tyr  Ser  Leu  Asp  Lys  Ile  Lys  Asn  Trp  Leu
   100               105               110
Ala  Ile  Ala  Asp  Pro  Lys  Gln  Lys  Gly  Lys  Tyr  Asp  Arg  Ile  Tyr  Asn
   115               120               125
Gly  Tyr  Thr  Phe  His  Arg  Tyr  Gln  Ser
   130               135

<210> SEQ ID NO 140
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Sequence of amino acids 24-67 of
avrL567 replacing theRXLR regio
n of mature AvrIb.

<400> SEQUENCE: 140
Met  Glu  His  Val  Pro  Ala  Glu  Leu  Thr  Arg  Val  Ser  Glu  Gly  Tyr  Thr
 1                5                10                15
Arg  Phe  Tyr  Arg  Ser  Pro  Thr  Ala  Ser  Val  Ile  Leu  Ser  Gly  Leu  Val
 20               25               30
Lys  Val  Lys  Trp  Asp  Asn  Glu  Gln  Met  Thr  Met  Thr  Phe  Ser  Val  Thr
 35               40               45
Asp  Leu  Trp  Asn  Lys  Val  Ala  Ala  Lys  Lys  Leu  Ala  Lys  Ala  Met  Leu
 50               55               60
Ala  Asp  Pro  Ser  Lys  Glu  Gln  Lys  Ala  Tyr  Glu  Lys  Trp  Ala  Lys  Lys
 65               70               75                80
Gly  Tyr  Ser  Leu  Asp  Lys  Ile  Lys  Asn  Trp  Leu  Ala  Ile  Ala  Asp  Pro
 85               90               95
Lys  Gln  Lys  Gly  Lys  Tyr  Asp  Arg  Ile  Tyr  Asn  Gly  Tyr  Thr  Phe  His
 100              105              110
Arg  Tyr  Gln  Ser
 115

<210> SEQ ID NO 141
<211> LENGTH: 116
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Sequence of amino acids 24-67 of
avrL567 replacing theRXLR region of mature AvrIb. RPYR-DE
mutated to AAAA-AA

<400> SEQUENCE: 141
Met  Glu  His  Val  Pro  Ala  Glu  Leu  Thr  Arg  Val  Ser  Glu  Gly  Tyr  Thr
 1                5                10                15
Ala  Ala  Ala  Ala  Ser  Pro  Thr  Ala  Ser  Val  Ile  Leu  Ser  Gly  Leu  Val
 20               25               30

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Lys Val Lys Trp Ala Asn Ala Gln Met Thr Met Thr Phe Ser Val Thr  
 35 40 45

Asp Leu Trp Asn Lys Val Ala Ala Lys Lys Leu Ala Lys Ala Met Leu  
 50 55 60

Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr Glu Lys Trp Ala Lys Lys  
 65 70 75 80

Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp Leu Ala Ile Ala Asp Pro  
 85 90 95

Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr Asn Gly Tyr Thr Phe His  
 100 105 110

Arg Tyr Gln Ser  
 115

<210> SEQ ID NO 142  
 <211> LENGTH: 169  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetic Sequence of the N-terminus of AvrM  
 replacing the RXLR region of secreted Avr1b.

&lt;400&gt; SEQUENCE: 142

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val  
 1 5 10 15

Val Thr Cys Asn Ala His Pro Val Tyr Asp Tyr Asn Pro Ile Pro Asn  
 20 25 30

His Ile His Gly Asp Leu Lys Arg Arg Ala Tyr Ile Glu Arg Tyr Ser  
 35 40 45

Gln Cys Ser Asp Ser Gln Ala Ser Glu Ile Arg Ala Ala Leu Lys Ser  
 50 55 60

Cys Ala Glu Leu Ala Ser Trp Gly Tyr His Ala Val Lys Asn Asp Asn  
 65 70 75 80

Arg Leu Phe Lys Leu Ile Phe Lys Thr Asp Ser Thr Asp Ile Gln Asn  
 85 90 95

Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys Leu  
 100 105 110

Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr Glu  
 115 120 125

Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp Leu  
 130 135 140

Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr Asn  
 145 150 155 160

Gly Tyr Thr Phe His Arg Tyr Gln Ser  
 165

<210> SEQ ID NO 143  
 <211> LENGTH: 192  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetic Sequence of the N-terminus of  
 AvrPita replacing theRXLR region of secreted Avr1b.

&lt;400&gt; SEQUENCE: 143

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val  
 1 5 10 15

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Val Thr Cys Asn Ala Asn Asn Leu Gly Thr Val Pro Asp Val Pro His
      20                               25                               30
Gln Ile Pro Asn Asp Lys Ser Gly Thr Pro Ala Ile Glu Asp Pro Lys
      35                               40                               45
Asp Met Lys Gly Phe Asn Lys Ala Leu Lys Ser Thr Pro Glu Ser Glu
      50                               55                               60
Lys Leu Gly Thr Ser Ser Val Glu Gly Ile Pro Gln Pro Glu Phe Asp
      65                               70                               75                               80
Arg Gly Phe Leu Arg Pro Phe Gly Ala Lys Met Lys Phe Leu Lys Pro
      85                               90                               95
Asp Gln Val Gln Lys Leu Ser Thr Asp Asp Leu Ile Thr Tyr Met Ala
      100                              105                              110
Glu Lys Asp Lys Asn Val Arg Thr Phe Ser Val Thr Asp Leu Trp Asn
      115                              120                              125
Lys Val Ala Ala Lys Lys Leu Ala Lys Ala Met Leu Ala Asp Pro Ser
      130                              135                              140
Lys Glu Gln Lys Ala Tyr Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu
      145                              150                              155                              160
Asp Lys Ile Lys Asn Trp Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly
      165                              170                              175
Lys Tyr Asp Arg Ile Tyr Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser
      180                              185                              190

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<210> SEQ ID NO 144
<211> LENGTH: 142
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

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<400> SEQUENCE: 144

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Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
 1                               5                               10                               15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
      20                               25                               30
Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile
      35                               40                               45
Ala Gly Gly Lys Arg Arg Ala Tyr Ile Glu Arg Ala His Glu Glu Asp
      50                               55                               60
Asp Ala Gly Glu Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val
      65                               70                               75                               80
Ala Ala Lys Lys Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu
      85                               90                               95
Gln Lys Ala Tyr Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys
      100                              105                              110
Ile Lys Asn Trp Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr
      115                              120                              125
Asp Arg Ile Tyr Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser
      130                              135                              140

```

```

<210> SEQ ID NO 145
<211> LENGTH: 139
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

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<400> SEQUENCE: 145

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```

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1           5           10           15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
                20           25           30
Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile
                35           40           45
Ala Gly Gly Arg Ala Ala Leu Lys Ala His Glu Glu Asp Asp Ala Gly
                50           55           60
Glu Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys
65           70           75           80
Lys Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala
                85           90           95
Tyr Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn
                100           105           110
Trp Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile
                115           120           125
Tyr Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser
                130           135

```

```

<210> SEQ ID NO 146
<211> LENGTH: 149
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

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```

<400> SEQUENCE: 146

```

```

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1           5           10           15
Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met
                20           25           30
Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile
                35           40           45
Ala Gly Gly Arg Leu Phe Lys Leu Ile Phe Lys Thr Asp Ser Thr Asp
                50           55           60
Ile Gln Ala His Glu Glu Asp Asp Ala Gly Glu Arg Thr Phe Ser Val
65           70           75           80
Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys Leu Ala Lys Ala Met
                85           90           95
Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr Glu Lys Trp Ala Lys
                100           105           110
Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp Leu Ala Ile Ala Asp
                115           120           125
Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr Asn Gly Tyr Thr Phe
                130           135           140
His Arg Tyr Gln Ser
                145

```

```

<210> SEQ ID NO 147
<211> LENGTH: 138
<212> TYPE: PRT
<213> ORGANISM: Phytophthora sojae

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<400> SEQUENCE: 147

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```

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val
1           5           10           15

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Ala Gly Gly Arg Phe Tyr Arg Ala His Glu Glu Asp Asp Ala Gly Glu  
 50 55 60

Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys  
 65 70 75 80

Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr  
 85 90 95

Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp  
 100 105 110

Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr  
 115 120 125

Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser  
 130 135

&lt;210&gt; SEQ ID NO 150

&lt;211&gt; LENGTH: 138

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

&lt;400&gt; SEQUENCE: 150

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val  
 1 5 10 15

Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met  
 20 25 30

Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile  
 35 40 45

Ala Gly Gly Arg Phe Ile Arg Ala His Glu Glu Asp Asp Ala Gly Glu  
 50 55 60

Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys  
 65 70 75 80

Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr  
 85 90 95

Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp  
 100 105 110

Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr  
 115 120 125

Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser  
 130 135

&lt;210&gt; SEQ ID NO 151

&lt;211&gt; LENGTH: 138

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

&lt;400&gt; SEQUENCE: 151

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val  
 1 5 10 15

Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met  
 20 25 30

Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile  
 35 40 45

Ala Gly Gly Arg Phe Met Arg Ala His Glu Glu Asp Asp Ala Gly Glu  
 50 55 60

Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys  
 65 70 75 80



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Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr  
 115 120 125

Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser  
 130 135

<210> SEQ ID NO 154

<211> LENGTH: 138

<212> TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

<400> SEQUENCE: 154

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val  
 1 5 10 15

Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met  
 20 25 30

Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile  
 35 40 45

Ala Gly Gly Arg Leu Gly Thr Ala His Glu Glu Asp Asp Ala Gly Glu  
 50 55 60

Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys  
 65 70 75 80

Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr  
 85 90 95

Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp  
 100 105 110

Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr  
 115 120 125

Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser  
 130 135

<210> SEQ ID NO 155

<211> LENGTH: 138

<212> TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

<400> SEQUENCE: 155

Met Arg Leu Ser Phe Val Leu Ser Leu Val Val Ala Ile Gly Tyr Val  
 1 5 10 15

Val Thr Cys Asn Ala Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met  
 20 25 30

Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile  
 35 40 45

Ala Gly Gly Arg Leu Thr Gln Ala His Glu Glu Asp Asp Ala Gly Glu  
 50 55 60

Arg Thr Phe Ser Val Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys  
 65 70 75 80

Leu Ala Lys Ala Met Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr  
 85 90 95

Glu Lys Trp Ala Lys Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp  
 100 105 110

Leu Ala Ile Ala Asp Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr  
 115 120 125

Asn Gly Tyr Thr Phe His Arg Tyr Gln Ser  
 130 135

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<210> SEQ ID NO 156  
<211> LENGTH: 53  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 156  
gcatcccatg gagccagcat agtctgggac gtcatatgga tagccggaca tgg 53

<210> SEQ ID NO 157  
<211> LENGTH: 32  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 157  
atgcagatct ctcaactgcg ccacctccga gc 32

<210> SEQ ID NO 158  
<211> LENGTH: 48  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 158  
catatggata gccggacatg gtggatctgt cgactctggt ttcaatcg 48

<210> SEQ ID NO 159  
<211> LENGTH: 46  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 159  
catatggata gccggacatg gtggatctgt cgactgcggt tgcaat 46

<210> SEQ ID NO 160  
<211> LENGTH: 27  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 160  
agtcagatct acaagagtcc ccgacga 27

<210> SEQ ID NO 161  
<211> LENGTH: 48  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 161  
catatggata gccggacatg gtggatctgt cgacttgcca ggattatg 48

<210> SEQ ID NO 162  
<211> LENGTH: 26  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 162  
atcgagatct atgggaatgg aacatg 26

<210> SEQ ID NO 163  
<211> LENGTH: 45  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 163  
gatagccgga catggtggat ctgtogaccc acaggtcagt cacgc 45

<210> SEQ ID NO 164  
<211> LENGTH: 57  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 164  
tcccatggag ccagcatagt ctgggacgtc atatggatag ccggacatgg tggatct 57

<210> SEQ ID NO 165  
<211> LENGTH: 28  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 165  
atcgagatct gataagtatg agaaagcg 28

<210> SEQ ID NO 166  
<211> LENGTH: 53  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 166

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catatggata gccggacatg gtggatctgt cgaccgtttg cttgcttttc cgg 53

<210> SEQ ID NO 167  
<211> LENGTH: 52  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning  
  
<400> SEQUENCE: 167

gattaccgct ttcgagagtc tgcagcagca gcagcaggcg aagatacctg cg 52

<210> SEQ ID NO 168  
<211> LENGTH: 52  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning  
  
<400> SEQUENCE: 168

atgcagatct gataagtatg agaaagcggg agattacggc tttegagagt ct 52

<210> SEQ ID NO 169  
<211> LENGTH: 23  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning  
  
<400> SEQUENCE: 169

atgcagatct tttacaata acc 23

<210> SEQ ID NO 170  
<211> LENGTH: 47  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning  
  
<400> SEQUENCE: 170

catatggata gccggacatg gtggatctgt cgacatctgc aacatgg 47

<210> SEQ ID NO 171  
<211> LENGTH: 46  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning  
  
<400> SEQUENCE: 171

ggcttgaacc tcaacaaggc agcagcagca gcaacacaag cacatg 46

<210> SEQ ID NO 172  
<211> LENGTH: 35

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<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 172

gtagtaagaa tgctaaaggc ttgaacctca acaag 35

<210> SEQ ID NO 173  
<211> LENGTH: 46  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 173

atgcagatct tttacaata acctgtgtag taagaatgct aaaggc 46

<210> SEQ ID NO 174  
<211> LENGTH: 28  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 174

atgcagatct agttacaaca agatcaac 28

<210> SEQ ID NO 175  
<211> LENGTH: 47  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 175

catatggata gccggacatg gtggatctgt cgacactatc atctaac 47

<210> SEQ ID NO 176  
<211> LENGTH: 54  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 176

catctactta tacacacagt gcagcagcag cagcattgga gtccatcaca ttgg 54

<210> SEQ ID NO 177  
<211> LENGTH: 58  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

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<400> SEQUENCE: 177

atgcagatct agttacaaca agatcaactc gtcactctact tatacacaca gtgcagca 58

<210> SEQ ID NO 178

<211> LENGTH: 29

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 178

gcgtggatcc ctcaacttgcg ccacctccg 29

<210> SEQ ID NO 179

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 179

tcgagtcgac tcagataatc atgatgctgt 30

<210> SEQ ID NO 180

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 180

gcgtggatcc acaagagtcc ccgacga 27

<210> SEQ ID NO 181

<211> LENGTH: 29

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 181

tcgagtcgac tcacttggcc tcttgcat 29

<210> SEQ ID NO 182

<211> LENGTH: 30

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 182

cagcggcggc ggcgctctcaa gctacgaacg 30

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<210> SEQ ID NO 183  
<211> LENGTH: 45  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 183

gttcgtagct tgagacgccg ccgccgctgt cgaggttgcc atgtc 45

<210> SEQ ID NO 184  
<211> LENGTH: 40  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 184

ggctaacggtt tcgattgcga acgcgggatg aacccttcag 40

<210> SEQ ID NO 185  
<211> LENGTH: 38  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 185

cgcaatcgaa acgtttagccg ccgcccaccac gttcgtag 38

<210> SEQ ID NO 186  
<211> LENGTH: 32  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 186

gcgtccatgg gagctcaactt gcgccacctc cg 32

<210> SEQ ID NO 187  
<211> LENGTH: 30  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 187

tcgaggtacc tcagataatc atgatgctgt 30

<210> SEQ ID NO 188  
<211> LENGTH: 52  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to

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amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 188

agatcccggg gggcagtgag atatgctcac ttgcccacc tccgagcaac ag 52

<210> SEQ ID NO 189

<211> LENGTH: 54

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 189

atcgccatgg gaatggaaca tgtaccagca gagttgacca gagtcagca aggg 54

<210> SEQ ID NO 190

<211> LENGTH: 58

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 190

accagagtca gcgaagggta tacacgattt taccggctcc caacggctag tgtaatac 58

<210> SEQ ID NO 191

<211> LENGTH: 59

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 191

caacggctag tgtaatactg tcaggattgg taaaggtaa atgggataat gaacaaatg 59

<210> SEQ ID NO 192

<211> LENGTH: 51

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 192

tgggataatg aacaaatgac gatgccgacc ttcagcgtga ctgacctgtg g 51

<210> SEQ ID NO 193

<211> LENGTH: 44

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 193

agatcccggg gggcaatgag atatgatgga acatgtacca gcag 44

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<210> SEQ ID NO 194  
<211> LENGTH: 58  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 194  
tcctgacagt attacactag ccgttgggga cgcagcggct gctgtatacc cttcgctg 58

<210> SEQ ID NO 195  
<211> LENGTH: 59  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 195  
gtgtaatact gtcaggattg gtaaaggtta aatgggctaa tgcacaaatg acgatgccg 59

<210> SEQ ID NO 196  
<211> LENGTH: 59  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 196  
atcgcaactcg agctttcgca gatccccggg ggcaatgaga tatgctcta tcttttctg 59

<210> SEQ ID NO 197  
<211> LENGTH: 34  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 197  
atcgccatgg gacaccagtt ttacgattac aatc 34

<210> SEQ ID NO 198  
<211> LENGTH: 58  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 198  
cacgccgta aaaatgacaa tcggttattt agattaatct ttaaaactga cagcacag 58

<210> SEQ ID NO 199  
<211> LENGTH: 52  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 199  
ctttaaact gacagcacag atattcaaaa caccttcagc gtgactgacc tg 52

<210> SEQ ID NO 200  
<211> LENGTH: 45  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 200  
ccacgaggcg agctcggcac aacttttttag cgcggcacga atttc 45

<210> SEQ ID NO 201  
<211> LENGTH: 50  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 201  
ccgattgtca tttttaacgg cgtgatagcc ccacgaggcg agctcggcac 50

<210> SEQ ID NO 202  
<211> LENGTH: 46  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 202  
atcgccatgg gaaacaacct tggaacagta ccggatgtgc cacatc 46

<210> SEQ ID NO 203  
<211> LENGTH: 57  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 203  
cagtaccgga tgtgccacat caaattccaa atgacaaaag tggactcct gccattg 57

<210> SEQ ID NO 204  
<211> LENGTH: 59  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 204

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caaaagtggt actcctgccca ttgaagaccc aaaagatatg aaaggattca ataaggctc 59

<210> SEQ ID NO 205  
<211> LENGTH: 56  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning  
  
<400> SEQUENCE: 205

ccacaggtca gtcacgctga aggttcgtac atttttatct tttttgccca tgtatg 56

<210> SEQ ID NO 206  
<211> LENGTH: 59  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning  
  
<400> SEQUENCE: 206

gacattgccg gcggaaaaag gcgggcttat attgaacgcg ctcacgaaga ggacgatgc 59

<210> SEQ ID NO 207  
<211> LENGTH: 53  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning  
  
<400> SEQUENCE: 207

gacattgccg gcggacgtgc cgcgctaaaa agtgctcatg aagaggacga tgc 53

<210> SEQ ID NO 208  
<211> LENGTH: 49  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning  
  
<400> SEQUENCE: 208

ctttaaact gacagcacag atattcaagc tcatgaagag gacgatgcg 49

<210> SEQ ID NO 209  
<211> LENGTH: 59  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning  
  
<400> SEQUENCE: 209

ctgtgctgtc agttttaaag attaatttaa ataaccgtcc gccggcaatg tcgcccgttc 59

<210> SEQ ID NO 210  
<211> LENGTH: 23

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<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 210

cattgccggc ggaaagt ttc 23

<210> SEQ ID NO 211  
<211> LENGTH: 47  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 211

cgacattgcc ggcggacact ttcttcgagc tcatgaagag gacgatg 47

<210> SEQ ID NO 212  
<211> LENGTH: 20  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 212

cattgccggc ggacaatttc 20

<210> SEQ ID NO 213  
<211> LENGTH: 47  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 213

cgacattgcc ggcggaagat tttctcgagc tcatgaagag gacgatg 47

<210> SEQ ID NO 214  
<211> LENGTH: 47  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 214

cgacattgcc ggcggaagat tttatcgagc tcatgaagag gacgatg 47

<210> SEQ ID NO 215  
<211> LENGTH: 47  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

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<400> SEQUENCE: 215

cgacattgcc ggcggaagat ttattcgagc tcatgaagag gacgatg 47

<210> SEQ ID NO 216

<211> LENGTH: 47

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 216

cgacattgcc ggcggaagat ttatgcgagc tcatgaagag gacgatg 47

<210> SEQ ID NO 217

<211> LENGTH: 23

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 217

cattgccggc ggaagatttg ttc 23

<210> SEQ ID NO 218

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 218

cattgccggc ggaagatttg ctgc 24

<210> SEQ ID NO 219

<211> LENGTH: 27

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 219

cattgccggc ggaagatttc ttcaagc 27

<210> SEQ ID NO 220

<211> LENGTH: 47

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 220

cgacattgcc ggcggaagat ttcttggagc tcatgaagag gacgatg 47

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<210> SEQ ID NO 221  
 <211> LENGTH: 47  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 221

cgacattgcc ggcggaagaa tggttcgagc tcatgaagag gacgatg 47

<210> SEQ ID NO 222  
 <211> LENGTH: 47  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 222

cgacattgcc ggcggaagac ttggaacagc tcatgaagag gacgatg 47

<210> SEQ ID NO 223  
 <211> LENGTH: 47  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 223

cgacattgcc ggcggaagac ttactcaagc tcatgaagag gacgatg 47

<210> SEQ ID NO 224  
 <211> LENGTH: 390  
 <212> TYPE: PRT  
 <213> ORGANISM: Magnaporthe grisea

<400> SEQUENCE: 224

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
 1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
 20 25 30

Arg Trp Gly Ser Arg Ser His Pro Val Tyr Asp Tyr Asn Pro Ile Pro  
 35 40 45

Asn His Ile His Gly Asp Leu Lys Arg Arg Ala Tyr Ile Glu Arg Tyr  
 50 55 60

Ser Gln Cys Ser Asp Ser Gln Ala Ser Glu Ile Arg Ala Ala Leu Lys  
 65 70 75 80

Ser Cys Ala Glu Leu Ala Ser Trp Gly Tyr His Ala Val Lys Asn Asp  
 85 90 95

Asn Arg Leu Phe Lys Leu Ile Phe Lys Thr Asp Ser Thr Asp Ile Gln  
 100 105 110

Asn Glu Phe Arg Ser Thr Met Ser Gly Tyr Pro Tyr Asp Val Pro Asp  
 115 120 125

Tyr Ala Gly Ser Met Gly Ser Gly Ile Gln Arg Pro Thr Ser Thr Ser  
 130 135 140

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Ser Leu Val Ala Ala Ala Thr Met Ser Lys Gly Glu Glu Leu Phe  
145 150 155 160

Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly  
165 170 175

His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly  
180 185 190

Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro  
195 200 205

Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser  
210 215 220

Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met  
225 230 235 240

Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly  
245 250 255

Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val  
260 265 270

Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile  
275 280 285

Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile  
290 295 300

Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg  
305 310 315 320

His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln  
325 330 335

Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr  
340 345 350

Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp  
355 360 365

His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly  
370 375 380

Met Asp Glu Leu Tyr Lys  
385 390

&lt;210&gt; SEQ ID NO 225

&lt;211&gt; LENGTH: 390

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Magnaporthe grisea

&lt;400&gt; SEQUENCE: 225

Met Arg Gly Ser His His His His His Gly Met Ala Ser Met Thr  
1 5 10 15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Lys Asp  
20 25 30

Arg Trp Gly Ser Arg Ser His Pro Val Tyr Asp Tyr Asn Pro Ile Pro  
35 40 45

Asn His Ile His Gly Asp Leu Ser Ala Ser Ala Ser Ile Glu Ala Ala  
50 55 60

Ser Ala Cys Ser Asp Ser Gln Ala Ser Glu Ile Ala Ser Ser Ala Ala  
65 70 75 80

Ser Cys Ala Glu Leu Ala Ser Trp Gly Tyr Ala Ser Ala Ala Asn Asp  
85 90 95

Asn Ala Ala Ser Ala Leu Ile Phe Lys Thr Asp Ser Thr Asp Ile Gln  
100 105 110

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Asn Glu Phe Arg Ser Thr Met Ser Gly Tyr Pro Tyr Asp Val Pro Asp  
 115 120 125  
 Tyr Ala Gly Ser Met Gly Ser Gly Ile Gln Arg Pro Thr Ser Thr Ser  
 130 135 140  
 Ser Leu Val Ala Ala Ala Ala Thr Met Ser Lys Gly Glu Glu Leu Phe  
 145 150 155 160  
 Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly  
 165 170 175  
 His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly  
 180 185 190  
 Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro  
 195 200 205  
 Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser  
 210 215 220  
 Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys Ser Ala Met  
 225 230 235 240  
 Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly  
 245 250 255  
 Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val  
 260 265 270  
 Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile  
 275 280 285  
 Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile  
 290 295 300  
 Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg  
 305 310 315 320  
 His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln  
 325 330 335  
 Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr  
 340 345 350  
 Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp  
 355 360 365  
 His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly  
 370 375 380  
 Met Asp Glu Leu Tyr Lys  
 385 390

&lt;210&gt; SEQ ID NO 226

&lt;211&gt; LENGTH: 363

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Puccinia striiformis

&lt;400&gt; SEQUENCE: 226

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
 1 5 10 15  
 Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
 20 25 30  
 Arg Trp Gly Ser Arg Ser Met Gly Thr Leu Leu Asn Val Thr Arg Arg  
 35 40 45  
 Leu Gln Asn Asp Gly Lys Pro Pro Asp Tyr Cys Val Asp Lys Trp Asp  
 50 55 60  
 Glu Met Met Lys Glu Arg Asn Lys Arg Leu Thr Gly Lys Pro Arg Gly



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Glu Met Met Lys Glu Arg Asn Ala Ala Ala Ala Ala Lys Pro Arg Gly  
 65 70 75 80  
 Gln Cys Val Asp Glu Ile Glu Phe Arg Ser Thr Met Ser Gly Tyr Pro  
 85 90 95  
 Tyr Asp Val Pro Asp Tyr Ala Gly Ser Met Gly Ser Gly Ile Gln Arg  
 100 105 110  
 Pro Thr Ser Thr Ser Ser Leu Val Ala Ala Ala Ala Thr Met Ser Lys  
 115 120 125  
 Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp  
 130 135 140  
 Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly  
 145 150 155 160  
 Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly  
 165 170 175  
 Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Ser Tyr Gly  
 180 185 190  
 Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe  
 195 200 205  
 Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe  
 210 215 220  
 Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu  
 225 230 235 240  
 Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys  
 245 250 255  
 Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser  
 260 265 270  
 His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val  
 275 280 285  
 Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala  
 290 295 300  
 Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu  
 305 310 315 320  
 Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro  
 325 330 335  
 Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala  
 340 345 350  
 Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys  
 355 360

&lt;210&gt; SEQ ID NO 228

&lt;211&gt; LENGTH: 413

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Melampsora lini

&lt;400&gt; SEQUENCE: 228

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
 1 5 10 15  
 Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
 20 25 30  
 Arg Trp Gly Ser Arg Ser Asn Asn Leu Gly Thr Val Pro Asp Val Pro  
 35 40 45  
 His Gln Ile Pro Asn Asp Lys Ser Gly Thr Pro Ala Ile Glu Asp Pro  
 50 55 60

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Lys Asp Met Lys Gly Phe Asn Lys Ala Leu Lys Ser Thr Pro Glu Ser  
 65 70 75 80  
 Glu Lys Leu Gly Thr Ser Ser Val Glu Gly Ile Pro Gln Pro Glu Phe  
 85 90 95  
 Asp Arg Gly Phe Leu Arg Pro Phe Gly Ala Lys Met Lys Phe Leu Lys  
 100 105 110  
 Pro Asp Gln Val Gln Lys Leu Ser Thr Asp Asp Leu Ile Thr Tyr Met  
 115 120 125  
 Ala Glu Lys Asp Lys Asn Val Arg Glu Phe Arg Ser Thr Met Ser Gly  
 130 135 140  
 Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Ser Met Gly Ser Gly Ile  
 145 150 155 160  
 Gln Arg Pro Thr Ser Thr Ser Ser Leu Val Ala Ala Ala Thr Met  
 165 170 175  
 Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu  
 180 185 190  
 Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly  
 195 200 205  
 Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr  
 210 215 220  
 Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe Ser  
 225 230 235 240  
 Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His  
 245 250 255  
 Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr  
 260 265 270  
 Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys  
 275 280 285  
 Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp  
 290 295 300  
 Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr  
 305 310 315 320  
 Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile  
 325 330 335  
 Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln  
 340 345 350  
 Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val  
 355 360 365  
 Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys  
 370 375 380  
 Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr  
 385 390 395 400  
 Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys  
 405 410

&lt;210&gt; SEQ ID NO 229

&lt;211&gt; LENGTH: 413

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Melampsora lini

&lt;400&gt; SEQUENCE: 229

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr

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1	5	10	15
Gly	Gln 20	Met	Gly Arg Asp 25
Arg	Trp 35	Gly Ser Arg Ser Asn 40	Leu Tyr Asp 45
Ala	Ala 50	Ala Asn Asp 55	Ala Ala Pro 60
Ala	Ala 65	Ala Gly Phe Asn 70	Ala Ala Ala 75
Glu	Ala 85	Ala Ser Ser Val 85	Glu Gly Ile 90
Asp	Ala 100	Ala Ala Pro Phe 105	Gly Ala Lys 110
Pro	Asp 115	Gln Val Gln Ala 120	Ala Asp Asp 125
Ala	Glu 130	Lys Asp Ala Ala 135	Ala Ala Glu 140
Tyr	Pro 145	Tyr Asp Val Pro 150	Asp Tyr Ala 155
Gln	Arg 165	Pro Thr Ser Ser 165	Leu Val Ala 170
Ser	Lys 180	Gly Glu Leu Phe 185	Thr Val Val 190
Leu	Asp 195	Gly Asp Val Asn 200	Gly His Lys 205
Glu	Gly 210	Asp Ala Thr Tyr 215	Lys Leu Thr 220
Thr	Gly 225	Lys Leu Pro Val 230	Pro Trp Pro 235
Tyr	Gly 245	Val Gln Cys Phe 245	Ser Arg Tyr 250
Asp	Phe 260	Phe Lys Ser Ala 265	Met Pro Glu 270
Ile	Phe 275	Phe Lys Asp Asp 280	Gly Asn Tyr 285
Phe	Glu 290	Gly Asp Thr Leu 295	Val Asn Arg 300
Phe	Lys 305	Glu Asp Gly Asn 310	Ile Leu Gly 315
Asn	Ser 325	His Asn Val Tyr 325	Ile Met Ala 330
Lys	Val 340	Asn Phe Lys Ile 345	Arg His Asn 350
Leu	Ala 355	Asp His Tyr Gln 360	Asn Thr Pro 365
Leu	Leu 370	Pro Asp Asn His 375	Tyr Leu Ser 380
Asp	Pro 385	Asn Glu Lys Arg 390	Asp His Met 395
Ala	Ala 405	Gly Ile Thr His 405	Gly Met Asp 410

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<210> SEQ ID NO 230
<211> LENGTH: 369
<212> TYPE: PRT
<213> ORGANISM: Melampsora lini

<400> SEQUENCE: 230

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr
1          5          10
Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp
20          25          30
Arg Trp Gly Ser Arg Ser Gln Tyr Val Val Asp Pro Gly Phe Gly Glu
35          40          45
Ile Glu Cys Met Cys Gly Gln Ile Ala Arg Leu Thr Gln Arg Pro Phe
50          55          60
Asp Val Glu Cys Glu Ala Thr Pro Ser Cys Glu Phe Arg Ser Thr Met
65          70          75          80
Ser Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Glu Phe Arg Ser
85          90          95
Thr Met Ser Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Ser Met
100         105         110
Gly Ser Gly Ile Gln Arg Pro Thr Ser Thr Ser Ser Leu Val Ala Ala
115         120         125
Ala Ala Thr Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
130         135         140
Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
145         150         155         160
Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
165         170         175
Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
180         185         190
Thr Thr Phe Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
195         200         205
Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
210         215         220
Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg
225         230         235
Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
245         250         255
Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu
260         265         270
Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln
275         280         285
Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp
290         295         300
Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
305         310         315         320
Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
325         330         335
Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
340         345         350
Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr

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340                      345                      350

Glu Phe Val Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr  
           355                                      360                                      365

Lys

<210> SEQ ID NO 232  
 <211> LENGTH: 350  
 <212> TYPE: PRT  
 <213> ORGANISM: Melampsora lini

<400> SEQUENCE: 232

Met Arg Gly Ser His His His His His His Gly Met Ala Ser Met Thr  
 1                                      5                                      10                                      15

Gly Gly Gln Gln Met Gly Arg Asp Leu Tyr Asp Asp Asp Asp Lys Asp  
                                     20                                      25                                      30

Arg Trp Gly Ser Arg Ser Met Gly Glu Phe Leu Glu Asp Ala Arg Asp  
                                     35                                      40                                      45

Ile Gln Gly Phe Ser Arg Lys Ser Gly Ser Lys Leu Glu Glu Glu Ser  
                                     50                                      55                                      60

Asp Ser Ser Arg Asp Arg Gln Glu Lys Glu Phe Arg Ser Thr Met Ser  
 65                                      70                                      75                                      80

Gly Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Ser Met Gly Ser Gly  
                                     85                                      90                                      95

Ile Gln Arg Pro Thr Ser Thr Ser Ser Leu Val Ala Ala Ala Ala Thr  
                                     100                                      105                                      110

Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val  
                                     115                                      120                                      125

Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu  
                                     130                                      135                                      140

Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys  
 145                                      150                                      155                                      160

Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe  
                                     165                                      170                                      175

Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln  
                                     180                                      185                                      190

His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg  
                                     195                                      200                                      205

Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val  
                                     210                                      215                                      220

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile  
 225                                      230                                      235                                      240

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn  
                                     245                                      250                                      255

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly  
                                     260                                      265                                      270

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val  
                                     275                                      280                                      285

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro  
                                     290                                      295                                      300

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser  
 305                                      310                                      315                                      320

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val





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<210> SEQ ID NO 239  
<211> LENGTH: 51  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning  
  
<400> SEQUENCE: 239

catatggata gccggacatg gtggatctgt cgacgttttg aatatctgtg c 51

<210> SEQ ID NO 240  
<211> LENGTH: 37  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning  
  
<400> SEQUENCE: 240

atgcagatct atgggaaaca accttgaac agtaccg 37

<210> SEQ ID NO 241  
<211> LENGTH: 62  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning  
  
<400> SEQUENCE: 241

catatggata gccggacatg gtggatctgt cgactcgtac attttatct tttctgcca 60

tg 62

<210> SEQ ID NO 242  
<211> LENGTH: 33  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning  
  
<400> SEQUENCE: 242

agtcagatct caccagttt acgattacaa tcc 33

<210> SEQ ID NO 243  
<211> LENGTH: 51  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning  
  
<400> SEQUENCE: 243

catatggata gccggacatg gtggatctgt cgacgttttg aatatctgtg c 51

<210> SEQ ID NO 244  
<211> LENGTH: 41

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<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 244

gaaacgcagc agctgcggca aaacctcgag ggcaatgtgt c 41

<210> SEQ ID NO 245  
<211> LENGTH: 40  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 245

gttttgagc cgctgctgcg tttctctctt tcatcatttc 40

<210> SEQ ID NO 246  
<211> LENGTH: 36  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 246

catttcgaga tgaagctggc acatccggta ctgttc 36

<210> SEQ ID NO 247  
<211> LENGTH: 48  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 247

cagcgctatt gaatcctgct gaggcaacttg ggtcttcaat ggcaggag 48

<210> SEQ ID NO 248  
<211> LENGTH: 37  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

<400> SEQUENCE: 248

gacgaagccg cagcggcttc ggattctgga gtagatg 37

<210> SEQ ID NO 249  
<211> LENGTH: 44  
<212> TYPE: DNA  
<213> ORGANISM: Artificial Sequence  
<220> FEATURE:  
<223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
amplify genes from genomes or plasmids with various restriction  
sites to facilitate cloning

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<400> SEQUENCE: 249

caaaaggcgc agaggctgca ctgtcaaatt ctggttgagg gatc 44

<210> SEQ ID NO 250

<211> LENGTH: 55

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 250

gatgctgaa cttggtccgg actagcggct gacatttttg ctccaaaagg cgcag 55

<210> SEQ ID NO 251

<211> LENGTH: 40

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 251

gtgccagctt catctgcaaa tgacaaaagt ggtactcctg 40

<210> SEQ ID NO 252

<211> LENGTH: 48

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 252

cagcaggatt caatagcgtc gccgcactca ctccagaatc cgaaaaac 48

<210> SEQ ID NO 253

<211> LENGTH: 37

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 253

gaagccgctg cggettctgc agttgaagg atccctc 37

<210> SEQ ID NO 254

<211> LENGTH: 34

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Synthetically constructed DNA sequence to amplify genes from genomes or plasmids with various restriction sites to facilitate cloning

<400> SEQUENCE: 254

cagtgcagcc tctgcctt ttggagcaaa aatg 34

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<210> SEQ ID NO 255  
 <211> LENGTH: 50  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
 amplify genes from genomes or plasmids with various restriction  
 sites to facilitate cloning

<400> SEQUENCE: 255  
 gtccggacca agttcaggca tcttcagccg atgatctcat cacatacatg 50

<210> SEQ ID NO 256  
 <211> LENGTH: 51  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
 amplify genes from genomes or plasmids with various restriction  
 sites to facilitate cloning

<400> SEQUENCE: 256  
 atcgccatgg gagaattctt agaggatgca gcgagcagcg cgggggttctc c 51

<210> SEQ ID NO 257  
 <211> LENGTH: 42  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
 amplify genes from genomes or plasmids with various restriction  
 sites to facilitate cloning

<400> SEQUENCE: 257  
 caaatcgctg ctgcccgcgc acgaccattt gatgtggaat gt 42

<210> SEQ ID NO 258  
 <211> LENGTH: 36  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Synthetically constructed DNA sequence to  
 amplify genes from genomes or plasmids with various restriction  
 sites to facilitate cloning

<400> SEQUENCE: 258  
 tgctgccgca gcagcgattt gtccgcacat acattc 36

<210> SEQ ID NO 259  
 <211> LENGTH: 24  
 <212> TYPE: PRT  
 <213> ORGANISM: *Phytophthora sojae*

<400> SEQUENCE: 259  
 Ala Met Val Glu Ser Pro Asp Leu Val Arg Arg Ser Leu Arg Asn Gly  
 1                    5                    10                    15  
 Asp Ile Ala Gly Gly Arg Phe Leu  
                   20

<210> SEQ ID NO 260  
 <211> LENGTH: 24  
 <212> TYPE: PRT  
 <213> ORGANISM: *Phytophthora sojae*

<400> SEQUENCE: 260

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Ser Leu Arg Asn Gly Asp Ile Ala Gly Gly Arg Phe Leu Arg Ala His  
 1 5 10 15

Glu Glu Asp Asp Ala Gly Glu Arg  
 20

<210> SEQ ID NO 261  
 <211> LENGTH: 117  
 <212> TYPE: PRT  
 <213> ORGANISM: *Phytophthora sojae*

<400> SEQUENCE: 261

Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met Val Glu Ser Pro Asp  
 1 5 10 15

Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile Ala Gly Gly Arg Phe  
 20 25 30

Leu Arg Ala His Glu Glu Asp Asp Ala Gly Glu Arg Thr Phe Ser Val  
 35 40 45

Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys Leu Ala Lys Ala Met  
 50 55 60

Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr Glu Lys Trp Ala Lys  
 65 70 75 80

Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp Leu Ala Ile Ala Asp  
 85 90 95

Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr Asn Gly Tyr Thr Phe  
 100 105 110

His Arg Tyr Gln Ser  
 115

<210> SEQ ID NO 262  
 <211> LENGTH: 117  
 <212> TYPE: PRT  
 <213> ORGANISM: *Phytophthora sojae*

<400> SEQUENCE: 262

Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met Val Glu Ser Pro Asp  
 1 5 10 15

Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile Ala Gly Gly Ala Ala  
 20 25 30

Ala Ala Ala His Glu Glu Asp Asp Ala Gly Glu Arg Thr Phe Ser Val  
 35 40 45

Thr Asp Leu Trp Asn Lys Val Ala Ala Lys Lys Leu Ala Lys Ala Met  
 50 55 60

Leu Ala Asp Pro Ser Lys Glu Gln Lys Ala Tyr Glu Lys Trp Ala Lys  
 65 70 75 80

Lys Gly Tyr Ser Leu Asp Lys Ile Lys Asn Trp Leu Ala Ile Ala Asp  
 85 90 95

Pro Lys Gln Lys Gly Lys Tyr Asp Arg Ile Tyr Asn Gly Tyr Thr Phe  
 100 105 110

His Arg Tyr Gln Ser  
 115

<210> SEQ ID NO 263  
 <211> LENGTH: 49  
 <212> TYPE: PRT  
 <213> ORGANISM: *Phytophthora sojae*

-continued

&lt;400&gt; SEQUENCE: 263

Ser Glu Asn Ala Phe Ser Ala Ala Thr Asp Ala Asp Gln Ala Thr Val  
 1 5 10 15  
 Ser Lys Leu Ala Ala Ala Glu Phe Asp Thr Leu Val Asp Val Leu Thr  
 20 25 30  
 Thr Glu Ser Lys Arg Ser Leu Arg Ala Thr Val Asp Asp Gly Glu Glu  
 35 40 45

Arg

&lt;210&gt; SEQ ID NO 264

&lt;211&gt; LENGTH: 44

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

&lt;400&gt; SEQUENCE: 264

Thr Glu Tyr Ser Asp Glu Thr Asn Ile Ala Met Val Glu Ser Pro Asp  
 1 5 10 15  
 Leu Val Arg Arg Ser Leu Arg Asn Gly Asp Ile Ala Gly Gly Arg Phe  
 20 25 30  
 Leu Arg Ala His Glu Glu Asp Asp Ala Gly Glu Arg  
 35 40

&lt;210&gt; SEQ ID NO 265

&lt;211&gt; LENGTH: 86

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

&lt;400&gt; SEQUENCE: 265

Leu Thr Cys Ala Thr Ser Glu Gln Gln Thr Arg Pro Glu Leu Cys Phe  
 1 5 10 15  
 Phe Phe Ser Val Arg Ser Ser Trp Pro Ser Thr Ile Ser Asp Gly Ala  
 20 25 30  
 Cys Leu Ala Leu Val Ser Ala Glu Gln Gly Ala Thr Ala Gly Arg Asn  
 35 40 45  
 Thr Leu Ser Leu Arg Ser Met Met Ala Thr Glu Asp Met Ala Thr Ser  
 50 55 60  
 Thr Arg Ser Leu Arg Ser Gln Ala Thr Asn Val Asp Asp Asp Ala Asn  
 65 70 75 80  
 Val Ser Ile Glu Asn Arg  
 85

&lt;210&gt; SEQ ID NO 266

&lt;211&gt; LENGTH: 40

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Phytophthora sojae*

&lt;400&gt; SEQUENCE: 266

Leu Ser Thr Thr Asn Ala Asn Gln Ala Lys Ile Ile Lys Gly Thr Ser  
 1 5 10 15  
 Pro Gly Gly His Ser Pro Arg Leu Leu Arg Ala Tyr Gln Pro Asp Asp  
 20 25 30  
 Glu Gly Asp Ser Pro Glu Asp Arg  
 35 40

&lt;210&gt; SEQ ID NO 267

-continued

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<211> LENGTH: 48  
 <212> TYPE: PRT  
 <213> ORGANISM: *Phytophthora sojae*

<400> SEQUENCE: 267

Val Glu Pro Ser Ala Thr Ser Thr Val Glu Val Ala Glu Val Gln Ala  
 1 5 10 15  
 Arg Gly Ala Asp Lys Arg Phe Leu Arg Ser Leu Gln Thr Glu Glu Glu  
 20 25 30  
 Gln Gly Asp Ser Asp Val Asn Glu Ala Glu Asp Gly Ser Glu Glu Arg  
 35 40 45

<210> SEQ ID NO 268  
 <211> LENGTH: 50  
 <212> TYPE: PRT  
 <213> ORGANISM: *Phytophthora sojae*

<400> SEQUENCE: 268

Ile Thr Asp Glu Ser Gln Pro Arg Asp Ala Thr Ile Val Asp Ala Pro  
 1 5 10 15  
 Leu Thr Gly Arg Gly Ala Asn Ala Arg Tyr Leu Arg Thr Ser Thr Ser  
 20 25 30  
 Ile Ile Lys Ala Pro Asp Ala Gln Leu Pro Ser Thr Lys Ala Ala Ile  
 35 40 45  
 Ala Ser  
 50

<210> SEQ ID NO 269  
 <211> LENGTH: 44  
 <212> TYPE: PRT  
 <213> ORGANISM: *Phytophthora sojae*

<400> SEQUENCE: 269

Thr Ala Glu Val Asp Ser Lys Thr Ala Leu Ala Ala Glu Val Pro Ala  
 1 5 10 15  
 Ala Ile Arg Ser Leu Glu Ser Asp Thr Pro Ala Ser Arg Leu Leu Arg  
 20 25 30  
 Thr Gly Thr Val Thr Ser Ala Asp Asn Glu Asp Arg  
 35 40

<210> SEQ ID NO 270  
 <211> LENGTH: 38  
 <212> TYPE: PRT  
 <213> ORGANISM: *Phytophthora infestans*

<400> SEQUENCE: 270

Ile Asp Gln Thr Lys Val Leu Val Tyr Gly Thr Pro Ala His Tyr Ile  
 1 5 10 15  
 His Asp Ser Ala Gly Arg Arg Leu Leu Arg Lys Asn Glu Glu Asn Glu  
 20 25 30  
 Glu Thr Ser Glu Glu Arg  
 35

<210> SEQ ID NO 271  
 <211> LENGTH: 33  
 <212> TYPE: PRT  
 <213> ORGANISM: *Phytophthora infestans*

<400> SEQUENCE: 271

-continued

Lys Ala Asp Ser Leu Ala Arg Thr Val Ser Val Val Asp Asn Val Lys  
1 5 10 15

Val Lys Ser Arg Phe Leu Arg Ala Gln Thr Asp Glu Lys Asn Glu Glu  
20 25 30

Arg

<210> SEQ ID NO 272  
<211> LENGTH: 50  
<212> TYPE: PRT  
<213> ORGANISM: *Phytophthora infestans*

&lt;400&gt; SEQUENCE: 272

Ala Val Ser Ser Asn Leu Asn Thr Ala Val Asn Tyr Ala Ser Thr Ser  
1 5 10 15

Lys Ile Arg Phe Leu Ser Thr Glu Tyr Asn Ala Asp Glu Lys Arg Ser  
20 25 30

Leu Arg Gly Asp Tyr Asn Asn Glu Val Thr Lys Glu Pro Asn Thr Ser  
35 40 45

Asp Glu  
50

<210> SEQ ID NO 273  
<211> LENGTH: 38  
<212> TYPE: PRT  
<213> ORGANISM: *Phytophthora infestans*

&lt;400&gt; SEQUENCE: 273

Val Ala Ala Phe Pro Ile Pro Asp Glu Ser Arg Pro Leu Ser Lys Thr  
1 5 10 15

Ser Pro Asp Thr Val Ala Pro Arg Ser Leu Arg Ile Glu Ala Gln Glu  
20 25 30

Val Ile Gln Ser Gly Arg  
35

<210> SEQ ID NO 274  
<211> LENGTH: 44  
<212> TYPE: PRT  
<213> ORGANISM: *Hyaloperonospora arabidopsidis*

&lt;400&gt; SEQUENCE: 274

Thr Glu Ser Ser Glu Thr Ser Gly Thr Ile Val His Val Phe Pro Leu  
1 5 10 15

Arg Asp Val Ala Asp His Arg Asn Asp Ala Leu Ile Asn Arg Ala Leu  
20 25 30

Arg Ala Gln Thr Ala Leu Asp Asp Asp Glu Glu Arg  
35 40

<210> SEQ ID NO 275  
<211> LENGTH: 48  
<212> TYPE: PRT  
<213> ORGANISM: *Hyaloperonospora arabidopsidis*

&lt;400&gt; SEQUENCE: 275

Leu Leu His Ala His Ala Leu His Glu Asp Glu Thr Gly Val Thr Ala  
1 5 10 15

Gly Arg Gln Leu Arg Ala Ala Ala Ser Glu Val Phe Gly Leu Ser Arg  
20 25 30

-continued

Ala Ser Phe Gly Leu Gly Lys Ala Gln Asp Pro Leu Asp Lys Phe Phe  
 35 40 45

<210> SEQ ID NO 276  
 <211> LENGTH: 92  
 <212> TYPE: PRT  
 <213> ORGANISM: Leptosphaeria maculans

<400> SEQUENCE: 276

Ser Pro Ala Thr Lys Asn Asn Val Asn Gln Pro Leu Asp Asn Ile Ser  
 1 5 10 15  
 Arg Arg Ser Glu Trp Lys Ser Val Gln Ile Ser Pro Val Lys Glu His  
 20 25 30  
 Ser Ala Lys Thr Ala Asp Asn Thr Glu Asn Asn His Asn Leu Glu Lys  
 35 40 45  
 Arg Val Phe Thr Ser Pro His Met Lys Arg Thr Phe Thr Leu Ala Leu  
 50 55 60  
 Glu Asn Thr Phe Tyr Ala Met Ala Trp Leu Ile Asp Phe Ser Phe Ser  
 65 70 75 80  
 Glu Glu Gly Glu Pro His Phe Ser Tyr Lys Leu Gln  
 85 90

<210> SEQ ID NO 277  
 <211> LENGTH: 50  
 <212> TYPE: PRT  
 <213> ORGANISM: Leptosphaeria maculans

<400> SEQUENCE: 277

Cys Arg Glu Ala Ser Ile Ser Gly Glu Ile Arg Tyr Pro Gln Gly Thr  
 1 5 10 15  
 Cys Pro Thr Lys Thr Glu Ala Leu Asn Asp Cys Asn Lys Val Thr Lys  
 20 25 30  
 Gly Leu Ile Asp Phe Ser Gln Ser His Gln Arg Ala Trp Gly Ile Asp  
 35 40 45  
 Met Thr  
 50

<210> SEQ ID NO 278  
 <211> LENGTH: 48  
 <212> TYPE: PRT  
 <213> ORGANISM: Leptosphaeria maculans

<400> SEQUENCE: 278

Gln Pro His Leu Leu Cys Ala Cys Glu Ser Gly Arg Arg Asp Gly Val  
 1 5 10 15  
 Asp Asp Thr Arg Thr Leu Lys Val Val Lys Gly Thr Gly Gly Arg Phe  
 20 25 30  
 Val Phe Ser Ser Arg Tyr Trp Thr Lys Ala Glu Gly Ala Pro His Glu  
 35 40 45

<210> SEQ ID NO 279  
 <211> LENGTH: 75  
 <212> TYPE: PRT  
 <213> ORGANISM: Magnaporthe oryzae

<400> SEQUENCE: 279

His Pro Val Tyr Asp Tyr Asn Pro Ile Pro Asn His Ile His Gly Asp

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1           5           10           15
Leu Lys Arg Arg Ala Tyr Ile Glu Arg Tyr Ser Gln Cys Ser Asp Ser
      20           25           30
Gln Ala Ser Glu Ile Arg Ala Ala Leu Lys Ser Cys Ala Glu Leu Ala
      35           40           45
Ser Trp Gly Tyr His Ala Val Lys Ser Asp Asn Arg Leu Phe Lys Leu
      50           55           60
Ile Phe Lys Thr Asp Ser Thr Asp Ile Gln Asn
      65           70           75

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<210> SEQ ID NO 280
<211> LENGTH: 50
<212> TYPE: PRT
<213> ORGANISM: Magnaporthe oryzae

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<400> SEQUENCE: 280

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Leu Pro Thr Pro Ala Ser Leu Asn Gly Asn Thr Glu Val Ala Thr Ile
1           5           10           15
Ser Asp Val Lys Leu Glu Ala Arg Ser Asp Thr Thr Tyr His Lys Cys
      20           25           30
Ser Lys Cys Gly Tyr Gly Ser Asp Asp Ser Asp Ala Tyr Phe Asn His
      35           40           45
Lys Cys
      50

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<210> SEQ ID NO 281
<211> LENGTH: 51
<212> TYPE: PRT
<213> ORGANISM: Magnaporthe oryzae

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<400> SEQUENCE: 281

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Arg Phe Cys Val Tyr Tyr Asp Gly His Leu Pro Ala Thr Arg Val Leu
1           5           10           15
Leu Met Tyr Val Arg Ile Gly Thr Thr Ala Thr Ile Thr Ala Arg Gly
      20           25           30
His Glu Phe Glu Val Glu Ala Lys Asp Gln Asn Cys Lys Val Ile Leu
      35           40           45
Thr Asn Gly
      50

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<210> SEQ ID NO 282
<211> LENGTH: 47
<212> TYPE: PRT
<213> ORGANISM: Magnaporthe oryzae

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<400> SEQUENCE: 282

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Ser Phe Val Gln Cys Asn His His Leu Leu Tyr Asn Gly Arg His Trp
1           5           10           15
Gly Thr Ile Arg Lys Lys Ala Gly Trp Ala Val Arg Phe Tyr Glu Glu
      20           25           30
Lys Pro Gly Gln Pro Lys Arg Leu Val Ala Ile Cys Lys Asn Ala
      35           40           45

```

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<210> SEQ ID NO 283
<211> LENGTH: 44
<212> TYPE: PRT
<213> ORGANISM: Magnaporthe oryzae

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-continued

&lt;400&gt; SEQUENCE: 283

Glu Thr Gly Asn Lys Tyr Ile Glu Lys Arg Ala Ile Asp Leu Ser Arg  
 1 5 10 15  
 Glu Arg Asp Pro Asn Phe Phe Asp His Pro Gly Ile Pro Val Pro Glu  
 20 25 30  
 Cys Phe Trp Phe Met Phe Lys Asn Asn Val Arg Gln  
 35 40

&lt;210&gt; SEQ ID NO 284

&lt;211&gt; LENGTH: 43

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Fusarium oxysporum

&lt;400&gt; SEQUENCE: 284

Leu Pro Lys Gly Glu Glu Gly Asp Ile Ile Gly Thr Phe Asn Phe Ser  
 1 5 10 15  
 Ser Ser Asp Ser Gln Pro Leu Lys Ile His Trp Val Asp Thr Pro Asp  
 20 25 30  
 Ser Ser Gly Ser Asn Leu Val Lys Arg Ser Ala  
 35 40

&lt;210&gt; SEQ ID NO 285

&lt;211&gt; LENGTH: 81

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Fusarium oxysporum

&lt;400&gt; SEQUENCE: 285

Leu Pro Val Glu Asp Ala Asp Ser Ser Val Gly Gln Leu Gln Gly Arg  
 1 5 10 15  
 Gly Asn Pro Tyr Cys Val Phe Pro Gly Arg Arg Thr Ser Ser Thr Ser  
 20 25 30  
 Phe Thr Thr Ser Phe Ser Thr Glu Pro Leu Gly Tyr Ala Arg Met Leu  
 35 40 45  
 His Arg Asp Pro Pro Tyr Glu Arg Ala Gly Asn Ser Gly Leu Asn His  
 50 55 60  
 Arg Ile Tyr Glu Arg Ser Arg Val Gly Gly Leu Arg Thr Val Ile Asp  
 65 70 75 80  
 Val

&lt;210&gt; SEQ ID NO 286

&lt;211&gt; LENGTH: 89

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Fusarium oxysporum

&lt;400&gt; SEQUENCE: 286

Gln Glu Ala Ala Val Arg Glu Pro Gln Ile Phe Phe Asn Leu Thr Tyr  
 1 5 10 15  
 Thr Glu Tyr Leu Asp Lys Val Ala Ala Ser His Gly Ser Pro Pro Asp  
 20 25 30  
 Lys Ser Asp Leu Pro Trp Asn Asp Thr Met Gly Ser Phe Pro Gly Asn  
 35 40 45  
 Glu Thr Asp Asp Gly Val Gln Thr Glu Thr Gly Ser Ser Leu Ser Arg  
 50 55 60  
 Arg Gly His Ile Val Asn Leu Arg Lys Arg Glu Pro Phe Gly Glu Glu  
 65 70 75 80

-continued

Ser Arg Asn Asp Arg Val Thr Gln Asp  
85

<210> SEQ ID NO 287  
<211> LENGTH: 50  
<212> TYPE: PRT  
<213> ORGANISM: Fusarium oxysporum

<400> SEQUENCE: 287

Asn Pro Ala Gly Asp Ser Leu Pro Asp Asp Ala His Leu Pro Asp Arg  
1 5 10 15  
Arg Leu Ser Pro Ser Glu Val Gln Ala Leu Lys Lys Ala Gln Ile Tyr  
20 25 30  
Pro Pro Gly Tyr Ile His Lys Arg Val Thr Phe Gly Glu Gly Lys Asp  
35 40 45  
Ala Val  
50

<210> SEQ ID NO 288  
<211> LENGTH: 48  
<212> TYPE: PRT  
<213> ORGANISM: Fusarium oxysporum

<400> SEQUENCE: 288

Arg Asp His Gln Tyr Cys Ala Cys Gln Ser Gly Ser Gly Asp Ser Ile  
1 5 10 15  
Asp Ile Asp Ala Thr Thr Gln Leu Gln Asn Asp Asn Ser Lys Ser Tyr  
20 25 30  
Leu Trp Ala Gln Thr Ser Pro Ala Tyr Trp Phe Ala Asp Arg His Lys  
35 40 45

<210> SEQ ID NO 289  
<211> LENGTH: 48  
<212> TYPE: PRT  
<213> ORGANISM: Fusarium oxysporum

<400> SEQUENCE: 289

Gly Pro Leu Ala Gln Thr Glu Ser Glu Ser Ala Asp Val Ala Glu His  
1 5 10 15  
Thr Ile Asn Tyr Ile Asp Ile Ala Pro Glu Glu Phe Glu Pro Pro Lys  
20 25 30  
Ala Asn Leu Ser Ser Leu Val Ser Arg Asp Thr Leu Pro Val Ser Thr  
35 40 45

<210> SEQ ID NO 290  
<211> LENGTH: 50  
<212> TYPE: PRT  
<213> ORGANISM: Melampsora lini

<400> SEQUENCE: 290

Met Glu His Val Pro Ala Glu Leu Thr Arg Val Ser Glu Gly Tyr Thr  
1 5 10 15  
Arg Phe Tyr Arg Ser Pro Thr Ala Ser Val Ile Leu Ser Gly Leu Val  
20 25 30  
Lys Val Lys Trp Asp Asn Glu Gln Met Thr Met Pro Leu Phe Lys Trp  
35 40 45  
Ile Gly

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50

<210> SEQ ID NO 291  
 <211> LENGTH: 100  
 <212> TYPE: PRT  
 <213> ORGANISM: Melampsora lini

<400> SEQUENCE: 291

Ser Leu Ser Asn Asn Leu Gly Thr Val Pro Asp Val Pro His Gln Ile  
 1 5 10 15  
 Pro Asn Asp Lys Ser Gly Thr Pro Ala Ile Glu Asp Pro Lys Ala Ala  
 20 25 30  
 Ile Glu Asp Pro Lys Asp Met Lys Gly Phe Asn Lys Ala Leu Lys Ser  
 35 40 45  
 Thr Pro Glu Ser Glu Lys Leu Gly Thr Ser Ser Val Glu Gly Ile Pro  
 50 55 60  
 Gln Pro Glu Phe Asp Arg Gly Phe Leu Arg Pro Phe Gly Ala Lys Met  
 65 70 75 80  
 Lys Phe Leu Lys Pro Asp Gln Val Gln Lys Leu Ser Thr Asp Asp Leu  
 85 90 95  
 Ile Thr Tyr Met  
 100

<210> SEQ ID NO 292  
 <211> LENGTH: 33  
 <212> TYPE: PRT  
 <213> ORGANISM: Melampsora lini

<400> SEQUENCE: 292

Gln Tyr Val Val Asp Pro Gly Phe Gly Glu Ile Glu Cys Met Cys Gly  
 1 5 10 15  
 Gln Ile Ala Arg Leu Thr Gln Arg Pro Phe Asp Val Glu Cys Glu Ala  
 20 25 30  
 Thr

<210> SEQ ID NO 293  
 <211> LENGTH: 31  
 <212> TYPE: PRT  
 <213> ORGANISM: Melampsora lini

<400> SEQUENCE: 293

Glu Phe Leu Glu Asp Ala Arg Asp Ile Gln Gly Phe Ser Arg Lys Ser  
 1 5 10 15  
 Gly Ser Lys Leu Glu Glu Glu Ser Asp Ser Ser Arg Asp Arg Gln  
 20 25 30

<210> SEQ ID NO 294  
 <211> LENGTH: 71  
 <212> TYPE: PRT  
 <213> ORGANISM: Ustilago maydis

<400> SEQUENCE: 294

Asn Gly Ser Ile Ser Asn Ala Ser His His His Gln Arg Arg Met Val  
 1 5 10 15  
 Arg Gln Arg His Ile Glu Ala Arg Ser Ala Met Ser Trp Leu Thr Lys  
 20 25 30  
 Ile Ser Ser Lys Ala Ser Asp Trp Met Phe Gly Ser Val His Ala Pro

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      35              40              45
Asn Leu Asp Lys Lys Asp Leu Pro Lys Pro Leu Val Gly Gly Val Ala
  50              55              60

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Val Met Pro Lys Met Pro Tyr
  65              70

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<210> SEQ ID NO 295
<211> LENGTH: 47
<212> TYPE: PRT
<213> ORGANISM: Laccaria bicolor

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<400> SEQUENCE: 295

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Ser Pro Val Pro Gly Glu Val Gly Leu Val Glu Arg Gly Pro Ile Pro
  1              5              10              15
Asn Ala Val Phe Arg Arg Val Pro Glu Pro Asn Phe Phe Lys Asp Leu
      20              25              30
Leu Arg Ala Leu Gly Gln Ala Ser Gln Gly Gly Asp Leu His Arg
      35              40              45

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<210> SEQ ID NO 296
<211> LENGTH: 59
<212> TYPE: PRT
<213> ORGANISM: Acyrthosiphon pisum

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<400> SEQUENCE: 296

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Ser Ala Ala Glu Pro Tyr Asp Glu Gln Glu Glu Ala Ser Val Glu Leu
  1              5              10              15
Pro Met Glu His Arg Gln Cys Asp Glu Tyr Lys Ser Lys Ile Trp Asp
      20              25              30
Lys Ala Phe Ser Asn Gln Glu Ala Met Gln Leu Met Glu Leu Thr Phe
      35              40              45
Asn Thr Gly Lys Glu Leu Gly Ser His Glu Val
      50              55

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<210> SEQ ID NO 297
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Mayetiola destructor

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<400> SEQUENCE: 297

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Ser Pro Leu Pro Leu Ala Tyr Thr Asp Gln Val Tyr Asp Ala Cys Asp
  1              5              10              15
Arg Gln Phe Asp Glu Thr Val Arg Asn Ser Gln Pro Leu
      20              25

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<210> SEQ ID NO 298
<211> LENGTH: 154
<212> TYPE: PRT
<213> ORGANISM: Mayetiola destructor

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<400> SEQUENCE: 298

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Leu Val Leu Asp Thr Arg Ala Met Pro Glu Thr Asp Phe Glu Lys Ala
  1              5              10              15
Leu Lys Glu Trp Asn Arg Val Gln Thr Leu Val Leu Ile Ala Pro Glu
      20              25              30
Gln Arg Arg Thr Met Val Leu Ile Ala Glu His Leu Thr Asn Leu Lys
      35              40              45

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Lys Met Asn Val Asp Ser Pro Gly Gly Ser Phe Leu Tyr Leu Lys Asp  
50 55 60

Gly Asp Pro Val Ile Lys Leu Pro Ser Val Glu His Phe Glu Ile Thr  
65 70 75 80

Phe Arg Gly Pro Tyr Gly Val Asp Lys Asn Phe Ser Phe Tyr Met Pro  
85 90 95

Lys Leu Lys Lys Leu Ile Val Arg Asp Ala Asp Ala Asn Asp Lys Lys  
100 105 110

Ile Ile Lys Phe Val Ser Gln His Ser Arg Thr Leu Lys Thr Leu Asp  
115 120 125

Leu Val Ala Ala Asn Tyr Arg Thr Leu Arg Thr Leu Gly Ala Met Lys  
130 135 140

His Ile Glu Glu Phe Val Thr Ser Pro Pro  
145 150

<210> SEQ ID NO 299  
 <211> LENGTH: 50  
 <212> TYPE: PRT  
 <213> ORGANISM: *Phytophthora sojae*

<400> SEQUENCE: 299

Thr Arg Val Pro Asp Asp Ala Asn Leu Gln Ser Val Asn Ala Pro Val  
1 5 10 15

Gln Thr Val Thr Arg Ser Arg Arg Phe Leu Arg Thr Ala Asp Thr Asp  
20 25 30

Ile Val Tyr Glu Pro Lys Val His Asn Pro Gly Lys Lys Gln Val Phe  
35 40 45

Ile Glu  
50

<210> SEQ ID NO 300  
 <211> LENGTH: 101  
 <212> TYPE: PRT  
 <213> ORGANISM: *Pythium ultimum*

<400> SEQUENCE: 300

Met Met Pro Ser Thr Asp Ala His Gly Tyr Ile Ala Phe Pro Pro Ala  
1 5 10 15

Gln Tyr Lys Asp Pro Ala Thr Ala Thr Asn Tyr Asn Ala Ile Ile Thr  
20 25 30

Ala Ser Ile Asn Thr Ala Phe Ala Gly Lys Lys Trp Asp Asp Asn Pro  
35 40 45

Thr Ala Asn Thr Lys Thr Phe Thr Ala Ala Phe Lys Lys Ser Gly Tyr  
50 55 60

Thr Ser Leu Lys Gln Met Leu Asp Lys Lys Val Pro Gly Cys Gln Asn  
65 70 75 80

Ser Arg Thr Asp Phe Thr Pro Ile Lys Thr Lys Thr Tyr Lys Thr Met  
85 90 95

Glu Trp Gln Asn Asp  
100

<210> SEQ ID NO 301  
 <211> LENGTH: 99  
 <212> TYPE: PRT  
 <213> ORGANISM: *Pythium ultimum*

-continued

&lt;400&gt; SEQUENCE: 301

His Ser Gln Met Thr Val Pro Asn Pro Lys Phe Ser Asp Val Ser Lys  
 1 5 10 15  
 Ala Asn Ser Pro Leu Gly Thr Ile Asp Gly Pro Thr Val Met Pro Pro  
 20 25 30  
 Pro Ala Gly Gln Ser Tyr Ala Met Gly Thr Asp Thr Asn Ile Lys Ala  
 35 40 45  
 Tyr Val Glu Ala Phe Ala Lys Gln Thr Lys Trp Lys Thr Leu Lys Asp  
 50 55 60  
 Leu Ile Met Asp Lys Tyr Val Glu Asp Gly Asn Ile Pro Asp Arg Ala  
 65 70 75 80  
 Cys Gly Leu Thr Asp Lys Thr Tyr Met Gln Pro Leu Pro Asp Lys Tyr  
 85 90 95  
 Val Val Trp

&lt;210&gt; SEQ ID NO 302

&lt;211&gt; LENGTH: 49

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Albugo candida*

&lt;400&gt; SEQUENCE: 302

Ser Thr Met Gly Leu Ser Asn Ser Arg His Leu Glu Asp Ala Val Glu  
 1 5 10 15  
 Arg Val Leu Gly Asp Leu Lys Leu Asn Gln Lys Asp Glu Lys Gln Asn  
 20 25 30  
 Glu Asn Glu Val Asp Lys Asn Asn Ser Lys Gly Lys Asp Arg Glu Ser  
 35 40 45  
 Ser

&lt;210&gt; SEQ ID NO 303

&lt;211&gt; LENGTH: 54

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Aspergillus flavus*

&lt;400&gt; SEQUENCE: 303

Ala Gly Leu Pro Val Phe Pro Asn Gln Ala Val Leu Arg Pro Ser Leu  
 1 5 10 15  
 Ala Leu Pro Gly Asp Asn Ser His Arg Tyr Ser Leu Pro Met Phe Asp  
 20 25 30  
 Leu Gln Pro Trp Glu Arg Val Asp Glu Ile Arg Leu Ala Arg Lys Gly  
 35 40 45  
 Tyr Leu Tyr Gly Ser Pro  
 50

&lt;210&gt; SEQ ID NO 304

&lt;211&gt; LENGTH: 43

&lt;212&gt; TYPE: PRT

<213> ORGANISM: *Pyrenophora tritici-repentis*

&lt;400&gt; SEQUENCE: 304

Gln Val Lys Gly Asn Ala Ile Arg Cys Gly Gln Asp Asp Lys Ser Asp  
 1 5 10 15  
 Gln Asp Thr Arg Asn Phe Cys Arg Phe Met Phe Thr Ser Asp Arg Thr  
 20 25 30  
 Leu Lys Ile Asn Gly Glu Phe Arg Gly Asn Ala



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      35              40              45
Arg Ala Pro Ser Trp Phe Thr Ser Thr Leu Met Ala Arg Arg Leu Leu
 50              55              60

Ala Leu Ser Thr Thr Gly Thr Val Ser Thr Ile Phe Pro Asp Pro Leu
65              70              75              80

Pro Gly Asn Ser His Ala Pro Pro Ser Val Ala Gly Leu Pro
      85              90

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<210> SEQ ID NO 309
<211> LENGTH: 77
<212> TYPE: PRT
<213> ORGANISM: Aspergillus fumigatus

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<400> SEQUENCE: 309

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Val Ala Met Gly Val Ser Glu Gln Arg Lys Ala Asn Glu Arg Lys Met
 1              5              10              15

Asp Ala Arg Arg Met Ala Arg Phe Asn Ile Asp Ile Glu Thr Ser Gly
 20              25              30

Glu Thr Gln Glu Glu Asp Glu Ile Arg Gly Lys Arg Ile Val Leu Arg
 35              40              45

Asp Asn Lys Val Tyr Leu Asp Asp Pro Leu Pro Ala Asn Arg Lys His
 50              55              60

Pro Ser His Thr Ala Glu Ser Phe Tyr Ile Asp Tyr Pro
 65              70              75

```

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<210> SEQ ID NO 310
<211> LENGTH: 49
<212> TYPE: PRT
<213> ORGANISM: Aspergillus fumigatus

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<400> SEQUENCE: 310

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Pro Val Val Pro Gly Gln Thr Val Met Glu Pro Ser Ala Ala Leu Pro
 1              5              10              15

Asp Asp Gly Asp His Leu Tyr Thr Leu Pro Met Phe Asp Ile Arg Pro
 20              25              30

Trp Glu Arg Val Ser Glu Val Arg Leu Ala Arg Glu Gly Tyr Leu Tyr
 35              40              45

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Gly

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<210> SEQ ID NO 311
<211> LENGTH: 89
<212> TYPE: PRT
<213> ORGANISM: Coccidioides immitus

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<400> SEQUENCE: 311

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Pro Ile Ala Pro Ala Gly Glu Phe Pro Phe Asp Ser Ser Pro Pro Glu
 20              25              30

Ala Arg Met Thr Ile Pro Tyr Ala Asp Asn Glu Pro Asp Ser Ser Leu
 35              40              45

Ser Ile Pro Ser Trp Pro Thr Thr His Leu Leu Ala Arg Arg Leu Leu
 50              55              60

Gly Leu Ser Thr Thr Gly Val Leu Ser Thr Val Phe Pro Arg Thr Asn
 65              70              75              80

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-continued

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Arg Asp Pro Ala Leu Val Gly Val Pro
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<210> SEQ ID NO 312
<211> LENGTH: 76
<212> TYPE: PRT
<213> ORGANISM: Coccidioides immitus

<400> SEQUENCE: 312

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Lys Met Asn Leu Ile Val Ser Cys Ser Asp Pro Ser Arg Lys Ser Lys
      20      25      30
Asp Val Asp Gly Cys Phe Val Val Leu Arg Asn His Lys Leu Trp Ile
      35      40      45
Ala Ser Arg Pro Ser Asp Gly Glu Ala Asn Glu Pro Ser Asp Asp Ala
      50      55      60
Thr Arg Phe Lys Ala Ser Leu His Gln Cys His His
65      70      75

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1. A method of inhibiting entry, into a cell, of a pathogenic effector protein, said entry of said pathogenic effector protein into said cell requiring binding of at least one motif of said effector protein to at least one polar lipid of said cell, comprising the step of

- i) binding a blocking compound to said at least one motif of said pathogenic effector protein; or
  - ii) binding a blocking compound to said at least one polar lipid of said cell,
- wherein said step of binding prevents entry of said pathogenic effector protein into said cell.

2. The method of claim 1, wherein said at least one motif comprises an amino acid sequence BXZ, where

B is an amino acid selected from arginine, lysine and histidine;

X is any amino acid and may be present or absent; and

Z is an amino acid selected from leucine, methionine, isoleucine, tryptophan, tyrosine and phenylalanine.

3. The method of claim 2, and wherein said at least one motif is selected from the group consisting of RxLR, RSLR, Pexel, RYWT, RIYER, RRLLR, RRFLR, and RFYR.

4. The method of claim 3, wherein said at least one motif is RIYER or RYWT.

5. The method of claim 1, wherein said blocking compound is a polypeptide that binds to said at least one motif.

6. The method of claim 5, wherein said polypeptide is a synthetic peptide.

7. The method of claim 1, wherein said blocking compound is a polar lipid.

8. The method of claim 7 wherein said polar lipid is selected from phosphoinositides, phospholipids, and sphingolipids.

9. The method of claim 8 wherein said phosphoinositide is selected from the group consisting of phosphatidyl-inositol-3-phosphate (PI-3-P), phosphatidyl-inositol-4-phosphate (PI-4-P), phosphatidyl-inositol-5-phosphate (PI-5-P), phosphatidyl-inositol-3,4-diphosphate (PI-3,4-P2), phosphatidyl-inositol-3,5-diphosphate (PI-3,5-P2), phosphatidyl-inositol-4,5-diphosphate (PI-4,5-P2), phosphatidyl-inositol-3,4,5-

triphosphate (PI-3,4,5-P3), lysophosphatidyl-inositol-3-phosphate (LPI-3-P), lysophosphatidyl-inositol-4-phosphate (LPI-4-P), lysophosphatidyl-inositol-5-phosphate (LPI-5-P), lysophosphatidyl-inositol-3,4-diphosphate (LPI-3,4-P2), lysophosphatidyl-inositol-3,5-diphosphate (LPI-3,5-P2), lysophosphatidyl-inositol-4,5-diphosphate (LPI-4,5-P2), and lysophosphatidyl-inositol-3,4,5-triphosphate (LPI-3,4,5-P3), and phosphatidyl-inositol (PI), and lysophosphatidyl-inositol (LPI).

10. The method of claim 7 wherein said polar lipid is selected from the group consisting of phosphatidyl-serine (PS), phosphatidyl-glycerol (PG), phosphatidyl-ethanolamine (PE), phosphatidyl-choline (PC), lysophosphatidyl-serine (LPS), lysophosphatidyl-glycerol (LPG), lysophosphatidyl-ethanolamine (LPE), lysophosphatidyl-choline (LPC), phosphatidic acid (PA), lysophosphatidic acid (LPA), sphingosine-1-phosphate (S-1-P), ceramide-1-phosphate (C-1-P), a glycosylphosphatidylinositol (GPI)-protein anchor, a glycosylsphingosylinositol (GSI)-protein anchor, a glycosyl phosphoryl inositol ceramide (GPIC) and sphingomyelin (SM).

11. The method of claim 1, wherein said blocking compound is selected from the group consisting of: an inositol phosphate, an inositol sulfate, an inositol carboxylate, an inositol arsenate, an inositol phosphorothioate, a hexose phosphate, a hexose sulfate, a hexose carboxylate, a hexose arsenate, a hexose phosphorothioate, a hexitol phosphate, a hexitol sulfate, a hexitol carboxylate, a hexitol arsenate, a hexitol phosphorothioate, a polyol phosphate, a polyol sulfate, a polyol carboxylate, a polyol arsenate, a polyol phosphorothioate, a phosphorylated glycan, a sulfated glycan, a carboxylated glycan, a glycan arsenate, or a glycan phosphorothioate.

12. The method of claim 11, wherein said blocking compound is a polypeptide that binds to said at least one polar lipid.

13. The method of claim 12, wherein said polypeptide is or comprises a portion of a lipid-binding protein.

14. The method of claim 13, wherein said polypeptide comprises a domain selected from the group consisting of C1, C2, PH, FYVE, PX, ENTH, ANTH, BAR, FERM, PDZ, and tubby domains.

15. The method of claim 12, wherein said polypeptide is a synthetic peptide.

16. The method of claim 1, wherein said host cell is a plant cell.

17. The method of claim 16, wherein said plant cell is of a type selected from the group consisting of wheat, maize, rice, sorghum, barley, oats, millet, soybean, common bean (*Phaseolus* species), green pea (*Pisum* species), cowpea, chickpea, alfalfa, clover, tomato, potato, tobacco, pepper, egg plant, grape, strawberry, raspberry, cranberry, blueberry, blackberry, hops, walnut, apple, peach, plum, pistachio, apricot, almond, pear, avocado, cacao, coffee, tea, pineapple, passionfruit, coconut, date and oil palm, citrus, orange, lemon, grapefruit, safflower, carrot, sesame, common bean, banana, citrus, papaya, macadamia, guava, pomegranate, pecan, *Brassica* species, canola, cabbage, cauliflower, mustard, cucurbits, pumpkin, cantalope, squash, zucchini, melon, cotton, sugar cane, sugar beets, sunflower, lettuce, onion, garlic, ornamental cut flowers; and grass.

18. The method of claim 1 wherein said host cell is an animal cell.

19. The method of claim 18, wherein said animal cell is from or in an animal selected from the group consisting of cattle, sheep, pigs, goats, horses, cats, dogs, chickens, turkeys, bees, salmon, trout, bass, catfish, shellfish, crayfish, lobsters, shrimp, and crabs.

20. The method of claim 19 wherein said animal cell is a human cell.

21. The method of claim 19 wherein said animal cell is a red blood cell, a lymphocyte, a macrophage, a neutrophil, a dendritic cell, a spleen cell, a thymus cell, a liver cell, a nerve cell, a brain cell, a lung cell, a muscle cell, or an epithelial cell.

22. The method of claim 1 wherein said pathogenic effector protein is from an oomycete.

23. The method of claim 22 wherein said oomycete is selected from the group consisting of: a *Phytophthora* species, *Phytophthora infestans*, *Phytophthora sojae*, *Phytophthora ramorum*, *Phytophthora parasitica*, *Phytophthora capsici*, *Phytophthora nicotianae*, *Phytophthora cinnamomi*, *Phytophthora cryptogea*, *Phytophthora drechsleri*, *Phytophthora cactorum*, *Phytophthora cambivora*, *Phytophthora citrophthora*, *Phytophthora citricola*, *Phytophthora megasperma*, *Phytophthora palmivora*, *Phytophthora megakarya*, *Phytophthora boehmeriae*, *Phytophthora kernoviae*, *Phytophthora erythroseptica*, *Phytophthora fragariae*, *Phytophthora heveae*, *Phytophthora lateralis*, *Phytophthora syringae*, a *Pythium* species, *Pythium ultimum*, *Pythium aphanidermatum*, *Pythium irregulare*, *Pythium graminicola*, *Pythium arrhenomanes*, *Pythium insidiosum*, a downy mildew species, a *Peronospora* species, *Peronospora tabacina*, *Peronospora destructor*, *Peronospora sparsa*, *Peronospora viciae*, a *Bremia* species, *Bremia lactucae*, a *Plasmopora* species, *Plasmopora viticola*, *Plasmopora halstedii*, a *Pseudoperonospora* species, *Pseudoperonospora cubensis*, *Pseudoperonospora humuli*, a *Sclerospora* species, *Sclerospora graminicola*, a *Peronosclerospora* species, *Peronosclerospora philippinensis*, *Peronosclerospora sorghi*, *Peronosclerospora sacchari*, a *Sclerophthora* species, *Sclerophthora rayssiae*, *Sclerophthora macrospora*, a *Albugo* species, *Albugo candida*, a *Aphanomyces* species, *Aphano-*

*myces cochlioides*, *Aphanomyces euteiches*, *Aphanomyces invadans*, a *Saprolegnia* species, *Saprolegnia parasitica*, and a *Achlya* species.

24. The method of claim 1 wherein said pathogenic effector protein is from a fungus.

25. The method of claim 24 wherein said fungus is selected from the group consisting of: a rust fungus, a smut fungus, a bunt fungus, a powdery mildew fungus, a *Puccinia* species, *Puccinia striiformis*, *Puccinia graminis*, *Puccinia triticina* (syn. *Puccinia recondita*), *Puccinia sorghi*, *Puccinia schedonardii*, *Puccinia cacabata*, a *Phakopsora* species, *Phakopsora pachyrhizi*, *Phakopsora gossypii*, a *Phoma* species, *Phoma glycincicola*, a *Ascochyta* species, *Ascochyta gossypii*, a *Cryphonectria* species, *Cryphonectria parasitica*, a *Magnaporthe* species, *Magnaporthe oryzae*, a *Gaeumannomyces* species, *Gaeumannomyces graminis*, a *Synchytrium* species, *Synchytrium endobioticum*, a *Ustilago* species, *Ustilago maydis*, *Ustilago tritici*, *Ustilagoideia vixens*, a *Tilletia* species, *Tilletia indica*, *Tilletia caries*, *Tilletia foetida*, *Tilletia barclayana*, a *Erysiphe* species, *Erysiphe necator*, a *Blumeria* species, *Blumeria graminis*, *Podosphaera oxyacanthae*, a *Alternaria* species, *Alternaria alternata*, a *Botrytis* species, *Botrytis cinerea*, a *Diaporthe* species, *Diaporthe phaseolorum*, a *Fusarium* species, *Fusarium graminearum*, *Fusarium oxysporum*, *Fusarium moniliforme*, *Fusarium solani*, a *Leptosphaeria* species, *Leptosphaeria maculans*, *Leptosphaeria maydis*, a *Macrophomina* species, *Macrophomina phaseolina*, a *Monilinia* species, *Monilinia fructicola*, a *Mycosphaerella* species, *Mycosphaerella graminicola*, *Mycosphaerella fijiensis*, *Mycosphaerella tassiana*, *Mycosphaerella zae-maydis*, a *Phialophora* species, *Phialophora gregata*, a *Phymatotrichopsis* species, *Phymatotrichopsis omnivora*, a *Taphrina* species, *Taphrina deformans*, a *Aspergillus* species, *Aspergillus flavus*, *Aspergillus parasiticus*, *Aspergillus fumigatus*, a *Verticillium* species, *Verticillium dahliae*, *Verticillium albo-atrum*, *Rhizoctonia solani*, *Ophiostoma ulmi*, *Ophiostoma novo-ulmi*, a *Septoria* species, *Septoria avenae*, a *Pyrenophora* species, *Pyrenophora tritici-repentis*, a *Colletotrichum* species, *Colletotrichum graminicola*, a *Sclerotinia* species, *Sclerotinia sclerotiorum*, a *Sclerotium* species, *Sclerotium rolfsii*, a *Thielaviopsis* species, *Thielaviopsis basicola*, a *Coccidioides* species, *Coccidioides immitis*, a *Paracoccidioides* species, *Paracoccidioides brasiliensis*, a *Pneumocystis* species, *Pneumocystis carinii*, a *Histoplasma* species, *Histoplasma capsulatum*, a *Cryptococcus* species, *Cryptococcus neoformans*, a *Candida* species, *Candida albicans*, a microsporidial species, a *Enterocytozoon* species, a *Encephalitozoon* species and *Encephalitozoon cuniculi*.

26. The method of claim 1, wherein said pathogen effector protein is from a protozoon.

27. The method of claim 26 wherein said protozoon is selected from the group consisting of: an apicomplexan parasite, a *Plasmodium* species, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, a *Babesia* species, *Babesia bovis*, *Babesia bigemina*, a *Cryptosporidium* species, *Cryptosporidium parvum*, a *Toxoplasma* species, *Toxoplasma gondii*, a *Trypanosomatid* species, a *Trypanosoma* species, *Trypanosoma brucei*, *Trypanosoma cruzi*, *Trypanosoma congolense*, *Trypanosoma vivax*, a *Leishmania* species, *Leishmania donovani*, an amebozoan parasite, an *Entamoeba* species, *Entamoeba histolytica*, a *Mastigamoeba* species, a *Schistosoma* species, a *Onchocerca* species, and a *Giardia* species.

**28.** The method of claim 1 wherein said step of binding includes the step of providing to a plant or animal a sufficient quantity of said blocking compound so that it is present to bind to said target molecule prior to entry of said pathogenic effector protein into cells of said plant or animal.

**29.** A method for screening compounds to identify whether they are potential blocking compounds for inhibiting entry of pathogenic effector proteins into a cell, comprising the steps of:

providing one or more proteins each of which has one or more motifs which are bound by polar lipids as a prerequisite to translocation;

exposing a candidate compound to said one or more proteins; and

determining whether said candidate compound binds to said one or more motifs of said one or more proteins, and, if binding occurs, determining that said compound is a potential blocking compound for inhibiting entry of pathogenic effector proteins into a cell,

wherein said one or more motifs comprises an amino acid sequence BXZ, where

B is an amino acid selected from arginine, lysine and histidine;

X is any amino acid and may be present or absent; and

Z is an amino acid selected from leucine, methionine, isoleucine, tryptophan, tyrosine and phenylalanine.

**30.** The method of claim 29 wherein said one or more proteins provided in said providing step are pathogenic effector proteins derived from a bacterial, protozoal, fungal, oomycete or nematode source.

**31.** A method for screening compounds to identify whether they are potential blocking compounds for inhibiting entry of pathogenic effector proteins into a cell, comprising the steps of:

providing one or more polar lipids, each of which binds to one or more motifs of an effector protein as a prerequisite to translocation of said effector protein into said cell; exposing a candidate compound to said one or more polar lipids; and

determining whether said candidate compound binds to said one or more polar lipids, and, if binding occurs, determining that said compound is a potential blocking compound for inhibiting entry of pathogenic effector proteins into said cell,

wherein said one or more motifs comprises an amino acid sequence BXZ, where

B is an amino acid selected from arginine, lysine and histidine;

X is any amino acid and may be present or absent; and

Z is an amino acid selected from leucine, methionine, isoleucine, tryptophan, tyrosine and phenylalanine.

**32.** The method of claim 31, wherein said effector proteins are pathogenic effector proteins derived from a bacterial, protozoal, fungal, oomycete or nematode source.

**33.** A method of inhibiting entry, into a cell, of a pathogenic effector protein, said entry of said pathogenic effector protein into said cell requiring binding of at least one motif of said effector protein to at least one polar lipid of said cell, comprising the step of

contacting a substrate which contains or is likely to contain a pathogen comprising said pathogenic effector protein with a blocking compound, said blocking compound being capable of

i) binding to said at least one motif of said pathogenic effector protein; or

ii) binding to said at least one polar lipid of said cell,

wherein binding of said blocking compound prevents entry of said pathogenic effector protein into said cell if said pathogen comes into contact with said substrate.

**34.** The method of claim 33, wherein said substrate is selected from the group consisting of plants, fabric, water, skin and fur.

\* \* \* \* \*

专利名称(译)	通过阻断病原体蛋白质的进入来保护细胞的组合物和方法		
公开(公告)号	<a href="#">US20110212541A1</a>	公开(公告)日	2011-09-01
申请号	US12/944345	申请日	2010-11-11
[标]申请(专利权)人(译)	弗吉尼亚科技知识产权公司		
申请(专利权)人(译)	弗吉尼亚理工大学知识产权, INC.		
当前申请(专利权)人(译)	弗吉尼亚理工大学知识产权, INC.		
[标]发明人	TYLER BRETT KALE SHIV ANTIGNANI VINCENZO		
发明人	TYLER, BRETT KALE, SHIV ANTIGNANI, VINCENZO		
IPC分类号	G01N33/53 C12N5/04 C12N5/07 C12N5/071 C12N5/077 C12N5/078 C12N5/079		
CPC分类号	A01N37/46 A01N57/12 A01N57/24 A01N61/00 C12N15/8282 A61K31/683 A61K31/685 G01N33/502 A61K38/00 A61K31/6615		
优先权	61/260227 2009-11-11 US 61/128080 2008-05-19 US 61/160059 2009-03-13 US		
外部链接	<a href="#">Espacenet</a> <a href="#">USPTO</a>		

摘要(译)

致病性效应蛋白包括氨基酸共有序列BXZ的一个或多个毒力基序, 其中 B = RK或H; X =任何氨基酸或不存在; Z = L, M, I, W, Y或F) 与宿主(植物或动物)细胞上的目标极性脂质结合, 作为致病效应蛋白易位到细胞中的先决条件。通过将阻断化合物与效应蛋白的一个或多个基序或宿主细胞的脂质配体结合来防止易位。阻断化合物包括结合极性脂质或基序的合成或天然存在的多肽, 各种极性脂质, 极性脂质的亲水性头部基团等。合适的阻断化合物可通过证明与基序或靶标结合的测定来鉴定。极性脂质。

