

Confirmation of Molecular Markers and Flower Color Associated with QTL for Resistance to Common Bacterial Blight in Common Beans

Soon O. Park,¹ Dermot P. Coyne,¹ Nedim Mutlu,¹ Geunhwa Jung,² and James R. Steadman³
University of Nebraska, Lincoln, NE 68583-0724

ADDITIONAL INDEX WORDS. *Phaseolus vulgaris*, *Xanthomonas campestris* pv. *phaseoli*, flower color, randomly amplified polymorphic DNA (RAPD) markers, quantitative trait loci (QTL)

ABSTRACT. Common bacterial blight, incited by *Xanthomonas campestris* pv. *phaseoli* (*Xcp*), is a serious disease of common bean (*Phaseolus vulgaris* L.). Randomly amplified polymorphic DNA (RAPD) markers and flower color (*V* gene) previously were reported to be associated with six quantitative trait loci (QTL) affecting leaf and pod resistance to *Xcp*. However, the markers for the QTL were not confirmed in different populations and environments to indicate their merit in breeding. The objective was to determine if the associations of RAPD markers and the *V* gene with QTL for leaf and pod resistance to *Xcp* in a recombinant inbred (RI) backcross population from the cross BC₂F₆ ‘PC-50’ x XAN-159 and for leaf resistance to *Xcp* in an F₂ population from a different cross pinto ‘Chase’ x XAN-159 could be confirmed. One or two genes from XAN-159 controlled leaf and pod resistance to *Xcp*. Among six QTL previously detected, five in the RI backcross population and three in the F₂ population were confirmed to be associated with resistance to *Xcp*. The *V* gene and RAPD marker BC437.1050 on linkage group 5 were most consistently associated with leaf and pod resistance to two to five *Xcp* strains in the RI backcross population and with leaf resistance to two *Xcp* strains in the F₂ population. One to three QTL affecting leaf and pod resistance to *Xcp* accounted for 22% to 61% of the phenotypic variation. Gene number (one to two) estimations and number of QTL (one to three) detected for leaf and pod resistance to *Xcp* in the RI backcross population were generally in agreement. The marker BC437.1050 and *V* gene, along with other resistance genes from other germplasm, could be utilized to pyramid the different genes into a susceptible or partially resistant bean line or cultivar to enhance the level of resistance to *Xcp*.

Common bacterial blight (CBB), caused by *Xanthomonas campestris* pv. *phaseoli* (*Xcp*), is an important seed-transmitted disease in common bean (*Phaseolus vulgaris* L.) (Saettler, 1989). Resistant common bean cultivars are the most effective and economical strategy to control this disease (Schwartz and Galvez, 1981). Leaf, pod, and seed reactions to *Xcp* in some bean crosses show quantitative inheritance (Ariyaratne, 1994; Aggour and Coyne, 1989; Arnaud-Santana et al., 1994). Varying numbers of genes control leaf and/or pod reactions to *Xcp* in different crosses and populations (Eskridge and Coyne, 1996; Silva et al., 1989). Leaf and pod reactions to *Xcp* in different common bean populations have shown no (Coyne and Schuster, 1983; Aggour and Coyne, 1989), low (Arnaud-Santana et al., 1994), and intermediate to high correlations (Ariyaratne, 1994; Rava et al., 1987).

Molecular markers associated with resistance to *Xcp* in common beans have been reported in several studies. Restriction fragment length polymorphism (RFLP) markers (Nodari et al., 1993) and randomly amplified polymorphic DNA (RAPD) markers (Jung et al., 1996, 1997) associated with quantitative trait loci (QTL) affecting resistance to *Xcp* were reported in three linkage maps. RAPD markers associated with QTL affecting resistance to *Xcp* were detected using bulked segregant analysis (Miklas et al., 1996; Bai et al., 1997). Recently, RFLP markers linked to genes for resistance to *Xcp* were also detected (Yu et al., 1998).

The expression of QTL may differ over environments or populations in various crops. Among 29 QTL affecting fruit size, soluble solids concentration or pH in a tomato cross, only four QTL were expressed in three environments and 10 QTL were expressed in two environments (Paterson et al., 1991). Of seven QTL for seed size found in a common bean population, only one QTL was expressed in three environments and two QTL were expressed in two environments (Park et al., 1999). No common genomic region associated with QTL affecting plant height was found in four maize populations (Beavis et al., 1991). Only one QTL affecting resistance to *Xcp* was consistently expressed in four common bean populations (Jung et al., 1999). These are examples of genotype × environment interaction and genetic background affecting QTL expression. Identifying markers associated with QTL based on one environment and one population may be erroneous, especially QTL with minor effects (Beavis et al., 1991; Paterson et al., 1991). In a recombinant inbred (RI) population derived from the common bean cross BAC 6 x HT 7719, RAPD markers were associated with nine QTL affecting resistance to *Xcp* on a linkage map (Jung et al., 1996). When these RAPD markers were tested for confirmation in other common bean populations, only three QTL affecting resistance to *Xcp* in three plant organs, with two *Xcp* strains, and in three populations were consistently confirmed (Jung et al., 1999). The results show the importance of confirmation of the marker–QTL associations in a breeding program, particularly for traits like CBB resistance that have complex inheritance patterns, low narrow-sense heritabilities, and a number of genes involved.

Flower color (*V* gene) and RAPD markers were associated with six QTL affecting leaf and pod resistance to *Xcp* in a RI population (70 F₆ lines) from the cross ‘PC-50’ x XAN-159 in greenhouse experiments (Jung et al., 1997). However, these marker–QTL associations have not been confirmed in other populations of the same cross and/or a different cross, in different

Received for publication 28 Sept. 1998. Accepted for publication 9 June 1999. Published as Nebraska Agricultural Research Division journal series paper 12097. Research was conducted under Projects 20-036 and 20-042. We acknowledge financial support from the Title XII Bean/Cowpea CRSP (AID contract DNA-1310-G-SS-6008-00). We also appreciate Anne K. Vidaver, Pat Lambrecht, Lisa Sutton, and James Reiser for their assistance. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

¹Department of Horticulture, University of Nebraska, Lincoln, NE 68583.

²Department of Horticulture, University of Wisconsin, Madison, WI 53706.

³Department of Plant Pathology, University of Nebraska, Lincoln, NE 68583.

environments or with other *Xcp* strains. Therefore, the objective of this study was to confirm the significant associations of RAPD markers and the *V* gene with QTL for leaf and pod resistance to two to five *Xcp* strains in a RI backcross BC₂F₆ 'PC-50' [susceptible to *Xcp* (S)] x XAN-159 [resistant to *Xcp* (R)] and in the F₂ pinto 'Chase' [susceptible to *Xcp* (S)] x XAN-159 (R). The number of genes controlling resistance and the correlation of leaf and pod reactions to *Xcp* were also calculated in the RI backcross population.

Materials and Methods

PLANT MATERIALS. Sixty-four RI lines derived from the cross 'PC-50' (S) x XAN-159 (R), after two backcrosses to 'PC-50', were developed using the single-seed-descent breeding method (Arnaud-Santana et al., 1994). XAN-159 [Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia] is resistant to *Xcp* and has purple flower color (*V*). 'PC-50' (Dominican Republic) is susceptible to *Xcp* and has pink flower color (*v*^{lae}). Eighty-nine F₂ plants derived from the cross 'Chase' (S) x XAN 159 (R) were also developed in the greenhouse. 'Chase' has moderate resistance to *Xcp* and white flower color (*v*) (Coyne et al., 1994). However, in preliminary studies it was susceptible to some *Xcp* strains in the greenhouse.

Sixty-three RI backcross lines and parents 'PC-50' and XAN-159 (Expt. 1), and 61 RI backcross lines and the parents (Expt. 2) were planted in the greenhouse, Lincoln, Nebr., in a randomized complete block design (RCBD) with three replications on 7 Apr. 1994 and 30 Oct. 1996, respectively. One line in Expt. 1 and three lines in Expt. 2 were not planted due to a lack of seeds. Eighty-nine F₂ plants from the cross 'Chase' x XAN 159 and parents (Expt. 3) were planted in the greenhouse, Lincoln, Nebr., on 7 Oct. 1997. Two plants were grown in each 15-cm pot. A nutrient solution containing 2N-10P-20K fertilizer was applied weekly. Pesticides were applied weekly to control white flies. The greenhouse day/night temperatures were 28 ± 3/23 ± 2 °C in Expt. 1 and 27 ± 2/22 ± 2 °C in Expts. 2 and 3, respectively. The natural day/night lengths ranged from 12/12 to 15/9 h in Expt. 1 and 11/13 to 9/15 h in Expts. 2 and 3, respectively.

Sixty-one RI backcross lines and parents 'PC-50' and XAN-159 (Expt. 4) were planted in the field, Lincoln, Nebr., on 19 June 1994 and were arranged in an RCBD with three replications. Three lines were not planted because of insufficient seeds. Single row plots were 1.2 m long and spaced 0.5 m apart. Five to eight seeds of each line were planted in single row plots per replication. Sprinkler irrigation was used to apply water as needed. The field day/night temperatures were 32 ± 3/23 ± 3 °C. The natural day/night lengths in the field were about 15/9 h from date (15 July 1994) of inoculation until recording the disease reactions.

INOCULATION. Two *Xcp* strains EK-11 and DR-7 (source: A.K. Vidaver, Dept. Plant Pathology, Univ. Nebraska, Lincoln) were used in Expts. 1 (greenhouse), 3 (greenhouse), and 4 (field). Three *Xcp* strains SC-4A, LB-2 and SX-114 (source: as above) were used in Expt. 2 (greenhouse). Three of the *Xcp* strains EK-11, SC-4A and LB-2 originated in Nebraska. Strains DR-7 and SX-114 originated in the Dominican Republic and South Africa, respectively. Except for SX-114, these strains were used previously in genetic and breeding studies in Nebraska. The *Xcp* strains were cultured on MXP, a semiselective medium for *Xcp* (Clafin et al., 1987), for 48 to 72 h at 27 °C under dark conditions and then transferred to 25 mL of 0.01 M potassium phosphate buffer (pH 7.1), and diluted to read 0.1 on a spectrophotometer (Spectronic

20; Bausch and Lomb, Rochester, N.Y.) at 640 nm. A final concentration of 10⁷ colony-forming units (CFU)/mL of each *Xcp* strain was prepared for inoculations by adding a measured bacterial suspension to potassium phosphate buffer. Inoculations were conducted within 30 min after the bacterial suspensions were prepared. The multiple needle method (Andrus, 1948) was used to inoculate leaves in the greenhouse and field experiments. The first fully expanded trifoliolate leaves were inoculated at 28 d after planting. Potassium phosphate buffer was a control for leaf inoculations. Acrylic paint was used to randomly mark trifoliolate leaves for inoculation. The percentage (%) of the inoculated leaf area with disease symptoms, such as necrosis, water-soaking and chlorosis, was recorded for each plant at 14 d after inoculations. Leaf disease rating scales used were as follows: no symptoms = highly resistant, 1% to 20% = moderately resistant, 21% to 40% = slightly resistant, 41% to 60% = moderately susceptible, 61% to 80% = susceptible and 81% to 100% = highly susceptible.

Pods were inoculated with *Xcp* strains EK-11 and DR-7 in Expts. 1 and 4 at 45 d after planting. Young pods were punctured by a dissecting needle and then 10 µL of bacterial suspension (10⁷ CFU/mL) was introduced through the wound using a Pipetteman (Arnaud-Santana et al., 1994). Acrylic paint was used on pods to identify *Xcp* strains. The lengths in millimeter of water-soaked lesions were measured from the wound site at 14 d after inoculation. Pod disease rating scales used were as follows: 1 = 0 to 1.0 mm (resistant), 2 = 1.1 to 2.0 mm (slightly resistant), 3 = 2.1 to 3.0 mm (moderately susceptible), 4 = 3.1 to 4.0 mm (susceptible) and 5 = over 4.0 mm (highly susceptible). Flower color was also recorded on all RI backcross lines and F₂ plants.

MARKERS. Noninoculated fully expanded trifoliolate leaves of the three parents, 63 RI backcross lines, and 89 F₂ plants were collected at 28 d after planting. Total genomic DNA was extracted from lyophilized leaf tissue using the method of Skroch and Nienhuis (1995). Nine primers were obtained from Operon Technologies (Alameda, Calif.) and three primers were obtained from the University of British Columbia (Vancouver, B.C.). Ten and seven primers were used for the RAPD analysis (Williams et al., 1990) in the RI backcross and F₂ populations, respectively. In the two populations, the 10 and seven primers produced 13 and eight markers, respectively, that were associated with QTL affecting leaf and pod resistance to *Xcp* identified previously in 70 RI lines by Jung et al. (1997). Polymerase chain reactions (PCR) were performed in an air thermalcycler (model 1605; the Idaho Technology, Idaho Falls) in thin-walled glass capillary tubes. PCR protocols and the composition of the final volume of reactants were similar to those described by Skroch and Nienhuis (1995). The name of each RAPD marker is derived from the letter identifying the Operon kit or a BC prefix, the primer number and the approximate length of the marker as described by Jung et al. (1997).

LINKAGE GROUP DEVELOPMENT. The linkage groups of RAPD markers and the *V* gene for flower color were developed using 63 RI backcross lines and 89 F₂ plants, respectively, with MAPMAKER Macintosh version 2.0 (Lander et al., 1987). The logarithm of odds (LOD) score of 3.0 was used as a linkage threshold with 0.3 as the maximum recombination fraction for linkage groups. Map distances (cM) between ordered loci of marker and gene were calculated using recombination fractions and Kosambi mapping function (Kosambi, 1944).

DETECTION OF QTL. Single-factor analysis of variance (ANOVA) for each pairwise combination of quantitative trait and marker locus was used to analyze the data for detection of QTL

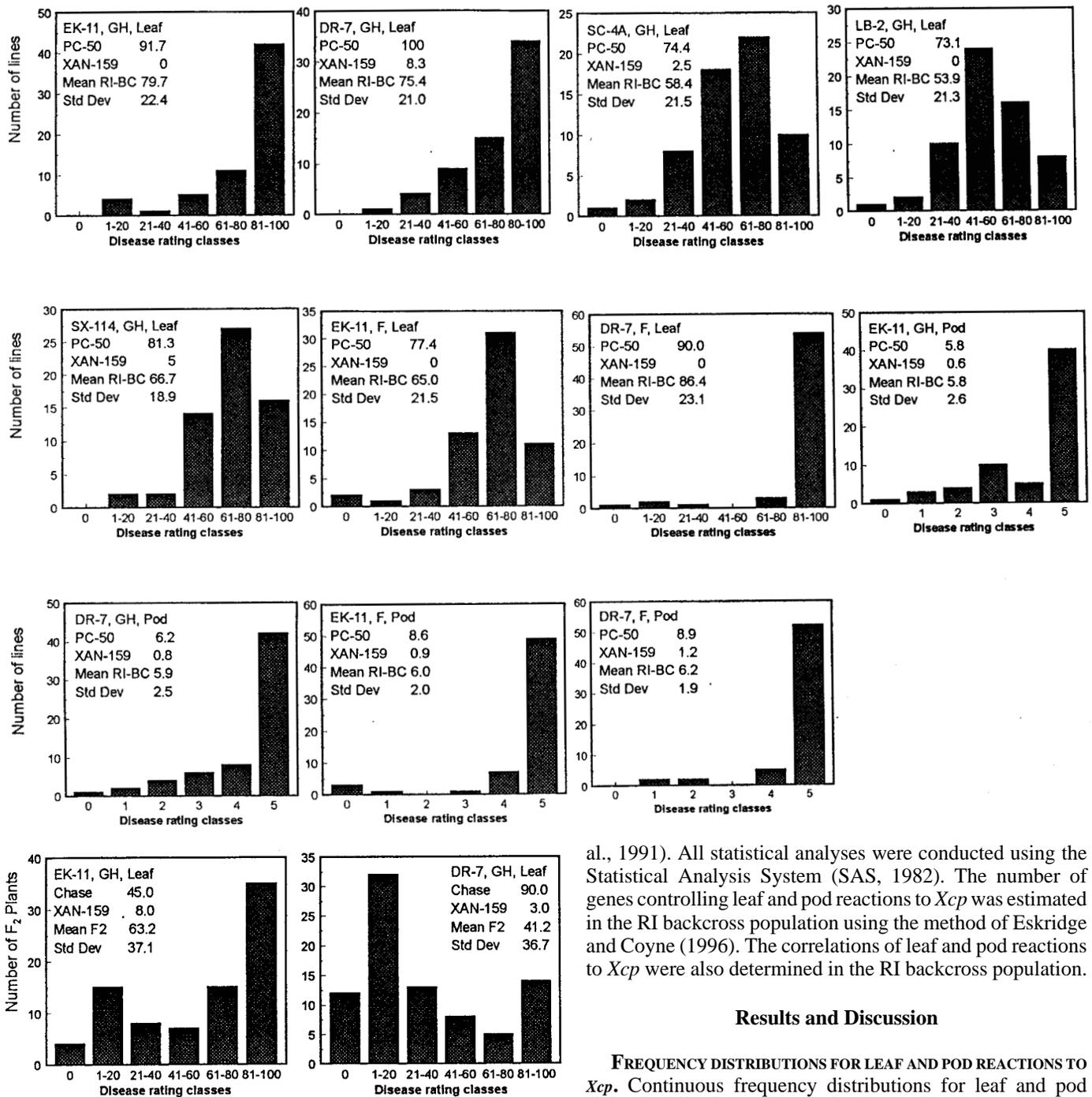


Fig. 1. Frequency distributions of disease rating classes for number of recombinant inbred backcross lines (RI-BC) derived from the cross BC₂F₆ 'PC-50' [susceptible to *Xanthomonas campestris* pv. *phaseoli* (*Xcp*)] × XAN-159 (resistant to *Xcp*) and F₂ plants derived from the cross pinto 'Chase' (susceptible to *Xcp*) × XAN-159. Each graph shows the mean and standard deviation of disease ratings for leaves (percentage of inoculated leaf area with disease symptoms) and pods (length (mm) of water-soaked lesions) of backcross lines or F₂ plants and the parents at 14 d after inoculation with *Xcp* strains EK-11, DR-7, SC-4A, LB-2 and SX-114 in the greenhouse (GH) and field (F).

affecting leaf and pod resistance to the *Xcp* strains. Significant differences in trait associations were based on F-tests ($P < 0.05$) (Edwards et al., 1987). Loci with the lowest P value per QTL were chosen and then were added in a stepwise regression to select the best set of markers ($P < 0.05$) for prediction of the total trait phenotypic variation explained by the detected QTL (Paterson et

al., 1991). All statistical analyses were conducted using the Statistical Analysis System (SAS, 1982). The number of genes controlling leaf and pod reactions to *Xcp* was estimated in the RI backcross population using the method of Eskridge and Coyne (1996). The correlations of leaf and pod reactions to *Xcp* were also determined in the RI backcross population.

Results and Discussion

FREQUENCY DISTRIBUTIONS FOR LEAF AND POD REACTIONS TO *Xcp*. Continuous frequency distributions for leaf and pod reactions to *Xcp* observed in greenhouse and field experiments, were skewed towards susceptibility as would be expected for lines developed after two backcrosses to the susceptible parent 'PC-50' (Fig. 1). Continuous frequency distributions for leaf reactions to *Xcp* also were observed for F₂ plants of the cross 'Chase' × XAN-159 in the greenhouse (Fig. 1). The quantitative inheritance patterns of leaf and pod reactions to *Xcp* in this study were similar to those reported previously (Ariyaratne, 1994; Aggour and Coyne, 1989; Coyne et al., 1965, 1966; Arnaud-Santana et al., 1994). However, Ariyaratne (1994) reported bimodal distributions for pod reactions to *Xcp* and Silva et al. (1989) and Musaana et al. (1993) reported a single gene controlling the resistance to *Xcp*.

GENE ESTIMATION CONTROLLING RESISTANCE TO *Xcp*. The number of genes controlling leaf and pod resistance to *Xcp* was

Table 1. Summary of the single-factor ANOVA analysis of molecular marker and phenotypic data for detection of QTL associated with resistance to *Xanthomonas campestris* pv. *phaseoli* (*Xcp*) strains in a recombinant inbred backcross population from the common bean cross BC₂F₆ 'PC-50' (susceptible to *Xcp*) x XAN-159 (resistant to *Xcp*) in greenhouse and field experiments.

Marker ^z	LG ^y	Leaf-greenhouse					Pod-greenhouse		Leaf-field		Pod-field	
		EK-11 ^x	DR-7	SC-4A	LB-2	SX-114	EK-11	DR-7	EK-11	DR-7	EK-11	DR-7
E4.1150	1						0.0081 ^w /11 ^v	0.0321/8				
E4.700	1						0.0043/13	0.0198/9				
AL7.650	1						0.0051/13	0.0133/10				
C7.900	1						0.0036/14	0.0128/10				
V	5	0.0000/36	0.0002/22	0.0000/30	0.0002/23	0.0000/34			0.0000/29	0.0000/61	0.0001/24	0.0000/28
BC437.1050	5	0.0000/31	0.0009/17	0.0000/36	0.0000/26	0.0000/28			0.0004/20	0.0000/45	0.0059/13	0.0020/16
BC420.900	5	0.0006/18	0.0304/8	0.0000/30	0.0001/23	0.0003/20			0.0174/10	0.0001/23		
AQ15.1300	5	0.0000/32	0.0002/21	0.0000/28	0.0002/21	0.0021/15			0.0380/7	0.0024/15		
BC420.600	5	0.0000/30	0.0002/21	0.0057/13	0.0347/8							
AP7.1800	9	0.0000/29	0.0002/21	0.0001/25	0.0012/17	0.0020/16	0.0127/10	0.0352/8	0.0097/11	0.0001/24		
D13.1000	UM ^u						0.0016/16	0.0037/14			0.0100/11	0.0289/8

^zMarker significantly associated with a trait. V is the flower color gene.

^yLinkage group, the assigned number for the linkage group of marker, developed by Jung et al. (1997), as defined in Fig. 2.

^x*Xcp* strain inoculated on the leaf or pod.

^wSignificance levels (*P*) determined for F tests on the basis of single-factor ANOVA.

^vPercentage (*R*²) of phenotypic variation explained by the marker locus on the basis of single-factor ANOVA.

^uUnassigned marker.

Table 2. Summary of the stepwise regression analysis of molecular marker and phenotypic data for detection of QTL associated with resistance to *Xanthomonas campestris* pv. *phaseoli* (*Xcp*) strains in a recombinant inbred backcross population from the common bean cross BC₂F₆ 'PC-50' (susceptible to *Xcp*) x XAN-159 (resistant to *Xcp*) in greenhouse and field experiments.

Marker ^z	LG ^y	Leaf-greenhouse					Pod-greenhouse		Leaf-field		Pod-field	
		EK-11 ^x	DR-7	SC-4A	LB-2	SX-114	EK-11	DR-7	EK-11	DR-7	EK-11	DR-7
E4.700	1						0.0474 ^w /5 ^v	0.0489/5				
V	5	0.0001/36	0.0002/22			0.0001/34			0.0001/29	0.0001/61	0.0001/24	0.0001/28
BC437.1050	5			0.0001/36	0.0001/26							
AP7.1800	9						0.0109/9	0.0334/7				
D13.1000	UM ^u						0.0016/16	0.0037/14			0.0420/6	0.0481/5
Cumulative <i>R</i> ²		36	22	36	26	34	30	26	29	61	30	33

^zMarker significantly associated with a trait. V is the flower color gene.

^yLinkage group, the assigned number for the linkage group of marker, developed by Jung et al. (1997), as defined in Fig. 2.

^x*Xcp* strain inoculated on the leaf or pod.

^wSignificance levels (*P*) determined for F tests on the basis of stepwise regression analysis.

^vPercentage (*R*²) of phenotypic variation explained by the marker locus on the basis of stepwise regression analysis.

^uUnassigned marker.

Table 3. Summary of the single-factor ANOVA and stepwise regression analyses of molecular marker and phenotypic data for detection of QTL associated with leaf resistance to *Xanthomonas campestris* pv. *phaseoli* (*Xcp*) strains in an F₂ population from the common bean cross pinto 'Chase' (susceptible to *Xcp*) x XAN-159 (resistant to *Xcp*) in the greenhouse.

Marker ^z	Linkage group ^y	Single-factor ANOVA		Stepwise regression	
		EK-11 ^x	DR-7	EK-11	DR-7
E4.1150	1	0.0039 ^w /10 ^v	0.0005/14		0.0167 ^w /5 ^v
E4.700	1	0.0014/12	0.0124/8		
V	5	0.0000/19	0.0001/18		
BC437.1050	5	0.0000/21	0.0000/31		0.0001/31
BC420.900	5	0.0000/24	0.0000/21	0.0001/24	
P8.1000	5	0.0006/14	0.0033/10		
Y7.1200	5	0.0005/14	0.0044/10		
AP7.1800	9	0.0002/15	0.0001/17		
Cumulative <i>R</i> ²				24	36

^zMarker significantly associated with a trait. V is the flower color gene.

^yThe assigned number for the linkage group of marker, developed by Jung et al. (1997), as defined in Fig. 2.

^x*Xcp* strain inoculated on the leaf.

^wSignificance levels (*P*) determined for F tests on the basis of single-factor ANOVA or stepwise regression analysis.

^vPercentage (*R*²) of phenotypic variation explained by the marker locus on the basis of single-factor ANOVA or stepwise regression analysis.

estimated in the RI backcross population. One major gene was estimated in XAN-159 for leaf resistance to each of strains EK-11, DR-7 and SX-114 in the greenhouse and field experiments. However, two genes were estimated for leaf resistance to each of strains SC-4A and LB-2 in the greenhouse experiment. One major gene was estimated in XAN-159 for pod resistance to each of the strains EK-11 and DR-7 in the greenhouse and field experiments similar to the findings of Eskridge and Coyne (1996) using RI backcross lines of the same cross. However, we found that the XAN-159 parent possessed one or two genes controlling resistance of leaf reactions to the *Xcp* strains used here, whereas five genes were estimated to determine the reaction to one *Xcp* strain by Eskridge and Coyne (1996). The differences in gene numbers may be due to the *Xcp* strains and environments used. One and six genes for resistance to *Xcp* were also reported by McElroy (1985) and Jung et al. (1997), respectively.

CORRELATION OF LEAF AND POD REACTIONS TO *Xcp*. A low (0.24, $P = 0.0483$) correlation was detected in the RI backcross population between leaf and pod reactions to *Xcp* strain EK-11 in the greenhouse experiment, while intermediate (0.57, $P = 0.0001$) and moderately high (0.75, $P = 0.0001$) correlations were noted between leaf and pod reactions to *Xcp* strains EK-11 and DR-7 in the field experiment. The low correlations between leaf and pod reactions to *Xcp* in the greenhouse were close to those found by Arnaud-Santana et al. (1994) in other bean crosses, while intermediate and high correlations between leaf and pod reactions to *Xcp* in the field experiment were nearly the same as those reported previously in three *P. vulgaris* crosses by Ariyaratne (1994). Other investigators reported no correlations between leaf and pod reactions to *Xcp* (Schuster and Coyne, 1981; Coyne and Schuster, 1983; Aggour and Coyne, 1989; Valladares-Sanchez et al, 1979, 1983).

DETECTION OF QTL FOR RESISTANCE TO *Xcp*. The *V* locus or marker BC437.1050 on linkage group (LG) 5 and marker AP7.1800 on LG 9 were associated with leaf resistance to five *Xcp* strains in the greenhouse and field in the RI backcross population based on single-factor ANOVA (Table 1). However, only one marker locus accounting for 22% to 61% of the phenotypic variation for this resistance was detected using the stepwise regression analysis (Table 2). Markers BC420.900, BC437.1050, E4.1150 and AP7.1800 were associated with leaf resistance to *Xcp* strains EK-11 and DR-7 in the F_2 population based on single-factor ANOVA (Table 3). In the stepwise regression analysis one and two markers explaining 24% and 36% of the phenotypic variation for leaf resistance to EK-11 and DR-7, respectively, were significant (Table 3).

Four markers (E4.700, C7.900, AP7.1800 and D13.1000) were significantly associated with pod resistance to *Xcp* strains EK-11 and DR-7 in the greenhouse in the RI backcross population using single-factor ANOVA (Table 1). In the stepwise regression analysis three markers provided significant associations and accounted for 30% and 26% of the phenotypic variation for pod resistance to EK-11 and DR-7, respectively, in the greenhouse (Table 2). The *V* locus and marker D13.1000 were associated with pod resistance to *Xcp* strains EK-11 and DR-7, respectively, in the field in the RI backcross population on the basis of single-factor ANOVA (Table 1). Using the stepwise regression analysis two marker loci were detected and explained 30% and 33% of the variation for pod resistance to EK-11 and DR-7, respectively, in the field (Table 2). Gene number (one to two) estimations and number of QTL (one to three) detected in the RI backcross population for resistance to *Xcp* generally agree.

CONFIRMATION OF QTL FOR RESISTANCE TO *Xcp*. Jung et al. (1997) found RAPD markers and the *V* gene on LG 5 associated with a single QTL affecting leaf and pod resistance to *Xcp* in the RI population (70 lines) from the cross 'PC-50' x XAN-159 in greenhouse experiments. For confirmation of this association four RAPD markers and the *V* gene on LG 5 were tested in the RI backcross population of the cross BC_2F_6 'PC-50' x XAN-159 and in the F_2 population of the cross pinto 'Chase' x XAN-159. RAPD markers AQ15.1300 and BC420.600 were detected in the susceptible parent 'PC-50' but not in pinto 'Chase', therefore the markers AQ15.1300 and BC420.600 were not tested in the F_2 population. The other RAPD markers used here occurred in the resistant parent XAN-159. All markers tested here were significantly associated with resistance to *Xcp* in both populations based on single-factor ANOVA (Tables 1 and 3). Marker BC437.1050 and the *V* gene were more associated with leaf and pod resistance to *Xcp* strains than other markers. The three most resistant lines and plants based on phenotypic evaluation in the RI backcross and F_2 populations were identified by the marker BC437.1050 and the *V* gene. Marker BC420.900 was consistently associated only with leaf resistance to *Xcp* strains, and accounted for 8% to 30% of the variation for the traits.

RAPD markers on LG 1 associated with two QTL affecting leaf and pod resistance to *Xcp* were also reported by Jung et al. (1997). Six and three RAPD markers on LG 1 were tested for confirmation in the RI backcross and F_2 populations, respectively. Markers U15.1000, C7.900 and BC432.1000 were present in 'PC-50' but not in pinto 'Chase' and thus were not evaluated in the F_2 population. The other markers tested here were present in XAN-159. Markers E4.1150 and E4.700 were significantly associated with a single QTL for pod resistance to two *Xcp* strains in the greenhouse in the RI backcross population and with leaf resistance to two *Xcp* strains in the F_2 population. These markers accounted for 8% to 14% of the variation for the traits. Markers AL7.650 and C7.900 were associated with another QTL only for pod resistance to 2 *Xcp* strains in the greenhouse-grown RI backcross population (Table 1). However, the marker AL7.650 was not associated in the F_2 population.

Molecular markers associated with two QTL for pod resistance to *Xcp* were found by Jung et al. (1997) on LGs 4 and 9. They also found an unassigned marker D13.1000 to be associated with leaf resistance to *Xcp* (Jung et al., 1997). For confirmation three and one RAPD markers were used in the RI backcross and F_2 populations, respectively. Since markers AL7.1050 and D13.1000 were present in 'PC-50' but not in 'Chase', they were not tested in the F_2 population. Marker AP7.1800 was present in XAN-159 and was consistently associated with leaf and pod resistance to *Xcp* strains, except for pod resistance in the field. This marker located on LG 9 accounted for 8% to 29% of the variation for the traits. Unassigned marker D13.1000 was associated only with pod resistance to two *Xcp* strains and explained 8% to 16% of the variation for the traits. However, marker AL7.1050 on LG 4 was not associated in the RI backcross population. Therefore, among the six QTL, five in the RI backcross population and three in the F_2 population were confirmed to be associated with resistance to *Xcp* in this study.

Five common markers on LG 1 were connected between the RI and the RI backcross populations by straight lines, while two common markers on LG 1 were connected between the RI and the F_2 populations (Fig. 2). Three common markers between the RI and the RI backcross populations and four between the RI and the F_2 populations on LG 5 were noted. Most common markers on

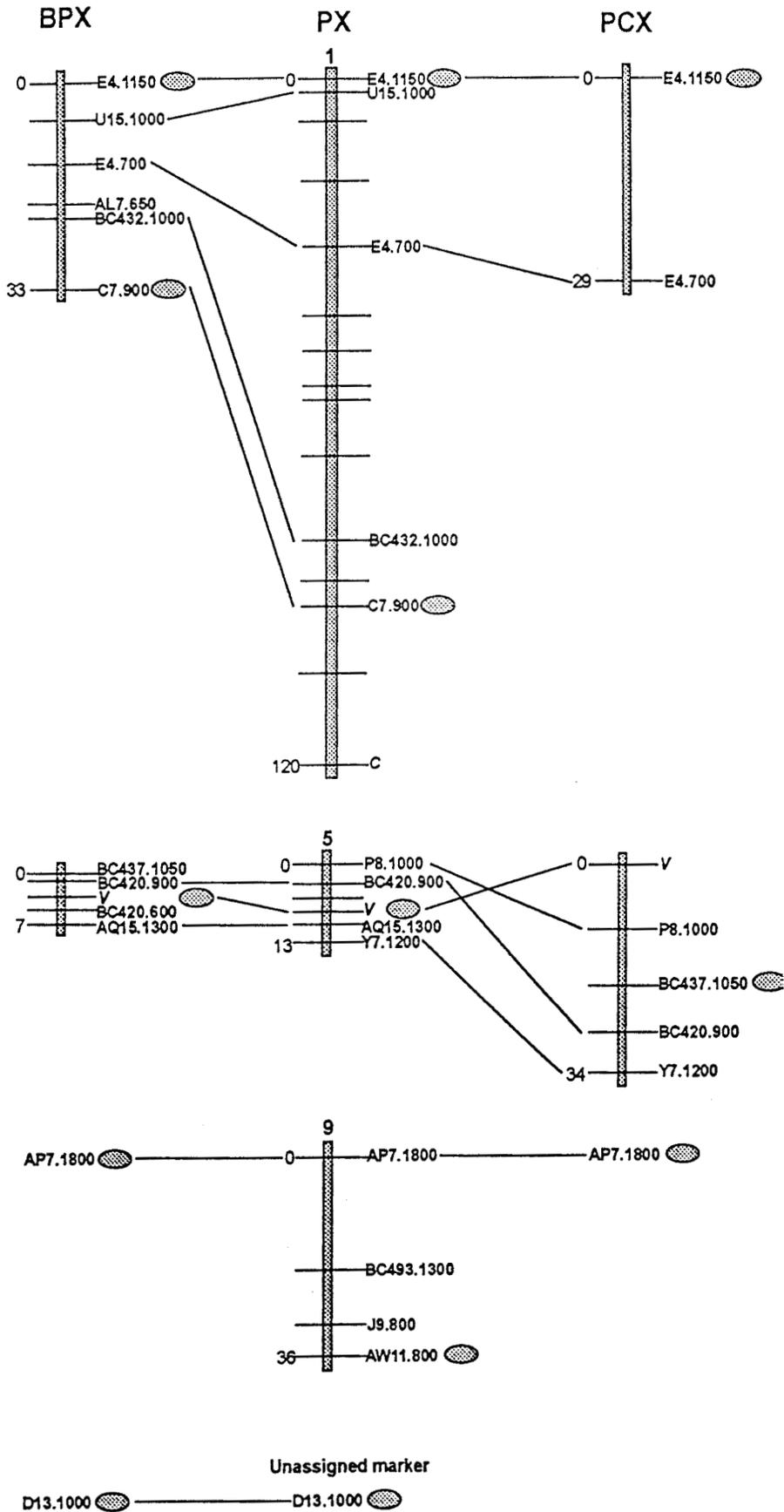


Fig. 2. Confirmation of markers associated with QTL controlling resistance to *Xanthomonas campestris* pv. *phaseoli* (*Xcp*) previously detected on linkage groups 1, 5 and 9 from a molecular marker-based partial linkage map using 70 recombinant inbred (RI) lines of the cross 'PC-50' x XAN-159 (PX) (Jung et al., 1997), a RI backcross population of the same cross BC₂F₆ 'PC-50' x XAN-159 (BPX) and an F₂ segregating population of the different cross pinto 'Chase' x XAN-159 (PCX). The gene and marker names are given on the right and the length in centiMorgans is indicated on the left of each linkage group. Markers significantly associated with QTL affecting resistance to *Xcp* on each linkage group in each population using single-factor ANOVA analysis are indicated by dotted oval forms. The common markers in each linkage group are connected between populations by straight lines.

scoring errors in the F₂ population. Distances between markers on LGs 1 and 5 in the two populations were different. Sampling variation in markers could contribute to variations in distances between markers.

Studies in tomato and common bean indicate that although not all QTL are detected in all environments, a few QTL are always expressed regardless of environmental conditions (Paterson et al., 1991; Park et al., 1999). Therefore, different environments should be tested to determine the consistency of associations of markers with QTL. Environment can also influence the quantitative patterns of inheritance of leaf and pod reactions to *Xcp* in common bean. Among six QTL detected by Jung et al. (1997), three were consistently expressed under greenhouse and field conditions in the RI backcross population while two were noted only under greenhouse conditions.

Among many detected QTL, only a few are consistently expressed in all populations (Beavis et al., 1991; Bubeck et al., 1993; Jung et al., 1999; Park et al., 1999). Jung et al. (1999) found only one common QTL affecting resistance to *Xcp* in common bean expressed consistently in four genetic populations. Three QTL on LGs 1, 5 and 9 were consistently expressed here in three (RI, RI backcross and F₂) populations, two QTL were expressed in two (RI and RI backcross) populations, and one QTL was only found in a single (RI) population.

Markers BC437.1050, BC420.900, AQ15.1300 and *V* locus on LG 5 and marker AP7.1800 on LG 9 were consistently associated with leaf resistance to the five *Xcp* strains from different origins (Nebraska, the Dominican Republic and South Africa) in the RI backcross population (Table 1). Thus, the markers are expected to be useful in breeding programs for resistance to a wide range of *Xcp* strains. The association of the *V* gene and RAPD markers with leaf resistance to the newly tested *Xcp* strains SC-4A, LB-2 and SX-114 in crosses of XAN-159 to the same or different susceptible common

LGs 1 and 5 were arranged in the same order. However, the order of the *V* locus on LG 5 was different, perhaps because of marker

tance to the newly tested *Xcp* strains SC-4A, LB-2 and SX-114 in crosses of XAN-159 to the same or different susceptible common

beans indicates nonspecific resistance to the five *Xcp* strains. This may not be the case in tepary bean (*P. acutifolius*) where Park et al. (1998) reported RAPD markers linked to the gene for specific resistance to each *Xcp* strain. This is consistent with phenotypic data on specificity for *Xcp* strains in tepary bean (Opio et al., 1996).

Correlated traits usually have common markers associated with them (Paterson et al., 1991). Where low correlations between leaf and pod reactions to *Xcp* strains EK-11 and DR-7 were observed in the greenhouse-grown RI backcross population, a common associated marker AP7.1800 on LG 9 was found (Table 1). Where intermediate and moderately high correlations between leaf and pod reactions to *Xcp* strains were observed in this population, the common associated *V* gene on LG 5 was noted (Table 1). These two markers should be tested for simultaneous selection of both of these traits.

The *V* gene on LG 5 confirmed here in the two populations was also reported by Park et al. (1999) to be associated with a single QTL affecting seed weight in the RI population of the cross 'PC-50' x XAN-159. The confirmed marker E4.1150 on LG 1 was also associated with a single QTL affecting seed weight in the RI population (Park et al., 1999). Among the six QTL affecting resistance to *Xcp* detected by Jung et al. (1997), two were also involved in controlling seed weight in the RI population. Thus, the *V* gene and marker E4.1150 could be used in bean breeding programs for larger seed size or resistance to *Xcp*.

Markers AQ15.1300 and BC420.600 on LG 5, marker C7.900 on LG 1 and unassigned marker D13.1000 associated with QTL for resistance to *Xcp* detected by Jung et al. (1997) were confirmed here in the RI backcross population. These markers are present in the susceptible parent 'PC-50' and should not be used for pyramiding different genes for improvement of resistance to *Xcp* in crosses between XAN-159 and other resistant parents.

The CBB resistant breeding lines, such as GN Nebr.#1 sel. 27 (Coyne and Schuster, 1983), navy bean lines (Scott and Michaels, 1988), and XAN lines (McElroy, 1985) that are frequent sources of CBB resistance are derived from interspecific crosses of common bean (susceptible to *Xcp*) x tepary bean (resistant to *Xcp*). The resistance to *Xcp* in the breeding lines is only partial and needs to be improved. Recombining additional genes for resistance to *Xcp* into common bean cultivars/lines will be necessary to increase the level of resistance in all plant organs to *Xcp*. Detection of molecular markers associated with different genes/QTL for resistance to *Xcp* will be useful to develop enhanced resistance to *Xcp*. RFLP markers associated with QTL for resistance to *Xcp* in BAT93 (Nodari et al., 1993), RAPD markers associated with QTL in BAC 6 (Jung et al., 1996), and RAPD markers associated with QTL in XAN 176 (Miklas et al., 1996) were identified. These germplasm sources derive their resistance from GN Nebraska #1 sel 27. RAPD markers linked to genes for resistance to *Xcp* from other tepary bean sources (Bai et al., 1997; Park et al., 1998), RFLP markers linked to genes for resistance in XR-235-1-1 derived from *P. coccineus* (Yu et al., 1998) were also detected. The *V* gene and the RAPD marker BC437.1050 on LG 5, originally associated with a single QTL in XAN-159 by Jung et al. (1997), were confirmed in two populations of the same cross and a different cross, in different environments and generations, and in different organs and *Xcp* strains. Therefore, the confirmed marker BC437.1050 and *V* gene, along with the markers associated with the above resistance genes from other germplasm, could be utilized to pyramid the different genes into a susceptible or partially resistant bean cultivar/line to enhance resistance to *Xcp*.

The resistance of pinto 'Chase' (recurrent parent) to *Xcp* was enhanced by backcrossing in QTL from the donor resistant parent XAN-159 using RAPD markers (Mutlu, 1998).

Literature Cited

- Aggour, A.R. and D.P. Coyne. 1989. Heritability, phenotypic correlations, and associations of the common blight disease reactions in beans. *J. Amer. Soc. Hort. Sci.* 114:828-833.
- Andrus, C.F. 1948. A method of testing beans for resistance to bacterial blights. *Phytopath.* 38:757-759.
- Ariyaratne, H.M. 1994. Inoculation procedures and heritabilities of the reactions to common bacterial blight in different plant parts in common beans. MS thesis, Univ. of Nebraska, Lincoln.
- Arnaud-Santana, E., D.P. Coyne, K.M. Eskridge, and A.K. Vidaver. 1994. Inheritance; low correlations of leaf, pod, and seed reactions to common blight disease in common beans; and implications for selection. *J. Amer. Soc. Hort. Sci.* 119:116-121.
- Bai, Y., T.E. Michaels, and K.P. Pauls. 1997. Identification of RAPD markers linked to common bacterial blight resistance genes in *Phaseolus vulgaris* L. *Genome* 40:544-551.
- Beavis, W.D., D. Grant, M. Albertsen, and R. Fincher. 1991. Quantitative trait loci for plant height in four maize populations and their associations with qualitative genetic loci. *Theor. Appl. Genet.* 83:141-145.
- Bubeck, D.M., M.M. Goodman, W.D. Beavis, and D. Grant. 1993. Quantitative trait loci controlling resistance to gray leaf spot in maize. *Crop Sci.* 33:838-847.
- Clafin, L.E., A.K. Vidaver, and M. Sasser. 1987. MXP, a semiselective medium for *Xanthomonas campestris* pv. *phaseoli*. *Phytopath.* 77:730-734.
- Coyne, D.P. and M.L. Schuster. 1983. Genetics of and breeding for resistance to bacterial pathogens in vegetable crops. *HortScience* 18:30-36.
- Coyne, D.P., M.L. Schuster, and L. Harris. 1965. Inheritance, heritability, and response to selection for common blight (*Xanthomonas phaseoli*) tolerance in *Phaseolus vulgaris* field bean crosses. *Proc. Amer. Soc. Hort. Sci.* 86:373-379.
- Coyne, D.P., M.L. Schuster, and L. Shaughnessy. 1966. Inheritance to reaction of halo blight and common blight bacteria in a *Phaseolus vulgaris* variety cross. *Plant Dis. Repr.* 50:29-32.
- Coyne, D.P., D.S. Nuland, D.T. Lindgren, and J.R. Steadman. 1994. 'Chase' pinto dry bean. *HortScience* 29:44-45.
- Edwards, M.D., C.W. Stuber, and J.F. Wendell. 1987. Molecular marker-facilitated investigations of quantitative trait loci in maize. I. Numbers, genomic distribution, and types of gene action. *Genetics* 116:113-125.
- Eskridge, K.M. and D.P. Coyne. 1996. Estimating and testing hypotheses about the number of genes using inbred-backcross data. *Heredity* 87:410-412.
- Jung, G., D.P. Coyne, P.W. Skroch, J. Nienhuis, E. Arnaud-Santana, J. Bokosi, H.M. Ariyaratne, J.R. Steadman, J.S. Beaver, and S.M. Kaeppeler. 1996. Molecular markers associated with plant architecture and resistance to common blight, web blight, and rust in common beans. *J. Amer. Soc. Hort. Sci.* 121:794-803.
- Jung, G., P.W. Skroch, D.P. Coyne, J. Nienhuis, E. Arnaud-Santana, H.M. Ariyaratne, S.M. Kaeppeler, and M.J. Bassett. 1997. Molecular-marker-based genetic analysis of tepary bean-derived common bacterial blight resistance in different developmental stages of common bean. *J. Amer. Soc. Hort. Sci.* 122:329-337.
- Jung, G., P.W. Skroch, J. Nienhuis, D.P. Coyne, E. Arnaud-Santana, and H.M. Ariyaratne. 1999. Confirmation of QTL associated with common bacterial blight resistance in four different genetic backgrounds in common bean. *Crop Sci.* (in press).
- Kosambi, D.D. 1944. The estimation of map distances from recombination values. *Ann. Eugenics* 12:172-175.
- Lander, E.S., P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, and L. Newburg. 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps with

- experimental and natural populations. *Genomics* 1:174–181.
- McElroy, J.B. 1985. Breeding dry beans, *Phaseolus vulgaris* L., for common bacterial blight resistance derived from *Phaseolus acutifolius* A. Gray. PhD diss., Cornell Univ., Ithaca, N.Y.
- Miklas, P.N., E. Johnson, V. Stone, J.S. Beaver, C. Montoya, and M. Zapata. 1996. Selective mapping of QTL conditioning disease resistance in common bean. *Crop Sci.* 36:1344–1351.
- Musaana, M.S., O.D. Mwandemele, and J.A. Kapuya. 1993. The number of genes controlling resistance in beans to common blight. *Annu. Rpt. Bean Improv. Coop.* 36:156–157.
- Mutlu, N. 1998. Enhancement of resistance to common bacterial blight in pinto beans and confirmation of markers for resistance from the cross pinto ‘Chase’ x XAN-159. MS thesis, Univ. Nebraska, Lincoln.
- Nodari, R.O., S.M. Tsai, P. Guzman, R.L. Gilbertson, and P. Gepts. 1993. Toward an integrated linkage map of common bean. III. Mapping genetic factors controlling host-bacteria interactions. *Genetics* 134:341–350.
- Opio, A.F., D.J. Allen, and J.M. Teri. 1996. Pathogenic variation in *Xanthomonas campestris* pv. *phaseoli*, the causal agent of common bacterial blight in *Phaseolus* beans. *Plant Pathology* 45:1126–1133.
- Park, S.O., D.P. Coyne, A. Dursun, and G. Jung. 1998. Identifying randomly amplified polymorphic DNA (RAPD) markers linked to major genes for common bacterial blight resistance in tepary bean. *J. Amer. Soc. Hort. Sci.* 123:278–282.
- Park, S.O., D.P. Coyne, G. Jung, E. Arnaud-Santana, J.R. Steadman, P.W. Skroch, J. Nienhuis, and H. M. Ariyaratne. 1999. Mapping of molecular markers associated with QTL affecting seed size and shape and their genetic relationships with common bacterial blight and rust resistance, and abaxial leaf pubescence in common bean. *J. Amer. Soc. Hort. Sci.* (in press).
- Paterson, A.H., S. Damon, J.D. Hewitt, D. Zamir, H.D. Rabinowitch, S.E. Lincoln, E.S. Lander, and S.D. Tanksley. 1991. Mendelian factors underlying quantitative traits in tomato: Comparison across species, generations, and environments. *Genetics* 127:181–197.
- Rava, C.A., M.J. de O. Zimmermann, and R. da S. Romeiro. 1987. Inheritance of resistance to *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye in *Phaseolus vulgaris* L. *J. Genet.* 4:709–727.
- Saettler, A.W. 1989. Common bacterial blight, p. 261–283. In: H.F. Schwartz and M.A. Pastor-Corrales (eds.). *Bean production problems in the tropics*. Ctr. Intl. Agr. Trop., Cali, Colombia.
- SAS Institute. 1982. *User’s guide: Statistics*. SAS Institute, Cary, N.C.
- Schuster, M.L. and D.P. Coyne. 1981. Biology, epidemiology, genetics, and breeding for resistance to bacterial pathogens of *Phaseolus vulgaris* L. *Hort. Rev.* 3:28–57.
- Schwartz, H.F. and G.E. Galvez. 1981. Bean production and pest constraints in Latin America, p. 3–14. In: H.F. Schwartz and G.E. Galvez (eds.). *Bean production problems in the tropics*. Ctr. Intl. Agr. Trop., Cali, Colombia.
- Scott, M.E. and T.E. Michaels. 1988. Inheritance of resistance to common bacterial blight in common bean. *Ann. Rpt. Bean Improv. Coop.* 31:72.
- Silva, L.O.E., S.P. Singh, and M.A. Pastor-Corrales. 1989. Inheritance of resistance to bacterial blight in common bean. *Theor. Appl. Genet.* 78:619–624.
- Skroch, P. and J. Nienhuis. 1995. Qualitative and quantitative characterization of RAPD variation among snap bean (*Phaseolus vulgaris*) genotypes. *Theor. Appl. Genet.* 91:1078–1085.
- Valladares-Sanchez, N.E., D.P. Coyne, and M.L. Schuster. 1979. Differential reaction of leaves and pods of *Phaseolus* germplasm to strains of *Xanthomonas phaseoli* and transgressive segregation for tolerance from crosses of susceptible germplasm. *J. Amer. Soc. Hort. Sci.* 104:648–655.
- Valladares-Sanchez, N.E., D.P. Coyne, and R.F. Mumm. 1983. Inheritance and associations of leaf, external, and internal pod reactions to common blight bacterium in *Phaseolus vulgaris* L. *J. Amer. Soc. Hort. Sci.* 108:272–278.
- Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalksi, and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* 18:6531–6535.
- Yu, Z.H., R.E. Stall, and C.E. Vallejos. 1998. Detection of genes for resistance to common bacterial blight of beans. *Crop Sci.* 38:1290–1296.