GENETICS AND BREEDING FOR RESISTANCE TO COMMON BACTERIAL BLIGHT AND WHITE MOLD IN COMMON BEAN, *Phaseolus vulgaris* L.

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Authorization to Submit Dissertation

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Abstract

Common bacterial blight (CBB) and white mold (WM) are important diseases in common bean. Identification of resistant genotypes and new genes/QTL are crucial for their control. The overall goals were to identify resistant genotypes for each disease, determine the genetics of WM resistance in A 195, identify new CBB resistance gene(s)/QTL in VAX 1, and determine the presence or absence of resistance QTL linked sequence characterized amplified region markers for both diseases. The specific objectives are given in each of the four chapters comprising this Doctoral Dissertation. Twenty three genotypes, 61 recombinant inbred lines from 'Othello'/VAX 1 and 100 from Othello/VAX 3 were evaluated against two *Xanthomonas campestris* pv. *phaseoli* (the cause of CBB) strains in the greenhouse at University of Idaho, Kimberly in 2011 and 2012. Similarly, 31 genotypes were inoculated with ARS12D and ND710 *Sclerotinia sclerotiorum* (the cause of WM) isolates in the greenhouse in Idaho in 2012, and CO460 and NY133 isolates at Colorado State University, Fort Collins in 2013. Also, the parents and F_1, F_2, and F_3 from Othello/A 195 and A 195/G 122 crosses were evaluated against ARS12D and ND710 isolates in the greenhouse in Idaho in 2011 and 2012. For CBB, the mean trifoliolate leaf score (4.8) was higher than the primary leaf score (2.5). The strain Xcp25 (5.2 trifoliolate) was more aggressive than ARX8AC (4.2 trifoliolate). RCS52-2, RCS53-3, and RCS63-5B with BC420 and SU91QTL, and 08SH840 with SAP6 and SU91 QTL were intermediate (3.5 to 6.2) to both bacterial strains. A novel QTL at Pv11.4 linkage group explained 13 to 23% phenotypic variance for resistance to ARX8AC and 26 to 51% to Xcp25 in leaves. Evaluations of WM at 28 days (Idaho) and 21 days (Colorado) post inoculation were optimum. Breeding line SE152-6 with pyramided resistance and WM2.2, WM7.1, and WM8.3 QTL was resistant (≤4) to all *S. sclerotiorum*

isolates after three inoculations/plant. Two independent complementary dominant genes controlled resistance of WM in Othello/A 195 to each isolate; and a single dominant gene different from the WM2.2, WM7.1, and WM8.3 QTL controlled the resistance in A 195/ G122 to ND710 isolate.

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Dedication

To my parents and nanny: Estela Dillon, Eduardo Viteri **(+)** and Teresa Dávila for being my support and motivation.

To my brother, Pablo Viteri, and sister Gabriela Viteri for their encouragement to continue my Graduate studies.

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Chapter 1

Response of 23 Common Beans to Two Strains of the Common Bacterial Blight Pathogen, *Xanthomonas campestris* pv. *phaseoli*

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Abstract

Common bacterial blight (CBB) is a severe disease of common bean and the use of resistant cultivars is the most effective control. The objectives of this study were to determine (i) the most appropriate leaf type for CBB evaluation, (ii) the aggressiveness of two bacterial strains, (iii) the presence or absence of SAP6, BC420, and SU91 resistance QTL linked markers, and (iv) the most resistant genotypes. The CBB response in the primary and trifoliolate leaves of 21 genotypes and two checks was evaluated in two greenhouses. Mean trifoliolate leaf score (4.8) was higher than the primary leaf (2.4). The strain Xcp25 (3.2 primary, 5.4 trifoliolate) was more aggressive than ARX8AC (1.6 primary, 4.2 trifoliolate). Andean 'Montcalm' with SAP6 marker was intermediate (6.0) to ARX8AC and susceptible (8.3) to Xcp25 in the trifoliolate leaf. New Andean breeding lines RCS52-2, RCS53-3, and RCS63-5B with BC420 and SU91, and 08SH840 with SAP6 and SU91 were intermediate (3.5 to 6.2) to both strains. But, Middle American VAX 3, VAX 4, and VAX 6 with SAP6 and SU91 were resistant (2.3 to 2.5) to ARX8AC and intermediate to Xcp25 (3.4 to 6.5) in the trifoliolate leaf. Further efforts are required to pyramid higher levels of resistance from across *Phaseolus* species and introgressed in Andean common bean.

Introduction

Common bacterial blight (CBB), caused by *Xanthomonas campestris* pv. *phaseoli* Smith (Dye) [*Xcp*, synonym: *X. axonopodis* pv. *phaseoli* (Smith) Vauterin et al.] and *X. fuscans* sbsp. *fuscans* sp. nov. (*Xff*) is an important disease of common bean (*Phaseolus vulgaris* L.)

in tropical and subtropical production regions worldwide (12, 43). The Gram-negative, aerobic, and motile bacteria infect leaves, pods, and seeds. Disease symptoms on leaves include water soaked spots that enlarge and coalesce causing necrotic lesions surrounded by a distinct yellow margin (36). In susceptible cultivars, the pathogen invades the entire leaf surface showing a burned appearance of the foliage. Early pod infection causes small, shriveled, and discolored seed adversely affecting their commercial quality and value, emergence, and seedling vigor (14, 36, 38). The disease is favored by temperatures between 28 to 30ºC with high humidity and frequent winds, and yield losses up to 50% have been reported in favorable environments (41, 43, 52, 54).

The virulence can vary among bacterial species and strains, but the *Xcp* strains are more variable compared with *Xff* (7, 20, 32). Duncan et al. (7), for example, reported differences in aggressiveness among eight bacterial strains from the Americas and East Africa. They concluded that some strains of *Xcp* (e.g., Xcp25) were more aggressive than *Xff* strains, and the small-seeded (<25 g 100 seed) Middle American common bean genotypes with tepary bean (*P. acutifolius* A. Gray) derived and pyramided or combined resistance such as VAX 3 to VAX 6 and XAN 309 were most resistant to both species.

Bacteria are seed-transmitted and their epiphytic populations may survive on non-host species and weeds (10, 13, 15). An integrated CBB management involves the use of resistant cultivars, pathogen-free seed, bactericides, deep plowing of crop residues, and crop rotation with non-host species (10, 13, 36). Of these, the use of resistant cultivars has been the most effective, economical, and environment friendly (41). Sources of CBB resistance have been identified in the common bean primary, secondary, and tertiary gene pools (31, 39, 41, 48, 56). Low levels of resistance occur in some small-seeded such as Colima 9, PI 207262

(synonymous with G1320), and Tamaulipas 9 (7, 41) and medium-seeded (25 to 40 g 100 seed) such as great northern Montana No. 5 (22, 41) common bean landraces of Middle American origin. Intermediate levels of resistance occur in scarlet runner bean (*P. coccineus* L.) (9, 31, 34, 41, 53). But, the highest levels of resistance are found in the tepary bean such as G 40001 and G 40020 (21, 39, 41, 48, 56).

The CBB resistance is inherited quantitatively and controlled by more than 20 QTL (quantitative trait loci) (3, 11, 23, 28, 46). Readers interested in more information on the major and minor effects CBB resistance QTL should also refer to reviews by Kelly et al. (17), Miklas and Singh (25), and Miklas et al. (24). The three major effects sequence characterized amplified region (SCAR) markers, namely SAP6 on Pv10 (23), SU91 on Pv08 (35), and BC420 on Pv06 (46, 55) linkage groups are associated with resistance QTL. In addition, more recently Viteri et al. (51) identified a new CBB resistance QTL on Pv11.4 linkage group in VAX 1 interspecific breeding line derived from tepary bean G 40001. The SAP6 is linked with the QTL from great northern Montana No. 5 and present in great northern Nebraska No. 1 Sel. 27 (22), and Colima 9 and Tamaulipas 9-B (7). SU91 (35) and BC420 (46, 55) are associated with resistance QTL derived from tepary bean PI 319443 (synonymous to G 40020). The SAP6, SU91, and/or BC420 markers have been used for marker-assisted selection (26, 27, 33, 55) and genetics studies (49, 50).

Although efforts for improving CBB resistance in large-seeded (>40 g 100 seed) Andean genotypes have been carried out since the 1960s, in general, these possess lower levels of resistance compared with some small-seeded Middle American counterparts (7, 18, 41). Thus, the development of large-seeded breeding lines and cultivars with higher levels of CBB resistance is extremely important for production areas with severe CBB problems such

as the Midwestern United States, Argentina, Spain, and Africa. Selection for higher CBB resistance may depend on aggressiveness of *Xcp* and *Xff* isolates, bacterial density used, plant parts inoculated, the post-inoculation time for evaluation, and the environment (7, 18). Bacterial densities ranging from 10^5 to 10^8 CFU/ml have been used for successful infection and disease development in genetic diversity (7, 19, 30), genetics (48, 49, 50), breeding (27, 33, 55), and pathology (7, 19, 30, 32) studies in common bean and other *Phaseolus* species. Differences in CBB response in leaves, pods, and seeds controlled by different genes/QTL with varying heritability were reported in the common and tepary beans (1, 5). Furthermore, CBB resistance QTL explained different percentages of the phenotypic variance for resistance depending upon the genotype, bacterial strain, plant parts inoculated, number of days post inoculation for evaluation, and environment (11, 28). However, often the breeding and pathology studies are based on the disease response in the trifoliolate leaves, evaluated from seven to 21 days post inoculation (7, 49, 50, 56). Researchers seldom used the disease response in the primary leaves (18). The objectives of this research were to determine (i) the most appropriate leaf type for CBB evaluation, (ii) the aggressiveness of two bacterial strains, (iii) the presence or absence of SAP6, BC420, and SU91 resistance QTL linked markers, and (iv) the most resistant genotypes.

Materials and Methods

Common Bean Genotypes. Twenty-one common bean genotypes of different origins with varying levels of CBB response and Andean ‘Montcalm’ and Middle American pinto ‘Othello’ as checks were evaluated. Montcalm, released by the Michigan Agricultural

Experiment Station in 1974 (Dr. James Kelly, personal communication, 2013), has large (>40 g 100 seeds) dark red kidney seed and determinate growth habit Type I (40). Othello has medium seed size and growth habit Type III (4). Interspecific breeding line XAN 159 derived from tepary bean PI 319443 has gray speckled seed, growth habit Type I (41), and other characteristics of Andean beans. The other 15 Andean breeding lines with tepary bean derived and pyramided resistance included 08SH840, CXR 1, GNX 6, RCS52-1, RCS52-2, RCS53-2, RCS53-3, RCS53-5, RCS63-3, RCS63-4, RCS63-5A, RCS63-5B, USDK-CBB-15, USWK-CBB-17, and Wilkinson 2. Of small-seeded Middle American genotypes the interspecific breeding line VAX 1 with cream striped seed and growth habit Type III was derived from tepary bean accession G 40001, and VAX 3, VAX 4, VAX 5, and VAX 6 have tepary bean derived (via VAX 1, which is synonymous with PVPA9576-1) and pyramided (via XAN 263 or XAN 309 that possess tepary PI 319443 and common great northern bean Montana No. 5 bean resistance) resistance (41, 42).

Greenhouse Evaluation. Split-plots in a randomized complete block design with three replications, and two simultaneous plantings in each of two different greenhouses were used. The main plots were bacterial strains ARX8AC and Xcp25, sub-plots were bacterial densities of 1.7 and 3.2 x 10^8 CFU/ml, and 23 common beans were randomized within the sub-plots. For each replicate and planting, a separate randomization was used. Three plants were sown in a 16.5 x 20.3 cm plastic pot for each genotype, bacterial strain, and bacterial density. Each plant was scored individually. Five disks punched out from the primary leaf from each plant (approximately 30 mg) were collected before inoculation with bacterial strains. Two strains of *Xcp*, namely ARX8AC and Xcp25, were used for CBB pathogen inoculation and disease evaluation. Bacterial cultures kept at -80ºC in XYT liquid media were activated on a Petri

plate containing the 523 media (16) and allowed to grow for 48 h at 30ºC. Subsequently, bacterial cultures were multiplied as needed, and cultures were suspended in distilled water and adjusted to densities of 1.7 and 3.2 x 10^8 CFU/ml for inoculation. The primary leaf was inoculated 10 days after sowing and the trifoliolate leaf at 23 days. A sterilized florist frog (i.e., multiple needles) was used for inoculation by pressing the leaf on top of a sponge submerged in a bacterial suspension in a Petri plate. Inoculated plants were kept in 70% humidity in the first and 85% in the second greenhouse. Humidifiers situated under the greenhouse benches and wetting of greenhouse floors after each inoculation were used to achieve high humidity. Plants were grown at a mean day temperature of 24ºC and mean night temperature of 16ºC in the first greenhouse. In the second greenhouse, mean day temperature was 26ºC and mean night temperature was 18ºC with 12 h of light in both greenhouses.

Disease severity in the primary leaf was evaluated 14 days post inoculation, whereas the trifoliolate leaf was evaluated at 21 days. A 1 to 9 scale, where 1= no visible symptoms; 3= necrotic lesions around the inoculated points and no more than two coalesced lesions together, 5= necrotic lesions coalesced together in the one-third of inoculated area, 7= necrotic lesions coalesced together in the entire inoculated area and lesions extended beyond the inoculated area, and 9= necrotic lesions extended to the leaf edge causing premature senescence and leaf drop (18). Genotypes with CBB scores of 1 to 3 were considered resistant, 4 to 6 intermediate, and 7 to 9 susceptible.

Molecular Marker Assays. The DNA extraction was carried out using the Dellaporta protocol (6). The DNA concentration was adjusted to 10 ug/ml, and 2.5 ul of this DNA was mixed with 12.5 ul of multiplex mastermix (Quiagen, Valencia, CA), 2.5 ul of primer mix, and 8 ul of purified sterile water. Primers were used in 10 uM concentrations with

oligonucleotide sequences (5'-GCAGGGTTCGAAGACACACTGG-3')/(5'-GCAGGGTTCGCCCAATAACG-3') for BC420 (55) and (5'-CCACATCGGTTAACATGAGT-3')/(5'- CCACATCGGTGTCAACGTGA-3') for the SU91 marker (26, 35). The amplification conditions involved a denaturation at 95ºC for 15 min, followed by 35 cycles at 94ºC for 30 s, 62ºC for 1.5 min (annealing); 72ºC for 1.5 min (extension) and a final extension of 72ºC for 10 min. The SAP6 marker assay was carried out by mixing the 2 ul of genomic DNA (adjusted to10 ug/ml) with 4 ul of 5x buffer, 2.4 ul of 25 mM $MgCl_2$, 0.8 ul of 5 mM dNTP, 2 ul of oligonucleotide primers (5'-GTCACGTCTCCTTAATAGTA-3')/(5'-GTCACGTCTCAATAGGCAAA-3') (23), 8.6 ul of purified sterilized water and 0.2 Go tag (DNA polymerase). The amplification conditions included 34 cycles at 94ºC for 10 min (denaturation), followed by 55ºC for 30 s (annealing), and 72ºC for 2 min (extension). The last step included a final extension of 72ºC for 5 min. All PCR reactions were performed in a PTC-100 thermocycler (MJ Research Inc., Walthman, MA) and PCR products were run in 1.4 % agarose gel stained with 2% of ethidium bromide. Due to the dominant characteristics of the markers, the presence or absence of different-sized fragments was recorded visually.

Statistical Analysis. Analysis of variance, mean disease score, and Fisher's least significant difference at $P \leq 0.05$ were calculated for each data set. Data was analyzed using the SAS 9.3 PROC GLM (37). Bartlett's test of the data from the two greenhouses displayed homogeneity of variances, therefore, a combined analysis of both greenhouses also was carried out (44).

Results

Mean squares due to greenhouse, leaf type, bacterial strain, bacterial density, genotype, and their interactions were significant ($P \leq 0.05$) (Table 1.1). Trifoliolate leaf had significantly higher CBB scores (4.8) compared to primary leaf (2.4). Similarly, bacterial strain Xcp25 (mean scores 3.2 in primary and 5.4 in trifoliolate) was significantly more aggressive than ARX8AC (mean scores 1.6 in primary and 4.2 in trifoliolate) in both leaves. Significant differences were noted only at higher bacterial density of Xcp25 in the primary leaf (Table 1.2). The CBB scores, in general, were higher in the second greenhouse (data not shown) and therefore, only data from this greenhouse were used for reporting the individual response of 23 common bean genotypes to both *Xcp* strains in this manuscript.

Othello was susceptible in primary and trifoliolate leaves to both *Xcp* strains (Table 1.3). Montcalm had intermediate or resistant score in response to ARX8AC, but was susceptible to Xcp25 in both leaves regardless of the bacterial densities. All genotypes, with the exception of Othello and Montcalm, had resistant (or near resistant) CBB scores in the primary leaf in response to ARX8AC at both bacterial densities. In contrast, at the high density of Xcp25, RCS52-1, RCS52-2, RCS53-2, RCS53-3, RCS63-5B, VAX 3, and VAX 5 had intermediate, and all other genotypes had susceptible (or near susceptible) scores in trifoliolate leaf (Table 1.3). Among the interspecific breeding lines derived from tepary bean, VAX 1 had significantly lower scores (2.2 to 6.5) compared to XAN 159 (3.0 to 7.2) in both leaves to both strains. Among the Andean breeding lines with tepary bean derived and pyramided resistance, USWK-CBB-17 and GNX 6 were intermediate to ARX8AC and susceptible to Xcp25 at both bacterial densities in the trifoliolate leaf. Thus, of 17 Andean

common beans tested 08SH840, RCS53-3, and RCS63-5B followed by RCS52-2 with either a resistant or intermediate CBB score were most resistant to both bacterial strains at both densities. But, Middle American VAX 3 had even higher levels of resistance than these recently developed Andean breeding lines (Table 1.3).

As could be expected, the susceptible check pinto Othello lacked the three CBB resistance QTL, while Montcalm and VAX 1 only possessed the SAP6 QTL (Table 1.3). Four each of Andean (USDK-CBB-15, USWK-CBB-17, 08SH840, CXR 1) and Middle American (VAX 3, VAX 4, VAX 5, VAX 6) genotypes had SAP6 and SU91 QTL. In contrast, all 12 genotypes with BC420 and SU91 were Andean common bean (Table 1.3).

Discussion

Differences observed in CBB severity between the two greenhouses could be due to variation in temperature, humidity, and light availability. Although the conditions for plant growth and disease development in both greenhouses were adequate, higher temperatures and higher moisture conditions (85%) due to humid dirt floor could have increased disease severity in the second greenhouse. Duncan et al. (7) also observed differences in CBB severity between the greenhouse plantings. Furthermore, CBB was reported to be highly destructive in warmer temperatures (28 to 30ºC), extended period of moisture, and prevalence of winds under field conditions (12, 36).

In common bean, most CBB evaluations are carried out in trifoliolate leaves in the greenhouse and/or field (7, 41). In this study, the trifoliolate leaf had significantly higher mean CBB scores, which facilitated separation among resistant, intermediate, and susceptible

genotypes. Lema et al. (18) also reported higher CBB scores in the trifoliolate leaf compared to the primary leaf. Trifoliolate leaves were more susceptible than primary leaves probably due to increasing plant age (5). However, inoculations in the primary leaf in common bean also are used for other bacteria (e.g., *Pseudomonas syringae* pv. *phaseolicola* Van Hall), fungi [e.g., *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib., *Uromyces appendiculatus* Pers.: Pers.) Unger], and viruses (e.g., *Bean common mosaic virus*, an aphid-vectored potyvirus) that readily reproduce in younger tissues resulting in a successful infection and disease development (29, 45, 47). The importance of evaluating CBB response in the primary leaf lies in the identification of susceptible genotypes such as Othello that could be eliminated in the earlier stages. Thus, only resistant genotypes would then be inoculated and evaluated in the trifoliolate leaf with the same and/or different pathogen isolates, reducing expense and work load especially when dealing with high plant populations. Similarly, genotypes such as Montcalm could be eliminated due to CBB response in the primary leaf to aggressive strains such as Xcp25. Thus, genotypes exhibiting lower CBB scores in both leaves to bacterial strains of different aggressiveness would be selected. They may have better and/or more genes/QTL conferring resistance in different plant parts for a prolonged growth period.

Bacterial strain Xcp25 was significantly more aggressive than ARX8AC in both leaves. Duncan et al. (7) and Lema et al. (18) also found Xcp25 to be most aggressive. Bacterial density was significant only for Xcp25 in the primary leaf in the second greenhouse where higher disease severity was noted. However, some genotypes (e.g., CXR 1, VAX 1, and VAX 3) were resistant at low density and intermediate at high density of ARX8AC in the trifoliolate leaf. Thus, highly disease-conducive environments may be required for

determining appropriate bacterial density for germplasm screening, breeding, and genetics studies. But, Lema et al. (18) reported that the use of higher densities of aggressive bacterial strains such as Xcp25 could eliminate valuable genotypes (e.g., GNX 6) with resistance to less aggressive strains such as ARX8AC. Thus, for separation of resistance, intermediate, and susceptible responses, appropriate bacterial density should be determined for the plant part to be inoculated.

The intermediate or resistant response of Montcalm and GNX 6 to ARX8AC in contrast to their susceptible response to Xcp25 in the trifoliolate leaf should be of interest to breeders and geneticists. But, there were no crossover interactions between the *Xcp* strains and the common bean genotypes. For example, all genotypes with a resistant or intermediate response to Xcp25 also had a similar or better response to ARX8AC. In general, only higher CBB scores were observed for Xcp25 compared to ARX8AC. Nonetheless, it is important to isolate and characterize bacterial strains from production regions of interest and use appropriate representative strains for germplasm screening (7, 19, 30). For example, use of less aggressive strains for regions with aggressive strains may lead to erroneous results. Therefore, use of at least one each of less aggressive and aggressive representative strains inoculated in different plant parts on the same plant should help select strain-specific as well as genotypes with broad-spectrum resistance.

Montcalm and VAX 1 only possessed the SAP6 QTL (7; this study). But, VAX 1 had higher levels of resistance than Montcalm. Thus, indicating that VAX 1 had additional CBB resistance genes/QTL, which may be worth identifying and tagging. In fact, as noted earlier VAX 1 possesses a new CBB resistance QTL located at Pv11.4 linkage group and identified by the closest marker SNP47467 that is derived from tepary bean accession G 40001 (51).

Furthermore, not all genotypes with the same set of SCAR markers (e.g., USDK-CBB-15, USWK-CBB-17, 08SH840, CXR 1, and VAX 3 to VAX 6 with SAP6 and SU91) had similar CBB response, especially to Xcp25. Therefore, it would be prudent to combine marker-assisted selection (33, 55) with direct CBB screening (2) to develop genotypes with the highest levels of resistance (8).

In summary, the use of contrasting bacterial strains of different aggressiveness, different bacterial densities, and inoculations in different leaves of the same plant facilitated separation of genotypes with different levels of CBB responses. Thus, the newly developed Andean genotypes (e.g., 08SH840, RCS52-2, RCS53-3, and RCS63-5B) were not susceptible to any bacterial strains in the primary and trifoliolate leaves, and had significantly higher levels of CBB resistance than Montcalm, XAN 159, Wilkinson 2, USDK-CBB-15, and USWK-CBB-17 developed earlier (27, 41). However, Middle American VAX 3, VAX 4, and VAX 6 with SAP6 and SU91 were resistant (2.3 to 2.5) to ARX8AC and intermediate to Xcp25 (3.4 to 6.5) in the trifoliolate leaf. Further efforts are therefore required to pyramid higher levels of resistance from across *Phaseolus* species and introgressed in Andean common bean.

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Table 1.1. A portion of analysis of variance for the response of 23 common bean genotypes in the primary and trifoliolate leaves to two strains of *Xanthomonas campestris* pv. *phaseoli* in two greenhouse environments at University of Idaho, Kimberly, Idaho in 2011.

Source	**df**	**Mean squares**
Greenhouse (H)	1	1473.54*
Leaf type (L)	1	3211.71*
Bacterial strain (B)	1	1157.38*
Bacterial density (D)	1	35.94*
Genotype (G)	22	182.71*
L x B	1	4.31*
L x D	1	35.24*
L x G	22	17.66*
B x D	1	39.57*
B x G	22	30.10*
G x D	22	3.60*
L x D x G	22	4.29*
L x B x D	1	17.59*
L x B x G	22	11.06*
B x D x G	22	5.26*
L x B x D x G	22	2.93*
H x L x B x D	22	33.68*
H x L x B x D x G	22	4.59*
Error	1464	0.5

*Significant at $P \leq 0.05$.

Table 1.2. Mean common bacterial blight scores for the primary and trifoliolate leaves for 23 common bean genotypes for two strains of *Xanthomonas campestris* pv. *phaseoli* at two bacterial densities evaluated at University of Idaho, Kimberly in 2011.

	ARX8				Xcp25				
Leaf type	**BD1[a]**	**BD2**	**Mean**	**LSD (*P*≤0.05)[b]**	**BD1**	**BD2**	**Mean**	**LSD (*P*≤0.05)[b]**	**Overall mean**
Primary	1.6	1.7	1.6	0.09	2.7	3.7	3.2	0.11	2.4
Trifoliolate	4.2	4.1	4.2	0.12	5.4	5.3	5.4	0.15	4.8
LSD (*P*≤0.05)[c]	0.11	0.10	0.07	**…**	0.13	0.13	0.09	**…**	0.06

[a]BD1, bacterial density of 1.7 x 10^8 CFU/ml , and BD2, bacterial density of 3.2 x 10^8 CFU/ml.

[b]To compare means between bacterial densities.

[c]To compare means within leaf types.

Table 1.3. Growth habit, seed type, marker composition, and mean common bacterial blight score for the primary and trifoliolate leaves of 23 common bean genotypes for two strains of *Xanthomonas campestris* pv. *phaseoli* at two bacterial densities evaluated in a greenhouse at University of Idaho, Kimberly in 2011.

				Mean common bacterial blight score								
				ARX08				Xcp25				
				1.7 x 10^8 CFU/ml		3.2 x 10^8		1.7 x 10^8		3.2 x 10^8		
Genotype	**GH[a]**	**Seed type**	**Marker**	**PL[b]**	**TL**	**PL**	**TL**	**PL**	**TL**	**PL**	**TL**	**Mean**
Andean												
Montcalm (check)	I	Dark red kidney	SAP 6	4.0[c]	6.0	2.7	4.8	7.9	8.0	8.7	8.3	6.3
XAN 159	I	Gray speckled	BC420, SU91	3.0	6.0	3.5	5.1	5.8	6.5	7.2	6.5	5.5
Wilkinson 2	I	White	BC420, SU91	2.4	6.5	2.4	6.5	3.0	7.5	5.0	6.0	4.9
USDK-CBB-15	I	Dark red kidney	SAP 6, SU91	1.4	6.9	1.0	4.0	4.2	7.5	6.7	8.0	5.0
USWK-CBB-17	I	White kidney	SAP 6, SU91	1.4	5.8	1.5	5.0	2.9	7.0	5.0	6.8	4.4
08SH840	I	Cranberry	SAP6, SU91	1.2	5.0	1.5	5.5	2.9	6.0	5.9	6.0	4.3
CXR 1	I	Cranberry	SAP 6, SU91	1.0	2.8	1.2	6.0	2.8	6.7	4.4	6.5	3.9
GNX 6	I	White	BC420, SU91	1.3	5.3	2.7	4.1	2.9	7.5	4.2	7.5	4.4
RCS52-1	I	White	BC420, SU91	1.9	7.8	1.9	6.2	3.9	7.0	6.9	4.5	5.0
RCS52-2	I	White	BC420, SU91	1.3	6.2	2.8	5.5	3.2	4.5	4.2	4.9	4.1
RCS53-2	I	White	BC420, SU91	1.2	6.9	1.4	6.3	1.5	6.0	3.7	5.7	4.1
RCS53-3	I	White	BC420, SU91	1.9	3.5	2.0	4.9	3.0	5.3	3.7	5.0	3.7
RCS53-5	I	White	BC420, SU91	1.7	6.9	2.2	5.5	1.9	6.5	3.8	6.7	4.4
RCS63-3	I	White	BC420, SU91	2.4	6.9	2.2	7.0	3.3	6.4	6.7	6.7	5.2
RCS63-4	I	White	BC420, SU91	2.4	6.5	1.3	6.2	4.2	6.5	7.2	6.3	5.1
RCS63-5A	I	White	BC420, SU91	1.0	6.0	1.2	7.5	2.4	5.7	5.2	7.5	4.6
RCS63-5B	I	White	BC420, SU91	1.3	5.9	1.9	3.5	1.5	4.5	4.7	5.3	3.6

				Mean common bacterial blight score								
				ARX08				Xcp25				
				1.7 x 10^8 CFU/ml		3.2 x 10^8		1.7 x 10^8		3.2 x 10^8		
Genotype	**GH[a]**	**Seed type**	**Marker**	**PL[b]**	**TL**	**PL**	**TL**	**PL**	**TL**	**PL**	**TL**	**Mean**
Middle American												
VAX 1	III	Cream striped	SAP 6	2.5	2.2	2.2	4.5	4.4	6.5	6.5	6.5	4.4
VAX 3	II	Red	SAP 6, SU91	1.0	1.9	1.0	3.9	1.0	4.0	2.2	5.2	2.5
VAX 4	II	Cream	SAP 6, SU91	1.0	2.3	1.0	2.5	1.0	5.0	4.7	6.5	3.0
VAX 5	II	Black	SAP 6, SU91	1.0	3.3	1.0	1.7	1.0	6.4	2.0	5.4	2.7
VAX 6	II	Red	SAP 6, SU91	1.0	3.0	1.0	2.5	1.0	3.4	4.9	6.5	2.9
Othello (check)	III	Pinto	None	7.6	9.0	7.5	9.0	8.9	9.0	8.9	9.0	8.6
Mean	…	…	…	2.0	5.3	2.0	5.1	3.2	6.2	5.3	6.4	4.5
LSD ($P \leq 0.05$)	…	…	…	0.8	0.8	0.8	0.8	0.9	0.4	1.0	0.7	0.3

[a]GH, Growth habit; I= determinate upright; II indeterminate upright; and III= indeterminate, prostrate, semiclimbing.

[b]PL, primary leaf, and TL, trifoliolate leaf.

[c]Common bacterial blight score, where 1= no visible symptoms ; 3= necrotic lesions around the inoculated points and no more than two coalesced lesions together, 5= necrotic lesions coalesced together in one-third of inoculated area, 7= necrotic lesions coalesced together in the entire inoculated area and lesions extended beyond the inoculated area, and 9= necrotic lesions extended to the leaf edge causing extended burning or premature senescence and leaf drop.

Chapter 2

A New Common Bacterial Blight Resistance QTL in VAX 1 Common Bean and Interaction of the New QTL, SAP6, and SU91 with Bacterial Strains

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Abbreviations: CBB, common bacterial blight; CIM, composite interval mapping; MAS, marker-assisted selection; QTL, quantitative trait locus or loci; RIL, recombinant inbred line or lines; SNP, single nucleotide polymorphism; SCAR, sequence characterized amplified region; *Xcp*, *Xanthomonas campestris* pv. *phaseoli*

Abstract

Common bacterial blight (CBB) is a severe disease in common bean. New resistance QTL (quantitative trait loci) should facilitate development of cultivars with high levels of resistance. Our objectives were to (i) identify new resistance QTL in VAX 1 and verify its presence in VAX 3, (ii) determine interaction of new QTL with existing SAP6 and SU91 QTL, and (iii) examine interaction of the QTL with less aggressive ARX8AC and aggressive Xcp25 bacterial strains. Sixty-one $F_{6:7}$ recombinant inbred lines (RIL) from 'Othello'/VAX 1 and 100 RIL from Othello/VAX 3 were screened in the greenhouse. Disease severity of inoculated primary leaves, 1st and 2nd trifoliolate leaves, and pods, was scored from 1= no symptoms to 9= completely diseased. Genotyping was carried out using 5398 single nucleotide polymorphism (SNP) BeadChip and CBB resistance QTL-linked sequence characterized amplified region (SCAR) markers, SAP6 and SU91. A novel resistance QTL with major effect was detected on Pv11 linkage group in Othello/VAX 1 and verified in Othello/VAX 3. This Pv11 QTL, defined by the closest marker SNP47467 (45,059,806 bp), explained 23% phenotypic variance for resistance in primary and 18% in trifoliolate leaves in Othello/VAX 1, and 13% and 22%, respectively, in Othello/VAX 3 to ARX8AC. The Pv11 QTL named $Xa11.4^{OV1,OV3}$ had greater influence against Xcp25, with respective values for variance for resistance explained in primary and trifoliolate leaves of 45% and 51% in Othello/VAX 1 and 26% and 37% in Othello/VAX 3. Conversely, SAP6 was only effective against ARX8AC in both populations and surprisingly SU91 was only effective against Xcp25 in Othello/VAX 3. SAP6 was the only QTL to condition resistance in pods. QTL interactions and differential reactions to strains indicate the new $Xa11.4^{OV1,OV3}$ QTL is critical

for breeding for stable and higher levels of foliage resistance to CBB in common bean.

Introduction

Common bacterial blight (CBB) is a severe disease limiting common bean (*Phaseolus vulgaris* L.) production and quality worldwide (Saettler, 1989; Schuster and Coyne, 1981; Schwartz et al., 2005; Singh and Schwartz, 2010). The disease is caused by the seed-borne pathogen *Xanthomonas campestris* pv. *phaseoli* Smith (Dye) (*Xcp*, synonym: *X. axonopodis* pv. *phaseoli* (Smith) Vauterin et al.) and *X. fuscans* sbsp. *fuscans* sp. nov. Some strains of *Xcp* are more aggressive compared to *X. fuscans* sbsp. *fuscans* (Duncan et al., 2011; Mahuku et al., 2006; Mutlu et al., 2008). Mutlu et al. (2008) observed differential pathogenicity of both groups of strains against a set of host genotypes with varying levels of resistance. But, no significant crossover responses were recorded.

Use of resistant cultivars is an essential component of an integrated CBB management strategy, which includes crop rotation, cultural practices, sowing pathogen free seed, and the use of bactericides (Gent et al., 2005; Gilbertson et al., 1990; Singh and Muñoz, 1999). Low to intermediate levels of CBB resistance occur in some common bean landraces from Mexico (Duncan et al., 2011; Singh and Muñoz, 1999) and the United States (Miklas et al., 2003) and *Phaseolus* species of the secondary gene pool (e.g., *P. coccineus* L. – scarlet runner bean, Mohan, 1982; Park and Dhanvantari, 1987; Singh and Muñoz, 1999; Welsh and Grafton, 2001), and the highest levels in the tertiary gene pool (e.g., *P. acutifolious* A. Gray – tepary bean, McElroy, 1985; Michaels, 1992; Parker, 1985; Singh and Muñoz, 1999; Urrea et al., 1999).

Common bacterial blight resistance is inherited quantitatively and more than 20

genes/QTL (quantitative trait loci) on all 11 linkage groups have been identified (Bai et al., 1997; Perry et al., 2013; Tar'an et al., 2001; also see reviews by Kelly et al., 2003; Miklas and Singh, 2007; Miklas et al., 2006). A major CBB resistance QTL on Pv10 linked with SAP6 SCAR marker **(**Miklas et al., 2000a) was derived from the medium-seeded (25 to 40 g 100 seeds^{-1}) common bean landrace great northern Montana No. 5 (Miklas et al., 2003). The SAP6 QTL also was found in some small-seeded (<25 g 100 seeds^{-1}) common bean landraces such as Colima 9 and Tamaulipas 9B from Mexico (Duncan et al., 2011).

Park and Dhanvantari (1987) found a continuous distribution for CBB resistance, indicating a quantitative inheritance in the backcross F_2 populations between the common bean and *P. coccineus*. Conversely, Welsh and Grafton (2001) reported a single recessive gene controlled resistance in crosses between the interspecific breeding lines derived from *P. coccineus* (Miklas et al., 1994) and susceptible common bean.

Thomas and Waines (1984) were the first to successfully cross the common and tepary (PI 319443 synonymous with G 40020) beans. From that interspecific population, McElroy (1985) selected CBB resistant interspecific breeding lines XAN 159, XAN 160, and XAN 161. The CBB resistance in XAN 159 derived from tepary PI 319443 is conditioned by one major QTL linked with SCAR marker SU91 located on Pv08 (Pedraza et al., 1997), and another major QTL linked with SCAR marker BC420 located on Pv06 (Yu et al., 2000). Parker (1985) and Parker and Michaels (1986) also reported interspecific hybridization between the common and tepary beans (e.g., PI 440795), and selection for common bacterial blight resistance. Moreover, Bai et al. (1997) reported CBB resistance QTL on Pv02, Pv03, Pv05, Pv06, and Pv08 derived from the tepary bean PI 440795 that was used in crosses by Parker (1985) and Parker and Michaels (1986).

Singh and Muñoz (1999) and Singh et al. (2001) developed CBB resistant interspecific breeding lines VAX 1 and VAX 2 from a multiple-parent interspecific cross between common and tepary bean G 41001 (A 769///A 775//'ICA Pijao'/G 40001) made by Mejía-Jiménez et al. (1994). Of the known CBB resistance QTL, VAX 1 and VAX 2 carry only the SAP6 linked QTL (Duncan et al., 2011; this article) found in great northern Montana No. 5 (Miklas et al., 2003), and Colima 9 and Tamaulipas 9B (Duncan et al., 2011), yet they possess significantly higher levels of CBB resistance than cultivars with only SAP6 linked QTL (e.g., Colima 9, 'Montcalm', Montana No. 5, Tamaulipas 9B, 'Tara'). However, VAX 3 with SAP6 and SU91 linked QTL (the latter derived from tepary bean PI 319443 via XAN 159 and XAN 309) possesses higher levels of CBB resistance even to more aggressive strains (Duncan et al., 2011; Lema et al., 2007; Singh et al, 2001). The SU91 QTL is associated with low yield (O'Boyle et al., 2007), and BC420 QTL is linked with undesirable seed coat color (Duncan et al., 2007; Mutlu et al., 2005; Park et al., 1999). Moreover, the homozygous recessive alleles at SU91 locus (i.e., su91/su91) are epistatic over the BC420 (Vandemark et al., 2008), thus reducing the frequency of CBB resistant recombinants in the segregating generations. Therefore, there is strong justification for the search for new CBB resistance genes/QTL from across *Phaseolus* species. The objectives of this research were to (i) identify new CBB resistance gene(s)/QTL in the VAX 1 interspecific breeding line and verify its presence in VAX 3, (ii) determine the individual and combined effects of the new CBB resistance QTL, SAP6, and SU91 QTL on CBB response, and (iii) examine the interaction of the resistance QTL with bacterial strains.

Materials and Methods

Parental Germplasm

Sixty-one $F_{6:7}$ recombinant inbred lines (RIL) from 'Othello'/VAX 1 and 100 RIL from Othello/VAX 3 were developed by single-seed-descent method from the F_2. Pinto Othello (Burke, et al., 1995) has an indeterminate prostrate growth habit Type III (Singh, 1982), early maturity, and is susceptible to CBB (this article). As noted above, VAX 1 is an interspecific breeding line derived from a multiple-parent interspecific cross between common bean and *P. acutifolious* (A 769///A 775//ICA Pijao/G 40001, Singh and Muñoz, 1999; Singh et al., 2001). VAX 1 has small cream-striped seed with growth habit Type III and possesses SAP6 SCAR marker (Duncan et al., 2011). VAX 3 was derived from a cross using VAX 1 and XAN 309 (A 769///A 775//ICA Pijao/G 40001/4/XAN 309) (Singh and Muñoz, 1999; Singh et al., 2001). VAX 3 has small Central American red seed with indeterminate upright growth habit Type II and possesses the SAP6 and SU91 SCAR markers (Duncan et al., 2011). Both VAX breeding lines are late maturing, and have resistance to CBB, the *I* gene for resistance to *Bean common mosaic virus* (an aphid-vectored potyvirus), and Fusarium root rot [caused by *Fusarium solani* f. sp. *phaseoli* (Burkholder) Snyder *et* Hansen] resistance (Singh et al., 2001).

The two parents and RIL of each population were planted in the greenhouse at University of Idaho, Kimberly Research and Extension Center in 2012. A separate randomized complete block design with three replications was used for each population. One plant was used for each replication. The experiment was repeated twice. The disease response was combined across replications and plantings, representing the mean score for six

plants.

Inoculum Preparation, Inoculation, and Common Bacterial Blight Evaluation

Two contrasting bacterial strains, less aggressive ARX8AC and more aggressive Xcp25 (Duncan et al., 2011; Lema et al., 2007) were used for inoculation. Bacterial inoculum frozen at -80ºC in the XYT liquid media was activated onto Petri plates containing 523 media (Kado and Heskett, 1970) and allowed to grow for 48 h at 30ºC. Bacterial cells were suspended in distilled water and adjusted to the density of 1.7 x 10^{8} CFU/ml. One primary leaf was inoculated with the strain ARX8AC (recovered from an Andean bean in Embarcación, Salta, Argentina in 2008) and the second with Xcp25 (recovered from an Andean bean in Wisconsin prior to 2003) 15 d after sowing. The three 3/4th expanded leaflets of the first trifoliolate leaf were inoculated with ARX8AC at 25 d. Similarly, the three leaflets of the second trifoliolate leaf were inoculated with Xcp25 at 30 d. Furthermore, two pods per plant were inoculated with each bacterial strain at mid-pod fill stage at 60 d. A sterilized florist frog (i.e., multiple needle method) was used for inoculation by pressing the leaves and pods on top of a sponge submerged in a liquid bacterial suspension in a Petri plate. Inoculated plants were kept under high humidity (~80%) using humidifiers situated under the greenhouse benches and by keeping the floor wet after inoculation. Plants were grown at mean day temperature of 26ºC and mean night temperature of 18ºC, with 12 h of light.

Common bacterial blight severity in the primary and trifoliolate leaves was evaluated at 21 d post inoculation, whereas the pods were evaluated at 7 d, using a 1 to 9 scale according to Lema el at. (2007). Genotypes were considered resistant with CBB scores of 1 to 3, intermediate 4 to 6, and susceptible 7 to 9.

Assays for Known Common Bacterial Blight Resistance QTL-linked SCAR Markers

Genomic DNA was extracted using the Fast DNA green spin kit (MP Biomedicals, Solon, OH). The DNA was extracted from a bulk sample of emerging trifoliolate leaves collected from three plants of each parent and RIL. The DNA concentration was adjusted to 10 ug/mL using a NanoDrop spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE) and mixed with 4 uL of 5x buffer, 2.4 uL of 25 mM $MgCl_2$, 0.8 uL of 5 mM dNTP, 2 uL of oligonucleotide primers at 10 uM concentrations, 8.6 uL of purified sterilized water, and 0.2 Go Taq DNA polymerase (Promega Corporation, Madison, WI). The forward 5'-GTCACGTCTCCTTAATAGTA-3' and reverse 5'-GTCACGTCTCAATAGGCAAA-3' SAP6 primers (Miklas et al., 2000a) and also the SU91-CG11 forward primer 5'-GGCGACGGCTTCTTTGAC- 3' and reverse 5'-TCCAAAGACCAAAGGGTGAG-3' primer (Shi et al., 2012) were used in separate reactions. The amplification conditions for SAP6 included 34 cycles at 94ºC for 10 s (denaturation), followed by 55ºC for 30 s (annealing), and 72ºC for 2 min (extension). The last step included a final extension at 72ºC for 5 min. For the SU91-CG11 marker the amplification conditions were 33 cycles at 94ºC for 10 s (denaturation), followed by 58ºC for 40 s (annealing), and 72ºC for 2 min (extension), followed by a final extension at 72ºC for 5 min. All PCR reactions were performed in a PTC-100 thermocycler (MJ Research Inc., Waltham, MA). The SU91 dominant marker was assayed, in part, to validate the new SU91-CG11 marker, using the same protocol and amplification conditions described for SU91-CG11. The forward 5'-CCACATCGGTTAACATGAGT-3' and reverse 5'-CCACATCGGTGTCAACGTGA-3' primers were used for SU91 marker (Pedraza et al., 1997). PCR amplicons were run in 1.4 % agarose gel stained with 2% of ethidium bromide. SAP6 is a dominant marker and therefore

the presence or absence of the amplified 820 bp fragment was recorded visually (Miklas et al., 2000b). We used SU91-CG11 co-dominant marker (Shi et al., 2012), in addition to SU91 dominant marker, because it enabled identification of heterozygous RIL. The QTL is referred to as the SU91 QTL regardless of the SCAR marker used to detect it.

Single Nucleotide Polymorphism Genotyping

Based upon the analysis of 192 common bean genotypes with 1307 SNP (single nucleotide polymorphism) using the Illumina GoldenGate assay (Hyten et al., 2010) a set of 15 diverse genotypes were identified. The 15 genotypes along with the genotypes BAT 93 and Jalo EEP 558 were prepared for whole genome DNA sequence analysis. Sequencing by synthesis on the Illumina GA IIx was conducted and two Illumina Infinium BeadChips with more than 5,000 SNP were developed. A third Infinium BeadChip referred to as the BARCBean6K_3 BeadChip with 5,398 SNP was developed with SNP selected from the first two BeadChips (manuscript in preparation). The BARCBean6K_3 BeadChip was used to genotype Othello, VAX 1, VAX 3 and the two RIL populations. SNP genotyping was conducted on the Illumina platform following the Infinium® HD Assay Ultra Protocol (Illumina, Inc. San Diego, CA). SNP allele calling was completed using the GenomeStudio Genotyping Module v1.8.4 (Illumina, Inc. San Diego, CA).

Molecular Linkage Mapping and QTL Analyses

Genetic linkage maps were constructed using default settings for the Regression method in JoinMap4 (Van Ooijen, 2006). There were 5398 SNP markers genotyped across both populations. The SNP used for genetic mapping were selected based on their polymorphism, lack of genetic distortion, and less than 5% missing data. The SAP6 and SU91 SCAR markers were included for the map construction.

For each population, linkage groups were selected based on independence logarithm of odds (LOD) score greater than 4.0. The linkage groups were aligned with the 11 chromosomes (Pv01 to Pv11) of the common bean genome based on physical map position of linked SNP. The physical map location of SNP markers was in reference to the 1.0 version of the whole genome *P. vulgaris* map (Goodstein et al., 2011). The SCAR marker positions were verified using published data (Miklas et al., 2003; Shi et al., 2012).

For each population analysis of variance of CBB scores combined across plantings was conducted using PROC GLM (SAS Institute, 2008). The Least-square CBB score means for each of primary and trifoliolate leaves and pods were generated for individual RIL from each population for use in the QTL analyses. Composite interval mapping (CIM) in QGene 4.0 (Joehanes and Nelson, 2008) was used for identification of QTL conferring CBB resistance. A permutation test (1000 permutations) at the 0.05 level of probability was used to determine the significance LOD level for declaration of a QTL. Only linkage groups with detected QTL were depicted in the results section. Estimates of the phenotypic variation (R^2) explained by individual QTL was determined by single factor analysis in PROC GLM (SAS Institute, 2008). The marker within the QTL peak with the highest R^2 and $P \leq 0.01$ was used to tag the QTL. These QTL-linked markers were analyzed by PROC REG (Stepwise) to determine significant interactions among the major QTL detected for each of primary and trifoliolate leaves and pods. A $P \leq 0.05$ was used to declare a significant interaction. For each of primary and trifoliolate leaves, pods, and population, the CBB score means for RIL with the presence of QTL-linked markers, individually and in combination, were pooled together. A Fisher's least significant difference at $P \leq 0.05$ for unbalanced means was used to detect differences among these pooled means to ascertain effects of individual QTL and

combinations of QTL on level of CBB resistance.

Results

Bacterial strains, common bean genotypes, and bacterial strains x genotypes interactions were significant ($P \leq 0.05$) in both leaves and pods for both RIL populations (Table 2.1). The aggressive bacterial strain Xcp25 induced greater disease severity than the less aggressive ARX8AC strain in leaves and pods for both populations as expected (Table 2.2). Of the primary and trifoliolate leaves and pods inoculated, disease severity was greatest in the trifoliolate leaves and least in the pods.

Of 5398 SNP markers genotyped, 1688 polymorphic SNP met the selection criteria for linkage mapping in Othello/VAX 1 and 1493 in Othello/VAX 3, with 1137 SNP in common between populations. Note that two RIL in Othello/VAX 1 and four in Othello/VAX 3 were not used for linkage mapping or subsequent QTL analysis because of missing marker data. When multiple SNP were located at the same locus, only one SNP was included for linkage group construction which reduced the number of SNP used for mapping by at least another 50%. For Othello/VAX 1, eleven linkage groups corresponding to the 11 Pv chromosomes were generated (not shown) with an average of 37 SNP per linkage group that ranged from 16 SNP in Pv05 to 50 for Pv11. The SAP6 SCAR mapped to Pv10 as previously reported (Miklas et al., 2000a). Eleven linkage groups were similarly generated for Othello/VAX 3 with an average of 43 SNP per linkage group, ranging from 19 SNP for Pv07 to 55 for Pv09. SAP6 mapped to Pv10 and SU91 dominant and SU91-CG11 co-dominant markers were tightly linked (2.2cM) and mapped to Pv08 as expected. Total map length was 904 cM for Othello/VAX 1 and 1275 cM for Othello/VAX 3, which matches closely with the estimated

length of ~1200 cM (Vallejos et al., 1992).

Composite interval mapping detected two QTL that conditioned resistance to CBB in the Othello/VAX 1 population, SAP6 on Pv10 and a new QTL on Pv11 (Figure 2.1, Tables 2.3 and 2.4). The resistance alleles for both QTL derived from the VAX 1 parent. The SAP6 QTL had a major effect against the less aggressive strain ARX8AC in primary and trifoliolate leaves and pods explaining 32% phenotypic variance for CBB resistance in the primary and 35% in the trifoliolate leaves, and 25% in pods in the Othello/VAX 1 population. However, SAP6 was ineffective against the aggressive Xcp25 strain in leaves and pods. In contrast, the new Pv11 QTL, with relative position defined by SNP47467 (45,059,806 bp), was effective against both strains in leaves but ineffective in pods. The genomic interval for the Pv11 QTL peak is ~9 cM and spans 44,897,729 (SNP47588) to 45,633,237 (SNP50599) bp. The interval has relatively poor SNP coverage, perhaps because the QTL region represents an interspecific introgression from tepary bean. The SNP47467 and the other Xa11.4 QTL-linked SNP were highly polymorphic across ~300 CBB susceptible genotypes surveyed from the BeanCAP (USDA National Institute of Food and Agriculture - project number 2009-01929) (unpublished data), indicating that these SNP markers will have absolutely no value for MAS of the Xa11.4 QTL. This result was expected because the SNP for the BARCBean6K_3 Infinium BeadChip were specifically selected for balanced polymorphism across a diverse array of genotypes.

Following QTL nomenclature guidelines for *P. vulgaris* (Miklas and Porch, 2010) this new QTL will be referred to as Xa11.4^{OV1}. The Xa represents the first two letters of the genus for the pathogen *Xanthomonas*. The 11.4 indicates that it is the fourth QTL for resistance to CBB identified on Pv11. A previous QTL on Pv11 was detected in BAC 6/HT 7719 by Jung

et al. (1996), and derived from the BAC 6 parent, which derives its CBB resistance from great northern Nebraska #1 Sel. 27 (Jung et al., 1999). Subsequently, Yu et al. (1998) identified two QTL in XR-235-1-1/'DIACOL Calima' linked with *Bng25a* and *Bng154* RFLP markers that derived from the susceptible parent DIACOL Calima. Unlike the Xa11.4 QTL in VAX 1 which likely derives from *P. acutifolius* G 40001 (Singh and Muñoz, 1999; Singh et al., 2001), none of these previously identified QTL on Pv11 was derived from common bean genotypes with tepary bean in their pedigree nor was any ever validated. The superscript ^{OV1} indicates the population of origin (Othello/VAX 1). The Xa11.4^{OV1} or Xa11.4 QTL for short had greater effect against the aggressive strain Xcp25 explaining 45% and 51% phenotypic variance for CBB resistance in the primary and trifoliolate leaves, respectively, compared to 23% and 18% in response to ARX8AC (Table 2.3). The interaction of SAP6 and Xa11.4 was only significant for primary and trifoliolate leaves inoculated with the ARX8AC bacterial strain, because SAP6 had no effect against Xcp25 and Xa11.4 was ineffective in pods. The pooled data (Table 2.4), however, suggests that Xa11.4 may have some effect in pod tissue when combined with SAP6.

Three QTL were detected by CIM in the Othello/VAX 3 population: SAP6, SU91, and Xa11.4 first identified in Othello/VAX 1 described above (Figure 2.2, Tables 2.3 and 2.4). All three QTL derived from the VAX 3 parent. The new QTL can now be listed as Xa11.4^{OV1,OV3} indicating it has been validated in a second population. A similar ~9 cM interval for the Xa11.4 QTL spanning 45,018,795 (SNP47465) to 45,688,346 (SNP48857) bp and once again with poor SNP coverage was observed. The peak of the QTL was nearest SNP47467 (45,059,806 bp) as observed before in the Othello/VAX 1 population. As previously noted, VAX 1 is a parent of VAX 3; therefore, it is not surprising that they both possess the new

Xa11.4 QTL. The SAP6 QTL in Othello/VAX 3, with major effect against ARX8AC in all three plant parts and no effect against Xcp25, exhibited the same pattern of effect as in the Othello/VAX 1 population. Othello had intermediate to susceptible CBB scores in leaves and pods to both strains. VAX 1 exhibited resistant scores to ARX8AC and intermediate scores to Xcp25 in both leaves. For pods, intermediate CBB scores were observed for both strains. VAX 3 had lower disease scores than VAX 1 in primary and trifoliolate leaves and pods to both strains as expected.

There were no differences in CBB response and percentages of phenotypic variance for CBB resistance explained between the dominant SU91 (data not shown) and co-dominant SU91-CG11 markers. Both markers are linked to a QTL on Pv08 (Figure 2.2), and conferred resistance to the aggressive strain Xcp25 but not the less aggressive strain ARX8AC in primary and trifoliolate leaves, nor in pods to either strain. This lack of effect against the weaker strain was unexpected because SU91 has been deployed into many different breeding lines (Duncan et al., 2011) and is thought to provide broad-spectrum resistance. SU91 QTL is known to derive from tepary bean PI 319443 and is present in breeding line XAN 309 (via XAN 159) which is the likely parental source of SU91 in VAX 3. Although an effect of SU91 against ARX8AC strain was not detected by CIM (Table 2.3), presence of the QTL did result in a significant minor reduction in mean disease severity in leaves (Table 2.4). This minor effect of SU91 against ARX8AC is supported by the significant interaction effect of SAP6 and SU91 for mean response against this strain.

The Xa11.4 QTL in Othello/VAX 3 (Figure 2.2, Table 2.3) exhibited a significant effect in leaves against both strains. The pooled mean data indicates Xa11.4 may also have a minor effect in pods alone and in combination with SAP6 (Table 2.4). The Xa11.4 QTL

interacted with both SAP6 QTL to increase resistance in leaves to ARX8AC and with SU91 to increase resistance in leaves to both strains. However, higher percentages of phenotypic variance for CBB resistance, 56% in primary and 58% in trifoliolate leaf, were explained in response to more aggressive strain Xcp25 when Xa11.4 interacted with SU91 QTL. The three QTL together did not have enhanced effects against either strain in primary and trifoliolate leaves and pods.

Discussion

The bacterial strain Xcp25 was more aggressive in leaves and pods than ARX8AC in both populations, but no significant crossover interactions occurred between the two bacterial strains and common bean genotypes (i.e., parents and RIL) in either population. Duncan et al. (2011) and Lema et al. (2007) also reported Xcp25 to be more aggressive compared with other strains from the Americas and Africa. Use of contrasting pathogen strains or races with different aggressiveness (or virulence) in breeding, genetics, and pathology studies helps to elucidate host-pathogen interactions or lack thereof. It also facilitates appropriate deployment of resistance genes/QTL and development of germplasm and cultivars with a broad spectrum of higher levels of pyramided durable resistance, especially for the production regions endemic with highly variable *Xcp* and/or *X. fuscans* sbsp. *fuscans* populations such as the Midwestern United States (Duncan et al., 2011).

Of known CBB resistance linked markers in VAX 1 only SAP6 was identified until now (Duncan et al., 2011), which is present in medium-seeded common bean landrace great northern Montana No. 5 (Miklas et al., 2003), and also present in small-seeded landraces (e.g.,

Colima 9, Tamaulipas 9B) from Mexico (Duncan et al., 2011). Thus, the likely donors of SAP6 in VAX 1, VAX 3, and VAX 4 to six breeding lines could be the small-seeded common bean parent(s) of VAX 1, namely A 775, A 769, or ICA Pijao, which had susceptible or near susceptible CBB responses in Colombia (Singh and Muñoz, 1999; Singh et al., 2001).

There was a SNP marker, namely SNP47912 that appeared to be closer to the QTL defined by the SAP6 SCAR on Pv10 in both populations. Although, pooled mean scores for individuals with the SNP47912 marker were sometimes lower than those with the SAP6 marker, the mean differences were inconsistent and non-significant (data not shown). Nonetheless, the physical map position for SNP47912 (40,517,575 bp) relative to SAP6 (39,938,699 - 39,939,569 bp; Perry et al., 2013) may guide further fine mapping of the region for development of a better marker for marker-assisted selection of the Pv10 QTL. But, it should be realized that the population size for the Othello/VAX 1 and Othello/VAX 3 RIL populations used in this study may be relatively small: therefore, the effects of the SAP6, SU91, and/or Pv11.4 QTL may be either under or over estimated. Thus, it may be advisable to use much larger populations to validate the individual and combined effects of each of these CBB resistance QTL in other populations. However, given that SAP6 and SU91 have been validated in many different breeding populations (Duncan et al., 2011; Miklas et al., 2006; Mutlu et al., 2005) and used successfully for marker-assisted selection in development of CBB resistant germplasm (Miklas et al. 2011), confidence should be gained in the validity of a QTL (Xa11.4) with even greater effect identified within their midst.

VAX 1 has significantly higher levels of CBB resistance than other common bean landraces (e.g., Colima 9, G 1320 or PI 207262, great northern Montana No. 5, Tamaulipas 9B), breeding lines (e.g., A 716, A 769, A 775, great northern #1 Sel 27, ICB 3, ICB 53, XAN

91), and cultivars (e.g., Jules, ICA Pijao, Montcalm, Tara) (Duncan et al., 2011; Lema et al. 2007; Singh and Muñoz, 1999; Singh et al., 2001) with or without SAP6 and without introgression of CBB resistance from the *Phaseolus* species of the tertiary (e.g., *P. acutifolius*) gene pool. Thus, the tepary G 40001, the male parent with 12.5% genetic contribution used in the initial interspecific cross (i.e., A 769///A 775//ICA Pijao/G 40001, Mejía-Jiménez et al., 1994) to develop VAX 1, is the likely source of the new CBB resistance Xa11.4 QTL. Tepary G 40001 is highly resistant to CBB (Singh and Muñoz, 1999), and it would be worth verifying the presence or absence of Xa11.4 in G 40001. VAX 3, in addition to SAP6, had SU91 marker linked QTL derived from tepary bean G 40020 (synonymous with PI 319443) via XAN 159 and XAN 309 (the additional parent used to develop VAX 3 with high levels of pyramided CBB resistance) (Singh and Muñoz, 1999; Singh et al., 2001). The SU91 and SU91-CG11 markers co-segregated in Othello/VAX 3 population.

Based on QTL identified by CIM (Figure 2.1 and 2.2, Table 2.3), bacterial strains with contrasting aggressiveness ARX8AC and Xcp25 detected a differential expression in primary and trifoliolate leaves among three QTL conferring resistance in the host: SAP6, SU91, and the new QTL Xa11.4 identified in this study. SAP6 had a major effect against ARX8AC but not Xcp25 in both populations. Conversely, SU91 had significant effect against Xcp25 but not ARX8AC in Othello/VAX 3, while Xa11.4 was effective against both strains in both populations. These results extend the host-pathogen interaction observed by Mutlu et al. (2008) to a host-QTL x *Xcp* strain aggressiveness interaction.

The availability of RIL with each CBB resistance QTL, SAP6 and Xa11.4 in Othello/VAX 1 and SAP6, SU91, and Xa11.4 in Othello/VAX 3, individually as well as in different combinations, offered additional opportunity to examine the main effects and

interactions of the QTL on mean CBB response to infection in primary and trifoliolate leaves and pods to the two strains with contrasting aggressiveness (Table 2.4). The QTL effects on mean CBB response mostly mirrored the phenotypic variance explained, but with some exceptions. The SAP6 QTL had slight effect against Xcp25 in primary and trifoliolate leaves in Othello/VAX 1 and only in the primary leaf in Othello/VAX 3. SAP6 in combination with SU91 or Xa11.4 provided slight increase in resistance to Xcp25 strain in both leaves in Othello/VAX 3. The SU91 QTL reduced disease score in both leaves to the ARX8AC strain alone and when combined with SAP6 or Xa11.4. The Xa11.4 QTL had slight effect on reducing disease score in pods in Othello/VAX 3 population to both strains. Xa11.4 interacted with SAP6 to further reduce pod infection to both strains in Othello/VAX 1 and to Xcp25 strain in Othello/VAX 3. These minor effects of the QTL undetected by CIM and R^2 analyses contributed to the lack of crossover interactions, meaning that all genotypes that were intermediate or resistant to the less aggressive strain ARX8AC did not exhibit a similar response to more aggressive Xcp25, and some were susceptible to the latter. But, all genotypes that were intermediate or resistant to Xcp25 also had either a similar or better resistant response to ARX8AC. Furthermore, all genotypes susceptible to ARX8AC were also susceptible to Xcp25.

The Xa11.4 and SU91 QTL interacted to confer significantly higher levels of CBB resistance in leaves against the more aggressive strain Xcp25. The Xa11.4 and SAP6 QTL interacted to provide higher level of resistance to less aggressive strain ARX8AC. Vandemark et al. (2009) observed a lack of interaction between SU91 and SAP6 QTL in their study because SAP6 QTL was ineffective against the aggressive strain they used. Our results confirm their observation that SAP6 had no effect against an aggressive strain (Xcp25) alone

or in combination with other QTL. A clear recessive epistatic interaction between CBB resistance QTL, as observed by Vandermark et al. (2008), was not observed in this study. Vandermark et al. (2008) reported recessive epistatic interaction between the two tepary bean PI 319443 derived CBB resistance QTL linked with SU91 and BC420 such that together they conferred higher levels of CBB resistance, but in the absence of SU91 (i.e., homozygous recessive su91/su91) the BC420 QTL was ineffective.

Moreover, SU91 was associated with a reduction in seed yield (O'Boyle et al., 2007) and BC420 was associated with the undesirable seed coat color (Duncan et al., 2007; Mutlu et al., 2005; Park et al., 1999), which restricts the use of BC420 QTL to black, brown, cream, gray, purple, and white seeded market classes. In that regard, use of Xa11.4 QTL alone or in combination with SAP6 QTL may be preferable over the use of SU91 and BC420 QTL. But, further research is needed to determine whether or not Xa11.4 QTL is linked with any undesirable agronomic trait(s). Moreover, interaction of Xa11.4 with SU91 and BC420, and joint effects of the three tepary bean resistance QTL and SAP6 also need to be determined.

Only the SAP6 QTL consistently conditioned resistance in pods against the less aggressive strain ARX08AC. The search for other major gene/QTL that confers higher levels of CBB resistance in pods against more aggressive strains from different *Phaseolus* gene pools should continue. For instance, Singh and Muñoz (1999) reported resistant response in pods in *P. acutifolius* accessions such as G 40029, G 40038, G 40155, and G 40156; and TARS VCI-4B. TARS VCI-4B is a pinto bean derived from an interspecific cross between *P. vulgaris* and *P. coccineus* (Miklas et al., 1994). The effects of genes/QTL found in these genotypes on CBB resistance in pods needs be evaluated against bacterial strains with different aggressiveness.

Conclusions

The new Xa11.4 QTL identified in Othello/VAX 1 and validated in Othello/VAX 3 RIL populations is a significant discovery because it exhibits broad spectrum CBB resistance against both ARX8AC and Xcp25 strains with contrasting aggressiveness. The new Xa11.4 QTL conferred higher levels of resistance than SU91 QTL in trifoliolate leaves and pods against both less aggressive and aggressive strains. Xa11.4 conditioned better resistance than SAP6 QTL in all plant tissues against the most aggressive strain. Conversely, the SAP6 QTL conferred better resistance than Pv11.4 QTL in all plant parts against the less aggressive strain. For less aggressive and aggressive strains, respectively, the SAP6 and Pv11.4 QTL and SU91 and Pv11.4 QTL combinations were better than the SAP6 and SU91 QTL combination. For breeders and geneticists practicing marker-assisted selection it would be advisable to develop an easily workable marker for the CBB resistance Xa11.4 QTL.

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Table 2.1. A portion of analysis of variance for common bean recombinant inbred lines from Othello/VAX 1 and Othello/VAX 3 populations and their parents for the response in primary and trifoliolate leaves and pods to ARX8AC and Xcp25 bacterial strains of *Xanthomonas campestris* pv. *phaseoli*, evaluated at 21 days post inoculation in leaves and 7 days in pods in the greenhouse at University of Idaho, Kimberly in 2012.

Source	Othello/VAX 1					Othello/VAX 3			
	Mean squares					Mean squares			
	df †	df ‡	PL§	TL¶	Pods	df	PL	TL	Pods
Strains (B)	1	1	766.0**	913.4**	178.5**	1	1790.8**	1806.5**	556.1**
Genotypes (G)	62	59	30.5**	31.0**	8.4**	101	28.0**	39.5**	14.9**
G x B	62	59	7.6**	8.6**	3.3**	101	6.7**	10.0**	6.8**
Error	496	472	0.9	0.6	0.6	808	0.7	0.6	0.5

**Significant at $P \leq 0.01$.

†Degrees of freedom for leaves.

‡Degrees of freedom for pods were reduced because three RIL did not have CBB scores for pods.

§PL, primary leaf.

¶TL, trifoliolate leaf.

Table 2.2. Range, and mean common bacterial blight score pooled over the parents and recombinant inbred lines for Othello/VAX 1 and Othello/VAX 3 common bean populations in response to bacterial strains ARX8AC and Xcp25 evaluated at 21 days post inoculation in leaves and 7 days in pods in the greenhouse at the University of Idaho, Kimberly in 2012.

Bacterial strain	Othello/VAX 1						Othello/VAX 3					
	Primary leaf		Trifoliolate leaf		Pod		Primary leaf		Trifoliolate leaf		Pod	
	range	score	range	score	range	score	range	score	range	score	range	score
ARX8AC	1-9	3.2†	1-9	4.5	1-7	3.8	1-9	2.7	1-9	3.5	1-7	3.5
Xcp25	2-9	5.2	1-9	6.7	2-6	4.7	1-9	5.1	1-9	6.0	2-6	4.9
LSD ($P \leq 0.05$)	…	0.14	…	0.11	…	0.11	…	0.09	…	0.09	…	0.07

†Common bacterial blight scored on a 1 to 9 scale, where 1= no visible symptoms, 3= necrotic lesions around the inoculated points and no more than two coalesced lesions together, 5= necrotic lesions coalesced together in one-third of inoculated area, 7= necrotic lesions coalesced in the inoculated area and lesions extended beyond the inoculated area, and 9= necrotic lesions extended to the leaf edge causing premature senescence and leaf drop, or reddish necrotic lesions extending to the edge of pods.

Table 2.3. Percentage of phenotypic variance for common bacterial blight resistance explained by SAP6, Xa11.4, and SU91 QTL in the primary and trifoliolate leaves and pods of common bean in response to ARX8AC and Xcp25 strains of *Xanthomonas campestris* pv. *phaseoli* in the Othello/VAX 1 and Othello/VAX 3 recombinant inbred line populations evaluated at 21 days post inoculation in leaves and 7 days in pods in the greenhouse at University of Idaho, Kimberly in 2012.

Population	QTL	Chromosome	Strain	Primary leaf		Trifoliolate leaf		Pods	
				R^2	Probability	R^2	Probability	R^2	Probability
				%	p value	%	p value	%	p value
Othello/VAX 1	SAP6	Pv10	ARX8AC	32	<0.0001	35	<0.0001	25	<0.0001
			Xcp25		NS[†]		NS		NS
	Xa11.4 [‡]	Pv11	ARX8AC	23	0.0001	18	0.0005		NS
			Xcp25	45	<0.0001	51	<0.0001		NS
	SAP6, Xa11.4	Pv10, Pv11	ARX8AC	61	<0.0001	60	<0.0001		NS
			Xcp25		NS		NS		NS
Othello/VAX 3	SAP6	Pv10	ARX8AC	38	<0.0001	41	<0.0001	51	<0.0001
			Xcp25		NS		NS		NS
	SU91[§]	Pv08	ARX8AC		NS		NS		NS
			Xcp25	33	<0.0001	23	<0.0001		NS
	Xa11.4	Pv11	ARX8AC	13	0.0003	22	<0.0001		NS
			Xcp25	26	<0.0001	37	<0.0001		NS
	SAP6, SU91	Pv08, Pv10	ARX8AC		NS		NS		NS
			Xcp25		NS		NS		NS

Population	QTL	Chromosome	Strain	Primary leaf		Trifoliolate leaf		Pods	
				R^2	Probability	R^2	Probability	R^2	Probability
	SAP6, Xa11.4	Pv10, Pv11	ARX8AC	49	<0.0001	62	<0.0001	57	0.001
			Xcp25		NS		NS		NS
	SU91, Xa11.4,	Pv08, Pv11	ARX8AC	17	0.03	26	0.02		NS
			Xcp25	56	<0.0001	58	<0.0001		NS
	SAP6,SU91,Xa11.4	Pv08,Pv10,Pv11	ARX8AC		NS		NS		NS
			Xcp25		NS		NS		NS

Table 2.4. Mean common bacterial blight scores for the primary and trifoliolate leaves and pods of common bean against ARX8AC and Xcp25 strains of *Xanthomonas campestris* pv. *phaseoli* in the Othello/VAX 1 and Othello/VAX 3 recombinant inbred line populations and their parents evaluated at 21 days post inoculation in leaves and 7 days in pods in the greenhouse at University of Idaho, Kimberly in 2012.

Genotypes	QTL present	Number	Primary leaf		Trifoliolate leaf		Pod	
			ARX8AC	Xcp25	ARX8AC	Xcp25	ARX8AC	Xcp25
Parents					score			
Othello	None	1	5.7† a	7.5 a	7.2 a	8.8 a	4.5 a	5.9 a
VAX 1	SAP6 (Pv10), 11.4 (Pv11)	1	3.0 b	4.5 b	3.0 b	5.5 b	3.4 b	4.3 b
VAX 3	SAP6 (Pv10), SU91 (Pv08), Xa11.4 (Pv11)	1	2.8 b	3.5 c	2.8 b	3.2 c	2.7 c	3.7 b
Recombinant inbred lines								
Othello/VAX 1	None	19	5.5 a	6.5 a	6.8 a	7.8 a	4.4 a	4.9 a
	SAP6	14	2.0 c	5.6 b	3.4 c	7.1 b	3.2 b	4.8 a
	Xa11.4‡	18(16) §	2.6 b	3.8 d	4.5 b	5.6 c	4.3 a	4.7 ab
	SAP6, Xa11.4	8 (7)	1.1 d	4.2 c	1.6 e	5.7 c	2.3 d	4.3 c
Othello/VAX 3	None	14	5.3 a	6.7 a	6.6 a	7.4 a	4.8 a	5.0 b
	SAP6	15	1.7 c	6.2 b	3.0 d	7.4 a	2.7 cd	4.9 bc
	SU91¶	10	3.7 b	5.0 c	5.4 b	6.7 b	4.7 a	5.3 a
	Xa11.4	16	3.8 b	5.1 c	4.9 c	6.0 c	4.1 b	4.6 cd
	SAP6, SU91	6	1.2 d	4.4 d	1.8 ef	5.9 d	2.6 d	4.8 bcd
	SAP6, Xa11.4	13	1.1 d	4.8 cd	1.4 h	5.4 e	2.5 d	4.5 d
	SU91, Xa11.4,	6	1.4 d	2.6 f	2.1 e	3.1 g	4.2 b	4.9 bc
	SAP6, SU91, Xa11.4	16	1.3 d	3.5 e	1.6 fg	4.0 f	2.7 cd	4.8 bcd

†Common bacterial blight scored on a 1 to 9 scale, where 1= no visible symptoms to 9= necrotic lesions extended to the leaf edge causing premature senescence and leaf drop, or reddish necrotic lesions extending to the edge of pods.
‡Xa11.4 was as detected by SNP47467.
§Number of RIL in parenthesis in Othello/VAX 1 represents those with pod reaction data.
¶SU91 QTL above represents the SU91-CG11 co-dominant marker.

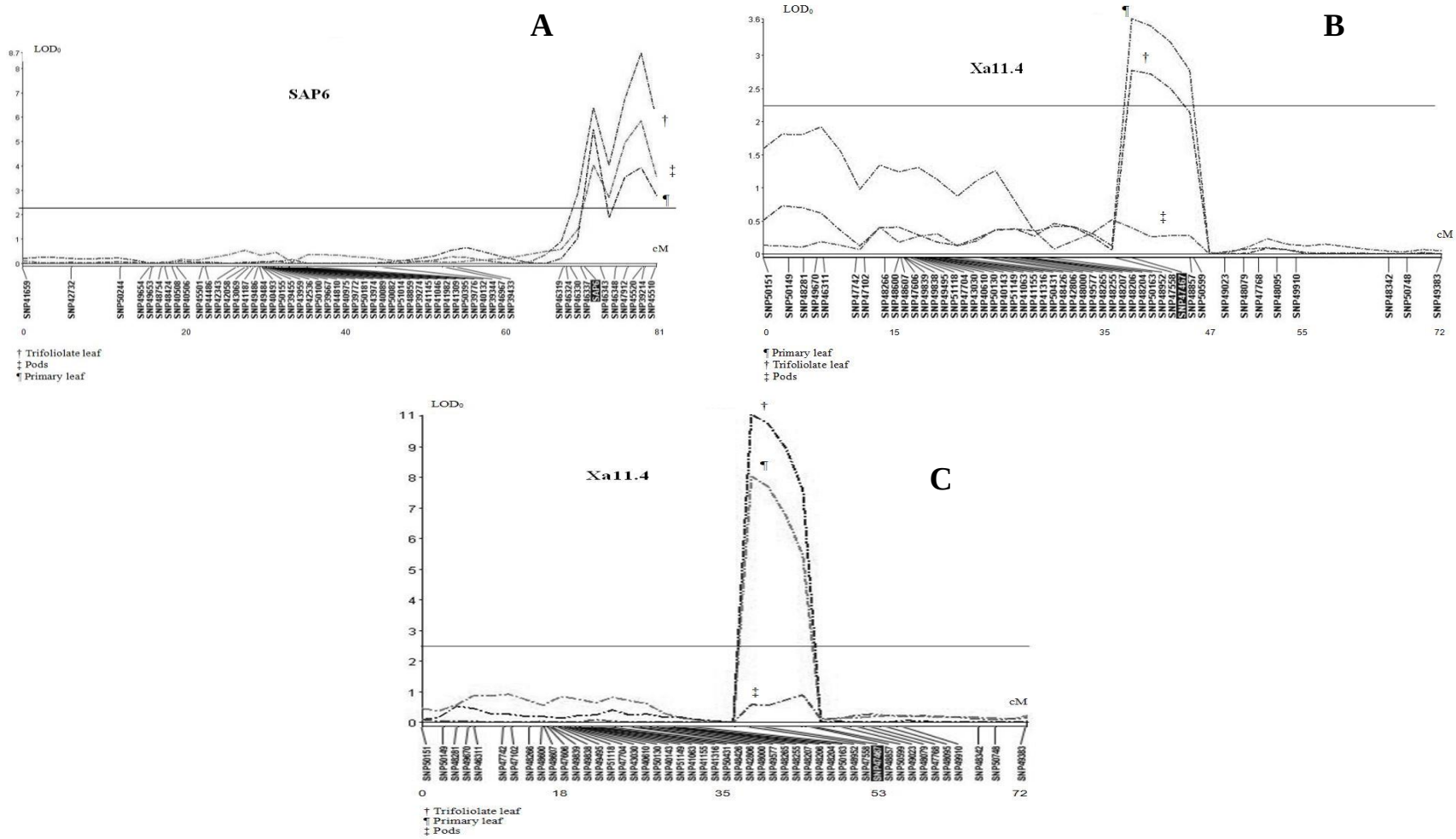

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Figure 2.1. Composite interval mapping logarithm of the odds (LOD_0) displaying molecular markers linked with QTL conferring common bacterial blight resistance in Othello/VAX 1 recombinant inbred line population of common bean. Traits showed different patterns according to the plant organ evaluated. (A) SAP6 on Pv10 against ARX8AC bacterial strain, (B) Xa11.4 on Pv11 against ARX8AC bacterial strain, and (C) Xa11.4 on Pv11 against Xcp25 bacterial strain.

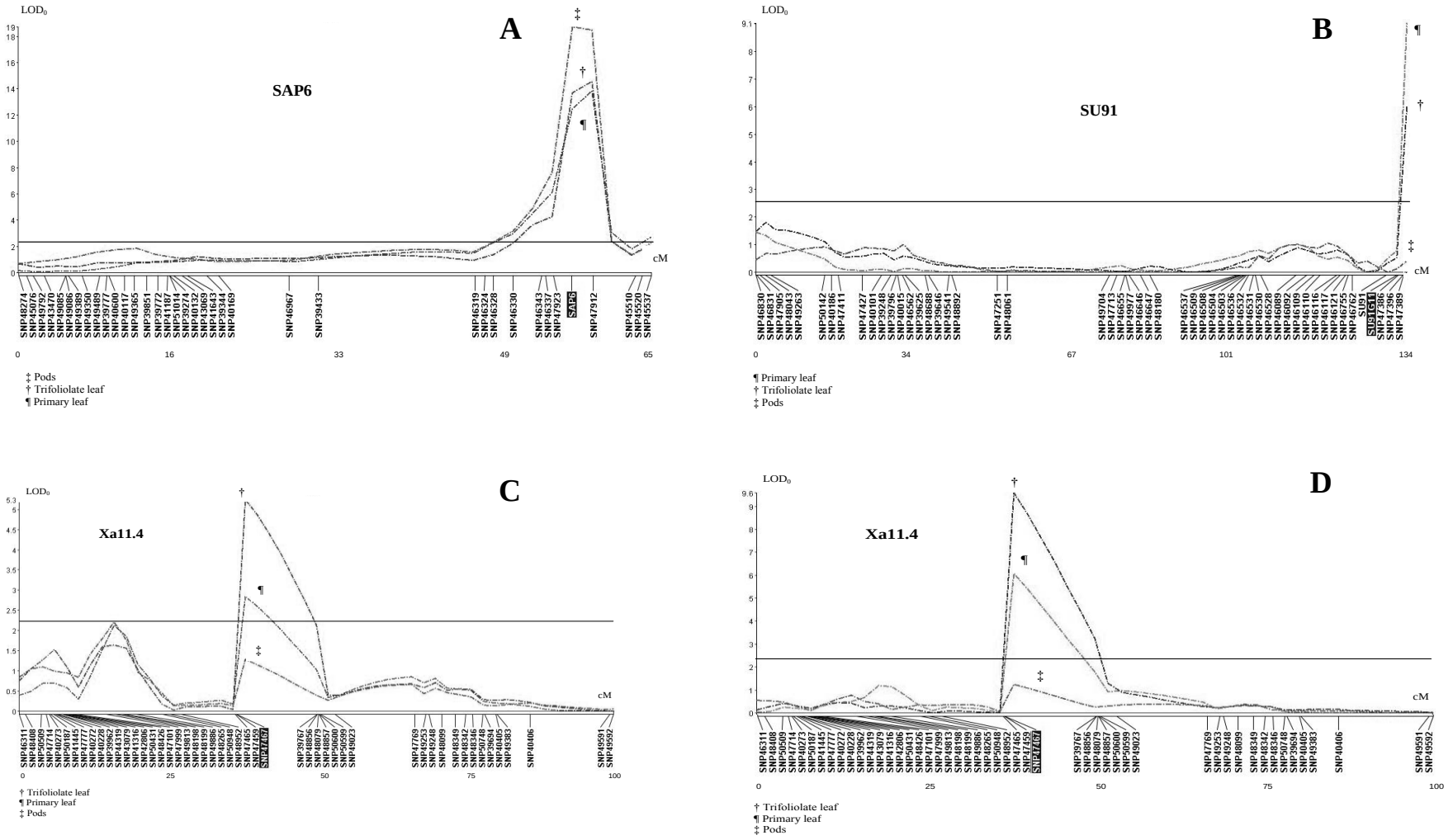

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Figure 2.2. Composite interval mapping logarithm of the odds (LOD_0) displaying molecular markers linked with QTL conferring common bacterial blight resistance in Othello/VAX3 recombinant inbred line population of common bean. Traits showed different patterns according to the plant organ evaluated. (A) SAP6 on Pv10 against ARX8AC bacterial strain, (B) SU91 on Pv08 against Xcp25 bacterial strain, (C) Xa11.4 on Pv11 against ARX8AC bacterial strain, and (D) Xa11.4 on Pv11 against Xcp25 bacterial strain.

Chapter 3

Response of Common and Scarlet Runner Beans to Four Isolates of *Sclerotinia sclerotiorum*

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Abstract

White mold is a severe disease of common bean in cool and wet production regions worldwide. Partial resistance is found in the primary and secondary gene pools. The objectives were to determine (i) the most appropriate post-inoculation evaluation date, (ii) the presence or absence of SCAR markers linked with WM2.2, WM6.1, WM7.1, WM 7.3, and WM8.3 resistance QTL, and (iii) the most resistant genotypes. Thirty common and one scarlet runner beans were inoculated one to three times/plant with ARS12D and ND710 isolates in the greenhouse in Idaho, and CO467 and NY133 isolates in Colorado. Evaluations at 28 days post inoculation in Idaho and 21 days in Colorado had the highest scores. Pinto 'Othello', with WM2.2 QTL, was susceptible to all isolates (scores 7 to 9) with one inoculation, and VC 13-5 with the same QTL was resistant (≤4.0) to three isolates and intermediate (>4 to <7) to ND710 after three inoculations/plant. SE155-9 with WM2.2, WM7.1, and WM8.3 QTL was resistant to CO467 and NY133 and intermediate to ARS12D and ND710; and SE152-6 with the same three QTL was resistant to all isolates after three inoculations per plant. Thus, use of multiple isolates and inoculations and delayed evaluations helped identify genotypes with higher levels of resistance.

Introduction

White mold caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most destructive diseases of common bean (*Phaseolus vulgaris* L.) in cool and wet production regions worldwide causing yield losses up to 100% (37, 39, 47). The fungus attacks more than

400 hosts, mostly dicotyledonous species (3, 4, 32, 48). In common bean, the fungus invades stems, branches, leaves, flowers, and pods. Dark-brown water soaked soft lesions are observed on stems. These lesions are caused by the presence of enzymes such as polygalacturonase, pectin methyl esterase, and oxalic acid (14, 15). Also, plant wilting and white mold growth followed by presence of black sclerotia is observed in and on the infected tissue (32, 48). A conditioned sclerotium in soil can divide carpogenically producing apothecia which contain asci with ascospores (sexual reproduction) or germinate directly through mycelia (asexual reproduction) (19, 35). Thus, the number of sclerotia in the field is crucial for the disease severity and it has been reported that one sclerotium per 5 Kg soil can cause over 40% of disease severity (1, 35). The disease is favored by low to moderate temperatures (<21ºC) and high moisture, and plants are more susceptible at pre-flowering and flowering stages (48).

Genetic studies of *S. sclerotiorum* showed polymorphism among different isolates from Brazil despite the fact that all isolates were of the same mycelial compatibility group (18). Moreover, different levels of aggressiveness have been reported among isolates from Spain (30) and United States (13). Disease management involved the use of cultural practices such as crop rotation, plant spacing, row orientation to prevailing winds, reduced frequency of irrigation, and deep plowing before planting (2, 31). Furthermore, the application of fungicides at initiation of and during flowering minimized the spread and severity of white mold (55). Physiological resistance, hereto referred as resistance (i.e., ability of the host genotype to stop white mold pathogen infection and disease in greenhouse inoculations either before reaching to or at the first post-inoculation node on the main stem and branches), and plant architectural avoidance traits (mostly expressed in the field) are used for cultivar

development (12, 21, 26). Resistance is related with oxalate sensitivity in common bean (11) and scarlet runner bean (*P. coccineus* L.) (6). Avoidance mechanisms are associated with plant architectural traits such as upright growth habit, porous canopy, and resistance to lodging, which help reduce the establishment and spread of the disease (12, 21, 26).

Navy bean 'ICA Bunsi' (synonymous with 'Ex-Rico 23') was the first cultivar reported to have partial resistance to white mold within the Middle American gene pool (23, 54). Moreover, breeding line USPT-WM-1 (25) and cultivars 'ElDorado' (10) and OAC Rex (20) derived resistance from ICA Bunsi. Andean genotypes G 122, PC 50, and NY6020-4 with major quantitative trait loci (QTL) have relatively higher levels of resistance (16, 21, 22, 29). Similarly, Andean A 195, CORN 601, MO 162, and VA 19 have higher levels of resistance (37, 46). Within the secondary gene pool, *P. coccineus* accessions PI 433246, PI 439534, and others possess higher levels of resistance (9, 36). Furthermore, interspecific breeding lines (BL) derived from *P. coccineus* such as 92BG-7, I9365-25, I9365-31, VCW 54, and VCW 55, and VRW 32 derived from *P. costaricensis* (24, 41, 43) also exhibit partial resistance depending on fungal isolate used. But, more recently developed interspecific pinto BL derived from *P. coccineus* PI 439534 (the VC13 series) have even higher levels of resistance (45). Similarly, breeding lines combining resistance from different gene pools displayed higher levels of resistance (44, 51, 53).

Resistance to white mold is inherited quantitatively and 27 quantitative trait loci (QTL) for resistance, and 36 QTL for avoidance mechanisms, in 21 regions across the nine linkage groups, have been identified (26, 46). Furthermore, 13 plant architectural avoidance QTL are co-located with 13 QTL for resistance (26). Out of nine sequence characterized amplified region (SCAR) markers, *Phs-T*, SAU.1350, SMe1Em5.110, SF12R9.350,

SF6Em3.220, and SF13R10.410 from Andean common bean and SF18R7.410/415 from I9365-31 derived from *P. coccineus* are associated with white mold resistance in greenhouse tests (21, 22, 46).

The greenhouse screening is used for detection of resistance. The greenhouse straw test or cut-stem method allows use of multiple *S. sclerotiorum* isolates, multiple inoculations per plant at different growth stages, and evaluations for prolonged period on the same plant, thus, selecting genotypes with higher levels of resistance (44, 45, 52). Previous studies used one to two inoculations per plant and evaluations from 8 to 33 days for greenhouse screening (30, 46, 53). The objectives of this study were to determine (i) the most appropriate post-inoculation evaluation date, (ii) the presence or absence of SCAR markers linked with WM2.2, WM6.1, WM7.1, WM 7.3, and WM8.3 resistance QTL, and (iii) the most resistant genotypes.

Materials and Methods

***Phaseolus* Bean Genotypes.** *P. coccineus* PI 439534 (36) and 30 common bean genotypes were included in this study. Of 30 common bean genotypes, four were previously developed pinto bean, namely ‘Chase’ (7), ‘UI 320’ (27), ‘Othello’ (5), and USPT-WM-1 (25); five were recently developed interspecific pinto breeding lines (VC13-1, VC13-3, VC13-4, VC13-5, VC13-6) derived from UI 320*2/PI 439534 (45); ICA Bunsi (20, 23, 54); five were previously reported white mold resistant Andean genotypes (A 195, G 122, NY6020-4, PC 50, VA 19) (33, 38, 40, 46); three were previously reported interspecific BL (92BG-7, I9365-31, VCW54) derived from the crosses between *P. vulgaris* and *P. coccineus* (24, 43); one

interspecific BL (VRW 32) was derived from *P. costaricensis* (41); and eleven were recently developed BL (SE 152-6, SE 152-8, SE153-1, SE153-3, SE153-6, SE153-7, SE154-1, SE154-5, SE154-9, SE154-10, SE155-9) with pyramided resistance (44). The sources of resistance for these BL were: SE152=CORN501/G 122//A 195/VCW 55, SE153=USPT-WM-1/CORN601//USPT-CBB-1/92BG-7, SE154=VA 19/MO 162//A 195/G 122, and SE155=A 195/4/NY6020-4/92BG-7///MO 162/I9365-25//ICA Bunsi/G 122 (44).

Inoculum Preparation and Greenhouse Evaluation. Four isolates of *S. sclerotiorum*, namely ARS12D, CO467, ND710, and NY133 of varying aggressiveness (17, 28, 49) were used for inoculation and evaluation of the response to white mold. Fresh (48 h old cultures kept at 22ºC) mycelium was multiplied as needed from sclerotia before each inoculation for each isolate (36, 42). A randomized complete block design with six replications was used in a factorial design. Three plants were sown in a 16.5 x 20.3 cm plastic pot for each genotype and replicate. The 31 genotypes were randomized within each isolate. Response to isolates ARS12D and ND710 was evaluated in the greenhouse at University of Idaho, Kimberly in 2012. Response to isolates CO467 and NY133 was evaluated in the greenhouse at Colorado State University, Fort Collins in 2013. Plants were inoculated beginning at the fifth internode (approximately one month after planting) leaving a 3 cm-long internode intact. A 200 ul eppendorf tip stacked with three plugs of fresh mycelia was used for each inoculation. The second and third inoculations were made 7 and 14 days after the first inoculation, respectively, only on the resistant (scores ≤ 4) plants. Inoculated plants were kept under high humidity (>80%) using humidifiers situated under the greenhouse benches and keeping greenhouse floors wet for one week after each inoculation. Plants were grown at mean day temperature of 24ºC and mean night temperature of 18ºC with 12 h of light. Disease severity

was evaluated at 7, 14, and 21 days post the first inoculation in Colorado and 7, 14, 21, 28, and 35 days in Idaho using a 1 to 9 scale according to Terán et al. (50). Plants were considered resistant with white mold scores of 1 to 4, intermediate >4 to <7, and susceptible 7 to 9. Data were recorded on a single-plant basis.

Molecular Marker Analysis. The DNA was extracted from the bulk sample of emerging trifoliolate leaves over all replicates for each genotype using the Dellaporta protocol (8). The DNA concentration was adjusted to 10 ug/ml and then mixed with 4 ul of 5x buffer, 2.4 ul of 25 mM $MgCl_2$, 0.8 ul of 5 mM dNTP, 2 ul of oligonucleotide primers at 10 uM concentration, 8.6 ul of purified sterilized water and 0.2 Go tag (DNA polymerase). Seven SCAR markers linked with QTL conferring resistance to white mold were assayed. They were the codominant *Phs-T* (5'-AGCATATTCTAGAGCCCTCC-3') (5'-GCTCAGTTCCTCAATCTGTTC-3') phaseolin seed protein gene marker associated with WM7.1 QTL from G 122 (21), SAU5.1350 (5'-GAGCTACCGTCAGTTTACTAA-3') (5'-GAGCTACCGTGGCTTTTTTCT-3') linked with WM6.1 QTL from NY6020-4 (22), and SMe1Em5.110 (5'-CCAAACCGGATAGTCTAAAC-3') (5'-GTACGAATTAACTGACTATG-3') linked with WM2.2 QTL from VA 19 (46). For SF12R9.350, SF6Em3.220, SF13R10.410, and SF18R7.410/415 markers, the DNA was mixed with 2.5 ul of 10X buffer, 3 ul of 25 mM $MgCl_2$, 1 ul of 5 mM dNTP, 0.6 ul of oligonucleotide primers at 20 uM concentration, 14.6 ul of purified sterilized water, and 0.2 of AmpliTag Stoffel. The primers used were SF12R9.350 (5'-ATCTTAGCCGGAGCTGAGAC-3') (5'-ACGAATTTGAGATGGTTTAC-3') and SF6Em3.220 (5'-GCGTACGAATTGACATACACC-3') (5'-CACAAGCCGGATATATCTTATC-3'), which amplify the WM2.2 allele from susceptible

Benton. Also, primers SF13R10.410 (5'-GACACCGTACGAATTAACTCATTTTG-3') (5'-CGAATCTTAGCCGGCACCGAAATGG-3') linked with WM8.3 QTL from VA 19, and SF18R7.410/415 (5'-ACCGTACGAATTTGCTTAAGTG-3') (5'-GATCCAGTTACCGGAAT-3'), which is a codominant marker for WM7.3 QTL detected in the Raven/I9365-31 population (46) were used. The amplification conditions for *Phs*, SMe1Em5.110, and SAU5.1350 included 34 cycles at 94ºC for 10 min (denaturation), followed by 55ºC (annealing for *Phs* and SMe1Em5.110), and 60ºC (annealing for SAU5.1350) for 40 s, and 72ºC for 2 min (extension). The last step included a final extension of 72ºC for 5 min. For the remaining markers, the amplification conditions consisted of initial denaturation at 94ºC for 2 min, followed by 35 cycles of 94ºC for 2 min, then 65ºC (annealing temperature for SF12R9.350), 60ºC (SF6Em3.220 and SF13R10.410) and 45ºC (SF18R7.410/415) for 40 s and 72ºC for 2 min. A final extension of 72ºC for 2 min was also performed. All PCR reactions were carried out in a PTC-100 thermocycler (MJ Research Inc., Walthman, MA) and PCR products were run in 1.5 % agarose gel stained with 1% of ethidium bromide.

Data Analysis. Analysis of variance, mean disease score, and Fisher's least significant difference at $P \leq 0.05$ were determined for each data set. The range and frequency of resistant plants for each genotype also were determined for each isolate at 21 days post inoculation in Colorado and 35 days in Idaho. Simple correlation coefficient was calculated between the mean white mold responses to four isolates for each evaluation date. Data were analyzed using the SAS 9.3 PROC GLM, FREQ, and CORR procedures (34).

Results

Mean squares were significant ($P \leq 0.05$) for number of post-inoculation days to evaluation, genotypes, and genotypes x isolates in Idaho and Colorado (Table 3.1). Pathogen isolates and genotypes x number of days to evaluation interaction mean squares were only significant in Idaho. Although the effects of number of inoculations per plant were confounded with the number of post-inoculation days to evaluation, white mold scores increased from 7 to 35 days post-inoculation in Idaho (Table 3.2) and from 7 to 21 days in Colorado (Table 3.3). Significant differences ($P \leq 0.05$) were not observed between the mean white mold scores at 28 and 35 days for both isolates in Idaho. The isolate ND710 with mean disease score of 5.6 was more aggressive than ARS12D with 5.0 score in Idaho (Table 3.2). But, significant differences were not observed between CO467 and NY133 at any evaluation dates in Colorado (Table 3.3). Furthermore, significant positive correlation coefficients (ranged from 0.7 to 0.9; $P \leq 0.01$) were observed between the mean white mold scores of all isolates at 7, 14, 21, 28, and 35 days (Table 3.4).

PI 439534 and 92BG-7 lacked the seven SCAR markers (Table 3.5). Pinto Othello had the two markers derived from Benton, while ICA Bunsi and USPT-WM-1 possessed only the SF12R9.350 marker. These results are similar to those of Soule et al. (46) who reported the presence of SCAR markers from Benton linked with WM2.2 QTL in these genotypes. Interspecific BL I9365-31, VCW 54, and VRW 32 derived from the secondary gene pool had the SF18R7.410/415 co-dominant marker first identified in Raven/I9365-31 linked with WM7.3 QTL (46). All Andean and pyramided breeding lines had the *Phs* marker (WM7.1 QTL) (Table 3.5). The green bean BL NY6020-4, in addition, had the SAU5.1360 (WM6.1

QTL), and SF13R10.410 (WM8.3 QTL), and SF6Em3.220 (WM2.2 QTL). This last marker was also observed in pinto UI 320 and the VC13 series interspecific BL. The SE 152 series BL also had WM2.2 QTL (SMe1Em5.110 marker) and WM8.3 QTL, while SE155-9 possessed all markers linked with WM2.2 QTL in addition to WM7.1 and WM8.3 QTL. The pinto SE 153 series BL carried *Phs* and SF12R9.350 markers, while Chase only had the marker linked with WM2.2 QTL.

Pinto Othello was susceptible to all fungal isolates with one inoculation/plant (mean scores 7.8-9.0). Chase, UI 320, ICA Bunsi, and USPT-WM-1 were susceptible to ARS12D, ND710, and NY133 with one inoculation/plant (6.5-9.0). In contrast, USPT-WM-1 was intermediate to CO467 (5.3) with two inoculations/plant. PI 439534 was intermediate to the four isolates at 21 days post-inoculation after three inoculations per plant. But, it had susceptible scores in Idaho at 35 days (Table 3.6). Among BL derived from the secondary gene pool, VCW 54 had significantly lower white mold scores compared to 92BG-7, I9365-31, and VRW 32 to the four isolates at 21 days post-inoculation. However, VCW 54 was susceptible to ND710 at 35 days after three inoculations per plant (Table 3.6). All newly developed BL of the SE152, SE153, SE154, and SE155-9 series were significantly more resistant than Andean G 122, PC 50, and NY6020-4 in both greenhouses (Table 3.6).

Newly developed interspecific pinto VC13 series BL derived from *P. coccineus* PI 439534 were in general resistant to ARS12D and intermediate to ND710 at 21 days post-inoculation (Table 3.6). In Colorado, all five had resistant or near resistant scores to both isolates at 21 days. Breeding lines SE152-6 and SE 153-7 were resistant or near resistant to the four isolates at 21 days with three inoculations (Table 3.6). These two and VC13-5 had the highest percentage of resistant plants. In contrast, previously developed Middle American

genotypes had lower percentages of resistant plants and broader range for white mold scores (Table 3.6).

Discussion

Significant differences in white mold severity among the number of post-inoculation days to evaluation occurred in both greenhouses. Because resistance for the entire crop growing season is essential for a successful cultivar, earlier evaluations (e.g., at 7 or 14 days) could lead to misidentification of genotypes with resistant or intermediate scores. For instance, ICA Bunsi had resistant scores in response to CO467 at 7 days, but was susceptible at 21 days. Therefore, selection of most resistant genotypes should be carried out at 21 days and beyond post-inoculation and verified at maturity (44, 45). Singh et al. (44, 45) and Terán and Singh (51, 53) identified genotypes with higher levels of resistance using delayed evaluations.

Although the number of inoculations per plant and number of days post-inoculation used for evaluation were confounded, the multiple inoculations per plant and delayed evaluations increased white mold severity. Thus, identification of highly resistant genotypes required at least three inoculations per plant and evaluations at 21 or more days post-inoculations. For example, Andean genotype SE152-6 exhibited resistant or near resistant scores up to 35 days post-inoculation and maintained that score until harvest. In contrast, one inoculation was enough to determine that pinto Othello was susceptible to all fungal isolates at 7 days. In practice, when dealing with higher plant populations, discarding susceptible

genotypes in earlier evaluations would optimize the use of greenhouse space. This should minimize expense, time, and labor used for the subsequent inoculum multiplications, inoculations, and evaluations.

Variation among *S. sclerotiorum* isolates in multi-site greenhouse and field screening nurseries of common bean have been reported (17, 28). Thus, if we only used the most aggressive isolate ND710 in Idaho, we would probably select only resistant genotypes SE152-6 and SE154-1. But, pinto VC13-5, VC13-6, SE153-1, SE153-6, and SE153-7 with higher levels of resistance to other isolates would be discarded. In contrast, if only less aggressive isolates ARS12D or CO467 were used the differentiation among the VC13 series of BL would have been difficult. Thus, using fungal isolates of varying aggressiveness enhances the selection of genotypes with higher isolate-specific as well as broad-spectrum resistance.

Among the Andean beans, A 195 and VA 19 were the most resistant genotypes to four isolates, supporting previous results (46, 51, 53). A 195 and VA 19 are derived from 'Red Kloud', and both possessed the WM2.2, WM7.1, and WM8.3 QTL (21, 46, this study). Thus, it would be important to determine the white mold response and confirm if Red Kloud carries these three and any other resistance QTL expressed in the greenhouse and field in future studies. Also, G 122 and PC 50 carried the WM2.2, WM7.1, and WM8.3 QTL, but they had higher mean white mold scores compared to A 195 and VA 19. Thus, A 195 and VA 19 may possess additional genes/QTL or different resistance allele(s) that would be important to identify and tag. The WM2.2 QTL from VA 19 was also observed in all Andean genotypes with the exception of NY6020-4 and the SE153 series pinto BL which did not have VA 19 in their pedigree. But, the WM2.2 QTL from Benton was observed in the SE153 series BL despite the fact that highly susceptible Benton (46) was not used as a parent in their

development. However, the QTL was derived from USPT-WM-1 which is one of the parents and also possesses the SF12R9.350 marker linked with WM2.2 QTL (46, this study). Similarly, this QTL was noted in SE155-9, which had ICA Bunsi as one of the parents. Nonetheless, the SF6Em3.220 marker linked with the WM2.2 QTL and SF13R10.410 linked with WM8.3 QTL derived from VA 19 were also observed in SE 155-9. Miklas and Delorme (22) and Soule et al. (46) also reported the presence of these three QTL in NY6020-4. Although the WM6.1 QTL was identified in NY6020-4 (22), we did not observe it in Andean SE155-9. The WM7.3 QTL from interspecific BL I9365-31 was observed in interspecific BL VCW 54 and VRW 32. Thus, WM7.3 QTL from I9365-31 derived from *P. coccineus* may also be present in *P. coccineus* G 35172 (43) used in VCW 54 and *P. costaricensis* G 40604 (41) used in VRW 32 as white mold resistant donor parents.

Breeding line SE152-6 with pyramided white mold resistance was significantly more resistant than A 195 in Idaho. Similarly, SE155-9 was more resistant than VA 19 in Colorado. Moreover, both genotypes had higher percentage of resistant plants compared to their other parents, namely G 122, NY6020-4, ICA Bunsi, USPT-WM-1, 92BG-7, I9365-31, and VCW 54 in both greenhouses. Thus, pyramiding white mold resistance from diverse germplasm sources is a sound strategy to maximize selection gains and breeding for higher levels of a broad-spectrum white mold resistance (51, 53).

Among pinto genotypes, the VC13 series interspecific BL derived from UI 320*2/PI 439534, had higher levels of resistance compared to UI 320, Chase, and USPT-WM-1. These new BL also had significantly higher levels of resistance than most of the previously developed interspecific BL (92BG-7, I9365-31, VCW 54, VRW 32). Although UI 320 had the WM2.2 QTL, it was susceptible to most isolates. Thus, *P. coccineus* PI 439534 very likely

contributed higher levels of white mold resistance in the VC13 series BL. Schwartz et al. (36) identified a dominant resistance gene in UI 320/PI 439534, which needs to be tagged and mapped for marker-assisted selection. The range and percentage of resistant plants, in addition to mean white mold scores, were used for development of the VC (44) and SE (45) series of BL. Consequently, some of these newly developed BL had 100% of resistant plants in response to some isolates (e.g., VC13-1 and VC13-5 in response to ARS12D) and comparatively low mean white mold scores. Thus, selection gains may be considerably enhanced by using together the range, percentage of resistant plants, and mean disease scores in the selection process.

In summary, identification of most resistant genotypes required use of multiple pathogen isolates per plant, three inoculations per plant, and delayed evaluations (21 to 35 days post inoculation). Furthermore, the range, percentage of resistant plants, and mean white mold scores were used in the selection process from the early segregating generations (44, 45). Thus, highly resistant interspecific pinto breeding lines (i.e., the VC13 series) from UI 320*2/PI 439534 and 11 BL with pyramided resistance (the SE series) were developed. Their white mold resistance was significantly higher than that of previously developed Middle American pinto BL and cultivars, Andean beans, and interspecific BL derived from secondary gene pool *Phaseolus* species. Effectiveness of these newly developed BL to combat white mold disease with and without fungicides and other control strategies should be determined. Also, their high levels of resistance should be transferred into cultivars of different market classes.

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Table 3.1. Analysis of variance for the response of 30 common and one scarlet runner beans to two isolates of *Sclerotinia sclerotiorum* in the greenhouse each at University of Idaho, Kimberly in 2012 and Colorado State University, Fort Collins in 2013.

	Idaho		Colorado	
Source	**df**	**Mean squares**	**df**	**Mean squares**
Pathogen isolate (I)	1	177.45*	1	5.55
Days post inoculation for evaluation (D)	4	185.65*	2	299.31*
I x D	4	2.04*	2	3.18*
Replicate (I x D)	50	1.21	30	18.19
Genotype (G)	30	101.31*	30	48.75*
G x I	30	2.90*	30	4.86*
G x D	120	1.41*	60	0.87
G x I x D	120	0.31	60	0.29
Error	1500	0.54	900	1.21

*Significant at $P \leq 0.05$.

Table 3.2. Mean white mold score at 7, 14, 21, 28, and 35 days post-inoculation in response to isolates ARS12D and ND710 of *Sclerotinia sclerotiorum* in common bean, evaluated in the greenhouse at University of Idaho, Kimberly in 2012.

Pathogen isolate	**Number of days post-inoculation**						
	7	**14**	**21**	**28**	**35**	**Mean**	**LSD *(P* ≤ 0.05)** [a]
ARS12D	4.1	4.6	5.0	5.5	5.7	5.0	0.4
ND710	4.6	5.1	5.6	6.2	6.5	5.6	0.4
Mean	4.4	4.9	5.3	5.9	6.1	5.3	0.2
LSD *(P* ≤ 0.05) [b]	0.1	0.1	0.1	0.1	0.1	0.1	

[a]To compare means between number of days post-inoculation.

[b]To compare means within isolates.

Table 3.3. Mean white mold score at 7, 14, and 21 days post inoculation in response to isolates CO467 and NY133 of *Sclerotinia sclerotiorum* in common bean, evaluated in the greenhouse at Colorado State University, Fort Collins in 2013.

Pathogen isolate	**Number of days post inoculation**				
	7	**14**	**21**	**Mean**	**LSD *(P* ≤ 0.05)** [a]
CO467	3.4	4.4	5.4	4.4	0.5
NY133	3.7	4.6	5.3	4.5	0.5
Mean	3.6	4.5	5.4	4.5	0.6
LSD *(P* ≤ 0.05) [b]	0.7	0.6	0.3	0.5	

[a]To compare means between number of days post-inoculation.
[b]To compare means within isolates.

Table 3.4. Simple correlation coefficient for the response of 30 common and one scarlet runner beans at different evaluation dates to two *Sclerotinia sclerotiorum* isolates in the greenhouse each at University of Idaho, Kimberly in 2012 and Colorado State University, Fort Collins in 2013.

Number of days post inoculation		**Pathogen isolate**		
		CO467	**ND710**	**NY133**
7	ARS12D	0.7**	0.9**	0.7**
	CO467	-	0.7**	0.8**
	ND710	-	-	0.6**
14	ARS12D	0.8**	0.9**	0.7**
	CO467	-	0.8**	0.8**
	ND710	-	-	0.7**
21	ARS12D	0.9**	0.9**	0.7**
	CO467	-	0.9**	0.8**
	ND710	-	-	0.7**
28	ARS12D	-	0.9**	-
35	ARS12D	-	0.9**	-

**Significant at $P \leq 0.01$.

Table 3.5. Presence or absence of seven sequence characterized amplified region markers linked with WM2.2, WM6.6, WM7.1, WM7.3, and WM8.3 resistance quantitative trait loci in 30 common and one scarlet runner beans determined at University of Idaho, Kimberly in 2013**.**

	WM2.2 QTL			**WM6.1 QTL**	**WM7.1 and 7.3 QTL**		**WM8.3 QTL**
Genotype	**SMe1Em5.110**	**SF12R9.350**	**SF6Em3.220**	**SAU5.1360**	***Phs-T***	**SF18R7.410/415**	**SF13R10.410**
Susceptible checks							
Othello	- [a]	+	+	-	-	-	-
Chase	-	+	-	-	-	-	-
UI 320	-	-	+	-	-	-	-
White mold resistance donor parents							
ICA Bunsi	-	+	-	-	-	-	-
USPT-WM-1	-	+	-	-	-	-	-
92BG-7	-	-	-	-	-	-	-
PI 439534	-	-	-	-	-	-	-
I9365-31	-	-	-	-	-	+	-
VCW 54	-	-	-	-	-	+	-
VRW 32	-	-	-	-	-	+	-
A195	+	-	-	-	+	-	+
G122	+	-	-	-	+	-	+
PC 50	+	-	-	-	+	-	+
NY 6020-4	-	-	+	+	+	-	+
VA 19	+	-	-	-	+	-	+
Breeding lines derived from *P. coccineus* PI 439534							
VC 13-1	-	-	+	-	-	-	-
VC 13-3	-	-	+	-	-	-	-
VC 13-4	-	-	+	-	-	-	-
VC 13-5	-	-	+	-	-	-	-

	WM2.2 QTL			WM6.1 QTL	WM7.1 and 7.3 QTL		WM8.3 QTL
Genotype	**SMe1Em5.110**	**SF12R9.350**	**SF6Em3.220**	**SAU5.1360**	***Phs-T***	**SF18R7.410/415**	**SF13R10.410**
VC13-6	-	-	+	-	-	-	-
Pyramided breeding lines							
SE 152-6	+	-	-	-	+	-	+
SE 152-8	+	-	-	-	+	-	+
SE 153-1	-	+	-	-	+	-	-
SE 153-3	-	+	-	-	+	-	-
SE 153-6	-	+	-	-	+	-	-
SE 153-7	-	+	-	-	+	-	-
SE 154-1	+	-	-	-	+	-	+
SE 154-5	+	-	-	-	+	-	+
SE 154-9	+	-	-	-	+	-	+
SE 154-10	+	-	-	-	+	-	+
SE 155-9	+	+	+	-	+	-	+

[a]A minus (-) indicates the absence of the marker and a plus (+) its presence.

Table 3.6. Seed type, range, mean score, and frequency of resistant plants for susceptible checks and resistant common and scarlet runner beans at 35 days post-inoculation to *Sclerotinia sclerotiorum* isolates ARS12D and ND710 at University of Idaho, Kimberly in 2012 and at 21 days post-inoculation to isolates C0467 and NY133 at Colorado State University, Fort Collins in 2013.

		Idaho						Colorado								
		ARS12D			ND710			CO467			NY133			Overall		
Genotype	**Seed type**	**Range**	**Mean**	**RP[a]**	**Range**	**Mean**	**RP**	**Range**	**Mean**	**RP**	**Range**	**Mean**	**RP**	**Range**	**Mean**	**RP**
Susceptible checks																
Othello	Pinto	9 [b]	9.0	0	9	9.0	0	8-9	8.9	0	3-9	8.2	5.6	8-9	8.8	1.4
Chase	Pinto	7-9	8.7	0	9	9.0	0	4-9	7.2	18.8	4-9	7.7	5.9	4-9	8.1	6.2
UI 320	Pinto	6-9	8.5	0	7-9	8.6	0	1-9	6.6	33.3	3-9	6.8	11.1	1-9	7.6	11.1
White mold resistance donor parents																
ICA Bunsi	Navy	6-9	7.5	0	7-9	8.6	0	4-9	7.3	6.3	4-9	7.7	6.3	4-9	7.8	3.1
USPT-WM-1	Pinto	8-9	8.9	0	7-9	8.9	0	4-9	6.5	22.2	3-9	5.3	38.9	3-9	7.4	15.3
92BG-7	Black	4-9	6.8	16.7	6-9	7.9	0	4-9	7.2	5.9	5-9	6.9	0	4-9	7.2	5.6
PI 439534	Beige mottled	3-9	6.7	25	7-9	8.2	0	3-8	5.7	17.6	3-8	4.4	42.9	3-9	6.3	21.4
I9365-31	Black	4-8	6.5	12.5	4-9	7.0	5.9	3-9	7.3	11.8	3-9	6.0	35.3	3-9	6.7	16.4
VCW 54	Black	4-8	5.6	35.3	4-9	7.5	5.6	3-8	5.8	23.5	3-7	4.1	77.8	3-9	5.8	35.5
VRW 32	Grayish brown	4-9	7.1	16.7	7-9	7.4	0	4-9	6.3	27.8	3-8	5.2	33.3	3-9	6.5	19.4
A 195	Beige	4-9	4.3	88.2	4-8	5.1	55.6	3-6	4.8	33.3	4-8	5.9	16.7	3-9	5.0	48.5
G 122	Cranberry	4-9	5.1	61.1	4-9	6.6	22.2	3-9	6.6	22.2	4-9	6.4	16.7	3-9	6.2	30.6
PC 50	Red mottle	4-9	6.8	28.6	4-9	6.8	17.7	4-9	6.3	31.3	4-9	7.8	5.9	4-9	6.9	20.8
NY 6020-4	White	4-9	6.2	33.3	4-9	6.7	25	4-7	4.9	53.3	4-9	6.3	26.7	4-9	6.0	34.6
VA 19	Light red kidney	4-8	4.9	62.5	4-8	6.0	25	2-9	4.8	52.9	3-9	5.0	50	2-9	5.2	47.6
Interspecific breeding lines derived from *P. coccineus* PI 439534																
VC13-1	Pinto	2-4	3.9	94.4	4-8	6.3	16.7	2-7	4.2	66.7	4-9	5.9	16.7	2-9	5.1	48.6

		Idaho			Colorado											
		ARS12D			ND710			CO467			NY133			Overall		
Genotype	**Seed type**	**Range**	**Mean**	**RP[a]**	**Range**	**Mean**	**RP**	**Range**	**Mean**	**RP**	**Range**	**Mean**	**RP**	**Range**	**Mean**	**RP**
VC13-3	Pinto	3-7	4.5	77.8	4-8	5.6	38.9	2-9	4.0	88.9	2-8	4.9	55.6	2-9	4.8	65.3
VC13-4	Pinto	3-9	4.7	77.8	4-9	6.2	27.8	3-6	4.5	55.6	3-7	3.8	83.3	3-9	4.8	61.1
VC13-5	Pinto	3-4	3.8	100	4-8	5.8	38.9	2-9	4.0	77.8	2-6	4.0	66.7	2-9	4.4	70.8
VC13-6	Pinto	3-8	4.7	66.7	4-9	5.6	50	3-5	4.0	83.3	3-7	4.1	83.3	2-9	4.6	70.8
Pyramided breeding lines																
SE152-6	Grayish brown	3-4	3.9	100	4-6	4.2	88.9	3-6	4.1	77.8	3-6	4.5	50	3-6	4.2	79.2
SE152-8	Grayish black	4-9	4.6	88.2	4-7	4.8	72.2	3-9	5.3	38.9	2-8	4.4	38.9	2-9	4.8	59.6
SE153-1	Pinto	4-7	5.4	50	4-9	6.7	16.7	3-7	4.1	81.3	3-5	3.8	72.2	3-9	5.0	55
SE153-3	Pinto	4-7	4.5	82.4	4-8	5.4	50	3-5	4.0	88.9	3-6	4.3	55.6	3-8	4.5	69.2
SE153-6	Pinto	3-8	4.4	83.3	4-9	6.1	22.2	3-7	4.3	66.7	3-7	4.3	44.5	3-9	4.8	54.2
SE153-7	Pinto	3-7	4.2	77.8	4-9	5.1	61.1	2-7	4.3	66.7	3-5	3.5	88.2	3-9	4.3	73.5
SE154-1	Cream mottle	4	4.0	100	4-7	4.3	88.9	3-8	4.7	61.1	3-9	5.3	50	3-9	4.6	75
SE154-5	Yellow opaque	4-9	5.2	50	4-8	5.0	55.6	3-9	5.4	50	2-8	5.1	55.6	2-9	5.2	52.8
SE154-9	Yellow opaque	4-9	5.4	50	4-9	5.6	44.4	3-8	4.7	61.1	3-8	4.7	44.4	3-9	5.1	50
SE154-10	Yellow opaque	4-8	4.6	77.8	4-9	5.0	61.1	3-9	5.5	38.9	3-7	4.8	44.5	3-9	5.0	55.6
SE155-9	Yellow opaque	4-9	5.5	50	4-9	5.6	50	3-6	4.1	72.2	3-7	4.0	77.8	3-9	4.8	62.5
Mean		…	5.7	51.8	…	6.5	30.3	…	5.4	46.3	…	5.3	41.9	…	5.7	42.6
LSD ($P \leq 0.05$)		…	1.1	…	…	1.0	…	…	1.4		…	1.3	…	…	…	…

[a]Percentage of resistant plants.

[b]White mold score, where 1= no sign of infection ; 3= infection did not reach the first internode, but passed more than 2.5 cm after the point of inoculation ; 5= infection passed the first post inoculation node but no more than 2.5 cm , 7= infection reached the second node after inoculation, and 9=fungus invasion passed the second node with or without causing plant death.

Chapter 4

Inheritance of White Mold Resistance in an Andean Common Bean A 195 and its Relationship with Andean G 122

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Abbreviation: QTL, quantitative trait loci; SCAR, sequence characterized amplified region.

Abstract

White mold caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a devastating disease of common bean in cool and wet production regions of the Americas and elsewhere. Partial resistance is found in common bean, wild bean, and *Phaseolus* species of the secondary gene pool. The objectives of this study were to determine (1) the inheritance of white mold resistance in a large-seeded Andean common bean A 195 in response to contrasting *S. sclerotiorum* isolates, and (2) the genetic relationship between A 195 and Andean G 122. White mold susceptible pinto 'Othello' was crossed with resistant A 195, and A 195 was crossed with resistant G 122. The F_1 and the three parents were inoculated at the 5^{th} internode with an aggressive isolate ND710 in 2011. Parents and F_2 from resistant F_1 plants (scores ≤ 4) were inoculated with the less aggressive isolate ARS12D, and only resistant plants were re-inoculated with ND710 and evaluated at 35 d in 2012. Two independent complementary dominant genes controlled resistance in the F_2 of Othello/A 195 in response to each of the two isolates. There was no segregation in the A 195/G 122 F_2 in response to ARS12D. But, a single dominant gene controlled the difference in resistance in the F_2 between A 195 and G 122 in response to ND710. The F_3 results corroborated the F_2 segregation ratios in both populations. This information should help introgress and pyramid higher levels of resistance into common bean cultivars of different market classes. Availability of tightly linked SCAR marker with resistance genes in A 195 should facilitate marker-assisted selection and development of white mold resistant cultivars.

Introduction

White mold caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a severe disease of common bean (*Phaseolus vulgaris* L.) in the cool and wet production regions of the Americas and elsewhere. Yield losses up to 100% have been reported (Singh and Schwartz, 2010). Also, differences in aggressiveness of *S. sclerotiorum* isolates occur (Otto-Hanson et al., 2011; Pascual et al., 2010). Use of resistant cultivars is an environmentally friendly, economical, and efficient strategy for white mold control.

Among the small-seeded (<25 g 100 seeds) Middle American gene pool, navy bean 'ICA Bunsi' was reported to have partial resistance to white mold (Miklas et al., 2004; Tu and Beversdorf, 1982). Small-seeded navy bean 'OAC Rex' (Michaels et al., 2006) and medium-seeded (25 to 40 g 100 seeds) pinto beans USPT-WM-1 (Miklas et al., 2006) and 'ElDorado' (Kelly et al., 2012) also derived their resistance from ICA Bunsi. Large-seeded (>40 g 100 seeds) Andean genotypes A 195, CORN 601, G 122, NY 6020-4, PC 50, and VA 19 have relatively high levels of white mold resistance compared to Middle American genotypes (Schwartz and Singh, 2013; Terán and Singh, 2010; Viteri et al., 2014). But, higher levels of resistance have been identified in the secondary gene pool such as *P. coccineus* accessions PI 433246 and PI 439534 (Gilmore et al., 2002; Schwartz et al., 2006; Singh et al., 2009a). Moreover, interspecific breeding lines derived from the secondary gene pool such as 92BG-7, I9365-25, and I9365-31 (Miklas et al., 1998), and VCW 54 and VCW 55 (Singh et al., 2009b) from *P. coccineus*, and VRW 32 derived from *P. costaricensis* (Singh et al., 2013) also exhibit partial resistance.

Most of the resistance (detected in the greenhouse) in primary and secondary gene

pools and plant architectural avoidance traits (mostly detected in the field tests) are inherited quantitatively (Miklas et al., 2013; Soule et al., 2011). However, a single recessive gene controlled white mold resistance in the greenhouse, and a dominant gene was responsible in the field evaluation in A 195/'Lime Light' Andean x Middle American inter-gene pool common bean population (Genchev and Kiryakov, 2002). Furthermore, Abawi et al. (1978) reported a single dominant gene responsible for white mold resistance in the greenhouse test in *P. coccineus* accession PI 175829. Schwartz et al. (2006) also reported a single dominant resistance gene in 'Othello'/PI 433246 and 'UI 320'/PI 439534 common bean x *P. coccineus* interspecific crosses in the greenhouse tests. But, within the secondary gene pool resistant x susceptible *P. coccineus* crosses, Myers and Stotz (2002) found two or three recessive alleles controlling white mold resistance.

Most recent studies using molecular markers identified >20 quantitative trait loci (QTL) for white mold resistance distributed across the eleven linkage groups on the common bean genome. White mold resistance is controlled by 27 QTL while avoidance mechanisms related with plant architectural traits are controlled by 36 QTL, which coalesced into 18 regions of the genome (Miklas et al., 2013; Soule et al., 2011). Of these two groups, 13 QTL are co-located across the common bean genome conferring resistance and plant architectural avoidance traits (Miklas et al., 2013). Readers interested in more detailed information on QTL analysis for white mold resistance and avoidance traits should refer to Ender and Kelly (2005), Miklas and Delorme (2003), Miklas et al. (2013), Mkwaila et al. (2011), Park et al. (2001), Pérez-Vega et al. (2012), and Soule et al. (2011).

Large-seeded Andean A 195 and G 122 possess high levels of white mold resistance to a broad spectrum of pathogen isolates (McCoy et al., 2014; Otto-Hanson et al., 2011; Singh

et al., 2014a, b; Viteri et al., 2014). The inheritance of white mold resistance in A 195 has never been verified against contrasting *S. sclerotiorum* isolates in North America. In the case of G 122, quantitative inheritance was reported with QTL on Pv01, Pv07, and Pv09 linkage groups conferring resistance in greenhouse and field evaluations (Maxwell et al., 2007; Miklas et al., 2001). Also, G 122 possesses three SCAR (sequence characterized amplified region) markers linked with white mold resistance QTL, including *Phs-T* (WM7.1 QTL) that is present in most large-seeded Andean beans (Gepts et al., 1986; Miklas et al., 2001). The objectives of this study were to determine (1) the inheritance of white mold resistance in a large-seeded Andean common bean A 195 in response to contrasting *S. sclerotiorum* isolates, and (2) the genetic relationship between A 195 and G 122, both Andean beans with high levels of white mold resistance.

Materials and Methods

Parental Selection and Population Development

Othello, A 195, and G 122 were screened in the greenhouse at University of Idaho, Kimberly in 2011, using the cut-stem inoculation method (Terán et al., 2006). Othello is a pinto bean cultivar (Burke et al., 1995) highly susceptible to white mold (Singh et al., 2014a, b; Viteri et al., 2014) with an indeterminate growth habit Type III (Singh, 1982). A 195 has large opaque beige (resembling “bayo” or “canela” market class in international trade) colored seed and G 122 is a cranberry bean type. Both Andean genotypes have determinate growth habit Type I and partial white mold resistance (Singh et al., 2007; Singh et al., 2014a, b; Viteri et al., 2014). Eighteen seeds of each parent were planted in six 16.5 x 20.3 cm plastic pots (3 seed

per pot). Plants were inoculated with an aggressive *S. sclerotiorum* isolate ND710 (Otto-Hanson et al., 2011) at the fifth internode leaving a 3 cm-long internode intact. Three mycelial plugs stacked in a 200 uL eppendorf tip from 48 h old culture at 22ºC in potato dextrose agar (Difco) amended with 200 mg/L of Streptomycin were used. Inoculated plants were kept under high humidity (>80%) with mean day temperature of 24ºC and night of 18ºC with 12 h of light. Plants were evaluated at 21 d post inoculation, using a 1 to 9 scale, where 1= no sign of infection in the inoculated internode, and 9=fungus invasion past the second node with or without plant death (Terán et al., 2006). Resistant (scores ≤ 4) A 195 plants were crossed with susceptible (scores 9) Othello and resistant G 122 plants. Only hybrid seed from A 195 female plants that were resistant until maturity were harvested for both F_1 crosses.

Screening of F_1, F_2, and F_3 for White Mold Response

Seventeen seeds of the Othello/A 195 F_1, 10 of A 195/G 122 F_1, and nine of each parent were planted in the greenhouse at University of Idaho, Kimberly in 2011. Plants were inoculated with an aggressive *S. sclerotiorum* isolate ND710 at the fifth internode, using the methodology described above. The response to white mold was scored at 35 d post inoculation and verified at maturity according to Terán et al. (2006). Only seed from resistant (scores ≤ 4) F_1 plants was harvested for both crosses.

Sixty-two F_2 seed from three resistant Othello/ A 195 F_1 plants, and 102 from five resistant A 195/ G 122 F_1 plants, and nine seed of each parent were inoculated first with a less aggressive pathogen isolate ARS12D (collected from a highly infected commercial field in Tartagal, Salta, Argentina in 2012) at the 5^{th} internode in 2012. Only resistant plants were re-inoculated one week later with aggressive isolate ND710, and evaluated at 35 d post inoculation.

The F_3 progeny test also was carried out in the greenhouse in 2012, using nine plants of each parent, 165 F_3 seeds from seven resistant F_2 plants against ARS12D, 47 F_3 seeds from two resistant F_2 to ND710, and 89 F_3 seeds from six susceptible F_2 plants (scores $\geq$ 5) against ND710 for Othello/ A 195. For A 195/G 122, only 132 F_3 seeds from six resistant F_2 plants to both isolates were tested. The F_3 was also first inoculated with ARS12D and only resistant plants were re-inoculated with ND710 and evaluated at 35 d post inoculation. Pooled data from resistant (scores $\leq$4.0) or susceptible (scores $\geq$5.0) plant-to-progenies with the same segregation ratio within each generation (i.e., F_2 or F_3) were subjected to the χ^2 test to determine the inheritance of white mold resistance in Othello/A 195 and A 195/G 122 populations.

Results

Pinto Othello with a mean white mold score of 9 was susceptible to both isolates (Table 4.1 and 4.2). A 195 and G 122 were variable to both isolates, but both had lower mean white mold scores in response to less aggressive isolate ARS12D. Similarly, both F_1 hybrids were variable in response to ND710 (Table 4.1 and 4.2). Thus, only three resistant F_1 plants in Othello/A 195 and five in A 195/G122 were harvested for the F_2 test.

The Othello/A195 F_2 segregated into 26 resistant to 36 susceptible (χ^2=5.31; $P \leq 0.05$) in response to the more aggressive isolate ND710. But, a ratio of 39 resistant to 23 susceptible was observed against ARS12D, giving a good fit of 9 resistant to 7 susceptible ratio (χ^2=1.04; $P \geq 0.05$). The F_3 from heterozygous resistant F_2 plants segregated into the same 9 resistant: 7 susceptible ratios in response to ARS12D. Furthermore, a segregation ratio of 73 resistant to

31 susceptible (3 resistant: 1 susceptible) in the F_3 was also noted with presumed single-locus heterozygote genotypes. In the F_3 progeny test, out of seven resistant F_2 plants, none produced all resistant plants: four segregated in 3 resistant: 1 susceptible, and three segregated in 9 resistant: 7 susceptible ratios against ARS12D. The data gave a good fit to the expected 1:4:4 ratios for $F_{2:3}$ progenies from the resistant F_2 plants (χ^2=1.2; $P \geq 0.05$). Furthermore, the F_3 progenies from susceptible F_2 plants were all susceptible, while F_3 from two resistant F_2 plants segregated into 20 resistant to 27 susceptible ratio against ND710 isolate (Table 4.1).

The 104 plants of the A 195/G 122 F_2 segregated into 75 resistant to 29 susceptible giving a good fit of 3 resistant: 1 susceptible ratio against ND710 (χ^2=0.46; $P \geq 0.05$). But, a total of 132 F_3 plants derived from six resistant F_2 plants, also segregated into 3 resistant: 1 susceptible ratio against ND710, and not in the expected 2 resistant: 1 susceptible ratio (χ^2=4; $P \leq 0.05$). This could have occurred because of a small sample size taken in the F_2, which did not include any homozygous resistant plants, and presumably only comprised heterozygous plants similar to the F_1. In contrast, out of 104 F_2 only one, and of 132 F_3 only eight plants were susceptible in response to ARS12D (Table 4.2).

Discussion

Pinto Othello had a uniform susceptible response to *S. sclerotiorum* isolates ARS12D and ND710. But, both A 195 and G 122 were variable in response to either isolate. Furthermore, despite the fact that only resistant plants of A 195 and G 122 were used to make Othello/ A 195 and A 195/G 122 crosses, both F_1 were variable in response to ND710. Thus, A 195 and G 122 did not behave as pure lines possibly contributing to extra susceptible plants especially

against ND710, which did not have a good fit to the 9 resistant: 7 susceptible ratios in the Othello/A 195 F_2. Also, the lack of good fit to the 9 resistant: 7 susceptible ratios might have occurred because of the relatively small population size and/or segregation distortion due to being an inter-gene pool cross. However, these results considered together support that there were two independent complementary dominant genes controlling white mold resistance in Othello/A 195. Thus, our results differed from those of Genchev and Kiryakov (2002) who reported a single recessive resistance gene in A 195/Lime Light population in the greenhouse, and a dominant gene in the field test. Differences in the two studies could be due to different susceptible parents used in crosses, a different pathogen isolate used, different post inoculation dates used for evaluation, and/or differences in the greenhouse and field environments. The response of common bean genotypes are known to vary across different *S. sclerotiorum* isolates, greenhouses and/or field environments (McCoy et al., 2012; Otto-Hanson et al., 2011; Steadman et al., 2006).

The A 195/G 122 F_2 segregation into 3 resistant to 1 susceptible ratio against ND710 suggested that the difference between A 195 and G 122 was controlled by a single dominant gene. Because both genotypes and their F_1 had a similar white mold score in response to ND710, the resistance gene may be derived either from A 195 or G 122. In the case of A 195, a qualitative inheritance of resistance was found (Genchev and Kiryakov, 2002; this study). In contrast, Maxwell et al. (2007) reported five QTL located on Pv01, Pv02, and Pv09 linkage groups accounted together for 48% of phenotypic variance in G 122/CO72548 population in the greenhouse test. Also, a QTL on Pv07 linked with *Phs-T* SCAR marker was responsible for 38% of phenotypic variance in the greenhouse in A 55/G 122 population (Miklas et al., 2001). Similarly, other QTL on Pv01 (close to the fin gene) explained 18% of phenotypic

variance in the field test in the same population (Miklas et al., 2001). Both A 195 and G 122 tested positive for the *Phs-T*, WM2.2, and WM8.3 QTL (Viteri et al., 2014), therefore, it is likely that the difference between the two Andean genotypes in white mold response to the ND710 isolate is either due to a different allele at one of these three loci or a different gene from the above three QTL. Thus, it would be worthwhile to identify (1) if any or all of the white mold resistance QTL on Pv01, Pv07, and Pv09 reported by Maxwell et al. (2007) and Miklas et al. (2001) are also present in A 195, and if not (2) what is the map position of the gene differentiating the two Andean genotypes for the response to ND710 isolate. Thus, further research is needed for mapping and tagging white mold resistance genes/QTL in A 195.

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Table 4.1. White mold response of common bean pinto Othello (susceptible parent), A 195 (resistant parent), and their F_1, F_2, and F_3 against ND710 and ARS12D isolates of *Sclerotinia sclerotiorum*, evaluated in the greenhouse at University of Idaho, Kimberly in 2011 and 2012.

Genotype	No. plants	Range	Mean	Ratio[†]		χ^2	*P*
				Observed	Expected		
	S. sclerotiorum isolate ND710						
Othello	27	9	9.0 [‡]	-	-	-	-
A 195	27	4-9	5.0	-	-	-	-
Othello/A 195							
F_1	17	4-9	5.6	-	-	-	-
F_2	62[§]	4-9	6.2	26R:36S	9R:7S	5.31	≤0.05
F_3	47[§]	4-9	5.9	20R:27S	9R:7S	3.09	>0.05
	89[¶]	6-9	8.0	0R:89S	All S	-	-
	S. sclerotiorum isolate ARS12D						
Othello	27	9	9.0	-	-	-	-
A 195	27	2-9	4.3	-	-	-	-
Othello/ A 195							
F_2	62[§]	4-9	5.5	39R:23S	9R:7S	1.04	>0.05
F_3	61[§]	3-9	5.7	35R:26S	9R:7S	0.07	>0.05
	104[§]	4-9	4.9	73R:31S	3R:1S	1.28	>0.05

[†]R= resistant, and S= susceptible.

[‡]White mold scored on a 1 to 9 scale, where 1= no symptoms, and 9= fungus invasion passed the second node with or without plant death.

[§]F_2 or F_3 from resistant F_1 and F_2 plants, respectively.

[¶]F_3 from susceptible F_2 plants.

Table 4.2. White mold response of Andean common bean A 195 (resistant parent), G 122 (resistant parent), and their F_1, F_2, and F_3 against ND710 and ARS12D isolates of *Sclerotinia sclerotiorum*, evaluated in the greenhouses at University of Idaho, Kimberly in 2011 and 2012.

Genotype	*S. sclerotiorum* isolate ND710						
	No. plants	Range	Mean	Ratio[†]		χ^2	*P*
				Observed	Expected		
A 195	27	4-9	5.0[‡]	-	-	-	-
G 122	27	4-8	5.3	-	-	-	-
A195/G122							
F_1	10	4-7	4.9	-	-	-	-
F_2	104[§]	2-9	4.8	75R:29S	3R:1S	0.46	>0.05
F_3	132[§]	4-9	4.9	98R:34S	3R:1S	0.04	>0.05
	S. sclerotiorum isolate ARS12D						
A 195	27	2-9	4.3	-	-	-	-
G 122	27	2-7	4.0	-	-	-	-
A195/G122							
F_2	104[§]	1-5	3.5	103R:1S	All R	-	-
F_3	132[§]	2-7	4.1	124R:8S	All R	-	-

[†]R= resistant, and S= susceptible.

[‡]White mold scored on a 1 to 9 scale, where 1= no symptoms, and 9= fungus invasion passed the second node with or without plant death.

[§]F_2 or F_3 from resistant F_1 and F_2 plants, respectively.