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Genotyping with real-time PCR reveals recessive epistasis between independent QTL conferring resistance to common bacterial blight in dry bean

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Abstract Resistance to common bacterial blight in common bean is a complex trait that is quantitatively inherited. Combining QTL is the current strategy for improving resistance, but interactions among different QTL are unknown. We examined the interaction between two independent QTL present in dry bean breeding line XAN 159. The QTL were studied in a near isogenic population consisting of 120 $BC_6:F_2$ plants. Each $BC_6:F_2$ plant was evaluated for disease reaction at several time points after pathogen inoculation and the dominant SCAR markers linked with QTL on linkage groups B6 (BC420 ∼ QTL) and B8 (SU91 ∼ QTL) were interpreted as codominant markers using real time PCR assays. This enabled assignment of $BC_6:F_2$ plants to all nine possible genotypes. Reaction to CBB in $BC_6:F_2$ plants was characterized by an epistatic interaction between BC420 and SU91 such that: 1) the expression of BC420 was epistatically suppressed by a homozygous recessive su91//su91 genotype; 2) SU91//SU91 and SU91//su91 genotypes conditioned an intermediate disease reaction when homozygous recessive for bc420// bc420; and 3) the highest level of disease resistance was conferred by genotypes with at least a single resistance allele at both QTL (BC420//-; SU91//-). Segregation for resistance among $BC_6:F_3$ plants derived from $BC_6:F_2$ plants that were heterozygous for both QTL did not deviate significantly from expected ratios of 9 resistant: 3 moderately resistant: 4 susceptible. This is consistent with a recessive epistatic model of inheritance between two loci. These results indicate breeders will realize greatest gains in resistance to CBB by selecting breeding materials that are fixed for both QTL. This is a first report of a qualitative digenic model of inheritance discerning an interaction between two QTL conditioning disease resistance in plants.

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Introduction

Common bacterial blight (CBB) of common bean (*Phaseolus vulgaris* L.), which is caused by *Xanthomonas axonopodis* pv. *Phaseoli* (*Xap*), is a disease of global importance to bean production in tropic, subtropic, and temperate regions (Coyne and Schuster 1974). CBB is seed-transmitted and contaminated seed is typically the primary source of inoculum for both local and global distribution of the disease (Saettler 1991). Control measures based on chemical applications do not significantly increase seed yield or reduce pod infection (Weller and Saettler 1976). The ability of the pathogen to grow as an epiphyte limits the effectiveness of crop rotation. The most effective method for controlling CBB is to grow resistant bean cultivars (Singh and Munoz 1999).

The majority of studies have indicated that resistance to CBB in common bean is quantitatively inherited (Beebe 1989; Silva et al. 1989; Musaana et al. 1993; Yu et al. 1998). Differences have been observed in the disease

reactions of leaves, pods and seeds of the same genotype (Aggour et al. 1989; Arnaud-Satana et al. 1994). Disease expression is also influenced by several environmental factors, including photoperiod, temperature and humidity (Saettler 1991). The complex inheritance of resistance to CBB, coupled with the influence of a range of environmental factors on the expression of disease makes it difficult for plant breeding programs to effectively screen for CBB resistance.

A promising alternative to disease screening for identifying genotypes with resistance to CBB has been to screen beans for molecular markers that are closely linked to disease resistance genes. Several different groups have employed linkage analysis to identify molecular markers that are closely associated with resistance to CBB (Miklas et al. 1996, 2006a; Bai et al. 1997; Tar'an et al. 1998, 2001; Yu et al. 1998, 2004; Jung et al. 1999; Park et al. 1999). At least 22 different quantitative trait loci (QTL) have been identified across all 11 linkage groups of common bean (Miklas et al. 2006b).

The molecular markers most used to identify QTL conditioning resistance to CBB are sequence characterized amplified regions (SCARs) (Paran and Michelmore 1993). Two SCAR markers of particular interest to breeding programs focused on enhancing resistance to CBB are the markers BC420 (Yu et al. 1998) and SU91 (Pedraza et al. 1997). The tepary bean (*P. acutifolius* A. Gray) accession PI 31944, via the interspecific dry bean line XAN 159 (Thomas and Waines 1984), is considered to be the source of both BC420- and SU91-linked QTL (Miklas et al. 2000a). BC420 was located on linkage group B6 (Yu et al. 2000) and SU91 on linkage group B8 (Pedraza et al. 1997). The different chromosome positions of these two SCAR markers make them attractive sources for introgressing independent QTL conditioning resistance to CBB into susceptible bean cultivars.

The presence of BC420 accounted for approximately 62% of the phenotypic variation in resistance to CBB expressed by a population derived from a cross between the *P. vulgaris* breeding lines HR67 (resistant) and W1744d (susceptible) (Yu et al. 2000). In F_5 recombinant inbred lines (RILs) derived from a cross between HR67 and OAC95-4 (susceptible), the presence of BC420 accounted for approximately 63% of the phenotypic variation in resistance to CBB (Yu et al. 2004). The presence of SU91 accounted for approximately 14% of the phenotypic variation for resistance to CBB in an F_2 population derived from a cross between XAN 159 (resistant) and Chase (moderately susceptible) (Park et al. 1999), and its presence accounted for 17% of the phenotypic variation for resistance in a BC_2F_1 population derived from the same cross (Mutlu et al. 2005a). SU91-linked QTL is perhaps the most useful QTL detected to date, as the CBB resistant dark red kidney bean line USDK-CBB-15 (Miklas et al. 2006c) and the CBB resistant pinto bean line ABCP-8 (Mutlu et al. 2005b) were both developed using marker assisted selection for SU91.

Unfortunately, both BC420 and SU91 are dominant markers. Dominant markers can identify plants that have at least one copy of the gene of interest, but cannot easily be used to distinguish plants with one copy of a gene (heterozygous) from plants with two copies of a gene (homozygous). No codominant markers have been detected that are sufficiently linked to SU91 to be of use in marker assisted selection. A codominant SSR marker has been identified that is linked to BC420 (Yu et al. 2004). However, in this work, Yu et al. (2004) mapped the SSR and BC420 to linkage group B7, whereas BC420 was originally (Jung et al. 1997) and subsequently mapped closely to the *V* locus on linkage group B6 (Liu et al. 2008). In addition, this SSR marker has not been reported to have been applied to marker assisted selection (MAS), so its association with the disease resistance QTL and its effectiveness for MAS has not been as rigorously validated as has the SCAR marker BC420 (Fourie and Hersellman 2002; Ibarra-Perez and Kelly 2005; Mutlu et al. 2005; Yu et al. 2000). Finally, the SSR marker has not been shown to be amplified from the tepary bean genome, unlike BC420. These observations suggest that alternatives to the SSR marker should be considered for the codominant interpretation of BC420.

A method has been developed based on the use of real time PCR (Holland et al. 1991; Heid et al. 1996) to discriminate between plants that are homozygous or heterozygous for dominant SCAR markers (Vandemark and Miklas 2002, 2005). This method was used to simultaneously genotype for both $SBD5_{1300}$ (Miklas et al. 2000b) and $SW13_{690}$ (Melotto et al. 1996) SCAR markers, which are linked to the independent $bc\text{-}1^2$ and I alleles conditioning resistance to *Bean common mosaic virus* (BCMV), respectively. The F_2 marker genotypes were verified by performing F_3 family progeny tests for disease reaction in a greenhouse screening. Agreement between the two methods was 100% (198/198) for the $bc\text{-}1^2$ allele, and 92.4% (183/198) for the I allele (Vandemark and Miklas 2005). Erroneous genotyping was due to recombination between $SW13_{690}$ and the I allele.

It has not been possible to use dominant SCAR markers to determine the allelic dosage effects of different numbers (one or two) of individual QTL on levels of resistance to CBB. Additionally, it is not possible to use dominant SCAR markers to investigate the effects of different numbers of copies of multiple QTL on levels of resistance to CBB. The objective of this study was to use real-time PCR assays to examine the genetic interaction between BC420 and SU91 QTL on expression of resistance to CBB.

Materials and methods

Real-time PCR primers and probes

The nucleotide sequence of BC420 (895 bp; GENBANK Accession EF553635) was analyzed with Primer Express software (Applied Biosystems, Foster City, CA) to identify sequences for real-time PCR primers and probes. The nucleotide sequences of the primers and the fluorochrome-labeled probe used in this study are as follows: forward primer BC420F20, 5′-d-TGGCTCAGGTGGTTTGCAA-3′; reverse primer BC420R111, 5′-d-GCGCCTGGGAACGATTT-3′; and probe BC420T59, 5′-d-CCCCATTCGCAGCGTCGCA-3′. The fluorochrome 6-carboxyfluorescein (6-FAM) was attached to the 5′ terminus of the probe (TaqMan; Applied Biosystems) and the quencher dye tetramethylcarboxyrhodamine (TAMRA) was attached to the 3′ terminus. The primer/probe set BC420F20–BC420T59–BC420R111 amplified a 92 bp fragment.

The nucleotide sequence of SU91 (669 bp; GENBANK Accession EF553635) was also examined with Primer Express software, and the sequences of the primers and the fluorochrome-labeled probe designed for this study are as follows: forward primer SU91F2, 5′-d-CACATCGGTTAACATGAGTGATTTC-3′; reverse primer SU91R86, 5′-d-CACACAAAGGAGGGATAAAAGAGATAA-3′; and probe SU9134, 5′-d-CATATATCATCGCCTATTGTGT-3′. The fluorochrome 6-carboxyfluorescein (6-FAM) was attached to the 5′ terminus of the probe (TaqMan; Applied Biosystems) and a non-fluorescent quencher coupled with a minor groove binder (MGB) was attached to the 3′ terminus. The primer/probe set SU91F2-SU9134-SU91R86 amplified an 85 bp fragment. All primers and probes were synthesized commercially (Applied Biosystems).

Real-time PCR assays

In separate reactions for each plant sample, the 92 bp amplicon from the SCAR marker BC420 was amplified with the primer/probe set BC420F20-BC420T59-BC420R111, while the primer/probe set SU91F2-SU9134-SU91R86 was used to amplify the 85 bp fragment from the SCAR marker SU91. PCR was done in 50 μl reactions containing 100 ng genomic DNA, 450 nM forward primer, 450 nM reverse primer, 250 nM fluorochrome-labeled probe and 25 μl of 2X TaqMan™ Universal PCR Master Mix (Applied Biosystems). PCR and detection of fluorescence were performed using the GeneAmp 7000 Sequence Detection System (Applied Biosystems). All PCR was conducted using a thermocycling profile consisting of an initial cycle of 2 min at 50°C, followed by a single cycle of 10 min at 95°C, and then 40 cycles of 15 s at 95°C and 1 min at 60°C. For each DNA sample, three replicate real-time PCR reactions were performed with each primer/probe set. To verify that reagents were not contaminated by template DNA each primer probe set was used in amplification reactions in which 5 ul of ddH$_2$0 was substituted for 100 ng DNA. For both primer/probe sets, standard curves were generated for each set of PCR reactions using three replicate reactions each of 5, 25, 50, 100, and 200 ng of purified genomic DNA of the homozygous parent XAN 159 [BC420 BC420 (BB)//SU91 SU91 (SS)]. The null genotype parent Teebus [bc420 bc420 (bb)//su91 su91 (ss)] was also amplified with each primer/probe set in three replicate reactions to confirm that the primer/probe sets did not amplify DNA from plants that did not possess at least one copy of both target templates.

Determining plant genotype with real-time PCR

The population examined in this study consisted of 120 BC$_6$-derived F$_2$ (BC$_6$:F$_2$) plants from a cross between XAN 159 (BBSS) × Teebus (bbss). Ten remnant BC$_6$-derived F$_1$ (BC$_6$:F$_1$) plants (BbSs) were examined to determine heterozygote probability distributions for BC420 and SU91. Three replicate PCR reactions were performed on each BC$_6$:F$_1$ plant for both BC420 and SU91. From this data a group mean (y) and standard deviation (σ_y) was calculated and a normal distribution of the data was verified by performing a Shapiro-Wilk test (Shapiro and Wilk 1965). Once a normal distribution was verified, a 99% probability distribution for heterozygotes was determined for both BC420 and SU91 using the formula $y \pm 2.58\sigma_y$ (Walpole and Myers 1978).

The genotype of each BC$_6$:F$_2$ plant for both BC420 and SU91 was determined based on the mean of three real-time PCR reactions for each marker. Plants with means within the 99% heterozygote probability distribution for BC420 were classified as heterozygous for BC420 (Bb). Plants with means that fell to the right of the heterozygote probability distribution were classified as homozygous for BC420 (BB). Plants for which no amplification was detected were scored as having the homozygous recessive genotype (bb). Chi-square (χ^2) analysis was conducted to determine if observed results were significantly different than the expected segregation ratios of 1 BB: 2 Bb: 1 bb).

Similarly, each BC$_6$:F$_2$ plant was genotyped for SU91 based on the position of its mean of three replicate real-time PCR reactions relative to the 99% heterozygote probability distribution determined for SU91. χ^2-analysis was conducted to determine if observed results were significantly different than the expected segregation ratios of 1 SS: 2 Ss: 1 ss. The independent assortment of alleles at both QTL was tested by conducting χ^2 analysis to determine if observed results were significantly different than the

expected segregation ratios of 1 BBSS: 2 BBSs: 1 BBss: 2 BbSS: 4 BbSs: 2 Bbss: 1 bbSS: 2 bbSs: 1 bbss.

Screening plants for resistance to CBB

Disease reaction of plants in response to inoculation by *Xap* was assessed for 10 plants of each parent (XAN 159 and Teebus), 10 BC_6:F_1 plants used as the comparative heterozygote sample, and all 120 BC_6:F_2 plants that were genotyped with real-time PCR for both BC420 and SU91. Seed of each line were planted in 20 L plastic bags (1 seed per bag) in sterile soil and maintained in a greenhouse at 18°C night/28°C day. Inoculum was prepared by suspending 48- to 72-h-old cultures (isolates Xf260 and Xf410) in sterile distilled water, and adjusting it to 10^8 CFU/ml using a Shimadzu UV-260 spectrophotometer. Fourteen to 20-day-old plants with fully expanded first trifoliate leaves were inoculated using the multiple needle inoculation method (Andrus 1948). Inoculated plants were kept in a greenhouse at 18°C night/28°C day and rated for CBB infection at 7, 10, 14, and 18 days after inoculation (DAI) using a 1–9 scale: 1 = no necrotic lesions and/or chlorosis; 2–3 = 1–25.5 leaf area affected; 4–6 = 26–64.5% leaf area affected, and 7–9 = 65–100% leaf area affected (Aggour et al. 1989). Data for CBB reaction of each genotype class was subjected to an analysis of variance and a pairwise *t* test using JMP Statistical Discovery Software (SAS Institute, Cary, NC) to determine if differences in CBB reaction were significant ($P \leq 0.05$) between different genotype classes.

Confirming genotypes based on real time PCR of BC_6:F_2 plants by BC_6:F_3 progeny testing for CBB reaction

The genotypes of all BC_6:F_2 plants were determined based on the results of real-time PCR and three plants of each of nine possible genotypes were randomly selected. These 27 plants were grown to maturity in the greenhouse and BC_6:F_3 progeny seed was harvested from each plant. Between 25 and 30 BC_6:F_3 progeny from each of these BC_6:F_2 plants were inoculated with *Xap* and scored at 7, 12, 14 and 19 DAI for reaction to CBB using the same protocol as described above. Plants that had CBB reaction ≤ 3 were classified as resistant; plants for which $3 < CBB$ reaction < 7 were classified as moderately resistant, and plants that had CBB reaction ≥ 7 were considered to be susceptible. Chi-square analyses were conducted to determine if observed phenotypic segregation for CBB reaction among F_3 within a BC_6F_2 genotype fit expected ratios based on digenic inheritance models suggested by the BC_6F_2 data. Data for CBB reaction of each genotype class was subjected to an analysis of variance and a pairwise t- test using JMP Statistical Discovery Software (SAS Institute, Cary,

NC) to determine if differences in CBB reaction were significant ($P \leq 0.05$) between different genotype classes.

Results

Precision of real-time PCR assays for SU91 and BC420

Standard curves were determined for both real-time PCR assays by amplifying target DNA in reactions using 5–200 ng of XAN 159 genomic DNA (BBSS). Standard curves determined with primer/probe set BC420F20-BC420T59-BC420R111 had R^2 values ranging between 0.993 and 0.998 for the relationship between the $\log_{10}$ of the initial DNA quantity and C_T value for all sets of reactions. R^2 values for this relationship ranged between 0.992 and 0.998 among all sets of reactions for primer/probe set SU91F2-SU9134-SU91R86. Neither real-time PCR assay amplified DNA from the null genotype parent Teebus (bbss).

Resolving plant genotypes for BC420 with real-time PCR

100 ng of genomic DNA from 10 remnant BC_6:F_1 plants (BbSs) amplified in replicated reactions using primer/probe set BC420F20-BC420T59-BC420R111 in order to determine a heterozygote probability distribution that was subsequently used to ascribe genotypes for BC420 to 120 BC_6:F_2 plants. Real-time PCR results among the sample of 10 remnant BC_6:F_1 plants resulted in a group mean and standard deviation of 58.13 ± 3.70. Since the 92 bp fragment amplified by primer/probe set BC420F20-BC420T59-BC420R111 represents such as small fraction of the entire bean genome, results are expressed without units. Analysis of the data from BC_6:F_1 plants with the Shapiro–Wilk test indicated that the data was normally distributed ($N = 30$, $W = 0.97$, Prob < $W = 0.48$). Based on these results the 99% heterozygote confidence interval was determined to be 48.57–67.69.

Real-time PCR results identified 18 plants for which the mean of three replicate PCR reactions fell to the right of the 99% heterozygote confidence interval, and these plants were classified as being homozygous dominant (BB). Fifty-one plants were identified that had mean PCR values that fell within the heterozygote confidence interval, and were classified as heterozygotes (Bb). No amplification with by primer/probe set BC420F20-BC420T59-BC420R111 was detected for an additional 51 plants, which were classified as being homozygous recessive (bb). These results (18 BB: 51 Bb: 51 bb) deviated significantly from the expected 1:2:1 segregation ratio ($\chi^2 = 20.85$; df = 2; $P < 0.0001$).

Resolving plant genotypes for SU91 QTL with real-time PCR

100 ng of genomic DNA from 10 remnant $BC_6:F_1$ plants (BbSs) was amplified in replicated reactions using primer/probe set SU91F2-SU9134-SU91R86 in order to calculate a heterozygote probability distribution that was used to genotype120 $BC_6:F_2$ plants for SU91. Real-time PCR results among the sample of 10 remnant $BC_6:F_1$ plants resulted in a group mean and standard deviation of 63.89 ± 5.06. Results are expressed without units since the 85 bp fragment amplified by primer/probe set SU91F2-SU9134-SU91R86 represents such as small fraction of the entire bean genome. Analysis of the data from $BC_6:F_1$ plants with the Shapiro–Wilk test indicated that the data was normally distributed ($N = 30$, $W = 0.97$, Prob $< W = 0.65$). Based on these results the 99% heterozygote confidence interval was determined to be 50.84–76.94.

Real-time PCR results identified 27 plants for which the mean of three replicate PCR reactions fell to the right of the 99% heterozygote confidence interval and these plants were classified as being homozygous dominant (SS). 66 plants were identified that had mean PCR values that fell within the heterozygote confidence interval and were classified as heterozygotes (Ss). No amplification by primer/probe set SU91F2-SU9134-SU91R86 was detected for an additional 27 plants, which were classified as homozygous recessive (ss). Based on real-time PCR the 120 $BC_6:F_2$ plants segregated as follows for SU91: 27 SS: 66 Ss: 27 ss, which did not significantly deviate from the expected 1:2:1 segregation ratio ($\chi^2 = 1.20$; df = 2; $P = 0.55$).

Relationships between plant genotype for BC420 and SU91 and CBB resistance

The observed numbers of $BC_6:F_2$ plants in each of nine possible genotype classes based on the assignment of genotype using real-time PCR assays for BC420 and SU91 is presented in Table 1. Chi-square analysis indicated that the observed results differed significantly from expected genotypic ratios, suggesting that the two QTL are not independently assorting (Table 1).

Means separations between $BC_6:F_2$ genotypes for reaction to CBB are presented for several time points during disease progression in Table 1. The means of the susceptible parent Teebus and the resistant parent XAN 159 are also presented. Teebus and XAN 159 expressed expected susceptible and resistant reactions to CBB, indicating that experimental and environmental conditions were conducive to disease development. At every time point the three genotypes that were homozygous recessive for SU91 (bbss, Bbss, and BBss) had significantly higher mean reactions (more susceptible) to CBB than the six other genotypes, all of which were either heterozygous (Ss) or homozygous dominant (SS) for SU91. At 10, 14 and 18 DAI, genotypes bbSs and bbSS expressed intermediate disease reactions that were significantly less than genotypes bbss, Bbss, and BBss, but significantly greater than genotypes BbSs, BbSS,

Table 1 Means separation of CBB response among $BC_6:F_2$ plants genotyped for BC420 and SU91 based on real-time PCR

Genotype[a]	Mean CBB reaction[c]					
	Observed (rt PCR)[b]	Expected (N = 120)	7 DAI	10 DAI	14 DAI	18 DAI
bbss	9	7.5	2.50 a	6.39 a	8.33 a	8.44 a
Bbss	14	15	2.75 a	6.64 ab	8.14 a	8.71 a
BBss	4	7.5	2.25 a	7.50 b	8.00 a	8.75 a
bbSs	28	15	1.30 b	2.57 c	3.16 b	3.59 b
bbSS	14	7.5	1.32 b	2.43 c	3.32 b	3.43 b
BbSs	29	30	1.09 b	1.48 d	1.64 c	1.67 c
BbSS	8	15	1.00 b	1.25 d	1.63 c	1.31 c
BBSs	9	15	1.00 b	1.06 d	1.17 c	1.16 c
BBSS	5	7.5	1.00 b	1.10 d	1.10 c	1.10 c
Teebus (bbss)	10		1.75	7.30	8.20	8.40
XAN 159 (BBSS)	10		1.00	1.05	1.05	1.05
$\sum \chi^2 = 25.43$; df = 8; $P = 0.0013$						

DAI days after inoculation

[a] B = BC420, b = bc420, S = SU91, and s = su91

[b] Plants were genotyped for BC420 using the primer/probe set BC420F20-BC420T59-BC420R111 and for SU91 using the primer probe set SU91F2-SU9134-SU91R86

[c] Reaction to CBB was evaluated using the following scale: 1 = no necrotic lesions and/or chlorosis; 2–3 = 1–25.5 leaf area affected; 4–6 = 26–64.5% leaf area affected, and 7–9 = 65–100% leaf area affected (Aggour et al. 1989)

Table 2 Means separation of CBB response among $BC_6:F_3$ plants produced from $BC_6:F_2$ parent plants of nonsegregating phenotypic and genotypic classes for BC420 and SU91

$BC_6:F_2$ genotype[a]	Mean CBB reaction[b] of $BC_6:F_3$ plants				
	N (BC_6F_3)	7 DAI	12 DAI	14 DAI	19 DAI
bbss	75	2.16 b	7.64 a	8.42 a	9.00 a
Bbss	81	2.36 a	7.61 a	8.48 a	9.00 a
BBss	80	2.17 b	7.55 a	8.49 a	9.00 a
bbSS	75	1.23 c	2.48 b	2.71 b	3.67 b
BBSS	78	1.00	1.00	1.00	1.01
Teebus (bbss)	25	2.62	8.00	9.00	9.00
XAN 159 (BBSS)	25	1.00	1.00	1.00	1.00

DAI days after inoculation

[a] B = BC420, b = bc420; S = SU91, and s = su91

[b] Reaction to CBB was evaluated using the following scale: 1 = no necrotic lesions and/or chlorosis; 2–3 = 1–25.5 leaf area affected; 4–6 = 26–64.5% leaf area affected, and 7–9 = 65–100% leaf area affected (Aggour et al. 1989)

BBSs, and BBSS. At every time point examined the four genotypes that possessed at least one copy of both BC420 and SU91 (BbSs, BbSS, BBSs, and BBSS) had the lowest mean reactions to CBB.

Segregation of resistance to CBB in $BC_6:F_3$ progeny

Segregation of resistance to CBB was examined in 27 $BC_6:F_3$ families derived from three different $BC_6:F_2$ plants for each nine possible $BC_6:F_2$ genotypic classes. Means separations for reaction to CBB between $BC_6:F_3$ progeny of five different genotypes of $BC_6:F_2$ plants that lacked segregation for disease reaction are presented for several time points during disease progression in Table 2. Teebus and XAN 159 expressed expected susceptible and resistant reactions to CBB, indicating that experimental and environmental conditions were conducive to disease development. Similar to results for reaction to CBB in $BC_6:F_2$ plants (Table 1), at every time point the means of $BC_6:F_3$ progeny produced from $BC_6:F_2$ plants that were homozygous recessive for SU91 (bbss, Bbss, BBss) were significantly higher than progeny produced from plants of all other six genotypes. By the time severe disease was observed in the susceptible parent Teebus (≥12DAI), no significant differences were observed among $BC_6:F_3$ progeny produced from $BC_6:F_2$ plants that were homozygous recessive for SU91.

Results of χ^2 analysis are presented (Table 3) for the four $BC_6:F_2$ genotypic classes of plants (BbSs, bbSs, BbSS, and BBSs) that produced F_3 progeny that segregated for resistance to CBB. Phenotypic segregation of $BC_6:F_3$ progeny derived from $BC_6:F_2$ plants that were heterozygous for both QTL (BbSs) did not significantly deviate from an expected ratio of 9 resistant: 3 moderately resistant: 4 susceptible. $BC_6:F_2$ plants with the BBSs genotype produced progeny that did not significantly deviate from an expected phenotypic ratio of 3 resistant: 1 susceptible. $BC_6:F_2$ plants with a BbSS genotype produced progeny that did not significantly deviate from expected phenotypic ratios of 3 resistant: 1 moderately resistant. However, in the case of $BC_6:F_2$ plants with the bbSs genotype, only two phenotypic classes, moderately resistant and susceptible were expected in progeny, but four resistant (CBB reaction < 3) progeny were also identified, two with CBB = 1.5 and in the other two CBB = 2.5. When these four plants were classified as moderately resistant the observed phenotypic ratios in the remaining $BC_6:F_3$ progeny did not deviate from an expected ratio of 3 moderately resistant: 1 susceptible.

Discussion

The examination of phenotypic reactions to CBB in $BC_6:F_2$ plants that were genotyped by real time PCR for two independent QTL enabled a digenic inheritance model to be formulated for the relationship between genotype and resistance to CBB. At 14 and 18 DAI, means separation revealed three significantly different reactions to CBB among the nine genotypes. The most susceptible reaction was observed in BC_6F_2 plants that had no copies of SU91 (bbss, Bbss, and BBss). An intermediate disease reaction was observed in plants that had no copies of BC420 but at least one copy of SU91 (bbSs and bbSS). The most resistant reaction was observed in plants that had at least a single resistance allele at both QTL (BbSs, BbSS, BBSs, and BBSS).

These results led us to make three preliminary observations of the interaction between BC420 and SU91 in the expression of resistance to CBB: 1) BC420 did not condition any significant level of resistance in genotypes that

Table 3 Segregation for CBB reaction in BC_6:F_3 progeny from BC_6:F_2 parent plants of segregating phenotypic and genotypic classes for BC420 and SU91

Genotype[b] (BC_6:F_2)	Segregation[a] for CBB reaction in BC_6:F_3 progeny						
	Expected			Observed			
	Resistant	Moderate resistant	Susceptible	Resistant	Moderate resistant	Susceptible	$\Sigma\chi^2$ (P)
BBSs	60		20	63		17	0.60 (0.44)
BbSS	62.25	15.75	20.75	61	22	22	0.10 (0.75)
BbSs	47.25	60	21	41	61	21	3.31 (0.19)
bbSs			20	4		15	1.67 (0.20)[c]

[a] Resistant = CBB reaction ≤ 3; moderately resistant = 3 < CBB reaction < 7; susceptible = CBB reaction ≥ 7

[b] B = BC420; b = bc420; S = SU91, and s = su91

[c] Chi-square value based on inclusion of the four resistant plants as moderately resistant

were homozygous recessive for SU91 (BBss and Bbss); 2) SU91 was capable of conditioning an intermediate disease reaction in genotypes that were homozygous recessive for BC420 (bbSs and bbSS), and 3) the highest level of disease resistance was in genotypes that had at least a single resistance allele at both QTL (BbSs, BbSS, BBSs, and BBSS). These three observations suggested the presence of a recessive epistatic interaction between BC420 and SU91 where by a homozygous recessive genotype at the SU91 locus (ss) suppresses the expression of a dominant QTL allele at the BC420 locus (BB, Bb).

This recessive epistatic model should have resulted in genotypes in the BC_6:F_2 at a ratio of 9 B_S_: 3 bbS_: 4 _ _ss, but the genotypes deviated significantly from this expected segregation ratio due to segregation distortion in favor of bb genotypes. The two QTLs are on different chromosomes (BC420 = B6 and SU91 = B8); therefore, the lack of independent assortment is most likely due to a lack of fitness, through either reduced seed or embryo viability, of genotypes that are homozygous for BC420. Nonetheless, the accuracy of genotyping based on real time PCR and the proposed model of recessive epistasis were both confirmed by the segregation of reaction to CBB in BC_6:F_3 progeny produced by all nine possible genotypic classes of BC_6:F_2 plants.

Examination of BC_6:F_3 progeny that did not segregate for disease reaction revealed that the highest mean CBB reactions, and therefore the most susceptible plants, were consistently found in progeny of BC_6:F_2 plants that were homozygous recessive for SU91 (Table 2). The recessive epistatic effect of SU91 upon BC420 is also supported by a lack of significant differences between progeny of bbss, Bbss, and BBss genotypes, despite there being segregation for BC420 in progeny from Bbss plants. Means separation between progeny from the most resistant fixed BC_6:F_2 genotypes also support an epistatic interaction between the two QTL, as progeny from BBSS had significantly lower means than progeny from bbSS plants which expressed intermediate disease reaction.

Inheritance of resistance to CBB was also examined by grouping BC_6:F_3 progeny which segregated for disease reaction into three phenotypic classes; resistant, moderately resistant, and susceptible. Chi-square analysis clearly showed that BC_6:F_2 plants with the BbSs genotype produced BC_6:F_3 progeny that did not significantly deviate from expected ratios of 9 resistant: 3 moderately resistant: 4 susceptible. BC_6:F_3 progeny of BC_6:F_2 plants with genotypes BbSS and BBSs also segregated according to expected phenotypic ratios. BC_6:F_3 progeny of bbSs plants were expected to segregate into two phenotypic classes, moderately resistant and susceptible, but four resistant plants were also detected in this sample. At the present it is difficult to determine if these results are due to experimental error or adverse environmental effects, such as a dry canopy, that could have reduced disease severity in plants that would normally be moderately resistant to CBB. The misclassification of a single BC_6F_2 bbSs genotype due to recombination between BC420 and the QTL is a remote possibility because one BC_6F_2 BbSs genotype would be expected to contribute 14–17 resistant plants to the cumulative progeny test across three BC_6F_2 bbSs genotypes.

The observed results are reminiscent of the inheritance of resistance to CBB observed in progeny from a cross between resistant and susceptible tepary bean lines, from which both BC420 (Yu et al. 1998) and SU91 (Pedraza et al. 1997) markers and respectively linked QTL are ultimately derived (Thomas and Waines 1984). Some F_2 progeny segregated 9 resistant to 7 susceptible, and it was proposed that resistance was conferred by a digenic epistatic interaction between two genes (Urrea et al. 1999). However, in the study of Urrea et al. (1999), plants were scored for CBB resistance using the same 1–9 scale employed in our study, but only two phenotypic classes were reported, with CBB reactions ranging from 1–4 being considered resistant and CBB reactions 5–9 being considered susceptible. In our study, plants that had CBB reaction ≤ 3 were classified as resistant; plants for which

3 < CBB reaction < 7 were classified as moderately resistant, and plants that had CBB reaction ≥ 7 were considered to be susceptible. If Urrea et al. (1999) had included a moderately resistant phenotypic class, it is possible that their results could have approached the 9 resistant: 3 moderately resistant: 4 susceptible segregation ratio observed in our study.

It should be noted that we minimized the confounding effects of minor QTL on the expression of resistance to CBB in this study by genotyping and phenotyping $BC_6{:}F_2$ plants that segregated for the presence of BC420 and SU91. Based on expected 99% $[1 - (0.5)^{6+1}]$ recovery of the recurrent parent using six backcrosses, QTL with minor effects would be predominately fixed in the $BC_6{:}F_2$ population. The effects of minor QTL can make it difficult to estimate the effects of specific QTL on the expression of resistance to CBB. Although the great northern landrace Montana No. 5 and the great northern cultivar GN#1 Sel 27 both possessed the QTL linked with SAP6 marker on linkage group B10, an F_2 population derived from this cross expressed a normal distribution for reaction to CBB (Miklas et al. 2003). This was proposed to be due to the segregation of multiple minor QTL in the population. Thus, studying the interaction between BC420 and SU91 QTL in a near-isogenic population contributed significantly to our ability to discern a specific digenic epistatic inheritance model.

The results presented in this study partially agree with those previously reported by O'Boyle et al. (2007), in which plants that had copies of both SU91 and BC420 had higher levels of CBB resistance than plants that had only one of either two QTL. The plants with just the BC420 QTL in our study were susceptible. Although O'Boyle et al. (2007) only genotyped the plants for the presence or absence of SU91 and BC420, they also suggested that resistance to CBB may be influenced by an epistatic interaction between BC420 and SU91. Park et al. (1999) similarly observed that there was an additive effect on resistance to CBB conferred to bean genotypes that possessed both SU91 and BC420. Our results support those observations in that the most resistant classes of $BC_6{:}F_2$ genotypes were those that had at least a single copy of both QTL. However, the genotyping results based on the analysis of $BC_6{:}F_2$ plants and phenotypic segregation of $BC_6{:}F_3$ progeny demonstrated that there was no significant difference in reaction to CBB between plants that were fixed for both QTL alleles (BBSS) and plants that were heterozygous for at least one QTL (BbSs, BBSs, and BbSS). These results suggest that resistance to CBB is not influenced by different dosages of these two QTL, provided that a plant has at least a single copy of both SU91 and BC420.

In this study we observed associations between phenotype and genotype indicating that BC420 and SU91 interact in the expression of resistance to CBB through the epistatic suppression of BC420 by a homozygous recessive SU91 genotype. The observed recessive epistatic interaction between the two QTL suggests that SU 91 is essential for the expression of an effective resistance mechanism, which does not function properly in plants that lack SU 91. This finding has serious implications in marker-assisted breeding for resistance to CBB, as derived breeding materials that possess only BC420, may be susceptible. Breeders need to also be aware that the association of BC420 with the *V* locus that influences seed color, restricts deployment of this QTL to white- and black-seed type market classes (Mutlu et al. 2005a).

At the present it is not possible to conclude that SU 91 will epistatically suppress the action of other QTL known to be associated with resistance to CBB that are derived from resistance sources other than tepary bean. The QTL SAP 6, which is derived from linkage group B10 of the common bean great northern landrace Montana #5 (Miklas et al. 2003), has been shown to be responsible for up to 35% of the phenotypic variation in CBB reaction in an F_2 population produced from the cross Montana #5/Othello (Miklas et al. 2003). We recently designed a real-time PCR assay for SAP 6 and are currently examining two different bean populations that segregate for both SAP 6 and SU 91 in an effort to determine how these two QTL interact in the expression of resistance to CBB.

Results of this study also emphasize the need for breeders to correctly identify plants that are homozygous for both SU91 and BC420. Breeding materials that are not fixed for BC420 may produce moderately resistant progeny in subsequent generations while plants that are not fixed for SU91 may produce susceptible progeny. The real time PCR assays described in this report accurately determined codominant genotypes for both SU91 and BC420. However, with current technologies, the real-time PCR assays may not be amenable for high throughput genotyping because of the need for precise quantification of template DNA in the PCR reaction. However, the possibility for multiplexing the markers in a single reaction (Vandemark and Miklas 2005) combined with the advantage of codominant interpretation still provides a viable assay for low to medium throughput marker assisted breeding applications. Moreover, breeders will be able to employ these PCR assays to greatly reduce progeny testing for reaction to CBB and realize increased gains in selection for resistance by selecting for breeding materials that are fixed for both QTL.

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