# Inheritance of Resistance to a Colorado Race of *Fusarium oxysporum* f. sp. *phaseoli* in Common Beans

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**ABSTRACT**

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Fusarium wilt of common bean *Phaseolus vulgaris* caused by *Fusarium oxysporum* f. sp. *phaseoli* (FOP) is a serious disease in many production areas of the world. Inheritance of resistance in common bean to a pathogenic race of FOP isolated from pinto bean, U.I. 114, in Colorado was investigated. Resistant (R) and susceptible (S) common bean lines and cultivars from diverse sources were used as parents. The parental material, and $F_2$ and $F_3$ progeny derived from crosses between R and S lines were evaluated for reaction to FOP using a seedling root-clip inoculation technique under controlled greenhouse conditions. Inheritance of resistance to FOP differed among the parental lines and cultivars. Three segregation patterns were observed in the $F_2$ progeny of crosses between R and S parents. In one group, segregation patterns fit a single completely-dominant gene model (3R:1S), whereas segregation in the other group fit a more complex inheritance pattern in which recessive gene action controlled resistance to FOP. Other resistance patterns were more indicative of a quantitative pattern. Resistant lines that possessed single dominant genes for resistance originated from the Durango race, while resistant lines having recessive genetic resistance were from the Mesoamerican race of the Middle American Center of Diversity.

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *phaseoli* (FOP) J. B. Kendrick & W. C. Snyder, is a serious disease of beans (*Phaseolus vulgaris* L.) throughout Latin America and Africa (1). Yield losses in the High Plains area of the U.S. have been estimated at a minimum of 10% (E. Kerr and D. Nuland, *personal communication;* 10,12). The disease affects most commercial pinto, great-northern, and navy bean cultivars of common beans (1,5,11).

Information regarding the number of FOP races and the variability within races is limited. Several strains have been classified into races based on host reaction. The classification is often related to geographical origin, since the strains from Colombia, Brazil, Italy, and the U.S. have been characterized as different races (2,3,6). Two strains from the U.S., including ATCC 18131 from South Carolina and ATCC 90245 from Colorado used in the present study, have been classified as belonging to the same race (8).

Resistance to FOP is usually race specific (1,5). Brazilian line HF 465-63-1 is resistant to four different races of FOP, including a Brazilian race. The cultivars Porrillo Sintetico and Jamapa are resistant to a Colorado race, but susceptible to Brazilian races (5,8,9). Resistance to a Brazilian race was controlled by a single completely-dominant gene in the cultivars Tenderette, Pintado, and Early Gallatin (7). Resistance to the U.S. race ATCC 18131 was controlled by a single incompletely-dominant gene in the cultivar Preto Uberabinha. Resistance to these races was conditioned by a restriction of fungal distribution at the basal part of the stem (7).

The mechanism of inheritance of resistance to the Colorado race of FOP in lines and cultivars from North and Central America is unknown. This study was conducted to determine the mode(s) of inheritance for resistance to the Colorado race among common bean cultivars and lines previously classified as resistant by Salgado et al (9).

## MATERIALS AND METHODS

All parental lines and cultivars and progeny were evaluated for their reaction to FOP using a modified root-dip inoculation technique adapted from that described by Pastor-Corrales and Abawi (5). The ATCC 90245 culture (Colorado race) of FOP was recovered from an infected pinto bean, cultivar U.I. 114, collected by H. F. Schwartz in northeast Colorado in 1990 (8). Single spores (macroconidia) from a culture of infected plant material were used to establish and maintain the pathogen in culture tubes containing antoclaved, finely sieved sandy soil mixed with 2% powdered oat meal and 15% distilled water (w/w). This stock culture was stored at 4 C. A few milligrams of the soil culture was distributed onto petri dishes containing potato-dextrose agar (Difco), pH 5.6±0.2 and incubated in the laboratory for 7 to 10 days at room temperature (22–25 C) and 12 hr dark-light cycle. Conidia were suspended in sterile distilled water, filtered through a double layer of cheesecloth, and washed twice at 2,700 rpm at 4 C for 10 min. The inoculum concentration was adjusted to $10^6$ conidia per milliliter by use of a hemacytometer. Four hundred milliliters of the conidial suspension was added to a 1,000-ml beaker and continually stirred during each test.

Cultivars and lines of common beans previously classified by Salgado et al (9) as resistant (R) or susceptible (S) to the Colorado race were used (Table 1). Origins of the entries are as follows: HF-465-63-1 is a line with cream-beige seed color from the International Center for Tropical Agriculture's (CIAT) Fusarium Wilt Nursery, and seed was provided by M. A. Pastor-Corrales at CIAT. Jamapa, Porrillo Sintetico, and Rio Tibagi are black-seeded cultivars grown in Central and South America and seed was provided by Shree P. Singh at CIAT. The pinto bean lines CO 22625, CO 33142, and CO 59196 are advanced lines provided by M. A. Brick at Colorado State University. Othello and Viva are pinto and pink-seeded cultivars, respectively, released by USDA/ARS; seed was provided by M. Silbernagel, USDA-ARS, Prosser, WA. Plants were grown and crosses were made in the greenhouse following hand emasculation. The $F_1$ seeds were planted in the greenhouse to produce $F_2$ seed. A portion of the $F_2$ seed was planted in the field at the Colorado State University Fruita Research Center, Fruita, CO, under disease-free conditions to obtain random $F_3$ families for segregation analyses. Seed color and morphological traits were used to determine the success or failure of hybridization.

Plant reactions to FOP were evaluated in the greenhouse during 1991–1992. Two seeds of each entry (parent or progeny) were planted in a 13-cm-diameter plastic pot (1 L volume) containing a commercial potting medium (Terralite, Metromix #200); 20 or more progeny of each entry were evaluated depending upon seed availability. Seedlings were

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grown in the greenhouse at 25±2 C for 10–13 days until the unifoliolate leaves were nearly fully expanded. Seedlings were selected for uniform size, removed from the pots, and immediately washed with tap water to remove residual planting medium. Plants were maintained for 5 min or less with their root systems immersed in tap water before inoculation. Subsequently, the distal one-third portion of the root system was clipped with a sterile pair of scissors. Root pruning was done to reduce the number of escapes and enhance uniform infection and disease development (15). The remaining root systems were then placed in the conidial suspension for 5 min, and immediately transplanted into a 13-cm-diameter plastic pot with potting medium. Noninoculated plants were handled in the same manner except roots were dipped in sterile distilled water. During inoculation, the greenhouse temperature was maintained at 21–25 C. A cool (≈16 C) postinoculation temperature was maintained for 3 days to reduce transplanting shock. Inoculated plants were then incubated in the greenhouse with a 21/32 C night/day temperature. Relative humidity ranged from 50 to 100% and supplemental halide lights provided a 12 hr photoperiod with photosynthetically active radiation at the bench level of approximately 350–400 $\mu E/m^2$ per second. Plants were fertilized every 3 days starting 4 days after inoculation with a liquid fertilizer (6 g/20 L of 15-30-15 NPK, 100 ml per pot) to maintain vigorous plant growth.

Disease symptoms were rated 21 days after inoculation according to the CIAT severity scale: 1 = no visible symptoms; 3 = 10%; 5 = 25%; 7 = 50%; and 9 = 75% of leaves wilted, chlorotic, or dead (16). Plants that rated 1–3 were classified as resistant, 4–6 as intermediate, and 7–9 as susceptible (5).

A bimodal distribution of $F_2$ plants (based on two discrete classes: resistant to intermediate/susceptible) allowed the use of chi-square tests to compare significance of fit between theoretical and observed ratios. Intermediate reactions were combined with susceptible reactions because we assumed that these reactions represented either a delay in the eventual death of plants or environmental variation. Plants inoculated on sequential days were then treated as blocks; however, data from the blocks were pooled since a chi-square test for heterogeneity showed that blocks were not different. Progeny testing was conducted to detect escapes and homozygous and heterozygous $F_2$ plants. In all experiments, inoculated resistant and susceptible parents were used to evaluate uniformity of inoculation tests and to confirm their disease classification.

## RESULTS AND DISCUSSION

Inheritance of resistance to FOP from Colorado differed among parental lines and cultivars. Two distinct segregation patterns were observed in $F_2$ progeny of crosses between R and S parents. In one group segregation patterns fit a single gene completely-dominant model, whereas in the other group, segregation patterns were more complex.

Previous classification of parental lines as resistant or susceptible to FOP from Colorado was confirmed during evaluation of $F_2$ and $F_3$ progenies (Table 1). The observed reactions for all parental lines and cultivars fit the expected patterns, except HF-465-63-1 and Rio Tibagi. There was a variable disease reaction among HF 465-63-1 plants as checks in the $F_3$ progeny test (Table 1); however, in the $F_2$ progeny test oniy two plants were susceptible (*data not shown*). These reactions are likely due to lack of uniform infection, seed contamination, or environmental conditions that influenced disease symptoms. This genotype by environment interaction also suggests that resistance may be quantitatively inherited.

Three of the $F_2$ families derived from crosses between the R (CO 33142, CO 59156, Jamapa) and S (CO 22625, Othello) lines fit a 3R:1S segregation ratio (Table 2). Segregation in the $F_2$ progeny from R × R and S × S crosses among the same parents fit 1R:0S and 0R:1S ratios, respectively. Resistant plants observed in the progeny of S × S crosses were likely escapes. Based on $F_3$ progeny tests from 71 randomly chosen resistant $F_2$ plants in these crosses, all were correctly classified in the $F_2$. Based on these results, we proposed that inheritance of resistance to FOP was controlled by a single dominant gene. The proposed hypothesis assumed that R parents are homozygous dominant, and S parents are homozygous recessive for the gene that controls reaction to FOP. According to this hypothesis, the $F_2$ progeny should segregate 3R:1S, and $F_3$ families derived from randomly chosen $F_2$ plants should segregate 1 R:2 segregating:1 S. Results indicated that all $F_2$ and $F_3$ families fit the expected ratios for the proposed hypothesis (Tables 2 and 3). These results are in agreement with a previous report that resistance for two strains of FOP was controlled by a dominant single gene in common bean (7).

The second pattern of segregation occurred in progeny that resulted from R × S crosses when Porrillo Sintetico, HF 465-63-1, and Rio Tibagi were used as resistant parents. None of the five $F_2$ families that resulted from these crosses fit the 3R:1S ratio for the dominant single-gene model ($P < 0.01$, $X^2$ values not shown). Since segregation patterns in the progeny of crosses with Porrillo Sintetico suggested that resistance may be controlled by recessive alleles, a recessive single-gene model was hypothesized and tested. The $F_2$ progeny from this cross did not provide an acceptable fit to this hypothesis (Table 2); however, the $F_3$ progeny from randomly selected $F_2$ plants fit the expected ratio of 1 R:2 segregating:1 S. Furthermore, $F_3$ progeny tests on seven of the 11 resistant $F_2$ plants in the Othello × Porrillo Sintetico cross revealed that only three were homozygous for resistance. These results indicate that resistance to FOP is controlled by more than one recessive

**Table 1.** Reaction to the Colorado race of *Fusarium oxysporum* f. sp. *phaseoli* (FOP) among $F_2$ and $F_3$ progeny of bean (*Phaseolus vulgaris*) cultivars and lines

| | | No. of plants observed[b] | | | | | |
|---|---|---|---|---|---|---|---|
| | | $F_3$ progeny test | | | $F_2$ progeny test | | |
| Cultivars and lines | Parental reaction[a] | Res. | Int. | Sus. | Res. | Int. | Sus. |
| CO33142 | R (2.22) | 35 | 1 | 0 | 17 | 2 | 1 |
| CO22625 | S (7.05) | 3 | 4 | 29 | 0 | 1 | 26 |
| CO59196 | R (1.64) | 35 | 1 | 0 | 17 | 3 | 0 |
| Othello | S (8.58) | 2 | 0 | 34 | 0 | 0 | 44 |
| Viva | S (8.86) | 0 | 0 | 36 | 0 | 0 | 39 |
| Jamapa | R (1.40) | 35 | 0 | 1 | 17 | 6 | 2 |
| Porrillo Sintetico | R (1.16) | 36 | 0 | 0 | 37 | 0 | 0 |
| HF 465-63-1 | R (3.69) | 22 | 2 | 12 | 40 | 1 | 1 |
| Rio Tibagi | R (3.30) | 26 | 4 | 6 | | | |

[a] R indicates resistant and S indicates susceptible parental line.
[b] Plants rated 1–3 classified as resistant (Res.); 4–6 as intermediate (Int.); and 7–9 as susceptible (Sus.) to FOP according to the CIAT standardized rating scale (16). Number in parenthesis refers to the mean disease rating.

**Table 2.** Segregation for reaction to the Colorado race of *Fusarium oxysporum* f. sp. *phaseoli* (FOP) among $F_2$ families of ten *Phaseolus vulgaris* crosses

| Cross | Disease reactions[a] | Plants observed[b] | | | Expected ratio | $X^2$ | Probability |
|---|---|---|---|---|---|---|---|
| | | Res. | Int. | Sus. | | | |
| CO 22625 × CO 33142 | S × R | 42 | 1 | 9 | 3:1 | 0.64 | 0.25–0.50 |
| CO 59196 × Othello | R × S | 64 | 1 | 23 | 3:1 | 0.13 | 0.50–0.75 |
| Viva × Othello | S × S | 1 | 1 | 93 | 0:1 | ... | ... |
| Viva × CO 22625 | S × S | 2 | 3 | 92 | 0:1 | ... | ... |
| Jamapa × CO 22625 | R × S | 71 | 9 | 22 | 3:1 | 1.31 | 0.25–0.50 |
| Jamapa × CO 33142 | R × R | 24 | 0 | 2 | 1:0 | ... | ... |
| Viva × Porrillo Sintetico | S × R | 18 | 5 | 20 | 1:3 | 5.66 | 0.01–0.05 |
| Othello × Porrillo Sintetico | S × R | 11 | 5 | 69 | 1:3 | 5.96 | 0.01–0.05 |
| Othello × HF 465-63-1 | S × R | 7 | 9 | 81 | 1:15 | 0.03 | 0.75–0.90 |
| Othello × Rio Tibagi | S × R | 2 | 0 | 62 | 1:15 | 0.60 | 0.25–0.50 |

[a] R indicates resistant and S indicates susceptible parental line.
[b] Plants rated 1–3 classified as resistant (Res.); 4–6 as intermediate (Int.); and 7–9 as susceptible (Sus.) to FOP according to the CIAT standardized rating scale (16).

**Table 3.** Segregation for reaction to the Colorado race of *Fusarium oxysporum* f. sp. *phaseoli* (FOP) in $F_3$ plants of six *Phaseolus vulgaris* crosses

| Cross | Reaction[a] | Plants observed[b] | | | Expected ratio (R/S) | $X^2$ | Probability |
|---|---|---|---|---|---|---|---|
| | | Res. | Segregating | Sus. | | | |
| CO 22625 × CO 33142 | S × R | 6 | 4 | 5 | 1:2:1 | 3.40 | 0.10–0.20 |
| CO 59196 × Othello | R × S | 4 | 5 | 6 | 1:2:1 | 2.19 | 0.30–0.50 |
| Jamapa × CO 22625 | R × S | 1 | 13 | 4 | 1:2:1 | 4.54 | 0.10–0.20 |
| Viva × Porrillo Sintetico | S × R | 4 | 8 | 4 | 1:2:1 | 0.00 | 1.00 |
| Othello × Porrillo Sintetico | S × R | 2 | 8 | 4 | 1:2:1 | 0.85 | 0.50–0.70 |
| HF 465-63-1 × Othello | R × S | 0 | 7 | 9 | None tested | | |

[a] R indicates resistant and S indicates susceptible parental line.
[b] Plants rated 1–3 classified as resistant (Res.); 4–6 as intermediate (Int.); and 7–9 as susceptible (Sus.) to FOP according to the CIAT standardized rating scale (16).

gene in Porrillo Sintetico.

Two $F_2$ families from the cross Othello × HF 465-63-1 were evaluated (Table 2). In the first evaluation, the $F_2$ progeny fit the two-gene recessive model (1R:15S). However, in the second evaluation this model did not provide an acceptable fit (*data not shown*). Consequently, we were unable to formulate a valid hypothesis to test the $F_3$ generation. In the $F_3$, no family had all resistant plants, which suggests that resistance is complex and may be quantitatively inherited. The $F_2$ progeny from the cross Othello × Rio Tibagi also fit the 1R:15S ratio. This segregation pattern suggests that resistance to FOP in the cultivar Rio Tibagi is also conditioned by more than one gene; however, due to the limited number of progeny evaluated, this suggestion cannot be validated. Polygenic inheritance for resistance to *F. oxysporum* has also been reported in flax (*Linum usitatissimum* L.) (4) and chickpea (*Cicer arietinum* L.) (13).

The simple mode of inheritance for the dominant genes for resistance to FOP should enable plant breeders to easily transfer this resistance into commercial dry bean cultivars using the backcross breeding method. Seeds of resistant parental lines CO 33142 and CO 59196 are available from the bean breeding program at Colorado State University.

An apparent association between the patterns of inheritance and evolutionary relationships among the parental lines and cultivars was observed. The resistant pinto lines CO 33142 and CO 59196, which each possessed dominant single-gene forms of resistance, originated from the Durango race of the Middle American Center of Diversity (14). However, the resistant lines and cultivars HF 465-63-1 and Porrillo Sintetico, which had more complex recessive genetic resistances, originated from the Mesoamerican race of the Middle American Center of Diversity. These associations suggest that the two races of bean germ plasm evolved separate and different forms of resistance to FOP. However, the dominant resistance gene found in the cultivar Jamapa may be an exception to this association, since Jamapa was classified into the Mesoamerican race by Singh et al (14). Further research with more diverse germ plasm and races of FOP is needed to qualify these observations.

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