

Quantitative Trait Loci Linked to White Mold Resistance in Common Bean

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ABSTRACT

White mold disease (WM) of common bean (*Phaseolus vulgaris* L.), caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, reduces crop yield and quality throughout the United States. The development of cultivars with resistance to WM would be facilitated by the identification and use of molecular markers linked to resistance genes. The objectives of this research were (i) to characterize WM reaction in a recombinant inbred line (RIL) population derived from a cross between resistant and susceptible germplasm, (ii) to validate the effect of a previously reported quantitative trait locus (QTL) for WM resistance, and (iii) to locate additional QTL associated with WM resistance. A RIL population that consisted of 94 lines was derived from a cross between G122 (resistant) and CO72548 (susceptible). The population was evaluated for WM reaction in three greenhouse tests and one field environment, and for molecular markers throughout the genome. Two RIL were identified with higher resistance levels ($P < 0.05$) than the resistant parent G122. A previously reported QTL on linkage group B7 was significant ($P < 0.01$) in single-factor analysis of variance, but not with composite interval mapping. Five QTL for resistance to WM were found (likelihood odds ratio [LOD] > 2.7) on linkage groups B1, B2b, B8, and B9. The QTL were contributed from both parents and together accounted for 48% of the phenotypic variation (R^2). For field resistance, one QTL ($R^2 = 12\%$) on linkage group B8 was detected. These results confirm polygenic resistance to WM in common bean.

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Abbreviations: AFLP, amplified fragment length polymorphism; ASI, average severity index; bp, base pair; CIM, composite interval mapping; CSU, Colorado State University; DI, disease incidence; DS, disease severity; LOD, likelihood odds ratio; NDSU, North Dakota State University; PCR, polymerase chain reaction; QTL, quantitative trait locus(i); RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; RIL, recombinant inbred line(s); SCAR, sequence characterized amplified region; SFA, Single factor analysis; SSR, simple sequence repeat; WM, white mold disease.

WHITE MOLD (WM), a disease of common bean (*Phaseolus vulgaris* L.) caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, reduces yield in most production regions in the United States. White mold lowers economic yield by reducing seed number, weight, and quality (Kerr et al., 1978; Steadman, 1979). Control methods for WM include cultural practices such as crop rotation, crop residue management, and reduced irrigation, fungicide application, and the use of resistant cultivars (Steadman, 1979). Development of bean cultivars with increased genetic resistance to WM would provide a mechanism to control yield losses, reduce the cost of production, and protect the environment by reducing the need to apply fungicides.

Two forms of resistance to WM in common bean are disease avoidance and physiological resistance. Avoidance mechanisms

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include upright plant architecture, a more porous canopy, and early maturity to escape late season infection (Blad et al., 1978). Physiological resistance is controlled by host genetic factors that may inhibit infection or spread of the pathogen in host tissue. Together, avoidance and physiological resistance are the most economical and effective control methods to reduce loss of yield and seed quality (Miklas et al., 2001). Because selection for physiological resistance is confounded by disease avoidance in field environments, it is difficult to select lines exclusively for physiological resistance (Hunter et al., 1982; Steadman, 1983). Therefore, artificial inoculation methods have been developed to identify physiological resistance independent of avoidance mechanisms. These methods include excised stem assay (Miklas et al., 1992a), limited-term inoculation (Coyne et al., 1981; Dickson et al., 1982; Hunter et al., 1981), callus assay (Miklas et al., 1992b), juvenile stem test (Dickson et al., 1981), and the oxalate test (Kolkman and Kelly, 2000). Each of these tests uses procedures in the greenhouse to identify physiological resistance. Repeatability of the tests varies within and among labs for the different evaluation procedures (Steadman et al., 2001). The most common inoculation test, the straw test, developed by Petzoldt and Dickson (1996), is a greenhouse test that uses mycelium applied with a soda straw to the severed stem of a seedling bean plant. The straw test evaluates physiological resistance, and has been shown to be among the most consistent and discriminative tests across laboratories (Kolkman and Kelly, 2003; Miklas et al., 2001; Park et al., 2001).

High levels of resistance to WM have been found in scarlet runner bean (*P. coccineus* L.) (Gilmore and Myers, 2003) and moderate levels in common bean (Adams et al., 1973). Resistance has been reported to be controlled both by single genes or polygenic. Abawi et al. (1978) and Schwartz et al. (2006) reported that resistance was controlled by a single gene that conditioned a high level of physiological resistance in progeny from crosses between scarlet runner and common bean. Many inheritance studies in common bean reported low levels of WM resistance controlled by several genes (Coyne et al., 1981; Ender and Kelly, 2005; Fuller et al., 1984; Kolkman and Kelly, 2003; Lyons et al., 1987; Miklas et al., 1992a, 2001; Park et al., 2001). Recurrent selection has been suggested to increase resistance to WM (Lyons et al., 1987), however, recurrent selection among derived lines becomes less efficient in advanced generations because genetic variation is reduced and environmental effects have a greater effect, consequently precision is reduced.

Molecular genetic markers have been used widely to detect quantitative trait loci (QTL) for a variety of traits in common bean, including plant morphology and agronomic traits (Kolkman and Kelly, 2003; Tarán et al., 2002), biochemical processes (Erdmann et al., 2002), and WM resistance (Ender and Kelly, 2005; Miklas et al., 2001, 2003;

Park et al., 2001). Reports regarding QTL associated with WM resistance have ranged from numerous weak QTL to single major QTL. Park et al. (2001) reported several restriction fragment length polymorphism (RFLP) markers linked with physiological and field resistance. They identified one RFLP marker inherited from the cultivar PC-50 that was associated with lower WM disease scores. The marker explained 5 to 16% of the phenotypic variation for resistance and was located on linkage group B2. Ender and Kelly (2005) reported markers for field disease severity located on linkage groups B2, B5, B7, and B8 of the integrated bean map. Miklas et al. (2001) reported a single major-effect QTL located on linkage group B7 (B7 QTL) that accounted for 38% of the phenotypic variation for disease score in the greenhouse straw test. Their results indicated that the resistant allele at the B7 QTL, derived from the parent line G122, was important for conditioning physiological resistance in both greenhouse and field evaluations. The B7 QTL was closely linked to the phaseolin locus (*Phs*), which can be identified by a sequence characterized amplified region (SCAR) marker (Kami et al., 1995); therefore, the authors suggested using the *Phs* SCAR marker to tag the B7 QTL.

The objectives of this research were (i) to characterize a RIL population derived from a single cross between G122 and Colorado pinto breeding line CO72548 for reaction to WM, (ii) to validate in this population the effect of the B7 QTL reported by Miklas et al. (2001), and (iii) to identify additional QTL associated with WM resistance through genomewide linkage mapping.

MATERIALS AND METHODS

Genetic Material

A RIL population that consisted of 94 $F_{5,6}$ lines derived from the cross G122/CO72548 was used in this study. G122 (PI 163120) is an upright, determinate bush (Type I) dry edible bean with elongated, cranberry-colored seeds. It is representative of the Andean gene pool and was the source of the resistant allele at a QTL located on linkage group B7 in a previous study (Miklas et al., 2001). CO72548 is an indeterminate, semi-vine (Type II), advanced pinto breeding line from race Mesoamerica of the Middle America gene pool. It was developed at Colorado State University (CSU) and is adapted to the western United States, but lacks adequate levels of resistance to WM. Ninety-four F_2 plants were advanced from the F_2 to the F_5 generation in the greenhouse by single seed descent. The seed produced on individual F_5 plants was planted in the field at Fruita, CO during summer 2004 to produce F_6 seed for testing of each RIL. All screening and marker work was conducted with this seed source. The cultivar Montrose (Brick et al., 2001) and PC-50 (Saladin et al., 2000) were used as susceptible and resistant checks, respectively, throughout the study. PC-50 is single plant selection in the Pompadour Checa land race and has been reported to be moderately resistant to WM (Park et al., 2001). Montrose is a pinto bean cultivar developed at CSU and is highly susceptible to WM.

White Mold Greenhouse Evaluation

Three separate straw tests (Petzoldt and Dickson, 1996) were conducted to evaluate reaction to WM in the RIL population, parental lines, and checks at the CSU greenhouse facilities in Fort Collins, CO. Straw test 1 (fall, 2003), straw test 2 (fall, 2004), and straw test 3 (spring, 2005) consisted of four, four, and three replicates, respectively. Each test used plants that were germinated in 15-cm plastic pots with commercial potting medium. Each pot contained two plants that represented one replicate of an entry, and each plant a sample within the replicates. Replicates were arranged in a randomized complete block design. For all tests, the greenhouse environment was maintained at 24 to 27°C with 12 to 14 h natural or supplemental light. Plants were watered and fertilized for optimal growth.

The *S. sclerotiorum* culture S20 (Steadman et al., 2004) used for all greenhouse straw tests was grown from sclerotia collected from a Colorado bean seed cleaning plant in 1996. The sclerotia were germinated on potato dextrose agar (PDA) in a Petri plate, and then subcultured to prepare plates of fresh actively growing mycelium. The entire Petri plate of growing mycelium was used as inoculum after mycelium had grown from the center to the periphery of the Petri plate. Agar plugs of inoculum were cut into commercial soda straws for application to the plants. The straws were 6 mm in diameter, cut into 3-cm length pieces, and heat sealed at one end. The open ends of the straws with a plug of agar and mycelium at the distal end were placed over the freshly cut fourth internode of 21 to 28 d old plants for all straw test inoculations.

Immediately after inoculation, plants were transferred to a mist chamber that was automatically misted for 30 s every 5 min and kept there for the remainder of the evaluation. Disease progression was evaluated on the 14th d post inoculation. Disease severity was rated on a 1 to 9 scale based on mycelial growth, where 1 = no mycelial growth past the point of inoculation, 2 = mycelial growth half way to the first internode, 3 = mycelial growth up to the first internode, 4 = mycelial growth into the first internode, but not past the first internode, 5 = mycelial growth past the first internode, but not half way to the second internode, 6 = mycelial growth over half way to second internode, 7 = mycelial growth into second internode, 8 = mycelial growth past second internode, but still had a green stem below infection, and 9 = total plant death.

Field Evaluation

A subset of 90 RIL, two parental lines, and two checks were grown in a field plot at the North Dakota State University (NDSU) Carrington Research/Extension Center during the summer of 2005. Experimental units consisted of single rows 3 m long with approximately 50 plants in each row, with three replicates arranged in a randomized complete block design. The plants were artificially inoculated with ascospores at the R2 growth stage to obtain uniform infection. Disease severity (DS) was visually scored as the percentage of the entire experimental unit that had disease symptoms (white mycelium on the foliage). Disease incidence (DI) was based on the percentage of plants among 10 consecutive plants at a location selected at random within the row. The DI was analyzed using analysis of variance procedures as a percentage of plants with mycelial

growth. Data obtained from the field evaluation were compared to the reaction using the straw test. Plant growth habit, which is controlled by a single locus (*fin*), was classified either as determinate or indeterminate at a nondiseased nursery at the CSU Western Colorado Research Center, Fruita CO in 2006.

Molecular Marker Analyses

We extracted DNA from three to four young trifoliolate leaves from a single plant for each RIL and parent using the method described by Skroch and Nienhuis (1995). Four DNA analysis systems were used to genotype parental lines and the RIL population: randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellite DNA or simple sequence repeats (SSR), and sequence characterized amplified region (SCAR). Parental lines were screened for polymorphisms before genotyping the 94 RIL used in this study. All polymerase chain reactions (PCR) were conducted in 96-well plates on a PTC-100 or PTC-200 thermal cycler (MJ Research, Inc., Hercules, CA), and used *Taq* DNA polymerase and 10X ThermoPol buffer (New England BioLabs, Inc., Beverly, MA).

Random Amplified Polymorphic Analysis

Nine primers (Operon Technologies, Alameda, CA) were used to genotype the RIL population. These primers corresponded to loci reported on previous common bean linkage maps. The PCR amplifications were conducted as described by Freyre et al. (1998) with slight modifications, which included the use of 4 ng total genomic DNA and a different source of *Taq* DNA polymerase. Amplification products were separated on 1.5% agarose gels in 1X TBE buffer at 100 V for 2.5 h, stained with ethidium bromide, and digitally photographed under ultraviolet light with an AlphaImager gel documentation system (Alpha Innotech Corp., San Leandro, CA). Band sizes that corresponded to previously mapped loci were scored and named as follows: the Operon primer serial name, an underscore, and band sized in base pairs (bp) estimated from a 1 kb molecular weight ladder (Promega, Madison, WI). For example, W6_600 identified a band of 600 bp amplified by the W6 Operon primer.

Amplified Fragment Length Polymorphism Analysis

The AFLP analyses were performed using the restriction enzyme combinations *EcoRI/MseI* and *PstI/MseI*. Digestion, ligation, pre-amplification, and selective amplification reactions were conducted as described by Vos et al. (1995) with slight modification. The selective amplification was performed in a 20 μ L reaction of 30 ng of a two-nucleotide selective primer for *PstI* and 30 ng of a three-nucleotide selective primer for *EcoRI* and *MseI*, 2 mM dNTPs, 1 unit of *Taq* DNA polymerase with 10X ThermoPol buffer, and 1 μ L of the pre-amplification product. The PCR program for the selective amplification was programmed for 36 cycles with the following cycle profile: 30 s at 65°C, subsequently reduced each cycle by 0.7°C for the next 12 cycles, then continued at 55°C for the remaining 23 cycles, and 1 min at 72°C. Amplified fragments were separated on 4% polyacrylamide gels run at 70 W for 2 h, then silver stained and scanned on a flat bed scanner at 150 dpi resolution.

Polymorphic bands were scored for each RIL in relation to the parental marker class. The AFLP loci were named by a capital letter corresponding to their respective digestion enzyme combination where $P = PstI$, $E = EcoRI$, and $M = MseI$. Lowercase letters correspond to the selective nucleotide sequence and the number represents the band size estimated from a 10 bp molecular weight ladder (BioRad, Hercules, CA).

Microsatellite Analysis

Microsatellite marker analysis was conducted with 33 SSR primer pairs developed by Yu et al. (2000), Gaitan-Solis et al. (2002), and Blair et al. (2003). In this study, PCR conditions were in accordance with Gaitan-Solis et al. (2002), with minor modifications. The PCR reactions were performed in a 20 μ L final volume containing 50 ng of genomic DNA, 0.1 μ M of each forward and reverse primer, 0.25 mM of dNTP, 10X ThermoPol buffer, and 1 unit of *Taq* DNA polymerase. The PCR temperature cycling profile was a hot start of 92°C for 5 min; 30 cycles of 92°C for 1 min, 47°C for 1 min, and 72°C for 2 min; followed by 1 cycle of 5 min at 72°C. When the size difference between parental polymorphic fragments was relatively large (>10 bp), PCR products were separated on 4% agarose SFR (Amresco, Solon, OH), and for smaller size differences they were separated on 4% polyacrylamide gels. Gels were visualized as previously described for RAPD and AFLP markers.

Phaseolin Seed Protein Evaluation

All RIL and parental lines were genotyped for their respective alleles at the phaseolin seed storage locus type (*Phs*). A SCAR marker developed by Kami et al. (1995) was used to distinguish lines that possessed either the Tendergreen (T) or Sanilac (S) allele. Seed protein composition of a subset of lines was confirmed by extraction and gel electrophoresis of seed proteins using the procedure described by Gepts et al. (1992).

Data Analysis

Phenotypic Data

All greenhouse experiments were analyzed as randomized complete block designs, using Proc GLM of SAS for Windows 9.1 (SAS Institute, Cary NC). Entries (RIL and checks) were considered as a fixed model factor, while blocks were considered a random factor. An average severity index (ASI) score was calculated as the average straw test score for the three repeated straw tests by the use of the LSMEANS statement of Proc GLM. Error variance was homogeneous ($P \geq 0.05$) among the three straw test evaluations. Deviations from normality among the ASI scores were determined by the Shapiro and Wilk test statistic W (SAS Proc Univariante). The same procedure was used for field data. Correlation analysis (SAS Proc Corr) was used to determine the associations among disease evaluations under greenhouse straw test runs and the field evaluation.

Molecular Marker Data

For each marker, we conducted a chi-square analysis to detect significant deviation of genotypic classes from the expected 1:1 Mendelian segregation ratio. Linkage maps were constructed with JoinMap 3.0 software (Van Ooijen and Voorrips, 2001). Pairwise linkage between markers was considered significant at a minimum

\log_{10} of the likelihood odds ratio (LOD) score of 3.0, and multi-point analyses were conducted to determine linkage distances with the Haldane mapping function. Microsatellite and RAPD markers that had been previously mapped, as well as the *fin* and *Phs* loci, were used to anchor linkage groups to the common bean core map (Beebe et al., 2006; Blair et al., 2003, 2006; Frei et al., 2005; Liao et al., 2004; Ochoa et al., 2006; Yu et al., 2000). When two or more of our initial linkage groups corresponded to a single core map linkage group based on common markers, we attempted to bring them together at a reduced LOD stringency of 1.0 in Join-Map, as suggested by the procedure used by Micic et al. (2005).

Quantitative Trait Locus Analysis

Markers significantly associated with resistance to WM were identified through single-factor analysis of variance (SFA) conducted with SAS Proc GLM and composite interval mapping (CIM) as implemented by QTL Cartographer (Wang et al., 2005). Analysis parameters for QTL Cartographer were Model 6 of the CIM procedure, stipulating an R11 population, window size of 10 cM, 10 cofactors determined by forward stepwise regression, and genome scanning every 2 cM. Genomewide empirical threshold levels for declaring QTL significance were obtained by 1000 permutations of the data set in accordance with Churchill and Doerge (1994). A chromosome location having a peak significance value exceeding the threshold was interpreted as the most likely position of a QTL, and the R^2 value at that point was considered as the percent phenotypic variance explained by the locus. A two-LOD support interval was drawn on each side of the QTL positions. Epistatic interactions among all pairwise combinations of markers were evaluated with SAS Proc GLM, as implemented in the program EPISTACY (Holland, 1998), using a threshold significance level of $P < 0.0001$. Finally, multiple-locus models including the most significant flanking marker of each QTL detected by CIM were evaluated with SAS Proc GLM.

RESULTS AND DISCUSSION

Characterization of Recombinant Inbred Line Population for Reaction to White Mold

Significant variation for WM reaction among lines in the RIL population and check cultivars was observed in both greenhouse and field tests. Reactions of the entries using the straw test indicated that results were consistent across the three evaluations ($r = 0.716$ to 0.789 , $P < 0.01$), and the mean ASI among entries combined over evaluations was highly correlated with each of the three evaluations ($r = 0.939$, 0.943 , and 0.907 ; $P < 0.01$) (Table 1). Field tests for disease severity (DS) scores differentiated the checks Montrose and PC-50, with mean DS 80% and 26%, respectively. Parental lines G122 and CO72548 were not significantly different in the field test with DS scores of 26 and 60%, respectively. The field DS scores for the RIL population ranged from 13 to 90%, and the frequency distribution was normally distributed with transgressive segregates at both the high and low ends of the distribution (distribution not shown). The greenhouse straw test ASI

Table 1. Simple correlation coefficients among lines across three separate greenhouse straw test evaluations, and the mean straw test average severity index (ASI) for the recombinant inbred line (RIL) population derived from the cross G122/CO72548 ($n = 94$).

	Straw test 1	Straw test 2	Straw test 3
	r		
Straw test 2	0.789**		
Straw test 3	0.716**	0.779**	
Mean ASI	0.939**	0.943**	0.907**

**Significant at the 0.01 probability level.

was correlated with field disease severity ($r = 0.54$; $P < 0.01$) and disease incidence ($r = 0.302$; $P < 0.05$) (Table 2). Significant correlations between the straw test and field disease severity indicate that the two tests provide similar estimates for reaction to WM. However, because the straw test does not detect avoidance mechanisms that are useful under field conditions, the results were not expected to be identical. The correlation between ASI and field disease incidence was likely lower than between ASI and field disease severity because there was more variation in the data set for disease incidence (CV = 41 and 65 for disease severity and disease incidence, respectively).

The straw tests effectively differentiated ($P \geq 0.05$) WM reactions between the resistant (PC-50) and susceptible (Montrose) checks with a mean ASI of 5.9 and 8.0, respectively, when averaged over the three evaluations (Table 3). The parental lines of the RIL population G122 and CO72548 did not differ for mean ASI, with values of 4.5 and 5.8, respectively. The ASI scores among lines in the RIL population ranged from 3.2 to 8.8. The frequency distribution for the mean straw test ASI among lines was normally distributed ($P > 0.05$) and showed evidence of transgressive segregation at both the high and low ends of the distribution (Fig. 1). A continuous frequency distribution and presence of transgressive segregates in both the straw test and field test suggest that physiological resistance is controlled quantitatively. Quantitative inheritance is consistent with earlier investigations on the inheritance of resistance to WM (Fuller et al., 1984; Kolkman and Kelly, 2003; Miklas and Grafton, 1992; Park et al., 2001). Two entries, RIL 31 and RIL 67, had lower ($P < 0.05$) mean ASI than the resistant parent G122 (Table 3). Since the parental lines are from different common bean centers of domestication, with G122 from the Andean center and CO72548 from the Middle American center, it is likely that the parents possess different resistance alleles.

Molecular Marker Analysis

Our marker analysis resulted in 198 scored polymorphic loci: 152 AFLPs, 31 microsatellites, 13 RAPDs, one SCAR (*Phs*), and one morphological locus (*fin*). Ninety-four RIL were genotyped, however only 89 were used for data analysis because of the incidence of heterozygosity found

Table 2. Simple correlation coefficients among lines for reaction to white mold in the greenhouse straw test with field incidence and field severity for the recombinant inbred line (RIL) population derived from the cross G122/CO72548 ($n = 63$).

	Field severity [†]	Field incidence [‡]
	r	
Straw test mean ASI	0.540**	0.302*
Field Incidence	0.714**	

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level.

[†]Disease severity was based on visual observations of severity, rated from 0 to 100%.

[‡]Disease incidence indicates the percentage of plants with mycelial growth in an inoculated field nursery.

using microsatellite markers. The linkage map constructed with JoinMap resulted in 13 linkage groups that could be identified with core map linkage groups based on known marker locations. The 13 groups (Fig. 2) included 126 loci and covered a total genome length of 733 cM. An additional three linkage groups, with a total of 14 markers and 77 cM, consisted solely of AFLP markers and could not be related to the core map. Compared to the 1200 cM linkage map reported by Freyre et al. (1998), our combined map

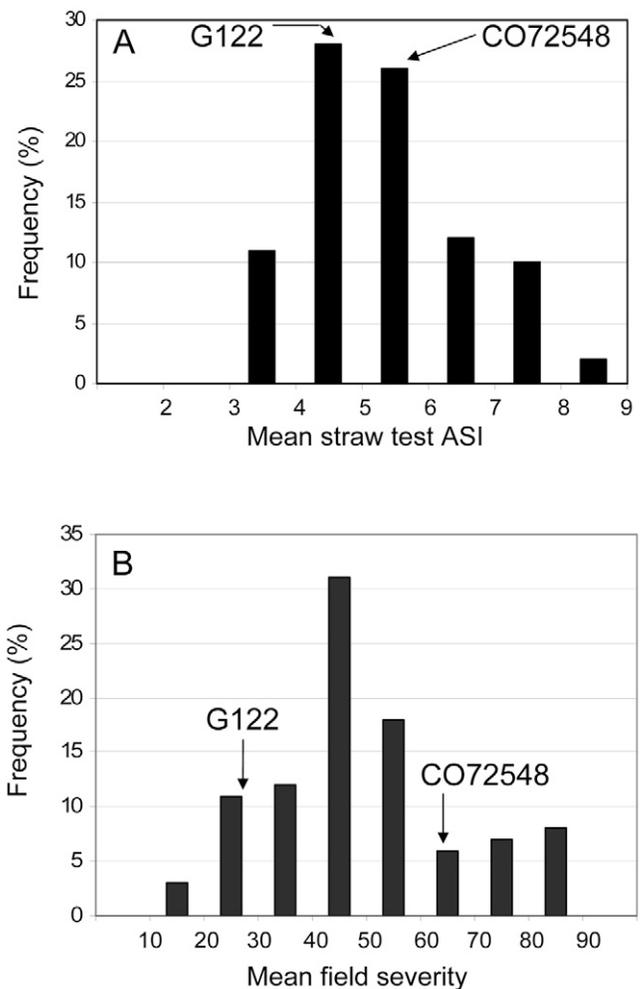


Figure 1. Frequency distribution of G122/CO72548 recombinant inbred lines based on (A) mean straw test average severity index and (B) field severity.

Table 3. Entry, greenhouse straw test average severity index (ASI), and phaseolin type among checks and recombinant inbred line (RIL) that had lower observed ASI than parent G122.

Entry	ASI	Phaseolin type [†]
RIL 31	3.2a [‡]	T
RIL 67	3.4a	T
RIL 32	3.5ab	T
RIL 7	3.5ab	T
RIL 21	3.6ab	T
RIL 35	3.7ab	S
RIL 130	3.8ab	S
RIL 125	3.8ab	T
RIL 118	3.8ab	S
RIL 47	3.9ab	T
RIL 103	3.9ab	S
RIL 17	4.1ab	T
RIL 9	4.2ab	T
RIL 111	4.2ab	T
RIL 164	4.2ab	T
RIL 41	4.2ab	S
RIL 102	4.3ab	T
RIL 20	4.3ab	S
RIL 26	4.4ab	T
G122	4.5bc	T
CO72548	5.8c	S
PC-50	5.9c	NT [§]
Montrose	8.0d	S

[†]Denotes the seed storage protein content for each entry, where T = Tendergreen and S = Sanilac.

[‡]Scores followed by the same letter are not significantly different ($P < 0.05$).

[§]NT, not tested. This entry was not tested for phaseolin type in this research project.

represents about two-thirds of the common bean genome, about the same coverage as the map used by Miklas et al. (2001) for QTL analysis of WM resistance.

Twenty-four of the 126 loci on the Fig. 2 linkage maps showed deviations from Mendelian segregation at $P < 0.01$. These included all 12 loci on linkage group B10 (B10a and B10b in Fig. 2), six loci on B5, two loci on B4, and one locus each on B6, B8, B9, and B11. All of the distorted loci on linkage groups B5, B10a, and B10b had an excess of CO72548 alleles, indicating the presence of factors that inhibited transmission of G122 alleles on those chromosomes. Paredes and Gepts (1995) examined segregation distortions in Andean \times Middle American gene pool crosses and also found that the distorted markers had an over-representation of Middle American alleles. The large number of distorted markers on B10 is consistent with previous reports for marker segregation distortion in common bean (Blair et al., 2003; Fall et al., 2001).

Validation of the Quantitative Trait Locus Linked to *Phs*

To validate the effect of the QTL linked to the *Phs* locus on B7 as reported by Miklas et al. (2001), the RIL popula-

tion was characterized with the *Phs* SCAR marker which distinguishes between T and S phaseolin alleles (Kami et al., 1995). We evaluated the ability of the *Phs* marker to identify the correct phaseolin allele by characterizing 10 RIL for their seed storage protein patterns according to Gepts et al. (1992). The *Phs* marker identified the correct phaseolin type for all 10 RIL evaluated. Therefore, the remainder of the RIL were characterized only with the *Phs* SCAR marker.

Because the B7 QTL was reported to condition resistance to WM, we conducted SFA to determine the effect of variation at the *Phs* locus on the resistance traits. The T and S genotypic classes that represent parents G122 and CO72548 (resistant and susceptible allele, respectively) were significantly different ($P < 0.01$) for ASI, with mean values of 5.0 and 5.7, respectively. The R^2 value for this analysis was 8.8%, compared to 38% for the B7 QTL reported by Miklas et al. (2001). The effect of variation at *Phs* was not significant ($P > 0.05$) for field severity in our study, although it was significant in Miklas et al. (2001). There are several potential reasons for this difference in results. First, the different genetic background of the susceptible parent used in the two studies may have altered the effect of the QTL. Because the two populations shared the parent G122, we anticipated similar results, however, the alternate parent used to develop the RIL differed in the Miklas population. Miklas et al. (2001) used a black seeded line from race Mesoamerica as the alternate parent, whereas we used a pinto line representative of germplasm from race Durango. Second, the effect of the QTL may have been overestimated because Miklas et al. (2001) cautioned readers that the magnitude of the QTL effect could have been an overestimation because of small population size ($n = 67$). A third potential explanation is the different *S. sclerotiorum* isolates used in the two studies. Miklas et al. (2001) used an isolate designated T001.1, which has been shown to be less aggressive than the Colorado isolate used in this study (Steadman et al., 2004). Park et al. (2001) reported different QTL for WM resistance when two different isolates were used on the same RIL population. In summary, the *Phs* marker alone may not be the best indicator of resistance, because it conditioned a low amount of resistance in our study.

Quantitative Trait Locus Analysis

Composite interval mapping revealed five QTL associated with physiological resistance to WM based on the greenhouse straw test (ASI), located on linkage groups B1, B2b, B8, and B9; and one QTL for field disease severity detected on linkage group B8 (Table 4, Fig. 2). The QTL positions identified by CIM were supported by significant results from the SFA for markers located in close proximity to the detected chromosome regions. No epistatic

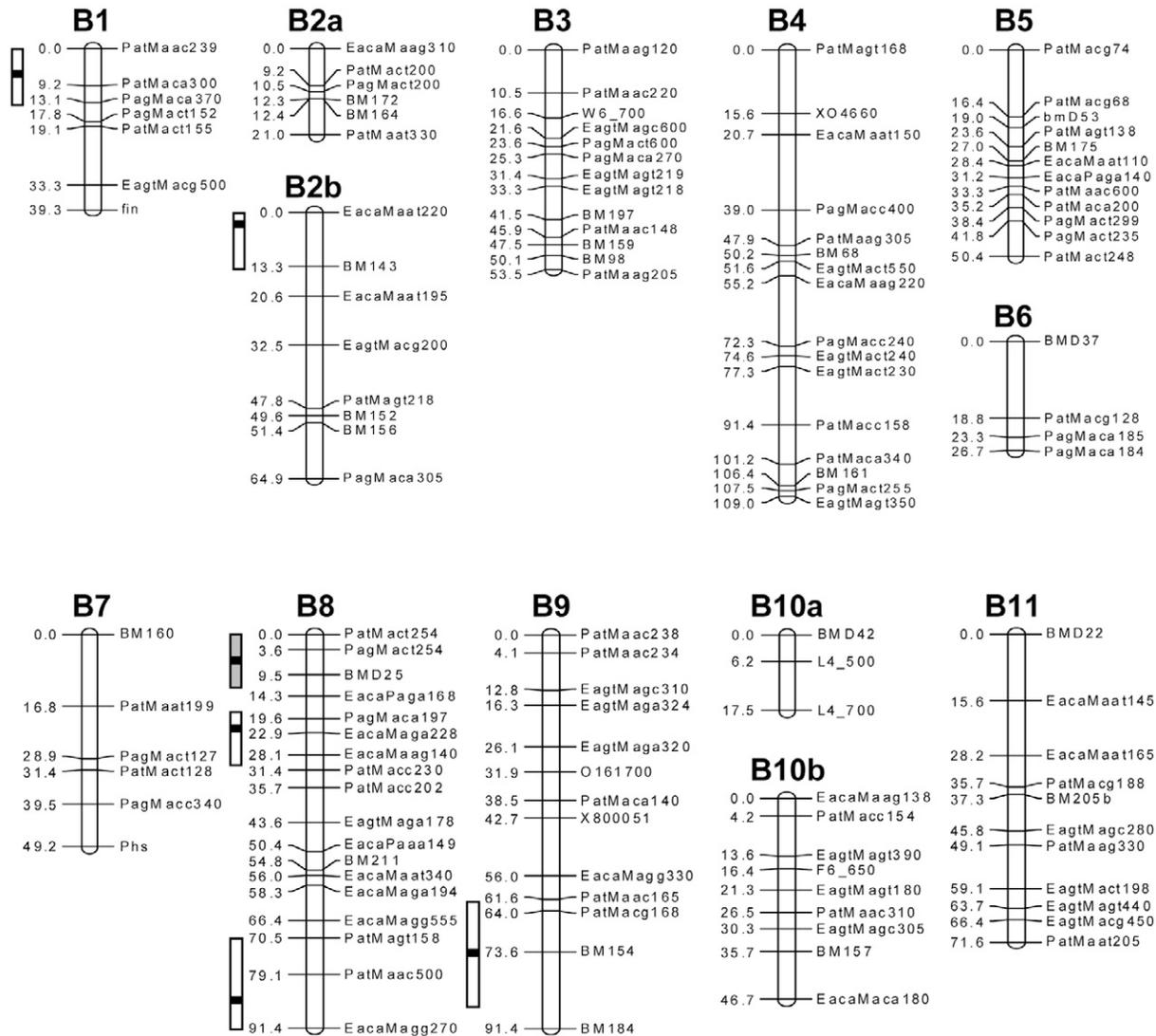


Figure 2. Linkage map and quantitative trait locus (QTL) locations for white mold resistance in the common bean recombinant inbred line (RIL) population G122/CO72548. Open bars are placed to the left of QTL positions for straw test average severity index (ASI, white bars), and the shaded bar indicates a field severity QTL. Horizontal lines within a bar mark the point of peak QTL significance and the bar length indicates the 2-likelihood odds ratio (LOD) support interval on either side of the peak.

interactions were detected at a comparisonwise level of $P < 0.0001$ for either ASI or field disease severity.

A QTL that accounted for 20% of the phenotypic variability (R^2) for ASI was identified on B1 between the AFLP markers PatMaac239 and PatMaca300. Another moderately large QTL for ASI ($R^2 = 14.8\%$) was located near AFLP marker EacaMaat220 on B2b. Other QTL associated with physiological resistance to WM have been mapped on B2. Ender and Kelly (2005) reported two QTL in close proximity on B2 that were associated with WM resistance and detected in two different RIL populations. Linkage group B2 has also been reported as an important region in the common bean genome for defense related genes for WM and other diseases (Ender and Kelly, 2005; Ryder et al., 1987; Walter et al., 1990). However, due to lack of common markers, a comparison between the loca-

tion of the B2 QTL reported here and prior QTL could not be made.

Two smaller-effect QTL for ASI were detected in different regions of linkage group B8 (Fig. 2, Table 4). For both of these QTL, the favorable allele (i.e., associated with a lower ASI rating) came from CO72548, in contrast to the QTL on other linkage groups, where G122 was the favorable allele donor. This finding could explain at least a portion of the transgressive segregates among the RILs that were observed both in the straw test and field experiment. Regions of B8 have been previously reported to be associated with resistance to WM in the field and greenhouse (Ender and Kelly, 2005; Miklas et al., 2003; Park et al., 2001).

The QTL on B9 mapped closest to the microsatellite marker BM154 and accounted for 12% of the phenotypic

Table 4. Position, significance, percent variance explained (R^2) and additive effects of quantitative trait locus (QTL) for greenhouse straw test average severity index (ASI) and field severity in the population G122/CO72548. Results were obtained by composite interval mapping with genomewide empirical threshold significance levels.

Trait	Linkage group	QTL position	Nearest locus	LOD score [†]	R^2 [‡]	Additive effect [§]	Favorable allele
		cM			%		
ASI	B1	6.0	PatMaca300	6.96**	20.2	-0.572	G122
	B2b	2.0	EacaMaat220	4.78**	14.8	-0.572	G122
	B8	21.6	EacaMaga228	2.73 [†]	7.0	0.353	CO72548
	B8	85.1	PatMaac500	3.14 [#]	10.9	0.449	CO72548
	B9	73.6	BM154	5.24**	12.7	-0.477	G122
Multiple locus model					48.4		
Field severity	B8	5.6	PagMact254	3.34 [#]	12.4	-6.81	G122

**Significant at the genomewide significance level of $P < 0.01$.

[†]LOD, likelihood odds ratio.

[‡]Percent phenotypic variance explained by the QTL.

[§]Additive effects indicate the effect of G122 alleles.

[#]Significant at the genomewide significance level of $P < 0.15$.

^{*}Significant at the genomewide significance level of $P < 0.10$.

variation for ASI (Table 4, Fig. 2). This QTL may be novel, since to our knowledge no other reports have identified this region as associated with WM resistance. If confirmed, the B9 QTL would be a good candidate for marker-assisted selection, because of the relative ease of using microsatellite markers for that purpose.

When analyzed together in a multiple-locus model, the most significant markers flanking the five QTL for ASI accounted for 48% of the phenotypic variance for the trait. When *Phs* was added to the model, its effect was not significant ($P > 0.05$).

For low field disease severity only one QTL, on linkage group B8 with an R^2 value of 12%, was detected at the genomewide significance level of 0.10. The QTL position is intriguingly close to the QTL for ASI in the same region of B8 (Fig. 2), however, the 2-LOD support intervals of the QTL do not overlap, and the source of the favorable alleles differ for the two QTL (Table 4). Multiple QTL on B8 for physiological and field resistance is similar to the results of Park et al. (2001), who reported several overlapping QTL in what is apparently the same region of this linkage group. A potential correspondence in genomic regions that influence the two traits occurred on B1, where there was a large-effect QTL for ASI with a peak at 6 cM, and a subthreshold region with a peak LOD of 2.05 at 9 cM for field disease severity (not shown on Fig. 2).

CONCLUSIONS

The combination of physiological resistance and avoidance mechanisms is the most economical and sustainable approach to control WM disease. Screening procedures that identify physiological resistance are needed to accomplish this goal. The results of this study show that the straw test provides a useful and repeatable method to select material with an increased level of physiological resistance

to WM without an extensive field test. The straw test also has the discriminating ability to identify transgressive segregates that indicate resistance can be obtained from moderately susceptible germplasm.

This study did not validate the same level of effect for the B7 QTL for WM resistance reported by Miklas et al. (2001) based on either CIM or SFA. With SFA the B7 QTL accounted for 8% of the variation for WM resistance in our study, whereas Miklas et al. (2001) reported that it accounted for 38% of the phenotypic variation for disease score in the greenhouse straw test.

We identified five QTL that together explained 48% of the variation in reaction to WM based on the straw tests. The combination of markers linked to QTL that conferred physiological resistance to WM on B1, B2b, B8, and B9, and one QTL for field resistance on linkage group B8 offer unique insights for breeding strategies, such as QTL pyramiding, and for greater understanding and improvement of resistance to WM. Breeding methods such as recurrent selection could be useful to develop WM resistant cultivars that possess both avoidance and physiological resistance. Using marker-assisted selection for physiological resistance alleles, together with morphological selection for avoidance, genetic gain per cycle could be maximized and improvements could be expected in a relatively short time.

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References

- Abawi, G.S., R. Provvidenti, D.C. Crosier, and J.E. Hunter. 1978. Inheritance of resistance to white mold disease in *Phaseolus coccineus*. *J. Hered.* 69:200-202.

- Adams, P.B., C.J. Tate, R.D. Lumsden, and J.P. Meiners. 1973. Resistance of *Phaseolus* species to *Sclerotinia sclerotiorum*. Annu. Rep. Bean Improve. Coop. 16:8–10.
- Beebe, S.E., M. Rojas-Pierce, X.L. Yan, M.W. Blair, F. Pedraza, F. Munoz, J. Tohme, and J.P. Lynch. 2006. Quantitative trait loci for root architecture traits correlated with phosphorus acquisition in common bean. *Crop Sci.* 46:413–423.
- Blad, B.L., J.R. Steadman, and A. Weiss. 1978. Canopy structure and irrigation influence white mold and microclimate of dry beans. *Phytopathology* 68:1431–1437.
- Blair, M.W., G. Iriarte, and S. Beebe. 2006. QTL analysis of yield traits in an advanced backcross population derived from a cultivated Andean x wild common bean (*Phaseolus vulgaris* L.) cross. *Theor. Appl. Genet.* 112:1149–1163.
- Blair, M.W., F. Pedraza, H.F. Buendia, E. Gaitan-Solis, S.E. Beebe, P. Gepts, and J. Tohme. 2003. Development of a genome-wide anchored microsatellite map for common bean (*Phaseolus vulgaris* L.). *Theor. Appl. Genet.* 107:1362–1374.
- Brick, M.A., H.F. Schwartz, J.B. Ogg, J.J. Johnson, and F. Judson. 2001. Registration of 'Montrose' pinto bean. *Crop Sci.* 41:260.
- Churchill, G.A., and R.W. Doerge. 1994. Empirical threshold values for quantitative trait mapping. *Genetics* 138:963–971.
- Coyne, D.P., J.R. Steadman, C. Campbell, and P. Fuller. 1981. Development of *Phaseolus vulgaris* with resistance and/or avoidance to white mold disease (*Sclerotinia sclerotiorum*). Annu. Rep. Bean Improve. Coop. 24:82–83.
- Dickson, M.H., J.E. Hunter, M.A. Better, and J.A. Cigna. 1982. Selection for resistance in *Phaseolus vulgaris* L. to white mold disease caused by *Sclerotinia sclerotiorum* (Lib.) de bray. *J. Am. Soc. Hortic. Sci.* 107:231–234.
- Dickson, M.H., J.F. Hunter, J.A. Gina, and M.A. Better. 1981. Resistance to white mold. Annu. Rep. Bean Improve. Coop. 24:126–128.
- Ender, M., and J.D. Kelly. 2005. Identification of QTL associated with white mold resistance in common bean. *Crop Sci.* 45:2482–2490.
- Erdmann, P.M., R.K. Lee, M.J. Bassett, and P.E. McClean. 2002. A molecular marker tightly linked to P, a gene required for flower and seed coat color in common bean (*Phaseolus vulgaris* L.), contains the Ty3-gypsy retrotransposon Tpv3g. *Genome* 45:728–736.
- Fall, A.L., P.F. Byrne, G. Jung, D.P. Coyne, M.A. Brick, and H.F. Schwartz. 2001. Detection and mapping of a major locus for Fusarium wilt resistance in common bean. *Crop Sci.* 41:1494–1498.
- Frei, A., M.W. Blair, C. Cardona, S.E. Beebe, H. Gu, and S. Dorn. 2005. QTL mapping of resistance to *Thrips palmi* Karny in common bean. *Crop Sci.* 45:379–387.
- Freyre, R., P.W. Skroch, V. Geffroy, A.F. Adam-Blondon, A. Shirmohamadali, W.C. Johnson, V. Llaca, R.O. Nodari, P.A. Pereira, S.M. Tsai, J. Tohme, M. Dron, J. Nienhuis, C.E. Vallejos, and P. Gepts. 1998. Towards an integrated linkage map of common bean: 4. Development of a core linkage map and alignment of RFLP maps. *Theor. Appl. Genet.* 97:847–856.
- Fuller, P.A., D.P. Coyne, and J.R. Steadman. 1984. Inheritance of resistance to white mold disease in a diallel cross of dry bean. *Crop Sci.* 24:929–932.
- Gaitan-Solis, E., M.C. Duque, K.J. Edwards, and J. Tohme. 2002. Microsatellite repeats in common bean (*Phaseolus vulgaris*): Isolation, characterization, and cross-species amplification in *Phaseolus* spp. *Crop Sci.* 42:2128–2136.
- Gepts, P., V. Llaca, R.O. Nodari, and L. Panella. 1992. Analysis of seed proteins, isozymes, and RFLPs for genetic and evolutionary studies in *Phaseolus*. p. 63–93. In H.-F. Linskens and J.F. Jackson (ed.) *Seed analysis. Modern methods of plant analysis, New Series, Vol. 14.* Springer, Berlin, Germany.
- Gilmore, B., and J.R. Myers. 2003. A preliminary molecular marker map for *Phaseolus coccineus*. Annu. Rep. Bean Improve. Coop. 46:87–88.
- Holland, J.B. 1998. EPISTACY: A SAS program to detect two-locus epistatic interactions using genetic marker information. *J. Hered.* 89:374–375.
- Hunter, J.E., M.H. Dickson, M.A. Boettger, and J.A. Cigna. 1982. Evaluation of plant introductions of *Phaseolus* spp. for resistance to white mold. *Plant Dis.* 66:320–322.
- Hunter, J.E., M.H. Dickson, and J.A. Cigna. 1981. Limited-term inoculation: A method to screen bean plants for partial resistance to white mold. *Plant Dis.* 65:414–417.
- Kami, J., V.B. Velasquez, D.G. Debouck, and P. Gepts. 1995. Identification of presumed ancestral DNA sequences of phaseolin in *Phaseolus vulgaris*. *Proc. Natl. Acad. Sci. USA* 92:1101–1104.
- Kerr, E.D., J.R. Steadman, and L.A. Nelson. 1978. Estimation of white mold disease reduction of yield and yield components of dry beans. *Crop Sci.* 18:275–279.
- Kolkman, J.M., and J.D. Kelly. 2000. An indirect test using oxalate to determine physiological resistance to white mold in common bean. *Crop Sci.* 40:281–285.
- Kolkman, J.M., and J.D. Kelly. 2003. QTL conferring resistance and avoidance to white mold in common bean. *Crop Sci.* 43:539–548.
- Liao, H., X.L. Yan, G. Rubio, S.E. Beebe, M.W. Blair, and J.P. Lynch. 2004. Genetic mapping of basal root gravitropism and phosphorus acquisition efficiency in common bean. *Funct. Plant Biol.* 31:959–970.
- Lyons, M.E., M.H. Dickson, and J.E. Hunter. 1987. Recurrent selection for resistance to white mold in *Phaseolus* species. *J. Am. Soc. Hortic. Sci.* 112:149–152.
- Micic, Z., V. Hahn, E. Bauer, A.E. Melchinger, S.J. Knapp, S. Tang, and C.C. Schon. 2005. Identification and validation of QTL for *Sclerotinia* midstalk rot resistance in sunflower by selective genotyping. *Theor. Appl. Genet.* 111:233–242.
- Miklas, P.N., R. Delorme, and R. Riley. 2003. Identification of QTL conditioning resistance to white mold in snap bean. *J. Am. Soc. Hortic. Sci.* 128:564–570.
- Miklas, P.N., and K.F. Grafton. 1992. Inheritance of partial resistance to white mold in inbred populations of dry bean. *Crop Sci.* 32:943–948.
- Miklas, P.N., K.F. Grafton, and B.D. Nelson. 1992a. Screening for partial physiological resistance to white mold in dry bean using excised stems. *J. Am. Soc. Hortic. Sci.* 117:321–327.
- Miklas, P.N., K.F. Grafton, G.A. Secor, and P.E. McClean. 1992b. Using of pathogen filtrate to differentiate physiological resistance of dry bean to white mold disease. *Crop Sci.* 32:310–312.
- Miklas, P.N., W.C. Johnson, R. Delorme, and P. Gepts. 2001. QTL conditioning physiological resistance to white mold in dry bean. *Crop Sci.* 41:309–315.
- Ochoa, I.E., M.W. Blair, and J.P. Lynch. 2006. QTL analysis of adventitious root formation in common bean under contrasting phosphorus availability. *Crop Sci.* 46:1609–1621.
- Paredes, O.M., and P. Gepts. 1995. Segregation and recombination in inter-gene pool crosses of *Phaseolus vulgaris* L. *J. Hered.* 86:98–106.

- Park, S.O., D.P. Coyne, J.R. Steadman, and P.W. Skroch. 2001. Mapping of QTL resistance to white mold in common bean. *Crop Sci.* 41:1253–1262.
- Petzoldt, R., and M.H. Dickson. 1996. Straw test for resistance to white mold in beans. *Annu. Rep. Bean Improve. Coop.* 39:142–143.
- Ryder, T.B., S.A. Hendrick, J.N. Bell, X. Liang, S.D. Clouse, and C.J. Lamb. 1987. Organization and differential activation of a gene family encoding the plant defense enzyme chalcone synthase in *Phaseolus vulgaris*. *Mol. Gen. Genet.* 210:219–233.
- Saladin, F., E. Arnaud-Santana, J.C. Nin, G. Goday-Gutz, J.S. Beaver, D.P. Coyne, and J.R. Steadman. 2000. Registration of ‘PC-50’ red mottled bean. *Crop Sci.* 40:858.
- Schwartz, H.F., K. Otto, H. Terán, M. Lema, and S.P. Singh. 2006. Inheritance of white mold resistance in *Phaseolus vulgaris* × *P. coccinius* crosses. *Plant Dis.* 90:1167–1170.
- Skroch, P.W., and J. Nienhuis. 1995. Qualitative and quantitative characterization of RAPD variation among snap bean (*Phaseolus vulgaris*) genotypes. *Theor. Appl. Genet.* 91:1078–1085.
- Steadman, J.R. 1979. Control of plant disease caused by *Sclerotinia* species. *Phytopathology* 69:904–907.
- Steadman, J.R. 1983. White mold: A serious yield limiting disease of bean. *Plant Dis.* 67:346–350.
- Steadman, J.R., J. Kelly, K. Kmieciak, J. Kolkman, J. Myers, and P. Miklas. 2001. Evaluation of source of resistance to *Sclerotinia sclerotiorum* in common bean with five test methods at multiple locations. *Annu. Rep. Bean Improve. Coop.* 44:89–90.
- Steadman, J.R., L.K. Otto-Hanson, and K. Powers. 2004. Resistance improvement of bean through multi-site screening and pathogen characterization. Available at <http://www.whitemoldresearch.com/html/abstractdetails.cfm?RID=104> (verified 14 Aug. 2007). National Sclerotinia Initiative, Fargo, ND.
- Tarán, B., T.E. Michaels, K. Peter, and K.P. Pauls. 2002. Genetic mapping of agronomic traits in common bean. *Crop Sci.* 42:544–556.
- Van Ooijen, J.W., and R.E. Voorrips. 2001. JoinMap 3.0, software for the calculation of genetic linkage maps. Plant Research Int., Wageningen, the Netherlands.
- Vos, P., M. Bleeker, M. Reijans, T. Vandele, M. Hornes, A. Frijter, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP: A new technique for DNA-fingerprinting. *Nucleic Acids Res.* 23:4407–4414.
- Walter, M.H., J. Liu, C. Grand, C.J. Lamb, and D. Hess. 1990. Bean-pathogenesis-related proteins deduced from elicitor-induced transcription are members of a ubiquitous new class of conserved PR proteins including pollen allergens. *Mol. Gen. Genet.* 222:353–360.
- Wang, S., C.J. Basten, and Z.-B. Zeng. 2005. Windows QTL cartographer 2.0. Available at <http://statgen.ncsu.edu/qtlcart/WQTLCart.htm> (verified 14 Aug. 2007). Dep. of Statistics, North Carolina State University, Raleigh.
- Yu, K., S.J. Park, V. Poysa, and P. Gepts. 2000. Integration of simple sequence repeat (SSR) markers into a molecular linkage map of common bean (*Phaseolus vulgaris* L). *J. Hered.* 91:429–434.