

# Marker-Assisted Backcrossing QTL for Partial Resistance to Sclerotinia White Mold in Dry Bean

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## ABSTRACT

White mold caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a major disease limiting dry bean (*Phaseolus vulgaris* L.) production. Genetic resistance provides some control but is difficult to breed for because of low heritability. We sought to determine if marker-assisted selection for quantitative trait loci (QTL) conferring partial resistance could facilitate breeding for resistance to white mold in dry bean. The *Phs* marker linked with a QTL derived from landrace G122 on linkage group B7 was backcrossed into 'Winchester' pinto, forming two BC<sub>3</sub>F<sub>4:6</sub> inbred line populations. The AW9.1200 and SS18.1650 markers linked with a QTL from snap bean breeding line NY6020-4 on B8 were backcrossed into 'Maverick' pinto and 'Matterhorn' great northern, forming BC<sub>2</sub>F<sub>4:6</sub> inbred line populations. The B7 QTL in the BC<sub>3</sub>F<sub>4:6</sub> populations on average explained 52% of the phenotypic variation for disease reaction in the greenhouse test and 10% across four field tests. The B8 QTL explained 30% in the greenhouse and 10% in the field. Averaged across tests and populations the B7 and B8 QTL conditioned 15 and 17% reduction in disease severity score, respectively. Linkage drag from selection of the B7 QTL was observed for yield. MAS for the B7 and B8 QTL was an effective breeding tool for introgressing partial resistance to white mold into susceptible pinto and great northern dry bean market classes, but further selection for agronomic performance may be required to obtain lines worthy of commercial production.

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**Abbreviations:** DAP, days after planting; QTL, quantitative trait loci; Pop-I, Population I; Pop-II, Population II; Pop-III, Population III; Pop-IV, Population IV; RAPD, random amplified polymorphic DNA; SCAR, sequence-characterized amplified region.

WHITE MOLD caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a devastating disease of dry bean (*Phaseolus vulgaris* L.) in temperate production regions worldwide. Complete resistance does not exist in dry bean, but lines with partial levels of resistance have been identified (Hunter et al., 1982; Miklas et al., 1998, 1999; Kolkman and Kelly, 2000; Steadman et al., 2001). A few improved lines with partial resistance to white mold have been developed through traditional breeding approaches (Coyne et al., 1976, 1994; Lyons et al., 1987; Miklas et al., 2006a), but breeding for resistance is difficult because partial resistance is quantitatively inherited with low to moderate heritability (Fuller et al., 1984; Miklas and Grafton, 1992; Kolkman and Kelly, 2002; Miklas et al., 2004). Evaluation of resistance in the field is further complicated by expression of both avoidance traits and physiological mechanisms (Schwartz et al., 1987; Miklas et al., 2001; Kolkman and Kelly, 2003;), and epidemics can be sporadic.

The recent identification of more than 10 independent quantitative trait loci (QTL) conditioning resistance (Miklas et al., 2001, 2003, 2007; Park et al., 2001; Kolkman and Kelly, 2003; Ender and Kelly, 2005) affirms the complexity and quantitative nature of white mold resistance in common bean. Those QTL identified with

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stable expression across environments and with relatively major effect (>10% of phenotypic variation explained), provide an opportunity for marker-assisted breeding to expedite development of cultivars with enhanced levels of white mold resistance. Two such QTL, with major effect and expression across multiple environments, were chosen for marker-assisted selection in this study.

A QTL derived from G122 resides on linkage group B7 near the *Phs* (phaseolin) seed protein locus and explained 38% of the phenotypic variation for disease reaction in the straw test and 26% in the field (Miklas et al., 2001). A QTL derived from NY6020-4 resides on linkage group B8 near the *Co-4* locus for resistance to anthracnose caused by *Colletotrichum lindemuthianum* (Miklas et al., 2006b) and explained 35% of the variation in the straw test and 15% in the field (Miklas et al., 2003). The partial resistance conferred by these two QTL is likely conditioned by physiological mechanisms versus disease avoidance traits because they exhibit greater expression in the greenhouse straw test (Petzoldt and Dickson, 1996) than in field trials.

Other QTL derived from G122 on linkage group B1 and from NY6020-4 on B6 had relatively minor effect or no expression in greenhouse tests and were conditioned by disease avoidance traits in the field due to more open and porous canopy, increased plant height, and/or reduced lodging. Upright architecture promotes air and sunlight penetration into the plant canopy, creating a drier microclimate less conducive to white mold epidemics (Schwartz et al., 1987).

Pinto and great northern dry bean market classes grown across the United States lack partial physiological resistance to white mold and are severely affected by the disease. The purpose of this study was to determine whether marker-assisted selection for the QTL on B7 from G122 and QTL on B8 from NY6020-4 could be used effectively to transfer partial resistance to white mold into the susceptible pinto and great northern bean market classes.

## MATERIALS AND METHODS

### Parental Materials and Population Development

Recurrent parents included 'Winchester' pinto, 'Maverick' pinto (Grafton et al., 1997), OT0630-17-5 pinto breeding line (USDA-ARS, Prosser, WA), CO8112034 pinto breeding line (Colorado State University), and 'Matterhorn' great northern (Kelly et al., 1999). Donor parents of QTL conditioning partial resistance to white mold were G122 (PI 163120), a large-seeded landrace from India named 'Jatu Rong', and NY6020-4, a snap bean breeding line from Cornell University.

An  $F_2$  population from a three-way cross CO8112034//G122/Winchester was obtained from Syngenta Seed Company (Nampa, ID). An  $F_{2:4}$  line from this population with high level of resistance to white mold, as detected by the greenhouse straw test (Petzoldt and Dickson, 1996), was obtained. This line possessed the *Phs* SCAR (sequence-characterized amplified region) marker (described below) linked with the B7 QTL for partial

resistance to white mold from G122 (Miklas et al., 2001). The  $F_{2:4}$  line, simulating a  $BC_1F_{2:4}$  line because it contained two doses of pinto bean as recurrent parent, was backcrossed twice more to Winchester pinto bean. A  $BC_2F_1$  with T-allele of the *Phs* marker was used as the parent for the subsequent backcross to Winchester. From the final backcross, two separate  $BC_3F_1$  plants [Winchester\*2/ $F_{2:4}$  line (CO8112034//G122/Winchester)] assayed for presence of the B7 QTL-linked SCAR marker were selfed. Fifty and 38  $BC_3F_2$  plants were advanced by single-seed descent for two generations followed by one generation of bulk seed increase to obtain 50 and 38  $BC_3F_{4:6}$  derived lines representing Population I (Pop-I) and Population II (Pop-II), respectively.

Population III (Pop-III) was derived from the pedigree Maverick/3/OT9630-17-5//NY6020-4/OT9630-17-5. A  $BC_1F_1$  plant with the B8 QTL-linked markers (AW9.1200 RAPD and SS18.1650 SCAR; Miklas et al., 2003) was crossed with Maverick pinto. From the final backcross, a  $BC_2F_1$  plant with the B8 QTL-linked markers was selfed to produce 33  $F_2$  seed that were advanced by single-seed descent to produce 33  $BC_2F_{4:6}$  lines for Pop-III.

Population IV (Pop-IV) was derived from a conventional backcross with NY6020-4 as the donor parent of the B8 QTL, for partial physiological resistance to white mold, and Matterhorn great northern as the recurrent parent (Matterhorn\*3/NY6020-4). A  $BC_1F_1$  plant with B8 QTL-linked markers was backcrossed to the recurrent parent. A  $BC_2F_1$  plant from the final backcross with the B8 QTL-linked markers was selfed to produce 41  $BC_2F_2$ , which were advanced by single-seed descent to produce 41  $BC_2F_{4:6}$  lines for Pop-IV.

### Greenhouse Straw Test

The straw test described by Petzoldt and Dickson (1996) was used to screen the  $BC_2F_{4:6}$  or  $BC_3F_{4:6}$  lines from each population for reaction to white mold. Each population was tested separately. An individual plant of each line represented a replicate, and there were six replicates randomized in complete blocks. The greenhouse environment was maintained at 18°C night to 25°C day with a 14-h daylength provided by sunlight and supplemental lighting. Plants were watered and fertilized for normal growth. The *S. sclerotiorum* culture T001.1, hyphal tip isolated from a sclerotia collected from 'Newport' navy bean in Quincy, WA in 1996, was the source of inoculum. The intact main stem, freshly cut above or below the fifth node, was fitted with a plastic straw containing an agar plug of mycelium approximately 28 d after planting (DAP). Mycelium germinated from a single sclerotium placed on a PDA plate was subcultured to prepare plates of growing mycelium for the inoculations. The entire 15- × 100-mm Petri plate of growing mycelium was used as inoculum once the mycelium reached the periphery of the plate, approximately 3 d after subculturing. Eight to 10 d after inoculation, reaction to white mold was scored from 1 to 9, where 1 = no symptoms, 2 = invasion of the stem past the site of inoculation but not to the first node, 3 = invasion of the stem to the first node, 4 = invasion of the internode slightly past the first node, 5 = invasion to the middle of the internode, 6 = invasion to the second node, 7 = invasion slightly past the second node, 8 = invasion to the middle of the second internode and beyond, and 9 = total plant collapse. Parents and checks utilized in the greenhouse straw tests and field

trials below included Winchester, G122, Maverick, Matterhorn, and 'Montrose' pinto (Brick et al., 2001).

## Field Trials

The BC<sub>3</sub>F<sub>4:6</sub> and BC<sub>2</sub>F<sub>4:6</sub> lines, parents, and checks for Pop-I, Pop-II, and Pop-III were examined for disease reaction to *S. sclerotiorum* in the field in 2004 and 2005. Pop-IV was only tested in 2005. Each population was tested in a separate experiment. The experiments, planted 19 June 2004 and 16 June 2005, were conducted at Paterson, WA. The field plot at the USDA-ARS Cropping Systems Research Farm at Paterson has a history of uniform *S. sclerotiorum* disease in common bean and most recently has been used successfully to differentiate among recombinant-inbred lines for field reaction to white mold for QTL studies (Miklas et al., 2001, 2003). The soil is a Quincy sandy loam (mixed, mesic Typic Torripsamments).

For each field test, a randomized complete-block design with three replications was used, except for Pop-II in 2004, which had only two replications planted due to a seed shortage. A plot consisted of one row 3 m long. Plot rows were spaced 0.56 m apart. Planting density was 234848 seeds ha<sup>-1</sup>. To promote white mold disease, approximately 6.3 mm of water was applied in two applications approximately 12 h apart, by overhead center-pivot irrigation on a daily basis from the onset of flowering to late pod-fill. To maintain vigorous plant growth, nitrogen was foliar applied by chemigation at a rate of 22 kg ha<sup>-1</sup> on a weekly basis for 8 wk from the early seedling growth stage (about 18 DAP) to mid pod-fill (about 74 DAP).

Disease reaction was scored both years from 1 to 9 based on combined incidence and severity of infection at physiological maturity, where 1 = no diseased plants, 2 = 1 to 20% diseased plants and/or 1 to 5% infected tissue, 3 = 20 to 30% diseased plants and/or 5 to 10% infected tissue, 4 = 30 to 40% diseased plants and/or 10 to 20% infected tissue, 5 = 40 to 50% diseased plants and/or 20 to 30% infected tissue, 6 = 50 to 60% diseased plants and/or 30 to 40% infected tissue, 7 = 60 to 70% diseased plants and/or 40 to 50% infected tissue, 8 = 70 to 80% infected plants and/or 50 to 60% infected tissue, and 9 = 80 to 100% diseased plants and/or 60 to 100% infected tissue. The plots were scored for lodging (1 to 9, where 1 = no lodging and 9 = >90% lodged) at physiological maturity in 2005 for Pop-I, Pop-II, and Pop-IV. Maturity was estimated in 2005 as the number of days after planting till harvest maturity (d). The entire plot was harvested to estimate seed yield (kg ha<sup>-1</sup>) in 2005 for Pop-I, Pop-II, and Pop-IV. Estimated seed yield for Pop-III was obtained in 2004 from a nondisease nursery used for seed increase on the WSU Roza experimental farm near Prosser, WA. Pop-I, Pop-II, and Pop-III had seed increased at this site in 2004 in single-row plots (3-m length with 0.56-m row spacing) with three replications. Seed weight (g 100 seeds<sup>-1</sup>) was measured from the nondisease nursery plots to estimate seed size for the lines from the three populations. Seed weight for Pop-IV was obtained from the 2005 disease nursery.

## Marker Assays

Genomic DNA was extracted using the FastDNA Kit (Bio 101, Vista, CA) according to the manufacturer's instructions. The DNA sample, representing a line or parent, was extracted from the emerging trifoliolate leaves collected (approximately 50 mg) from three separate plants macerated together. The procedure



Figure 1. Depiction of the codominant (sequence-characterized amplified region) SCAR marker of the *Phs* (phaseolin seed protein) locus run on a long 2% agarose gel for 12 h at 3V cm<sup>-1</sup>. T-phaseolin (three bands: 249, 264, and 285 bp) allele in coupling phase linkage with the B7 QTL conditioning partial resistance to white mold derived from G122 is in lane 2 = G122 donor parent, and lanes 4, 5, and 7 = partially resistant BC<sub>3</sub>F<sub>4:6</sub> lines from Population I. S-phaseolin (two bands: 249 and 270 bp) allele linked with susceptibility is in lane 3 = Winchester recurrent parent, and lanes 6, 8, and 9 = susceptible BC<sub>3</sub>F<sub>4:6</sub> lines from Population I. Lane 1 = 100-bp ladder.

involved placing the leaf tissue in a microcentrifuge tube with DNA extraction buffer and homogenizing the tissue for two 30-s periods at an intensity setting of 5.0 in the FastPrep FP 120 homogenizer (BIO 101/Savant, Vista, CA). The DNA was bound to a matrix using a binding buffer, washed, and then eluted with 100 µL of sterile distilled water. The purified DNA was adjusted to 10 ng µL<sup>-1</sup> using a fluorometer before all PCR reactions.

The codominant SCAR marker previously developed (Kami et al., 1995) for the phaseolin seed protein locus (*Phs*) was used to assay individual BC<sub>2</sub>F<sub>1</sub> and BC<sub>3</sub>F<sub>1</sub> plants, and the 50 (Pop-I) and 38 (Pop-II) BC<sub>3</sub>F<sub>4:5</sub> lines for presence of the B7 QTL derived from G122 (Miklas et al., 2001). The SCAR marker for the T-phaseolin allele at the *Phs* locus amplifies three homoduplex bands 249, 264, and 285 nucleotides in length, which are in coupling phase linkage with the QTL (Fig. 1). The SCAR is actually a codominant marker as it amplifies two other bands 249 and 270 nucleotides in length, for the susceptible S-phaseolin allele at the *Phs* locus.

The PCR reaction for amplifying the *Phs* SCAR consisted of 25-µL reactions containing 2.5 units of *Taq* polymerase (Promega, Madison, WI), 1X buffer, 3 mM MgCl<sub>2</sub>, 200 µM of each dNTP, 0.5 µM each primer, and 25 ng of template DNA. The forward 5'-AGCATATTCTAGAGGCTCC-3' and reverse 5'-GCTCAGTTCCTCAATCTGTTC-3' primers reported by Kami et al. (1995) were used. The amplification profile consisted of 34 cycles of 94°C for 10s, 50°C for 40s, and 72°C for 2 min, followed by a final cycle extension at 72°C for 5 min.

Two markers linked with the QTL on B8 were assayed (Miklas et al., 2003). The SS18.1650 SCAR and AW9.1200 (5'-ACTGGGTCGG-3') RAPD (random amplified polymorphic DNA) markers, 1.9 cM apart in the original Benton/NY6020-4 RIL mapping population, were assayed across the

**Table 1.** Amount of phenotypic variation explained by markers linked with quantitative trait loci (QTL) conferring partial physiological resistance to white mold on linkage group B7 derived from G122 and on B8 from NY6020-4 that were backcrossed into pinto and great northern bean backgrounds using marker-assisted selection.

| BC <sub>n</sub> F <sub>4:6</sub> lines |     |     |           | Observed marker  | Straw test (1–9) <sup>†</sup> |                | Field (1–9) <sup>†</sup> 2004 |                 | Field (1–9) <sup>†</sup> 2005 |                |
|----------------------------------------|-----|-----|-----------|------------------|-------------------------------|----------------|-------------------------------|-----------------|-------------------------------|----------------|
| Population                             |     | QTL | Marker    |                  | R <sup>2</sup>                | Probability    | R <sup>2</sup>                | Probability     | R <sup>2</sup>                | Probability    |
|                                        | no. |     |           | present+/absent– | %                             | <i>p</i> value | %                             | <i>p</i> value  | %                             | <i>p</i> value |
| I                                      | 50  | B7  | Phs       | 32+/18–          | 42                            | 0.000          |                               | NS <sup>‡</sup> | 17                            | 0.004          |
| II                                     | 38  | B7  | Phs       | 18+/20–          | 62                            | 0.000          | 12                            | 0.030           | 10                            | 0.048          |
| III                                    | 33  | B8  | SS18.1650 | 16+/17–          | 28                            | 0.002          |                               | NS              |                               | NS             |
|                                        |     |     | AW9.1200  | 15+/18–          | 34                            | 0.000          | 13                            | 0.040           |                               | NS             |
| IV                                     | 41  | B8  | SS18.1650 | 15+/26–          | 28                            | 0.000          |                               | NT <sup>‡</sup> | 22                            | 0.002          |
|                                        |     |     | AW9.1200  | 14+/27–          | 25                            | 0.001          |                               | NT              | 28                            | 0.000          |

<sup>†</sup>Disease severity for greenhouse straw test and field scored from 1 to 9, where 1 = most resistant and 9 = completely susceptible (see text).

<sup>‡</sup>NS = not significant; NT = trial not conducted.

BC<sub>1</sub>F<sub>1</sub> and BC<sub>2</sub>F<sub>1</sub> individuals and the 33 and 41 BC<sub>2</sub>F<sub>4:5</sub> lines for Pop-III and Pop-IV, respectively, for detection of the QTL on linkage group B8 derived from NY6020-4.

The PCR protocol for the SS18.1650 SCAR marker was same as for the *Phs* SCAR except that a 63°C annealing temperature was used. The forward 5'-CTGGCGAACTGTAC ATGCAACATAC-3' and reverse 5'-CTGGCGAACTGATTTCATACATTG-3' primers used for the SS18.1650 SCAR were the same as those published by Miklas et al. (2003).

The RAPD protocol for amplifying the AW9.1200 marker consisted of 25-μL reactions containing 2 U Stoffel fragment DNA polymerase (Applied Biosystems, Foster City, CA), 1X Stoffel buffer, 0.2 μM primer, 5 mM MgCl<sub>2</sub>, 200 μM each dNTP, and 25 ng template DNA. Amplifications were performed on a Peltier Thermal Cycler PTC-200 (MJ Research Inc., Waltham, MA) programmed for an initial cycle at 94°C for 2 min; three cycles at 94°C for 1 min, 32°C for 1 min, 72°C for 2 min; followed by 30 cycles of 94°C for 10 s, 37°C for 20 s, and 72°C for 2 min; with a final 5 min extension period at 72°C. Amplified products from the PCR reactions for the AW9.1200 and SS18.1650 markers were separated on 1.4% agarose gels containing ethidium bromide (0.5 μg mL<sup>-1</sup>) for 5 h at 3V cm<sup>-1</sup> constant voltage. Resolution of the *Phs* marker was better in a 2% agarose gel run at 12 h at 3V cm<sup>-1</sup>.

## Statistical Analysis

The experimental data were analyzed by a general linear model using PROC GLM (SAS Institute, 1987). Least-square means were computed for all traits. The effect of the QTL on disease reaction in the four backcross populations was obtained by regression of the marker (presence = 1, absence = 0) and mean disease scores from each line, similar to a one-way ANOVA procedure, using PROC GLM (SAS Institute, 1987). The effect or phenotypic variation for disease score explained by the QTL-linked marker(s) was estimated by R<sup>2</sup> value, and significance of the QTL on disease score was determined by an *F*-test.

## RESULTS AND DISCUSSION

### Marker-Assisted Selection for the B7 QTL

The B7 QTL conferring partial physiological resistance to white mold and derived from G122, as detected by the *Phs* SCAR marker (T-phaseolin allele), had a major effect

on resistance reaction in the pinto bean backcross populations Pop-I and Pop-II, respectively, explaining 42% and 64% of the phenotypic variation for disease reaction in the greenhouse straw test (Table 1). The BC<sub>3</sub>F<sub>4:6</sub> lines with the T-*Phs* marker on average had a 15% (Pop-I) and 19% (Pop-II) reduction in disease severity score in the greenhouse test compared with those lines absent the marker (Table 2). As determined by chi-square tests expected 1:1 segregation ratios for presence and absence of the B7 and B8 QTL-linked markers among the BC<sub>n</sub>F<sub>4:6</sub> lines were observed for all populations (Pop-II, Pop-III, and Pop-IV) except Pop-I, which had more lines with the T-*Phs* marker than expected.

The B7 QTL exhibited less effect in the field, explaining 12% of the phenotypic variation for disease reaction for Pop I in 2004, and 17% and 10% for Pop I and II, respectively, in 2005. The B7 QTL in the original A55/G122 mapping population had less disparity in expression between greenhouse (38%) and field (26%) trials (Miklas et al., 2001). In field trials, disease avoidance confounds expression of physiological resistance to white mold, which likely explains the reduced effect of the QTL in the field. Furthermore, expression of partial physiological resistance conferred by the QTL may be overcome by high disease pressure. Moderate to severe disease pressure was observed in 2004 and 2005 as indicated by the high disease scores obtained for the susceptible check Montrose (Table 2). The amount of reduction in disease severity score among BC<sub>3</sub>F<sub>4:6</sub> lines in the field due to presence of the marker was similar to levels observed in the greenhouse tests, 5% (Pop-I) and 15% (Pop-II) in 2004 and 15% (Pop-I) and 20% (Pop-II) in 2005.

The effect of selection for the B7 QTL on agronomic traits is presented in Table 2. On average, the BC<sub>3</sub>F<sub>4:6</sub> lines with the T-*Phs* marker yielded 4 (Pop-I) and 9% (Pop-II) less than those lines without the marker, indicating a slight yield drag effect from selection for the marker. However, the populations overall had 21 (Pop-I) and 24% (Pop-II) less yield than the recurrent parent suggesting that genomic

**Table 2. Disease reaction and field performance of select pinto and great northern lines with partial white mold resistance obtained by marker-assisted backcrossing quantitative trait loci (QTL) derived from G122 landrace (QTL linked with *Phs* marker T-allele on linkage group B7) and NY6020-4 snap bean breeding line (QTL linked with AW19.1200 RAPD on linkage group B8).**

|                                                      | Straw   | Field 2004 | Field 2005 | Maturity | Lodging | Seed wt                   | Yield               |                                                      | Straw   | Field 2004 | Field 2005 | Maturity | Lodging | Seed wt                   | Yield               |
|------------------------------------------------------|---------|------------|------------|----------|---------|---------------------------|---------------------|------------------------------------------------------|---------|------------|------------|----------|---------|---------------------------|---------------------|
|                                                      | 1 to 9† | 1 to 9†    | 1 to 9†    | d        | 1 to 9† | g 100 seeds <sup>-1</sup> | kg ha <sup>-1</sup> |                                                      | 1 to 9† | 1 to 9†    | 1 to 9†    | d        | 1 to 9† | g 100 seeds <sup>-1</sup> | kg ha <sup>-1</sup> |
| Population I (B7 QTL from G122)                      |         |            |            |          |         |                           |                     | Population III (B8 QTL from NY6020-4)                |         |            |            |          |         |                           |                     |
| 011A-3 select BC <sub>3</sub> F <sub>4,6</sub> line  | 3.9     | 2.8        | 4.0        | 90‡      | 5.5‡    | 44.4‡                     | 2470‡               | 006C-10 select BC <sub>2</sub> F <sub>4,6</sub> line | 4.4     | 5.7        | 5.2        | 114/99   | NM      | 42.5                      | 3150                |
| 011A-29 select BC <sub>3</sub> F <sub>4,6</sub> line | 3.9     | 2.2        | 3.0        | 90       | 6.2     | 42.5                      | 2310                | 006C-15 select BC <sub>2</sub> F <sub>4,6</sub> line | 5.5     | 4.3        | 5.2        | 117/100  | NM      | 42.3                      | 3130                |
| 011A-35 select BC <sub>3</sub> F <sub>4,6</sub> line | 4.2     | 2.3        | 3.5        | 89       | 5.2     | 44.6                      | 2980                | 006C-26 select BC <sub>2</sub> F <sub>4,6</sub> line | 5.4     | 4          | 4          | 109/98   | NM      | 42.0                      | 3570                |
| 011A-39 select BC <sub>3</sub> F <sub>4,6</sub> line | 4.2     | 1.8        | 3.8        | 87       | 4.0     | 40.3                      | 2430                | Mean of lines with AW19.1200 marker                  | 5.3     | 4.4        | 5.7        | 111/99   | NM      | 40.7                      | 2970                |
| Mean of lines with <i>Phs</i> marker                 | 4.5     | 3.3        | 3.9        | 88       | 5.2     | 40.1                      | 2430                | Mean of lines absent marker                          | 6.2     | 5.3        | 5.8        | 112/98   | NM      | 42.8                      | 2940                |
| Mean of lines absent marker                          | 5.3     | 3.5        | 4.8        | 87       | 5.4     | 37.3                      | 2540                | Population mean                                      | 5.8     | 4.9        | 5.7        | 111/98   | NM      | 41.8                      | 2950                |
| Population mean                                      | 4.8     | 3.4        | 4.2        | 88       | 5.3     | 38.9                      | 2470                | Maverick recurrent parent                            | 4.7     | 6.5        | 7.0        | 92/92    | NM      | 43.4                      | 2840                |
| Winchester recurrent parent                          | 4.9     | 4.9        | 5.0        | 87       | 3.5     | 39.7                      | 3150                | NY6020-4 donor parent                                | 3.8     | 3.0        | 4.3        | 118/107  | NM      | 38.6                      | 1900                |
| G122 donor parent                                    | 4.7     | 1.7        | 2.2        | 104      | 4.2     | NT§                       | NM§                 | Montrose-susceptible check                           | NT      | 8.2        | 8.0        | NT/93    | NM      | NT                        | NT                  |
| Montrose susceptible check                           | NT      | 7.9        | 8.5        | 93       | 9.0     | NT                        | 860                 | LSD 0.05 for select lines, check, and parents        | 1.2     | 1.6        | 1.5        | 6.5/4.5  |         | 2.4                       | 1220                |
| LSD 0.05 for select lines, check, and parents        | 0.9     | 1.5        | 1.9        | 3.4      | 1.9     | 2.5                       | 750                 | Population IV (B8 QTL from NY6020-4)                 |         |            |            |          |         |                           |                     |
| Population II (B7 QTL from G122)                     |         |            |            |          |         |                           |                     | 029C-1 select BC <sub>2</sub> F <sub>4,6</sub> line  | 4.2     | NT         | 3.7        | 93       | 6.3     | 36.7                      | 1910                |
| 011D-3 select BC <sub>3</sub> F <sub>4,6</sub> line  | 4.5     | 4.8        | 5.3        | 91       | 6.8     | 45.6                      | 2640                | 029C-6 select BC <sub>2</sub> F <sub>4,6</sub> line  | 3.2     | NT         | 4.5        | 95       | 5.7     | 38.7                      | 2080                |
| 011D-17 select BC <sub>3</sub> F <sub>4,6</sub> line | 5.1     | 4.8        | 3.5        | 91       | 5.3     | 40.2                      | 2130                | 029C-20 select BC <sub>2</sub> F <sub>4,6</sub> line | 3.5     | NT         | 4.5        | 89       | 4.7     | 32.4                      | 2290                |
| 011D-21 select BC <sub>3</sub> F <sub>4,6</sub> line | 4.9     | 5.5        | 3.0        | 93       | 5.0     | 40.1                      | 2060                | 029C-26 select BC <sub>2</sub> F <sub>4,6</sub> line | 3.1     | NT         | 3.7        | 93       | 6.0     | 36.2                      | 1830                |
| 011D-25 select BC <sub>3</sub> F <sub>4,6</sub> line | 4.9     | 3.0        | 3.7        | 88       | 5.0     | 43.0                      | 2300                | 029C-40 select BC <sub>2</sub> F <sub>4,6</sub> line | 3.9     | NT         | 2.7        | 89       | 4.0     | 35.2                      | 1980                |
| 011D-27 select BC <sub>3</sub> F <sub>4,6</sub> line | 4.7     | 3.2        | 4.3        | 91       | 5.3     | 43.0                      | 1940                | Mean of lines with AW9.1200 marker                   | 4.4     | NT         | 5          | 91       | 5.9     | 33.1                      | 1971                |
| 011D-35 select BC <sub>3</sub> F <sub>4,6</sub> line | 5.0     | 4.8        | 3.2        | 91       | 7.0     | 41.0                      | 1730                | Mean of lines absent marker                          | 5.4     | NT         | 6.2        | 89       | 6.0     | 31.8                      | 1810                |
| Mean of lines with <i>Phs</i> marker                 | 5.3     | 4.9        | 4.6        | 90       | 6.0     | 41.7                      | 1940                | Population mean                                      | 5.1     | NT         | 5.6        | 89       | 5.9     | 32.4                      | 1880                |
| Mean of lines absent marker                          | 6.6     | 5.8        | 5.8        | 87       | 5.5     | 39.3                      | 2140                | Matterhorn recurrent parent                          | 7.3     | NT         | 5.7        | 93       | 4.7     | 39.2                      | 2220                |
| Population mean                                      | 6.0     | 5.3        | 5.0        | 88       | 5.7     | 40.4                      | 2050                | NY6020-4 donor parent                                | 3.4     | NT         | 3.7        | 104      | 6.7     | NM                        | NM                  |
| Winchester recurrent parent                          | 6.7     | 6.0        | 5.8        | 88       | 4.7     | 40.4                      | 2700                | Montrose susceptible check                           | 6.5     | NT         | 8.3        | 93       | 8.7     | 36.0                      | 1200                |
| G122 donor parent                                    | 4.5     | 2.0        | 1.8        | 99       | 3.2     | NM                        | NM                  | LSD 0.05 for select lines, check, and parents        | 1.2     |            | 1.7        | 2.8      | 0.7     | 2.9                       | 810                 |
| Montrose susceptible check                           | NT      | 8.0        | 8.0        | 93       | 8.5     | 43.2                      | 1770                |                                                      |         |            |            |          |         |                           |                     |
| LSD 0.05 for select lines, check, and parents        | 0.9     | 1.7        | 2.1        | 3        | 1.8     | 0.3                       | 750                 |                                                      |         |            |            |          |         |                           |                     |

†Disease severity for greenhouse straw test and field scored from 1 to 9, where 1 = most resistant and 9 = completely susceptible (see text). Lodging was scored from 1 to 9, where 1 = no lodging and 9 = completely lodged.

‡Means for maturity, lodging, seed weight, and seed yield were measured in the 2005 disease nursery trial with the following exceptions: mean maturity for Population III was measured in 2004 seed increase plots and 2005 disease nursery; mean seed weight for Population I and Population III was measured in 2004 seed increase plots and for Population II from 2004 disease nursery; seed yield for Population III was measured in 2004 seed increase plots.

§NT, line, parent, or check not tested in trial; NM, trait not measured.

regions independent of the target *T-Phs* locus had not been fully recovered from Winchester pinto with only three backcrosses. It is imperative that residual donor genome be reduced as much as possible because few if any lines derived from intergene pool (Andean/Middle American) hybridizations attain the yield potential or commercial phenotype of the Middle American parent (Kornegay et al., 1992; Singh, 1995; Welsh et al., 1995). G122 is of Andean origin and Winchester is of Middle American origin.

The wide intergene pool cross and too few backcrosses that contributed to reduced yield also likely contributed to an increase in lodging, 32% for Pop-I overall and 17% for Pop-II, in comparison to the recurrent parent. Lodging is an undesirable trait in dry bean that promotes white mold disease. The  $BC_3F_{4,6}$  lines with *T-Phs* marker, across Pop-I and Pop-II, respectively, had harvest maturity delayed by 1 and 3 d, and seed weight increased by 7 to 6%, compared to lines absent the marker, which suggests that selection for the donor *T-Phs* allele had some linkage drag effects on these traits also. The *Phs* locus was reported by Johnson et al. (1996) to affect from 18 to 33% of total seed weight depending on environment and population. Thus, when using *Phs* locus for MAS of the B7 QTL for partial physiological resistance to white mold derived from G122, it advisable to periodically monitor selected lines for commercial seed size.

The  $BC_3F_{4,6}$  lines with *T-Phs* marker listed in Table 2 were selected for consistently low disease scores across tests and favorable agronomic characteristics such as higher yield, adequate seed size, reasonable maturity, and moderate lodging. These select lines will be further evaluated for disease reaction and agronomic performance to identify candidate pinto bean lines with partial resistance to white mold for official germplasm release and registration. The partially resistant lines 011A-35 from Pop-I and 011D-3 and 011D-25 from Pop-II are noteworthy for their high yield potential under moderate white mold disease pressure. The low yields for Montrose pinto, an otherwise high-yielding pinto in the absence of white mold disease (Brick et al., 2001), emphasizes the devastating effect white mold can have on pinto bean yield. Winchester, the recurrent parent, maintains yield performance under white mold pressure because of avoidance traits (upright architecture, minimal lodging) and perhaps other factors like the stay green trait, which contributes to physiological resistance (Miklas et al., 2007). Plants with branches and stems that remain green as the pods reach harvest maturity are still likely to be physiologically active and engaged in plant defense response (Miklas et al., 2004). Data on stay green trait was not collected in this study.

### Marker-Assisted Selection for the B8 QTL

The B8 QTL conditioning partial physiological resistance to white mold and derived from the snap bean breeding line NY6020-4, as detected by the AW19.1200 RAPD marker, had major expression in the pinto Pop-III and great north-

ern Pop-IV backcross populations, explaining 37 and 25%, respectively, of the phenotypic variation for disease reaction in the greenhouse straw test (Table 1). Results for the B8 QTL are based on selection for the AW19.1200 RAPD marker because it is closer to the QTL than the SS18.1650 SCAR (Table 1), as also observed by Miklas et al. (2003) in the original Benton/NY6020-4 mapping population. The markers do not flank the QTL (Miklas et al., 2003), which is another reason for only including results for the AW19.1200 marker from here on.

The B8 QTL had less effect in field trials for Pop-III, with only 13% of the variation in disease severity score explained in 2004 and none in 2005. A similar disparity of expression of B8 QTL between environments, 35% greenhouse and 15% field, was observed in the original Benton/NY6020-4 mapping population (Miklas et al., 2003). The effect of a QTL conferring partial physiological resistance in the field may be influenced by many factors, as stated above. The higher field expression for the B8 QTL in Pop-IV (28%) in comparison with the greenhouse straw test (25%) may be due to an unknown genetic background effect contributed by the recurrent parent (Matterhorn great northern).

Disease severity for  $BC_2F_{4,6}$  lines with the B8 QTL, as implied by presence of the AW9.1200 marker, was reduced by 14 (Pop-III, 2004) and 18% (Pop-IV) in the greenhouse tests compared to those lines absent the QTL-linked marker, and was reduced by 17 (Pop-III, 2004) and 19% (Pop-IV) in the field. Marker-assisted selection for the B8 QTL had no adverse affect on yield, seed weight, maturity, or lodging. In fact the  $BC_2F_{4,6}$  lines in Pop-IV with the B8 QTL-linked marker yielded 8% more than those lines without the marker and had 4% larger seed size.

Maturity for Pop-III overall in comparison to the recurrent parent Maverick was delayed by 19 d in a seed increase plot in 2004 and by 6 d in the 2005 disease nursery (Table 2). Conversely, for Pop-IV, seed weight and yield were less, and lodging increased compared to the recurrent parent. These comparisons suggest that the wide cross and residual donor genome of the snap bean parent NY6020-4, apart from the genomic region flanking the B8 QTL, are inhibiting recovery of agronomic performance at the same level of the recurrent parent for both market classes. A few  $BC_2F_{4,6}$  lines with better agronomic performance and higher levels of partial resistance across tests were selected for further evaluation. Line 006C-26 (Pop-III), with high yield potential, and line 029C-40 (Pop-IV), with early maturity and reduced lodging, represent good candidate lines for use as donor parents in subsequent crosses with elite pinto and great northern bean.

### CONCLUSIONS

Results obtained herein verify the existence of QTL for partial resistance to white mold on linkage group B7 near the *Phs* locus and on linkage group B8 near AW9.1200

RAPD marker. The small population sizes used in this study likely caused an overestimation of QTL effects. Nonetheless, the QTL were stably expressed across environments and in different genetic backgrounds and are amenable to marker-assisted selection.

When the B7 and B8 QTL were introgressed separately into pinto and great northern backcross populations by marker-assisted selection, they explained a major portion of the phenotypic variation for disease reaction in the greenhouse test but explained less in the field. Field expression of the partial physiological resistance conferred by the QTL is confounded by disease avoidance traits and may be overcome by too severe disease pressure. The level of resistance conferred by the QTL was fairly consistent across the four backcross populations resulting in a ~15 to 20% reduction in disease severity or by a score of ~1 on the 9-point scale (reduced from 6 to 5, 5 to 4, etc.) in the greenhouse and field tests alike.

Less negative linkage drag effects were observed for agronomic traits, such as harvest maturity, seed weight, and yield, with the B8 QTL. Besides linkage drag from selection of the QTL, other remnant donor genome from the exotic parental sources likely contributed to poor agronomic performance. Thus, further breeding and germplasm development will be needed to recover pinto bean and great northern beans possessing both B7 and B8 QTL for partial resistance to white mold with acceptable maturity, seed quality, and yield. Initially marker-assisted selection among segregating progeny from crosses between select lines from Pop-I and Pop-II with those from Pop-III and Pop-IV would facilitate combining two unique and independent sources of partial resistance to white mold in a pinto bean or great northern bean market type. Eventually marker-assisted selection will be useful for combining the B7 QTL from G122 and B8 QTL from NY6020-4 with other sources of resistance and disease avoidance traits to attain even higher levels of resistance to white mold in dry bean.

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