

Supplemental Figure 1.

**Supplemental Figure 1.** 2D Gel Images of Proteins in Pepper Leaves Uninfected and Infected by *Xcv*.

Proteins were separated in the first dimension on IPG strips (24 cm, p/ 3-10 nonlinear) and in the second dimension on a 12.5% polyacrylamide SDS gel. Differentially expressed proteins are numbered: N1-N11 for newly induced proteins, U1-U7 for upregulated proteins, and D1 for downregulated proteins. These spot numbers are assigned arbitrarily, and are shown in Table 1. The p/ range of the p/ gradient strips is shown above the gel images, and the positions of protein molecular weight markers, MW (kDa), are indicated on the left.

(A) Proteins in healthy pepper leaves.

(B and C) Proteins in pepper leaves 15 h after inoculation with the virulent (compatible) Ds1

(B) and avirulent (incompatible) Bv5-4a (C) strains of Xcv, respectively.



**Supplemental Figure 2.** The Proportion of Proteins that are Differentially Expressed in Pepper Leaves Infected by the DS1 and Bv5-4a Strains of *Xcv*.

Using *Arabidopsis* putative orthologs of identified pepper proteins, these proteins were functionally categorized based on the SOLANACEAE EST Analysis System and the MIPS *Arabidopsis thaliana* genome database (MatDB).



Supplemental Figure 3. Relative Protein Expression Changes in Pepper Leaves Infected by Xcv.

The changes in protein spots were calculated with ImageMaster<sup>TM</sup> 2D Platinum 6.0 and are plotted as the relative densities of spots in Supplemental Figure 1. The values are the means  $\pm$  standard deviations of percentage volumes of proteins from three independent experiments.



Supplemental Figure 4. Differentially Expressed Protein Spots.

The relative intensities of the protein spots are shown at the right. The highest levels are shown as 100.

	Healthy	Compatible	Incompatible	<ul><li>Healthy</li><li>Compatible</li><li>Incompatible</li></ul>
N7, N8 Peroxidase		N7 → N8 →	N7 ≯ N8 →	100 N7 N8   60 40 20   0 0 0
N9 Endochitinase		-	×	100 80 60 40 20 0
N10 Osmotin-like protein precursor		*		100 80 60 40 20 0
U1 Acetyl-CoA carboxylase	*		*	100 80 60 40 20 0
U2 Light harvesting chlorophyll a/b-binding protein				100 80 60 40 20 0

Supplemental Figure 4 (continued).



Supplemental Figure 4 (continued).

## Α

MTGTTEENQP	KVQESEPQAP	SIPTSSSSDE	KEKQPEMDQQ	KWGTHIMGPP
AVPTSHPDNQ	KAAALWRAAD	QKEEFHPQPY	VVYTPVDHRP	TNNPLESVVN
FNSWSNRAET	IARNI <u>WHNLR</u>	<u>TGPSVTEAAW</u>	<mark>gk</mark> lnltak <mark>al</mark>	<u>TEGGFEPLYK</u>
QIFSTDPNEQ	<u>lkktfacyls</u>	<u>tttgpvagtl</u>	<u>YLSSTKVAFC</u>	<u>Sdrplsfk<mark>ap</mark></u>
<u>SGQEAWSYYK</u>	<u>VAIPLTNIGT</u>	<u>Mnpivmrenp</u>	<u>PER</u> YIQIVTI	DGHDFWFMGF
VNFEKATHHL	LDALSNFKAQ	PPHVGEVPQP	ASN*	

## В

gi|25038635 Mass: 13009 Score: 230 Queries matched: 3 Frame: 2 KS12040A12 KS12 Capsicum annuum cDNA, mRNA sequence Start - End Observed Mr(expt) Mr(calc) Delta Miss Sequence 6 - 17 602.3937 1202.7729 1202.5931 0 R.TGPSVTEAAWGK.L 0.1798 (Ions score 89) 24 - 35 662.9277 1323.8408 1323.6710 0.1698 K.ALTEGGFEPLYK.Q 0 (Ions score 49) 84 - 95 693.9164 1385.8182 1385.6251 0.1930 0 K.APSGQEAWSYYK.V (Ions score 95) e 0.19 Error 0.18 0.17 0.16 1200 1250 1300 1350 RMS error 139 ppm Mass (Da)

## **Probability Based Mowse Score**

Ions score is -10\*Log(P), where P is the probability that the observed match is a random event. Individual ions scores > 67 indicate identity or extensive homology (p<0.05). Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.



Supplemental Figure 5. Identification of ABR1 by LC/MS-MS.

(A) The identified peptides and their positions in the ABR1 sequence are marked in yellow. Previously reported EST sequences are underlined.

**(B)** The corresponding peptide list, delta mass error of the observed ions, and the probability based MOWSE (for Molecular Weight Search) score by MASCOT MS/MS ion search (http://www.matrixscience.com).

cttgaaaatagttttccgcacttaaatcagttaatcATGACAGGCACAACAGAAGAAAAAT 60 MTGTTEEN 8 CAACCCAAAGTTCAAGAATCAGAGCCTCAAGCACCCTCTATTCCTACATCTTCTTCT 120 Q P K V Q E S E P Q A P S I P T S S S S 28 180 DEKEKQPEMDQQKWGTHIMG 48 CCACCAGCAGTTCCAACAAGTCATCCAGATAATCAGAAAGCTGCTGCGTTATGGAGAGCT 240 P P A V P T S H P D N Q K A A A L W R A 68 GCAGACCAAAAAGAAGAGTTTCACCCACAGCCTTACGTTGTTTATACTCCAGTTGATCAT 300 A D Q K E E F H P Q P Y V V Y T P V D H 88 AGGCCTACTAATAATCCACTTGAATCTGTTGTTAATATGTTTAATTCTTGGAGTAATCGA 360 108 GCTGAGACCATCGCCCGCAACATCTGGCATAATCTGAGAACTGGACCATCAGTGACAGAA 420 A E T I A R N I W H N L R T G P S V T E 128 GCAGCGTGGGGAAAGCTTAATTTGACAGCCAAGGCCTTAACAGAAGGCGGATTCGAGCCG 480 A A W G K L N L T A K A L T E G G F E P 148 CTTTACAAGCAGATTTTCTCTACGGACCCTAATGAGCAGCTGAAGAAGACATTTGCTTGT 540 LYKQIFSTDPNEQLKKTFAC 168 TATCTTTCAACAACTACTGGTCCTGTTGCTGGAACACTCTATTTGTCATCTACTAAGGTT 600 A F C S D R P L S F K A P S G Q E A W S 208 TACTACAAGGTAGCAATACCATTGACAAACATTGGGACTATGAACCCAATAGTGATGAGA 720 Y Y K V A I P L T N I G T M N P I V M R 228 GAGAATCCACCAGAGAGGTACATTCAGATAGTTACAATCGATGGTCATGACTTCTGGTTC 780 ENPPERYIQIVTIDGHDFWF 248 ATGGGGTTTGTCAATTTTGAGAAAGCAACACATCATCTCCTTGATGCCTTGTCTAATTTT 840 MGFVNFEKATHHLLDALSNF 268 AAGGCCCAACCTCCTCATGTTGGGGAAGTGCCACAACCAGCTAGTAACTAGgaaaaaatg 900 K A Q P P H V G E V P Q P A S N \* 285 cctgcattagatatatgtattctctgtatgtatttttattgcaacattatttgtattata 1020 1172

**Supplemental Figure 6.** Nucleotide and Deduced Amino Acid Sequences of the Pepper (*Capsicum annuum*) ABA-Responsive Protein (*ABR1*) Gene cDNA.

The nucleotide and amino acid residues are numbered at the right. The initiation codon is indicated in bold and the termination codon is indicated by an asterisk (\*). The gray box represents the GRAM domain present in glucosyltransferases, myotubularin and other putative membrane-associated proteins.

Α

ABR1 Grapevine (XP_002263365; Grapevine (XP_002263309; Arabidopsis (NP_196824); Rice (ABA98234) Barley (AAD09343)	ATGTTEE	11 19 14 10 23 60
ABR1 Grapevine (XP_002263365 Grapevine (XP_002263309) Arabidopsis (NP_196824) Rice (ABA98234) Barley (AAD09343)	VQESEMDQQKWG1H1X AKESDAKKWG1H1X AKESDDAKKWG1HW AKESDDTKWG1HW AKESDDTKWG1HW AEPQTQFAKWG1HW AEPQTQFAKWG1RQ APPHPSSPLTQQAMELEGKVAAPQPGTGATPSPLTQQAVDGKDAAPTPPAESARWG1RQ	47 52 43 44 54 120
ABR1 Grapevine (XP_002263365 Grapevine (XP_002263309) Arabidopsis (NP_196824) Rice (ABA98234) Barley (AAD09343)	GEPAWETSHEDNUKAAALUWRAADQKEEFHPQEYVVVTPVDHRPTNNLL GADAMETVHEDNUK-AALUWRADEHQHTVH-QPYVQVSPVE-KPS	96 98 88 91 107 177
ABR1 Grapevine (XP_002263365 Grapevine (XP_002263309) Arabidopsis (NP_196824) Rice (ABA98234) Barley (AAD09343)	SVVNMENSWSNEAETIARSIWHNURTGPSVTEAAWGRUNUTAKALTEGEEFIVKCULS- OPVIHTENSWSRKAETIGRIWHNUKMGFSVSETAWGRVNUTAKALTEGEFSUVKUTEA- OPVIHTENSWSRKAETIARSIWHNUKMGFSVSETAGGRVNURAKAMTREGEFSUVKUTEA- PVIGMEHTWSRKAETVARLUHNUKGFSVSETAWGRVNURAKAITKGGEFSUFRUIGG- HILDFENTWSRKAETVARLUHNUKTAFSMSDAAMGRUSIGAKALSEGGEDKUVKUTEAG HILDFENTWSRKAEELASIWUNUKTAFSMSDAAMGRUSIGAKALSEGGEDKUVKUTEG-	155 157 147 150 167 236
ABR1 Grapevine (XP_002263365 Grapevine (XP_002263309) Arabidopsis (NP_196824) Rice (ABA98234) Barley (AAD09343)	TOPNEQLKKTFACVLSTTTCFVAGILVLSSTKVAECSURELSEKAESCKEAUSVVKVAIE DIDPNEKLKKTFACVLSTSTEFVAGILVLSSTACVAECSURELSEKAESCREAUSVVKVIE DIDPNEKLKKTFACVLSTSTEFVAGILVLSTARLAECSURELSETAESCREAUSVVKVVIE TEPNELLKKTFACVLSTTTCFVAGILVLSNARVAECSURELSEAAPSCREAUSVVKVVV AGADERLRKTFACVLSTTTCFVAGILVLTNRSVAECSURELSEAAPSCREAUSVVKVVIE SGPDEHVKKTFACVLSTTTCFVAGILVLTNRNAECSURELSEAAPSCREAUSVVKVMIE SGPDEHVKKTFACVLSTTTCFVAGILVLTNRNAECSURELSEAAPSCREAUSVKVVMIE	215 217 207 210 227 296
ABR1 Grapevine (XP_002263365 Grapevine (XP_002263309) Arabidopsis (NP_196824) Rice (ABA98234) Barley (AAD09343)	LINIGIMN, IVMRENPPEN, IUIVIIUGHUFWEMGEVNEEKAIHHLLUALSNEKAUPPHV DENIGTVNEVVMRENSSEKUIQILTIDGHDFWEMGEVNEEKAVHHLLSSTSEFRAHENAV DENIGTVNEVTMRENSSEKUIQILTIDGHDFWETGEVNEKATOHLLSIVSEFRAHENAV LANVATVNEVVKETPPEKUIQILTTVDGHDFWEMGEVNYEKATOHLLSIVSEFRAHA	275 277 264 270 287 326
ABR1 Grapevine (XP_002263365 Grapevine (XP_002263309) Arabidopsis (NP_196824) Rice (ABA98234) Barley (AAD09343)	GEVPQPASN 284 RPVAGASS 285 (66%) ) 264 (65%) SG 272 (63%) DTAAAVPGDGK 298 (57%) 326 (51%)	
В	Rice (BAD36095)	
	Rice (NP_001064915) Rice (EAZ09419)	
Rice (NP_0010475	24)	
	Rice (NP_001051196)	
	Group II	
Rice (NP_001047525)	Arabidopsis (NP_192070	))
Barley (AAI	009343) Grapevine (XP_002263365)	
	Rice (ABA98234) Grapevine (XP_002263369)	
	Arabidopsis (NP 196824) CaABR1	
0.1	Group I	

**Supplemental Figure 7.** Comparison of ABR1 with Other ABA-Responsive Proteins.

(A) Comparison of the deduced amino acid sequences of pepper (*Capsicum annuum*) ABAresponsive protein (ABR1) with those of grapevine unidentified protein products, *Arabidopsis* ABA-responsive protein, rice ABA-responsive protein, and barley ABA-responsive protein (Liu et al., 1999). Gaps introduced for optimal alignment are indicated by hyphens (-). The numbers at the right refer to amino acid residue positions.

**(B)** Phylogenetic analysis of ABR1 and related plant ABA-responsive protein homologs. The unit at the bottom of the tree indicates the number of substitution events. Group I, ABA-responsive proteins; Group II, unidentified proteins.



**Supplemental Figure 8.** Immunoblot analysis of Expression of ABR1 in Leaves of Empty Vector (TRV:00) and *ABR1*-Silenced (TRV:ABR1) Pepper Plants.

Total proteins were extracted 24 h after infection by *Xcv. H*: Healthy, C: Compatible, I: Incompatible, CBB: Coomassie brilliant blue.



**Supplemental Figure 9.** T-DNA Insertion Sites and Expression of *Arabidopsis* ABA-Responsive Protein-Like At *ABR1*.

(A) T-DNA insertion sites in At ABR1.

(B) RT-PCR analysis of expression of At *ABR1* in the *abr1-1* and *abr1-2* mutants.

Supplemental Table 1. Pathogen Proteins Newly Expressed in Pepper Leaves Infected by Virulent (compatible) Strain Ds1 and Avirulent (incompatible) Strain Bv5-4a of *Xanthomonas campestris* pv. *vesicatoria* 

Spot number	Protein (organism)	Accession number	Experimental pl/MW(kDa)	Theoretical pl/MW(kDa)	SC (%) <sup>a</sup>	MS used for identification
P1	Cation/multidrug efflux pump (Magnetospirillum magnetotacticum MS-1)	ZP_00055164	5.77 / 38	6.4 / 93.21	13	MALDI-TOF
P2	60 kDa chaperonin ( <i>Xanthomonas axonopodis</i> pv. <i>citri</i> strain 306)	AAM35431	5.04 / 66	5.0 / 57.15	33	MALDI-TOF
Ρ3	Distinct helicase family with a unique C-terminal domain including a metal-binding cysteine cluster ( <i>Magnetococcus</i> sp. MC-1)	ZP_00291359	4.71 / 42	5.7 / 187.88	10	MALDI-TOF

<sup>a</sup> SC: Sequence coverage.

Supplemental Table 2. Gene-Specific Primers for qRT-PCR Used in This Study

Gene	Forward and reverse primers
18S rRNA	F:5'-TATGGTGTGCACCGGTCGTCTCGT-3'
	R:5'-GCAGTTGTTCGTCTTTCATAAATCCAA-3'
CaABR1	F:5'-ATGACAGGCACAACAGAAGAAAAT-3'
	R:5'-AATAAGTTATGACAGAGCCATTTT-3'
CaBPR1	F:5'-CAGGATGCAACACTCTGGTGG-3'
	R:5'-ATCAAAGGCCGGTTGGTC-3'
CaPO2	F:5'-ATGGCAGAGAAAACCACCAGCA-3'
	R:5'-TCAAAAAAAGTGACCTCCTTTCTGT-3'
CaSAR82	F:5'-CAGGGAGATGAATTCTGAGGC-3'
	R:5'-CATATGAACCTCTATGGATTTCTG-3'

Gene	Forward and reverse primers
CaABR1	F:5'-TCTAGAATGACAGGCACAACAGAAGAA-3'
	R:5'GGATCCAATAAGTTATGACAGAGCC-3'
CaABR1 <sup>N</sup>	F:5'-TCTAGAATGACAGGCACAACAGAAGAA-3'
	R:5'-GGATCCCTCGAATCCGCCTTC-3'
CaABR1 <sup>GRAM</sup>	F:5'-TCTAGAATGCCGCTTTACAAGCAG-3'
	R:5'-GGATCCCACTATTGGGTTCATAGTCC-3'
CaABR1 <sup>C</sup>	F:5'-TCTAGAATGAGAGAGAATCCACCAGAG-3'
	R:5'GGATCCAATAAGTTATGACAGAGCC-3'
CaABR1 <sup>∆C</sup>	F:5'-TCTAGAATGACAGGCACAACAGAAGAA-3'
	R:5'-GGATCCCACTATTGGGTTCATAGTCC-3'
CaABR1 <sup>∆N</sup>	F:5'-TCTAGAATGCCGCTTTACAAGCAG-3'
	R:5'-GGATCCAATAAGTTATGACAGAGCC-3'

Supplemental Table 3. Primers for Generation of GFP Fusion Constructs Used in This Study.