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ORIGINAL PAPER

Genetic mapping of the bean golden yellow mosaic geminivirus resistance gene *bgm*-1 and linkage with potyvirus resistance in common bean (*Phaseolus vulgaris* L.)

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Abstract *Bean golden yellow mosaic virus* (BGYMV) is a whitefly-transmitted geminivirus of the *Begomovirus* family that causes important yield losses to common beans grown in tropical and sub-tropical countries of Latin America and the Caribbean. A major resistance gene that has been widely deployed in this region is the recessive locus *bgm*-1 that prevents the development of severe yellowing typical of the disease. In this study, we developed a co-dominant sequence-characterized amplified region (SCAR) marker, SR2, based on a previously identified random amplified polymorphic DNA (RAPD) marker that is tightly linked to the *bgm*-1 resistance gene and identified the position of the locus in the common bean genome through comparative mapping using two genetic maps for the species. The SR2 marker was mapped relative to *bgm*-1 in a segregating population of recombinant inbred lines developed from the resistant × susceptible cross of DOR476 × SEL1309. Polymorphism was shown to be based on a 37 bp insertion event in the SR2 allele associated with susceptibility compared to the allele associated with resistance and the marker mapped at a distance of 7.8 cM from the resistance gene. The SR2 marker was significantly associated with overall disease symptoms and with three of the four symptoms associated with the disease (yellowing or chlorosis, flower abortion, foliar deformation) in a greenhouse trial in Colombia with the mechanically transmissible BGYMV–Guatemala strain. In both the DOR364 × G19833 and BAT93 × Jalo EEP558 mapping populations, SR2 was located near the end of linkage group b03 (chromosome 5) suggesting a sub-telomeric position. The position of the *bgm*-1 resistance gene was estimated to be close to that of *bc*-1, a strain-specific resistance gene for *Bean common mosaic virus* (BCMV), based on linkage of SR2 with the SCAR marker SBD5 in the DOR364 × G19833 mapping population. The implications of linkage between these two recessive resistance genes are discussed, as this is the first association between resistance genes against both a begomovirus and a potyvirus.

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Introduction

Bean golden yellow mosaic virus (BGYMV) is the most serious viral disease of common bean (*Phaseolus vulgaris* L.) in Central America and the Caribbean (Morales and Anderson 2001; Morales 2005). The disease is devastating in bean production areas below 1,000 masl in many countries especially Dominican Republic, Guatemala, El Salvador, Haiti, Honduras and Nicaragua. BGYMV is also prevalent in parts of Costa Rica and southern Mexico (Morales 2005) and has been reported in Florida (Blair et al. 1995). BGYMV is transmitted in a semi-persistent manner by different biotypes of the whitefly *Bemisia tabaci* Genn., a widespread and

cosmopolitan pest that is found on a range of horticultural crops such as tomato, pepper, cucumber, and melon as well as on tobacco, soybean, and common bean (Mansoor et al. 2003; Morales 2005). The prevalence of BGYMV has increased with the widespread diffusion of a more aggressive biotype (B) of *B. tabaci* (Anderson et al. 2005). BGYMV is endemic throughout the region but is known to cause serious epidemics when conditions are favorable for the insect vector, such as low rainfall, sequential plantings, or pesticide abuse (Morales and Anderson 2001). Yield losses can be from 45 to 100% during epidemics and the disease continues to expand its range into new seasons and environments at higher altitudes and latitudes (Morales and Anderson 2001; Morales and Jones 2004).

BGYMV is a bipartite begomovirus with two molecules of single-stranded DNA encapsidated in twin (geminate), quasi-icosahedral particles (Goodman 1977; Haber et al. 1981; Mansoor et al. 2003; Fauquet et al. 2003). Related begomoviruses include *Bean golden mosaic virus* (BGMV), a virus once considered to be the same as BGYMV but which is limited to South America, predominantly in Brazil, Bolivia and Argentina, and which is not mechanically transmissible. BGYMV and BGMV induce similar symptoms but differ in their pathogenicity on certain bean genotypes with symptoms of both diseases including intense yellowing, pod deformation, stunting, and flower abortion (Morales 2005). The first of the symptoms to appear is yellowing along the veins spreading to form a reticulate pattern that coalesces across the leaf. The most severe yellowing symptoms can occur on young leaves. The yellowing typical of the disease has often been called chlorosis (Velez et al. 1998) although the etiology of the yellowing symptom is not well understood. Overall symptom severity is related to the physiological age of the plant at time of infection and varietal resistance or susceptibility. Other damaging begomoviruses affecting bean production are *Bean calico mosaic virus*; *Bean dwarf mosaic virus*, *Tomato yellow leaf curl virus* (Morales and Anderson 2001).

Breeding for resistance to the virus has been the most effective strategy for controlling the disease (Beebe 1994; Beebe et al. 1995; Morales 2001; Singh et al. 2000). This is especially true given that other forms of disease control, such as elimination of the whitefly vector with systemic insecticides, are expensive or impractical due to the relatively rapid transmission of the virus and the repeated development of insecticide resistance (Anderson et al. 2005). A few resistance genes for BGYMV have been identified in common beans and these function by attenuating symptoms and yield losses when the plant is infected (Blair and Beaver 1993; Blair et al. 1994; Beebe 1994; Urrea et al. 1996; Velez et al. 1998; Miklas et al. 1996, 2000a; Acevedo et al. 2004). One resistance source, A429, derived from the Durango landrace Garrapato (G2402), and subsequent DOR lines developed at CIAT, were shown to contain a single recessive gene, *bgm-1*, which reduces mosaic and yellowing symptoms (Morales and Niessen 1988; Blair and Beaver 1993; Velez et al. 1998). A RAPD marker was reported as linked to this resistance gene (Urrea et al. 1996) and the gene has been used for breeding resistant genotypes (Beebe 1994; Beebe et al. 1995; Singh et al. 2000; Kelly et al. 2003; Beaver et al. 2003; Miklas et al. 2006). While *bgm*-1 has been deployed in both Mesoamerican and Andean beans (Blair et al. 2006b), most other resistance genes for the disease remain comparatively under-utilized or have only been bred into a limited pool of advanced breeding materials or cultivars (Morales 2001). Given this, there is an important role for marker-assisted selection to play in the efficient transfer of BGYMV resistance genes. In this regard it is important to have well-characterized genes and marker combinations with an understanding of their inheritance in order to carry out marker-assisted selection. Our objective for this study therefore was to genetically map the *bgm*-1 resistance gene based on the development and mapping of a co-dominant SCAR marker. Although this gene-marker combination has been among the most useful for common bean breeding little was known about the locus until this research. In this study we fully characterize the SCAR marker and genetically position the locus in the bean genome using a combination of cleaved amplified polymorphic sequence (CAPS) marker analysis and comparative mapping between well-studied genetic populations.

Materials and methods

Population development

A recombinant inbred line (RIL) population was developed from the cross of DOR476 × SEL1309 through single seed descent from the F2 generation until the F5 generation. Subsequently the harvests for each of the 100 RILs were bulked until the F7 generation. The source of BGYMV resistance in the cross came from the paternal parent, DOR476 [pedigree: (DOR367 × (DOR364 × BAT1298)) where DOR367 resulted from the cross A429 × RAO30] that contains the *bgm*-1 resistance gene and the associated marker alleles derived from the A429 source, while the maternal parent SEL1309 [pedigree: SEL1152 (RAB489 ×

(A686 × UI36))] is susceptible to BGYMV. DOR476 has small red seed while SEL1309 has reddish-brown colored seed but both are of the Mesoamerican genepool and have type II growth habits. The set of 100 RILs was tested in the greenhouse at the International Center for Tropical Agriculture (CIAT) with mechanical inoculation using a BGYMV strain from Guatemala maintained by the Virology Unit of CIAT in a susceptible genotype ('Topcrop') grown in whitefly-proof cages. For each RIL treatment, ten replicate plants were grown individually in 10 cm diameter pots and evaluated for four separate symptom characteristics (chlorosis, dwarfing, flower abortion, and foliar deformation) according to Morales and Niessen (1988) using a 1–9 scale where 1 was equivalent to very resistant and 9 to very susceptible for each of the symptoms. DNA was extracted from the DOR476 × SEL1309 RILs by the method of Afanador and Haley (1993) and quantified with a Hoefer DyNA Quant 2000 fluorometer for dilution to a standard concentration of 10 ng/ul for marker amplification. Two bulks were created with DNAs from the six most susceptible and three most resistant genotypes in the DOR476 × SEL1309 population (average chlorosis scores of 5.8 and 2.0, respectively) to use along with the two parents of this population in all marker analysis. A larger number of genotypes were used in the susceptible bulk compared to the resistant bulk so as to avoid the detection of false positives in the polymorphism assays. In addition to the primary RIL population, DNAs from two other RIL populations, namely DOR364 × G19833 and BAT93 × Jalo EEP558 (Blair et al. 2003b; Freyre et al. 1998) were used for marker locus placement.

SCAR marker development and testing

SCAR development was carried out based on the RAPD band (R2) identified by Urrea et al. (1996). Briefly, amplification of the RAPD fragment was carried out in 25 µl volume reactions with the same reaction components as in this previous study. The polymorphic bands associated with resistance and susceptibility from this previous study were cut out of 1.5% low melting point agarose gels and cleaned individually with the Wizard PCR prep purification system (Promega, Madison, WI). The purified insert DNAs were cloned into the pPCR-Script Amp SK(+) plasmid vector (Stratagene, La Jolla, CA) using T4 DNA ligase (Promega) in a reaction at 20°C for 1 h followed by 16 h incubation at 4°C. The ligation reaction was transformed by heat shock into XL1-Blue MRF′ host cells and the bacteria were plated onto selective media. Several recombinant clones were picked per ligation reaction and their DNA extracted with a standard miniprep protocol (Sambrook et al. 1989). These minipreps were checked for insert size with PCR amplification and *Sac*I and *Eco*RI digestions. Clones having the expected fragments were selected and end sequenced with T7 and T3 primers and the Sequenase™ Version 2.0 DNA sequencing kit. Sequences were compared and searched for inverted repeats and SNP polymorphisms using the software Sequencher v.4.1 (Gene Codes corporation, Ann Arbor, MI) or DNAman (Lynnon Biosoft) and analyzed for sequence homology to each other and to NCBI database using BLAST (http://www.ncbi.nlm.nih.gov/BLAST) on April, 10, 2005. Specific primers were designed from the fragment ends and at an internal location using Primer 3.0 software (http://www.frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) and were tested for ability to amplify single-copy SCAR products as described below.

PCR reactions for the SCAR markers were carried out in 25 µl reaction volumes containing 50 ng of genomic DNA, 0.2 uM each of forward and reverse primers, 20 mM of total dNTP and 1 unit of *Taq* polymerase in 1X PCR buffer [10 mM of Tris–HCl (pH 7.2), 50 mM of KCl]. Adding this component separately to the PCR reaction mix tested final MgCl2 concentrations of 1.5–2.5 mM. PCR amplifications were carried out in a PTC200 thermocycler (MJ Research, presently Biorad). After amplification, 12 µl of the PCR products were combined with 5 µl of loading buffer (30% glycerol; 0.25% bromphenol blue dye) and run on 1.5% agarose gels in 0.5× TBE buffer. A total of 0.5 µg/ml ethidium bromide was added to the gels for visualization of the DNA bands on an ultraviolet transilluminator. The new SCAR was tested for amplification on a panel of genotypes representing parents of the test population described above and mapping populations used for marker placement: DOR476, SEL1309, DOR364, G19833, BAT93, Jalo EEP558 (Blair et al. 2003b; Freyre et al. 1998). Segregation for the SCAR was evaluated on the entire population of 100 individual RILs from the DOR476 × SEL1309 population where the marker was co-dominant. For the other two populations polymorphism was uncovered by CAPS marker analysis as described below.

CAPS marker development and testing

A restriction analysis was carried out on the sequenced fragment from above using the software DNAman (Lynnon Biosoft, Inc.) and nine restriction enzymes (*Alu*I, *Bam*HI, *Dpn*I, *Eco*RI, *Hae*III, *Hinf*I, *Mse*I,

*Rsa*I, and *Taq*I). These enzymes were then used to digest 12.5 µl of the SCAR product with five units of each enzyme in separate 20 µl reaction volumes for periods of 4 h at the recommended temperatures for each enzyme. The restriction fragment patterns were analyzed on 2% agarose gels run in 0.25× TBE using the undigested PCR product as a control reaction as described above. Polymorphisms were scored between the parents described above and segregation of the CAPS marker was tested on 87 individuals of the DOR364 × G19833 and 78 individuals of the BAT93 × JaloEEP558 mapping populations from Blair et al. (2003) and Freyre et al. (1998), respectively. Results from agarose gel evaluation were confirmed by mixing 3 µl of the CAP reaction with an equal volume of loading buffer (5 µl formamide, 0.4% bromophenol blue and 0.25% w/v xylene cyanol FF), denaturing at 96°C for 4 min and running on 4% polyacrylamide gels (29:1 acrylamide:bis-acrylamide) in Sequi-Gen GT electrophoresis units (Bio-Rad, USA) at 100 constant watts for approximately 1.5 h followed by silver staining.

Data analysis

Genotypic data for the SCAR and CAPS markers were used for linkage analysis in the DOR476 × SEL1309 population and in two additional populations for which genetic maps have been constructed, DOR364 × G19833 (Blair et al. 2003) and BAT93 × JaloEEP558 (Freyre et al. 1998). All genetic mapping was conducted using the software Mapmaker 2.0 for Macintosh (Lander et al. 1987) and a minimum LOD of three under the 'assign' command. Segregation distortion was evaluated with Chi-square tests and marker-trait associations in the DOR476 × SEL1309 population were determined with analysis of variance and *F* tests with the software program qGENE (Nelson 1997). Based on this last analysis, *F* test values are reported for the significant marker trait associations with a probability threshold of $P > 0.001$.

Results

SR2 development and association with the resistance gene

As a first step in the development of a SCAR marker for *bgm*-1 we evaluated the DOR476 × SEL1309 population bulks for the resistance linked RAPD polymorphism OR2 identified by Urrea et al. (1996). We identified the polymorphism in both parents and bulks and confirmed that the bands $OR2_{530}$ and $OR2_{570}$ associated with the resistant and susceptible alleles were present in DOR476 and SEL1309, respectively. Based on these results we proceeded to clone and sequence the polymorphic fragments and found the DOR476 and SEL1309 alleles to be exactly 533 and 570 bp in length, respectively. These results indicated that the larger fragment associated with susceptibility had an insertion event of 37 bp compared to the smaller fragment associated with resistance. The sequences for these bands were deposited in Genbank under the entry numbers BV681857 and BV681858. The insertion event along with the homology of the two fragments was confirmed in the sequence alignment (Fig. 1) where in addition to the indel polymorphism, we identified single nucleotide polymorphisms (SNP) and restriction enzyme (RE) digestion sites that would be useful for CAPS marker development. A total of three SNPs were found throughout the sequence of which two were C–G transversions (at nucleotide sites 142 and 321 as indicated in the figure) and one was a C–T transition (at nucleotide site 183). In a BLASTn search of the NCBI database we found that a small portion of both sequences near the second C–G transversion was homologous to a tRNA gene from *P. vulgaris* (Genbank Y15080.1; *E* value = 10–7). Several short (6–8 bp) inverted and direct repeats were found near, flanking and within the insertion event (at nucleotide sites 314, 380, 417, 454, and 507 in the sequence for SEL1309), however we were not able to find homology with any expressed genes or transposon sequences in BLASTx and BLASTn queries to the NCBI protein and EST databases (with threshold *E* values > 10^{-5}). The production of sequence information for the co-dominant RAPD bands was critical for the development of a SCAR marker as it allowed us to evaluate the actual polymorphisms underlying the co-dominant RAPD band and to design primers around key sequence differences.

Two sets of SCAR primers were developed based on the sequence information for the cloned fragments. The first set of primers (forward: CACAGCTGCCA CAGGTGGGA; reverse: CACAGCTGCCCTAAC AAAAT) were anchored to the ends of the fragment and including the decamer sequence (underlined nucleotides) that generated the original RAPD polymorphism. The second set of primers was designed to flank the insertion/deletion event with the forward primer anchored at its 3′ end to the second SNP site (CATGAGGGGCATGAGATGCG) and the reverse primer equivalent to that of the first set (CACAG CTGCCCTAACAAAAT). The marker amplified by the first set of primers was named SR2 and amplified

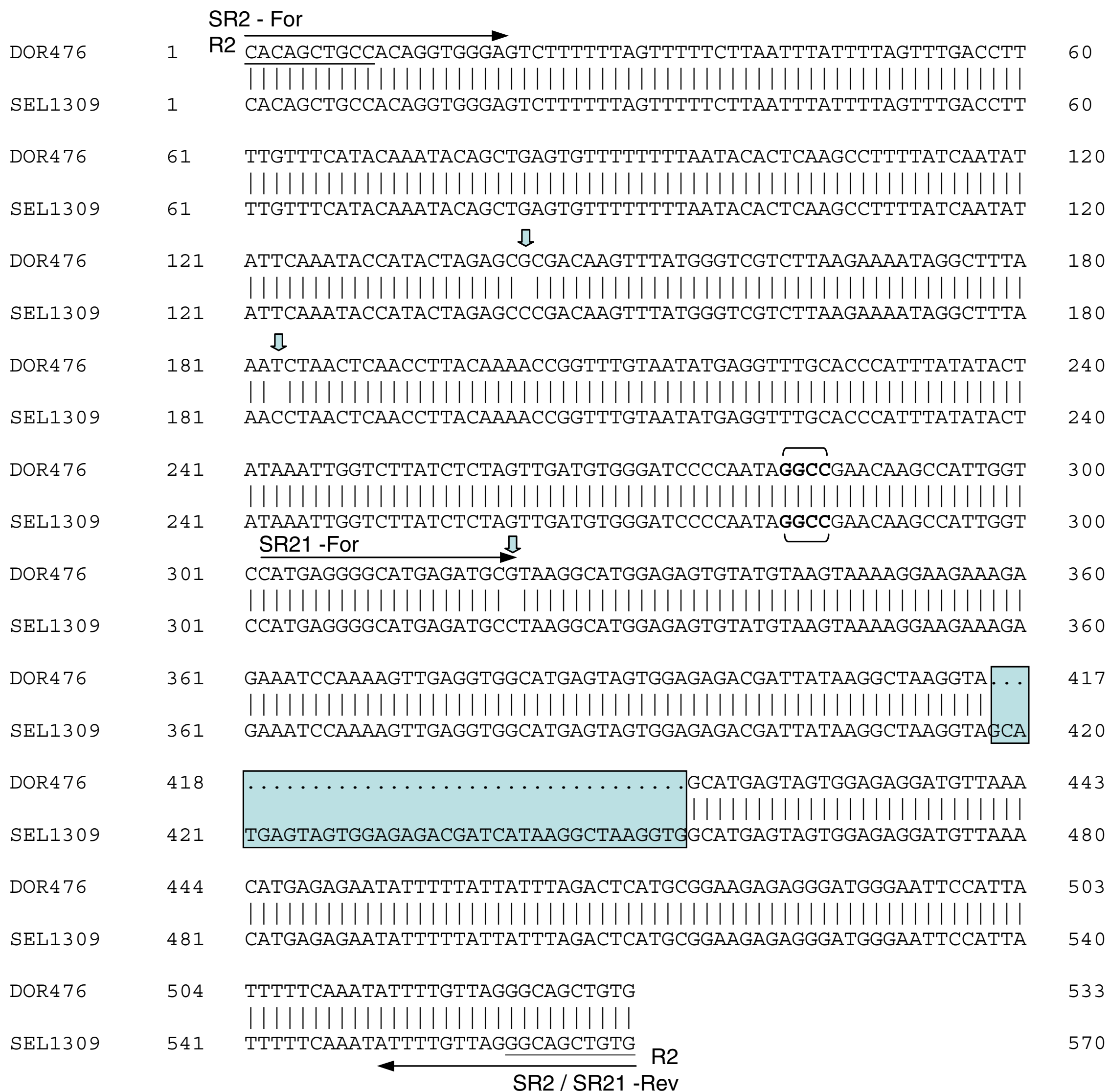

Fig. 1 Sequence alignment of the SR2 fragments cloned from SEL1309 (570 nt) and DOR 476 (533 nt). Single nucleotide polymorphisms (*arrow* above the SNP site), critical *Hae*III restriction site (*brackets*) and an insertion/deletion of 40 pb (*gray box*) are indicated. The two primer sets designed for SCAR amplification (SR2 and SR21) are shown with *right* and *left arrows* above the sequence with the RAPD primers (R2)

products of 533 bp in DOR476 and 570 bp in SEL1309 (Fig. 2). The marker amplified by the second set of primers was named SR21 and amplified products of 232 bp in DOR476 and 269 bp in SEL1309. Both SCAR markers produced strong amplification products and were diagnostic for the *bgm*-1 gene showing clear polymorphisms between resistant and susceptible parents of the DOR476 × SEL1309 population as well as with the original sources of resistance, A429 and Garrapato. The development and testing of the SR2 and SR21 markers is summarized in Fig. 2 while the central panel of Fig. 3 shows the amplification of SR2 for the parents of three mapping populations. While the SCAR marker was polymorphic for the parents of the DOR476 × SEL1309 population as expected, it was not polymorphic for the well-saturated genetic mapping populations represented by the parents DOR364 and G19833 as well as BAT93 and Jalo EEP558. Therefore we proceeded to develop a CAPS marker from SR2 as described below.

Conversion to a CAPS marker

Our strategy to detect and genetically map a polymorphism for the SR2 marker in the Andean × Mesoamerican mapping populations DOR364 × G19833 and BAT93 × Jalo EEP 558 was to develop a CAPS marker based on digestion of the PCR product with a set of restriction enzymes that have been useful previously in generating polymorphisms in common beans

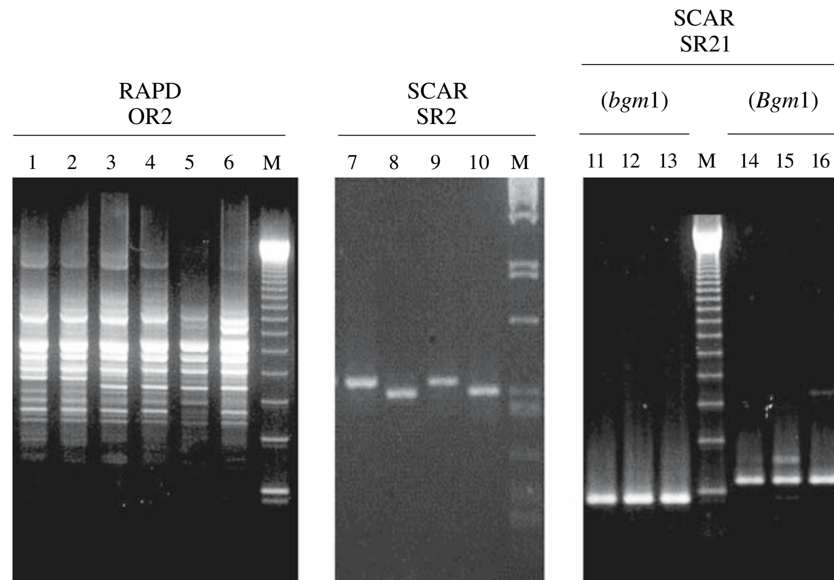

Fig. 2 Conversion of RAPD marker OR2 (*left panel*) to SCAR markers SR2 (*middle panel*) and SR21 (*right panel*). Lane genotypes are: susceptible RIL bulk (1, 9, and 15), SEL1309 (2, 7, and 14), DOR476 (3, 8, and 13), A429 (breeding line source of *bgm-1*) (4 and 12), G24404 (Garrapato–landrace source of *bgm-1*) (5 and 11), Resistant RIL bulk (6 and 10) and DOR364 (negative control) (16). *Lanes* labeled as *M* are molecular weight standards (100 bp ladder for OR2 and SR21; lambda *Pst*I for SR2)

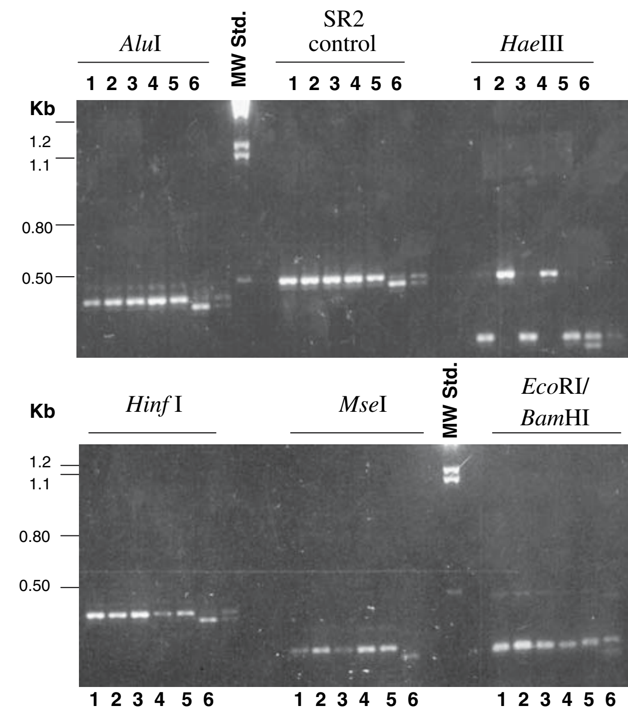

Fig. 3 Development of CAPS marker for SR2 using five restriction enzymes based on 4 or 5 bp recognition sites (*Alu*I, *Dpn*I, *Hae*III, *Hinf*I and *Mse*I) and a double digestion with two restriction enzymes with 6 pb recognition sites (*Eco*RI and *Bam*HI). Parental survey represented by genotypes DOR 364 (*lane 1*), G19833 (*lane 2*), BAT93 (*lane 3*), JaloEEP558 (*lane 4*), SEL 1309 (*lane 5*), DOR 476 (*lane 6*)

(Blair et al. 2006a). SR2 was chosen for CAPS marker development over SR21 because it gave a larger amplification fragment. The development of the CAPS marker and the control amplification of the SCAR marker are shown in Fig. 3. Results of restriction digestions agreed with the predicted restriction sites from the sequence analysis of the cloned SR2 bands for DOR476 and SEL1309. As predicted by this sequence analysis, the restriction enzymes *Alu*I, *Eco*RI/*Bam*HI, *Hinf*I, and *Mse*I generated digestion products smaller than the control amplification (Fig. 3). In the comparison with four other genotypes representing the parents of the DOR364 × G19833 and BAT93 × Jalo EEP 558 mapping populations no polymorphism was found for these five enzymes.

On the other hand, the restriction enzyme *Hae*III was successful in generating a polymorphism that distinguished the Andean and Mesoamerican parents within each of these two populations. In this case, the restriction pattern for SR2 with *Hae*III was the same for the two Mesoamerican genotypes, DOR364 and BAT93, indicating presence of the restriction site at nucleotide position 283 of the 570 bp long band associated with susceptibility for these amplified products; while the restriction site was absent at this nucleotide position for the alleles amplified for both Andean genotypes, G19833 and Jalo EEP558 in the same sized PCR product. The fragment sizes for the bands produced by the digestion of SR2 with *Hae*III in the Mesoamerican susceptible parents were 283 and 287 bp. Since these bands are nearly the same length they overlap in agarose gels and appear as a single strong band allowing the CAPS marker to be easily interpreted as a co-dominant marker (Fig. 3). The bands are separable in polyacrylamide but resolution is similar. Just like the two Mesoamerican genotypes above, DOR476 and SEL1309 both had the restriction site but due to the overall smaller size of the amplified PCR product in DOR476 this genotype gave two bands of 283 and

250 bp. Two other frequently cutting restriction enzymes which were not predicted to have recognition sites within the fragment (*Rsa*I and *Taq*I) were also tried as negative controls for CAPS development and as expected did not show restriction patterns within the SR2 PCR product. Therefore based on the parental survey with DOR476, SEL1309, DOR364, G19833, BAT93, and JaloEEP558, *Hae*III was selected as the restriction enzyme for the genetic mapping of the CAPS marker.

Genetic mapping and positioning of the bgm-1 locus

The SCAR and CAPS markers were used for genetic mapping and validated by applying them to the three recombinant inbred line populations discussed above. In the case of the SCAR markers, SR2 was amplified on the DOR476 × SEL1309 population and used to determine the association of the marker with the phenotypic data for disease resistance, with histograms showing the population distributions for disease symptom reactions of the recombinant inbred lines in this population (supplementary figure). In the single point analysis of the association between marker genotypes and phenotypic data, the *F* tests from the analysis of variance were highly significant for the overall symptom score (*F* value = 46.18; $P < 0.0001$) and for three of the four symptoms evaluated including chlorosis (*F* value = 46.18; $P < 0.0001$), foliar deformation (*F* value = 7.38; $P = 0.0078$) and flower abortion (*F* value = 23.98; $P < 0.0001$) but not dwarfing (*F* value = 0.14; $P = 0.7091$). The calculation of the genetic distance between the SR2 marker and the *bgm*-1 gene as evaluated phenotypically based on segregation data for chlorosis symptoms in the DOR476 × SEL1309 population was 7.8 cM, which is similar to the reported distance between the marker and gene reported by Urrea et al. (1996) for F2 populations of snap and dry beans. Segregation was skewed towards the SEL1309 allele (60%) over the DOR476 allele (40%), however distortion was not significant based on a Chi-square test ($X^2_{1:1} = 3.00$; $P = 0.0833$).

In the case of the CAPS marker, the *Hae*III digestion of the SR2 amplification product was used to position the locus on the genetic maps developed for the DOR364 × G19833 and BAT93 × Jalo EEP558 populations (Blair et al. 2003b; Freyre et al. 1998). In both populations, the CAPS marker mapped close to the end of linkage group b03 as shown in Fig. 4**.** The CAPS marker was 4.2 cM from the RFLP marker D1151 in the BAT93 × Jalo EEP558 population and 8.2 cM from the RAPD marker I161G in the

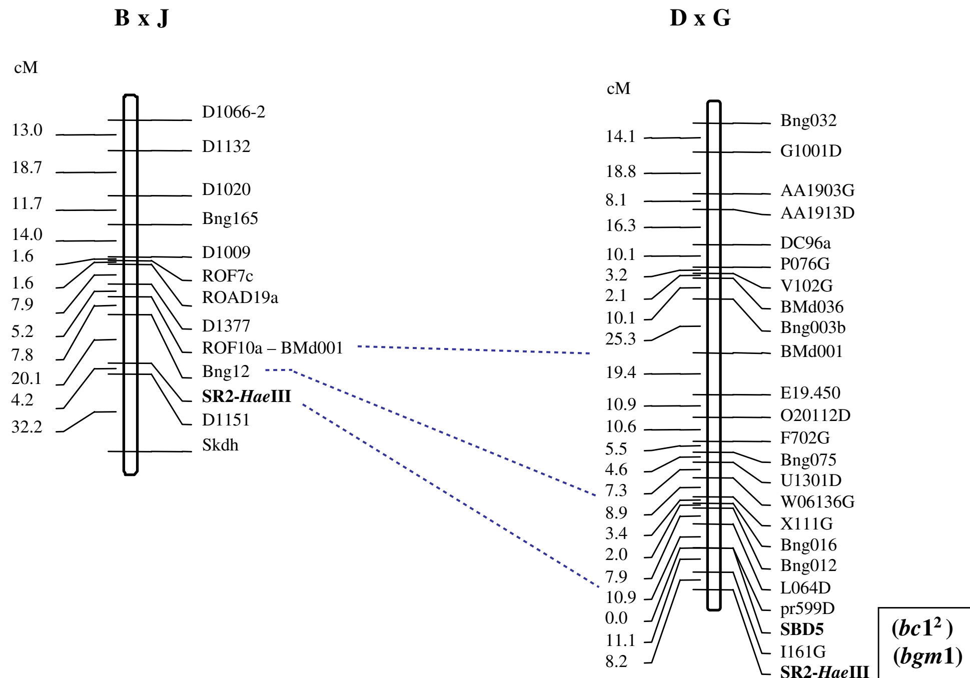

Fig. 4 Comparative mapping of the SR2/*Hae*III CAPS marker on linkage group B3 of the DOR364 × G19833 (D × G) and BAT93 × Jalo EEP558 (B × J) recombinant inbred line population maps from Blair et al. (2003) and Freyre et al. (1998). Orientation of the linkage groups according to these previous studies. Marker names indicated to the right of the linkage group with position of the CAPS marker in *bold* and *dashed* lines connecting shared markers between maps. Approximate location of *bgm*-1 and *bc*-1^2 resistance genes indicated in parenthesis based on this study and mapping of the SBD5 marker in the D × G population (Miklas et al. 2000b). Distances in Kosambi centimorgans (cM) indicated to the left of the linkage groups

DOR364 × G19833 population. In both populations the CAPS marker was distal to the RFLP marker Bng12. The RFLP Bng12 and the microsatellite marker BMd001 were mapped in both populations allowing for orientation of the linkage groups and comparison between the two maps. Segregation distortion was greater for the SR2-*Hae*III marker in the Andean × Mesoamerican mapping populations than it was for the SR2 marker in the DOR476 × SEL1309 population. Segregation distortion was toward the Mesoamerican allele in the DOR364 × G19833 population ($X^2_{1:1} = 8.24$; $P = 0.0041$) but towards the Andean allele in BAT93 × Jalo EEP558 population ($X^2_{1:1} = 4.50$; $P = 0.0339$).

Discussion

During the course of this study we created two reliable SCAR markers (SR2 and SR21) for the detection of the *bgm*-1 resistance gene. Furthermore we mapped the SR2 marker locus in two well saturated mapping populations and showed its association with resistance to the geminivirus BGYMV in the DOR476 × SEL1309 population created for this study. It was notable that the SR2 marker was especially predictive of resistance to chlorosis and associated symptoms that are the hallmark of the *bgm*-1 resistance gene (Blair and Beaver 1993; Velez et al. 1998). On the other hand, the marker was not significantly associated with resistance to dwarfing which is a reaction not associated with the *bgm*-1 resistance gene but rather other resistance factors (eg. *Dwf*; Blair and Beaver 1993; Velez et al. 1998). Other genes for normal pod development during infection (eg. *Bgp*1; Molina and Beaver 1998; Acevedo et al. 2004) or quantitative trait loci for resistance to the disease (eg. W12 locus; Miklas et al. 1996, 2000a) have been shown to exist in common beans, but most of these are related to yield tolerance under disease pressure and are most effective when present with the *bgm*-1 gene. Therefore, it was not surprising that the other traits evaluated such as flower abortion and foliar deformation were also associated with the SR2 marker along with tolerance to chlorosis symptoms. In practical terms for plant breeding, the *bgm*-1 gene is the most important gene for resistance needed in most breeding programs for BGYMV infected regions (Beebe et al. 1994, 1995; Singh et al. 2000) especially given that no systemic hypersensitive response has ever been observed for the disease (Morales and Niessen 1988) and resistance genes from other species such as those found in scarlet runner bean (*Phaseolus coccineus*) have not been widely transferred within dry beans (*P. vulgaris*) (Osorno et al. 2003).

In practical terms for marker assisted selection and laboratory analysis, the marker evaluation showed that both the SR2 and SR21 SCARs were co-dominant and in comparison to the OR2 marker detected by Urrea et al. (1996) both produced single copy bands that made them more reliable and easier to interpret than the original RAPD. Sequence comparisons between the resistant and susceptible alleles of the SR2 marker showed the source of the co-dominant polymorphism to be the presence of a 37 bp insertion/deletion event flanked by direct repeats which are hallmarks of retrotransposon activity (Kumar and Bennetzen 1999). Retrotransposons have been suggested as generators of RAPD polymorphism in several previous SCAR development studies (Erdmann et al. 2002; Blair et al. 2006a) and have also been found in genomic and cDNA libraries of common bean (Garber et al. 1999; Melotto et al. 2005). The SR21 marker was designed as an alternative to SR2 given that smaller amplification products can be useful for multiple gel loading. In addition although SR21 was designed to be co-dominant marker based on the insertion/deletion event described above it could also be converted to a dominant marker through the use of higher annealing temperatures, this due to the placement of the 3′ end of the forward primer at a single nucleotide polymorphism between DOR476 and SEL1309. The high frequency of single nucleotide and insertion/deletion based polymorphisms that occurs between these parents for this short PCR product may be a result of the introgression of the SR2 marker from resistant race Durango accessions into a race Mesoamerica background (Miklas et al. 2006).

In addition to the SCAR markers, we developed a CAPS marker from *Hae*III restriction of the SR2 amplification product which was useful for uncovering polymorphism between genotypes of the Andean and Mesoamerican gene pools and for genetic mapping of the *bgm*-1 locus in the two saturated mapping populations DOR364 × G19833 and BAT93 × Jalo EEP558 (Blair et al. 2003b; Freyre et al. 1998). The CAPS marker was based on a polymorphism located within the cloned sequence that resulted in the presence of the restriction site for the Mesoamerican genotypes not present in the Andean genotypes tested. This marker was co-dominant since the alleles with and without the restriction site could be detected in heterozygous individuals. The linkage analysis in the two Mesoamerican × Andean mapping populations allowed us to definitively place the CAPS marker associated with the *bgm*-1 locus to linkage group b03. The two mapping populations

we employed have also been useful for positioning SCAR markers in previous studies (Miklas et al. 2000b; Blair et al. 2003b; Miklas et al. 2006) and comparative mapping between populations is a valuable way to confirm genetic map locations for markers that are linked with resistance loci (Meinie et al. 2005; Blair et al. 2006a).

In both populations, the *bgm*-1 linked marker was mapped near the end of the linkage group b03. This linkage group has been shown to correspond to chromosome 5 as defined in the integrated cytogenetic and genetic map of the species (Pedrosa et al. 2003). Given the distal location of the gene and the good coverage of markers in both genetic maps for this linkage group, it is likely that the *bgm*-1 gene could be sub-telomeric. Other resistance genes have been found at sub-telomeric locations and these genomic regions are suspected to have generally greater recombination, a characteristic that is potentially important for host-pathogen resistance (Blair et al. 2003a).

The genetic location of the *bgm*-1 gene as revealed by the CAPS marker was also interesting given that the gene mapped to a region known to contain an additional locus for recessive virus resistance; namely the strain specific *bc*-1/*bc*-1^2 alleles for BCMV and BCMNV resistance (Miklas et al. 2000b). These results are based on the mapping of tightly linked SCAR marker for the *bc*-1^2 allele, SBD5 that was also placed to linkage group b03 in the DOR364 × G19833 mapping population (Miklas et al. 2000b; Fig. 4). Clusters of dominant resistance genes with multiple fungal, bacterial or viral resistance functionalities have been located in several regions of the common bean genome (reviewed in Miklas et al. 2006) and are known to be correlated with the position of resistance gene analogs that are homologous to NBS-LRR sequences (Lopez et al. 2003); while a previous report from Strausbaugh et al. (1999) suggested linkage between *bc*-1/*bc*-1^2 and *bc–u*, another bean common mosaic virus resistance gene. However, this may be the first example in common bean of linkage between recessive genes that control resistances to different viral pathogens from different viral taxa, namely the Geminiviridae and Potyviridae families. Clusters of recessive virus resistance genes with varying specificities are found for other plant species including other legumes but often involve resistance genes for potyviruses (Kang et al. 2005). We cannot estimate the exact genetic distance between the *bgm*-1 and *bc*-1/*bc*-1^2 genes since we were not able to map the SBD5 locus in the DOR476 × SEL1309 population, however given that SR2 is estimated to be 7.8 cM away from *bgm*-1 and that in the DOR364 × G19833 map the two markers are 19.3 cM apart, it is possible that the two genes are within 10 cM of each other. The genetic distance between SR2 and *bgm*-1 agree with those of Urrea et al. (1996) who found a range of 0–11 cM distance between R2 and *bgm*-1. Further dissection of the relationship between *bgm*-1 and *bc*-1/*bc*-1^2 will require allelism studies using parents that differ at both loci. Interestingly, the geminivirus and potyvirus resistance genes *bgm*-1 and *bc*-1/*bc*-1^2 are known to originate in the Durango race of common beans and some cultivars from this race such as Pinto 114, and Great Northern 31 which possess *bc*-1/*bc*-1^2 genes have tolerance to BGYMV (Morales and Niessen 1988). Further dissection of this genomic region will also be of interest since as in other studies of recessive viral resistance genes in common beans, the genomic region associated with the *bgm*-1 and *bc*-1/*bc*-1^2 loci does not contain any dominant resistance genes or QTLs for BGYMV / BCMV resistance, or other resistance genes against fungal or bacterial pathogens (Miklas et al. 2000a, 2006).

In terms of symptom development both *bgm*-1 and *bc*-1/*bc*-1^2 provide resistance to yellowing or chlorosis (Morales and Niessen 1988; Blair and Beaver 1993; Velez et al. 1998; Miklas et al. 2000b) suggesting that they may share mechanistic properties. Further studies will be required to investigate how closely linked the genes are or whether they are allelic. It would also be interesting to evaluate the distribution of the resistance genes in other common bean genotypes since the *bgm*-1 gene is thought to be exclusively from Durango landraces (Morales and Niessen 1988) while the *bc*-1/*bc*-1^2 alleles are found in Durango and other differentials (Miklas et al. 2000b). Further studies could also evaluate the mechanism of resistance provided by these genes since *bgm*-1 appears to function alone in providing BGYMV resistance while *bc*-1/*bc*-1^2 are epistatic with other recessive BCMV resistance genes and with the dominant *I* gene (Blair and Beaver 1993; Velez et al. 1998; Strausbaugh et al. 1999; Miklas et al. 2000b). Cloning the underlying genes involved in these resistances and their further characterization would be needed to resolve their mode of action.

In terms of plant breeding, the discovery that the two recessive resistance genes are linked may allow plant breeders to use multiple markers developed for each of the genes to select for both or either of the loci. For example the co-dominant marker developed for *bc*-1^2 by Vandemark and Miklas (2002) could be useful for selection of *bgm*-1 and vice versa the CAPS marker developed here could be useful for the selection of either the *bc*-1 or *bc*-1^2 alleles. While CAPS markers themselves have been useful for genetic mapping in common bean they have been less often used than

SCAR markers for marker assisted selection (Miklas et al. 2006). Additional assays could be developed to convert the polymorphism detected by us into markers that do not require restriction digestion as was done for the SBD5 marker (Vandemark and Miklas 2002). It will be important to determine whether the two genes are ever found in coupling or if linkage between the two genes can be broken given the interest in incorporating resistance to both BCMV and BCMNV with BGYMV into varieties for certain regions of production such as the Caribbean (Blair et al. 2006b). Meanwhile, the *bgm*-1 gene has been especially important and effective in Central America (Beebe et al. 1995; Beaver et al. 2003) showing that it is a stable and valuable gene.

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