Genetic Characterization and Molecular Mapping *Pse-2* Gene for Resistance to Halo Blight in Common Bean

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ABSTRACT

Pseudomonas syringae pv. *phaseolicola* (Burkholder) Young et al. (*Psp*) causes halo blight, which is a serious bacterial disease of common bean (*Phaseolus vulgaris* L.). Several resistance (R) genes have been discovered in host differential cultivar ZAA 12. Our objectives were to further characterize and enable marker-assisted selection (MAS) of the *Pse-2* gene in ZAA 12 purported to have broad effect against multiple *Psp* races. A recombinant inbred population, ZAA 12 × 'Canadian Wonder', was challenged by the halo blight pathogen differential set consisting of nine *Psp* races. The resistance conferred by *Pse-2* to races 2, 3, 4, 5, 7, 8, and 9 expanded the effect known for this gene from three to seven races. Segregation in F_2 populations confirmed dominant inheritance for *Pse-2* against all races except Race 8. Resistance to Race 8 was recessively inherited and most closely fit a 7 resistant to 9 susceptible segregation ratio in the F_2 generation. Perhaps other genes are segregating that modify the effect of *Pse-2* against Race 8. A sequence characterized amplified region (SCAR) marker tightly linked with *Pse-2* was generated. The marker was used to integrate *Pse-2* to chromosome 10. A survey of lines and cultivars revealed the SCAR will have broad utility for MAS of *Pse-2*.

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Abbreviations: BA, BelNeb-RR-1 × A 55; BCMNV, *Bean common mosaic necrosis virus*; DG, DOR 364 × G 19833; HR, hypersensitive resistance; MAS, marker-assisted selection; PCR, polymerase chain reaction; *Psp*, *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young et al.; R, resistance; RAPD, random amplified polymorphic DNA; RH, relative humidity; RIL, recombinant inbred line; S, susceptible; SCAR, sequence characterized amplified region; Z12C, ZAA 12 × Canadian Wonder.

Halo blight caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young et al. (*Psp*) plagues common bean (*Phaseolus vulgaris* L.) production in moderately cool and wet regions of Africa, Europe, North America, and South America. Genetic resistance within the host can provide effective control of halo blight but is not completely understood. Five putative resistance (R) genes, 1 to 5, were tentatively identified in a host differential series developed for the disease (Taylor et al., 1996a). The host–pathogen differential series consists of one tepary bean (*Phaseolus acutifolius* A. Gray) and seven common bean host cultivars and nine *Psp* races (Table 1).

Miklas et al. (2009) confirmed earlier findings (Taylor et al., 1996a, b; Teverson, 1991; Walker and Patel, 1964) that the *Pse-1* gene derived from UI-3 (also known as Red Mexican #3) host differential cultivar conditioned resistance to races 1, 5, 7, and 9 and

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Table 1. Host–pathogen differential set for halo bacterial blight adopted from Teverson (1991) (Taylor et al., 1996a, b), as modified by Miklas et al. (2009), and updated based on results from this study, consisting of nine *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young et al. (Psp) races and seven *Phaseolus vulgaris* L. differential cultivars and one *Phaseolus acutifolius* A. Gray (tepary bean) differential cultivar 1072, with resistance (R) genes listed.

		Psp races								
Host differential	R-genes†	1	2	3	4	5	6	7	8	9
Canadian Wonder	None	+‡	+	+	+	+	+	+	+	+
ZAA 54 (A52)	*Pse-4*	+	+	+	+	–	+	+	+	+
Tendergreen	*Pse-3*	+	+	– HR	– HR	+	+	+	+	+
Red Mexican #3 (UI-3)	*Pse-1*, *Pse-4*	–	+	+	+	–	+	–	+	–
1072	?	+	–	+	–	–	+	–	+	+
ZAA 55 (A53)	*Pse-3*, *Pse-4*	+	+	– HR	– HR	–	+	+	+	+
ZAA 12 (A43)	*Pse-2*, *Pse-3*, *Pse-4*	+	–	– HR	– HR	–	+	–	–	–
Guatemala 196-B	*Pse-1*, *Pse-3*, *Pse-4*	–	+	– HR	– HR	–	+	–	+	–

†The *Pse-1* gene, conferring resistance to races 1, 5, 7, and 9, is located on linkage group 10; *Pse-2* conferring resistance to races 2, 3, 4, 5, 7, 8, and 9, which is also located on linkage group 10 (this study); *Pse-3* is completely linked with the *I* gene on linkage group 2; and *Pse-4* is unmapped and confers resistance solely to Race 5. The R gene in tepary 1072, formally listed as *Pse-2*, is no longer valid given the broader effect described for *Pse-2* from ZAA 12, and thus was replaced with a question mark.

‡+, compatible (susceptible ratings from 2 to 5); –, incompatible (resistant rating of 1); – HR, incompatible reaction with severe hypersensitive response.

had dominant inheritance. They identified sequence characterized amplified region (SCAR) markers tightly linked with *Pse-1* and used them to locate the gene to linkage group 10 of the core map (Freyre et al., 1998). A second gene in UI-3 reported by Teverson (1991), *Pse-4*, which confers resistance solely to Race 5, was also observed.

Teverson (1991) reported that the host differential cultivar ZAA 12 (A43) possessed four resistance genes: *R2* (*Pse-2*) for resistance to races 2, 5, and 7; *R3* (*Pse-3*) for resistance to races 3 and 4; *R4* (*Pse-4*) for resistance to Race 5; and *R5* (*pse-5*) conditioning recessive resistance to Race 8 and suspected to be involved in recessive resistance to Race 2. A dominant gene (unnamed) for resistance to Race 9 was also noted but not fully characterized because this race was discovered at the end of her practical studies. In addition to *Pse-3*, which was linked with the *I* gene that conditions resistance to *Bean common mosaic virus* (BCMV) and hypersensitive resistance to *Bean common mosaic necrosis virus* (BCMNV), Teverson (1991) noted the presence of a second gene from ZAA 12 conferring resistance to Race 3 but did not assign a symbol to the gene. Note that the symbols assigned to these putative R genes in parentheses above fit gene nomenclature guidelines set forth by the Bean Genetics Committee (Bassett and Myers, 1999; Myers and Weeden, 1988) and follow the precedent set by Miklas et al. (2009) in renaming the R1 and R4 genes in the host differential cultivar UI-3, *Pse-1* and *Pse-4*, respectively.

We sought to further characterize the putative R genes (*Pse-2*, *Pse-3*, *Pse-4*, and *pse-5*) in ZAA-12 discovered by Teverson (1991) and validate effects of the genes against seven differential races of the pathogen. Because *Pse-2* is effective against multiple races, and thus has high breeding value, our ultimate goal was to tag this gene with DNA markers having utility for marker-assisted selection (MAS) in bean breeding programs across the globe.

MATERIALS AND METHODS

ZAA 12, also known as A43, is a host differential cultivar that possesses resistance to races 2, 3, 4, 5, 7, 8, and 9 (Table 1). ZAA 12, from the cross G8106 × NY76-2812-15 was bred at the International Center for Tropical Agriculture (CIAT) in Cali, Colombia. Canadian Wonder is susceptible to halo blight and is one of the seven host differential cultivars (Taylor et al., 1996a). To facilitate further characterization of the resistance present in ZAA 12 and enable tagging of the *Pse-2* with broad effect, a recombinant inbred population consisting of 79 $F_{6:8}$ lines was derived from the cross ZAA 12 × Canadian Wonder (Z12C) by the single seed descent method. Futhermore, ZAA 12 was crossed with each of the *P. vulgaris* host differential cultivars Canadian Wonder, ZAA 54 (A52), Tendergreen, ZAA 55 (A53), UI-3, and Guatemala 196-B to generate F_2 populations for (i) analyzing the inheritance of the R genes present in ZAA 12, (ii) verifying the races controlled by each gene, and (iii) conducting tests of allelism with the R genes present in the other host differential cultivars. ZAA 12 was not crossed with the '1072' tepary bean (*P. acutifolius*) host differential cultivar because recovery of fertile progeny from an interspecific hybridization between *P. acutifolius* and *P. vulgaris* is extremely difficult. Residual F_1 seed from these crosses were set aside for inoculation with specific races to further discern recessive versus dominant inheritance of the R genes present in ZAA 12.

Reaction of the Z12C recombinant inbred lines (RILs) to each race (2, 3, 4, 5, 7, 8, and 9) was tested in separate greenhouse experiments by inoculating 10 plants of each RIL and parent against the corresponding race for the respective experiment. Inoculum (10^8 colony forming units ml^{-1}) was applied to 7- to 10-d-old seedlings with fully expanded primary leaves using the method of Taylor et al. (1996a). Likewise, for the F_2 populations, separate subgroups of individual F_2 plants were inoculated with the different races. Inoculated plants were kept in a humidity chamber (19°C and 100% relative humidity [RH]) for 48 h before being transferred to a greenhouse (18/25°C night/day and 70% RH). Plants were rated for infection based on leaf reaction 10 d after inoculation on a 1 to 5 scale with 1 being highly resistant and 5 being highly susceptible (Taylor et al., 1996b). Lines rated between 2 and 5 were considered susceptible.

Bulked-segregant analysis was used to identify DNA markers in the Z12C population putatively linked with *Pse-2* gene. Genomic DNA was extracted from the RILs and parents using the FastDNA Kit (Bio 101, Vista, CA) according to the manufacturer's instructions. Purified DNA was adjusted to 10 ng μl^{-1} using a fluorometer before all polymerase chain reactions (PCRs). Equal amounts of DNA from eight Z12C RILs with resistance to all seven races (2, 3, 4, 5, 7, 8, and 9) were combined to form the R DNA bulk and DNA from eight Z12C RILs susceptible to these seven races were combined to form the susceptible (S) DNA bulk. These DNA bulks were used to screen 700 decamer primers for random amplified polymorphic DNA (RAPD) bands present in the R and absent in the S bulks. Only those RAPD markers identified between the bulks that cosegregated with disease reaction across at least 13 of the 16 individual RILs comprising the bulks were subsequently assayed across the entire Z12C mapping population consisting of 79 RILs.

The RAPD protocol consisted of 25 μL reactions containing 2 U Stoffel fragment DNA polymerase (Applied Biosystems, Foster City, CA), 1x Stoffel buffer, 0.2 μM primer, 5 mM $MgCl_2$, 200 μM each deoxyribonucleotide triphosphate (dNTP), and 25 ng template DNA. Amplifications were performed on a Peltier Thermal Cycler PTC-200 (MJ Research Inc., Waltham, MA) programmed for an initial cycle at 94°C for 2 min; three cycles at 94°C for 1 min, 32°C for 1 min, and 72°C for 2 min; followed by 30 cycles of 94°C for 10 s, 37°C for 20 s, and 72°C for 2 min; with a final 5 min extension period at 72°C. Amplified products from all PCRs were separated on 1.4% agarose gels containing ethidium bromide (0.5 μg mL^{-1}) for 5 h at 3 V cm^{-1} constant voltage.

Linkage analysis of markers and categorical disease reaction to each race (a plant with a score of 1 was categorized as resistant and with a score from 2 to 5 as susceptible), for the Z12C RIL population, was performed by Mapmaker 3.0 (Lander et al., 1987) using Group and Order commands. Centimorgan distances between linked loci were based on recombination fractions using the Kosambi mapping function. One RAPD marker found tightly linked (0 cM) with the putative *Pse-2* resistance gene was cloned into the vector pCR4-Topo using the Topo TA cloning kit (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Specific primers were designed based on the terminal sequences of the cloned RAPD marker for conversion to a SCAR marker. Thermocycling parameters were optimized and a final profile was employed that consisted of a single cycle of 5 min at 95°C; 30 cycles of 1 min at 94°C, 1 min at optimum annealing temperature 60°C, and 1 min at 72°C; and a final extension for 7 min at 72°C. The SCAR, using this protocol, was assayed across a few common bean breeding lines and cultivars representative of bean genotypes from both the Andean and Middle American gene pools to determine potential utility for MAS.

To further discern application of the SCAR for MAS, the DNA from an F_2 population consisting of 122 individuals from the cross ZAA 12 × Canadian Wonder was extracted and then assayed for the marker as described above. Subsets of 10 to 15 F_3 progeny from each F_2 plant were inoculated separately with races 2, 7, 8, and 9 to determine *Pse-2* genotype (*Pse-2//Pse-2*, *Pse-2//pse-2*, or *pse-2//pse-2*) of individual F_2 plants. Disease screening followed the same protocol as above. Therefore, cosegregation between the marker and disease resistance in this F_2 population was based on $F_{2:3}$ progeny tests for halo blight reaction.

To integrate the *Pse-2* resistance gene into the *Phaseolus* linkage map (Freyre et al., 1998; Kelly et al., 2003), the SCAR marker tightly linked with *Pse-2* was assayed across the DOR 364 (also known as Dorado) × G 19833 (DG) core mapping population (Blair et al., 2003; Beebe et al., 2006) at CIAT. Further optimization of the PCR protocol was necessary for amplification of the SCAR marker in the DG population. Changes made to the PCR protocol included a greater number of cycles (35), lower denaturation (92°C for 40 s) and annealing (53°C for 40 s) temperatures, and a longer final extension (10 min) period at 72°C using 100 ng of DNA in 20 μL reaction volumes as described above.

RESULTS

ZAA 12 × Canadian Wonder Recombinant Inbred Line Mapping Population – All Races

The parent ZAA 12 had a disease score rating of 1 for reaction to races 2, 5, 7, 8, and 9 and a hypersensitive resistance (HR) response to races 3 and 4. The HR in bean to halo blight infection with races 3 and 4 is characterized by dry, red-brown necrotic lesions distributed evenly across the entire inoculated leaf. The susceptible parent Canadian Wonder had susceptible ratings of 3 and 4 to races 2, 3, 4, 7, 8, and 9, and susceptible ratings of 2 and 3 against Race 5. The disease reaction ratings for the Z12C RILs that were resistant and susceptible to the seven races corresponded to the ratings observed for the resistant and susceptible parents.

Table 2 summarizes segregation for disease reaction among the 79 RILs. Cosegregation for reaction to races 2, 3, 4, 5, 7, 8, and 9 was observed, as half of the RILs (37) were either resistant (presumptively conditioned by *Pse-2*) to all seven races or susceptible (36 RILs) to races 2, 7, 8, and 9. The 36 RILs susceptible to races 2, 7, 8, and 9 were either susceptible or resistant to races 3, 4, and 5 due to absence or presence of other R genes (*Pse-3* and *Pse-4*). Six RILs with plants that segregated for disease reaction to individual races were excluded from further analysis.

Twenty-six of 36 RILs lacking *Pse-2* (i.e., susceptible to races 2, 7, 8, and 9) were resistant to both races 3 and 4 as conditioned by the *Pse-3* gene, nine were susceptible to both races, and one RIL was segregating exhibiting both resistant and susceptible plants to each race. Cosegregation of reaction to races 3 and 4 agrees with Teverson's (1991) observations based on coinoculation of individual F_2 plants with both races.

Segregation for HR to NL-3 strain of BCMNV in the Z12C population was skewed toward presence of the *I* gene, with 53 and 26 lines showing resistance and susceptibility, respectively, to the pathogen (Table 2). All 53 RILs that possessed the *I* gene also expressed HR to races 3 and 4. For those 26 RILs lacking the *I* gene, segregation for reaction to races 3 and 4 fit the expected 1:1 ratio for

Table 2. Segregation in 'ZAA 12' × 'Canadian Wonder' $F_{6:8}$ recombinant inbred line (RIL) population for reaction to seven differential races of the halo blight pathogen (*Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young et al. [*Psp*]), and *I* gene segregation based on reaction to NL-3 strain of *Bean common mosaic necrosis virus* (BCMNV).

Putative resistance (R) gene	Pathogen races controlled	Recombinant inbred lines (no.)					χ^2 probability values‡
			Total	R	S†	Segregating	
Pse-2	Cosegregation for reaction to races 2, 3, 4, 5, 7, 8,§ and 9	Observed	79	37	36	6	0.91, 1 df
		Expected		36.5	36.5		
Pse-3	Segregation for reaction to races 3 and 4 in lines lacking resistance to races 2, 7, 8, and 9	Observed	36	26	9	1	0.004, 1 df
		Expected		17.5	17.5		
I gene	Segregation for reaction to races 3 and 4 in lines with the *I* gene	Observed	53	53	0		
		Expected		53	0		
i gene	Segregation for reaction to races 3 and 4 in lines lacking the *I* gene	Observed	26	13	9	4	0.39, 1 df
		Expected		11	11		
Pse-4	Segregation for reaction to Race 5 in lines lacking resistance to races 2, 7, 8, and 9	Observed	36	15	19	2	0.49, 1 df
		Expected		17	17		

†S, susceptible.

‡Chi-square probability values based on expected 1:1 ratio for number of resistant and susceptible lines.

§Recessive resistance to Race 8 conferred by *pse-5* gene as observed by Teverson (1991) was inseparable from *Pse-2* gene due to cosegregation of reaction to all seven races.

the number of HR to susceptible lines (Table 2). The HR response to races 3 and 4 in the 13 RILs lacking the *I* gene cosegregated with *Pse-2* (i.e., resistance to races 2, 5, 7, 8, and 9). Thus, resistance to races 3 and 4 as conferred by the *Pse-3* gene and presence of the *I* gene as inferred by HR to BCMNV completely cosegregated in the 36 RILs that lacked *Pse-2* (data not shown).

Segregation for reaction to Race 5 among RILs lacking *Pse-2* fit the expected 1:1 ratio for number of resistant (15) to susceptible (19) RILs (Table 2). Resistance to Race 5 is putatively conditioned by the *Pse-4* locus, which is further investigated in the F_2 populations.

F_2 Populations – Races 2, 7, and 9

Results from inheritance and allelism tests involving reactions to seven *Psp* differential races in F_2 populations derived from ZAA 12 crossed with each of the host differential cultivars are presented in Table 3. Dominant inheritance (3 resistant to 1 susceptible ratio) for monogenic resistance to races 2, 7, and 9 was observed in 12 of 14 tests involving F_2 populations from crosses between ZAA 12 and host differentials that lacked resistance to these races. Canadian Wonder, ZAA 54, Tendergreen, and ZAA 55 lack resistance to races 7 and 9 and all the common bean host differentials except ZAA 12 lack resistance to Race 2 (Table 1). The two exceptions were reactions to Race 7 (111 resistant to 58 susceptible individuals) in Z12C and to Race 9 (46 resistant to 26 susceptible individuals) in ZAA 12 × Tendergreen F_2 populations, where more susceptible individuals were observed than would be expected by chance alone for an expected 3 resistant to 1 susceptible segregation ratio. All residual F_1 plants from the crosses above were resistant to either Race 2, 7, or 9, confirming dominant inheritance.

Segregation for reaction to races 7 and 9 in F_2 populations from crosses between ZAA 12 and host differentials UI-3 and Guatemala 196-B possessing the *Pse-1* gene that conditions resistance to these same races indicated the presence of two independent dominant genes (15 resistant to 1 susceptible) in only one (Race 7 [48 resistant to 1 susceptible individual] reaction in ZAA 12 × UI-3) of the four allelism tests conducted. All plants were resistant to either Race 7 or 9 in the other three allelism tests. These results suggest that if individual dominant genes *Pse-2* from ZAA 12 and *Pse-1* from UI-3 and Guatemala 196-B condition resistance to these same races as expected then they are linked in repulsion. Further evidence for linkage between *Pse-1* and *Pse-2* is described below (Fig. 1).

F_2 Populations – Races 3 and 4

The F_2 populations from crosses between ZAA 12 and host differentials Canadian Wonder, ZAA 54, and UI-3, which lack resistance to races 3 and 4, segregated 15 resistant to 1 susceptible for reaction to races 3 and 4 (Table 3). These 15:1 ratios support that ZAA 12 possesses two independent dominant genes, *Pse-2* and *Pse-3*, for HR to races 3 and 4. Interestingly, the *Pse-2* gene conditions an HR response to races 3 and 4 and an immune (no symptom) response to races 2, 5, 7, 8, and 9. The F_2 plants were all resistant to races 3 and 4 as expected from crosses between ZAA 12 and host differentials ZAA 55, Tendergreen, and Guatemala 196-B, which possess *Pse-3*. Teverson (1991) observed a 63:1 ratio for the F_2 population ZAA 12 × ZAA 55, indicating the two genes conferring resistance to Race 3 in ZAA 12 were independent of the gene

conditioning resistance to Race 3 in ZAA 55. The size of our F_2 population for this cross (n = 30) was not large enough to confirm her observation.

F_2 Populations – Race 5

The F_2 segregation observed for reaction to Race 5 in Z12C and ZAA 12 × Tendergreen populations fit a 13 resistant to 3 susceptible ratio indicating presence of two independent genes, one dominant and the other recessive (Table 3). The F_2 individuals from crosses between ZAA 12 and host differentials ZAA 54 and ZAA 55 purported to possess *Pse-4* gene were all resistant to Race 5. These results indicate that ZAA 12 has a gene for resistance to Race 5 in common with ZAA 54 and ZAA 55. Teverson (1991) observed similar results, and concluded that the gene in common among ZAA 12, ZAA 54, and ZAA 55 was *Pse-4*.

The F_2 plants from crosses between ZAA 12 (*Pse-2* and *Pse-4*) and the host differential cultivars UI-3 and Guatemala 196-B (*Pse-1* and *Pse-4*) were all resistant as expected given that both pairs of parents possess two genes governing resistance to Race 5. Further investigation of the inheritance of resistance to Race 5 in the host differential cultivars is ongoing.

F_2 Populations – Race 8

Observed reactions to Race 8, for all the F_2 populations from crosses between ZAA 12 and the other six host differentials, fit 7 resistant to 9 susceptible segregation ratios. The consistent 7:9 segregation ratios observed in this study (Table 3) suggest that two independent recessive genes condition resistance to Race 8. All 58 residual F_1 plants from the above crosses inoculated with Race 8 were susceptible, confirming recessive inheritance.

Pse-2 Gene Tagging and Mapping

Four RAPD markers, three dominant V10.300, D13.1600, and AE15.955, and one codominant AM10.450/425, identified by bulked-segregant analysis, were linked with the *Pse-2* gene (Fig. 1) in the Z12C RIL population. One RAPD marker, AE15.955, was completely linked (no recombinants) to *Pse-2*. This marker was converted to a SCAR marker SAE15.955 (forward 5′-TGCCTGGACCCAAAAGCTATTCAC-3′, reverse 5′-TGCCTGGACCACTCTTAACG-3′, with original decamer underlined, 60°C annealing), which was subsequently modified (see Materials and Methods section) for assay in the DG RIL mapping population (Blair et al., 2003; Beebe et al., 2006) where it mapped to linkage group 10. Thus, by inference *Pse-2* gene is located on linkage group 10.

The RAPD marker V10.300 that was 9 cM from *Pse-2* in the original Z12C mapping population mapped toward one end of linkage group 10 in the BelNeb-RR-1 × A 55 (BA) RIL mapping population (Ariyarathne et al., 1999). The V10.300 marker similarly mapped to the distal end of

Table 3. Segregation for disease reaction (resistant or susceptible) to *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young et al. (*Psp*) race differentials in F_2 populations from crosses between 'ZAA 12' and the other common bean host differentials for halo bacterial blight.

F_2 populations		Race 2		Race 3 or 4		Race 5		Race 7		Race 8		Race 9	
		(R:S)†	Probability	(R:S)	Probability	(R:S)	Probability	(R:S)	Probability	(R:S)	Probability	(R:S)	Probability
ZAA 12 × Canadian Wonder	Observed	97:31		35:2		146:25		111:58		138:166		98:34	
	Expected	3:1	0.84	15:1	0.84	13:3	0.17	3:1	0.005	7:9	0.57	3:1	0.86
ZAA 12 × ZAA 54	Observed	33:13		32:3		61:0		29:10		41:40		52:26	
	Expected	3:1	0.61	15:1	0.89	all R	1.00	3:1	0.92	7:9	0.22	3:1	0.09
ZAA 12 × Tendergreen	Observed	47:15		37:0		66:23		58:12		28:32		46:26	
	Expected	3:1	0.89	all R	1.00	13:3	0.09	3:1	0.13	7:9	0.65	3:1	0.04
ZAA 12 × ZAA 55	Observed	54:13		30:0		89:0		61:13		40:49		56:20	
	Expected	3:1	0.29	all R	1.00	all R	1.00	3:1	0.14	7:9	0.34	3:1	0.79
ZAA 12 × UI-3	Observed	47:12		19:2		105:0		48:1		34:38		96:0	
	Expected	3:1	0.41	15:1	0.53	all R	1.00	15:1	0.22	7:9	0.55	15:1	0.01
ZAA 12 × Guatemala 196-B	Observed	26:15		43:0		21:0		57:0		23:34		58:0	
	Expected	3:1	0.08	all R	1.00	all R	1.00	15:1	0.05	7:9	0.59	15:1	0.049

†R, resistant; S, susceptible.

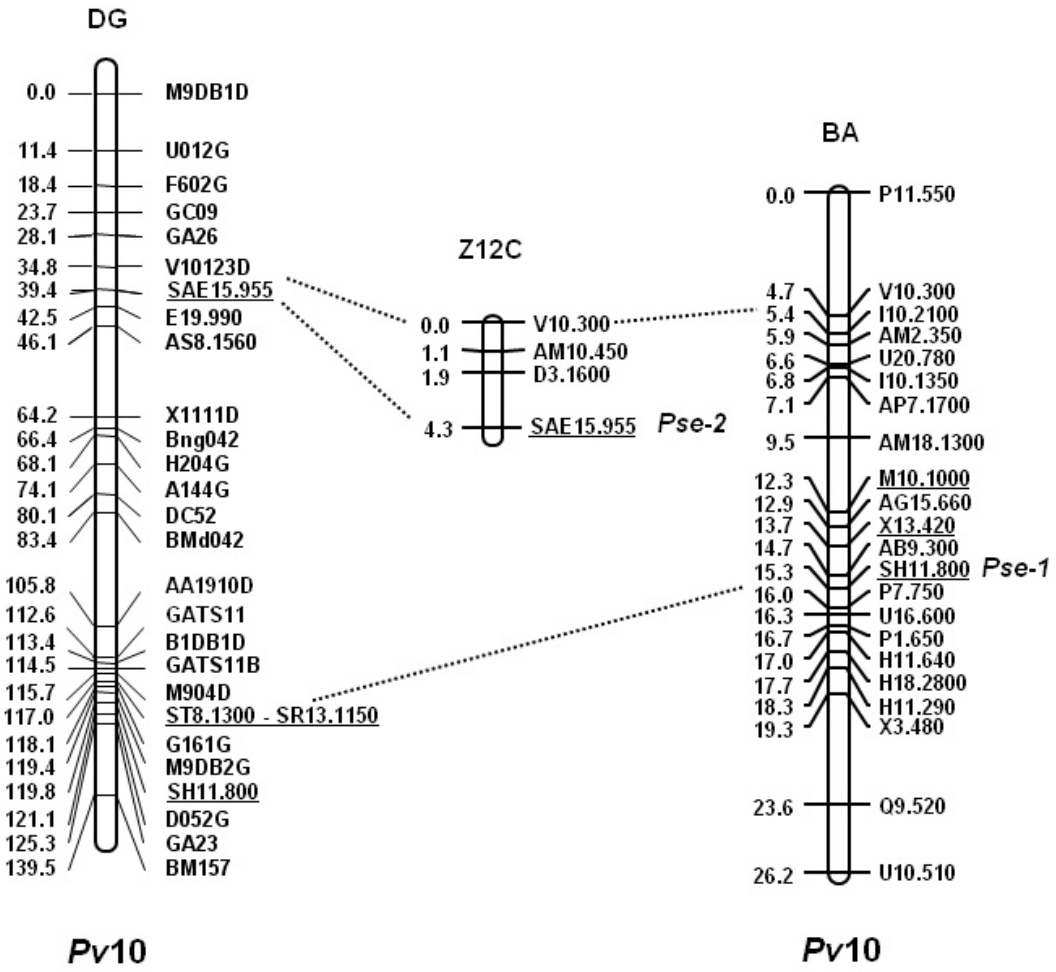

Figure 1. Integration of halo blight resistance gene *Pse-2*, which conditions resistance to *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young et al. (*Psp*) races 2, 3, 4, 5, 7, 8, and 9, on linkage group 10 of the *Phaseolus vulgaris* L. core map. BA, BelNeb-RR-1 × A 55 recombinant inbred line (RIL) mapping population (Ariyarathne et al., 1999) consisting of random amplified polymorphic DNA (RAPD) markers and sequence characterized amplified region (SCAR) markers linked with *Pse-1* underlined (Miklas et al., 2009); Z12C, 'ZAA 12' × 'Canadian Wonder' RIL mapping population (this study) consisting of RAPD markers and SCAR marker SAE15.955 linked with *Pse-2* gene underlined; DG, DOR 364 × G 19833 RIL mapping population with SCAR markers linked with *Pse-1* and *Pse-2* genes underlined and consisting of simple sequence repeat (SSR), restriction fragment length polymorphism (RFLP), and other markers (Blair et al., 2003; Beebe et al., 2006); Numbers on the left for BA, Z12C, and DG represent centimorgans, based on Kosambi mapping function, with the scale for DG reduced by ~70% compared to scale used for BA and Z12C. Note that the Pv10 linkage groups presented for BA and Z12C depicts the distal portion and for DG the entire linkage group is shown.

linkage group 10 in the A 55 × G122 recombinant inbred mapping population (Miklas et al., 2001) (data not shown). A RAPD marker, V10123D, also based on amplification with the V10 decamer primer 5′-TGTACCCGTC-3′, was linked at 4.6 cM to the SAE15.955 SCAR marker on linkage group 10 in the DG population, further confirming linkage relationships between V10 RAPD markers and placement of *Pse-2* on linkage group 10 (Fig. 1).

Further evidence for linkage of *Pse-2* with *Pse-1* loci is provided by BLASTing (Altschul et al., 1990) the whole nucleotide sequences for the respectively linked SAE15.955 (GenBank no. FJ938209) and SH11.800 (GenBank no. FJ938208) (Miklas et al., 2009) SCAR markers against the soybean [*Glycine max* (L.) Merr.] genome (Gepts and Lin, 2011). Sequences for framework markers for linkage group 10 from other maps including restriction fragment length polymorphism (RFLP) markers (Bng) (Murray et al., 2002), legume markers (Leg) (Hougaard et al., 2008), gene-based markers (g) (McConnell et al., 2010) and microsatellite markers (BM) (Gaitán-Solís et al., 2002; Grisi et al., 2007) were similarly aligned with the soybean genomic map (Fig. 2; Table 4). Soybean is a diploidized ancient tetraploid (Shoemaker et al., 2006); hence, one expects two homologous soybean sequences for every common bean sequence, unless further chromosomal changes postgenome duplication either increase or decrease the number of homologous sequences. The SAE15.955 and SH11.800 sequences both aligned with chromosome Gm03 of soybean at approximately Mbp position 27 and Mbp position 43, respectively, although the E-value for SAE15.955 was markedly lower (higher sequence similarity) than that for the SH11.800 marker

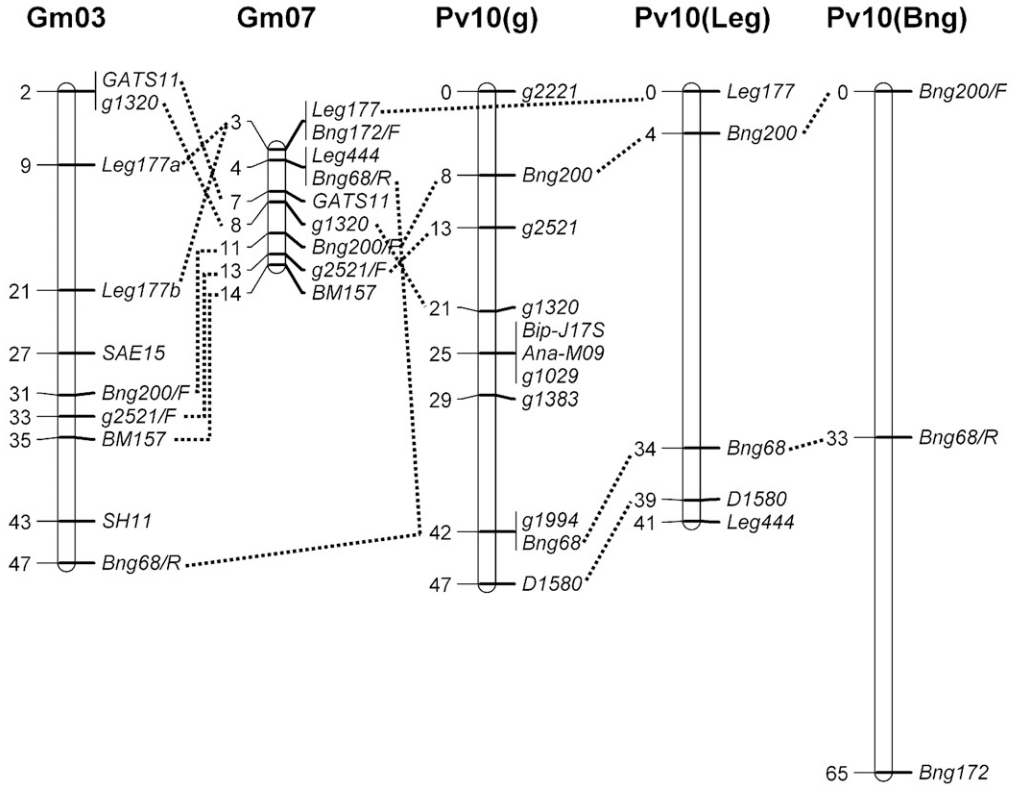

Figure 2. Synteny between *Phaseolus vulgaris* L. and *Glycine max* (L.) Merr. in the *Pse1* and *Pse2* region. The sequences of the SAE15 and SH11 markers, as well as linked markers on linkage group 10, were BLASTed (Altschul et al., 1990) onto the whole-genome sequence of soybean as included in the PhaseolusGenes genome database (Gepts and Lin, 2011) (see Table 4 for soybean chromosome locations and E-values). Note that numbers on the left of *Glycine max* (L.) Merr. (Gm) linkage groups represent physical position on the pseudomolecule measured in megabase pairs (millions of nucleotide base pairs) and on the left of *Phaseolus vulgaris* L. (Pv) linkage groups represent genetic positions expressed in Kosambi centimorgans.

Table 4. Homology of *Phaseolus vulgaris* L. marker sequences from chromosome Pv10 with the soybean genome based on BLAST algorithm (Altschul et al., 1990).

Chromosome Gm03			Chromosome Gm07		
Phaseolus vulgaris marker	Position (Mbp)	E-value	*Phaseolus vulgaris* marker	Position (Mbp)	E-value
GATS11	2001	1.10×10^{-12}	Bng172/F	2639	4.80×10^{-46}
g1320	2116	6.60×10^{-89}	Leg177	2639	9.10×10^{-12}
Leg177a	9119	1.40×10^{-47}	Leg444	3837	3.50×10^{-87}
Leg177b	21,151	1.40×10^{-47}	Bng68/R	4353	2.90×10^{-32}
SAE15	27,275	1.80×10^{-32}	GATS11	7441	1.70×10^{-14}
Bng200/F	31,428	2.30×10^{-85}	g1320	7553	6.80×10^{-92}
g2521.F	33,201	1.70×10^{-11}	Bng200/F	11,213	5.50×10^{-80}
BM157	34,719	3.80×10^{-13}	g2521.F	12,707	9.90×10^{-07}
SH11	42,602	4.10×10^{-02}	BM157	14,240	9.30×10^{-11}
Bng68/R	47,415	9.90×10^{-20}			

(Table 2). The same marker sequences, however, were not present in the homologous genomic region on chromosome Gm07 (Fig. 2). Sequences for the framework markers linked to the two SCAR markers on chromosome Pv10 did also map generally on the homologous Gm03 and Gm07 chromosomes of soybean with low E-values but with some chromosome rearrangements (Fig. 2; Table 4). Overall, these results suggest that the genome region carrying the *Pse-1* and *Pse-2* genes is conserved in gene content between common bean and soybean.

A survey of 32 lines and cultivars representing germplasm of Andean and Middle American origin and known to lack *Pse-2* revealed the absence of the SAE15.955 SCAR marker (data not shown). The SAE15.955 SCAR completely cosegregated with resistance conferred by *Pse-2* to the four races (2, 7, 8, and 9) tested among 122 $F_{2:3}$ (data not shown). All F_2 plants with F_3 progeny that were resistant or segregating for resistance to the four races possessed the SAE15.955 SCAR marker. The 33 all-resistant, 60 segregating, and 29 all-susceptible $F_{2:3}$ progeny fit a 1:2:1 ratio ($p = 0.87$).

DISCUSSION

Inheritance of resistance to halo bacterial blight in the host differential cultivar ZAA 12 was investigated in a RIL population Z12C and in numerous F_2 populations derived from crosses with the host differential cultivars, challenged by seven differential races of the pathogen. Disease reaction to races 2, 3, 4, 5, 7, 8, and 9 among the

RILs in the Z12C population revealed presence of three independent resistance genes *Pse-2*, *Pse-3*, and *Pse-4*. The three R genes were contributed by the host differential cultivar ZAA 12.

Cosegregation for resistance to races 2, 3, 4, 5, 7, 8, and 9 in the Z12C RIL population provided definitive evidence that *Pse-2* confers resistance to all seven *Psp* races. It is plausible that a cluster of genes associated with the *Pse-2* locus combine to effect resistance to all seven races; however, recombinant lines from the Z12C RIL population or the $F_{2:3}$ population (Z12C) to support this hypothesis were not recovered. Stavely (1984) found that the *Ur-5* locus consisted of a cluster of genes conditioning race-specific resistance to bean rust [*Uromyces appendiculatus* (Pers.:Pers.) Unger]. This locus is clustered with other R genes conditioning resistance to anthracnose [*Colletotrichum lindemuthianum* (SACC. & Magnus) Lams.-Scrib.] on linkage group 4 (Miklas et al., 2006).

The F_2 data from this study indicates *Pse-2* has dominant inheritance for resistance to races 2, 7, and 9. Teverson (1991) reported ratios of 3:1, 13:3, and 9:7 for reaction to races 2 and 7 across F_2 populations; however, observed ratios were inconsistent between repeated tests, which prevented definitive conclusions from being made about the inheritance of resistance to races 2 and 7. Although Teverson (1991) suspected there were two genes from ZAA 12, one with dominant (*Pse-2*) and the other recessive inheritance (*pse-5*), that conditioned resistance to Race 2, our overall data support that only a single dominant gene *Pse-2* confers resistance to Race 2.

The resistance to Race 8 conferred by *Pse-2* gene conflicts with Teverson's (1991) results. She observed that resistance to Race 8 was conditioned by an independent gene (*pse-5*) with recessive inheritance. Our F_1 data (all susceptible plants) support Teverson's (1991) finding that resistance to Race 8 is recessively inherited. However, the cosegregation of resistance to races 2, 3, 4, 5, 7, 8, and 9 in the Z12C RIL population clearly indicates *Pse-2* confers resistance to Race 8. This result contradicts the F_2 data, which fit a consistent 7:9 ratio. Teverson (1991) observed 1:3 ratios for four of six tests conducted for four of the same F_2 populations. For two repeated tests, 7:9 ratios provided a better fit for the observed data. Nonetheless, Teverson (1991) concluded that a single recessive gene (*pse-5*) governed resistance to Race 8. A better explanation for Teverson's results and ours is that *Pse-2* confers resistance to Race 8, but it is influenced by other genes, which partially suppress expression, resulting in a perceived recessive inheritance for resistance to this particular race in F_1 and segregating F_2 populations. Genetic background effects on expression of halo blight resistance have been documented (Taylor et al., 1978). Additional studies are planned to test this hypothesis.

Among Z12C RILs lacking *Pse-2*, additional segregation for resistances to races 3, 4, and 5 was detected, supporting the presence of additional genes in ZAA 12 that contributed to pyramided resistance to these three races. Teverson (1991) observed two genes from ZAA 12 for resistance to Race 3, *Pse-3* and an independent gene thought to represent a copy of *Pse-3*. Among RILs without the *I* gene as determined by inoculation with NL-3 strain of BCMNV, the HR response to races 3 and 4 cosegregated with the *Pse-2* locus. Thus, it is clear from our results that this second gene conferring resistance to races 3 and 4 is *Pse-2*. Both *Pse-2* and *Pse-3* condition HR to races 3 and 4; however, in the case of *Pse-2*, this is a first report of HR to races 3 and 4 independent of the *Pse-3* and *I* genes.

There was complete cosegregation of *Pse-3* with the *I* gene, which is in agreement with Teverson's (1991) observations. Segregation distortion in favor of the *I* gene occurs frequently in RIL populations (Ariyarathne et al., 1999; P. Miklas, unpublished data, 2003), so skewed segregation as observed in the Z12C recombinant inbred population toward presence of *Pse-3*, which is completely linked with *I*, was no surprise.

Segregation for reaction to Race 5 in those Z12C RILs lacking *Pse-2* did not deviate significantly from the expected 1:1 ratio indicating presence of a single resistance gene (*Pse-4*). This finding supports Teverson's (1991) observation for a second gene, *Pse-4*, in ZAA 12, in addition to *Pse-2*, which provides resistance solely to Race 5. The F_2 population data from this study supports a 13:3 segregation ratio, which suggests that either *Pse-2* or *Pse-4* confers dominant resistance and the other recessive resistance to Race 5. The F_2 data collected by Teverson (1991) for Z12C fit a 13:3 ratio (43 resistant to 6 susceptible) and for ZAA 12 × Tendergreen fit a 15:1 ratio (37 resistant to 3 susceptible). Miklas et al. (2009) observed that the UI-3 host differential cultivar possessed two genes (*Pse-1* and *Pse-4*) with similar gene action (one dominant and the other recessive) for resistance to Race 5 in the UI-3 × Canadian Wonder F_2 population, but a 15:1 segregation ratio was observed for the UI-3 × Tendergreen F_2 population. Perhaps an alternate explanation for the ~13:3 segregation ratios is that they result from the presence of an additional gene(s) in some susceptible lines, which modify or partially suppress the effect of either *Pse-2* or *Pse-4* against Race 5. Further analysis of Race 5 resistance in the host differential cultivars is planned to test this hypothesis.

The SCAR marker SAE15.955 tightly linked with *Pse-2* (0 cM) in the Z12C RIL population cosegregated (0 cM) with *Pse-2* in a separate F_2 population (Z12C) and was absent across all lines and cultivars assayed that lacked the *Pse-2* gene, indicating the marker will have broad application for MAS across a wide range of germplasm.

Refinement of the PCR protocol enabled faint amplification of SAE15.955 in the DG mapping population, which integrated to linkage group 10, and by inference *Pse-2* maps to the same location. The RAPD V10.300 linked with *Pse-2* (9 cM) integrated to the same linkage group 10 but in a different mapping population (BA). The mapping of SAE15.955 and V10.300, albeit via different mapping populations, to the distal portion of the same linkage group provides evidence that *Pse-2* resides on linkage group 10.

The map location for *Pse-2* inferred by linked markers suggests possible linkage with the *Pse-1* gene, which likewise resides on linkage group 10 (Fig. 1). The SCAR (SH11.800) and RAPD (X13.425) markers tightly linked with *Pse-1* gene were mapped to linkage group 10 in the BA population by Miklas et al. (2009) but were proximal from V10.300, at distances of 15.4 and 13.9 cM, respectively. This location for *Pse-1* in relation to V10.300 places *Pse-1* near the *Pse-2* locus. The SCAR markers SAE15.955 and SH11.800 were homologous with genomic regions on the same linkage group (Gm03) in soybean (Fig. 2), providing additional evidence that *Pse-1* and *Pse-2* reside on the same chromosome in dry bean. This result reflects the high level of synteny between the genomes for these two species (McConnell et al., 2010). The homology of Gm07 to this same region of Pv10 was previously identified by McClean et al. (2010).

Genetic mapping in the DG population places the SCAR markers ST8.1300, SH11.800, and SR13.1150 developed by Miklas et al. (2009) for *Pse-1* at the opposite end of linkage group 10 from the SAE15.955 marker for *Pse-2*. Note that ST8.1300, described as a dominant marker originally, amplified as a codominant marker in the DG population. This position corresponds with several disease resistance quantitative trait loci (QTL) identified in previous studies including several for fungal and bacterial pathogens (Miklas et al., 2006). The lack of independence between *Pse-1* and *Pse-2*, caused by linkage in repulsion, is supported by the skewed segregation toward resistance to races 7 and 9 observed in the F_2 populations. Teverson (1991) similarly observed all resistant plants in ZAA 12 × UI-3 F_2 population for reaction to Race 7. However, the large distance between the markers for *Pse-1* and *Pse-2* in the DG mapping population as well as in the syntenic region on chromosome Gm03 of soybean suggests that the linkage is not tight.

SUMMARY

The inheritance of resistance to halo bight in host differential cultivar ZAA 12 was further characterized in this study. The presence of three dominantly inherited genes, *Pse-2*, *Pse-3*, and *Pse-4*, was confirmed. Although recessive inheritance against Race 8 suggests a reversal of dominance for *Pse-2*, this interaction remains unresolved. Nonetheless, *Pse-2* gene has broad effect conferring resistance to seven differential races (2, 3, 4, 5, 7, 8, and 9) and appears to be the most useful qualitative gene available from any source for global deployment by breeders to combat this pathogen. To facilitate breeding for *Pse-2*, a SCAR marker, SAE15.955, tightly linked with the gene on linkage group 10 was generated. A survey for SAE15.955 among susceptible common bean genotypes revealed that the marker will be useful for MAS of the *Pse-2* gene across a broad array of common bean market types and gene pools.

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