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CHARACTERIZATION OF *MACROPHOMINA PHASEOLINA* ISOLATES COLLECTED FROM THE DOMINICAN REPUBLIC AND PUERTO RICO

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ABSTRACT

Charcoal rot or ashy stem blight caused by the soil-borne fungus *Macrophomina phaseolina* (Mp) is recognized as an important disease of bean (*Phaseolus vulgaris*) in Puerto Rico (PR) and the Dominican Republic (DR). Severe symptoms occur especially when bean seedlings or adult plants are stressed from moisture deficits. Differences in virulence and morphological, physiological, and biochemical characteristics were obtained among PR and DR isolates of Mp in this study. PRMp2 was more virulent than PRMp1. However, DRMp2 was more virulent than either PRMp2 or DRMp1. Mycelia morphological characteristics and sclerotia size were similar in the DR isolates but different from those of the PR isolate. There were not significant differences in sclerotia size and mycelia radial growth of each isolate under light or dark conditions. Analysis of protein patterns of the PR and DR isolates was performed by polyacrylamide gel electrophoresis (PAGE). Protein profiles of DR isolates were similar but different from that of PR isolate (PRMp2).

RESUMEN

La pudrición carbonosa o gris del tallo causada por el hongo del suelo *Macrophomina phaseolina* (Mp) es considerada como una enfermedad de la habichuela (*Phaseolus vulgaris*) de importancia económica en la República Dominicana (RD) y en Puerto Rico (PR). Los síntomas severos ocurren especialmente cuando plántulas o plantas adultas sufren de estrés por sequía. Diferencias en virulencia y en características morfológicas, fisiológicas y bioquímicas entre aislados de Mp colectados en RD y en PR son informados en este estudio. PRMp2 fue más virulento que PRMp1. Sin embargo, RDMp2 fue más virulento que PRMp2 y RDMp1. Las características morfológicas y el tamaño de los esclerocios fueron similares en aislados de RD, pero diferentes al aislado de PR. No hubo diferencias significativas para el tamaño de los esclerocios y el crecimiento radial del micelio cuando los aislados se expusieron a la luz o a la oscuridad. Se utilizó la electroforesis del gel de poliacrilamida (PAGE) para el análisis de proteínas de los aislados de PR y de RD. Los perfiles de proteínas de los aislados de RD fueron similares pero diferentes al del aislado de PR (PRMp2).

INTRODUCTION

Macrophomina phaseolina (Tassi) Goid. is the causal agent of the charcoal rot or ashy sten blight of common bean (*Phaseolus vulgaris* L.). The disease is prevalent in warmer bean growing areas of the Dominican Republic (DR) (Sanchez, 1989), and Puerto Rico (PR) (Echavez and Beaver, 1987). Severe symptoms occur when bean seedlings or adult plants are stressed from moisture deficits (Echavez and Beaver, 1987; Olaya *et al.*, 1996). The pathogen attacks more than 500 plant species, including, soybean, cotton, corn, sorghum, and edible legumes (Dhingra and Siclair, 1977). The patogenicity is obtained for most isolates near 30° C. The fungus affects the hypocotyl of seedlings or the base of cotyledons. *Macrophomina phaseolina* is a soil-borne fungus, and can affect the seeds, resulting in a seed-borne pathogen (Zaumeyer and Thomas, 1957). Variation in morphology and virulence among isolates of *M. phaseolina* were reported in soybean, and other crops (Dhingra and Sinclair, 1977). Soil inoculation techniques with microsclerotia or mycelia were reported by Dhingra and Sinclair (1973). The toothpick inoculation method has been effective for greenhouse screening of common bean germplasm resistant to charcoal rot (Echavez an Beaver, 1987) and was used in this study. Breeding works for charcoal resistant bean germplasm have not been already made in PR and DR because the pathogenic variation of the fungus isolates is unknown. The objectives of this work were to study the morphological, physiological and biochemical characteristics of Mp isolates collected in DR and PR and determine their variation in pathogenicity on common bean.

MATERIALS AND METHODS

Morphological characteristics: Two assays were performed, one using the PR isolates, Mp1 and Mp2, and the other using three isolates, one from PR and two from DR. PRMp1 and PRMp2 were obtained from infected stems of bean plants grown in the south and on the northwestern coast of the island, respectively. Isolates from DR (DRMp1 and DRMp2) were collected in the south and southwestern areas of the country. All isolates were grown in potato dextrose agar (PDA) supplemented with an antibiotic or in yeast extract mannitol dextrose (YEMD) liquid media and incubated at 28° C. An Mp-colonized disk (5mm) was plated on the center surface of the PDA dish or placed into the liquid medium. Radial growth were measured (mm) at 24, 48, 72 and 96 h after inoculation on PDA and microsclerotia size (mm) determined when the culture was seven days old. Mycelia texture, color and form were observed in the liquid medium. A completely randomized design with five (first essay) and four (second assay) replications was used.

Physiological characteristics: PDA cultures of PRMp2, DRMp1, and DRMp2 were incubated for 14 h under fluorescent light or in the dark. Each treatment was replicated four times in a completely randomized design with a split plot arrangement. The main plots were the artificial light (14 h period) and dark treatments, and subplots were the isolates. Data were analyzed by analysis of variance and means separated by an LSD test.

Biochemical characteristics: Mycelia of PRMp1, DRMp1, and DRMp2 were grown in YEMD liquid medium for two wks incubation in a shaker incubator at 28° C, then the mycelia were collected and ground in potassium phosphate buffer (0.1 M, pH 7). A modified May and Lilly (1988) technique was used to extract the mycelial protein. Sample concentrations were 0.5 µg protein/µl using the Esen colorimeter method (Esen, 1978). Polyacrylamide gels were run at 37 V for 15 h with tris-glycine and SDS buffer (pH 8.8). TEMED (N,N,N',N'-tetramethylethylene-diamine) was used in polymerization of the gel. Myosin,, _-galactosidase, phosphorylase, albumin bovine, ovalbumin, and carbonic anhydrase were used as standard proteins.

Pathogenicity of PR isolates: The pathogenicity of PR isolates (PRMp1 and PRMp2) was tested on three bean genotypes (8437-22, Cuarentena and RIZ 44) 30 days after planting in 20-cm plastic pots containing pasteurized soil. Both Mp isolates were grown in acidified PDA medium. Inoculum was applied by inserting infested toothpicks into stems near the cotyledonary nodes. A visual scale of 0 (no symptoms) to 4 (plant death) was used to measure the charcoal rot severity at 10, 20, and 30 days after inoculation. Treatments were replicated four times, data were analyzed by analysis of variance and Fischer's Least Significant Difference (LSD) test.

Pathogenicity of the PR and DR isolates: Thirteen bean cvs of landrace Pompadour were used in a factorial arrangement of a randomized complete block with five replications. Three bean seeds/cv were planted in 20-cm diameter plastic pot containing Promix ® and pasteurized soil. Two *M. phaseolina* isolates from DR (DRMp1, DRMp2) and the most virulent PR isolate determined in the previous test (PRMp2), were used as inocula. We used the same procedure (the inocula preparation and the inoculation technique), and the severity scale as mentioned above. All data were statistically analyzed.

RESULTS AND DISCUSSION

Morphological characteristics: Results of the first assay showed that microsclerotia size and the radial growth were not significantly different for the two PR isolates (Table 1). In the second assay, there were significant differences between the mycelium radial growth of PRMp2 and that of the DRMp1 and DRMp2 isolates (Table 2). Microsclerotia mean size of DR isolates were significantly larger than that of PRMp2 (Table 2). Mycelia color and texture and morphological characteristics were similar between DR isolates but were different from those of the PR isolate.

Physiological characteristics: There were not significant differences in microsclerotia size and mycelia radial growth of each isolate under light or dark conditions (Table 2). Similar results were obtained by Johnson (8) with this fungus using different light intensities and x rays.

Biochemical characteristics: Protein concentrations obtained from DRMp1 (225 µl) and DRMp2 (400 µl) were higher than that of PRMp2 (150µl). The analysis of protein by the polyacrylamide gel electrophoresis (PAGE) indicates that the protein patterns of DR isolates were similar, but different than that of the PRMp2 (Fig. 1). DR and PR isolates showed 16 and 17 proteins, respectively. Thirteen proteins were common among isolates (Table 3). It is important to note that the photograph (Figure 1) lacks of some protein bands but all bands, including the standars, were clearly defined in the gel.

Pathogenicity of PR isolates: Pathogenicity was highly variable between PRMp1 and PRMp2. The PRMp2 isolate caused significantly higher charcoal rot severity on the common bean plantings than the PRMp1 isolate (Table 4).

Pathogenicity of the PR and DR isolates: Genotype-disease severity interactions were highly significant (P0.01). This demonstrates that distinct pathogenic variation existed among PRMp2, DRMp1, and DRMp2. The host variation also was observed among landrace Pompadour cvs. Similar results were obtained by Dhingra and Sinclair (3) and Abawi and Pastor-Corrales (1) with varietal differences in charcoal rot in soybean and common bean, respectively. The DRMp2 isolate was the most virulent (Table 5).

The basic information obtained in this study will facilitate proper planning of local level bean breeding programs in developing charcoal rot-resistant varieties.

Table 1. Morphological and physiological characteristics of *Macrophomina phaseolina* isolates collected in Puerto Rico

Isolates	Microsclerotia size (mm)	Radial growth (mm)	
		24 h [1]	48 h
PRMp1	83	40	80
PRMp2	79	38	75

1/ Incubation period

Table 2. Morphological and physiological characteristics of *M. phaseolina* isolates collected in Puerto Rico and the Dominican Republic under light and dark conditions.

Isolates	Radial growth (mm)		Microsclerotia size (mm)	
	Light	Dark	Light	Dark
Puerto Rico PRMp2	78 a [1]	78 a	95 a	91 a [2]
Dominican Rep DRMp1	85 b	80 b	126 b	141 b
Dominican Rep DRMp2	85 b	80 b	138 b	140 b
LSD (0.05)	4.67	4.67	21.98	21.98
C.V. (%)	2.08	2.08	2.2	2.2

Mean values followed by the same letters within the same row for radial growth[1] and for microsclerotia size[2] are not significantly different (P=0.05) according to an LSD test.

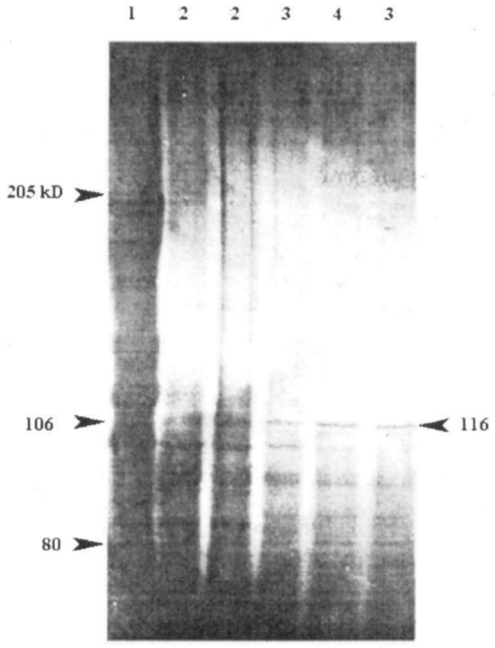

Figure 1. Polyacrylamide gel electrophoresis of proteins extracted from mycelia of PR and DR isolates. 1=standards protein, 2= PRMp2, 3= DRMp1, 4= DRMp2. Arrows points to specific bands of known molecular weight (kD).

Table 3. Molecular weight (kD) of proteins from *Macrophomina phaseolina* isolates analyzed by polyacrylamide gel electrophoresis (PAGE).

Molecular weight (kD)	Puerto Rico (PRMp2)	Dom. Rep. (DRMp1)	Dom. Rep. (DRMp2)
67	X	X	X
69	X	X	X
74	X	X	X
77	X	X	X
79	X	X	X
82	X	X	X
84	X	X	X
86	X	—	—
89	X	X	X
90	X	—	—
93	—	X	X
97	X	—	—
100	X	X	X
103	X	X	X
110	X	X	X
116	—	X	X
127	X	—	—
136	—	X	X
158	X	X	X
270	X	X	X

kD= kilodalton; X= protein band; —= no protein band

Table 4. Pathogenic variation in Puerto Rico isolates (PRMp1 and PRMp2) on three common bean genotypes.

	Charcoal rot severity [1]		
Isolates	**8437-22**	**Cuarentena**	**RIZ 44**
PRMp1	1.70 a [2]	2.00 a	1.25 a
PRMp2	3.40 b	3.70 b	3.50 b

1/ *Visual severity scale: 0= no symptoms; 4= plant death*

2/ *Mean values followed by different letters within the same column are significantly different (P=0.05) according to an LSD test.*

Table 5. Pathogenic variation in isolates of Puerto Rico (PRMp2) and Dominican Republic (DRMp1 and DRMp2) on some bean landrace Pompadour cultivars.

	Charcoal rot severity [1]		
Landrace cultivar	**DRMp1**	**DRMp2**	**PRMp2**
Pompadour E	1.8	2.4	1.4
Pompadour T	2.5	3.5	1.3
Pompadour S	2	3.4	2.5
Pompadour K	3.5	4	2.3
Pompadour V	2.8	3.5	2.7

1/ *Visual severity scale: 0= no symptoms; 4= plant death*

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