

Inheritance of Resistance to Common Bacterial Blight in Four Tepary Bean Lines

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ABSTRACT. High levels of resistance to common bacterial blight caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye (*Xcp*) have been observed for tepary bean (*Phaseolus acutifolius* A. Gray var. *latifolius* Freeman). However, the inheritance of resistance from this source is unknown for many lines. The inheritance of common bacterial blight resistance was studied in four tepary bean lines crossed with the susceptible tepary bean MEX-114. Progenies were inoculated with a single *Xcp* strain 484a. Segregation ratios in the F₂ generation suggested that resistance in Neb-T-6-s and PI 321637-s was governed by one dominant gene, and Neb-T-8a-s had two dominant genes with complementary effects. These hypotheses for inheritance of resistance were supported by various combinations of F₁, F₃, BC₁P_n segregation data in all lines except PI 321637-s where an additional minor-effect gene with recessive inheritance was indicated. Generation means analyses corroborated that multiple resistance genes were present in PI 321638-s. Lack of segregation for susceptibility among testcrosses for allelism between Neb-T-6-s/PI 321637-s, Neb-T-6-s/Neb-T-8a-s, PI 321637-s/Neb-T-8a-s, and PI 321637-s/PI 321638-s, suggested that one or more loci conditioning resistance to common bacterial blight were in common across the four tepary lines.

The value of tepary bean as a potential gene donor of resistance to common bacterial blight and other useful traits to common bean (*Phaseolus vulgaris* L.) via interspecific hybridization has been noted (Mejía-Jiménez et al., 1994; Pratt and Gordon, 1994). For common bacterial blight resistance introgressed from tepary to common bean, both quantitative (Honma, 1956) and qualitative (Michaels, 1992) patterns of inheritance were observed. Mixed inheritance was corroborated by genetic marker analyses revealing that the tepary-derived resistance was conditioned by multiple loci, and with one locus usually exhibiting a prominent effect (Bai et al., 1997; Jung et al., 1997; Miklas et al., 1996; Nodari et al., 1993).

The genetics of resistance to common bacterial blight within tepary bean itself has been investigated by several researchers. Drijfhout and Blok (1987) observed that the resistance in PI 319443 was governed by one dominant gene. Conversely, McElroy (1985) working with PI 319443 and Scott (1988) working with PI 440795 both found three genes conditioned resistance with one having a major effect and two having modifying effects. A series of tightly linked genes were observed when multiple *Xcp* strains were included in the genetic analysis of tepary bean resistance to common bacterial blight (Cafati and Kimati, 1972; Dursun et al., 1995; Freytag, 1989). Park et al. (1998) found molecular markers tightly linked to three genes, one for resistance to each of three

different *Xcp* strains in a tepary cross. Generally, each combination of a linked gene conditioning resistance to a specific strain segregated 3:1 as expected for a dominant gene in the F₂ generation.

Our objective was to study the inheritance of resistance in other tepary bean lines identified by Miklas et al. (1994) as having high levels of field resistance to common bacterial blight. Information gained should facilitate continued introgression of common bacterial blight resistance from tepary to the highly susceptible common bean.

Materials and Methods

Four cultivated tepary bean lines: Neb-T-6-s (P₁), Neb-T-8a-s (P₂), PI 321637-s (P₃), and PI 321638-s (P₄), expressing high levels of field resistance to common bacterial blight in Puerto Rico (Miklas et al., 1994) were crossed to the susceptible tepary bean MEX-114 (P₅) (Freytag, 1989). The Neb-lines originated from a University of Nebraska tepary bean collection likely obtained from Freeman (S. Honma, personal communication, 1993), and the PIs were collected in Arizona. All four resistant lines represent selections (-s) from the original sources for uniform disease reaction, seed type, and growth habit (Miklas et al., 1994). Seeds of these lines may be requested from P.N. Miklas. For most crosses, disease reactions of individuals within F₁, F₂, BC₁P_n, and F₃, progenies were examined. Only the F₂ and F₃ generations were evaluated from the cross involving PI 321637-s. Allelism among potentially different sources of resistance were tested in F₂ progenies generated from crosses among resistant lines: Neb-T-6-s/PI 321637-s, Neb-T-6-s/Neb-T-8a-s, PI 321637-s/Neb-T-8a-s, and PI 321637-s/PI 321638-s.

All parents and progenies were inoculated by a single strain of the pathogen, *Xcp* 484a (courtesy of M. Zapata, University of Puerto Rico, Mayagüez, PR) that was isolated from dry bean growing in Juana Díaz, Puerto Rico. Plants were grown under

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Table 1. Average disease score for the parents and F_1 progeny and distribution of individual F_2 and backcross plants for reaction to common bacterial blight in four populations derived from resistant x susceptible tepary bean (F_2 distributions for four crosses between resistant tepary bean are also included).

Population	Mean score (1–9) ^z			Generation	Distribution for disease score (no. of plants)								
	P _{1–4}	P ₅	F ₁		1	2	3	4	5	6	7	8	9
Resistant X susceptible													
Neb-T-6-s (P ₁)/MEX-114 (P ₅)	1.1	9.0	2.0	F ₂	91	82	26	23	19	6	16	17	19
				BC ₁ P ₁	6	12	2	1	0	0	0	1	3
				BC ₁ P ₅	0	0	1	2	0	0	2	1	3
PI 321637-s (P ₂)/MEX-114 (P ₅)	1.8	9.0	---	F ₂	24	26	24	5	4	2	7	4	10
Neb-T-8a-s (P ₃)/MEX-114 (P ₅)	1.1	9.0	1.0	F ₂	17	36	33	14	10	7	19	8	19
				BC ₁ P ₃	5	13	11	6	2	0	0	0	1
				BC ₁ P ₅	1	0	3	2	0	0	1	0	33
PI 321638-s (P ₄)/MEX-114 (P ₅)	1.0	9.0	1.6	F ₂	94	44	23	7	10	2	5	0	2
				BC ₁ P ₄	12	3	0	0	0	0	0	0	0
				BC ₁ P ₅	1	2	5	5	1	0	1	0	14
Resistant X resistant													
Neb-T-6-s/PI 321637-s				F ₂	79	1	1	0	0	0	0	0	0
Neb-T-6-s/Neb-T-8a-s				F ₂	73	1	2	0	0	0	0	0	0
PI 321637-s/Neb-T-8a-s				F ₂	34	6	3	1	0	0	0	0	0
PI 321637-s/PI 321638-s				F ₂	68	2	0	0	0	0	0	0	0

^zPercentage diseased area based on a 1–9 scale (CIAT, 1987), with 1, 2, 3, 4, 5, 6, 7, 8, and 9 representing (in %) 0–11, 12–22, 23–33, 34–44, 45–55, 56–66, 67–77, 78–88, and 89–100 necrosis or chlorosis of the inoculated area (4 cm²), respectively.

Table 2. Segregation for resistant (1–4) and susceptible (5–9) disease severity scores^z among individuals within populations derived from resistant by susceptible tepary bean crosses, that exhibited bimodal distributions in the F_2 (see Table 1).

Cross or generation	Individuals (no.)	Observed 1–4:5–9	Expected 1–4:5–9	Goodness of fit statistics χ^2	<i>P</i>
Neb-T-6-s(P_1)/MEX-114(P_5) ^y					
F_1 (1–4)	11	11:0	All resistant		
BC_1P_1	25	21:4	All resistant		
BC_1P_5	9	3:6	1:1	1.00	>0.20
F_2	299	222:77	3:1	0.09	>0.50
F_3 (1–4)	149(6) ^x	148:1	All resistant		
(1–4)	161(8)	110:51	3:1	3.82	>0.05
(5–9)	39(4)	0:39	All susceptible		
F_3 families	18	6:8:4	1:2:1	0.67	>0.70
PI 321637-s(P_2)/MEX-114(P_5)					
F_2	106	79:27	3:1	0.01	>0.90
F_3 (1–4)	50(6)	50:0	All resistant		
(1–4)	143(7)	105:38	3:1	0.19	>0.50
(4)	43(1)	12:31	1:3	0.19	>0.50
(5–9)	11(1)	2:9	1:3	0.28	>0.50
(5–9)	24(1)	0:24	All susceptible		
F_3 families	16	6:7:2:1	1:2:1 ^w	1.03	>0.50
Neb-T-8a-s(P_3)/MEX-114(P_5)					
F_1	7	7:0	All resistant		
BC_1P_3	38	37:1	All resistant		
BC_1P_5	40	6:34	1:3	2.13	>0.10
F_2	163	100:63	9:7	1.50	>0.20
F_3 (1–4)	136(7)	136:0	All resistant		
(1–4)	186(11)	148:38	3:1	2.02	>0.10
(1–4)	120(7)	74:46	9:7	1.42	>0.20
(5–9)	147(4)	0:147	All susceptible		
F_3 families	29	7:11:7:4	1:4:4:7	22.78	<0.01

^zPercentage diseased area based on a 1–9 scale (CIAT, 1987), with 1, 2, 3, 4, 5, 6, 7, 8, and 9 representing (in %) 0–11, 12–22, 23–33, 34–44, 45–55, 56–66, 67–77, 78–88, and 89–100 necrosis or chlorosis of the inoculated area (4 cm²), respectively.

^yThe resistant ($P_{1,2,3}$) and susceptible (P_5) parents of each cross averaged 1 and 9 for disease score, respectively.

^xNumber of combined progenies in parentheses. Only progenies exhibiting homogeneous segregation patterns as indicated by heterogeneity interaction χ^2 tests (not shown) of $P > 0.05$ were combined.

^wTo test the goodness of fit to a 1:2:1 ratio, the 1:3 segregation class was included as all susceptible (S).

ambient conditions in the greenhouses and screenhouses at the USDA-ARS, Tropical Agriculture Research Station, in Mayagüez, PR. The natural photoperiod ranged from 11 (December) to 13 (June) h. The average day and night temperatures ranged from 26 (December) to 35 °C (June) and 17 (December) to 23 °C (June), respectively. Four to five plants were grown per 23-cm-diameter pot containing an artificial soil medium (Sunshine Mix No. 1, Fision, Hort., Vancouver, B.C.). Pots were watered and fertilized as needed to promote healthy plants. Inoculations were conducted from January 1994 to December 1995. The central leaflet of the first trifoliolate leaf was inoculated, with a suspension of strain *Xcp* 484a that had been diluted with sterile 0.01 M phosphate buffer to 3 to 6 × 10⁷ CFU/mL, ≈14 d after planting by the multiple-needle method (Andrus, 1948; Zapata et al., 1985). Control plants were inoculated with just buffer.

Disease reactions were evaluated 14 d after inoculation by scoring the percentage of diseased area based on a 1–9 scale [Centro Internacional Agricultura Tropical (CIAT), 1987], with 1, 2, 3, 4, 5, 6, 7, 8, and 9 representing (in %) 0–11, 12–22, 23–33, 34–44, 45–55, 56–66, 67–77, 78–88, and 89–100 necrosis or chlorosis of the inoculated area (4 cm²), respectively. In this study, bimodal distributions in the F₂ generation generally indicated that plants scoring from 1–4 were resistant and from 5–9 susceptible (Table 1). This demarcation between resistance and susceptibility was supported by F₃ progenies derived from F₂ plants scoring 1–4, either segregating for resistance or having uniformly resistant reactions; whereas, plants with scores from 5–9 always had uniformly susceptible progenies (Table 2). Of each F₂ population 75 to 200 plants were inoculated. About 20 individual plants within F₃ progenies derived from 16 to 34 randomly selected F₂ plants were subsequently inoculated.

For F₂ populations exhibiting bimodal distributions for disease reaction (Table 1), the F₁, F₂, BC₁P_{1,2,3} and BC₁P₅, and F₃ generations were analyzed by chi-square (χ²) tests to compare significance of fit between theoretical and observed Mendelian segregation ratios for resistant and susceptible plants. Generation means analysis, involving the P₄ and P₅ parents, F₁, F₂, BC₁P₄, and BC₁P₅ generations, was conducted for the PI 321638-s/MEX-114 cross which exhibited a continuous F₂ distribution for disease reaction (Table 1). A simple additive-dominance model (Ng, 1990) based on mean disease severity scores for the respective generations was used to examine the inheritance of common bacterial blight resistance in this cross.

Results and Discussion

The F₂ populations from crosses involving Neb-T-6-s and PI 321637-s by the susceptible tepary MEX-114 exhibited 3:1 segregation ratios for numbers of resistant to susceptible plants, suggesting the hypothesis that resistance in these lines was conditioned by a single dominant gene (Tables 1 and 2). A 1:1 ratio for

segregation in the BC₁P₅, all resistant F₁ plants, and a 1:2:1 segregation for all resistant, segregating 3:1 for resistant and susceptible, and all susceptible individuals among F₃ progenies supported dominant monogenic resistance in Neb-T-6-s. A few susceptible individuals occurred within otherwise all resistant F₃ and BC₁P₁ progenies. Perhaps these individuals were resistant but succumbed to an excessive amount of bacterial suspension inserted via the multiple-needle inoculation procedure. Some inoculations, performed during the summer months under higher temperatures ideal for severe *Xcp* infection (Saettler, 1989), may also have contributed to the resistance of a few individuals being overcome. The few susceptible BC₁P₁ individuals could have resulted from self-pollination. Modifier genes having a minor effect on expression of resistance could also have caused a few outlier reactions to occur in otherwise homozygous progenies.

For PI 321637-s some F₃ progenies from previously resistant or susceptible plants in the previous generation segregated 1:3 for resistance, indicating a minor-effect gene with recessive inheritance also conditioned resistance in this line. Interestingly, only progenies from resistant individuals scoring a 4 in the PI 321637-s/MEX-114 population segregated 1:3. For all other populations, progenies from plants previously scoring 4 were completely resistant or had segregation ratios in favor of resistance and progenies from plants previously scoring 5 or above were all susceptible, indicating that our classification of disease severity scores from 1–4 as resistant and 5–9 as susceptible was appropriate. Similar dominant monogenic resistance to *Xcp* 484a was observed for two additional tepary bean lines GN-605-s and PI 440806-s (data not shown; Urrea, 1996).

A digenic 9:7 segregation pattern for numbers of resistant to susceptible individuals in the F₂ generation suggested the presence of two complementary dominant genes conditioned resistance in Neb-T-8a-s (Tables 1 and 2). Combined 1:3 segregation in the BC₁P₅ generation, all resistant plants in the F₁ generation, and individuals within F₃ progenies either all resistant, segregating 3:1 or 9:7 for resistance and susceptibility, or all susceptible, supported the hypothesis for inheritance conditioned by two dominant genes with complementary effects. The F₃ generation did not fit the expected 1:4:4:7 segregation for progenies with all resistant, 3:1, 9:7, or all susceptible individuals. The inoculated F₂ plants set aside for generation of F₃ progenies, that were susceptible, were less likely to produce enough F₃ seed for testing; thus, probably contributed to the observed F₃ segregation skewed toward all resistant progenies. A similar 9:7 ratio was observed by Scott and Michaels (1988) for tepary-derived resistance in F₂ populations of common bean.

Significant additive epistatic effects and estimated presence of three resistance genes by the generation means analysis (Table 3) corroborated the quantitative inheritance suggested by the continuous F₂ distribution (Table 1) for disease reaction in the PI 321638-s/MEX-114 cross. High heritability and F₂ segregation

Table 3. Adequacy of the additive/dominance model, broad sense heritability (h), estimated number of genes conditioning resistance (k), and genetic effects^z (±SE): d = additive effects and i = epistasis between additive effects, obtained from generation means analyses of common bacterial blight reaction^y to *Xcp* strain 484a in a resistant × susceptible tepary bean cross that exhibited a continuous F₂ distribution (Table 1).

Cross	Model (3df)	h	k	d	i
PI 321638-s/MEX-114	72.0**	0.96	3.16	−219.2(0.02)**	5.13(1.22)*

^zAll dominance effects, epistasis between dominance effects, and epistasis between additive and dominance effects within this model were nonsignificant.

^yPercentage diseased area based on a 1–9 scale (CIAT, 1987), with 1, 2, 3, 4, 5, 6, 7, 8, and 9 representing (in %) 0–11, 12–22, 23–33, 34–44, 45–55, 56–66, 67–77, 78–88, and 89–100 necrosis or chlorosis of the inoculated area (4 cm²), respectively.

*,**Significant at P = 0.05 or 0.01, respectively.

skewed toward resistance suggested that environment had a minimal affect on expression of this quantitative resistance. An F_2 segregation of 168 resistant (disease scores from 1–4) to 19 susceptible (disease scores from 5–9) individuals, combined with 3:1, 13:3, and 55:9 resistant to susceptible segregation ratios observed in some F_3 progenies (data not shown), suggests that the quantitative resistance in PI 321638-s may be conditioned by one dominant and two recessive genes. This speculative trigenic inheritance for resistance to common bacterial blight parallels the inheritance described by McElroy (1985) and Scott (1988) where resistance was conditioned by one major and two minor or modifying genes. Urrea (1996) also observed quantitative inheritance, involving from two to five resistance genes, in the tepary lines Neb-T-1s, GN-610-s, PI 440788-s, and PI 502217-s.

No segregation for susceptibility was observed in any of the F_2 populations derived from crosses between resistant tepary bean lines (Table 1), indicating that at least one resistance gene was probably in common across Neb-T-6-s, Neb-T-8a-s, PI 321637-s and PI 321638-s. A similar limited variability for resistance to rust was observed in cultivated tepary bean by Miklas and Stavely (1998), providing further support for the occurrence of a bottleneck effect during domestication of this species. Conversely, the mono-, di-, and trigenic inheritance observed here and elsewhere (Drijfhout and Blok, 1987; McElroy, 1995; Scott and Michaels, 1992) suggests that the variability for resistance to common bacterial blight in cultivated tepary bean has probably not been fully exploited in the improvement of common bean.

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