Tagging and Mapping *Pse-1* Gene for Resistance to Halo Blight in Common Bean Differential Cultivar UI-3

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ABSTRACT

Halo blight [caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkh.) Young et al. (*Psp*)] is a serious seed-borne bacterial disease of common bean (*Phaseolus vulgaris* L.). A few resistance (R) genes and quantitative trait loci provide control to one or more races of the pathogen. To better understand monogenic resistance and improve breeding efficiency, we sought to tag and map a gene (*Pse-1*) in host differential cultivar UI-3 (previously named 'Red Mexican UI-3') that provides resistance to races 1, 5, 7, and 9 of *Psp*. Cosegregation for resistance to races 1, 5, 7, and 9, in a recombinant inbred population, 'Canadian Wonder'/UI-3 (CU), confirmed the effect of *Pse-1* against multiple races of the pathogen. Bulked-segregant analysis in the CU population identified six random amplified polymorphic DNA (RAPD) markers tightly linked (0–3.3 cM) to *Pse-1*. Three of the RAPDs completely linked with *Pse-1* in the CU population were converted to sequence characterized amplified region (SCAR) markers SH11.800, SR13.1150, and ST8.1350. The linked markers were used to integrate *Pse-1* to linkage group B10 of the core map. Allelism tests (F_2) confirmed relationships of *Pse-1* and *Pse-4* derived from UI-3 with R genes in the other host differential cultivars. A survey of advanced lines and cultivars revealed that the SCAR markers generated in this study will have utility for marker-assisted selection of *Pse-1* in germplasm from the Andean gene pool (e.g., kidney, calima) and from race Mesoamerican within the Middle American gene pool (black, carioca).

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Abbreviations: BA, BelNeb-RR-1/A55 recombinant inbred population; BJ, BAT 93/Jalo EEP558 core mapping population; CU, Canadian Wonder/UI-3 recombinant inbred population; GT-196-B, Guatemala 196-B; MAS, marker-assisted selection; PCR, polymerase chain reaction; *Psp*, *Pseudomonas syringae* pv. *phaseolicola*; QTL, quantitative trait locus or loci; RAPD, random amplified polymorphic DNA; R, resistance or resistant; RIL, recombinant inbred line; S, susceptible; SCAR, sequence characterized amplified region.

Halo blight [caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkh.) Young et al. (*Psp*)] is a seed-borne bacterial disease that limits common bean production. Although *Psp* has worldwide distribution, halo blight occurs mostly on beans grown in production regions with cool to moderate temperatures. Genetic resistance provides an effective means for combating this disease, but the basis for resistance is not completely understood. As reviewed by Schwartz (1989), breeding for resistance is complicated by qualitative and quantitative modes of inheritance, independent genes for expression of resistance in leaves and pods, and separate mechanisms for resistance to bacterium growth per se vs. suppression of toxin production. Once characterized, deployment of halo blight resistance in common bean is complicated by the virulence diversity of *Psp*.

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Pseudomonas syringae pv. *phaseolicola* races 1 and 2 were available in the 1960s and 1970s for characterizing resistance (R) genes present in common bean germplasm. Inheritance studies conducted with each race indicated presence of recessive and dominantly inherited monogenic R genes, partial polygenic resistance, and independent R genes across different sources (Schwartz, 1989). Since then, a host–pathogen differential series was developed by Taylor et al. (1996a) based on leaf reaction of eight *Phaseolus* lines to nine *Psp* races. The differentials provide a host–pathogen interaction framework (Table 1) from which to identify new R genes and pathogen races.

Five putative R genes, R1, R2, R3, R4, and R5, were tentatively identified in the differential series (Teverson, 1991; Taylor et al., 1996a). Based on gene nomenclature conventions outlined by the Bean Genetics Committee (Myers and Weeden, 1988; Bassett and Myers, 1999), R genes conditioning resistance to halo blight, on verification henceforth will be preceded by the symbol *Pse* if dominant and *pse* if recessively inherited (Bassett, 2004). Only, the *Pse-3* (~R3; Teverson, 1991; Crute and Pink, 1996) gene, which conditions localized necrotic hypersensitive resistance reactions to races 3 and 4, has been located on the genetic linkage map (Gepts, 1999) by virtue of its association with the *I* gene on linkage group B2, which conditions resistance to *Bean common mosaic virus*.

The *Pse-1* and *Pse-4* genes are purported to occur in the differential cultivar UI-3, which possesses resistance to *Psp* races 1, 5, 7, and 9 (Teverson, 1991; Taylor et al., 1996a). The full name for this cultivar in the past was Red Mexican UI-3. The name was shortened to UI-3 herein because the industry name for the red-seeded market type, formerly 'Red Mexican', was changed to 'small red'. UI-3 is from the University of Idaho and derives from a cross between two landrace cultivars, Great Northern UI-1 and Common Red Mexican. Common Red Mexican landrace is the source of *Pse-1* gene (Patel and Walker, 1965). A more recent study (Teverson, 1991) reported that the *Pse-1* gene conditioned resistance to races 1, 5, and 7. The *Pse-4* gene is reported to condition resistance only to race 5. UI-3 was shown to possess a dominant gene for resistance to race 9 (Teverson, 1991; Taylor et al., 1996a), but a direct relationship between *Pse-1* and resistance to race 9, although inferred, was never directly observed. With a long-term goal of genomic mapping of resistance to halo blight, we sought to tag and map the *Pse-1* and *Pse-4* resistance genes present in UI-3, and to validate the relationship between the R genes in UI-3 with those in the host differential series (Table 1) by allelism tests and chromosome positioning.

MATERIALS AND METHODS

A recombinant inbred line (RIL) population consisting of 52 $F_{5:7}$ recombinant inbred lines was derived from the cross 'Canadian Wonder'/UI-3 (henceforth the CU population) by the single-seed descent method. Canadian Wonder is susceptible to halo blight and is one of the eight differential cultivars (Taylor et al., 1996a). UI-3 was crossed to *P. vulgaris* host differential cultivars ZAA 54 (A52), Tendergreen, ZAA 55 (A53), ZAA 12 (A43), and Guatemala 196-B (GT-196-B) to generate F_2 populations for allelism tests to test the hypotheses that *Pse-1* and *Pse-4* in UI-3 are independent of other putative R genes *Pse-2*, *Pse-3*, and *pse-5*, in the host differential series. UI-3 was also crossed with Canadian Wonder, to further examine inheritance of the putative *Pse-1* and *Pse-4* genes in the F_2 generation. The cross of UI-3 with the tepary bean (*Phaseolus acutifolius* A. Gray) 1072 host differential was not conducted because it represents an interspecific hybridization that is extremely difficult to obtain through embryo rescue.

Reaction to each race (1, 5, 7, and 9) was tested separately by inoculating 10 plants of each RIL and parent. Tests were repeated for any RIL with both resistant and susceptible plants to an individual race to confirm that the RIL segregated for disease reaction. Because race 5 infection occasionally resulted in some plants exhibiting a weaker susceptible rating of 2, 10 additional plants of each RIL were inoculated with race 5 in a separate test to confirm disease reaction. Inoculum [10^8 colony forming units mL^{-1}] was applied to 7- to 10-d-old seedlings with fully expanded primary leaves by the method of Taylor et al. (1996a). Likewise, separate individuals from the F_2 populations were inoculated with each of the different races. Inoculated plants were kept in a humidity chamber (19°C, relative humidity = 100%) for 48 h before being transferred to a greenhouse (18°C night/25°C day, relative humidity = 70%). Plants were rated for infection based on leaf reaction 10 d after inoculation on a scale of 1 to 5 (Innes et al., 1984; Taylor et al., 1996a), where 1 = red-brown necrotic reaction in the area of maximum inoculation; 2 = red-brown necrotic reaction with a trace of water soaking; 3 = some necrosis but extensive water-soaking in the area of maximum inoculation; 4 = small water-soaked lesions, <1 mm diam., distributed at random across the leaf underside; and 5 = larger water-soaked lesions, 1 to 3 mm

Table 1. Host–pathogen differential set for halo blight (taken and modified from Teverson, 1991; Taylor et al., 1996a,b) consisting of nine *Pseudomonas syringae* pv. *phaseolicola* (*Psp*) races, seven common bean lines–cultivars, and one tepary bean differential cultivar 1072 with putative resistance (R) genes listed.

Host differential	Proposed R genes	*Psp* race†								
		1	2	3	4	5	6	7	8	9
Canadian Wonder	–	+	+	+	+	+	+	+	+	+
ZAA 54 (A52)	*Pse-4*	+	+	+	+	–	+	+	+	+
Tendergreen	*Pse-3*	+	+	–HR	–HR	+	+	+	+	+
UI 3 (Red Mexican)	*Pse-1, Pse-4*	–	+	+	+	–	+	–	+	–
1072	*Pse-2*	+	–	+	–	–	+	–	+	+
ZAA 55 (A53)	*Pse-3, Pse-4*	+	+	–HR	–HR	–	+	+	+	+
ZAA 12 (A43)	*Pse-2, Pse-3, Pse-4, pse-5*	+	–	–HR	–HR	–	+	–	–	–
Guatemala 196-B	*Pse-1?, Pse-3, Pse-4*	–	+	–HR	–HR	–	+	–	+	–

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

diam., distributed at random across the leaf underside. For this study, plants rated 1 were considered resistant (− incompatible reaction) and plants rated between 2 and 5 were considered susceptible (+ compatible reaction). Conversely, Teverson (1991) and Taylor et al. (1996a) used ratings of 1 and 2 to indicate resistance and 3 to 5 to indicate susceptibility; however, unlike this study in which race-specific resistance was the focus, they were also interested in characterizing sources of non–race-specific resistance, which required less discrete demarcation between resistant and susceptible reactions.

Approximately 50 mg of leaf tissue was collected from the emerging trifoliate leaf of each of four individual plants within a line and combined into a single tube. The genomic DNA was then extracted using the FastDNA Kit (Bio 101, Vista, CA) according to the manufacturer's instructions. The purified DNA samples were adjusted to 10 ng μL^{-1} using a fluorometer before all polymerase chain reactions (PCRs).

For the CU population, equal amounts of DNA from eight RILs with resistance to races 1, 5, 7, and 9 were combined to form the "R" DNA bulk and DNA from eight RILs susceptible to races 1, 5, 7, and 9 were combined to form the "S" DNA bulk. These DNA bulks were used to tag the *Pse-1* gene by screening 700 decamer primers for random amplified polymorphic DNA (RAPD) markers 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 assayed across the entire mapping population of 52 RILs. DNA bulks for identifying markers linked with *Pse-4* gene conferring resistance to race 5 could not be developed because only three RILs resistant to race 5 and susceptible to races 1, 7, and 9 existed in the population of 52 RILs.

The RAPD protocol consisted of 25-μL reactions containing 2 U Stoffel fragment DNA polymerase (Applied Biosystems, Foster City, CA), 1× Stoffel buffer, 0.2 μM of primer, 5 mM of $MgCl_2$, 200 μM of each dNTP, and 25 ng of 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, then three cycles at 94°C for 1 min, 32°C for 1 min, and 72°C for 2 min. This was 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 at 72°C. Amplified products from all PCR reactions 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.

For the CU RIL population, linkage analysis of markers and categorical disease reaction to each race (resistance = score of 1 vs. susceptible = score of 2–5) was performed by JoinMap 4 (Van Ooijen, 2006) using default settings for the maximum likelihood mapping option. Four RAPD markers found tightly linked (0 cM = no recombinants) with the putative *Pse-1* resistance gene that conditioned resistance to races 1, 5, 7, and 9 were excised from agarose gels. DNA was purified from the excised gel bands using GeneLute Minus agarose spin columns (Sigma-Aldrich, St. Louis, MO) and cloned into the vector pCR4 using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA) according to the manufacture's instructions. Plasmid DNA was purified from *Escherichia coli* with alkaline lysis (Sambrook et al., 1989), and clones containing the PCR product were identified by digestion with *Eco*R1 and visualized by agarose gel electrophoresis. The DNA product was sequenced using the dideoxy-chain termination method, and extended primers were subsequently designed based on the terminal sequences of the cloned marker for conversion to a sequence characterized amplified region (SCAR) primer pair. Thermocycling parameters were optimized, and a final profile was employed that consisted of a single cycle of 5 min at 95°C, followed by 30 cycles of 1 min at 94°C, 1 min at optimum annealing temperature, 1 min at 72°C, and a final extension for 7 min at 72°C.

To integrate the *Pse-1* resistance gene segregating in the CU $F_{5:7}$ mapping population with the *Phaseolus* core map (Freyre et al., 1998; Kelly et al., 2003), SCAR and RAPD markers linked with *Pse-1* were assayed across 71 RILs from the BAT 93/Jalo EEP 558 (BJ) mapping population (Freyre et al., 1998) provided by P. Gepts (Univ. of California, Davis) and the BelNeb-RR-1/A 55 (BA) recombinant inbred mapping population using 70 of the 77 RILs provided by Ariyarathne et al. (1999). JoinMap 4 (Van Ooijen, 2006) was used to visualize integration of the individual linkage groups from the CU, BJ, and BA populations that possessed the *Pse-1* linked makers. The regression mapping option with Kosambi mapping function was used to construct the BJ and BA linkage groups for this purpose. The SCARs were also assayed across several common bean breeding lines and cultivars representative of bean genotypes from both the Andean and Middle American gene pools (Singh et al., 1991) to assess potential utility for marker-assisted selection (MAS) of the linked *Pse-1* halo blight resistance gene in different genetic backgrounds. To validate linkage intensity of the SCAR markers with *Pse-1* gene, they were assayed across 121 and 120 F_2 individuals from UI-3/ZAA 55 and UI-3/Canadian Wonder crosses, respectively. Inoculation with *Psp* race 7, disease reaction rating, DNA extraction, and SCAR marker amplification were as described above, except they were applied to individual F_2 plants.

RESULTS AND DISCUSSION

CU Population

The parent UI-3 had a resistant disease score rating of 1 for reaction to races 1, 5, 7, and 9. The universal susceptible parent Canadian Wonder had susceptible ratings of 3 and 4 to races 1, 5, 7, and 9, and an occasional susceptible rating of 2 against race 5. The resistant RILs from the CU population had a rating of 1, and the susceptible RILs expressed a similar range of ratings to the four races as the susceptible parent.

Table 2 summarizes segregation for disease reaction among the 52 RILs. Cosegregation for resistance to races 1, 5, 7, and 9, conditioned by the *Pse-1* gene, was observed for 33 RILs. This result represents all 40 plants tested within a RIL with a resistant disease rating of 1 across the four races. This result also verifies that *Pse-1* conditions resistance to race 9, which was unclear from earlier studies (Teverson, 1991; Taylor et al., 1996a). Sixteen RILs cosegregated for susceptibility to races 1, 7, and 9. This result represents 30 plants tested to races 1, 7, or 9 within a RIL with susceptible disease ratings of 3 or 4. Three RILs were heterozygous with mixed R and S reactions to all four races and were omitted from further analysis. Three of the 16 CU RILs cosegregating for susceptible reaction to races 1, 7, and 9 expressed

homozygous resistance to race 5 as conferred by the putative *Pse-4* gene, and two RILs expressed mixed heterozygous R and S reactions to race 5. This result represents ratings based on 20 plants within a RIL. The 14 RILs with homozygous segregation for the putative *Pse-4* gene in this CU population were deemed too few to be useful for tagging the gene.

The observed segregation among lines in the CU population for reaction to races 1, 7, and 9 conferred by *Pse-1* was skewed toward resistance, differing significantly from the expected 1:1 ratio for number of resistant to susceptible lines. The observed segregation for reaction to race 5 conferred by *Pse-4* among those RILs absent *Pse-1* also deviated from expected 1:1 ratio, but in this case there were significantly more susceptible than resistant lines. This skewed segregation in RILs lacking *Pse-1* in favor of susceptibility to race 5 (lacking *Pse-4*) suggests that perhaps *Pse-1* and *Pse-4* could be linked in coupling. The deviation from expected segregation for *Pse-1* could be due to preferential transmission of gametes or zygotes possessing *Pse-1* because of its linkage with some unknown gene affecting fitness. Nonetheless, these reasons presented for the significant deviations from expected 1:1 segregations are speculative because the small population size for CU precludes definitive conclusions from being made.

F_2 Populations—Races 1, 7, and 9

Results from allelism tests involving UI-3 crossed with each of the host differential cultivars (Table 3) support the presence of a dominant gene (*Pse-1*) in UI-3 that conditions resistance to races 1, 7, and 9. Resistance to race 5 is reported separately because it is conditioned by two genes, the same gene (*Pse-1*) that conditions resistance to races 1, 7, and 9, and an independent gene (*Pse-4*) that conditions resistance to race 5 only. Segregation for reaction in the F_2 generation to races 1, 7, and 9 in crosses with differential cultivars ZAA 54, Tendergreen, and ZAA 55 that lack resistance to races 1, 7, and 9, fit a single dominant resistance gene model (3 resistant to 1 susceptible ratio) with only a few exceptions. For unknown reasons, segregation for reaction to race 1 was skewed toward more susceptible individuals in the UI-3/Tendergreen F_2 population and segregation to race 7 was skewed toward more resistant individuals in UI-3/Canadian Wonder and UI-3/Tendergreen populations. Teverson (1991) observed ratios of 3 resistant to 1 susceptible in the F_2 generation for these same crosses except that segregation in UI-3/ZAA 54 was skewed toward resistance to race 1 but not race 7. These deviations from 3:1 segregation, predominantly due to more resistant individuals than expected, could be caused by a gene linked with

Table 2. Segregation in common bean CU (Canadian Wonder/UI-3) $F_{5:7}$ recombinant inbred line (RIL) population for halo blight reaction to four differential races of the causal pathogen *Pseudomonas syringae* pv. *phaseolicola*.

Pathogen races and putative resistance genes as proposed by Teverson (1991)	Ratios	Recombinant inbred lines†				χ^2/*P* value
		Total	R	S	Seg	
			no.			
Cosegregation for reaction to races 1, 5, 7, and 9 (~*Pse-1*)	Observed	52	33	16	3	5.6/0.02, 1 df
	Expected (1:1)		24.5	24.5		
Reaction to race 5 (~*Pse-4*), in lines lacking resistance to races 1, 7, and 9	Observed	16	3	11	2	5.1/0.02, 1 df
	Expected (1:1)		7	7		

†R, resistant—incompatible disease reaction score of 1; S, susceptible—compatible disease reaction score of 2, 3, 4, or 5; Seg, segregation among plants within a RIL for R and S disease reactions.

Table 3. Allelism tests (F_2 generation) for examining the relationship of *Pse-1* and *Pse-4* genes for halo blight resistance in differential common bean cultivar UI-3 with putative resistance genes present in the other common bean differential cultivars (see Table 1) by reaction to four *Pseudomonas syringae* pv. *phaseolicola* races.

UI-3 crossed with	Ratios	Race 1					Race 5					Race 7					Race 9				
			R†	S	χ^2	*P*		R	S	χ^2	*P*		R	S	χ^2	*P*		R	S	χ^2	*P*
Canadian Wonder	Observed		104	33				114	16				68	12				65	21		
	Expected	3:1	102.75	34.25	0.06	0.81	13:3	105.6	24.4	3.56	0.06	3:1	60	20	4.30	0.04	3:1	64.5	21.5	0.01	0.92
							15:1	121.9	8.1	8.21	0.00										
ZAA 54 (A52)	Observed		71	20				128	0				63	23				48	21		
	Expected	3:1	68.25	22.75	0.44	0.51	All R	128	0			3:1	64.5	21.5	0.13	0.72	3:1	51.75	17.25	1.08	0.39
Tendergreen	Observed		23	17				59	6				40	4				64	17		
	Expected	3:1	30	10	6.50	0.01	13:3	52.8	12.2	3.87	0.05	3:1	33	11	5.93	0.01	3:1	60.75	20.25	0.69	0.41
							15:1	61.9	4.1	0.88	0.35										
ZAA 55 (A53)	Observed		14	6				22	0				15	5				16	3		
	Expected	3:1	15	5	0.30	0.58	All R	22	0			3:1	15	5	0.0	1.0	3:1	14.3	4.75	0.9	0.34
ZAA 12 (A43)	Observed		40	15				105	0				48	1				96	0		
	Expected	3:1	41.25	13.75	0.14	0.71	All R	105	0			15:1	45.9	3.1	1.52	0.22	15:1	90	6	6.4	0.01
Guatemala 196-B	Observed		70	0				70	0				70	0				70	0		
	Expected	All R	70	0			All R	70	0			All R	70	0			All R	70	0		

†R, resistant—incompatible disease reaction score of 1; S, susceptible—compatible disease reaction score of 2, 3, 4, or 5.

Pse-1 affecting fitness, which is further supported by the skewed segregation in favor of *Pse-1* as observed in the CU recombinant inbred population.

No segregation was observed in the F_2 generation for reaction to races 1, 7, and 9 in the UI-3/GT-196-B population, as all individuals tested were resistant to these races, indicating that GT-196-B possesses the same *Pse-1* gene (Table 3). For this same cross, Teverson (1991) observed segregation (41 R and 5 S) for reaction to race 1 but not to race 7. Teverson (1991) observed segregation in other crosses (GT-196-B/ZAA 55 = 7 R and 37 S to race 1; GT-196-B/TG = 3 R and 94 S to race 1) which suggested resistance to race 1 in GT-196-B was recessively inherited. These mixed results explains why Teverson (1991) placed a question mark (Table 1) in the list of putative genes present in GT-196-B (*Pset1?*, *Pse-3*, and *Pse-4*). Seed sources of GT-196-B have been observed by us and other researchers to be heterozygous for disease reaction (unpublished data, 2006) which may have influenced her results.

Segregation in the UI-3/ZAA 12 F_2 population fit a single dominant gene model for reaction to race 1 because ZAA 12 lacks resistance to race 1 (Table 1). Segregation in the UI-3/ZAA 12 population fit a digenic duplicate dominant epistasis model (15:1) for resistance to race 7. This result would be expected if each parent contributed independent dominant genes for resistance to race 7, *Pse-1* from UI-3 and *Pse-2* from ZAA 12 (Table 3). Conversely, all F_2 plants tested against race 9 were resistant in our study, whereas all plants tested against race 7 by Teverson (1991) were resistant. The observation of more resistant individuals could be the result of a repulsion phase linkage between *Pse-1* and *Pse-2*. Preliminary results indicate that *Pse-2* is located on linkage group B10 (M. Blair, personal communication, 2007), same as *Pse-1* (see below), but linkage distance between *Pse-1* and *Pse-2* has not yet been discerned.

Overall, the allelism tests support the findings of Teverson (1991) that resistance in UI-3 to races 1 and 7 was conditioned by a single dominant gene, *Pse-1*. Race 9 was not used by Teverson (1991) in many of her allelism tests because it was not discovered until the end of her practical studies.

F2 Populations—Race 5

The F_2 segregation for reaction to race 5 (Table 3), fit a 13 resistant to 3 susceptible, independent dominant and recessive gene model, for both the UI-3/Canadian Wonder and UI-3/Tendergreen populations. Segregation in the latter population did not deviate from a 15 resistant to 1 susceptible, duplicate dominant gene model, either. Teverson (1991) observed a 15:1 segregation in the former and did not test the latter cross. Observation of the 13:3 ratio suggests that it is plausible that either *Pse-1* or *Pse-4* confers dominant resistance and the other recessive resistance to race 5. The segregation of resistant to susceptible F_2 plants fitting somewhere between 13:3 and 15:1 ratios may reflect a loose coupling linkage between two dominant genes, which is supported by the segregation data observed for the CU population (Table 2).

The F_2 individuals from crosses between UI-3 and host differentials ZAA 54 and ZAA 55 purported to possess *Pse-4* gene were all resistant to race 5. These results indicate that UI-3 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 UI-3, ZAA 54, and ZAA 55 was *Pse-4*. Further investigations of the inheritance of resistance to race 5 in all the host differential cultivars are ongoing.

Gene Tagging and Mapping

Six RAPD markers identified by bulked-segregant analysis were linked with the *Pse-1* gene (Fig. 1) in the CU RIL population. Four of the markers, R13.1150, T8.1350, X13.420, and H11.800, were completely linked (no recombinants) to the *Pse-1* gene and with each other. The F9.1000 and M10.1000 markers were tightly linked to one side of the gene. Three of the four markers completely linked to *Pse-1* were successfully converted to SCAR markers (Table 4).

Some RAPD (F9.1000 and X13.420) and SCAR (SR13.1150 and ST8.1350) markers linked with *Pse-1* in the CU population were polymorphic in the BJ core mapping population. Map location for four of the *Pse-1*-linked markers in the BJ map places *Pse-1* gene on linkage group B10 (Fig. 1) (Freyre et al., 1998; Ariyarathne et al., 1999; Blair et al., 2003). The four markers were tightly linked, within 0.7 cM of each other in the BJ population, which is similar to the tight linkage observed among this set of markers in the original CU population. The *Pse-1*-linked markers (X13.420, SH11.800, and M10.1000) polymorphic in the BA RIL population similarly mapped to linkage group B10. In a preliminary study, Fourie et al. (2004) mapped a gene on linkage group B4 that also conferred resistance to *Psp* races 1, 5, 7, and 9. This gene was derived from BelNeb-RR-1 (Stavely et al., 1999) and was thought to be the same *Pse-1* gene present in UI-3. Chromosome location and allelism tests (unpublished data, 2008), however, indicate that *Pse-1* from UI-3 and the unnamed gene from BelNeb-RR-1, with similar specificity for resistance to races 1, 5, 7, and 9, are in fact independent genes.

The location for *Pse-1* on linkage group B10 (Fig. 1) occurs in the vicinity of resistance gene analog polymorphism markers mapped in a previous study (Mutlu et al., 2006), and near other disease resistance quantitative trait loci (QTL) conferring resistance to Fusarium wilt [*Fusarium oxysporum* Schlechtend.:Fr. f. sp. *phaseoli* (Kendrick and Snyder)] and halo blight in the BA mapping population (Ariyarathne et al., 1999, Fall et al., 2001), and to angular leaf spot [*Phaeoisariopsis griseola* (Sacc.) Ferraris] in the core map (López et al., 2003; Miklas et al., 2006). The occurrence of *Pse-1* in the

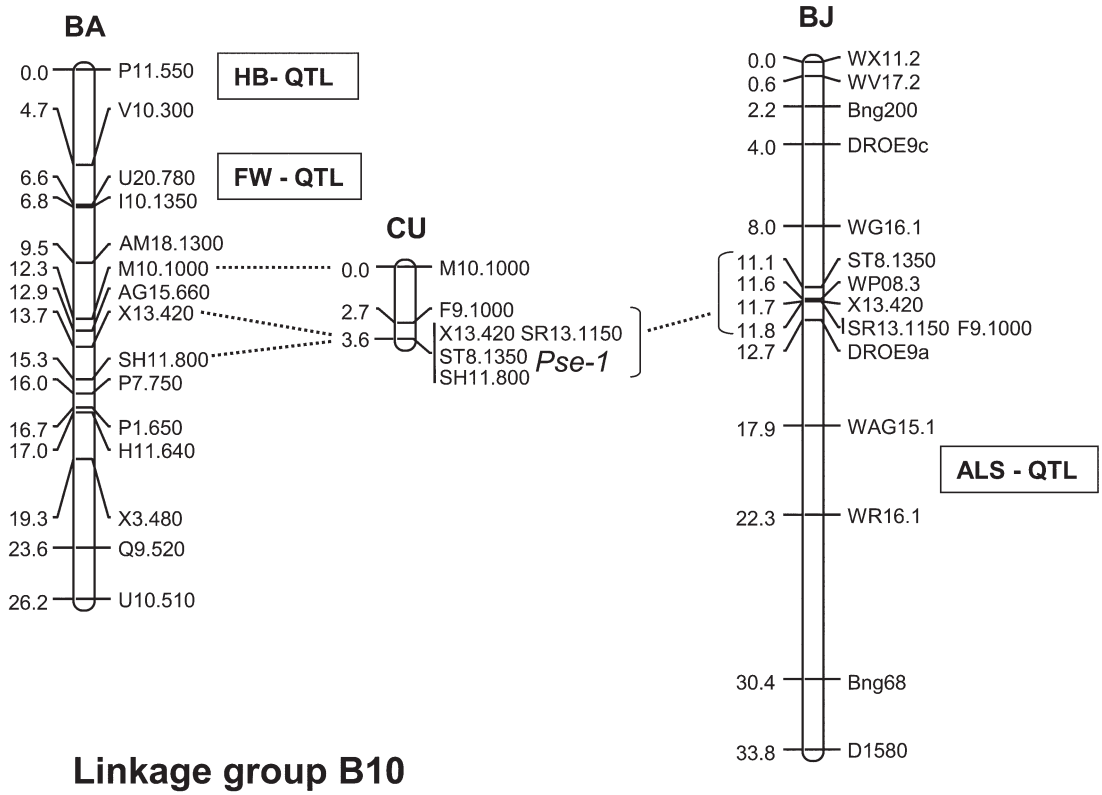

Figure 1. Map integration of the *Pse-1* halo bacterial blight resistance gene, derived from host differential common bean cultivar UI-3, which conditions resistance to *Pseudomonas syringae* pv. *phaseolicola* races 1, 5, 7, and 9. The gene is located on linkage group B10 of CU, original mapping population (Canadian Wonder/UI-3); BA, secondary mapping population (BelNeb-RR-1/A 55; Ariyarathne et al., 1999); and BJ, primary core mapping population (BAT 93/Jalo EEP558; Freyre et al., 1998). All markers are random amplified polymorphic DNAs (RAPDs), except Bng designate is a restriction fragment length polymorphism (RFLP), D1580 is an RFLP, and markers preceded by the letter S are sequence characterized amplified region markers. Generally, primer designation and length (kb) of the marker is separated by a period, except for RAPDs preceded by a W or DRO in the BJ map (see Freyre et al. 1998 for details). Boxes to the right of the linkage group indicate quantitative trait loci (QTL) for resistance to HB, halo blight; FW, Fusarium wilt; or ALS, angular leaf spot. Linkage distances (cM) represent Kosambi map units.

genomic vicinity of a QTL conditioning resistance to halo blight suggests that the *Pse-1* locus could affect quantitative resistance to some *Psp* races. Geffroy et al. (2000) observed similar colocalization of R genes and QTL for resistance to bean anthracnose in the bean core map (Freyre et al., 1998). Presence of QTL conferring resistance to fungal pathogens in the same general genomic region as *Pse-1* on the B10 linkage group infers potential duplication of an ancestral resistance gene followed by divergence for resistance to different pathogens (Michelmore and Myers, 1998).

The *Ur-5* locus for resistance to bean rust [*Uromyces appendiculatus* (Pers.) Unger var. *appendiculatus*)] in dry bean line B-190 consists of at least six tightly linked genes with different race specificities which, when combined, provide broad resistance to the hypervariable rust pathogen (Stavely, 1984). Similarly, the resistance to races 1, 5, 7, and 9 conditioned by *Pse-1* could be due to a group of tightly linked genes (gene block) with specificities for the different races; however, no recombinants were observed in the CU population to test such a hypothesis.

A survey of the SCAR markers SR13.1150, ST8.1350, and SH11.800 across a subset of lines and cultivars known to lack *Pse-1* gene revealed absence of the SR13.1150 and ST8.1350 markers in germplasm from the Andean gene pool and presence in germplasm from the Middle American gene pool (Table 5). The SH11.800 SCAR was present in germplasm

Table 4. Primer and recombination information for sequence characterized amplified region (SCAR) markers linked with *Pse-1* gene derived from UI-3 in Canadian Wonder/UI-3 common bean recombinant inbred line mapping population.

RAPD† marker	SCAR	Product size	Forward primer	Reverse primer	Annealing temperature	Linkage with *Pse-1*
		bp			°C	
R13.1150	SR13.1150	1150	5′-GGACGACAAGGAACATATTCA-3′	5′-GGACGACAAGGCTGCAAGAACCAT-3′	60	0 cM
T8.1350	ST8.1350	1350	5′-AACGGCGACATCAGTGTAAAGG-3′	5′-AACGGCGACAACCGACCATGTTTTAC-3′	65	0 cM
H11.800	SH11.800	800	5′-CTTCCGCAGTCGAGAGAT-3′	5′-CTTCCGCAGTAGCACC-3′	67	0 cM

†RAPD, random amplified polymorphic DNA.

of race Durango origin but absent in germplasm of the Andean gene pool and of Mesoamerican origin except ND88-106-04. Races Durango and Mesoamerican are divisions within the Middle American gene pool (Singh et al., 1991). Thus, MAS for *Pse-1* using the available linked SCAR markers will be restricted to the Andean gene pool and Mesoamerican race within the Middle American gene pool. The limited utility of disease-resistance linked markers for MAS due to ubiquitous presence of the marker in a specific gene pool regardless of the presence or absence of the resistance gene in the same gene pool is well documented in common bean (Miklas et al., 1996, 2006).

Linkage of the three SCAR markers with *Pse-1* was verified in two separate F_2 populations (Table 5). Linkages between the gene and marker were not as tight as in the CU RIL, in which no recombination between the markers and gene were observed. Fewer RIL lines vs. the larger number of F_2 plants tested, and the possible escape of a few F_2 individuals from infection, could have contributed to the differences in linkage intensity between the population types. All three markers occurred on the same side of the gene in the UI-3/ZAA 55 F_2 population, but ST8.1350 flanked the gene in the UI-3/Canadian Wonder F_2 population, which suggests errors from escapes or in the marker assays themselves contributed to this lack of consistency in marker alignment with the gene. Moreover, this assay was conducted in a completely different laboratory from which the SCARs originated. The linkage of the SCARs with the gene in separate populations and laboratories confirms utility of the markers for MAS.

Table 5. Assay of sequence characterized amplified region (SCAR) markers linked with *Pse-1* gene conferring resistance to halo blight in a survey of bean lines representative of different gene pools, and linkage intensity between the markers and gene in two F_2 populations.

Lines	Market type		SCAR marker†		
		Pse-1	SR13.1150	ST8.1350	SH11.800
Middle American—race Durango					
UI-3	Red	Yes	+	+	+
BelNeb-RR-1	Great northern	No	+	+	+
Weihing	Great northern	No	+	+	–
GN No.1 sel 27	Great northern	No	+	+	+
Montana No. 5	Great northern	No	+	+	+
Chase	Pinto	No	+	+	–
Olathe	Pinto	No	+	+	–
Aztec	Pinto	No	+	+	+
Middle American—race Mesoamerican					
VAX 4	Tan	No	+	+	–
VAX 6	Red	No	+	+	–
Dorado	Red	No	+	+	–
XAN 176	Black/tan	No	+	+	–
BAT 93	Tan	No	+	+	–
Raven	Black	No	+	+	–
A 55	Black	No	+	+	–
I9365-31	Black	No	+	+	–
Guatemala 196-B	Black	Yes	+	+	+
ND88-106-04	Navy	No	+	+	+
Andean					
Montcalm	Dark red kidney	No	–	–	–
Red Hawk	Dark red kidney	No	–	–	–
Canadian Wonder	Dark red kidney	No	–	–	–
Red Kloud	Light red kidney	No	–	–	–
G122	Cranberry	No	–	–	–
Jalo-EEP558	Yellow	No	–	–	–
CAL143	Calima	No	–	–	–
Moncayo	Snap	No	–	–	–
Primo	Snap	No	–	–	–
Benton	Snap	No	–	–	–
Hystyle	Snap	No	+	–	–
Tendergreen	Snap	No	+	–	–
NY6020-4	Snap	No	–	–	–
ZAA 12	Red mottled	No	–	–	–
ZAA 54	Dark red kidney	No	–	–	–
ZAA 55	Dark red kidney	No	–	–	–
AND 277	Calima	No	–	–	–
F_2 populations	**No. individuals**	**R:S**	**Linkage distance (cM)**		
UI-3/ZAA 55	121	94:27	4.4 (3,2)‡	8.6 (9,1)	6.9 (7,1)
UI-3/Can. Wonder	120	96:24	4.5 (4,1)	9.0 (9,1)	3.6 (2,2)

†+, marker present; –, marker absent.

‡Linkage distance between the marker and *Pse-1* followed by the number of recombinants for each phenotypic class in parentheses. For example, (3,2) indicates three resistant plants lacked the marker and two susceptible plants possessed the marker. The 94:27 and 96:24 ratios for number of resistant to susceptible plants fit expected 3:1 segregation ratios (chi-squares not shown).

CONCLUSIONS

Cosegregation for resistance to races 1, 5, 7, and 9 in the CU RIL population provides definitive evidence that a single gene conditions resistance to all four *Psp* races. Teverson (1991) previously showed by coinoculation of Canadian Wonder/UI-3 F_2 individuals with races 1 and 5, and 1 and 7, respectively, that a single gene conferred resistance to these three races. Race 9 was not included in many of her tests because it became available toward the end of her studies. Segregation in the F_2 populations from the crosses of UI-3 with the host differentials that lack resistance to races 1, 5, 7, and 9 support Teverson's (1991) findings that the gene conferring resistance to these four races has dominant inheritance. We further tested 11 F_1 progeny from a UI-3/ZAA 12 cross with race 1 and all were resistant, which further supports Teverson's findings. The dominant gene conferring resistance to races 1, 5, and 7 was designated R1 by Teverson (1991) and is renamed *Pse-1* in conformation with current rules governing gene

nomenclature. Our results also show that *Pse-1* does in fact confer resistance to race 9.

The lack of segregation for plants susceptible to race 5 in UI-3/ZAA 54 and UI-3/ZAA 55 F_2 populations indicated that UI-3 and the host differentials ZAA 54 and ZAA 55 have a resistance gene locus in common that confers resistance to race 5. We were unable to discern if this locus in common among the three differentials was *Pse-4* or an allele at *Pse-1.* Based on race specificity, Teverson (1991) concluded that *Pse-4* was the gene in common among UI-3, ZAA 54, and ZAA 55.

The *Pse-1* gene, which conditions broad resistance to races 1, 5, 7, and 9, has greater value to plant breeders than the *Pse-4* gene with sole resistance to a single race. The SCAR markers linked with *Pse-1* will have the greatest utility for MAS in large-seeded kidney, calima, cranberry, and other dry and snap bean market types of Andean origin. In addition, SCAR SH11.800 will be useful for MAS of *Pse-1* in navy, black, carioca, and other small-seeded market types of race Mesoamerica origin within the Middle American gene pool.

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