Inheritance of web blight resistance in common bean[1]

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ABSTRACT

Expanded production of common bean (*Phaseolus vulgaris* L.) in the humid tropics requires the development of cultivars with greater levels of resistance to web blight, a destructive disease caused by *Thanatephorus cucumeris* Frank Donk (anamorph: *Rhizoctonia solani* Kuhn). The objectives of this study were 1) to estimate the heritability of resistance to web blight in common bean and 2) to measure the effect of web blight on seed yield and quality. Sixty advanced lines from the cross 'MUS 83/DOR 483//Tío Canela 75' were evaluated in replicated field and laboratory tests for reaction to web blight. Heritability estimates for leaf damage (LD) scores, seed yield and percentage seed damage (SD) were intermediate in magnitude, ranging from 0.32 to 0.53. Although the LD on many lines was severe, the SD was less than 10% for most of the advanced lines, all of which suggests that resistance to LD and SD may be inherited separately. A simple field evaluation technique permitted a preliminary screening for resistance to web blight. The laboratory technique induced more severe symptoms and thus would be appropriate for identifying lines with high levels of physiological resistance expressed in the leaves.

Key words: *Phaseolus vulgaris, Thanatephorus cucumeris, Rhizoctonia solani*, heritability, disease screening techniques

RESUMEN

Herencia de la resistencia a la mustia hilachosa de la habichuela

Para lograr una mayor producción de frijol común (*Phaseolus vulgaris* L.) en los trópicos húmedos, se requiere el desarrollo de cultivares con mayores niveles de resistencia a la mustia hilachosa, una enfermedad destructiva causada por *Thanatephorus cucumeris* (anamorph: *Rhizoctonia solani* Kuhn). Los objetivos de este estudio fueron 1) estimar la heredabilidad de la resistencia a la mustia hilachosa del frijol común y 2) medir el efecto de la mustia hilachosa en el rendimiento y en la calidad de la semilla. Sesenta líneas del cruce MUS 83/DOR 483// Tío Canela 75 se evaluaron en el

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campo y en el laboratorio para reacción a la mustia hilachosa. Los estimados de heredabilidad para lecturas de la magnitud del daño en las hojas (DH) y el porcentaje de daño en la semilla (DS) fueron intermedios con un rango de 0.32 a 0.53. Aunque el DH en muchas líneas fue severo, el DS fue menos de 10% para la mayoría de las líneas avanzadas evaluadas. Estos resultados sugieren que la resistencia a DH y a DS podría tener una herencia independiente. Las evaluaciones en el campo fueron sencillas ya que permitieron la evaluación preliminar para resistencia a la mustia hilachosa. La técnica de inoculación en el laboratorio indujo síntomas más severos, por lo cual es apropiada para identificar líneas con altos niveles de resistencia fisiológica en las hojas.

Palabras clave: frijol, *Phaseolus vulgaris, Thanatephorus cucumeris, Rhizoctonia solani*, heredabilidad

INTRODUCTION

Web blight (WB) caused by the basidiomycete fungus *Thanatephorus cucumeris* (Frank) Donk (anamorph: *Rhizoctonia solani* Kühn) can reduce both the yield and seed quality of common bean (*Phaseolus* vulgaris L.) grown in the humid tropics (Gálvez et al., 1989; Godoy-Lutz et al., 1996). In Panama, minimum tillage and systemic fungicides are used to reduce crop damage caused by web blight (Rodríguez et al., 1997). Although breeding lines and cultivars with moderate levels of resistance have been developed (Beebe, 1987; Nin et al., 2000), greater levels of resistance are needed to alleviate dependence on fungicides to control the disease. The objectives of this study were 1) to estimate the heritability of resistance to web blight in common bean and 2) to measure the effect of web blight on seed yield and quality.

MATERIALS AND METHODS

Field Trials

Field trials were conducted for two years at the University of Puerto Rico Agricultural Experiment Station substation in Isabela. Planting dates of the field trials were 10 June 1998 and 15 June 1999. A population of 60 randomly selected F_5 and F_6 lines from the cross 'MUS 83/DOR 483//Tío Canela 75', resistant checks DOR 557 and PR9607-29, and susceptible checks Tío Canela 75 and PR9418-2 were evaluated by using a randomized complete block design with six replications. The experimental units were single 0.5-m rows in 1998 and single 1.0-m rows in 1999. The spacing between rows was 0.6 m and the spacing between plants within rows was approximately 0.1 m. Means of lines were compared by using Least Significant Differences ($P < 0.05$).

The WB isolate used in the laboratory and field trials in Puerto Rico belongs to anastomosis groups AG-1-IE (macrosclerotia > 1 mm), (Godoy-Lutz et al., 2002). A pure culture of the mycelial state of

R. solani (AG-1-IE) and a potato dextrose (PDA) medium were used to prepare the inoculum. Eighteen 4-mm diameter disks, collected from the margin of the fungal colonies, were placed in a liquid medium containing 10 g of peptose, 15 g of dextrose, 0.5 g of KH_2P0_4, and 0.25 g of $MgS0_4$ per liter of water (Olaya and Abawi, 1994). The solution was agitated in a shaker-incubator (New Brunswick Scientific)[7] at 27 °C for a period of 14 days. The mycelial growth was blended (Waring blender) for 30 seconds at low speed, filtered, and washed twice in distilled water. The filtrate was dried for 24 h before water was added. The final concentration of the inoculum was obtained by using a spectrophotometer (Bausch and Lomb Spectronic 20). Water was added to the solution until 25% transmittance was obtained at a wavelength of 640 nm. The final concentration was approximately 1 g of mycelial mass per 300 ml of water. Tween 80 was added at a concentration of 1 ml/L to increase adhesion of the inoculum to the surface of the leaves.

The leaves of the bean plants were inoculated in the field with a 3.8-L Flomaster sprayer (Model 1201) using a pressure of 138 kPa. Each experimental unit received 10 to 15 ml of inoculum. The inoculum was applied late in the afternoon 35 days after planting. The trial received 15 minutes of overhead irrigation each morning during the first week after inoculation, and plants were evaluated at eight and 15 days after inoculation. Leaf damage scores for each row in the trial were estimated by using a 1 to 9 scale published by CIAT (Schoonhoven and Pastor-Corrales, 1987) where 1 = no visible symptoms; 3 = 6 to10% of the leaf area with symptoms; 5 = 21 to 30% of the leaf area with symptoms; 7 = 41 to 60% of the leaf area with symptoms; and 9 = > 85% of the leaf area with symptoms. Seed yield per plant was estimated as follows: seed weight per row/number of plants harvested per row. In addition, to calculate the percentage of damaged seed, the seed from each plot was separated into commercially acceptable and blemished seed catagories.

Near narrow sense heritabilities were based on variance component estimates on a progeny mean basis by the following formula:

$$\text{Narrow sense heritability} = h^2_{NS} = \sigma^2_A/\sigma^2_P$$

$$\text{Additive genetic variance for } F_5 \text{ lines} = \sigma^2_A = \sigma^2_{(\text{Among F5 lines})} / (15/8)$$

$$\text{Additive genetic variance for } F_6 \text{ lines} = \sigma^2_A = \sigma^2_{(\text{Among F6 lines})} / (31/16)$$

[7]Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment or materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

$$\text{Phenotypic variance} = \sigma^2_P = [\sigma^2_{A\,+}\,(\sigma^2_E/r)]$$

where σ^2_E = error mean square and r = number of replications. Standard errors of variance component heritability estimates were calculated as described by Hallauer and Miranda (1988).

The additive genetic correlations were calculated as follows:

$$r = \sum L_1L_2/(\delta^2_{L1*}\delta^2_{L2})^{1/2}$$

where δ^2_{L1} and δ^2_{L2} are the additive genetic variances of the dependent variables to be correlated and $\sum L_1L_2$ is the sum of crossproducts of the variables. The significance of the additive genetic correlations was evaluated by using t tests ($P < 0.05$).

Laboratory Trials

The detached leaf tests were conducted at the University of Puerto Rico Mayagüez Campus during the winter of 1999 and at the University of Nebraska-Lincoln during the summer of 2000. In Puerto Rico, we evaluated 60 F_5 lines from the cross MUS 83/DOR 483//Tío Canela 75; two resistant checks, DOR 557 and PR9607-29; and two susceptible checks, Tío Canela 75 and PR9418-2.

One seed of each line was planted in 15-cm-diameter pots containing a mixture of peat moss and vermiculite. Plants were grown in a greenhouse at 26 ± 2 °C. A replication within set arrangement of a randomized complete block was the experimental design. Each set contained four replications of 20 F_5 lines and the four check lines. The first set was planted 20 November 1998; the second set was planted 9 January 1999 and the third set, 26 February 1999. The fully expanded trifoliolate leaves were collected in the greenhouse and evaluated in the laboratory for reaction to the web blight pathogen by using a detached leaf inoculation technique (Bautista-Pérez and Echávez-Badel, 2000). Leaves were inoculated with a virulent AG-1-1E (macroclerotia) isolate of *R. solani* from Puerto Rico (Echávez-Badel et al., 2000). The inoculation method consisted of the placement of a petiole of a detached trifoliolate leaf in an orchid tube filled with water. The leaflets were positioned in 42 × 30 × 6-cm aluminum trays. Each leaflet was placed on top of a petri plate to avoid immersion in water. One 4-mm-diameter disk of agar colonized with *R. solani* was placed on the adaxial side and centered on each leaflet. To create a high-humidity environment favorable for the development of the fungus, the aluminum trays were placed inside plastic bags after inoculation. The trays were incubated in a laboratory at 27 ± 1 °C. The experimental units were trifoliolate leaves and the three leaflets were samples. Mean ratings of the symptom severity (within the range of 1 = no symptoms and 9 = very severe damage) to the leaflet, were taken at 24, 48 and 72 h after inoculation. Mean lesion size (cm^2) was also measured at 24, 48 and 72 h after inoculation. A Bartlett's test was used to compare the homogeneity of the experimental errors of the sets. A pooled analysis of variance was conducted and means of the lines were compared by using LSD ($P \leq 0.05$).

The detached leaf technique was used at the University of Nebraska-Lincoln to evaluate the trifoliolate leaf reaction of 10 advanced generation lines to *R. solani* isolate BV-1 AG-1-IF (microsclerotia < 1 mm), from the Dominican Republic (Godoy-Lutz et al., 2002). The experimental design was a randomized complete block with three replications. The inoculation techniques and system of evaluation were the same as those described for the laboratory experiments conducted in Puerto Rico except that laboratory temperatures were 22 ± 1 °C.

RESULTS AND DISCUSSION

Climatic conditions at the Isabela Substation during the 1998 and 1999 growing seasons were favorable for the development of web blight. Mean maximum temperatures were greater than 30 °C and mean minimum temperatures exceeded 22 °C. During the months of July and August mean precipitation was 517 mm in 1998 and 474 mm in 1999.

It was possible to identify several bean breeding lines with moderate levels of resistance to web blight. Approximately 5% of the lines had a disease score similar to that of the resistant parent PR9607-29 (Table 1). PR9807-82, PR9807-5 and PR9807-50 were among the most resistant lines both in 1998 and 1999 whether readings were taken at eight or 15 days after inoculation. Mean disease scores of these lines were significantly lower than that of the susceptible parent Tío Canela 75 and similar to that of the resistant parent PR9607-29. Narrow sense heritability estimates for disease scores at eight and 15 days after inoculation were similar in magnitude to estimates of seed yield/plant, ranging from 0.35 to 0.52. Selection for web blight resistance in this population would be effective in replicated trials of advanced lines. Field selection for web blight resistance in the Dominican Republic was effective when climatic conditions favored disease development (Arnaud-Santana et al., 1994).

The use of a randomized complete block design with six replications provided sufficient precision to detect significant differences among breeding lines for disease scores (Table 1). Coefficients of variation for the mean disease scores ranged from 18.8 to 29.4%. The use of single row 1-m plots would facilitate the screening of a large number of breeding lines. A short period of irrigation early each morning during the

TABLE 1. *Mean disease scores from field trials planted at Isabela, Puerto Rico, of the parents, a susceptible check and three of the most resistant bean breeding lines.*

	8 d after inoculation[1]		15 d after inoculation	
Line	1998	1999	1998	1999
PR9807-82	3.3	4.5	4.3	5.3
PR9807-50	3.7	3.7	4.8	5.7
PR9807-5	3.7	3.3	4.3	5.3
Mean of all breeding lines	5.4	4.3	6.3	6.4
PR9607-29 (Res.)	3.2	3.4	4.0	4.6
Tío Canela 75 (Susc.)	5.3	4.8	6.8	7.1
PR9418-2 (Susc.)	6.0	4.5	7.2	7.4
LSD (0.05)	1.8	1.1	1.8	1.3
CV (%)	29.4	22.8	25.8	18.8

[1]Disease score scale: 1 = no symptoms; 3 = 6 to10% of leaf area with symptoms; 5 = 21 to 30% of leaf area with symptoms; 7 = 41 to 60% of leaf area with symptoms; and 9 = >85% of leaf area with symptoms.

week after inoculation extended the dew period and favored the development of web blight.

PR9807-82, PR9807-5 and PR9807-50 produced mean seed yields similar to those of the heat tolerant parents Tío Canela 75 and PR9607-29 (Table 2). Narrow sense heritabilities for seed yield/plant were 0.32 in 1998 and 0.42 in 1999 (Table 3). Heritability estimates for resistance to web blight in bean leaves have ranged from moderate to high (Rodríguez et al., 1995; Montoya et al., 1997).

TABLE 2. *Mean seed yield/plant and percentage of damaged seed from field trials planted at Isabela, Puerto Rico, of the parents, a susceptible check and three of the most resistant bean breeding lines.*

	Seed yield/plant (g)		Percentage seed damaged[1]	
Line	1998	1999	1998	1999
PR9807-82	22.7	16.8	3.1	6.9
PR9807-50	17.0	13.2	3.4	4.4
PR9807-5	20.8	13.7	1.9	3.6
Mean of breeding lines	17.5	14.3	5.3	7.7
PR9607-29 (Res.)	14.6	13.8	2.9	7.2
Tío Canela 75 (Susc.)	17.4	13.1	4.8	7.8
PR9418-2 (Susc.)	21.6	18.3	17.5	23.0
LSD (0.05)	8.7	5.7	—	—
CV (%)	44.2	36.0	37.4	22.0

[1]LSD not presented because the data were not normally distributed.

TABLE 3. *Heritability estimates and standard errors for web blight disease scores, seed yield/plant and seed damage from field trials planted at Isabela, Puerto Rico.*

Year	Disease scores at 8 d after inoculation	Disease scores at 15 d after inoculation	Seed yield per plant (g)	ln (% seed damage + 1)
1998	0.35 ± 0.24	0.42 ± 0.25	0.32 ± 0.24	0.44 ± 0.18
1999	0.52 ± 0.27	0.47 ± 0.26	0.42 ± 0.25	0.53 ± 0.18

Both parents and the resistant breeding lines PR9807-82, PR9807-5 and PR9807-50 had < 10% blemished seed (Table 2). Tío Canela 75 is considered by bean producers in Central America as a bean cultivar with good seed quality. In contrast, the susceptible check PR9418-2 had 17.5% blemished seed in 1998 and 23.0% in 1999. Narrow sense heritabilities for ln (percentage seed damage +1) were 0.44 in 1998 and 0.53 in 1999. These results suggest that there may be different mechanisms controlling resistance in leaves and seed damage caused by web blight. Therefore, plant breeders should evaluate bean lines for both leaf reaction and seed quality when screening for web blight resistance. Coyne and Schuster (1974) found that different genes conferred resistance to common bacterial blight in bean leaves and pods.

Additive genetic correlations between disease readings taken at eight and 15 days after inoculation (DAI) were 0.95 in 1998 and 0.91 in 1999 (Table 4), thus indicating that similar results were obtained from the first and second readings. In both 1998 and 1999, there were negative additive genetic correlations between seed yield and the severity of web blight infection. However, the additive genetic correlations between the web blight readings at eight DAI and seed yield were greater than the correlations between the reading at 15 DAI and seed yield. These results suggest that the most informative web blight readings in the field were taken at eight DAI. In all cases, there was a negative correlation between the severity of the web blight readings and seed yield.

TABLE 4. *Additive genetic correlations between disease evaluations at eight and 15 days after inoculation in the field at Isabela, Puerto Rico, and between disease evaluations and seed yield/plant.*

	1998	1999
8 vs. 15 days after inoculation	0.95*	0.91*
8 days after inoculation vs. seed yield/plant	-0.68*	-0.42*
15 days after inoculation vs. seed yield/plant	-0.42*	-0.23

*Significant at $P \leq 0.05$.

Both leaf and seed damage caused by web blight can contribute to reduced seed yields.

When web blight reaction was evaluated in the laboratory by using the detached leaf technique, there were no significant differences in mean lesion scores and mean lesion sizes among PR9807-82, PR9807-5, PR9807-50, the resistant and susceptible parents, and the susceptible check cultivar PR9418-2 (Table 5). Differences in web blight reaction in the field may have been due to differences in plant architecture rather than physiological resistance. Plant architecture has been found to be an important trait for the avoidance of web blight damage (Jung et al., 1996). It is possible that the detached leaf inoculation technique may have been too severe to detect partial resistance to web blight. However, this technique has been successful in identifying partial resistance to another necrotrophic fungal pathogen, *Sclerotinia sclerotiorum*, in soybean (Arahana et al., 2001).

Mean lesion size in the trial conducted in Puerto Rico was greater than the lesion size of most lines in the trial conducted in Nebraska (Tables 5 and 6). The lower temperature used in Nebraska (22 ± 1 °C) may have reduced the rate of growth of the fungus. Differences in virulence of the isolates of the web blight pathogen from the Dominican Republic and those of Puerto Rico may also have contributed to differences in mean lesion size (Godoy-Lutz et al., 2002). It would be desirable, however, to identify breeding lines with resistance to isolates of diverse geographic origins.

TABLE 5. *Mean web blight scores of the parents, a susceptible check and three of the most resistant bean breeding lines using the detached leaf technique in Puerto Rico.*

	Mean score[1] of the lesion at 24 h after inoculation		Mean lesion size (cm^2) at 48 h after inoculation	
Line	First trifoliolate	Third trifoliolate	First trifoliolate	Third trifoliolate
PR9807-82	3.8	2.8	24.6	31.6
PR9807-50	2.3	3.5	13.6	25.7
PR9807-5	3.3	3.0	18.9	29.1
Mean of all breeding lines	3.4	3.0	25.6	27.3
PR9607-29 (Res.)	3.0	3.1	24.5	29.1
Tío Canela 75 (Susc.)	3.2	3.1	24.2	29.7
PR9418-2 (Sucs.)	3.6	3.0	20.7	24.5
LSD (0.05)	1.3	1.0	15.1	14.5
CV (%)	27.3	27.9	27.9	28.0

[1]Rated on a scale of 1 to 9 where 1 = no symptoms and 9 = very severe symptoms. Mean of four replications of leaves inoculated with an AG-1-1F isolate.

TABLE 6. *Mean web blight lesion size of bean lines evaluated at the University of Nebraska 48 h after inoculation using the detached leaf technique.*

Line	Mean lesion size[1] (cm^2)
Pinto 114	23.8
HT 7719	14.0
Arroyo Loro Negro	12.0
MUS 138	10.7
PR 9607-29	10.6
VAX 5	5.3
EAP 9503-32-A	4.8
MUS 132	4.1
G 14241	4.0
BAT 93	2.8
L.S.D. (0.05)	7.4

[1]Mean of three replications of leaves inoculated with isolate BV-1 (AG-1-1F).

Pinto 114 is a line susceptible to web blight and had a significantly greater lesion size than the other lines in the trial when evaluated by using the detached leaf technique (Table 6). HT7719, Arroyo Loro Negro, MUS 138 and PR9607-29, which were selected in the field in Central America and the Caribbean for web blight resistance (Nin et al., 2000; Beebe, 1987), had mean lesion sizes that were <50% of the size of Pinto 114 lesions. BAT 93 is a line which has exhibited resistance to web blight in both Central America and the Caribbean and had a mean lesion size significantly smaller than that of HT7719, Arroyo Loro Negro, MUS 138 or PR9607-29. On the basis of these results, BAT 93 has been used as a parent to attempt to combine enhanced levels of physiological resistance to web blight with plant architectural traits that contribute to field avoidance of web blight infection.

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