AN ABSTRACT OF THE THESIS OF

Jack Erron Haggard for the degree of Master of Science in Horticulture presented on April 27, 2006.
Title: Characterization of Physiological Resistance to White Mold and Search for Molecular Markers Linked to Resistance via Advanced Backcross QTL Analysis in an Interspecific Cross between *Phaseolus coccineus* and *P. vulgaris*

Abstract approved:

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James R. Myers

White mold, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, results in severe losses in the production of common bean, *Phaseolus vulgaris*, especially snap bean varieties. Advanced backcross QTL analysis was used to identify quantitative trait loci for resistance to white mold in an interspecific cross of *P. vulgaris and P. coccineus* and to select resistant, well-adapted germplasm for development of superior common bean cultivars. A population of 115 BC_2F_4 lines from a cross of snap bean cultivar OR 91G x runner bean accession PI 255956 was genotyped using amplified fragment length polymorphism (AFLP®) and simple sequence repeat (SSR) markers. Corresponding BC_2F_5 progeny were evaluated for resistance to white mold in a greenhouse straw test and for tolerance to oxalate in a laboratory test. BC_2F_6 lines were then tested for resistance under field conditions. Single factor analysis of variance identified 29 marker loci contributing to response in at least one phenotypic test. One QTL conditioning 6% of the variance for field resistance was identified by composite interval mapping on linkage group 09, anchored to the consensus linkage group b09 by SSR loci. Significant deviation from expected segregation ratios was observed at all but 3 SSR loci and at all but 7 AFLP® loci ($p < 0.05$). Codominant

SSR markers revealed excessive heterozygosity, and an underrepresented donor homozygous marker class. Several SSR markers polymorphic between parents failed to segregate in the progeny, particularly those corresponding to bean core map linkage groups b01, b04, and b05. Several lines were identified as possessing resistance superior to the recurrent parent. These will be incorporated into our efforts to produce highly resistant snap bean varieties.

Characterization of Physiological Resistance to White Mold and Search for Molecular Markers Linked to Resistance via Advanced Backcross QTL Analysis in an Interspecific Cross between *Phaseolus coccineus* and *P. vulgaris*

by
Jack Erron Haggard

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Jack Erron Haggard, Author

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DEDICATION

In loving memory of James G. Haggard, Sr., my grandfather, whose great love for and humble service to his creator, his family, and every person he encountered are a constant inspiration.

Characterization of Physiological Resistance to White Mold and Search for Molecular Markers Linked to Resistance via Advanced Backcross QTL Analysis in an Interspecific Cross between *Phaseolus coccineus* and *P. vulgaris*

Literature Review

The ascomycetous fungi *Sclerotinia sclerotiorum* (Lib.) de Bary has been described as a "cosmopolitan pathogen," because it causes white mold disease in over 400 species of both monocots and dicots with worldwide distribution (Bolton et al., 2006). In the production of common bean, *Phaseolus vulgaris*, infection reduces pod and seed quality and yield. Kerr et al. (1978) found a 44% reduction in yield of totally infected dry bean plants versus healthy plants, and estimated the yield loss in field conditions from 6% to 19% over a four-year study. In snap beans, the economic impact of white mold is worsened, as low processor thresholds result in the discard of entire truckloads of beans from fields with as low as 3% infection.

White mold in beans is controlled using a combination of protectant chemicals, biological control agents, cultural practices, and resistant or tolerant cultivars (Steadman, 1979). In the past the industry workhorse fungicides, Benomyl and Ronilan, were relied upon heavily for control; however, environmental concerns have resulted in their removal from the market. Farmers now rely on other chemicals, such as Topsin® M and Endura®, but they require multiple well-timed applications to be effective (Bolton et al., 2006) and they are expensive to purchase and apply. Mycoparasites, such as *Coniothyrium minitans* and *Sporidesmium sclerotivorum* are available to reduce survival of sclerotia, but have not been widely accepted by growers. Cultural practices such as crop rotation, residue management, and moisture regulation can reduce the incidence of infection, but sclerotia can survive in the soil for at least 3 years (Steadman, 1979) and very little inoculum is necessary to initiate a

devastating outbreak of white mold. Peachey et al. (2006) found an increase in between-row spacing resulted in reduced severity of infection, but in the absence of disease also resulted in reduced overall yields. To date, only moderate levels of resistance to white mold have been identified in common bean (Fuller et al., 1984; Miklas and Grafton, 1992; Kolkman and Kelly, 1999). To reduce grower dependence upon fungicides and to reduce the costs associated with biological and cultural controls requires the development of cultivars of common bean with greater levels of genetic resistance.

Sclerotinia sclerotiorum is a necrotrophic pathogen and requires an exogenous source of nutrition and hydration to infect healthy tissue (Bolton et al., 2006). In beans, infection generally begins on senescent flower petals that fall to the ground or onto lower surfaces of the bean plant (Cook et al., 1975) where they are easily colonized by windblown ascospores from nearby sclerotial apothecia or by mycelial infection either through plant-to-plant contact or germinating directly from soil-bound sclerotia. Plants generally become infected from 8 weeks after emergence through maturity (Kerr et al., 1978). Infected leaves, pods, and stems develop water-soaked lesions that spread rapidly. Lesions generally turn necrotic and, with sufficient moisture, produce fluffy white mycelium. This mycelium will eventually aggregate and melanize, forming sclerotia which overwinter and produce inoculum for the next growing season (Bolton et al, 2006).

In *S. sclerotiorum* both the ability to form sclerotia and pathogenicity are dependent upon oxalic acid, which is produced in large quantities during infection

(Godoy et al., 1990; Zhou and Boland, 1999; Maxwell and Lumsden, 1970). Foliar concentrations of oxalate up to 10 mM were reported in *Vicia faba* at 2 days post-inoculation, more than 25 times the normal level (Guimarães and Stotz, 2004). Oxalic acid precedes hyphal infection, deregulating stomatal guard cell function (Guimarães and Stotz, 2004), lowering the pH of plant tissues to a favorable range for production and activity of polygalacturonase, pectolytic and proteolytic enzymes (Bateman and Beer, 1965; Marciano et al., 1983; Rollins and Dickman, 2001; Billon-Grand et al., 2002), and suppressing the plant's defenses such as the oxidative burst (Cessna et al., 2000) and polygalacturonase-inhibiting protein (PGIP) (Favaron et al., 2004).

Bean breeding for field resistance to white mold is complicated by a number of avoidance factors. Miklas et al. (2001) found lower white mold levels in field trials of an A55/G122 population of recombinant inbred lines (RILs) associated with the *fin* locus for determinate bush habit, a Quantitative Trait Locus (QTL) for canopy porosity, and generally with increased plant height. Park et al. (2001) also reported an association between QTL for partial field resistance and for canopy porosity in a PC-50/XAN-159 RIL population. Kolkman and Kelly (2002) found growth habit, canopy dimensions, and lodging all significantly associated with lower white mold severity in Bunsi/Newport and Huron/Newport RIL populations.

In attempts to isolate the physiological components of resistance to *S. sclerotiorum*, many tests have been devised. Adams et al. (1973) used colonized oat seed as inoculum in temperature tank experiments. Hunter et al. (1981) used a limited-term inoculation method. The excised-stem inoculation technique was used by Miklas

et al. (1992a). Miklas et al. (1992b) used pathogen filtrate in callus culture medium. Steadman et al. (1997) used a leaf-agar plug assay. The straw test, a greenhouse evaluation developed by Petzholdt and Dickson (1996) using agar plugs in drinking straw sections placed over cut stems of bean plants has been adopted widely by programs breeding for white mold resistance due to its consistency (Miklas et al., 1999; Gilmore and Myers, 2000; Park et al., 2001; Gilmore et al., 2002; Genchev and Kiryakov, 2002; Miklas and Delorme, 2003; Gilmore and Myers, 2004; Schwartz et al., 2004; Schwartz et al 2006). The oxalate test was developed by Kolkman and Kelly (2000) as a method to directly test the tolerance of common bean lines to oxalic acid and has seen limited experimental use (Chipps et al., 2005).

What little genetic variability common bean has for white mold resistance is quantitatively inherited with low to moderate heritability (Fuller et al., 1984; Miklas and Grafton, 1992; Kolkman and Kelly, 1999, 2003; Park et al. 2001). The complexity of white mold resistance breeding has led many researchers to employ the analysis of quantitative trait loci (QTL) to locate molecular markers useful in marker assisted selection. QTL analysis is an extension of the method described by Sax (1923), who associated seed coat pigment and pattern with seed weight and size. The utility of this concept for characterizing polygenic traits was expanded greatly with the advent of molecular markers. Park et al. (1999) reported three QTL on linkage groups (LG) B1 and B4 explaining 33% of the variance for reaction to *S. sclerotiorum* isolate 152 and four QTL on LGs B1, B3, and B4 explaining 54% of the variance for reaction to isolate 279 in a PC-50/XAN-159 RIL population. Park et al. (2001) identified three

QTL explaining 26% of the variance for reaction to isolate 152 on LGs B4, B7, and B8, and one QTL explaining 24% of the variance for reaction to isolate 279 in straw tests of the PC-50/XAN-159 RIL population. Three QTL were also identified on LGs B4, B7, and B8, explaining 27% of field reaction to white mold. Miklas et al. (2001) detected a QTL on LG B7 near the *Phaseolin* (*Phs*) locus explaining 38% and 26% of the variance for straw test and field response, respectively in an A 55/G 122 RIL population. A QTL on B1 conditioning 18% of the variance for field response was associated with the *fin* locus and a QTL explaining 34% of the variance for canopy porosity, suggesting a role of this locus in disease avoidance. In Bunsi/Newport and Huron/Newport RIL populations, Kolkman and Kelly (2003) found QTL on B2 and B7 that account for 12% and 17% of the variance in disease severity, respectively. A QTL conditioning 16% of the variance in oxalate resistance was also detected on B7. In a Benton/NY 6020-4 RIL, Miklas and Delorme (2003) identified QTL on B6 and B8 that account for 12% and 38% of the variance in straw test disease reaction and 13% and 26% of the variance in field disease reaction, respectively. Ender and Kelly (2005) detected QTL on B2, B5, B7, and B8 that accounted for 10.1%, 10.7%, 14.7%, 9.2 % of the variance in field disease reaction, respectively, in a Bunsi/Raven RIL population. Miklas et al. (2007) report significant QTL on B2 over three of four trials and on B3 over two of four trials explaining from 8.7-22.7% and 5.3-15.7% of the variance in field white mold response, respectively. Despite the many successes in locating markers associated with white mold resistance in *Phaseolus vulgaris*, no highly-resistant common bean cultivars have yet been released.

In 1887, Anton de Bary was the first to observe genetic resistance to *Sclerotinia sclerotiorum* in the runner bean, *Phaseolus coccineus*. Runner bean remains the best source of resistance to white mold in the secondary gene-pool of common bean (Abawi et al., 1978; Adams et al., 1973, Gilmore and Myers, 2000; Gilmore et al., 2002; Hunter et al., 1982; Lyons et al. 1987; Schwartz et al., 2004, Schwartz et al., 2006; Debouck, 1999). Abawi et al. (1978), Schwartz et al. (2004), and Schwartz et al. (2006) reported a single dominant resistance gene in interspecific crosses between *P. vulgaris* and *P. vulgaris*. Adams et al. (1973) and Gilmore and Myers (2004), however, found resistance to be quantitatively inherited.

Interspecific crosses for specific traits typically suffer many complications, and the combination of *P. vulgaris* and *P. coccineus* is no exception. Embryos resulting from the cross only develop normally when *P. vulgaris* is the maternal parent (Shii et al., 1982). The F_1 hybrids from such a cross have only 25% viable (stainable) pollen, and later generations tend to revert to one parental phenotype or the other (Guo et al, 1991). Other problems such as linkage drag and distorted segregation, due to lower incidence of crossing-over and elimination of lethal or undesirable recombinants, hinder the interspecific transfer of traits (Guo et al., 1994).

Eshed and Zamir (1995) suggested that backcross inbred lines, such as those Wehrhahn and Allard described in wheat (1965), would allow researchers to "dissect" quantitative traits using molecular markers. Tanksley and Nelson (1996) refined this concept to propose advanced backcross QTL analysis (AB-QTL) as a technique for

integrating QTL discovery and the development of superior varieties, a strategy that has many advantages over mapping QTL in a more balanced population. The lines being evaluated are much more similar to their elite recurrent parent, allowing more accurate assessment of traits. Selection is possible to remove undesirable alleles. Epistatic effects between donor alleles are reduced, allowing more exact estimation of each allele's additive effect. The resulting lines are nearly isogenic, or could be converted to QTL-NILs with little additional effort. The additional opportunities for meiotic recombination in advanced generations reduce the effect of linkage drag, especially important in wide crosses. Research using the AB-QTL method has been successful in several crop species, including barley (Yun et al., 2006; Li et al., 2006), pepper (Rao et al., 2003), tomato (Tanksley and Nelson, 1996; Bernacchi et al., 1998; Fulton et al., 2000; Frary et al., 2004), wheat (Huang et al., 2003; Huang et al., 2004), and rice (Xiao et al., 1998; Yamamoto et al., 1998; Moncada et al., 2001). In common bean, AB-QTL has been employed successfully by Blair et al. (2006b) in a cultivated x wild cross to determine loci conditioning phenological traits, plant architecture, seed weight, yield, and yield components.

AB-QTL analysis relies on the association of phenotypic traits with markers, usually those based on molecular polymorphisms. Many different molecular marker systems exist today that are useful for the construction of linkage maps. Selection of an appropriate system is critical, as the types of markers employed determine not only the resolution but also the usefulness of the map for QTL analysis. Prior to 2000, the common bean consensus mapping effort relied primarily on restriction fragment

length polymorphisms (RFLPs) and randomly-amplified polymorphic DNA (RAPDs) (Freyre et al., 1998). While RAPDs produce large amounts of polymorphic bands, they have the limitation of dominant inheritance, reducing the information content of these markers to one quarter that of codominant markers. In addition, RAPD results are often difficult to reproduce within a population and not usually transferable between populations. Though more reproducible, RFLPs are more labor intensive, require large amounts of DNA and often show limited polymorphism (Collard et al., 2005). Microsatellites, or simple-sequence repeats (SSRs), are tandem repeats of short nucleotide motifs, usually 1 to 6 bases in length. SSRs have the advantages of high reproducibility, high throughput, codominant inheritance, high degree of polymorphism, and random distribution throughout the genome (Pejic et al., 1998; Tautz, 1989; Weber and May, 1989). SSRs were first applied to research in human health, but their utility for linkage mapping in common bean has been exploited beginning with Yu et al. (2000). The reproducibility of SSRs make them especially useful as anchoring loci for comparison of linkage maps from different common bean populations (Beattie et al., 2003; Blair et al., 2006a; Blair et al., 2003; Yu et al., 2000). Another valuable piece of molecular marker technology is amplified fragment-length polymorphism (AFLP®) (Vos et al., 1995). AFLP® combines digestion of genomic DNA by restriction enzymes with PCR-based amplification using selective primers. This results in amplification of multiple fragments, many of which are generally polymorphic. Even though the procedure requires an extra step beyond that required for most other systems, its high multiplex ratio makes AFLP® one of the most high-

throughput marker systems in common usage. A major disadvantage of AFLP® over other marker systems is that the fragments generated show dominant inheritance (Collard et al., 2005). Because individuals heterozygous for a locus producing an amplified fragment are indistinguishable from those homozygous for the fragment's presence, only the individuals that do not amplify a particular fragment can be scored with certainty. Nevertheless, AFLP® has gained wide acceptance, especially for crops where little DNA sequence data are known. AFLP® have been successfully employed for mapping QTL for white mold resistance and avoidance in common bean (Kolkman and Kelly, 2000; Ender and Kelly, 2005).

The objective of this research is to apply the AB-QTL method to an interspecific *Phaseolus* population, associating scores from the straw test, oxalate test, and a field trial with a linkage map constructed from AFLP® and SSR markers, in order to identify and transfer all resistance alleles from the *P. coccineus* accession PI 255956 into *P. vulgaris* cultivar OR 91G.

Materials and Methods

Parental material

The mapping population was created from OR 91G (the *P. vulgaris* recurrent parent) crossed to PI 255956 (the *P. coccineus* donor parent). OR 91G is a bush blue lake green bean released by J. R. Baggett, W. A. Frazier, and G. W. Varseveld at Oregon State University (OSU) in 1980, bred using material from the Mesoamerican center of common bean domestication (Baggett, 1995). OR 91G, the Oregon snap bean industry's standard variety, is very susceptible to infection from *S. sclerotiorum* due to its poor growth habit and lack of physiological resistance, resulting in heavy losses during seasons with environmental conditions favorable to growth of the fungus.

PI 255956 ("Mayan White Runner") is an USDA plant introduction from Guatemala. PI 255956 is a vigorous climber with white flowers and large, white, round seed. Gilmore et al. (2002) tested the entire USDA National Plant Germplasm System collection of *P. coccineus* for white mold response and found PI 255956 to be among the most resistant accessions.

Population development

The development of this advanced backcross population proceeded in three stages: (1) a cross was made between OR 91G and PI 255956 using the common bean as the maternal parent and the runner bean as the pollen source; (2) five plants resulting from the first cross, obviously hybrid from their morphology, were then

backcrossed to OR 91G; (3) each of the 46 resulting progeny were then backcrossed to OR 91G again. All hybridizations were performed during early morning hours (before 10 AM) in a greenhouse in Corvallis, Oregon (23°C average daytime temperature). BC_2F_1 seed was harvested and planted in the field at the OSU vegetable farm, resulting in 115 lines that were advanced to the BC_2F_4 through single plant selections with bush habit as the only selection criterion. From the BC_2F_4, the population was advanced in the greenhouse through single seed descent, with remnant seed from each line bulked for testing in greenhouse and field trials. The BC_2F_4 was chosen for QTL analysis because two backcrosses would restore the majority of the lines to the common bean genotype at most loci, resulting in more suitable germplasm for developing bush blue lake green bean cultivars, and because later generations of inbreeding favor the detection of additive effects (Tanksley and Nelson, 1996). Analysis in later generations also allows seed increase for replicated trials, an important consideration, as beans have a relatively low rate of increase.

Greenhouse straw test

The BC_2F_5 population was tested using the straw test described by Petzholdt and Dickson (1996) in three different environments. The first test was inoculated with a strain of *Sclerotinia sclerotiorum* collected from the OSU vegetable farm, while the second and third test used T001.01, a hyphal tip isolate from a sclerotium collected from 'Newport' navy bean in Quincy, WA in 1996, obtained from Phil Miklas (USDA-ARS, Prosser, WA). Three plants of each of 115 lines and 6 check varieties were grown for each test in a single 15 cm diameter pot using Sunshine® brand SB40

professional growing mix. Check varieties included G 122 (PI 163120, also known as 'Jatu Rong') – a cranberry type dry bean landrace from India with a QTL conferring moderate physiological resistance (Miklas et al., 2001), M0162 (PI 527856) – a yellow-brown seeded hybrid of *P. vulgaris* and *P. coccineus* developed by H. Lamprecht with moderate resistance (unpublished data), OR 91G – the susceptible recurrent parent, Minuette, a parent in the OSU 5630 / Minuette population with extreme susceptibility, OSU 5630 – a highly susceptible OSU breeding line derived from an OSU 5402 / OR 91G cross, and OSU 5613 – another highly susceptible OSU variety derived from a Rapier / OSU 5163 cross. The donor parent, PI 255956, was not included due to extreme differences in growth habit and development. Each test was performed in a greenhouse at OSU in Corvallis, OR. Greenhouse conditions were maintained at approximately 18°C/night and 24°C/day. Plants were grown under artificial HID lighting to maintain 16 hour daylength and were drip-irrigated as needed. Approximately one week after emergence, a dry volume of 2.5 ml of Scott's Osmocote® 14-14-14 slow-release fertilizer was applied to each pot as topdressing. At 28 days after planting (DAP), the main stem of each plant was cut approximately 4 cm above the third node and a 4 cm length section of drinking straw containing an agar plug of mycelium was placed over the cut stem. Actively growing mycelium was obtained for inoculation through subculture of mycelium obtained from single sclerotia placed on 15 x 100 mm plates of potato dextrose agar (PDA). Only the outer edge of the mycelium was used for inoculation, just as the mycelium reached the edge of the plate. In each test, reaction to white mold was rated at 8, 22, and 28 days after

inoculation on a 1-9 scale, where 1=no symptoms, 2=stem lesion forms, but not to the first node, 3= lesion progress down the stem to the first node, 4= lesion progress slightly beyond the first node, 5= lesion progress to the middle of the internode, 6= lesion progress beyond middle of the internode, but not to second node, 7= lesion progress to second node, 8= lesion progress beyond second node, and 9= total plant collapse (modified from Petzholdt and Dickson, 1996).

Oxalate test

One hundred and ten BC_2F_5 lines were also tested in the greenhouse at Corvallis using a modification of the oxalate test described by Kolkman and Kelly (2000). All plants were grown as described previously. Five varieties, including the two parents of the population, G 122, M0162, and NY 6020-5 – a Cornell University snap bean release with high levels of white mold resistance, were used as checks. Temperatures were maintained at a minimum of 24°C during the day and 18°C at night. Plants were grown under artificial HID lighting to maintain 16 hour daylength and were drip-irrigated as needed. At 20 DAP, the seedlings were cut at the base and inserted into 25 mm (50 ml) test tubes containing 40 ml 20mM oxalic acid dihydrate (Sigma-Aldrich®), adjusted to pH 4.0 with NaOH. Control tubes containing 40 ml distilled water adjusted to pH 4.0 with HCl were used to verify the oxalate response. The cut seedlings were then subjected to total darkness at an ambient temperature of approximately 24°C. After 12 hours, the seedlings were rated for wilting symptoms on a 0-9 scale, where 0= no symptoms, 1= one leaf with slight wilting symptoms, 2= one leaf wilting, 3=second leaf wilting slightly, 4= two leaves wilted, 5= three leaves

wilting, 6= four leaves wilting, 7= petioles collapsing, 8=flaccid stems, and 9= total plant collapse.

Field trial

107 lines from the OR 91G x PI 255956 BC_2F_6 population were tested in a replicated field trial in the summer of 2006 on the OSU Vegetable Research Farm at Corvallis, OR. The trial was planted June 28th, and scored for white mold response on September 11th. The field used for the trial has a history of white mold infestation. The soil is a Chehalis silty clay loam. Normal cultural practices for production of the trial were followed.

Entries were arranged in a randomized complete-block design with four blocks. Some lower yielding lines could only produce sufficient seed for two or three replications, however. A plot in this trial is defined as a single row 2.4 m long. In addition to the 107 BC_2F_6 lines, each of four check varieties was included twice in each block. The checks were G 122, M0162, 91G, and OSU 5630. The donor parent, PI 255956, was not included, as it would likely invade neighboring plots due to its growth habit. All plot and border rows were planted 0.76 m apart. Planting density was approximately 483,596 seed/ha. To provide optimal conditions for white mold infection, 3 mm of water was applied by sprinkler to the field daily in the mid-afternoon from the first appearance of flowers until near physiological maturity. Fifty-six kg/ha^{-1} of urea (46-0-0) was incorporated into the field and each row was fertilized by banding prior to planting at a standard rate of 616.22 kg/ha^{-1} of complete fertilizer (12-29-10-8). White mold incidence and severity were combined into a single rating

from 1 to 9, where 1 = no diseased plants and 9= 80% to 100% diseased plants and/or 60% to 100% infected tissue (Miklas et al., 2001).

DNA extraction and molecular marker analysis

DNA was extracted from young leaves of BC_2F_4 single plants in the field at the OSU vegetable farm using a modification of the protocol described by Miklas, et al. (1993) for *Phaseolus*. DNA quality was checked on 1% agarose 0.5x TBE gel, quantified using a NanoDrop ND-1000 UV-Vis spectrophotometer, and diluted to 10 ng/µl for use in PCR. One hundred seventy-two previously published microsatellite markers (Blair et al., 2003; Buso et al., 2006; Gaitán-Solís et al., 2002; Yu et al., 2000) were screened for polymorphism in the parents. Markers identified as polymorphic between the parents were surveyed again in a sub-sample of the population selected to represent both highly resistant and highly susceptible lines.

Microsatellite markers were amplified in a GeneAmp® PCR System 9700 (Applied Biosystems) thermocycler using standard reagents (18 ng genomic DNA, 0.68 µl 50mM MgCl, 1.35 µl 2mM dNTP, 0.18 µl of each 10 mM primer, 0.15 µl AmpliTaq® polymerase (Applied Biosystems), and 2.25 µl 10x reaction buffer in a total volume of 22.5 µl) and conditions (2 min at 94°C, then 35 cycles of 15 sec at 94°C, 15 sec at 50°C, and 15 sec at 72°C.). Microsatellite markers were visualized for screening using standard laboratory techniques, first on ethidium bromide stained 2% agarose 0.5x TBE gels to verify amplification, and then on silver stained 6% polyacrylamide gels to find polymorphism. Final scoring of microsatellite markers

was performed using fluorescently labeled primers on an ABI 3100 capillary sequencer with ABI Genescan® software (Applied Biosystems).

AFLP® analysis proceeded according to Vos et al. (1995) using AFLP® Analysis System I (Invitrogen Co., CA). Briefly, approximately 250 ng of genomic DNA was digested by 2 µL of EcoR I/Mse I in 5 µL of 5X restriction buffer [50 mM Tris-HCl (pH 7.5), 50 mM Mg-acetate, 250 mM K-acetate] in a final volume of 25 µL at 37 °C for three hours. Then the restriction endonucleases were inactivated by incubation at 70° C for 15 minutes, after which the digested samples were stored at 4° C briefly, until adaptor ligation. 24 µL of adapter/ligation solution [EcoR I/Mse I adapters, 0.4 mM ATP, 10 mM Tris-HCl (pH 7.5), 10 mM Mg-acetate, 50 mM K-acetate] and 1 µL T4 DNA Ligase [1 unit/µl in 10 mM Tris-HCL (pH 7.5), 1mM DTT, 50 mM KCl, 50% (v/v) glycerol] were then added to 24 µL of each double-digested DNA sample, and the mixture incubated at 16°C overnight. 10 µL of the reaction mixture was added to 90 µL TE buffer for a 1:10 dilution. Pre-amplification with primers complementary to the adaptor sequences, but carrying an additional selective nucleotide (EcoR I+ A and Mse I + C) proceeded by combining 2.5 µL of 10X Biolase buffer, 0.75 µL (50 mM) $MgCl_2$, 20 µL Primer Mix I, and 0.2 µL (5 U) Biolase Taq DNA polymerase (Bioline USA Inc., Randolph, MA), and 5 µL of diluted restriction/ligation product. Pre-amplification reactions were conducted in a GeneAmp® PCR System 9700 (Applied Biosystems) thermocycler with the following cycling parameters: 72° C for 2 minutes; 20 cycles of 94°C for 30 seconds, 56°C for

30 seconds, and 72°C for 120 seconds, then 72°C for 120 seconds and 60°C for 30 minutes.

The preselective amplification product was diluted 1:40, 5 µl preselective product to 195 µl TE (pH 8.0), and used as template DNA for selective amplification with EcoR I and Mse I primers with three selective bases at the 3' end. The selective reactions consisted of 1.5 µL of 10X PCR buffer, 1.2 µL of 25 mM MgCl2, 1.2 µL of 2.5 mM of each dNTP, 0.9 µL of labeled 10 µM EcoRI+ 3 primer, 0.9 µL of unlabeled 10 µM MseI+3 primer, 0.2 µL of 0.75 U Platinum Taq DNA Polymerase (Invitrogen Co., CA) and 3.8 µL of the diluted preselective template. Selective amplification reactions were conducted in a GeneAmp® PCR System 9700 (Applied Biosystems) thermocycler with the following cycling parameters: 94°C for 2 minutes; 10 cycles of 94°C for 20 seconds, 66°C (decreasing by 1°C /cycle) for 30 seconds and 72°C for 2 minutes followed by 25 cycles of 94°C for 30 seconds, 56°C for 30 seconds and 72°C for 2 minutes; followed by 72°C for 3 minutes and a final stage at 60° C for 30 minutes. Only the E32/M59 primer pair combination was tested. The EcoR I-based primers were fluorescently labeled with FAM (Invitrogen Co., CA) for detection on an ABI 3100 capillary sequencer with ABI Genescan® software (Applied Biosystems).

Trial data analysis

Area Under the Disease Progress Curve (AUDPC) was calculated for each straw test entry as described in Shaner and Finney (1977). AUDPC for the three straw test dates were then analyzed as a randomized complete block design (RBD), using PROC GLM of SAS for Windows 9.1 (SAS Institute, Cary NC). The oxalate test and

field trial were also analyzed as RBDs using Proc GLM (SAS). Our models in the analyses of data from the straw test, the oxalate test, and the field trial defined the advanced backcross lines and checks as fixed factors and blocks as random factors. For each experiment, homogeneity of variance was verified using both Bartlett's test and Levene's test and normality was verified with a Shapiro and Wilk W statistic (SAS Proc Univariate). Pearson correlation coefficients for the three individual straw test AUDPC scores, the mean straw test AUDPC scores for all three tests, the oxalate test, and the field trial were calculated using Proc Corr (SAS).

Molecular marker analysis

For each marker locus, chi-square analysis was conducted to determine significant deviation of marker classes from the expected codominant 55:2:7 and dominant 57:7 and 55:9 Mendelian segregation ratios for BC_2F_4 populations. JoinMap 4.0 (Van Ooijen and Voorips, 2006) was employed to generate linkage maps. Significant pairwise marker linkages exceeded a minimum $\log_{10}$ of the likelihood of odds ratio (LOD) score of 3.0. Haldane's mapping function was used to determine linkage distances in multipoint analysis. Linkage groups were assigned to the common bean core map based on previously mapped SSR markers (Beebe et al., 2006; Blair et al., 2003, 2006b; Frei et al., 2005; Liao et al., 2004; Ochoa et al., 2006; Yu et al., 2000).

QTL analysis

Single factor analysis of variance (SFA) was performed using Proc GLM (SAS) for each marker locus and phenotypic score combination. Interval mapping (IM), Composite interval mapping (CIM), and Bayesian interval mapping (BIM) in WinQTLCartographer (Wang et al., 2005) were then employed. IM was executed with a 1 cM walkspeed. CIM was executed using Model 6, a B12 population structure, 10 cM window size, and forward and backward stepwise regression with genome scanning every cM. 1000 permutations of the data were performed to ascertain an empirical threshold for QTL significance for both IM and CIM (Churchill and Doerge, 1994). Loci found significant at this threshold were considered the probable location of QTL and the percentage of variance for white mold or oxalate response explained by each locus is estimated based upon the peak R^2 value. A support interval of 2 LOD was calculated on both sides of each QTL. BIM was employed using WinQTLCartographer's default parameters with 99900 iterations to confirm the location of QTL.

Results and Discussion

Greenhouse straw test

Variation for white mold reaction by genotype in the straw test was significant (Table 1). There were significant differences between blocks in this trial, likely due to environmental differences in the greenhouse between trial dates as well as the use of different isolates of *S. sclerotiorum* for the first versus the second and third blocks. Nevertheless, the straw test was able to significantly differentiate ($p < 0.01$) between the resistant check G 122, the moderately resistant M0162, the susceptible recurrent parent, OR 91G, and the extremely susceptible OSU 5630, OSU 5613, and Minuette with LS mean AUDPC scores of 62, 76, 102, 116, 116, and 118, respectively (Appendix A, Table 1). The frequency distribution for LS mean AUDPC scores was not normally distributed ($p < 0.05$), and many of the progeny performed significantly worse than the recurrent parent (Fig. 1). This continuous distribution is consistent with previous reports of quantitative resistance to white mold (Fuller et al., 1984; Kolkman and Kelly, 2003; Miklas and Grafton, 1992; Park et al., 2001).The lines 762/2-6, 897/18-1, 823/47-2, 825/47-4, 861/13-14, 837/3-18, 760/2-3, 822/46-6, 787/35-1, 782/33-2, and 853/6-9 exhibited a high degree of resistance, significantly improved over the recurrent parent ($p < 0.05$) and not significantly different from the response of the resistant check G 122 ($p >$

Table 1. Analysis of Variance for response of OR 91G x PI 255956 BC_2F_5 population to a greenhouse straw test at OSU in 2005.

Source	DF	SS	MS	F Value	Pr > F
Line	120	183147	1526	5.2	<.0001
Block	2	57021	28510	97.5	<.0001
Error	961	280928	292		
Total	1083	521097			
R^2=0.46		CV=18.40			

0.05). While we were unable to test PI 255956, Gilmore and Myers reported a straw test score from a single reading of 3.1 for it in 2000, while G122 scored 6.6, and OR 91G rated 8.2.

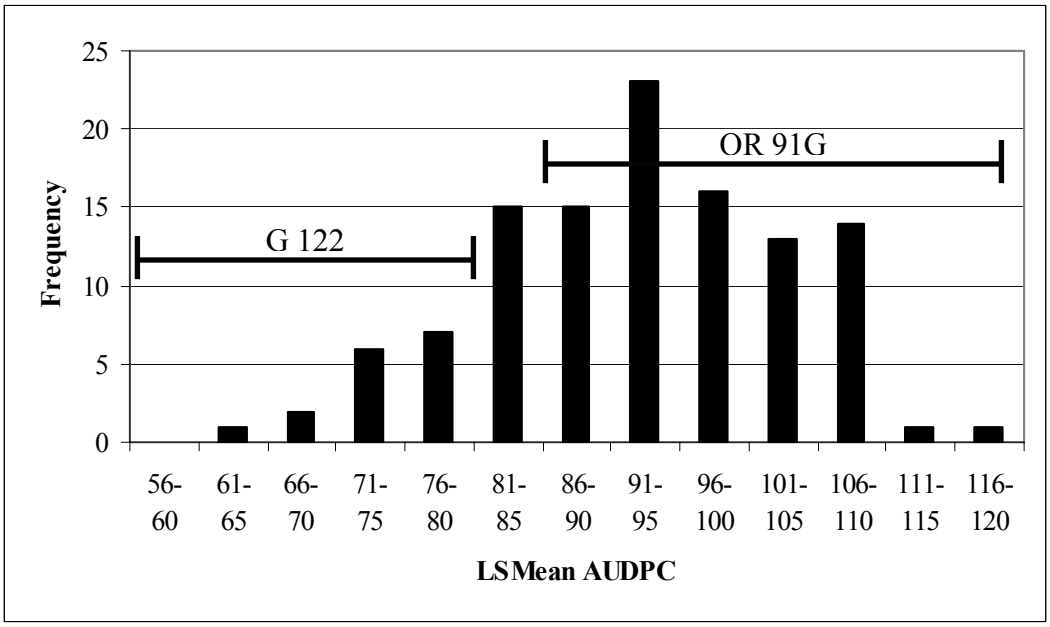


Figure 1. Response of OR 91G x PI 255956 BC_2F_5 population to a greenhouse straw test at OSU in 2005. Horizontal bars indicate 95% CI for check LS mean.

Oxalate test

Significant variation was found for oxalate tolerance between genotypes (Table 2). There were significant differences between blocks in this trial, likely due to variation in either the greenhouse or laboratory

Table 2. Analysis of variance for response of OR 91G x PI 255956 BC_2F_5 population to an oxalate test at OSU Vegetable Breeding Laboratory in 2006.

Source	DF	SS	MS	F Value	Pr > F
Line	114	865.0	7.6	4.5	<.0001
Block	3	24.0	8.0	4.7	0.003
Error	354	596.3	1.7		
Total	471	1485.3			

R^2=0.60 CV=29.34

environments, or both. Oxalate test data were normally distributed ($p > 0.05$), and 11 lines were found to transgress the susceptibility of OR 91G (Fig. 2). The donor parent

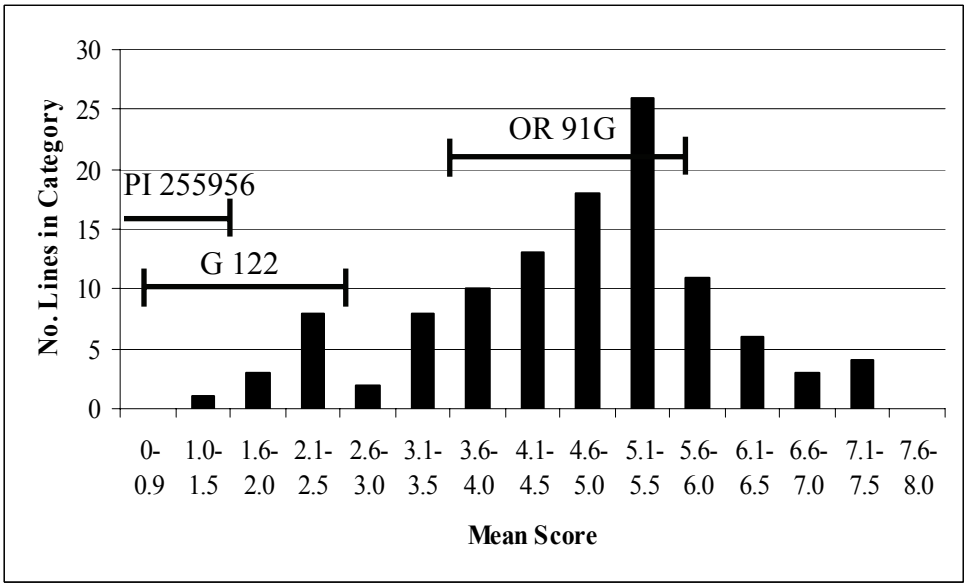


Figure 2. Response of OR 91G x PI 255956 BC_2F_5 population to an oxalate test at OSU Vegetable Breeding Laboratory in 2006.
Oxalate rating scale: 0= no symptoms, 1= one leaf with slight wilting symptoms, 2= one leaf wilting, 3=second leaf wilting slightly, 4= two leaves wilted, 5= three leaves wilting, 6= four leaves wilting, 7= petioles collapsing, 8=flaccid stems, and 9= total plant collapse. Horizontal bars indicate 95% CI for check means.

PI 255956 was particularly tolerant of oxalate, with a mean score of 0.07 (Appendix A, Table 2). G 122 and M0162 were tolerant, with mean scores of 1.6 and 2.6, respectively. NY 6020-5 and the recurrent parent, OR 91G, had intermediate responses to oxalate, with mean scores of 3.75 and 4.50, respectively. The lines 913/23-18, 851/9-13, 903/20-2, and 919/25-3 exhibited oxalate tolerance not significantly different from that of the donor parent PI 255956 ($p > 0.05$). In addition, 879/10-15, 849/8-3, 908/21-5, 912/23-8, 917/24-7, and 800/39-4 were significantly more tolerant of oxalate than the recurrent parent OR 91G. The continuous variation

observed in oxalate response in this population is also consistent with previous reports of quantitative inheritance of white mold resistance.

Field trial

Significant variation was found for white mold incidence and severity in the field trial (Table 3). Frequency distribution for the field trial was non-normal ($p > 0.05$) (Fig. 3). Various transformations were attempted, with none producing a

Table 3. Analysis of variance for response of OR 91G x PI 255956 BC_2F_6 population to a white mold field trial grown at the Vegetable Research Farm, Corvallis Oregon in 2006.

Source	DF	SS	MS	F Value	Pr > F
Entry	110	503.6	4.6	7.36	<.0001
Block	3	1.5	0.5	0.81	0.488
Error	168	104.4	0.6		
Total	281	609.5			
R^2=0.83		CV=14.26			

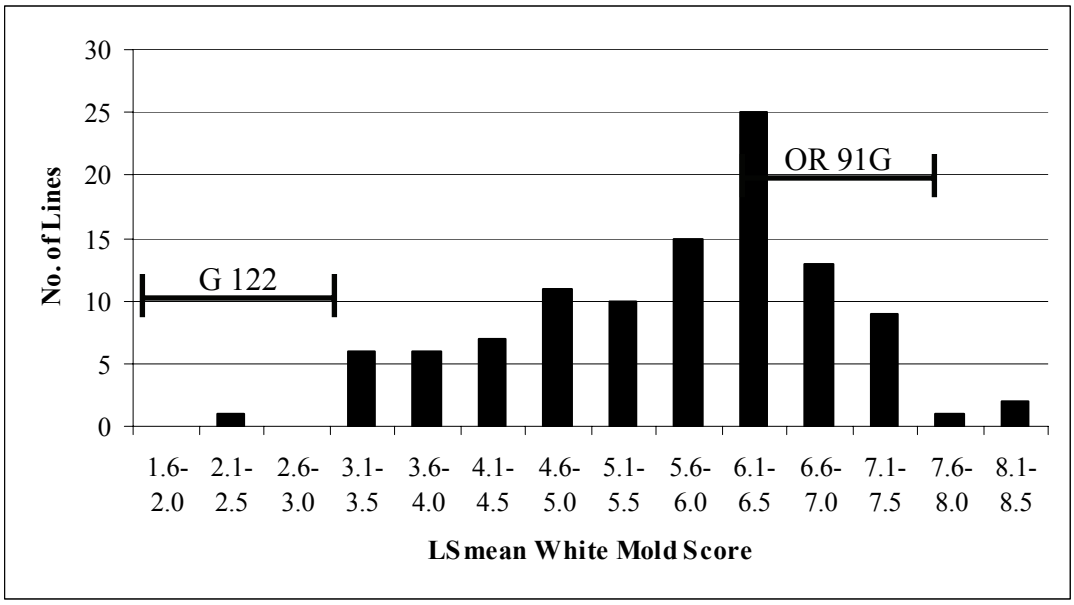


Figure 3. Response of OR 91G x PI 255956 BC_2F_6 population to a white mold field trial grown at the Vegetable Research Farm, Corvallis Oregon in 2005.
Field rating scale: 1 = no diseased plants, 9= 80% to 100% diseased plants and/or 60% to 100% infected tissue (Miklas et al., 2001) Horizontal bars indicate 95% CI for check LS mean.

normal distribution. ANOVA and interval mapping are generally considered robust to violations of the assumption of normality. The kurtosis present in the data increases the chances of type II error. Though the power of our test to identify QTL is slightly diminished, raw data was used for QTL analysis. G 122 and M0162 showed relatively high levels of resistance, with mean white mold scores of 2.3 and 2.7, while OR 91G and OSU 5630 were significantly more susceptible ($p < 0.05$), with means of 6.9 and 7.0, respectively. The responses of 7 lines, 897/18-1, 861/13-14, 859/13-8, 903/20-2, 904/20-3, 845/6-2, and 762/2-6, were not significantly different from that of G 122 ($p < 0.05$), while 55 lines were not significantly different in response from OR 91G ($p < 0.05$). No transgressive segregation for field white mold response was observed (Appendix A, Table 3). The continuous distribution found in the field trial provides yet more support for the assertion of quantitative inheritance of white mold resistance.

Trial correlation

Oxalate was not strongly correlated with the field trial, and was not significantly correlated with the straw test (Table 4). Similar results were observed by Kolkman and Kelly (2003) in the Bunsi / Newport and the Huron / Newport populations. Straw test and field trial scores were significantly, though weakly correlated. This is expected, as avoidance mechanisms induce additional variation in white mold response under field conditions not present in the greenhouse straw test.

Table 4. Pearson correlation coefficients for OR 91G x PI 255956 phenotypic evaluations.

	Field Trial	Oxalate Test
Oxalate Test	-0.19	
	p = 0.060	
Straw Test	0.32	-0.06
	p = 0.001	p = 0.505

Molecular marker analysis

Of 172 SSR primer pairs tested, 98 were polymorphic between the parents (Appendix B, Table 1). Of those, only 77 polymorphic SSRs were scorable in the progeny, and 2 revealed single introgressions. The remaining 21 SSRs were either monomorphic between progeny, or would not amplify in the progeny and were discarded. The single pair of selective AFLP® primers amplified 56 scorable segregating fragments. The linkage map constructed with JoinMap 4.0 consisted of 11 linkage groups (LGs) that correspond to 9 of the 11 core map LGs, based on known SSR marker locations, and a single LG with no anchoring loci (Fig. 4). The 11 LGs included 59 loci, covering a total genome length of 140 cM, or approximately 12% of the estimated length of the common bean genome. Thirty-one AFLP® markers formed a single linkage group with no SSR loci, however many linkages were suspect, therefore the group is not included in this report. Chi-square statistics revealed significant divergence ($p < 0.05$) from the expected Mendelian segregation ratios at most loci (Appendix B, Table 2). The only linked SSR marker that fit the expected ratio was BMd-52 on LG 09. Of the unlinked SSR markers, only PVag004 and PVat007 fit the expected ratio ($p > 0.05$). While the homozygous recurrent parental marker class was represented at the expected rate over most loci, the heterozygotic marker class tended to be overrepresented and the homozygous donor parental marker class tended to be underrepresented (Fig. 5).

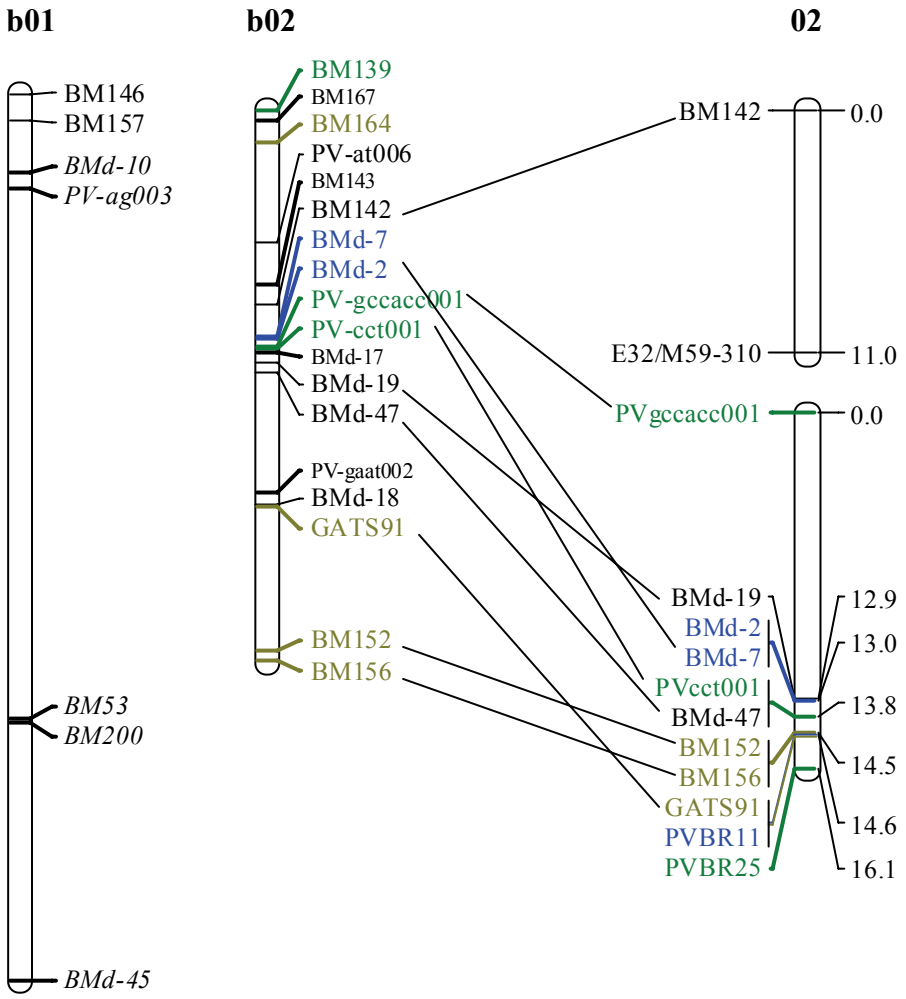
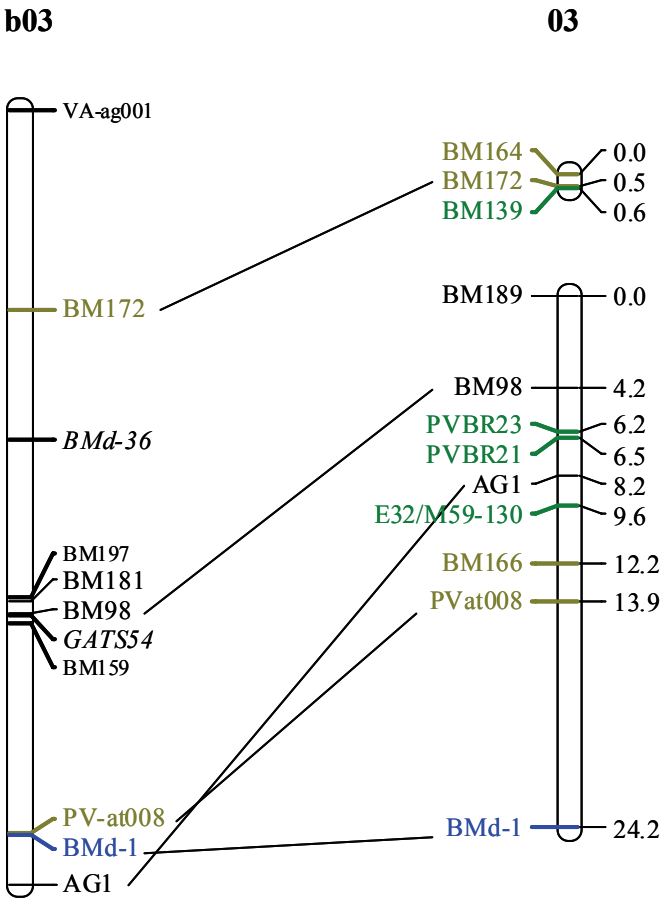


Fig. 4. Linkage map for OR 91G x PI 255956 and its relation to common bean consensus map anchor loci. Consensus groups b01-b11 are derived from Blair et al., 2003, with additional loci from Blair et al, 2006.Colored loci have significant association ($p < 0.05$) with response in field, straw, field and straw, and all three types of phenotypic evaluation, based on single factor analysis of variance. A single QTL for field response is identified as a vertical bar and depicted by composite interval mapping with threshold at LOD 1.5, determined by 1000 permutations. Loci identified as polymorphic between parents, but monomorphic among progeny are listed in *italics*. Loci listed with reduced font are those unscorable in this population.



Colored loci are associated ($p < 0.05$) with reaction in single factor ANOVA

Green = field trial
Blue = straw test
Gold = field and straw test
Violet = all three tests

Italics = polymorphic between parents, but no introgression in progeny

Reduced font = unscorable loci

Fig. 4 (Continued).

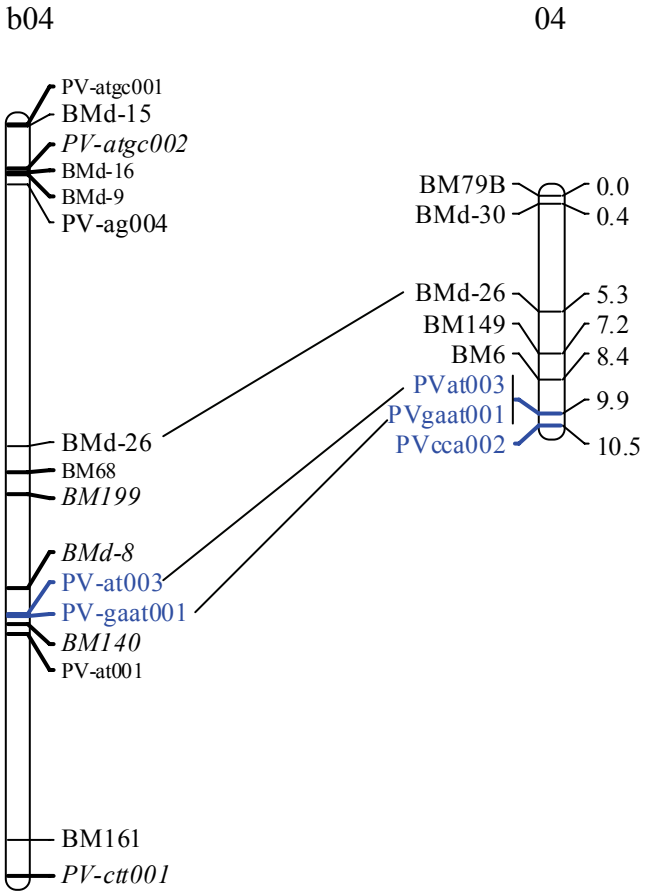


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Fig. 4 (Continued).

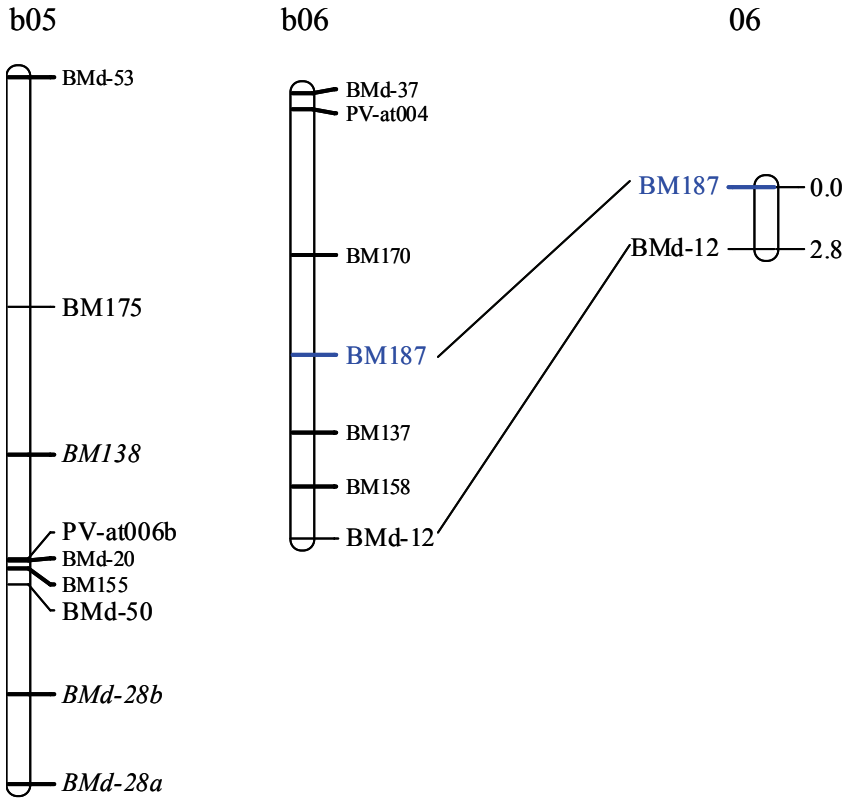

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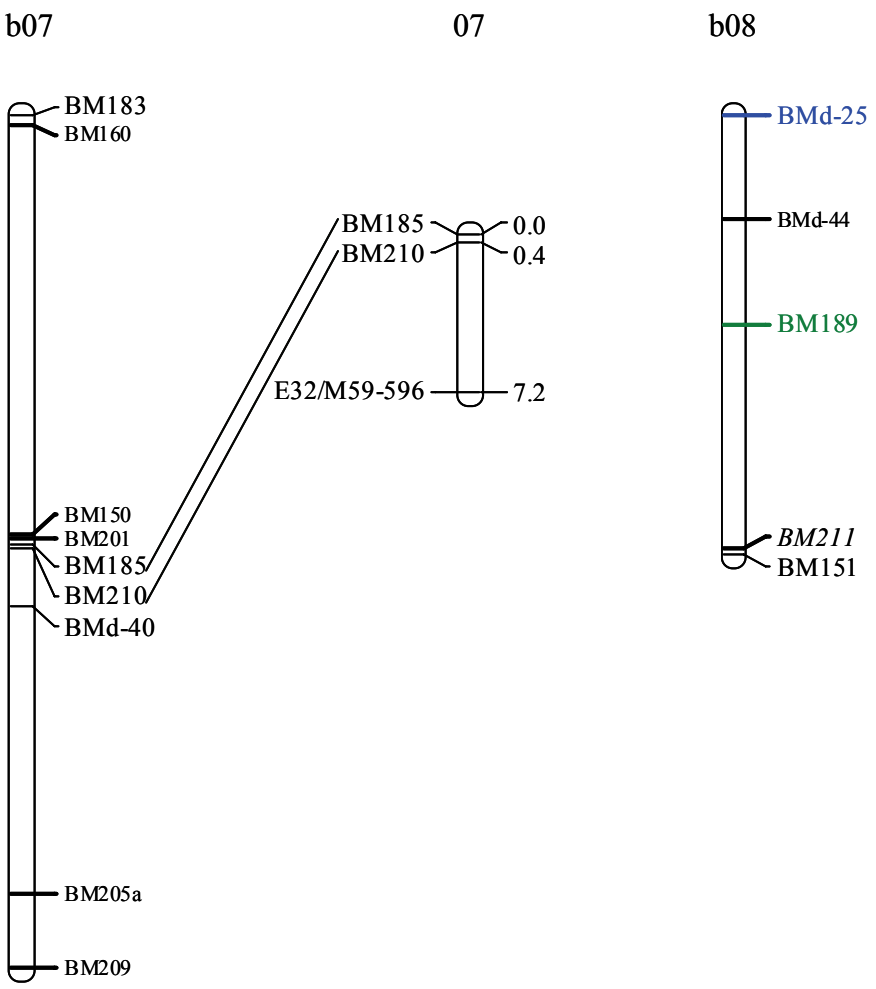
Fig. 4 (Continued).



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Green = field trial
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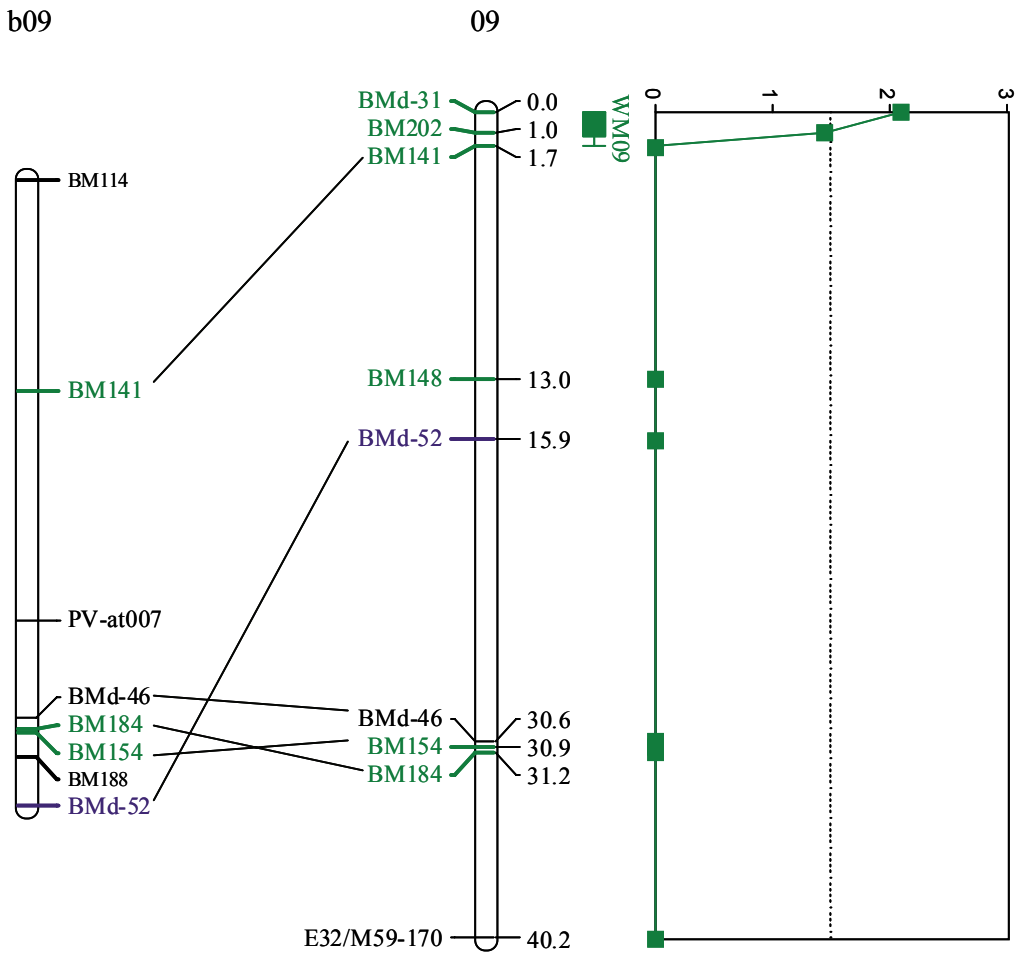
Reduced font = unscorable loci

Fig. 4 (Continued).



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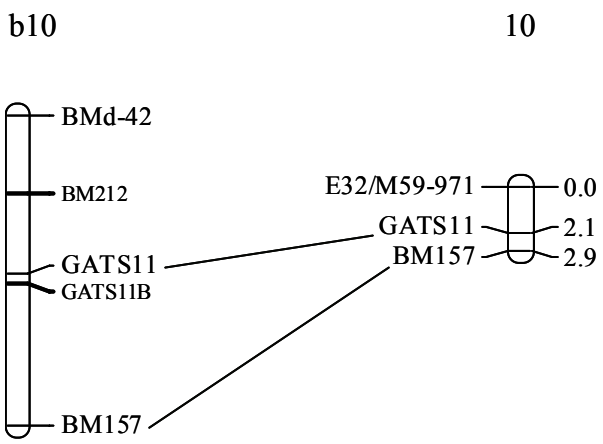
Reduced font = unscorable loci

Fig. 4 (Continued).



Colored loci are associated ($p < 0.05$)
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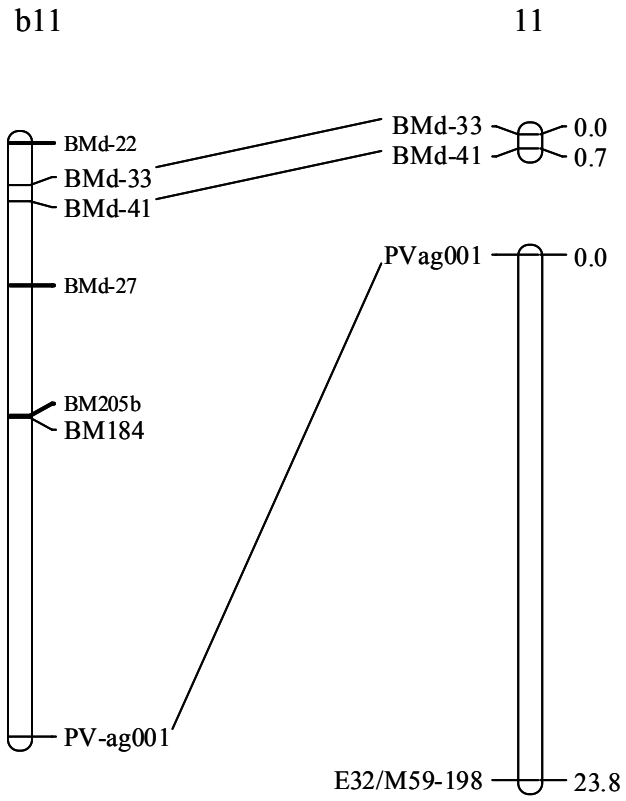
but no introgression in progeny

Reduced font = unscorable loci

Fig. 4 (Continued).



Colored loci are associated ($p < 0.05$)
with reaction in single factor ANOVA

Green = field trial
Blue = straw test
Gold = field and straw test
Violet = all three tests

Italics = polymorphic between parents,
but no introgression in progeny

Reduced font = unscorable loci

Fig. 4 (Continued).

Exceptions can be found on LGs 7, 10, and 11 at the SSR loci BM185, BM210, BM142, BMd-33, and BMd-41, the unlinked loci BM171, BM175, BM 181, and BM183 where the homozygous recurrent parental marker class was underrepresented, the homozygous donor parental marker class was slightly overrepresented, and the heterozygotic class was present at 7 to 14 times the expected frequency. The AFLP® markers E32/M59-971 on LG 10b and E32/M59-198 on LG 11 fit their expected Mendelian ratios; however, at E32/M59-130 on LG 03 the donor allele was underrepresented, while E32/M59-596 on LG 07, E32/M59-170 on LG 09, and E32/M59-310 on LG 10 each show an excess of the donor allele. The unlinked loci E32/M59-126, E32/M59-305, E32/M59-343, E32/M59-422, and E32/M59-633 each segregated as expected ($p > 0.05$), but all other AFLP® markers significantly deviated from expectation (Appendix B, Table 2). Regardless of the parent contributing the dominant allele, an excess of the dominant marker class was observed in 49 of 56 loci. This suggests an excess of heterozygosity at the AFLP® loci similar to that directly observed at the SSR loci. Excessive heterozygosity and segregation distortion at SSR loci was reported by Blair et al. (2003) in the wide intraspecific common bean recombinant inbred populations derived from BAT93 x JaloEEP558 and DOR364 x G19833; however the distortion was mostly limited to specific chromosomal regions. There are several possible sources for the observed segregation distortion in this population, involving gametic, zygotic, or post-zygotic selection. Interspecific incompatibility affecting pollen viability and development, such as that observed by Guo et al. (1991), could explain the preferential transfer of *P. vulgaris* alleles. It is also

possible that homologous chromosome pairing was not complete, effectively blocking entire regions from crossing-over. Lethal factors with recessive inheritance that are masked within the highly heterozygous *P. coccineus* background may have influenced segregation as this population's homozygosity increased over generations of selfing. We have observed hardseededness in early generations of *P. vulgaris* / *P. coccineus* populations, a trait resulting in prolonged seed dormancy which could result in selection against loci linked to the trait. All BC_2F_4 lines had type I determinate growth habit, suggesting selection against the donor allele at the *fin* locus and consequently against linked loci. Additional research into the mechanisms influencing segregation in the development of these types of populations would contribute to our understanding and could enhance their usefulness for the transfer of beneficial alleles into commercially superior common bean lines.

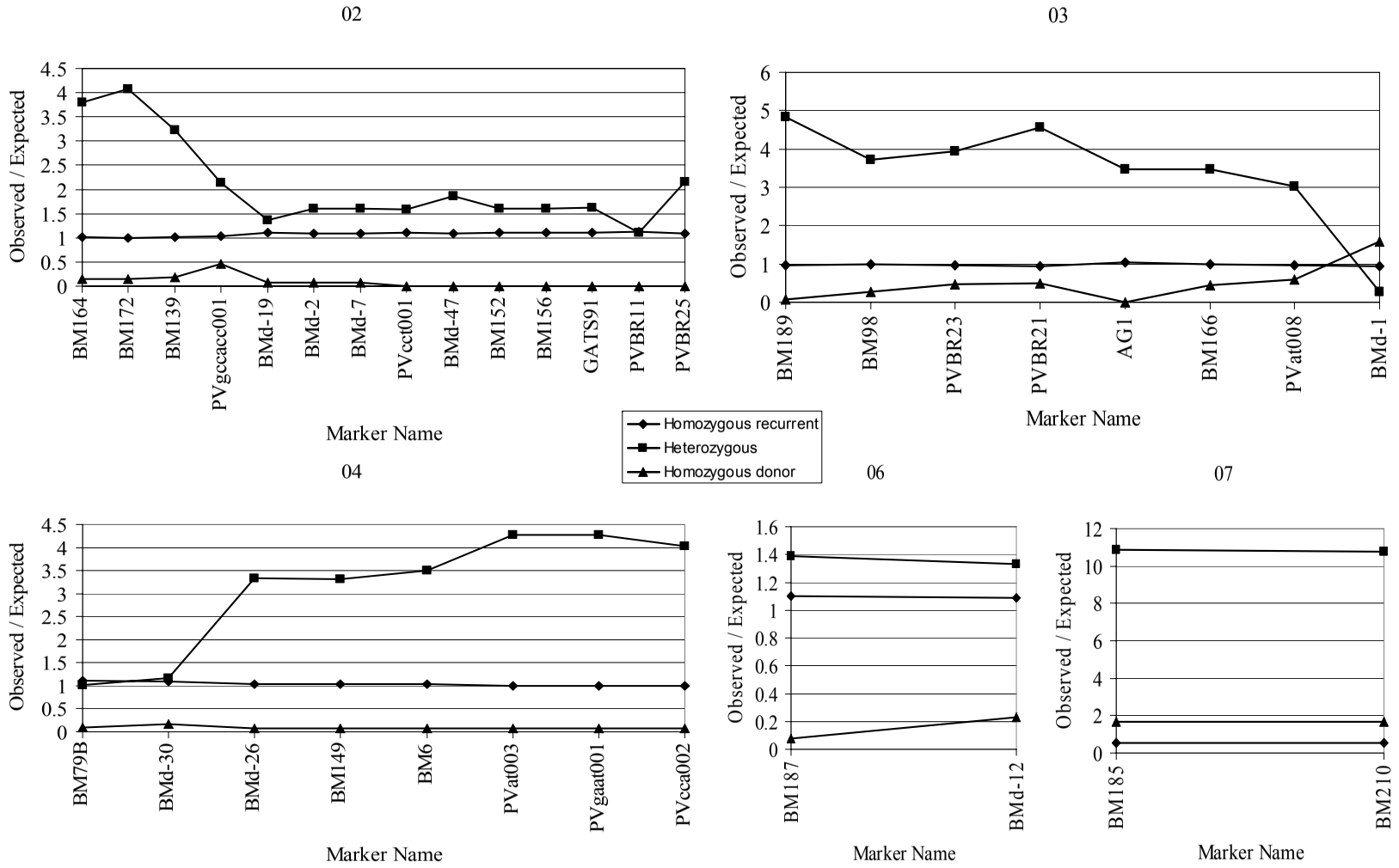


Fig. 5. Ratio of observed vs. expected frequencies for marker classes by linkage group for OR91G x PI 255956 BC_2F_4 population

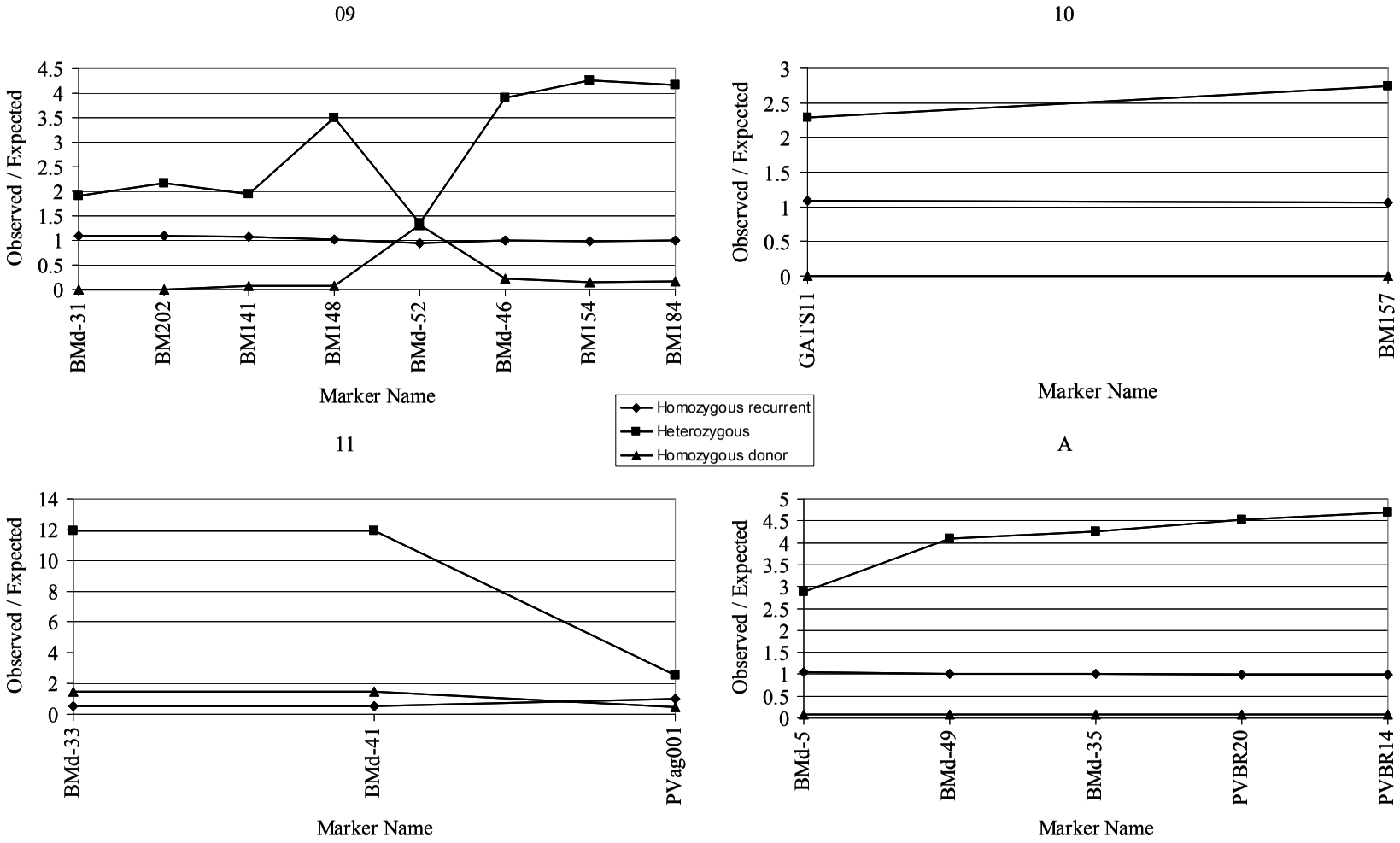


Fig. 5 (Continued). Ratio of observed vs. expected frequencies for marker classes by linkage group for OR91G x PI 255956 BC_2F_4 population.

QTL analysis

Single-factor analysis revealed 24 SSR markers associated with the white mold response in the field trial, 23 in the straw test, and 2 in the oxalate test ($p < 0.05$). Two AFLP® markers were associated with field resistance, 1 with straw test response, and 4 with oxalate tolerance. Eleven of these markers conditioned resistance in both field and straw tests, and one had significant influence on all three phenotypic evaluations (Table 5). OR 91G contributed several positive alleles, including the AFLP® E32/M59-298 allele, conditioning field resistance ($p < 0.05$); the SSR markers BM171, PVat003, PVgaat001, BMd-25, and PVat007, associated with resistance in the straw test ($p < 0.05$); and the SSR marker BMd-52, associated with improved tolerance to oxalate ($p < 0.05$). Paradoxically, the OR 91G allele at BMd-52 is associated with lower levels of resistance in field and straw tests. Along with the observation of the lack of correlation between the oxalate trial and the other phenotypic evaluations, these findings suggest the possible presence of multiple linked factors, one or more conditioning increased oxalate tolerance while one or more others negatively impact other aspects of white mold resistance.

Composite Interval Mapping (CIM) with WinQTLCartographer revealed the presence of a single QTL accounting for 6% of the variance for field resistance (R^2) on LG 09, between the SSR loci BMd-31 and BM202 (Fig. 3). This QTL is significant at the threshold LOD 1.5, determined by 1000 permutations. IM and BIM results confirm these findings and are not presented due to their similarity. Maxwell et al. (2007, in press) also found a QTL near BM154 on LG 09 explaining 12% of the variance for

white mold response in the straw test. Terpstra and Kelly (2006) also found a QTL for resistance to white mold in the straw test on LG 09 near the SSR markers BM114 and PV-at007.

Table 5. Single factor analysis of variance for OR 91G x PI 255956 SSR and AFLP® loci in a field trial, a straw test, and an oxalate trial.

	Field			Straw			Oxalate			Linkage
Marker	R^2	p value	Source	R^2	p value	Source	R^2	p value	Source	Group
BM139	0.06	0.026	*P. c.*							b02
BM152	0.05	0.029	*P. c.*	0.05	0.021	*P. c.*				b02
BM156	0.05	0.029	*P. c.*	0.05	0.021	*P. c.*				b02
BM164	0.06	0.014	*P. c.*	0.06	0.011	*P. c.*				b02
BMd-2				0.04	0.045	*P. c.*				b02
BMd-7				0.04	0.045	*P. c.*				b02
GATS91	0.05	0.028	*P. c.*	0.05	0.022	*P. c.*				b02
PVcct001	0.05	0.029	*P. c.*							b02
PVgccacc001	0.04	0.042	*P. c.*	0.03	0.057	*P. c.*				b02
PVBR11				0.04	0.049	*P. c.*				b02*
PVBR25	0.06	0.013	*P. c.*							b02*
BM172	0.06	0.010	*P. c.*	0.05	0.023	*P. c.*				b03
BMd-1	0.03	0.072	*P. c.*	0.05	0.019	*P. c.*				b03
PVat008	0.08	0.006	*P. c.*	0.07	0.010	*P. c.*				b03
AAC-CTA130	0.09	0.002	*P. c.*							b03*
BM166	0.07	0.006	*P. c.*	0.06	0.012	*P. c.*				b03*
PVBR21	0.04	0.041	*P. c.*							b03*
PVBR23	0.05	0.037	*P. c.*							b03*
PVat003	0.03	0.058	*P. c.*	0.03	0.047	*P. v.*				b04
PVgaat001	0.03	0.058	*P. c.*	0.03	0.047	*P. v.*				b04
PVcca002				0.04	0.042	*P. v.*				b04*
BM187				0.04	0.045	*P. c.*				b06
BM189	0.04	0.040	*P. c.*	0.03	0.071	*P. c.*				b08
BMd-25				0.04	0.040	*P. v.*				b08

Table 5 (continued). Single factor analysis of variance for OR 91G x PI 255956 SSR and AFLP® loci in a field trial, a straw test, and an oxalate trial.

	Field			Straw			Oxalate			Linkage
Marker	R^2	p value	Source	R^2	p value	Source	R^2	p value	Source	Group
BM141	0.08	0.003	*P. c.*							b09
BM154	0.05	0.018	*P. c.*	0.03	0.083	*P. c.*				b09
BM184	0.04	0.038	*P. c.*							b09
BMd-52	0.11	0.001	*P. c.*	0.05	0.019	*P. c.*	0.05	0.023	*P. v.*	b09
BM148	0.10	0.001	*P. c.*				0.03	0.068	*P. v.*	b09*
BM202	0.09	0.003	*P. c.*							b09*
BMd-31	0.09	0.002	*P. c.*							b09*
AAC-CTA211							0.05	0.025	*P. c.*	X
AAC-CTA239							0.05	0.023	*P. c.*	X
AAC-CTA284							0.06	0.013	*P. c.*	X
AAC-CTA393							0.04	0.038	*P. c.*	X
AAC-CTA298	0.06	0.011	*P. v.*							X
AAC-CTA354				0.04	0.033	*P. v.*				X
BM171				0.05	0.013	*P. v.*				X
BMd-35	0.04	0.041	*P. c.*	0.09	0.001	*P. c.*				X
BMd-4	0.04	0.039	*P. c.*							X
BMd-49	0.35	0.057	*P. c.*	0.08	0.003	*P. c.*				X
BMd-5	0.03	0.078	*P. c.*	0.09	0.002	*P. c.*				X
PVBR14	0.04	0.045	*P. c.*	0.10	0.001	*P. c.*				X
PVBR20	0.03	0.060	*P. c.*	0.10	0.001	*P. c.*				X
PVat002				0.05	0.055	*P. c.*	0.05	0.050	*P. v.*	X

* Map location inferred from placement by JoinMap in OR 91G x PI255956 population.

[X] Denotes linked loci not placed on consensus map.

Source is either *Phaseolus coccineus* (*P. c.*) or *P. vulgaris* (*P. v.*).

Conclusions

Critical to the success of this project and any other attempting to assess white mold resistance is the reliability of phenotypic data. While the straw test has been shown to produce very consistent results, significant environmental effects were evident between test dates. More extensive measures will be taken in future trials to control environmental variables, especially relative humidity, within the greenhouse trial area. Lack of correlation between the oxalate trial and the other two phenotypic evaluations could be explained if physiological factors such as those affecting stomatal regulation or morphological aspects such as vascular conductance or abaxial pubescence that could confound the wilting response were transferred from *P. coccineus* that condition drought tolerance traits. In our opinion, oxalate tolerance is certainly an important component of white mold resistance, but more research is required to separate the effects of oxalate tolerance from the confounding factors present in the oxalate test in order for this data to be useful for QTL mapping efforts.

Limited population size reduced our ability to identify QTL with small effects. Population size was restricted in several ways: 1) it was difficult to obtain large numbers of progeny at each crossing generation, 2) in later selfing generations, attrition, likely from embryo abortion, sterility, and general lack of thrift due to interspecific incompatibility, reduced the number of lines available for testing, 3) it was difficult to obtain sufficient seed for replication of some lines in our phenotypic trials 4) bound by budgetary constraints, the high costs associated with genotyping large populations resulted in competition between population size and marker

saturation. These restrictions must be overcome in order to fully exploit the resistance present in *P. coccineus* for the improvement of *P. vulgaris.* While issues related to interspecific incompatibility requires a great deal more research to overcome, genotyping cost is a factor which can be improved upon, based upon current research. Vales et al. (2005) suggest selective genotyping as a method for achieving maximum efficiency and economy in QTL mapping populations of between 100 and 300 individuals. Future QTL mapping efforts in these interspecific *Phaseolus* crosses could benefit from using these techniques to restrict marker analysis to highly informative individuals.

In addition to its effect upon population size, the high cost of genotypic evaluation also has a detrimental effect upon marker saturation. While a practically infinite number of molecular markers exist, the number which can be affordably scored in a large population is still quite small. SSRs require less labor to score, but have low multiplex ratio, whereas AFLPs® produce much more data per reaction, but require a great deal of effort to interpret and make use of. Newer marker technology, such as sequence-related amplified polymorphisms (SRAP), which combines the ease of SSRs with a multiplex ratio comparable to AFLP® without the need for restriction digestion (Li and Quiros, 2001), may provide an affordable means to saturate linkage maps based around a framework of SSR anchor loci. Marker saturation in this population was also reduced by the presence of large regions either lacking recombination or exhibiting extreme segregation distortion.

Segregation distortion had a major effect upon our ability to identify QTL conditioning white mold resistance. We were unable to construct linkage groups corresponding to the consensus groups b01, b05, and b08, all of which contain loci we identified as polymorphic between OR 91G and PI 255956, but did not segregate in our population. It is possible that one or more of these correspond to the chromosomes Shii et al. (1982) report as frequently forming univalents during metaphase I of microsporogenesis. Even in chromosomes forming bivalents, incomplete homology may result in regions of reduced recombination. Selection against donor alleles conditioning domestication-related traits and those resulting in sterility or lethality also contributed to segregation distortion. More research is necessary to determine the reasons for genomic incompatibility between *Phaseolus* spp. and to find ways to overcome it, allowing recombination at these loci.

Several lines were identified with superior response to the recurrent parent OR 91G in each of the three phenotypic evaluations. 762/2-6, 861/13-14, and 897/18-1 performed especially well in both the straw test and field trial. 903/20-2 possesses high levels of tolerance to oxalate and resistance to white mold in the field. These lines, as well as the top performing lines for each trial should provide valuable germplasm for breeding common bean lines with superior resistance to white mold. The absence of donor introgression in these elite lines for the LG 09 QTL further supports the conclusion that multiple factors conditioning white mold resistance are segregating in this population.

We hypothesized that AB-QTL would allow rapid identification and transfer of desirable alleles from unadapted *P. coccineus* into elite *P. vulgaris* varieties. Based upon these results, we have succeeded in this endeavor. Although we had originally sought to locate and transfer multiple QTL conditioning white mold resistance, factors such as uncontrolled environmental variation, small population size, limited marker saturation, and extreme segregation distortion reduced our power to identify such loci. Nevertheless, superior lines have been identified which will now be integrated into our breeding program to produce highly resistant common bean cultivars.

References

Abawi GS, Provvidenti R, Crosier DC, Hunter JE (1978) Inheritance of resistance to white mold disease in *Phaseolus coccineus*. J Hered 69:200-202

Adams PB, Tate CJ, Lumsden RD, Meiners JP (1973) Resistance of *Phaseolus* species to *Sclerotinia sclerotiorium*. Annu Rep Bean Improv Coop 16:8-9

Baggett JR (1995) A Historical Summary of Vegetable Breeding. Proc. Oregon Horticultural Society, 86:89-99

Bateman DF, Beer SV (1965) Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis of *Sclerotium rolfsii*. Phytopathology 55: 204-211

Beattie, AD , Larsen J, Michaels TE, Pauls KP (2003) Mapping quantitative trait loci for a common bean (*Phaseolus vulgaris* L.) ideotype. Genome 46:411-422

Beebe SE, Rojas-Pierce M, Yan XL, Blair MW, Pedraza F, Munoz F, Tohme J, Lynch JP (2006) Quantitative trait loci for root architecture traits correlated with phosphorus acquisition in common bean. Crop Sci 46:413-423

Bernacchi D, Beck-Bunn T, Emmatty D, Eshed Y, Inai S, Lopez J, Petiard V, Sayama H, Uhlig J, Zamir D, Tanksley S (1998) Advanced backcross QTL analysis of tomato. II. Evaluation of near-isogenic lines carrying single-donor introgressions for desirable wild QTL-alleles derived from *Lycopersicon hirsutum* and *L. pimpinellifolium*. Theor Appl Genet 97: 170-180

Billon-Grand G, Poussereau N, Fèvre M (2002) The extracellular proteases secreted *in vitro* and *in planta* by the phytopathogenic fungus *Sclerotinia sclerotiorum*. Phytopathology 150:507-511

Blair MW, Giraldo MC, Buendia HF, Tovar E, Duque MC, Beebe SE (2006a) Microsatellite marker diversity in common bean (*Phaseolus vulgaris*). Theor Appl Genet 113:100-109

Blair MW, Iriarte G, Beebe S (2006b) 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 MW, Giraldo MC, Buendia HF, Tovar E, Duque MC, Beebe SE (2005) Microsatellite marker diversity in common bean (*Phaseolus vulgaris* L.). Theor Appl Genet 113:100-109

Blair MW, Pedraza F, Buendia HF, Gaitan-Solis E, Beebe SE, Gepts P, Tohme J (2003) Development of a genome-wide anchored microsatellite map for common bean (*Phaseolus vulgaris* L.). Theor Appl Genet 107:1362–1374

Bolton MD, Thomma BPHJ, Nelson BD (2006) *Sclerotinia sclerotiorum* (Lib.) De Bary: biology and molecular traits of a cosmopolitan pathogen. Mol Plant Pathol 7:1-16

Buso GSC, Amaral ZPS, Brondani RPV, Ferreira ME (2006) Microsatellite markers for the common bean *Phaseolus vulgaris*. Mol Ecol Notes 6:252-254

Cessna SG, Sears VE, Dickman MB, Low PS (2000) Oxalic acid, a pathogenicity factor for *Sclerotinia sclerotiorum*, suppresses the oxidative burst of the host plant. Plant Cell 12:2191-2199

Chipps TJ, Gilmore B, Myers J, Stotz HU (2005) Evidence for oxalate insensitivity and oxalate oxidase in determining partial resistance of *Phaseolus coccineus* to *Sclerotinia sclerotiorum*. Phytopathology 95:292-299

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963-971

Collard BCY, Jahufer MZZ, Brouwer JB, Pang ECK (2005) Introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica 142: 169-196

Cook GE, Steadman JR, Boosalis MG (1975) Survival of *Whetzelinia sclerotiorum* and initial infection of dry edible beans in western Nebraska. Phytopathology 65:250-255

Debouck DG (1999) Diversity in *Phaseolus* species in relation to the common bean. p.25-52. In: Singh SP (ed.) Common Bean Improvement in the Twenty-First Century. Kluwer Academic Publ., Dordrecht

Ender M, Kelly JD (2005) Identification of QTL associated with white mold resistance in common bean. Crop Sci 45:2482–2490

Eshed Y, Zamir D (1995) An introgression line population of *Lycopersicon pennellii* in the cultivated tomato enables the identification and fine mapping of yield-associated QTL. Genetics 141:1147-1162

Favaron F, Sella L, D'Ovidio R (2004) Relationships among endo-polygalacturonase, oxalate, pH, and plant polygalacturonase-inhibiting protein (PGIP) in the interaction between *Sclerotinia sclerotiorum* and soybean. Mol Plant Microbe Interact 17:1402-1409

Frary A, Fulton TM, Zamir D, Tanksley SD (2004) Advanced backcross QTL analysis of a *Lycopersicon esculentum* x *L. pennellii* cross and identification of possible orthologs in the Solanaceae. Theor Appl Genet 108:485-496

Frei A, Blair MW, Cardona C, Beebe SE, Gu H, Dorn S (2005) QTL mapping of resistance to Thrips palmi Karny in common bean. Crop Sci 45:379-387

Freyre R, Skroch PW, Geffroy V, Adam-Blondon AF, Shirmohamadali A, Johnson WC, Llaca V, Nodari RO, Pereira PA, Tsai SM, Tohme J, Dron M, Nienhuis J, Vallejos CE, Gepts P (1998) Towards an integrated linkage map of common bean. 4. Development of a core linkage map and alignment of RFLP maps. Theor App Genet 97:847-856

Fuller PA, Coyne DP, Steadman JR (1984) Inheritance of resistance to white mold disease in a diallel cross of dry beans. Crop Sci 24:929-933

Fulton TM, Grandillo S, Beck-Bunn T, Fridman E, Frampton A, Lopez J, Petiard V, Uhlig J, Zamir D, Tanksley SD (2000) Advanced-backcross QTL analysis of a *Lycopersicon esculentum* x *Lycopersicon parviflorum* cross. Theor Appl Genet 100:1025-1042

Gaitán-Solís E, Duque MC, Edwards KJ, Tohme J (2002) Microsatellite repeats in common bean (*Phaseolus vulgaris*): isolation, characterization, and cross-species amplification in *Phaseolus* ssp. Crop Sci 42:2128-2136

Genchev D, Kiryakov I (2002) Inheritance of resistance to white mold disease (*Sclerotinia sclerotiorum* (Lib.) de Bary) in the breeding line A 195 of common bean (*Phaseolus vulgaris* L.). Bulgarian J Ag Sci 8:181-187

Gilmore B, Myers JR (2000) Examining the *Phaseolus coccineus* collection for white mold resistance. Hortscience 35:399 (abstract)

Gilmore B, Myers JR, Kean D (2002) Completion of testing of *Phaseolus coccineus* plant introductions (PIs) for white mold, *Sclerotinia sclerotiorum,* resistance. Annu Rep Bean Improv Coop 45:64-65

Gilmore B, Myers JR (2004) A preliminary molecular marker map for *Phaseolus coccineus.* Annu Rep Bean Improv Coop 47:87-88

Godoy G, Steadman JR, Dickman MB, Dam R (1990) Use of mutants to demonstrate the role of oxalic acid in pathogenicity of *Sclerotinia sclerotiorum* on *Phaseolus vulgaris.* Physiol Mol Plant Pathol 37:179-191

Guimarães RL, Stotz HU (2004) Oxalate production by *Sclerotinia sclerotiorum* deregulates guard cells during infection. Plant Physiol 136:3703-3711

Guo M, Mok MC, Mok DWS (1994) RFLP analysis of preferential transmission in interspecific hybrids of *Phaseolus vulgaris* and *P. coccineus*. J Hered 85:174-178

Guo M, Lightfoot DA, Mok MC, Mok DWS (1991) Analyses of *Phaseolus vulgaris* L. and *P. coccineus* Lam. Hybrids by RFLP: preferential transmission of *P. vulgaris* alleles. Theor Appl Genet 81:703-709

Huang XQ, Cöster H, Ganal MW, Röder MS (2003) Advanced backcross QTL analysis for the identification of quantitative trait loci alleles from wild relatives of wheat (*Triticum aestivum* L.). Theor Appl Genet 106:1379-1389

Huang XQ, Kempf H, Ganal MW, Röder MS (2004) Advanced backcross QTL analysis in progenies derived from a cross between a German elite winter wheat variety and a synthetic wheat (*Triticum aestivum* L.). Theor Appl Genet 109:933-943

Hunter JE, Dickson MH, Cigna JA (1981) Limited-term inoculation: a method to screen plants for partial resistance to white mold. Plant Dis 65:414-417

Hunter JE, Dickson MH, Boettger MA (1982) Evaluation of plant introductions of *Phaseolus* spp. for resistance to white mold. Plant Dis 66:320-322

Kerr ED, Steadman JR, Nelson LA (1978) Estimation of white mold disease reduction of yield and yield components of dry edible beans. Crop Sci 18:275-279

Kolkman JM, Kelly JD (2003) QTL conferring resistance and avoidance to white mold in common bean. Crop Sci 43:539–548

Kolkman JM, Kelly JD (2002) Agronomic traits affecting resistance to white mold in common bean. Crop Sci 42:693-699

Kolkman JM, Kelly JD (2000) An indirect test using oxalate to determine physiological resistance to white mold in common bean. Crop Sci 40:281–285

Kolkman JM, Kelly JD (1999) Inheritance of resistance to white mold in common bean. Annu Rep Bean Improv Coop 42:47-48

Li G, Quiros CF (2001) Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theor Appl Genet 103:455-461

Li JZ, Huang XQ, Heinrichs F, Ganal MW, Röder MS (2006) Analysis of QTLs for yield components, agronomic traits, and disease resistance in an advanced backcross population of spring barley. Genome 49:454-466

Liao H, Yan X, Rubio G, Beebe SE, Blair MW, Lynch JP (2004) Genetic mapping of basal root gravitropism and phosphorous acquisition efficiency in common bean. Funct Plant Biol 31:959-970

Lyons ME, Dickson MH, Hunter JE (1987) Recurrent selection for resistance to white mold in *Phaseolus* species. J Amer Soc Hort Sci 112:149-152

Marciano P, Di Lenna P, Magro P (1983) Oxalic acid, cell-wall degrading enzymes and pH in pathogenesis and their significance in the virulence of two *Sclerotinia sclerotiorum* isolates on sunflower. Physiol Plant Pathol 22:339-345

Maxwell DP, Lumsden RD (1970) Oxalic acid production by *S. sclerotiorum* in infected bean and in culture. Phytopathology 60:1395-1398

Maxwell JJ, Brick MA, Byrne PF, Schwartz HF, Shan X, Ogg JB, Hensen RA (2007) Quantitative trait loci linked to white mold resistance in common bean. Crop Sci (In press)

Miklas PN, Larsen KA, Terpstra KA, Hauf DC, Grafton KF, and Kelly JD (2007) QTL analysis of ICA Bunsi-derived resistance to white mold in a pinto x navy bean cross. Crop Sci. 47:174-179

Miklas PN, Kelly JD, Beebe SE, Blair MW (2006) Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. Euphytica 147:105-131

Miklas PN, Hauf DC, Henson RA, Grafton KF (2004) Inheritance of ICA Bunsi-derived resistance to white mold in a navy x pinto bean cross. Crop Sci 44:1584-1588

Miklas PN, Delorme R, Riley R (2003) Identification of QTL conditioning resistance to white mold in a snap bean population. J Am Soc Hort Sci 128:564-270

Miklas PN, Johnson WC, Delorme R, Gepts P (2001) QTL conditioning physiological resistance and avoidance to white mold in dry bean. Crop Sci 41:309-315

Miklas PN, Delorme R, Hannan R, Dickson MH (1999) Using a subsample of the core collection to identify new sources of resistance to white mold in common bean. Crop Sci 39:569-573

Miklas PN, Stavely JR, Kelly JD (1993) Identification and potential use of a molecular marker for rust resistance in common bean. Theor Appl Genet 85:745-749

Miklas PN, Grafton KF, Nelson BD (1992a) Screening for partial physiological resistance to white mold in dry bean using excised stems. J Am Soc Hort Sci 117:321-327

Miklas PN, Grafton KF, Secor GA, McClean PE (1992b) Use of pathogen filtrate to differentiate physiological resistance of dry bean to white mold disease. Crop Sci 32:310-312

Miklas PN, Grafton KF (1992) Inheritance of partial resistance to white mold in inbred populations of dry bean. Crop Sci 32:943-948

Moncada PP, Martinez CP, Borrero J, Chatel M, Gauch H, Guimaraes, E, Tohme J, McCouch SR (2001) Quantitative trait loci for yield and yield components in an *Oryza sativa* × *Oryza rufipogon* BC_2F_2 population evaluated in an upland environment. Theor Appl Genet 102: 41–52

Ochoa IE, Blair MW, Lynch JP (2006) QTL analysis of adventitious root formation in common bean under contrasting phosphorous availability. Crop Sci 46:1609-1621

Park SO, Coyne DP, Steadman JR, Jung G (1999). Mapping of molecular markers and seedcoat pattern associated with QTL affecting white mold resistance in common bean. Annu Rep Bean Improv Coop 42:45-46

Park, SO, Coyne, DP, Steadman, JR, Skroch PW (2001) Mapping of QTL for resistance to white mold disease in common bean. Crop Sci 41:1253–1262

Peachey E, Ludy RL, Powelson ML, McGrath DM (2006) Modification of plant arrangement suppresses white mold of snap beans. Hort Sci 41:1298-1302

Pejic I, Ajmone-Marsan P, Morgante M, Kozumplick V, Castiglioni P, Taramino G, Motto M (1998) Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs. Theor App Genet 97:1248-1255

Petzholdt, R, Dickson, MH (1996) Straw test for resistance to white mold in beans. Annu Rep Bean Improv Coop 39:142-143

Rao GU, Ben Chaim A, Borovsky Y, Paran I (2003) Mapping of yield-related QTLs in pepper in an interspecific cross of *Capsicum annuum* and *C. fructescens*. Theor Appl Genet 106:1457-1466

Rollins JA, Dickman MB (2001) pH signaling in *Sclerotinia sclerotiorum*: Identification of a *pacC/RIM1* homolog. App Environ Microbiol 67:75-81

Sax, K (1923) The association of size differences with seed coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics 8:552-560

Schwartz HF, Otto K, Teran H, Singh SP (2004) Inheritance of white mold resistance in the interspecific crosses of pinto cultivars Othello and UI 320 and *Phaseolus coccineus* L. accessions PI 433246 and PI 439534. Annu Rep Bean Improv Coop 47:279-280

Schwartz HF, Otto K, Teran H, Lema M, Singh SP (2006) Inheritance of white mold resistance in Phaseolus vulgaris x P. coccineus crosses. Plant Dis 90:1167-1170

Shaner G, Finney RE (1977) The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051-1056

Shii CT, Rabakoarihanta A, Mok MC, Mok DWS (1982) Embryo development in reciprocal crosses of *Phaseolus vulgaris* L. and *P. coccineus* Lam. Theor Appl Genet 62: 59-64

Steadman JR (1979) Control of plant diseases caused by *Sclerotinia* species. Phytopathology 69:904-907

Tanksley SD, Nelson JC (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. Theor Appl Genet 92:191-203

Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acid Res 17:6463-6471

Terpstra KA, Kelly JD (2006) Preliminary QTL analysis for white mold resistance in a black bean x wild Mexican bean inbred backcross mapping population. Annu Rept Bean Improv Coop 49:103-104

Vales MI, Schön CC, Capettini F, Chen XM, Corey AE, Mather DE, Mundt CC, Richardson KL, Sandoval-Islas JS, Utz HF, Hayes PM (2005) Effect of population size on the estimation of QTL: a test using resistance to barley stripe rust. Theor Appl Genet 111: 1260-1270

Van Ooijen J, Voorrips R (2006) JoinMap® 4.0, Software for the calculation of genetic linkage maps in experimental populations. Plant Research International, Wageningen, Netherlands

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Freijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res 23:4407-4414

Wang S, Basten CJ, and Zeng Z-B (2005) Windows QTL Cartographer 2.5. Dep. of Statistics, North Carolina State University, Raleigh, NC (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)

Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388-396

Wehrhahn C, Allard W (1965) The detection and measurement of the effects of individual genes involved in the inheritance of a quantitative character in wheat. Genetics 51:109-119

Xiao J, Li J, Grandillo S, Ahn S, Yuan L, Tanksley SD, McCouch SR (1998) Identification of trait-improving quantitative trait loci alleles from a wild rice relative, *Oryza rufipogon*. Genetics, 150: 899–909

Yamamoto T, Kuboki Y, Lin SY, Sasaki T, Yano M (1998) Fine mapping of quantitative trait loci Hd-1, Hd-2 and Hd-3, controlling heading date of rice, as single Mendelian factors. Theor Appl Genet 97:37–44

Yu K, Park SJ, Poysa V, Gepts P (2000) Integration of simple sequence repeat (SSR) markers into a linkage map of common bean (*Phaseolus vulgaris* L.). J Heredity 91:429-434

Yun SJ, Gyenis L, Bossolini E, Hayes PM, Matus I, Smith KP, Steffenson BJ, Tuberosa R, Muehlbauer GJ (2006) Validation of quantitative trait loci for multiple disease resistance in barley using advanced backcross lines developed with a wild barley. Crop Sci 46:1179-1186

Zhou T, Boland GJ (1999) Mycelial growth and production of oxalic acid by virulent and hypovirulent isolates of *Sclerotinia sclerotiorum*. Can J Plant Pathol 21:93-99

APPENDICES

APPENDIX A Phenotypic data for OR 91G x PI 255956 population.

Table 1. Straw test AUDPC LS means for OR 91G x PI 255956 BC_2F_5 population and p-values for tests of LS mean equal to LS means of check varieties.

Line	AUDPC LS mean	G 122	M0162	OR 91G	Minuette	OSU 5613	OSU 5630
762/2-6	54.8	0.28	0.00	<.0001	<.0001	<.0001	<.0001
897/18-1	65.4	0.71	0.16	<.0001	<.0001	<.0001	<.0001
823/47-2	66.9	0.56	0.20	<.0001	<.0001	<.0001	<.0001
825/47-4	70.4	0.25	0.37	<.0001	<.0001	<.0001	<.0001
861/13-14	71.6	0.19	0.47	<.0001	<.0001	<.0001	<.0001
837/3-18	71.6	0.19	0.47	<.0001	<.0001	<.0001	<.0001
760/2-3	71.9	0.19	0.52	<.0001	<.0001	<.0001	<.0001
822/46-6	71.9	0.17	0.50	<.0001	<.0001	<.0001	<.0001
787/35-1	72.4	0.35	0.70	0.01	<.0001	<.0001	<.0001
782/33-2	73.2	0.14	0.64	<.0001	<.0001	<.0001	<.0001
853/6-9	76.2	0.05	0.96	0.00	<.0001	<.0001	<.0001
881/11-4	78.9	0.02	0.74	0.00	<.0001	<.0001	<.0001
850/9-10	80.2	0.01	0.62	0.00	<.0001	<.0001	<.0001
903/20-2	80.5	0.01	0.58	0.00	<.0001	<.0001	<.0001
805/41-2	80.5	0.01	0.58	0.00	<.0001	<.0001	<.0001
840/4-6	80.7	0.01	0.58	0.00	<.0001	<.0001	<.0001
884/12-3	80.9	0.01	0.54	0.00	<.0001	<.0001	<.0001
913/23-18	81.1	0.01	0.54	0.00	<.0001	<.0001	<.0001
764/3-3	81.3	0.01	0.50	0.00	<.0001	<.0001	<.0001
828/48-5	81.3	0.01	0.50	0.00	<.0001	<.0001	<.0001
836/3-15	81.7	0.01	0.47	0.00	<.0001	<.0001	<.0001
831/49-5	82.0	0.01	0.46	0.01	<.0001	<.0001	<.0001
818/45-2	82.0	0.01	0.44	0.00	<.0001	<.0001	<.0001
894/17-4	82.4	0.00	0.40	0.01	<.0001	<.0001	<.0001
878/10-4	82.8	0.00	0.37	0.01	<.0001	<.0001	<.0001
769/27-1	83.2	0.00	0.34	0.01	<.0001	<.0001	<.0001
906/21-1	83.6	0.00	0.32	0.01	<.0001	<.0001	<.0001
849/8-3	84.0	0.00	0.29	0.01	<.0001	<.0001	<.0001
761/2-5	84.4	0.00	0.27	0.01	<.0001	<.0001	<.0001
763/3-2	85.3	0.00	0.22	0.02	<.0001	<.0001	<.0001
885/12-5	85.4	0.00	0.22	0.02	<.0001	<.0001	<.0001
846/6-3	85.6	0.00	0.20	0.02	<.0001	<.0001	<.0001
918/25-1	86.3	0.00	0.16	0.02	<.0001	<.0001	<.0001
777/29-9	86.4	0.00	0.18	0.03	<.0001	<.0001	<.0001
792/36-4	86.7	0.00	0.15	0.03	<.0001	<.0001	<.0001
779/31-3	87.5	0.00	0.12	0.04	<.0001	<.0001	<.0001
814/44-3	88.1	0.00	0.12	0.05	<.0001	<.0001	0.00
912/23-8	88.2	0.00	0.15	0.09	0.00	0.00	0.00
757/1-1	88.3	0.00	0.10	0.05	<.0001	<.0001	<.0001
759/1-11	88.3	0.00	0.10	0.05	<.0001	<.0001	<.0001

Table 1 (Continued). Straw test AUDPC LS means for OR 91G x PI 255956 BC_2F_5 population and p-values for tests of LS mean equal to LS means of check varieties.

Line	AUDPC LS mean	G 122	M0162	OR 91G	Minuette	OSU 5613	OSU 5630
860/13-11	88.3	0.00	0.10	0.05	<.0001	<.0001	<.0001
834/50-5	89.1	0.00	0.07	0.06	<.0001	<.0001	0.00
803/40-3	89.4	0.00	0.08	0.08	0.00	0.00	0.00
780/31-6	89.4	0.00	0.07	0.07	<.0001	0.00	0.00
904/20-3	89.9	0.00	0.08	0.11	0.00	0.00	0.00
810/42-5	90.6	<.0001	0.05	0.10	0.00	0.00	0.00
781/33-1	90.7	0.00	0.05	0.12	0.00	0.00	0.00
905/20-8	91.0	<.0001	0.04	0.11	0.00	0.00	0.00
768/26-3	91.0	<.0001	0.04	0.11	0.00	0.00	0.00
902/19-10	91.4	0.00	0.05	0.16	0.00	0.00	0.00
833/50-4	91.4	<.0001	0.03	0.13	0.00	0.00	0.00
806/41-7	91.8	<.0001	0.03	0.14	0.00	0.00	0.00
856/7-2	91.8	<.0001	0.03	0.14	0.00	0.00	0.00
845/6-2	92.1	<.0001	0.03	0.17	0.00	0.00	0.00
804/40-6	92.6	<.0001	0.02	0.17	0.00	0.00	0.00
900/19-3	92.9	<.0001	0.02	0.19	0.00	0.00	0.00
908/21-5	92.9	<.0001	0.02	0.19	0.00	0.00	0.00
917/24-7	93.3	<.0001	0.02	0.21	0.00	0.00	0.00
813/43-1	93.3	<.0001	0.02	0.21	0.00	0.00	0.00
851/9-13	93.3	<.0001	0.02	0.21	0.00	0.00	0.00
819/45-3	93.4	<.0001	0.02	0.23	0.00	0.00	0.00
886/14-9	93.7	<.0001	0.01	0.23	0.00	0.00	0.00
789/35-7	93.7	<.0001	0.01	0.23	0.00	0.00	0.00
839/4-4	93.8	<.0001	0.02	0.25	0.00	0.00	0.00
817/45-1	93.9	<.0001	0.02	0.28	0.00	0.00	0.00
790/36-1	94.1	<.0001	0.01	0.25	0.00	0.00	0.00
808/42-1	94.7	<.0001	0.01	0.31	0.00	0.00	0.00
774/28-4	94.9	<.0001	0.01	0.30	0.00	0.00	0.00
896/17-6	95.3	<.0001	0.01	0.33	0.00	0.00	0.00
854/7-8	96.0	<.0001	0.01	0.40	0.00	0.00	0.01
901/19-5	96.1	<.0001	0.01	0.39	0.00	0.00	0.00
848/8-2	96.1	<.0001	0.01	0.39	0.00	0.00	0.00
815/44-4	96.4	<.0001	0.00	0.42	0.00	0.00	0.01
821/46-5	96.4	<.0001	0.00	0.42	0.00	0.00	0.01
771/27-13	97.2	<.0001	0.00	0.49	0.00	0.01	0.01
800/39-4	97.6	<.0001	0.00	0.52	0.00	0.01	0.01
920/25-6	98.0	<.0001	0.00	0.56	0.00	0.01	0.01
891/15-2	98.4	<.0001	0.00	0.63	0.01	0.02	0.02
841/5-15	98.8	<.0001	0.00	0.64	0.01	0.01	0.01
809/42-3	99.9	<.0001	0.00	0.76	0.01	0.02	0.02
889/15-1	100.3	<.0001	0.00	0.80	0.01	0.02	0.02
799/39-3	100.3	<.0001	0.00	0.80	0.01	0.02	0.02
910/22-3	100.4	0.03	0.18	0.93	0.31	0.36	0.37
788/35-5	100.4	0.00	0.03	0.88	0.10	0.13	0.14
766/26-1	100.7	<.0001	0.00	0.85	0.01	0.02	0.03

Table 1 (Continued). Straw test AUDPC LS means for OR 91G x PI 255956 BC_2F_5 population and p-values for tests of LS mean equal to LS means of check varieties.

Line	AUDPC LS mean	G 122	M0162	OR 91G	Minuette	OSU 5613	OSU 5630
830/49-2	100.7	<.0001	0.00	0.85	0.01	0.02	0.03
802/40-1	101.4	<.0001	0.00	0.92	0.03	0.05	0.05
844/6-1	101.5	<.0001	0.00	0.93	0.02	0.03	0.04
857/6-7	101.5	<.0001	0.00	0.93	0.02	0.03	0.04
847/8-1	102.3	<.0001	0.00	0.98	0.02	0.04	0.05
824/47-3	103.1	<.0001	0.00	0.89	0.03	0.05	0.06
883/12-2	103.8	<.0001	0.00	0.80	0.04	0.07	0.08
826/48-3	103.8	<.0001	0.00	0.80	0.04	0.07	0.08
855/7-15	103.8	<.0001	0.00	0.80	0.04	0.07	0.08
882/11-6	104.6	<.0001	<.0001	0.72	0.06	0.09	0.10
784/34-1	104.6	<.0001	<.0001	0.72	0.06	0.09	0.10
880/11-1	105.4	<.0001	<.0001	0.64	0.07	0.11	0.13
770/27-3	105.6	<.0001	<.0001	0.63	0.09	0.14	0.15
812/43-3	105.8	<.0001	<.0001	0.60	0.08	0.13	0.14
772/28-1	106.2	<.0001	<.0001	0.56	0.09	0.14	0.16
915/24-1	106.6	<.0001	<.0001	0.52	0.10	0.16	0.17
852/9-16	106.6	<.0001	<.0001	0.52	0.10	0.16	0.17
859/13-8	106.9	<.0001	<.0001	0.49	0.11	0.17	0.19
919/25-3	106.9	<.0001	<.0001	0.49	0.11	0.17	0.19
797/38-5	106.9	<.0001	<.0001	0.49	0.11	0.17	0.19
807/41-11	106.9	<.0001	<.0001	0.49	0.11	0.17	0.19
838/4-1	107.3	<.0001	<.0001	0.45	0.12	0.19	0.21
835/3-12	107.8	<.0001	<.0001	0.43	0.16	0.23	0.25
827/48-4	108.2	<.0001	<.0001	0.40	0.18	0.26	0.28
798/38-6	108.5	<.0001	<.0001	0.36	0.17	0.25	0.28
816/44-5	109.3	<.0001	<.0001	0.30	0.21	0.30	0.33
811/43-4	110.1	<.0001	<.0001	0.25	0.25	0.36	0.39
778/31-1	110.4	<.0001	<.0001	0.23	0.27	0.39	0.42
832/50-3	115.1	<.0001	<.0001	0.06	0.66	0.85	0.89
879/10-15	120.2	<.0001	<.0001	0.01	0.79	0.60	0.56
G122	62.4		0.01	<.0001	<.0001	<.0001	<.0001
M0162	76.6	0.01		<.0001	<.0001	<.0001	<.0001
OR91G	102.1	<.0001	<.0001		0.01	0.01	0.01
OSU5630	116.1	<.0001	<.0001	0.01	0.71	0.95	
OSU5613	116.5	<.0001	<.0001	0.01	0.76		0.95
Minuette	118.3	<.0001	<.0001	0.01		0.76	0.71

Table 2. Oxalate test scores for OR 91G x PI 255956 BC_2F_5 population and pairwise comparisons with check varieties using Fisher's LSD.

Line	Mean	Std Dev	PI 255956	G122	M0162	NY6020-5	91G
913/23-18	1.3	0.65				**	**
851/9-13	1.5	0.65				**	**
903/20-2	1.5	0.65				**	**
919/25-3	1.5	0.65				**	**
879/10-15	2.0	0.65	**			**	**
849/8-3	2.3	0.65	**				**
908/21-5	2.3	0.65	**				**
912/23-8	2.3	0.65	**				**
917/24-7	2.3	0.65	**				**
800/39-4	2.8	0.65	**				**
918/25-1	2.8	0.65	**				**
784/34-1	3.0	0.65	**				
856/7-2	3.0	0.65	**				
860/13-11	3.0	0.65	**				
861/13-14	3.0	0.65	**				
878/10-4	3.0	0.65	**				
790/36-1	3.3	0.65	**	**			
805/41-2	3.3	0.65	**	**			
859/13-8	3.3	0.65	**	**			
802/40-1	3.5	0.65	**	**			
826/48-3	3.5	0.65	**	**			
883/12-2	3.5	0.65	**	**			
771/27-13	3.8	0.65	**	**			
772/28-1	3.8	0.65	**	**			
799/39-3	3.8	0.65	**	**			
808/42-1	3.8	0.65	**	**			
812/43-3	3.8	0.65	**	**			
847/8-1	3.8	0.65	**	**			
884/12-3	3.8	0.65	**	**			
759/1-11	4.0	0.65	**	**			
779/31-3	4.0	0.65	**	**			
810/42-5	4.0	0.65	**	**			
821/46-5	4.0	0.65	**	**			
823/47-2	4.0	0.65	**	**			
839/4-4	4.0	0.65	**	**			
782/33-2	4.0	0.75	**	**			
780/31-6	4.3	0.65	**	**	**		
818/45-2	4.3	0.65	**	**	**		
834/50-5	4.3	0.65	**	**	**		
850/9-10	4.3	0.65	**	**	**		
817/45-1	4.4	0.75	**	**			
885/12-5	4.4	0.92	**	**	**		
763/3-2	4.5	0.65	**	**	**		
789/35-7	4.5	0.65	**	**	**		

Table 2 (Continued). Oxalate test scores for OR 91G x PI 255956 BC_2F_5 population and pairwise comparisons with check varieties using Fisher's LSD.

Line	Mean	Std Dev	PI 255956	G122	M0162	NY6020-5	91G
797/38-5	4.5	0.65	**	**	**		
804/40-6	4.5	0.65	**	**	**		
832/50-3	4.5	0.65	**	**	**		
854/7-8	4.5	0.65	**	**	**		
889/15-1	4.5	0.65	**	**	**		
902/19-10	4.5	0.65	**	**			
905/20-8	4.5	0.65	**	**	**		
807/41-11	4.8	0.65	**	**	**		
809/42-3	4.8	0.65	**	**	**		
814/44-3	4.8	0.65	**	**	**		
822/46-6	4.8	0.65	**	**	**		
831/49-5	4.8	0.65	**	**	**		
896/17-6	4.8	0.65	**	**	**		
901/19-5	4.8	0.65	**	**	**		
915/24-1	4.8	0.65	**	**	**		
920/25-6	4.8	0.65	**	**	**		
762/2-6	5.0	0.65	**	**	**		
764/3-3	5.0	0.65	**	**	**		
766/26-1	5.0	0.65	**	**	**		
769/27-1	5.0	0.65	**	**	**		
770/27-3	5.0	0.65	**	**	**		
774/28-4	5.0	0.65	**	**	**		
778/31-1	5.0	0.65	**	**	**		
819/45-3	5.0	0.65	**	**	**		
830/49-2	5.0	0.65	**	**	**		
841/5-15	5.0	0.65	**	**	**		
848/8-2	5.0	0.65	**	**	**		
857/6-7	5.0	0.65	**	**	**		
880/11-1	5.0	0.65	**	**	**		
881/11-4	5.0	0.65	**	**	**		
886/14-9	5.0	0.65	**	**	**		
906/21-1	5.0	0.65	**	**	**		
757/1-1	5.3	0.65	**	**	**		
803/40-3	5.3	0.65	**	**	**		
806/41-7	5.3	0.65	**	**	**		
816/44-5	5.3	0.65	**	**	**		
835/3-12	5.3	0.65	**	**	**		
837/3-18	5.3	0.65	**	**	**		
840/4-6	5.3	0.65	**	**	**		
846/6-3	5.3	0.65	**	**	**		
855/7-15	5.3	0.65	**	**	**		
900/19-3	5.3	0.65	**	**	**		
761/2-5	5.5	0.65	**	**	**	**	
815/44-4	5.5	0.65	**	**	**	**	

Table 2 (Continued). Oxalate test scores for OR 91G x PI 255956 BC_2F_5 population and pairwise comparisons with check varieties using Fisher's LSD.

Line	Mean	Std Dev	PI 255956	G122	M0162	NY6020-5	91G
838/4-1	5.5	0.65	**	**	**	**	
852/9-16	5.5	0.65	**	**	**	**	
760/2-3	5.8	0.65	**	**	**	**	
777/29-9	5.75	0.65	**	**	**	**	
827/48-4	5.75	0.65	**	**	**	**	
828/48-5	5.75	0.65	**	**	**	**	
844/6-1	5.75	0.65	**	**	**	**	
897/18-1	5.75	0.65	**	**	**	**	
904/20-3	5.75	0.65	**	**	**	**	
781/33-1	6.00	0.65	**	**	**	**	
813/43-1	6.00	0.65	**	**	**	**	
836/3-15	6.00	0.65	**	**	**	**	
768/26-3	6.25	0.65	**	**	**	**	**
792/36-4	6.25	0.65	**	**	**	**	**
798/38-6	6.25	0.65	**	**	**	**	**
811/43-4	6.50	0.65	**	**	**	**	**
833/50-4	6.75	0.65	**	**	**	**	**
894/17-4	6.75	0.65	**	**	**	**	**
824/47-3	7.00	0.65	**	**	**	**	**
825/47-4	7.00	0.65	**	**	**	**	**
853/6-9	7.00	0.65	**	**	**	**	**
845/6-2	7.25	0.65	**	**	**	**	**
PI 255956	0.07	0.75			**	**	**
G122	1.63	0.46				**	**
M0162	2.66	0.43	**				**
NY6020-5	3.75	0.46	**	**			
91G	4.50	0.46	**	**	**		

** Significant LSD at $p < 0.05$

Oxalate rating scale: 0= no symptoms, 1= one leaf with slight wilting symptoms, 2= one leaf wilting, 3=second leaf wilting slightly, 4= two leaves wilted, 5= three leaves wilting, 6= four leaves wilting, 7= petioles collapsing, 8=flaccid stems, and 9= total plant collapse.

Table 3. 2006 Field trial scores for OR 91G x PI 255956 BC_2F_6 population and p values for pairwise comparisons with check varieties.

Entry	WM LS mean	G 122	M0162	OR 91G	OSU 5630
897/18-1	2.06	0.83	0.47	<.0001	<.0001
861/13-14	3.04	0.21	0.58	<.0001	<.0001
859/13-8	3.04	0.21	0.58	<.0001	<.0001
903/20-2	3.06	0.33	0.65	<.0001	<.0001
904/20-3	3.06	0.33	0.65	<.0001	<.0001
845/6-2	3.06	0.33	0.65	<.0001	<.0001
762/2-6	3.31	0.05	0.26	<.0001	<.0001
814/44-3	3.54	0.04	0.19	<.0001	<.0001
846/6-3	3.54	0.04	0.19	<.0001	<.0001
853/6-9	3.54	0.04	0.19	<.0001	<.0001
828/48-5	3.64	0.01	0.09	<.0001	<.0001
860/13-11	3.98	0.00	0.02	<.0001	<.0001
817/45-1	3.98	0.00	0.02	<.0001	<.0001
889/15-1	4.04	0.00	0.04	<.0001	<.0001
906/21-1	4.04	0.00	0.04	<.0001	<.0001
838/4-1	4.04	0.00	0.04	<.0001	<.0001
777/29-9	4.06	0.03	0.11	0.00	0.00
822/46-6	4.06	0.03	0.11	0.00	0.00
881/11-4	4.31	0.00	0.00	<.0001	<.0001
841/5-15	4.31	0.00	0.00	<.0001	<.0001
844/6-1	4.50	<.0001	0.00	<.0001	<.0001
878/10-4	4.54	0.00	0.00	0.00	0.00
797/38-5	4.54	0.00	0.00	0.00	0.00
798/38-6	4.54	0.00	0.00	0.00	0.00
805/41-2	4.54	0.00	0.00	0.00	0.00
815/44-4	4.54	0.00	0.00	0.00	0.00
824/47-3	4.54	0.00	0.00	0.00	0.00
884/12-3	4.64	<.0001	0.00	<.0001	<.0001
778/31-1	4.64	<.0001	0.00	<.0001	<.0001
806/41-7	4.64	<.0001	0.00	<.0001	<.0001
830/49-2	4.64	<.0001	0.00	<.0001	<.0001
827/48-4	5.00	<.0001	<.0001	0.00	<.0001
835/3-12	5.04	<.0001	0.00	0.00	0.00
836/3-15	5.04	<.0001	0.00	0.00	0.00
854/7-8	5.04	<.0001	0.00	0.00	0.00
886/14-9	5.06	0.00	0.01	0.03	0.02
894/17-4	5.06	0.00	0.01	0.03	0.02
799/39-3	5.06	0.00	0.01	0.03	0.02
816/44-5	5.06	0.00	0.01	0.03	0.02
840/4-6	5.31	<.0001	<.0001	0.00	0.00
821/46-5	5.31	<.0001	<.0001	0.00	0.00
812/43-3	5.50	<.0001	<.0001	0.01	0.00
856/7-2	5.50	<.0001	<.0001	0.01	0.00
900/19-3	5.54	<.0001	<.0001	0.03	0.02

Table 3 (Continued). 2006 Field trial scores for OR 91G x PI 255956 BC_2F_6 population and p values for pairwise comparisons with check varieties.

Entry	WM LS mean	G 122	M0162	OR 91G	OSU 5630
780/31-6	5.54	<.0001	<.0001	0.03	0.02
813/43-1	5.54	<.0001	<.0001	0.03	0.02
766/26-1	5.64	<.0001	<.0001	0.02	0.01
802/40-1	5.64	<.0001	<.0001	0.02	0.01
819/45-3	5.64	<.0001	<.0001	0.02	0.01
825/47-4	5.64	<.0001	<.0001	0.02	0.01
857/6-7	5.64	<.0001	<.0001	0.02	0.01
850/9-10	5.75	<.0001	<.0001	0.02	0.01
901/19-5	5.98	<.0001	<.0001	0.10	0.06
781/33-1	5.98	<.0001	<.0001	0.10	0.06
784/34-1	5.98	<.0001	<.0001	0.10	0.06
818/45-2	5.98	<.0001	<.0001	0.10	0.06
759/1-11	6.00	<.0001	<.0001	0.07	0.04
920/25-6	6.00	<.0001	<.0001	0.07	0.04
803/40-3	6.00	<.0001	<.0001	0.07	0.04
831/49-5	6.00	<.0001	<.0001	0.07	0.04
908/21-5	6.04	<.0001	<.0001	0.18	0.13
919/25-3	6.04	<.0001	<.0001	0.18	0.13
790/36-1	6.04	<.0001	<.0001	0.18	0.13
834/50-5	6.04	<.0001	<.0001	0.18	0.13
880/11-1	6.06	<.0001	0.00	0.34	0.27
885/12-5	6.06	<.0001	0.00	0.34	0.27
758/1-7	6.06	<.0001	0.00	0.34	0.27
763/3-2	6.06	<.0001	0.00	0.34	0.27
800/39-4	6.06	<.0001	0.00	0.34	0.27
826/48-3	6.06	<.0001	0.00	0.34	0.27
848/8-2	6.06	<.0001	0.00	0.34	0.27
851/9-13	6.06	<.0001	0.00	0.34	0.27
757/1-1	6.25	<.0001	<.0001	0.20	0.12
771/27-13	6.25	<.0001	<.0001	0.20	0.12
837/3-18	6.25	<.0001	<.0001	0.20	0.12
918/25-1	6.31	<.0001	<.0001	0.29	0.20
770/27-3	6.31	<.0001	<.0001	0.29	0.20
779/31-3	6.31	<.0001	<.0001	0.29	0.20
807/41-11	6.31	<.0001	<.0001	0.29	0.20
839/4-4	6.31	<.0001	<.0001	0.29	0.20
852/9-16	6.38	<.0001	<.0001	0.36	0.25
883/12-2	6.54	<.0001	<.0001	0.59	0.46
761/2-5	6.54	<.0001	<.0001	0.59	0.46
808/42-1	6.54	<.0001	<.0001	0.59	0.46
809/42-3	6.54	<.0001	<.0001	0.59	0.46
847/8-1	6.54	<.0001	<.0001	0.59	0.46
849/8-3	6.54	<.0001	<.0001	0.59	0.46
902/19-10	6.64	<.0001	<.0001	0.66	0.51

Table 3 (Continued). 2006 Field trial scores for OR 91G x PI 255956 BC_2F_6 population and p values for pairwise comparisons with check varieties.

Entry	WM LS mean	G 122	M0162	OR 91G	OSU 5630
789/35-7	6.64	<.0001	<.0001	0.66	0.51
917/24-7	6.98	<.0001	<.0001	0.85	0.96
769/27-1	6.98	<.0001	<.0001	0.85	0.96
792/36-4	6.98	<.0001	<.0001	0.85	0.96
811/43-4	6.98	<.0001	<.0001	0.85	0.96
832/50-3	6.98	<.0001	<.0001	0.85	0.96
855/7-15	7.01	<.0001	<.0001	0.87	0.99
905/20-8	7.04	<.0001	<.0001	0.80	0.95
768/26-3	7.04	<.0001	<.0001	0.80	0.95
764/3-3	7.04	<.0001	<.0001	0.80	0.95
916/24-4	7.06	<.0001	<.0001	0.82	0.94
772/28-1	7.06	<.0001	<.0001	0.82	0.94
796/38-3	7.06	<.0001	<.0001	0.82	0.94
915/24-1	7.25	<.0001	<.0001	0.44	0.61
804/40-6	7.31	<.0001	<.0001	0.42	0.57
896/17-6	7.54	<.0001	<.0001	0.29	0.39
774/28-4	8.04	<.0001	<.0001	0.07	0.10
810/42-5	8.04	<.0001	<.0001	0.07	0.10
879/10-15	8.06	<.0001	<.0001	0.16	0.21
91G	6.88	<.0001	<.0001		0.75
G122	2.25		0.32	<.0001	<.0001
M0162	2.68	0.32		<.0001	<.0001
OSU5630	7.00	<.0001	<.0001	0.75	

Field rating scale: 1 = no diseased plants, 9= 80% to 100% diseased plants and/or 60% to 100% infected tissue (Miklas et al., 2001).

APPENDIX B Genotypic data for OR 91G x PI 255956 population.

Table 1. Polymorphic SSR markers for OR 91G and PI 255956 parents.

Marker	Expected Allele Size	Recurrent Allele Size	Donor Allele Size(s)
AG1	132	134	136
BM6	153	147	142
BM79B	125	298	296
BM98	247	247	249
BM139	115	79	85
BM141	218	216	179
BM142	157	153	151
BM146	281	284	211
BM148	295	289	291
BM149	273	245	242
BM151	153	145	147
BM152	127	90	69
BM154	218	216	190
BM156	267	212	205
BM157	113	109	94, 114
BM160	211	180	183
BM161	185	187	175
BM164	182	144	151
BM166	151	145	143
BM171	149	145	147
BM172	107	75	81
BM175	170	189	155,141
BM181	192	185	179
BM183	149	142	128,148
BM184	160	161	155
BM185	105	100,101	104,105;92,93
BM187	191	170	145,158
BM189	114	107	105
BM201	102	99	92,112
BM202	156	134	156
BM210	166	166	158,181
BMd-1	165	159,172,201	139,168
BMd-2	106	100	92
BMd-4	146	137	128
BMd-5	122	116	120
BMd-7	166	169	172
BMd-12	167	158	149,161
BMd-15	166	163	160
BMd-17	116	111	93
BMd-18	156	239	236
BMd-19	154	150	153

Table 1 (Continued). Polymorphic SSR markers for OR 91G and PI 255956 parents.

Marker	Expected Allele Size	Recurrent Allele Size	Donor Allele Size(s)
BMd-25	118	111	115
BMd-26	141	140	128
BMd-30	134	130	140
BMd-31	161	144	156
BMd-33	110	95	86,104
BMd-35	128	121	124
BMd-40	197	192	198
BMd-41	250	237	224,245
BMd-42	95	145	107
BMd-46	158	323	320
BMd-47	150	147	153
BMd-49	95	87	90
BMd-50	124	116	119
BMd-52	151	180	147
GATS11	306	228	225
GATS91	229	257	224
PV-aaat001	203	201	192,211
PV-ag001	157	145	137
PV-ag004	201	201	199
PV-at002	254	245	239
PV-at003	139	131	133
PV-at004	163	163	161
PV-at005	305	290	268
PV-at006	132	127	160,169
PV-at007	192	192	190
PV-at008	161	173	141
PVBR11	142	184	196
PVBR14	196	170	166
PVBR20	197	171	167
PVBR21	229	257	235
PVBR23	345	376	354
PVBR25	250	158	140,152
PV-cca002	179	174	177
PV-cct001	149	145	148
PV-gaat001	163	156	153
PV-gccacc001	95	90	72
BM16	149	301	?
BM53	287	298	?
BM138	203	194	?
BM140	190	156	?
BM199	304	276	?
BM211	186	179	?
BMd-8	176	173	?
BMd-10	139	136	?
BMd-28	151	125,149	?

Table 1 (Continued). Polymorphic SSR markers for OR 91G and PI 255956 parents.

Marker	Expected Allele Size	Recurrent Allele Size	Donor Allele Size(s)
BMd-36	164	160	?
BMd-43	176	173	?
BMd-45	129	126	?
BMd-55	188	182	?
GATS54	114	110	?
PV-atgc002	144	139	?
PVBR5	195	168	?
PVBR16	198	189	?
PV-cca001	151	202	?
PV-cca003	115	113	?
PV-ctt001	152	158	?
PV-ggc001	238	163	?

? Indicates loci monomorphic in progeny.

Table 2. Chi-square values for SSR and AFLP® marker loci in OR 91G x PI 255956 BC_2F_4 population.

Marker	χ^2	p value
AG1	36.09	0.000
BM6	34.33	0.000
BM79B	9.43	0.009
BM98	30.02	0.000
BM139	22.66	0.000
BM141	14.56	0.001
BM142	458.76	0.000
BM146	17.42	0.000
BM148	34.33	0.000
BM149	30.23	0.000
BM151	17.10	0.000
BM152	15.62	0.000
BM154	49.46	0.000
BM156	15.62	0.000
BM157	24.22	0.000
BM161	11.28	0.004
BM164	38.06	0.000
BM166	26.73	0.000
BM171	155.02	0.000
BM172	43.92	0.000
BM175	409.99	0.000
BM181	244.19	0.000
BM183	537.24	0.000
BM184	45.11	0.000
BM185	373.90	0.000
BM187	12.25	0.002
BM189	65.99	0.000
BM202	18.68	0.000
BM210	380.21	0.000
BMd-1	6.34	0.042
BMd-2	13.50	0.001
BMd-4	15.31	0.000
BMd-5	22.74	0.000
BMd-7	13.42	0.001
BMd-12	8.99	0.011
BMd-15	11.43	0.003
BMd-18	15.14	0.001
BMd-19	12.46	0.002
BMd-25	12.80	0.002
BMd-26	30.42	0.000
BMd-30	9.40	0.009
BMd-31	16.79	0.000
BMd-33	392.02	0.000

Table 2 (Continued). Chi-square values for SSR and AFLP® marker loci in OR 91G x PI 255956 BC_2F_4 population.

Marker	χ^2	p value
BMd-35	51.22	0.000
BMd-40	14.68	0.001
BMd-41	464.82	0.000
BMd-42	10.36	0.006
BMd-46	27.00	0.000
BMd-47	16.89	0.000
BMd-49	46.07	0.000
BMd-50	16.46	0.000
BMd-52	2.05	0.358
GATS11	18.66	0.000
GATS91	15.46	0.000
PVag001	11.67	0.003
PVag004	2.15	0.341
PVat003	51.22	0.000
PVat006	12.35	0.002
PVat007	2.64	0.267
PVat008	15.37	0.000
PVBR11	14.25	0.001
PVBR14	60.14	0.000
PVBR20	58.03	0.000
PVBR21	48.24	0.000
PVBR23	34.10	0.000
PVBR25	18.69	0.000
PVcca002	45.32	0.000
PVcct001	13.10	0.001
PVgaat001	51.22	0.000
PVgccacc001	8.76	0.012
PVat002	7.40	0.025
E32/M59-89	33.01	0.000
E32/M59-91	110.35	0.000
E32/M59-100	475.65	0.000
E32/M59-104	110.35	0.000
E32/M59-107	280.63	0.000
E32/M59-121	158.44	0.000
E32/M59-126	0.25	0.616
E32/M59-153	462.76	0.000
E32/M59-159	40.20	0.000
E32/M59-166	366.03	0.000
E32/M59-170	18.13	0.000
E32/M59-174	136.77	0.000
E32/M59-177	754.14	0.000
E32/M59-178	629.09	0.000
E32/M59-193	251.53	0.000

Table 2 (Continued). Chi-square values for SSR and AFLP® marker loci in OR 91G x PI 255956 BC_2F_4 population.

Marker	χ^2	p value
E32/M59-198	0.15	0.696
E32/M59-205	6.68	0.010
E32/M59-211	40.20	0.000
E32/M59-216	820.91	0.000
E32/M59-228	23.55	0.000
E32/M59-231	855.36	0.000
E32/M59-239	300.92	0.000
E32/M59-250	40.20	0.000
E32/M59-252	872.84	0.000
E32/M59-263	528.97	0.000
E32/M59-284	332.68	0.000
E32/M59-286	56.70	0.000
E32/M59-293	44.06	0.000
E32/M59-298	66.02	0.000
E32/M59-310	116.69	0.000
E32/M59-324	11.33	0.001
E32/M59-343	0.15	0.696
E32/M59-354	104.19	0.000
E32/M59-368	151.04	0.000
E32/M59-418	136.77	0.000
E32/M59-422	1.65	0.200
E32/M59-492	737.89	0.000
E32/M59-533	181.70	0.000
E32/M59-564	488.71	0.000
E32/M59-580	86.77	0.000
E32/M59-584	599.60	0.000
E32/M59-635	8.31	0.004
E32/M59-659	599.60	0.000
E32/M59-669	366.03	0.000
E32/M59-811	40.20	0.000
E32/M59-971	0.64	0.424
E32/M59-130	7.59	0.006
E32/M59-134	6.19	0.013
E32/M59-142	16.73	0.000
E32/M59-188	18.98	0.000
E32/M59-216	6.19	0.013
E32/M59-231	9.13	0.003
E32/M59-282	4.22	0.040
E32/M59-305	1.33	0.249
E32/M59-393	198.02	0.000
E32/M59-464	10.81	0.001
E32/M59-521	16.73	0.000
E32/M59-530	18.98	0.000

Table 2 (Continued). Chi-square values for SSR and AFLP® marker loci in OR 91G x PI 255956 BC_2F_4 population.

Marker	χ^2	p value
E32/M59-577	18.98	0.000
E32/M59-596	4.22	0.040
E32/M59-607	18.98	0.000
E32/M59-633	1.33	0.249
E32/M59-637	14.61	0.000
E32/M59-643	18.98	0.000