Resistance to Fusarium oxysporum f. sp. phaseoli in Tepary Beans (Phaseolus acutifolius)

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ABSTRACT

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More than 60 accessions of tepary bean (*Phaseolus acutifolius*) and five cultivars of common bean (*P. vulgaris*) were evaluated for disease reactions to different races of wilt-causing *Fusarium* (*F. oxysporum* f. sp. *phaseoli*) under glasshouse conditions; 95% of the tepary accessions were resistant. Resistant sources included both cultivars and wild accessions. We propose that a systematic screening of diverse germ plasm sources such as tepary beans be conducted to complement Fusarium wilt and multiple disease resistance information currently available for common beans.

Fusarium wilt, caused by Fusarium oxysporum Schlechtend.:Fr. f. sp. phaseoli J.B. Kendrick &. W.C. Snyder, occurs in common beans (Phaseolus vulgaris L.) in the United States, South America, Europe, and Africa (1,3,4,8, 9,14). Four physiological races have been identified among strains from Brazil, Colombia, Italy, and the United States (3,6,19,20). The host range of F. o. phaseoli in three related domesticated Phaseolus species—lima (P. lunatus L.), scarlet runner (P. coccineus L.), and tepary (P. acutifolius Gray) beans—has not been adequately described. The scarlet runner bean accessions PI 319449 and PI 321088 were reported susceptible to races of F. o. phaseoli from Italy and the United States and resistant to a Brazilian race (3,19). A F. o. phaseoli strain from Holland was reported pathogenic on scarlet runner beans in the Netherlands and pathogenic to common bean germ plasm tested by Ribeiro and Hage-

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dorn (19). Kendrick and Snyder (14) observed a resistant response on the lima bean cultivars Henderson's Bush and Wilbur, which were inoculated with six different F. o. phaseoli strains from California. However, no information is available about F. o. phaseoli pathogenicity on tepary bean germ plasm.

The tepary bean is an annual, cleistogamous species that probably originated in Central America, and wild forms extend from Guatemala to Arizona (12,15). Pratt and Nabhan (18) recognize two types of tepary germ plasm: acutifolius, which has lanceolate leaflets, and tenuifolius, which has narrow leaflets. The tepary bean is commercially cultivated in Mexico and is a valuable germ plasm source for resistance or tolerance to drought, heat, salt, and pests (22). In addition, variable to complete resistance responses among accessions of tepary bean have been reported to ashy stem blight caused by Macrophomina phaseolina (Tassi) Goidanich, common bacterial blight caused by Xanthomonas campestris pv. phaseoli (Smith) Dye, root rot caused by Fusarium solani (Mart.) Sacc. f. sp. phaseoli (Burkholder) W.C. Snyder & H.N. Hans., and rust caused by Uromyces appendiculatus (Pers.:Pers.) Unger. However, accessions of tepary beans are susceptible to infection by bean common mosaic and bean golden mosaic viruses, halo blight caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkholder) Young et al, and white mold caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, among other pathogens (13,18; M. A. Pastor Corrales and H. F. Schwartz, *unpublished*). Interest in tepary beans as sources of resistance to abiotic and biotic stresses (13,18) led us to question if genetic variability exists for reaction of this species to different races of *F. o. phaseoli*.

The objective of this research was to evaluate different accessions of tepary bean and hybrid progeny between common and tepary bean to three races of *F. o. phaseoli* under controlled conditions.

MATERIALS AND METHODS

Three races of F. o. phaseoli from P. vulgaris hosts were used to screen for resistance among diverse tepary germ plasm sources—one race (ATCC 18131 and ATCC 90245) from the United States (20) and one race each from Colombia and Brazil. The ATCC 18131 culture of F. o. phaseoli (FOP-SC) was obtained from M. Silbernagel, USDA/ARS, IAREC, Prosser, Washington; this race was originally isolated by G. M. Armstrong and J. K. Armstrong (4) in South Carolina. The ATCC 90245 culture (FOP-CO1) was recovered from one of several infected pinto beans, cultivar U.I. 114, collected by H. F. Schwartz in northeast Colorado in 1990 (20). Races from Colombia (FOP-CL25) and Brazil (FOP-BR24) were provided by M. A. Pastor Corrales (6). Inocula of FOP-SC and FOP-CO1 were derived from singlespore macroconidia stock cultures that were grown at room temperature in culture tubes containing autoclaved finely sieved sandy soil mixed with 2% powdered oatmeal and 15% distilled water (w/w) and stored at 4 C until use. Inocula of FOP-CL25 and FOP-BR24 were also derived from single macroconidia stock cultures but were grown in culture tubes containing potato-dextrose agar and stored at 4 C until use.

Seeds of tepary bean accessions were provided by S. P. Singh (10 G lines, CIAT Genetic Resources Unit), R. Hannan (49 PI lines, USDA-ARS Western Regional Plant Introduction Station, Pullman, WA), M. A. Brick (one X and three MAS lines, Colorado State University Bean Breeding Program, Fort Collins), and G. F. Freytag (tepary 2 and 8, USDA/ARS, Mayaguez, Puerto Rico). Seeds of hybrid progeny of common × tepary beans were provided by T. E. Michaels (20 BLT lines, University of Guelph, Ontario, Canada). The CIAT lines consisted of domesticated tepary lines with white and brown seed coats, and seed size varied from 11 to 17 g/100 seeds.

Seeds were sown in noninfested potting soil (Terralite, Metromix No. 350), and seedlings were grown at 21-25 C with a minimum daily light cycle of approximately 12 hr. Two inoculation procedures were used: 1) a soil chlamydosporeinoculum procedure adapted from R. Baker's laboratory at Colorado State University for preliminary experiments with race FOP-SC and 2) a clipped-root conidial inoculation technique for subsequent experiments with the other races. The soil-chlamydospore inoculum procedure utilized seven to 20 transplants (depending on seed availability) in chlamydospore-infested (10⁴⁻⁵/gm) soil. The transplants were previously grown in pots containing noninfested potting soil and were immediately washed with tap water. Subsequently, one-third of the distal root system was clipped prior to transplantation of two plants per 13-cmdiameter plastic pot (to conserve limited bench space) containing uniformly infested potting soil. The procedure utilized for the clipped-root conidial inoculum technique (20) was modified from that reported by Pastor Corrales and Abawi (16). In this procedure, 10–14 uniform seedlings were grown in soil in pots. After the seedlings were removed from the pots, the roots were washed for 5 min before being clipped as described above. The root portion was then placed in a suspension of 10⁶ conidia per milliliter for 5 min. Two inoculated plants were transplanted to a 13-cm-diameter plastic pot containing noninfested potting soil. Two pots with noninoculated checks were also included in each experiment.

Depending on seed availability, 18 of the 38 tepary lines evaluated with the soil inoculum technique, five additional tepary lines, and the susceptible common bean cultivar U.I. 114 were subsequently evaluated for reaction to FOP-CO1 using

the conidial suspension root inoculation technique. During inoculation, the greenhouse temperature was maintained at 21-25 C. The postinoculation temperature was maintained at 16-20 C for 24 hr to reduce the effects of transplanting shock. Inoculated plants were incubated in the greenhouse on a 21/32 C night/ day cycle. The relative humidity ranged from 50 to 100%. Supplemental halide lighting was provided for 5 hr (from 3:00 to 8:00 p.m.) to provide plants with a minimum daily light cycle of approximately 12 hr. Photosynthetically active radiation measured at the bench level inside the greenhouse with artificial light

was approximately 350-400 $\mu \text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

Plants were fertilized once a week in the case of the soil-chlamydospore-in-oculum experiment and every 3 days for the root-dip inoculum experiment with a liquid fertilizer (6 g/20 L of 15-30-15 N-P-K, 100 ml per pot) to maintain vigorous plant growth. Plants that did not recover from transplanting were eliminated 4-7 days after inoculation,. Plants were watered daily. After plants were inoculated, pots were rotated every 3 days on the bench to more uniformly expose plants to temperature and light variations within the greenhouse.

Reactions to FOP-SC were based on

Table 1. Disease reactions to Fusarium oxysporum f. sp. phaseoli among tepary and common bean accessions/cultivars from CIAT and other sources grown in soil infested with 10⁴⁻⁵ chlamydospores per gram of FOP-SC or root-clip inoculated with 10⁶ conidia per milliliter of FOP-CO1^a

Identification ^b	Origin	FOP-SC° (%)	FOP-CO1d		
			Mean	Rating	
PI 200749	El Salvador	0	1.4	R	
PI 209480	Nicaragua	10	1.3	R	
PI 239056	Morocco	0	3.6	Ĩ	
PI 310606	Mexico	6	2.1	Ŕ	
PI 310801	Nicaragua	0	2.3	R	
PI 310802	Nicaragua	0	1.5	R	
PI 319438	Mexico	NT	1.0	R	
PI 319443	Mexico	22	1.4	R	
PI 321638	Arizona	21	1.3	Ř	
PI 331181	Argentina	5	1.4	R	
PI 430027		0	NT		
PI 430208		0	NT	•••	
PI 440789	Mexico	11	NT	•••	
PI 440790	Mexico	0	NT	•••	
PI 440791	Mexico	12	2.1	v	
PI 440792	Mexico	11	NT		
PI 440793	Mexico	10	1.6	 R	
PI 440794	Mexico	5	1.4	R	
PI 440804	Arizona	0	1.7	R	
PI 458873	Maryland	50	1.4	R	
PI 458874	Puerto Rico	NT	1.7	R	
PI 462026	Mexico	0	NT		
PI 476856		0	NT	• • •	
PI 476858	Mexico	10	2.2	 R	
PI 477032	Arizona	25	NT	K	
PI 477033	Arizona	0	NT	• • •	
PI 477034	Arizona	0	NT	• • •	
PI 477036	Arizona	11	NT	• • •	
PI 477037	Arizona	0	NT	• • •	
PI 477038	Arizona	0	NT	• • •	
PI 477039	Arizona	0	1.1		
PI 477040*	Arizona	NT	1.1	R R	
PI 485595	United States	0	NT	K	
PI 488874	Puerto Rico	18	NT NT	• • •	
PI 510636	Mexico	14	NT	• • • •	
PI 527334	Mexico	0	1.0		
PI 535214**	Arizona	0	1.6	R	
PI 535242*	Mexico	NT		R	
PI 535244*	Arizona	NT NT	1.0 1.0	R	
X-15918		0	NT	R	
MAS-Y-LCC62	•••	0	NT NT	• • •	
MAS-Y-LCC90	•••	25		• • •	
MAS-Y-LCL969	•••	23 14	NT NT	• • •	
Mayacoba	Mexico	100	N I NT	• • •	
Pinto U.I. 114	Idaho	91	N 1 8.9	 S	

^a Seven to 20 plants per entry were inoculated with FOP-SC and six to 30 with FOP-CO1.

^b All identified as *Phaseolus acutifolius* accessions except Mayacoba and U.I. 114, which are P. vulgaris cultivars. $\dot{} = P$. a. var. tenuifolius, $\ddot{} = P$. a. var. acutifolius.

^c Percentage of plants with external wilt symptoms and/or visible internal vascular discoloration 21 days after inoculation.

^d Mean disease severity recorded 21 days after inoculation on a CIAT scale of 1 (no external symptoms) to 9 (plant >75% diseased). R = resistant (1-3), I = intermediate (3.1-6), S = susceptible (6.1-9), V = variable reactions between plants; NT = not tested.

percentage of plants with external wilt symptoms and/or the presence or absence of internal vascular discoloration. External symptoms of each plant inoculated with the other races were rated 21 days after inoculation according to the CIAT severity scale of 1 (no external symptoms) to 9 (plant foliage >75% diseased). Ratings of 3, 5, and 7 correlated with 10, 25, and 50%, respectively, of the plant's foliage with wilted and/or chlorotic symptoms. Average disease severities of 1-3, 3.1-6, and 6.1-9 indicated resistant, intermediate, and susceptible reaction classes, respectively (16). Internal ratings were recorded 21 days after inoculation by cutting the main stem at the primary node and noting the presence or absence of internal discoloration. Internal discoloration was rated with the aid of a 10× hand lens because of the characteristically thin stems of the tepary germ plasm entries. Susceptible common bean cultivars specific for each race were included as inoculated checks: Pinto U.I. 114 for FOP-SC and FOP-CO1, Porrillo Sintetico for FOP-BR24, and Mortino for FOP-CL25.

The experimental design was completely randomized, with seven to 20 plants (replicates) for the soil-chlamy-dospore procedure with race FOP-SC and three to 15 plants (replicates) for the conidial-inoculum procedure with the other races. Replicate number per entry varied according to seed availability. Conidial-inoculum evaluations were repeated, and the results combined, since heterogeneity of chi-square tests determined that error terms were homogeneous among evaluations.

RESULTS

Reactions to F. o. phaseoli in soil infested with chlamydospores of FOP-SC among 38 tepary bean accessions/ cultivars and two common bean cultivars are shown in Table 1. No tepary entry had more than 50% of the plants that showed both plant wilt and internal vascular discoloration 21 days after inoculation. Furthermore, 19 tepary accessions did not show any external or vascular symptoms. The variable reaction among some accessions could be due to the heterogeneous nature of the seed source, i.e., population, disease escape, or both. The common bean cultivar Mayacoba was susceptible to FOP-SC as indicated by >90\% of the plants expressing both plant wilt and vascular discoloration. The common bean cultivar U.I. 114 and one tepary line (PI 239056) were susceptible and intermediate to FOP-CO1, respectively (Table 1). Four of the tepary lines received a mean severity rating of 1, which indicated that none of the plants exhibited external or vascular symptoms of F. o. phaseoli.

Reactions to FOP-CO1 and FOP-BR24 among domesticated tepary germ plasm obtained from CIAT and three

common bean cultivars are shown in Table 2. All tepary entries were resistant to both races. However, G40023 expressed a variable reaction to FOP-CO1 (some plants had vascular discoloration) but was resistant to FOP-BR24. G40001, G40063, and G40138 also exhibited some variability in terms of external symptoms but no vascular discoloration. In inoculations with FOP-BR24, no vascular discoloration at the stem level was observed. Lines G40066 and G40068 also were resistant to FOP-CL25 (M. A. Pastor Corrales, unpublished).

An additional set of accessions from the U.S. Plant Introduction collection was screened against FOP-BR24 and FOP-CL25 (Table 3). All tepary entries were resistant, whereas the common bean cultivars were susceptible to their respective races. No vascular discoloration was observed in most tepary lines, except for a few plants of PI 440787, PI 440791, PI 462025, and PI477034.

DISCUSSION

The high level of resistance among tepary accessions/cultivars to several races of the pathogen indicates that tepary beans may be a good source of resistance to F. o. phaseoli. Because known resistance sources of F. o. phaseoli in common bean are strain-specific (6,20), tepary bean may represent a more stable form of resistance. Because the mode of inheritance for resistance to F. o. phaseoli is not known in tepary bean, a genetic study is needed to determine the mode of inheritance so that appropriate breeding procedures can be planned.

In our study, most tepary entries were highly resistant to several races of F. o. phaseoli. All Plant Introduction lines tested with the conidial inoculum showed high resistance to the Colorado, Colombian, and Brazilian races. The CIAT germ plasm, which consisted of domesticated tepary lines, was highly resistant

Table 2. Disease reactions to Fusarium oxysporum f. sp. phaseoli among tepary and common bean accessions/cultivars from CIAT root-clip inoculated with 10⁶ conidia per milliliter of FOP-CO1 or FOP-BR24^a

Entry	FOP-CO1 ^b		FOP-BR24 ^b		
	Mean	Rating	Mean	Rating	
G40001	1.5	R	2.7	V	
G40020	1.4	R	NT		
G40021	1.2	R	NT		
G40023	3.8	V	1.7	R	
G40043	2.0	R	1.0	R	
G40063	1.8	V	1.3	R	
G40066	1.1	R	1.9	R	
G40068	1.1	R	1.0	R	
G40138	1.9	V	1.0	R	
G40159	1.1	R	1.0	R	
U.I. 114	9.0	S	NT		
Porrillo Sintetico	NT		8.8	S	
Jamapa	NT	•••	9.0	S	

^a A total of 22-30 plants per entry were inoculated with FOP-CO1 and 12-20 with FOP-BR24. ^b Mean disease severity recorded 21 days after inoculation on a CIAT scale of 1 (no external symptoms) to 9 (plant >75% diseased). R = resistant (1-3), I = intermediate (3.1-6), S = susceptible (6.1-9), V = variable reactions between plants; NT = not tested.

Table 3. Disease reactions to Fusarium oxysporum f. sp. phaseoli among tepary and common bean accessions/cultivars from the USDA root-clip inoculated with 10⁶ conidia per milliliter of FOP-BR24 or FOP-CL25^a

Entry	Origin	FOP-BR24b		FOP-CL25 ^b	
		Mean	Rating	Mean	Rating
PI 310800	Nicaragua	1.0	R	1.2	R
PI 312122	Salvador	1.2	R	1.0	R
PI 319442	Mexico	1.7	R	1.0	R
PI 440787	Mexico	1.3	R	1.1	R
PI 440791	Mexico	1.4	R	1.0	R
PI 440803	Arizona	1.3	R	1.0	R
PI 462025	Arizona	1.0	R	1.1	R
PI 477033	Arizona	1.2	R	1.0	R
PI 477034	Arizona	1.1	R	1.0	R
PI 477035	Arizona	1.0	R	1.0	R
Porrillo Sintetico	Central America	9.0	S	NT	
Mortino	Colombia	NT		9.0	S

^a A total of nine to 20 plants per entry were inoculated with FOP-BR24 and 19-20 with FOP-CL25.

^b Mean disease severity recorded 21 days after inoculation on a CIAT scale of 1 (no external symptoms) to 9 (plant >75% diseased). R = resistant (1-3), I = intermediate (3.1-6), S = susceptible (6.1-9); NT = not tested.

to the Brazilian and Colorado races. Intermediate disease responses observed in PI 239056 and G40023 with the Colorado race are the exceptions to resistant reactions observed with the other 61 tepary lines screened.

The broad resistance observed in this group of wild and domesticated teparies of diverse origin led us to question the relationship between the observed resistant response with specific incompatibility. Apparent immunity has been observed in many lines inoculated with different races. However, infection of vascular tissue was observed in PI 319443, PI 458873, and G40043 with all F. o. phaseoli races used, but mainly with the Colorado race. Browning of vascular tissue is accepted by some researchers and questioned by others as an indicator of host/pathogen compatibility (11). We agree with the criterion of vascular discoloration as a symptom of wilt disease. However, lack of a truly susceptible tepary accession until the evaluation of tepary 8 suggested that further research was needed to discard a case of heterologous interaction (observed basic incompatibility of all plant genotypes within a species with all members of a formae specialis, and where basic incompatibility is the absence of parasitism on any member of a plant species) (2,5, 10,14). Identification of and transfer from tepary beans of a single gene or closely linked genes for resistance based on heterologous interactions may provide a stable and universal source of resistance in common beans to F. o. phaseoli.

Therefore, the tepary bean has practical significance in a breeding context as a source of disease resistance for multiple resistance to root diseases such as Fusarium wilt, ashy stem blight, and root rot and to foliar diseases such as rust and common bacterial blight. Entries G40020, G40021, G40023, G40063, G40066, G40068, G40138, and G40159 (Table 2) also have a high degree of resistance to ashy stem blight and an intermediate to resistant reaction to common bacterial blight and leaf rust (except for G40159) in Colombia (M. A. Pastor Corrales, unpublished). Hence, many of these accessions would be useful sources of multiple disease resistance. Likewise, the following tepary materials were highly resistant to a Colorado collection of rust races: PI 239056, PI 319443, PI 476858, PI 477040, PI 535242, and PI

535244 (H. F. Schwartz and M. O. Salgado, unpublished). Scott and Michaels (21) indicated that most of their interspecific hybrids were resistant to common bacterial blight in Canada. However, all 20 hybrids (BLT 87-1 to 87-20) and the common bean parents (Ex Rico 23 and ICA Pijao) were susceptible (CIAT severity ratings of 8.6-9.0) to FOP-CO1 in our studies. The tepary parent (PI 440795) was resistant (rating of 2.2). Tepary 2 and 8 lines from G. F. Freytag were resistant (1.7 rating) and susceptible (6.9), respectively, to FOP-CO1.

Interspecific pathogenic relationships between important bean pathogens and cultivated Phaseolus species other than common beans have not been thoroughly studied. We propose a systematic screening of these diverse germ plasm sources to complement disease resistance currently utilized within the common bean gene pool. Interspecific crosses of susceptible common beans should be made with resistant and susceptible tepary bean lines to understand the genetics of Fusarium wilt resistance conferred "above" the cultivar level (7). The experiment should also be conducted with adult rather than seedling plants because of the reported increase in host specificity with older plants (11). Tepary PI lines screened in this experiment and successful hybrids with common beans (PI 310800, PI 321443, PI 321638, PI 406633, and PI 440790) in other experiments (17) should be considered for the crosses.

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