Resistance to Common Bacterial Blight of Bean Introgressed from *Phaseolus coccineus*

M.M. Welsh¹

U.S. Department of Agriculture–Agricultural Research Service, Western Regional Plant Introduction Station, P.O. Box 46402, Pullman, WA 99164-6402

K.F. Grafton

Department of Plant Sciences, North Dakota State University, Fargo, ND 58105

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Abstract. Common bacterial blight, incited by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye, is a major bacterial disease of dry bean (*Phaseolus vulgaris* L.). Resistance to common bacterial blight has been identified in other *Phaseolus* species and resistance genes have been introgressed into *P. vulgaris*. The objective of this study was to characterize in dry bean the inheritance pattern of common bacterial blight–resistance genes derived from *P. coccineus*. Two common, bacterial blight–susceptible, dry bean cultivars were crossed with different common, bacterial blight–resistant dry bean lines with resistance derived from *P. coccineus*. F₂ progeny were inoculated with *Xanthomonas campestris* pv. *phaseoli* strain F19-W and were scored for disease reaction. The ratio of resistant to susceptible plants for F₂ populations did not differ significantly from a 1 resistant : 3 susceptible ratio. The F₃ segregation was obtained for only one cross and did not differ significantly from a 1 resistant : 2 heterozygous : 1 susceptible ratio, suggesting that the resistance introgressed from *P. coccineus* into dry bean was controlled by one recessive gene. Additionally, the range of symptom expression within the susceptible class provided evidence of other genes modifying the expression of resistance.

Common bacterial blight (CBB) is one of the major bacterial diseases of common bean (Saettler, 1991). Planting disease-free seed (certified seed) is the primary cultural control measure (Coyne and Schuster, 1980), but genetic resistance would provide the best solution (Singh and Muñoz, 1999; Zapata et al., 1985). Limited genetic variation for resistance to CBB in *P. vulgaris* underscores the need for other sources of resistance (van Schoonhoven and Voysest, 1991). Common bacterial blight resistance has been identified in other *Phaseolus* species and has been introgressed into *P. vulgaris* (Burkholder and Bullard, 1946; Coyne et al., 1965; Freytag et al., 1982; Miklas et al., 1994; Park and Dhanvantari, 1986; Schuster, 1955).

A greater level of resistance to CBB has been noted in some *P. coccineus* L. accessions (Coyne and Schuster, 1973; Coyne et al., 1963; Freytag et al., 1982; Singh and Muñoz, 1999). Previous genetic analyses have suggested a quantitative model of inheritance (Park and Dhanvantari, 1986; Yu et al., 1998), but a full

characterization of the inheritance of CBB resistance derived from *P. coccineus* has not been completed. Therefore, the objective of this study was to characterize the inheritance pattern of CBB–resistance genes derived from *P. coccineus*.

Materials and Methods

All disease screening was conducted in greenhouses at Fargo, N.Dak. Two separate populations were produced: ‘Midland’ (CBB–susceptible navy bean) × ‘92BG-191’ [population #1] and ‘Aztec’ (CBB–susceptible pinto bean) × ‘92BG-139’ [population #2]. ‘92BG-139’ is a sister line to ‘TARS VCI-4B’ (a multiple disease–resistant, small-seeded pinto dry bean from a recurrent, interspecific cross selection program that included *P. coccineus* accessions PI 311950 and PI 311977) (Miklas, et al., 1994). ‘92BG-191’ was produced by similar breeding methods using the *P. coccineus* accessions PI 311950 and PI 273667 (P. Miklas, personal communication, 1997).

F₂ plants of populations #1 and #2, 181 and 129 plants, respectively, were inoculated with *Xanthomonas campestris* pv. *phaseoli*. Two F₂ seeds per 15-cm-diameter pot, including 10 pots of each parental line as a control, were sown in Sunshine Mix medium (SunGro Horticulture, Bellevue, Wash.), augmented with 7.1 g per pot Osmocote fertilizer (Scott’s, Marysville, Ohio) (14N–7P–7K). The greenhouse was maintained at a 14- to 16-h photoperiod using natural light supplemented by high-pressure sodium lamps (1300 μmol·m⁻²·s⁻¹); greenhouse

temperatures ranged from 22 to 27 °C and relative humidity from 20% to 65%. Before inoculation, plants were watered and placed in an inoculation chamber at 100% relative humidity for 2 to 3 h. The middle leaflet of the fully expanded, fourth trifoliate leaf of each F₂ plant was inoculated by the multiple puncture method (Coyne et al., 1965) at the preflowering stage. After 30 h the plants were returned to the greenhouse. Disease reaction was evaluated 7, 14, and 21 d after inoculation and scored from 1 to 5, based on a previous inoculation study (Welsh, 1997). Susceptible parents scored in a range of 4–5, and resistant parents had scores of 1–2. Sixteen seeds (Hanson, 1959) of 122 F₃ families in population #1 were evaluated for resistance. The planting medium, fertilizer regime, and greenhouse conditions, and the incubation, evaluation, and scoring procedures were similar to those used for the F₂ populations. However, for F₃ plants, the fully expanded unifoliate leaf was inoculated on the underside by tissue infusion with a hypodermic syringe (Adams et al., 1989). A previous study indicated similar response when using either the multiple puncture or tissue infusion method (Welsh, 1997). A susceptible F₃ family contained individuals with scores ranging from 3 to 5 and with symptom expression increasing over time. Resistant families contained individuals with scores ranging from 1 to 2 and symptom expression did not increase over time. Heterozygous families contained individuals with scores ranging from 1 to 5, and symptom expression of the plants scoring >2 increased over time. Analysis of the data by the chi square test of goodness of fit and heterogeneity at the *P* < 0.05 level of significance was performed.

X. campestris pv. *phaseoli* strain F19-W (J. Venette, Dept. of Plant Pathology, North Dakota State Univ.) was grown on yeast–dextrose–carbonate growth media for 24 to 48 h at 27 °C. Bacteria were scraped from the surface of the medium and suspended in sterile distilled water to a concentration of 1 × 10⁹ cells/mL. Concentration was determined by absorption at OD 660 and compared with a previously established standard growth curve, relating cell number to absorbency. The inoculum was prepared by diluting this suspension with sterile distilled water to 1 × 10⁷ cells/mL.

Results and Discussion

Resistance to CBB, as represented by scores of 1 and 2, was expressed as no visible symptom development, or the leaf tissue developed a narrow, nonexpanding, chlorotic band around the inoculation site. Parents ‘92BG-191’ and ‘92BG-139’ had scores of 1–2. In the susceptible reaction, as represented by scores of 3–5, chlorosis and water soaking were present, and necrosis developed and expanded in some cases until the entire leaflet was dead or had abscised from the plant. Parents ‘Midland’ and ‘Aztec’ had scores of 4–5.

The ratio of resistant to susceptible F₂ plants did not differ significantly from a 1 resistant : 3 susceptible ratio, and the ratio of resistant to

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¹To whom reprint requests should be addressed. E-mail address: mmwelsh@wsu.edu

susceptible F₃ families did not differ significantly from a 1 resistant : 2 heterozygous : 1 susceptible ratio (Table 1), as expected if resistance is controlled by one recessive gene.

To prevent loss of desired characters from the *P. vulgaris* genome, transfer of a small piece of the *P. coccineus* genome containing the gene of interest into the *P. vulgaris* genome would be most desirable. The resistant parents used were advanced lines derived from *P. vulgaris* x *P. coccineus* crosses; they expressed good CBB resistance in field tests in Puerto Rico (P. Miklas, personal communication, 1992), yet possessed morphological traits associated with *P. vulgaris*. A small introgression from *P. coccineus* could explain the presence of resistance in the material used in this study.

The recessive resistance gene from *P. coccineus* already incorporated into *P. vulgaris* germplasm lines '92 BG-191' and '92BG-139' probably represents a potential source of CBB resistance not previously utilized in breeding programs; it confers a high level of resistance, indicating a major gene.

The level of resistance, conferred by a recessive gene, expressed in this experiment appeared to be similar to that conferred by gene(s) from *P. acutifolius* A. Gray, but the latter have been described as dominant (Michaels, 1992). Resistant parental lines in this experiment have similar sources for the CBB resistance, sharing one *P. coccineus* ancestor. Tests for allelism could provide clarification on these points.

The range of symptom expressions within the susceptible classes of F₂ plants and F₃ families showed a progression of reactions: chlorosis, water-soaking, tissue collapse, and necrosis expanding at slow rates out from the inoculation site through a more rapid development of all symptoms combined with eventual leaflet death. Some level of quantitative resistance, e.g., modifier genes, might explain this

Table 1. Segregation ratios in dry bean for resistance to common bacterial blight strain F19-W introgressed from *P. coccineus*.

Segregating population	Resistant	Heterozygous	Susceptible	Ratio	χ^2	P
Midland/92 BG 191 F ₂	38	---	143	1:3	1.55	0.2131
Aztec/92 BG 139 F ₂	24	---	105	1:3	2.81	0.0937
Midland/92 BG 191 F ₃ families	26	65	31	1:2:1	1.21	0.2713

result. Segregation within both F₂ populations showed bias toward the susceptible parent, but heterozygotic influence was indicated in F₃ families. Further consideration of these observations could help explain such discrepancies, and add to the understanding and value of this source of resistance.

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