Expression of the *Bs2* pepper gene confers resistance to bacterial spot disease in tomato

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The *Bs2* resistance gene of pepper specifically recognizes and confers resistance to strains of *Xanthomonas campestris* pv. *vesicatoria* that contain the corresponding bacterial avirulence gene, *avrBs2*. The involvement of *avrBs2* in pathogen fitness and its prevalence in many *X. campestris* pathovars suggests that the *Bs2* gene may be durable in the field and provide resistance when introduced into other plant species. Employing a positional cloning strategy, the *Bs2* locus was isolated and the gene was identified by coexpression with *avrBs2* in an *Agrobacterium*-mediated transient assay. A single candidate gene, predicted to encode motifs characteristic of the nucleotide binding site–leucine-rich repeat class of resistance genes, was identified. This gene specifically controlled the hypersensitive response when transiently expressed in susceptible pepper and tomato lines and in a nonhost species, *Nicotiana benthamiana*, and was designated as *Bs2*. Functional expression of *Bs2* in stable transgenic tomatoes supports its use as a source of resistance in other Solanaceous plant species.

Bacterial spot disease of tomato and pepper, caused by *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*), can be devastating to commercial production of these crops in areas of the world with high humidity and heavy rainfall. Control of *Xcv* is based largely on the application of pesticide; however, genetic resistance has been described in pepper and tomato (1–4). In pepper, several single loci (*Bs1*, *Bs2*, and *Bs3*) that confer resistance in a ''gene-for-gene'' manner have been identified (5) and the corresponding avirulence genes (*avrBs1*, *avrBs2*, and *avrBs3*) have been cloned from *Xcv* and shown to be essential for controlling resistance (6, 7).

Of particular interest is the genetic interaction that is governed by the avirulence gene *avrBs2* and the resistance (R) gene *Bs2.* The avrBs2 gene encodes a protein with homology to the *Agrobacterium tumefaciens* agrocinopine synthase and *Escherichia coli* UgpQ, suggesting a possible enzymatic function as a phosphodiesterase (8). Mutant *Xcv* strains in which the *avrBs2* gene has been disrupted or replaced are less virulent on susceptible hosts, growing 10–100 times less than the wild-type strain (8, 9). A survey of various races of *Xcv* and other pathovars of *X. campestris* also has shown that *avrBs2* is widespread (9). These studies suggest that *avrBs2* plays a highly conserved role in the fitness of *X. campestris*, and the effectiveness of the *Bs2* resistance gene in the field may be based on the fact that it controls both virulence and avirulence in *Xcv* (9).

To date, more than 15 R genes have been isolated (10). Although the genes that have been cloned are involved in specific interactions with a diverse array of pathogens, with the exception of the *Pto* kinase (11), they encode a number of common motifs at the protein level (12, 13). This is somewhat surprising given that the majority of the avirulence genes, which along with the R genes govern the specificity of these interactions, encode apparently unrelated proteins. The significance of the observed similarities among most R gene products and how these domains function to confer resistance is still unknown.

We report here the map-based cloning and characterization of the *Bs2* gene of pepper. Confirmation of the isolation of the *Bs2* gene was obtained by an *Agrobacterium*-mediated transient coexpression assay and stable transformation of tomato and *Nicotiana benthamiana*. Transgenic tomato plants expressing the pepper *Bs2* gene suppress the growth of *Xcv* in an *avrBs2*-dependent manner, indicating that the gene is able to function in a heterologous system. The *Bs2* gene is a member of the nucleotide binding site–leucine-rich repeat (NBS-LRR) class of R genes. Interestingly, the *Bs2* gene exhibits restricted taxonomic functionality (RTF), conferring a resistance response only to related genera in the Solanaceae.

Materials and Methods

Plants, Bacterial Strains, and Disease Resistance Scoring. Pepper cultivars (cv.) used were Early Calwonder (ECW; *bs1/bs1, bs2/bs2, bs3/bs3*) and the near-isogenic cv. ECW-20R (*bs1/bs1, Bs2/Bs2, bs3/bs3*) and ECW-123R (*Bs1/Bs1, Bs2/Bs2, Bs3/Bs3*). In addition, the susceptible tomato cv. VF36 and the nonhost *N. benthamiana* were used for functional assays. Disease resistance phenotypes were determined by hand infiltration of suspensions of *Xcv* (pepper race 4 strain P38 at 1×10^8 to 2×10^8) colony-forming units per ml in 10 mM $MgCl_2$ as described (6). Resistance, indicated by a HR, was scored 24–48 hr postinfiltration.

Isolation of Genomic DNA and Recombinant DNA Methods. Isolation of pepper genomic DNA was as described (14), except fresh tissue samples were used. Other molecular biological techniques were performed by using standard protocols (15, 16) unless otherwise noted.

Identification and Analysis of Yeast Artificial Chromosome (YAC) Clones. After isolation of total genomic yeast DNA and plasmid rescue of the YAC ends, probes were isolated by digesting the rescued fragments with the cloning-site enzyme, *Eco*RI, and either *Sac*I, *Bam*HI, or *Sph*I. Using previously identified recombinants (17), end probes were mapped and used to orient the YAC clones. One end probe, 22D8Sac7, was used to screen 47 *Eco*RI-digested YAC DNA pools (384 clones per pool) by DNA gel blot analysis (18), and the clone YCA92F5 was identified by colony hybridization.

cDNA Library Screening. A pepper leaf Lambda ZAP II cDNA library was constructed from ECW-20R infected with *Xcv*

Abbreviations: NBS-LRR, nucleotide binding site–leucine-rich repeat; RTF, restricted taxonomic functionality; ECW, Early Calwonder; YAC, yeast artificial chromosome; HR, hypersensitive reaction.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF202179).

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(*avrBs2*) following manufacturer's instructions (Stratagene). About 1×10^6 plaques were screened with radiolabeled YCA22D8. Lifts were prehybridized with sheared total pepper genomic DNA, YAC vector DNA, and DNA from a YAC not at the *Bs2* locus.

Cosmid Contig. *Sau*3AI and *Taq*I partially digested YCA22D8 and YCA80H11 DNA was ligated to the vector pCLD04541 (19, 20). Transduced *E. coli* DH5α were plated on medium with tetracycline (10 μg/ml) and cycloheximide (50 μg/ml). Initially, colony lifts were probed with the A2 and F1 markers. Cosmid clones were analyzed by restriction digestion with *Eco*RI and *Bam*HI. Single- and low-copy-number fragments were identified by probing cosmid blots with total pepper genomic DNA. *Eco*RI fragments not hybridizing to the probe were used to identify additional cosmids and for mapping. To confirm clone fidelity, fragments from each cosmid were used to probe DNA gel blots of the other YACs and pepper genomic DNA.

Physical Delimitation of the Locus. Sequence information from regions internal to previously identified recombinants (17) was used to design primers for the L1 marker (5′-AAGGGCCTA-CATTGGTTACC-3′ and 5′-AGCCAAAGACCGGAAT-GACG-3′) and the R1 marker (5′-GAAAACTTCCACTG-GTCTGC-3′ and 5′-CTCAGATAGACCTTGAAGG-3′). An F_2 mapping population of 1,800 plants (from an F_1 plant of an ECW $\times$ ECW-123R cross) was screened with L1 and R1 to identify recombinants between these markers and the *Bs2* locus.

Sequencing of the *Bs2* Locus. pBluescript KS(+) libraries from the cosmids C17, C18, C19, C20, C21, and C22 were constructed by sonication of cosmids. One kb size selected DNA fragments were then subcloned into the *Eco*RV site of Bluescript KS(+). Colonies containing pepper genomic DNA clones were identified by hybridization and subjected to PCR by using T3 and T7 primers. Products purified with QIAquick PCR purification kit (Qiagen) were sequenced by using SK and KS primers and the ABI Prism FACS kit (dyedeoxy terminators; PE Biosystems, Foster City, CA). Sequencing reactions were analyzed by using the ABI 377 DNA sequencer (PE Biosystems). Sequence alignment and analysis was performed by using the SEQUENCHER 3.0 software (Gene Codes, Ann Arbor, MI). Identification of coding regions was performed by using the LASERGENE DNA sequence analysis software package (DNAstar, Madison, WI), and DNA sequence similarities were identified by using BLAST, IDENTIFY, and PSORT (21–24).

5′ and 3′ Rapid Amplification of cDNA Ends (RACE) Analysis. Total RNA was isolated from pepper leaf tissue by using Trizol reagent according to supplier's instructions (GIBCO/BRL). RACE analysis was performed by using the GIBCO/BRL Life Technologies 5′ and 3′ RACE systems (Version 2.0) according to the manufacturer. For 5′ RACE, the first-strand primer was 5′-CCATCCCACACTTCACAACTCCA-3′. The nested gene-specific primers used for the PCR amplifications were 5′-CTGCTTCACCAATCATCTTAACCC-3′ and 5′-AACCTTC-GACGCGCCTTTTTTTTC-3′. For 3′ RACE analysis, the first-strand cDNA was amplified by using the primer 5′-GTCTAGTCCTCGTCAGC-3′ and reamplification was performed by using a nested primer 5′-GTCCTTGAGCGCCT-CATG-3′. The final PCR products of the 5′ and 3′ RACE reactions were cloned into the pCRII-TOPO vector (Invitrogen), and 5–10 independent clones were sequenced for each end.

***Agrobacterium*-Mediated Transient Coexpression Assay.** Constructs for the transient assays were made in pMD1, a derivative of pBI121 (CLONTECH) in which the GUS reporter gene has been replaced with a synthetic polylinker (courtesy of M. Dixon, Sainsbury Laboratory, Norwich, England). The 35S-*avrBs2* construct consists of the *avrBs2* ORF (8) cloned between the CaMV 35S promoter and the nopaline synthase 3′ sequences of pMD1 (D.D. and B. Savidge, unpublished results). The *Bs2* candidate gene construct was generated by ligating a 5′ and 3′ RACE product onto an internal, 2.3-kb *Sal*I-*Eco*RI genomic fragment from a cosmid subclone (Fig. 1*D*). These *Bs*2 sequences then were cloned between the CaMV 35S promoter and the nopaline synthase 3′ sequences of pMD1. Clones were sequenced for verification. Constructs were mobilized into *A. tumefaciens* C58C1 (pCH32) by triparental matings using standard methods. The pCH32 plasmid (courtesy of A. Hamilton, Sainsbury Laboratory, Norwich, England) was constructed by cloning the VirE operon from pSW108 (25) into the *Pvu*II site of pCH30, a derivative of the binary vector pCC113 (26). Cells were grown overnight on Luria agar (LA) containing rifampicin at 100 μg/ml, tetracycline at 5 μg/ml, and kanamycin at 50 μg/ml. Bacteria were collected and suspended in 10 mM $MgCl_2$/10 mM Mes/150 μM acetosyringone to a final OD_{600} of 0.6. After a few hours of induction in acetosyringone, *A. tumefaciens* containing the 35S-*Bs2* construct was mixed with an equal volume of cells containing the 35S-*avrBs2* construct. This mixture of cells was hand-infiltrated into the intercellular leaf spaces of ECW, VF36, and *N. benthamiana* plants with a plastic transfer pipette. As controls, suspensions of cells containing only one construct were infiltrated in comparable areas of the same leaves.

Plant Transformation. The same 35S-*Bs2* construct used in the transient assays was mobilized into the *A. tumefaciens* LBA4404 and used for the generation of stably transformed tomato (cv. VF36) and *N. benthamiana* plants by using standard procedures (27, 28). Putative transformants were analyzed by PCR using the following primers: *Bs*2 L1 (5′-CTGCTTCACCAATCATCT-TAAC-3′) and *Bs*2 R1 (5′-TGAGACTAACAGGAAC-TGTACT-3′), which amplify a 0.5-kb fragment of the *Bs2* gene. Tomato transformants were assayed for *Bs2* function by infiltration of *Xcv* P38 (*avrBs2*−) and the isogenic *Xcv* P38:*avrBs2* (*avrBs2*+). *N. benthamiana* transformants were assayed with *X. campestris* pv. *campestris* (*Xcc*) 8004 (*avrBs2*+) and the isogenic *Xcc* 8004:Δ*avrBs2* (*avrBs2*−). The *A. tumefaciens* strain containing the 35S-*avrBs2* construct also was used to detect functional expression of the *Bs2* gene in transgenic lines by using the *Agrobacterium* transient assay. The concentrations of bacteria and assay conditions were as described above. Standard bacterial growth curve assays were performed by using transgenic tomato plants (7).

Results

Identification of an Expressed Sequence at the *Bs2* Locus. Two YAC clones, YCA22D8 and YCA80H11, were isolated previously by using the cosegregating AFLP marker A2 and shown to be approximately 550 kb and 1.2 Mb, respectively (29). Mapping of end probes derived from these clones indicated that they span the *Bs2* locus (Fig. 1*A*). To identify expressed sequences in the region covered by the smaller clone, a cDNA library made from resistant pepper leaves infected with *Xcv* expressing *avrBs2* was screened with YCA22D8 DNA. Seven cDNA clones were isolated and, based on cross-hybridization and sequence, were shown to represent members of a multigene family. Southern analysis of YCA22D8 indicated that only one member of this family is located within the *Bs2* locus. This gene encodes a protein with a high degree of homology to *Rlk1* of *Arabidopsis* (30). Preliminary northern analysis of this gene, which we have designated *Prlk1*, indicates that it is induced upon infection by an avirulent *Xcv* strain and to a lesser extent with a virulent strain (T. Tai and M. Whalen, unpublished). However, *Prlk1* did not function in an *avrBs2*-dependent manner to induce a HR when assayed in the *Agrobacterium*-transient resistance assay discussed below. Given this result and the size of YCA22D8, it seemed

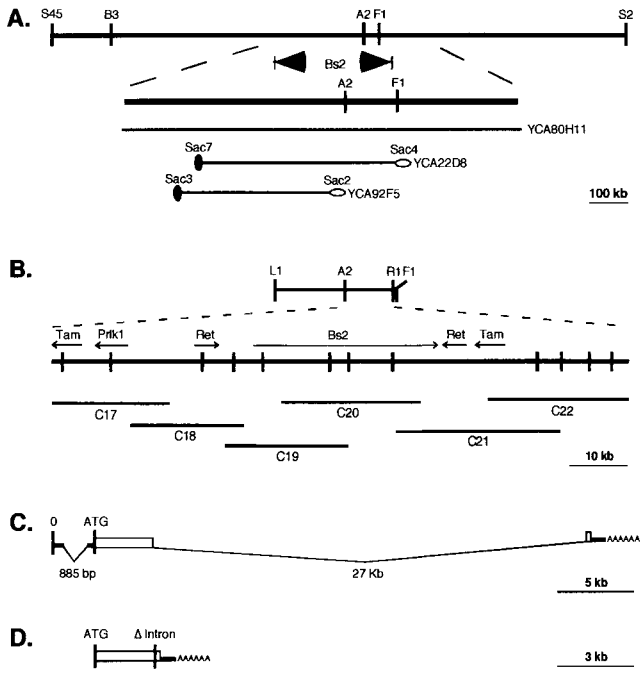

Fig. 1. Map-based cloning of the bacterial spot resistance gene *Bs2* from pepper. (*A*) Contig of the three ECW-123R pepper library YAC clones (YCA80H11, YCA22D8, and YCA92F5) that span the *Bs2* locus. (*B*) Contig of cosmid clones (C17-C22) that span the *Bs2* region from the YAC contig. This region was sequenced. Arrows show ORFs and direction of transcription for *Bs2*, *Prlk1*, *Tam* (homologous to transposon TAM), and Ret (homologous to retrotransposons). (*C*) *Bs2* transcript before splicing. (*D*) *Bs2* ORF with deletion of untranslated upstream sequences and 27-kb intron that was used in 35S:*Bs2* promoter constructs.

likely that additional expressed sequences should be present in this region that would contain *Bs2* activity.

Defining the *Bs2* Locus. A chromosome walk was initiated using cosmid libraries made from YCA22D8 and YCA80H11. Clones from the region were identified using AFLP markers, YAC ends, and *Prlk1* as probes. Additional probes were obtained from the cosmids and used to construct a contig of about 300 kb spanning the *Bs2* locus (Fig. 1*A*). Two PCR-based markers, L1 and R1, were used to screen 1,800 progeny from an F_2 population. Nine new recombinants were detected, and fine mapping with these recombinants delimited the *Bs2* locus to a region of about 100 kb spanned by cosmids C17 through C22 (Fig. 1*B*).

Sequence Analysis Identifies a *Bs2* Gene Candidate. To identify all the ORFs in the region, plasmid libraries of the cosmid contig clones were made and used for sequencing the locus. Six coding regions were identified including two retroelements, two Tam-like transposons, a sequence with homology to *Prlk1* gene, and a gene encoding a tripartite NBS and a LRR motif (Fig. 1*B*). The NBS-LRR gene was tentatively designated as the *Bs2* gene. Southern analysis indicated that it is a member of a multigene family (Fig. 2*A*) and is the only one at the locus as defined by the recombinants. Comparison of several clones obtained from 5′ RACE with the genomic sequence of the candidate *Bs2* gene revealed the presence of an 885-bp intron in the 5′ untranslated region (Fig. 1*C*). The 5′ splice site of the intron is located 52 bases downstream from the transcriptional start. A TATA box is located 34 bases upstream of the transcriptional start. 3′ RACE analysis revealed the presence of a 27-kb intron followed by

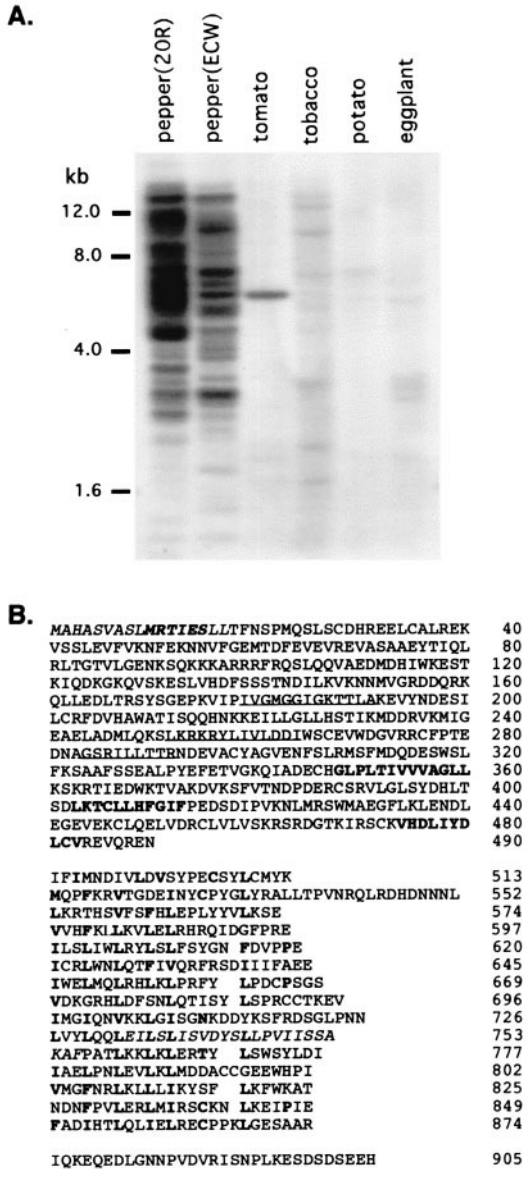

Fig. 2. (*A*) Homologs of the *Bs2* gene in DNA gel blots of various Solanaceous plants; *Eco*RI cut genomic plant DNA probed with the *Bs2* 0.5-kb PCR product of *Bs2* L1 and *Bs2* R1. (*B*) Predicted amino acid sequence of the *Bs2* gene product. Residues of significance are indicated by italics (hydrophobic domains), bold italics (mt targeting sequence), underlining (NBS), and bold (disease resistance signatures including GLPL, CFLY, MHD, and LRR).

sequence encoding 10 aa and a 280-bp untranslated 3′ region containing a polyadenylation site (Fig. 1*C*).

The sequence of the *Bs2* gene product is most similar to Rx virus resistance gene from potato (38% identity; ref. 31). The predicted protein has a putative NBS domain consisting of P-loop, kinase 2, and kinase 3a sequences followed by a domain with unknown function including sequences similar to the conserved sequence motifs GLPL, CFLY (CLLH in BS2), and MHD (VHD in *Bs2*; Fig. 2*B*). The putative LRR domain of *Bs2* has 14–15 imperfect copies of the repeat and matches the cytoplasmic LRR consensus sequence (32). *Bs2* has a hydrophobic N terminus containing a consensus sequence for mitochondrial sorting (24) and is similar to the human apoptotic protease activating factor-1 (APAF-1) through its central NBS domain (33). Unlike some other R proteins that contain a

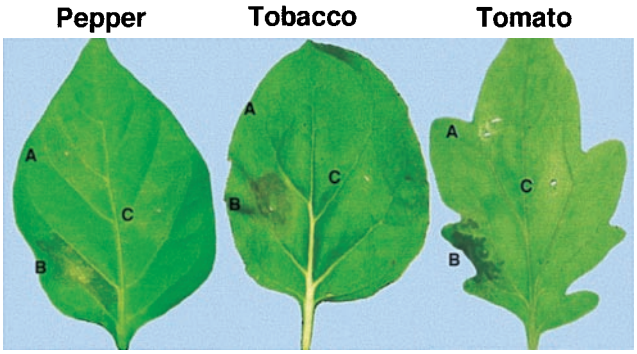

Fig. 3. Pepper (ECW), tobacco (*N. benthamiana*), and tomato (VF36) are shown 48 hr postinoculation with *Agrobacterium* containing binary plasmid 35S:*avrBs2* (*A*), mixture of 35S:*avrBs2* (*B*), and 35S:*Bs2* (*C*).

cytoplasmic LRR domain, *Bs2* does not appear to contain either leucine zipper or TIR motifs (10).

An *Agrobacterium*-Mediated Transient Coexpression Assay Confirms the *Bs2* Gene Isolation. Infiltration of *A. tumefaciens* containing a CaMV 35S-*avrBs2* construct into pepper leaves results in the induction of a HR only in plants containing the *Bs2* gene (D.D. and B. Savidge, unpublished results). Based on this result, a transient coexpression assay was developed to identify the candidate gene. In this assay, an *A. tumefaciens* strain containing the 35S-*avrBs2* construct and an *Agrobacterium* strain containing a 35S-candidate *Bs2* construct were mixed together and infiltrated into the intracellular leaf space of susceptible pepper or nonhost plants. Plants infiltrated with strains containing either the 35S-*avrBs2* or the 35S-candidate *Bs2* construct alone did not exhibit any response on susceptible pepper plants. However, plants that were infiltrated with a mixture of the strains developed a HR 24–48 hr postinfiltration (Fig. 3). The HR reactions were characteristic of *avrBs2*-induced HR in peppers with the *Bs2* gene. Similar reactions were observed when *avrBs2* and *Bs2* were transiently expressed in other Solanaceous plants including potato and eggplant; however, coinfiltration of non-Solanaceous species including *Arabidopsis*, turnip, cucumber, and broccoli did not result in a HR (data not shown). Furthermore, homology to *Bs2* was observed only in these Solanaceous plants (Fig. 2*A*). Finally, no response was observed when *A. tumefaciens* strains containing 35S-*avrBs2* and 35S-*Prlk1* constructs were coinfiltrated in pepper, tomato, or *N. benthamiana* (data not shown). The requirement of the candidate *Bs2* gene and the *avrBs2* gene for the elicitation of the HR in susceptible pepper and tomato plants and the nonhost *N. benthamiana* clearly confirms the identity of the *Bs2* gene.

Expression of the *Bs2* Gene in Tomato Confers Resistance to Bacterial Spot Disease. Tomato and *N. benthamiana* plants expressing the *Bs2* gene were generated by *Agrobacterium*-mediated plant transformation. A total of 16 tomato and 14 *N. benthamiana* primary transformants (T1) were obtained and shown to contain the *Bs2* transgene by PCR. All the T1 plants exhibited *Bs2* activity as indicated by the specific induction of a HR upon infiltration with either *Agrobacterium*- or *Xcv*-expressing *avrBs2*. Secondary transformants (T2) derived from selfing T1 plants of each species were assessed for the presence of the transgene by PCR and functional activity by bacterial infiltration. Functional activity cosegregated with the presence of the *Bs2* transgene for three different VF36 T2 populations and three different *N. benthamiana* T2 populations. To determine the effect of the *Bs2* gene on bacterial growth, bacterial growth curve assays were performed on selected T2 tomato plants by using avirulent

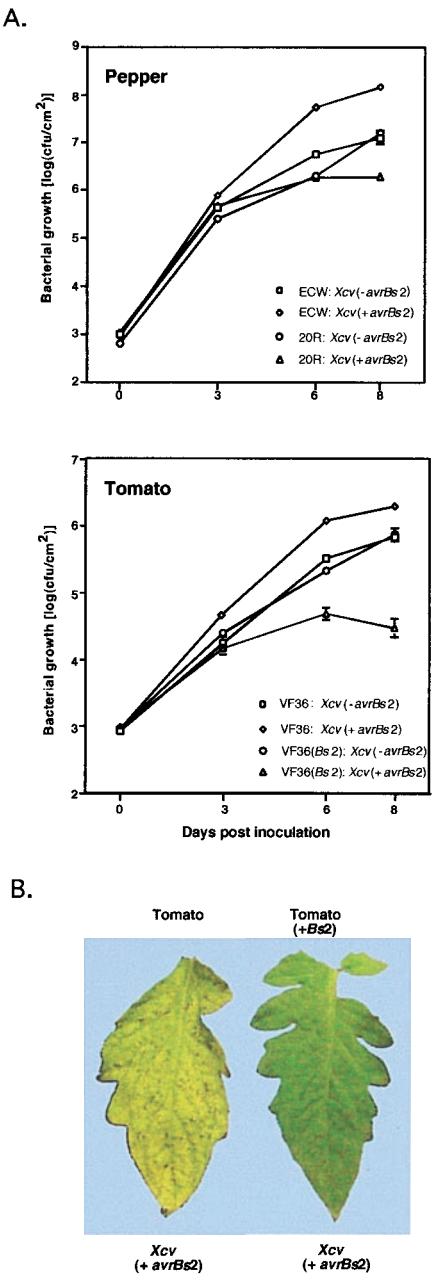

Fig. 4. (*A*) Kinetics of bacterial growth in tomato plants transformed with 35S:*Bs2*. Plants were vacuum-infiltrated with *Xcv* (with or without *avrBs2*) at a concentration of 1×10^5 colony-forming units (cfu)/ml. Bacterial concentrations in plant leaves were assayed after 0, 3, 6, and 8 days. Data points represent the mean of three replicate experiments plus or minus SE. (*B*) Disease symptoms on tomato (VF36) transformed with or without 35S:*Bs2* 2 days postinoculation of *Xcv*(*avrBs2*) at 1×10^6 cfu/ml.

(*avrBs2*+) and virulent (*avrBs2*−) strains of *Xcv* (Fig. 4*A*). These assays indicate that the *Bs2* gene suppresses the growth of *Xcv*-expressing *avrBs2* in tomato to levels characteristic of resistant plants. In addition, the effect of the *Bs2* gene can be observed by the lack of chlorotic disease symptoms on leaves inoculated with *Xcv* (Fig. 4*B*). In the case of the nonhost *N. benthamiana*, infiltration of *Xcc* containing *avrBs2*, which normally exhibits no response 24–48 hr postinfiltration, resulted in the elicitation of a HR in the transformed *N. benthamiana* plants whereas an isogenic *Xcc* strain lacking the *avrBs2* gene did not elicit a HR (data not shown).

Discussion

In agriculture, the most efficient form of protection against pathogens is genetic resistance. Like most traits manipulated by breeders, the genetic resistance typically employed is based on single, dominant or semidominant genes. These R genes usually confer race-specific resistance, and, as such, their effectiveness and durability are based on their interaction with complementary pathogen avirulence genes. The breakdown in effectiveness of a R gene often occurs as a result of the emergence of strains no longer expressing the specific avirulence gene product. In the case of the pepper *Bs2* gene, the avirulence gene *avrBs2* has been shown to be involved in the fitness of the *Xcv* pathogen and to be highly conserved among other *X. campestris* pathovars (9). Because the fitness contribution of *avrBs2* may offset the virulence of strains lacking *avrBs2*, the *Bs2* gene may provide effective, durable field resistance. Although there have been some reports of *Bs2* resistance breaking down in the field (34), these peppers are typically hybrids that are heterozygous at the *Bs2* locus and have an intermediate level of resistance (R. E. Stall, personal communication). Because the *avrBs2* gene is in other *X. campestris* pathovars that infect different plant species, transfer of the *Bs2* gene into these species may serve as a novel source of resistance. Evidence from our laboratory (D.D. and B.J.S., unpublished observations) indicates that *avrBs2* also contributes to the fitness of these other *X. campestris* pathovars, which suggests that if the *Bs2* gene is functional in other plant species, it may provide a durable resistance there as well.

In this study, we have used a positional cloning approach to isolate the *Bs2* gene. Although expression library screening using spanning YACs has been employed successfully to identify genes of interest (11), only one candidate, a putative receptor-like kinase gene *Prlk1*, was isolated when the spanning YAC clone YCA22D8 was used as a probe. The presence of such a gene in the *Bs2* region was of interest given that at least one R gene, *Xa21* from rice, has been shown to be a receptor-like kinase, although its putative receptor domain consists of a LRR motif (35). Other receptor-like kinases also have been implicated in disease resistance (36, 37), and, recently, a gene from *Brassica oleracea*, *SRF2*, was shown to be induced upon wounding and infection with *X. campestris* pv. *campestris* (38). Interestingly, Northern blot analysis has suggested that the *Prlk1* gene is induced upon infection with *Xcv* (T.H.T. and M.C.W., unpublished results).

To ensure the detection of all the putative ORFs at the *Bs2* locus, a sequencing approach was taken that resulted in the identification of an ORF encoding a putative protein with very high homology to the NBS-LRR class of R genes. RACE analyses revealed the presence of a short intron in the 5′ untranslated region and an extremely long (27-kb) intron positioned near the end of the coding region.

To verify that this was the *Bs2* gene, a transient coexpression assay was used with susceptible pepper and tomato cultivars and the nonhost *N. benthamiana.* In this assay, functional activity was defined as the induction of a HR in an *avrBs2*-dependent manner. Development of the assay provided a rapid method for testing *Bs2* gene function. Application of the assay to various plant species indicates that the *Bs2* gene that originates from pepper, a member of the Solanaceae, functions only in Solanaceous plants. This phenomenon, which we refer to as RTF, has also been observed when the *RPS2* gene of *Arabidopsis thaliana* was shown to be nonfunctional in transgenic tomatoes (D.D. and B.J.S., unpublished observations). It remains to be determined whether RTF can be overcome to allow cloned R genes, such as *Bs2*, to provide resistance in unrelated species.

Elicitation of a race-specific HR is a hallmark of many R genes; however, the HR itself has been separated from resistance (39). To show that the candidate *Bs2* gene actually confers resistance by suppressing the growth of *Xcv*, transgenic tomatoes were generated and bacterial growth assays were performed. The results show that the candidate *Bs2* gene functions in tomato to suppress the growth of an *Xcv* strain expressing *avrBs2* to levels indicative of resistance, and infected leaves showed no symptoms of disease (Fig. 4). These results confirm the identity of the *Bs2* gene and demonstrate its use as a novel source of genetic resistance to bacterial spot disease in tomato.

The putative protein encoded by the *Bs2* gene is most similar to the *Rx* virus resistance gene of potato (31) and then to various R gene products including *Prf, Mi-1,* and *I2C* from tomato (40–42) and *RPP8* from *Arabidopsis* (43). *Bs*2 has several structural features that are common to many R gene products including a putative NBS domain (P-loop, kinase 2, and kinase 3a sequences), a domain with unknown function including sequences similar to GLPL, CFLY, and MHD sequence motifs, and a cytoplasmic LRR domain (Fig. 2*B*). The N-terminal region of *Bs2* is hydrophobic and contains a mitochondrial sorting signal sequence (MRTIES) but does not contain an apparent leucine zipper (LX_6L) motif. *Bs2* is similar to APAF-1 throughout its central NBS domain, suggesting a link to mammalian regulators of apoptosis (33). Given the modular nature of proteins containing caspase recruitment domains (CARD; ref. 44), it is not surprising that the similarity of *Bs2* and APAF-1 does not extend to the N-terminal CARD or to the C-terminal WD-40 regulatory domain. The homology of NBS-LRR type R genes with APAF-1 has been noted previously (45). The presence of a mitochondrial sorting sequence in *Bs2* may be consistent with a role similar to that of APAF-1 in interacting with mitochondrial factors to regulate cell death (46). Identifying proteins that interact with BS2 will aid in understanding the relevance of this similarity.

The cloning of *Bs2* is major step in understanding its interaction with *avrBs2* and should contribute to the elucidation of the mechanism(s) involved in race-specific disease resistance. The ability of the *Bs2* gene to function in tomato provides a novel source of resistance against devastating outbreaks of bacterial spot disease in this economically important crop. Understanding the molecular basis for the RTF exhibited by *Bs2* and devising strategies to overcome it will be key to using *Bs2* in non-Solanaceous species.

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