

CHARACTERIZATION OF MEXICAN AND NON-MEXICAN ISOLATES OF *MACROPHOMINA PHASEOLINA* BASED ON MORPHOLOGICAL CHARACTERISTICS, PATHOGENICITY ON BEAN SEEDS AND ENDOGLUCANASE GENES

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SUMMARY

The diversity patterns of 30 isolates of the fungal pathogen *Macrophomina phaseolina*, 15 from Mexico and 15 from other countries were analyzed based on morphological characteristics, pathogenicity on bean seeds and presence or absence of endoglucanase genes. The anastomosis frequencies among all isolates were determined under *in vitro* conditions and finally, two endoglucanases (*egl1* and *egl2*) were investigated by Southern analysis. No relationship between microsclerotia size and pathogenicity was found. The most aggressive isolates on bean seeds were from Mexico, although they exhibited the lowest microsclerotia sizes and growth rate on *in vitro* conditions. A low frequency (less than 24%) of successful anastomosis among *M. phaseolina* isolates was found, while *egl1* and *egl2* were detected in 29 and 20 isolates, respectively. The results obtained indicate a different virulence between Mexican and non-Mexican isolates, but no clear differences between isolates were found when they were compared based on anastomosis capability or presence/absence of endoglucanase genes. Thus, we suggest that pathogenic specialization of *M. phaseolina* is related to the geographic origin of the isolates, whereas specialization could not be explained by analyses of pathogenicity genes or anastomosis frequencies.

Key words: charcoal rot, pathogenic specialization, endoglucanase genes.

INTRODUCTION

The fungus *Macrophomina phaseolina* (Tassi) Goid is a soil-borne pathogen distributed worldwide with a host range of more than 500 plant species (Mihail and Taylor, 1995). The pathogen is a Basidiomycete, mostly known in two anamorph forms belonging to *Macro-*

phomina and *Rhizoctonia*, respectively. It attacks particularly common beans (*Phaseolus vulgaris*) in both arid and tropical regions of Mexico and provokes charcoal rot or ashy stem blight diseases (Abawi and Pastor-Corrales, 1990).

Two aspects play a major role in disease development: 1) *M. phaseolina* shows a great morphological (Mihail and Taylor, 1995; Mayek-Pérez, 1999), physiological (Manici *et al.*, 1995; Mihail and Taylor, 1995), pathogenic (Manici *et al.*, 1995; Mihail and Taylor, 1995; Miklas *et al.*, 1998; Mayek-Pérez *et al.*, 2001; Su *et al.*, 2001), and genetic (Chase *et al.*, 1994; Mihail and Taylor, 1995; Jones *et al.*, 1998; Mayek-Pérez *et al.*, 2001; Su *et al.*, 2001) variability which increase its adaptability to diverse environmental conditions; 2) the fungus exists in two forms, either of which represents its own life-cycle, one saprophytic (named *R. bataticola*) where the fungus mainly produces microsclerotia, and another pathogenic (*M. phaseolina*), where the pathogen mainly produces pycnidia. In the pathogenic stage the fungus is a non-specific pathogen and attacks a broad spectrum of economically important crops such as common beans, maize, soybean, sorghum, sesame, cotton, safflower, sunflower or cucurbits (Dhingra and Sinclair, 1978).

The pathogen causes dark and irregular lesions on epicotyls and hypocotyls of common bean seedlings and the symptoms extend to the cotyledons causing death of seedlings. In adult plants, charcoal rot symptoms are followed by wilting due closing of the xylem vessels, microsclerotia are formed and roots show discoloration or red to brown lesions. Subsequently, gray-black mycelia and microsclerotia are produced. The plant is defoliated and appears chlorotic, severe infections cause severe grain yield losses. Incidence and development of charcoal rot are favored under high temperature and drought stresses (Abawi and Pastor-Corrales, 1990). Mayek-Pérez *et al.* (2001) found the pathogenic and genetic differentiation between *M. phaseolina* Mexican isolates collected from tropical or arid regions, while Su *et al.* (2001) analyzed isolates from four plots which had been cultivated during 10 years with cotton, soybean, sorghum and maize, and suggested the host specialization of *Macrophomina* based on physiological, pathogenic and genetic differentiation among isolates.

Fungal plant pathogens typically secrete an array of cell-wall degrading enzymes capable of depolymerizing each cell wall component such as carbohydrates (cellulose, xylan, pectin, polygalacturonic acid) and different classes of proteins (Walton, 1994; Roncero *et al.*, 2000) where outstand pectinases, xylanases, cellulases and proteases (Roncero *et al.*, 2000; Tonukari, 2003). Cellulases are divided in endoglucanases and in cellobiohydrolases and play a key role on parasitic ability of *M. phaseolina* (Dhingra and Sinclair, 1978; Jones *et al.*, 1998). Two β -1,4 endoglucanase genes (*egl1* y *egl2*) have been cloned and characterized from *M. phaseolina*, and both have been found in other saprophytic and pathogenic fungi. The *egl1* gene has a cellulolytic activity and facilitates the penetration of charcoal rot hyphae in the host, while *egl2* has similar enzymatic properties to *egl3* gene isolated from *Trichoderma reesei* (Wang and Jones, 1995a,b).

This work was conducted in order to (i) characterize the morphological, pathogenic and genetic variability in 30 isolates of *M. phaseolina* from Mexico and other countries; (ii) demonstrate the existence of pathogenic specialization in the fungus; (iii) obtain information which explains the mechanisms of host specialization and genetic exchange between fungal populations.

MATERIALS AND METHODS

Origin of isolates. Thirty isolates of *M. phaseolina* were used in the present study; fifteen of them were obtained from different locations of Mexico (states of Sinaloa, Tamaulipas, Coahuila, Aguascalientes, Veracruz, Puebla, Guerrero) and 15 others from other countries (Italy, Australia, Argentina, United States of America, Brazil). Geographical and host origins of the isolates are reported in Table 1. The isolates were cultured in potato-dextrose-agar (PDA). Pure culture of each isolate obtained from a single microsclerotium was cultured in acidified PDA medium.

Morphological analysis. One mycelial block (10 mm diameter) out from a seven days-old pure culture was transferred to the center of 9 cm Petri dish that was sealed and incubated in the dark at 30°C for 96 h. Each treatment was replicated four times. Mean diameter of colony (DC, mm) was recorded each 24 h and these data were used to calculate the relative growth rate of colony (RGR, mm d⁻¹) (Mayek-Pérez *et al.*, 1997). At the fifth day, certain characteristics of colony were registered (color of colony, presence/absence of aerial mycelium, color of mycelium, shape and color of microsclerotia, abundance of microsclerotia in the Petri dish, colony shape). Finally, mycelia and microsclerotia of each isolate were recovered and placed on synthetic resin; length and width of 40 microsclerotia were regis-

tered using a micrometer adapted to optical microscope (Carl Zeiss, Oberkochen, Germany), and shape and color of microsclerotia were recorded at 40X.

Hyphal fusion. The hyphal interactions among isolates were investigated by recording all paired combinations among them. The protocol of Mihail and Taylor (1995) was modified by setting up each co-culturing on 25 x 75 mm glass-slides for microscope covered with a thin film (around 2 mm) of diluted PDA. Each combination was established twice and rated positive if a minimum of three hyphal fusions were observed after incubation for 45-50 h at 30°C.

Pathogenicity on common bean cultivars. Pathogenicity of *M. phaseolina* isolates was evaluated using seeds of 12 common bean differential cultivars (Mayek-Pérez *et al.*, 2001). Seeds were sterilized with 2% sodium hypochlorite for 2 min and then rinsed in sterile tap water. Isolates of *M. phaseolina* were cultured in 9 cm Petri dishes at 30°C in the dark. When PDA of the dishes was completely colonized 10 seeds of each resistant (BAT 477, TLP 19, SEQ 12, Negro 8025, G 4523, G 19428) and susceptible (Pinto UI-114, Pinto Villa, Bayo Durango, Azufrado Tapatío, Bayo Mecentral, Rio Tibagi) cultivars were placed on the colony of each *M. phaseolina* isolate. Petri dishes were incubated at 28°C in the dark. After five days, seeds were evaluated for symptoms caused by the pathogen using one 0-5 scale where 0 = healthy seed; 1 = discoloration of a portion of the seedling in contact with the mycelium; 2 = seed teguments invaded by mycelium and sclerotia but healthy seedling; 3 = seed teguments free from the fungus, but seedling infected; 4 = seed tegument and seedling infected; 5 = seed infected and not germinated (Manici *et al.*, 1995). After, the virulence index by isolate was calculated by multiplying the number of seeds by the degree of virulence (*i.e.* six seeds with disease severity of 4 and four with disease severity of 5 = 24 + 20/10 = 4.4).

Southern analysis of endoglucanase genes. Fungal DNA was isolated according to Raeder and Broda (1985). Samples (20 µg) were digested overnight with *Sa*I (Roche Diagnostic, Indianapolis, USA), phenol:chloroform extracted, ethanol precipitated, dried, and separated by electrophoresis in 1.2% agarose gels. Transfer and probing were carried out as previously described Jones *et al.* (1998) except that the probe was chemiluminescent labeled by random-priming method (Roche Diagnostic, Indianapolis, USA). Two genes (*egl1* and *egl2*) supplied by Dr. Richard Jones (USDA-ARS, Beltsville, USA) were analyzed, representing the two β -1,4 endoglucanases produced by *M. phaseolina* (Wang and Jones, 1995a,b). One blot was probed with *egl1* and

the other blot with *egl2*. Film (X-ray film, Eastman-Kodak, Rochester, USA) exposure was 1 h. The actual activity of each enzyme was not measured.

Statistical analysis. Mean \pm standard error (ES) was calculated for morphological (length, width and length/width of microsclerotia and RGR) and pathogenic (virulence index) data by isolate. All morphological data were subjected to Principal Component Analysis (PCA) in order to identify the most stable morphological characteristics and *M. phaseolina* isolates were dispersed in a two-dimension graph on basis of the two principal components constructed by PCA. Means of virulence by isolate were used to perform a cluster analysis, where Euclidean distances among isolates were calculated to construct a dendrogram based on UPGMA algorithm.

RESULTS

The shape of colony varied from radial to irregular; mycelium production was intermediate in the most of isolates and colour of mycelium was from white to black (Table 1). Production of microsclerotia was higher in most Mexican isolates than in non-Mexican isolates. However, no significant differences between Mexican and non-Mexican isolates were found in length, width and length/width ratios of microsclerotia (Table 2). PCA indicated that three qualitative (colony colour, aerial mycelium, colour of mycelium) and four quantitative (RGR of colony at 24 h and average RGR; length and width of microsclerotia) characteristics were the most stable variables on *in vitro* conditions (Table 3). The three major principal components explained 79% of the

Table 1. Isolate number, place of origin, host and colony characteristics of 30 isolates of *M. phaseolina*.

Isolate	Origin	Host	Colony characteristics ^a		
			Shape	Aerial Mycelium	Mycelium color
México					
MEX01	Sinaloa	<i>Sesamum indicum</i>	Irregular	+	Gray
MEX02	Puebla	<i>Phaseolus vulgaris</i>	Radial	++	Gray
MEX03	Sinaloa	"	Radial	++	Gray
MEX04	Guerrero	<i>Solanum melongena</i>	Irregular	+	Gray
MEX05	Sinaloa	<i>Sesamum indicum</i>	Radial	+	Gray
MEX06	Coahuila	<i>Phaseolus vulgaris</i>	Irregular	++	Black
MEX07	"	<i>Zea mays</i>	Radial	++	Gray
MEX08	Tamaulipas	<i>Glycine max</i>	Radial	+	Gray
MEX09	Aguascalientes	<i>Phaseolus vulgaris</i>	Radial	++	Gray
MEX10	"	"	Radial	+	Black
MEX11	"	"	Radial	++	Gray
MEX12	Tamaulipas	<i>Sorghum bicolor</i>	Radial	++	Gray
MEX13	Aguascalientes	<i>Phaseolus vulgaris</i>	Radial	+	Gray
MEX14	"	"	Irregular	+	Gray
MEX15	Veracruz	"	Irregular	++	Gray
Other Countries					
AUS01	Australia	<i>Cicer arietinum</i>	Radial	++	White
AUS02	"	<i>Capsicum annuum</i>	Irregular	++	Gray
AUS03	"	<i>Cicer arietinum</i>	Radial	+	Gray
AUS04	"	<i>Phaseolus vulgaris</i>	Radial	+	White
AUS05	"	<i>Vigna mungo</i>	Radial	++	Gray
ARG01	Argentina	<i>Gossypium hirsutum</i>	Radial	++	Gray
ARG02	"	"	Irregular	++	Gray
BRA01	Brazil	<i>Glycine max</i>	Radial	++	Gray
ITA01	Italy	<i>Helianthus annuus</i>	Radial	+	Gray
ITA02	"	"	Irregular	++	White
EUA01	USA	<i>Euphorbia lathyris</i>	Irregular	+	Black
EUA02	"	"	Irregular	+	Gray
EUA03	"	<i>Parthenium agentatum</i>	Irregular	+	Black
EUA04	"	"	Radial	+	Gray
EUA05	"	<i>Ligustrum texanum</i>	Irregular	+	White

^a + = poor; ++ = intermediate.

Table 2. Microsclerotia growth, color, shape and size of 30 isolates of *M. phaseolina* from Mexico and from other countries.

Isolate	Abundance ^a	Color	Shape	Length (µm) ^b	Width (µm)	Length/width
México						
MEX01	+++	Black	Ovoid	238 ± 22	180 ± 7	1.3 ± 0.2
MEX02	++	Black	Ovoid	175 ± 13	125 ± 11	1.4 ± 0.3
MEX03	+	Black	Irregular	172 ± 15	114 ± 10	1.5 ± 0.2
MEX04	+++	Brown	Ovoid	224 ± 24	179 ± 11	1.3 ± 0.3
MEX05	+++	Black	Round	190 ± 12	134 ± 14	1.4 ± 0.2
MEX06	+	Brown	Ovoid	230 ± 23	170 ± 16	1.4 ± 0.1
MEX07	+++	Black	Ovoid	251 ± 32	156 ± 11	1.6 ± 0.2
MEX08	+	Brown	Ovoid	112 ± 12	78 ± 6	1.4 ± 0.2
MEX09	+++	Black	Ovoid	270 ± 22	193 ± 11	1.4 ± 0.2
MEX10	+	Black	Irregular	162 ± 32	117 ± 8	1.4 ± 0.1
MEX11	+++	Black	Ovoid	240 ± 21	169 ± 9	1.5 ± 0.3
MEX12	+	Brown	Ovoid	176 ± 11	110 ± 10	1.7 ± 0.3
MEX13	+++	Black	Round	264 ± 21	213 ± 14	1.3 ± 0.1
MEX14	+	Brown	Ovoid	195 ± 15	136 ± 11	1.4 ± 0.2
MEX15	+++	Brown	Irregular	247 ± 21	181 ± 14	1.4 ± 0.3
Mean				210 ± 45	150 ± 37	1.4 ± 0.1
Other Countries						
AUS01	+	Brown	Ovoid	160 ± 12	111 ± 9	1.4 ± 0.2
AUS02	+	Black	Ovoid	223 ± 18	152 ± 11	1.5 ± 0.4
AUS03	+	Black	Ovoid	164 ± 16	105 ± 10	1.6 ± 0.4
AUS04	+	Brown	Irregular	145 ± 11	95 ± 4	1.6 ± 0.2
AUS05	+	Black	Ovoid	324 ± 28	196 ± 13	1.7 ± 0.2
ARG01	+	Black	Ovoid	249 ± 22	157 ± 11	1.6 ± 0.2
ARG02	+	Black	Ovoid	225 ± 21	149 ± 12	1.6 ± 0.1
BRA01	++	Black	Round	217 ± 17	178 ± 14	1.2 ± 0.2
ITA01	+++	Brown	Irregular	234 ± 21	140 ± 11	1.7 ± 0.3
ITA02	+	Black	Ovoid	166 ± 12	110 ± 8	1.5 ± 0.2
EUA01	+	Black	Round	280 ± 17	181 ± 7	1.6 ± 0.3
EUA02	+	Brown	Round	135 ± 11	96 ± 5	1.4 ± 0.2
EUA03	+	Black	Round	312 ± 33	186 ± 9	1.7 ± 0.2
EUA04	+	Black	Round	299 ± 21	170 ± 14	1.8 ± 0.3
EUA05	+	Black	Round	280 ± 21	168 ± 16	1.7 ± 0.2
Mean				228 ± 63	146 ± 35	1.6 ± 0.2

^a + = poor; ++ = intermediate; +++ = high.

^b Mean ± standard error.

total variance. Mexican isolates were grouped together on basis of morphological characteristics and then were different to foreigner isolates (Fig. 1). Mexican isolates showed higher virulence on the 12 common bean cultivars (Table 4). When virulence data were used to perform a cluster analysis, a clear differentiation of isolates was found on the basis of geographical origin of the individual isolate, since Mexican isolates formed a group excluding the foreign isolates (Fig. 2). However, with the exception of one Mexican isolate, the gene *egl1* was found in all isolates and amplified a band of about 2 kb (Table 4; Fig. 3); while *egl2* gene was found in 11 out of 15 non-Mexican isolates and in 9 out of 15 Mexican isolates and amplified a band of about 4 kb (Table 4). Finally, hyphal fusions showed low frequencies (less than 24%) for all combinations of isolates (data not shown).

DISCUSSION

Mexican isolates were different from the other *M. phaseolina* isolates concerning *in vitro* growth rate and virulence on bean seeds. Mexican isolates showed the highest RGR of colony under *in vitro* conditions, and virulence on bean seeds, while non-Mexican isolates showed the biggest microsclerotia. No positive associations between *M. phaseolina* isolates were found before on the basis of morphological characteristics and pathogenicity on common beans (Mayek-Pérez *et al.*, 1997; Mayek-Pérez, 1999). Our data emphasize that no relationships between microsclerotia size and virulence are found in *M. phaseolina*, as Dhingra and Sinclair (1978) suggested, although Ayanru and Green (1978) and Mikhail *et al.* (1994) indicated that greater microsclerotia

Table 3. Eigenvalues associated to the three principal components from PCA based on *in vitro* characteristics of 30 *M. phaseolina* isolates.

Characteristic	Principal component		
	1	2	3
Colony shape	0.15	-0.03	-0.03
Colony color	<u>-0.93</u>	0.10	0.06
Aerial mycelium	<u>0.80</u>	0.15	-0.10
Mycelium color	<u>-0.92</u>	0.07	-0.08
Production of microsclerotia	-0.69	0.55	-0.03
Microsclerotia color	0.07	0.66	-0.19
Microsclerotia shape	0.13	0.22	0.14
Relative rate growth of colony (24 h)	-0.28	-0.05	<u>0.79</u>
Relative rate growth of colony (48 h)	0.08	-0.24	0.40
Relative rate growth of colony (72 h)	0.04	0.09	0.15
Relative rate growth of colony (96 h)	0.03	0.19	0.10
Average relative rate growth of colony	-0.20	-0.13	<u>0.88</u>
Length of microsclerotia (LM)	0.03	<u>-0.95</u>	-0.17
Width of microsclerotia (WM)	0.17	<u>-0.90</u>	0.06
LM/WM	-0.34	-0.31	-0.50
Variation (%)	31	28	20

have higher possibilities to quickly germinate and to produce germ tubes and finally, to infect the susceptible host. Our data suggest that *Macrophomina* may base its pathogenic capacity on the production of small but abundant microsclerotia. Moreover, the close association between high RGR of colony and virulence are according to the higher capability to assimilate the nutrients available on the culture medium by the most aggressive isolates (Rayner, 1991). As Mayek-Pérez (1999) found on *M. phaseolina* isolates from different geographical areas of México, microsclerotia size and RGR at 24 h on incubation were the quantitative characteristics most explicative from total variance. Our data emphasize that quantitative characteristics are more appropriate than qualitative characteristics for *Macrophomina* description and classification, as been suggested by Mayek-Pérez *et al.* (1997) and Mayek-Pérez (1999). We found a clear separation between Mexican isolates from the other *Macrophomina* isolates concerning virulence range, but no clear differences were found as for either presence/absence of endoglucanase genes or anastomosis ability. Mayek-Pérez *et al.* (1997) and Mayek-Pérez *et al.* (2001)

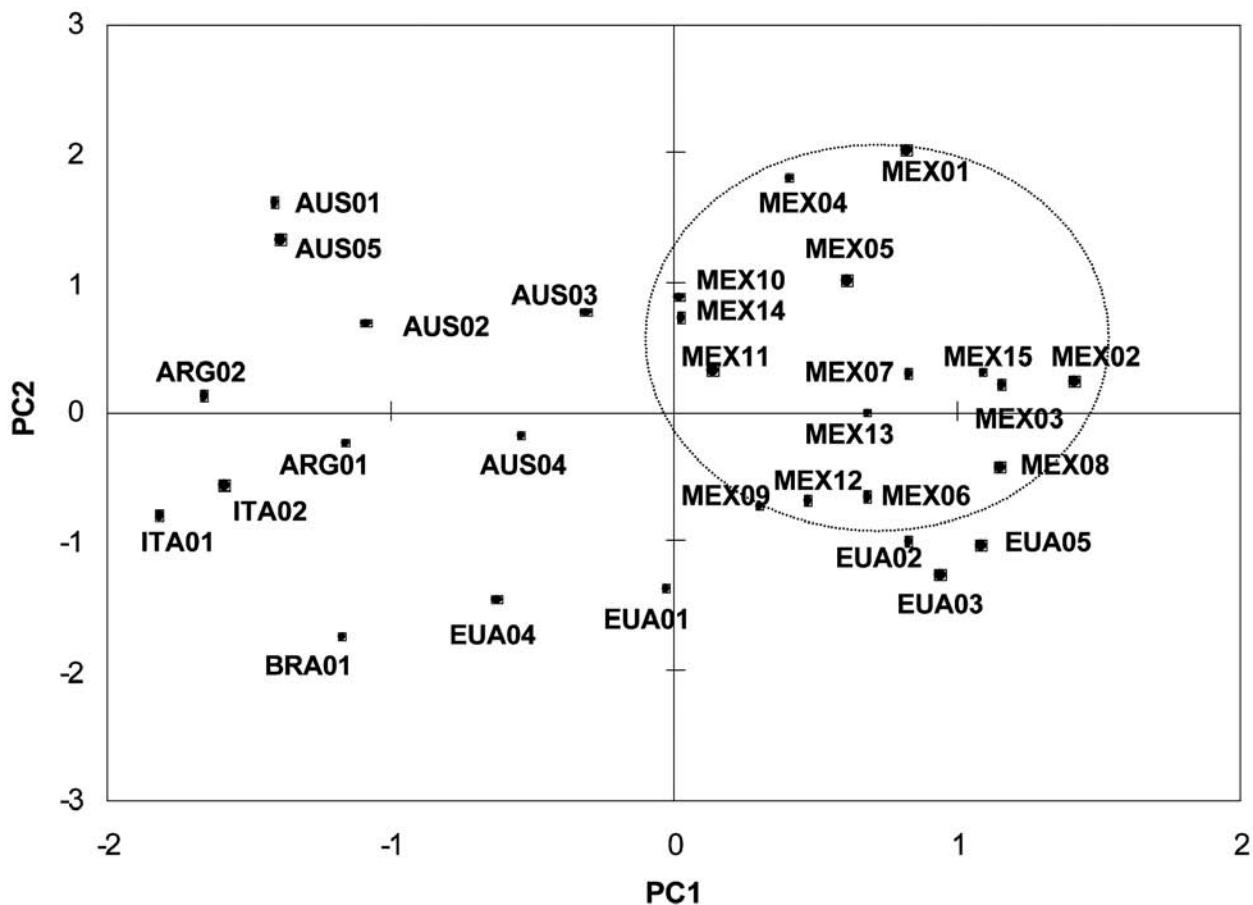


Fig. 1. PCA analysis of 30 isolates of *M. phaseolina* isolates based on the two principal components calculated from *in vitro* morphology analysis.

reported a clear pathogenic differentiation among *Macrophomina* isolates of different geographical origin (arid and tropical regions). However, the endoglucanase genes were not closely associated to pathogenicity capability as Jones *et al.* (1998) suggested when they analyzed *M. phaseolina* isolates from contrasting countries (USA and Somalia). It was clear that the presence of genes coding endoglucanases is not strong evidence about the actually enzymatic activity in the fungus. Further works where the enzymatic activity or endoglucanase gene expression could be characterized are needed.

Despite no clear evidences for free genetic exchange among *M. phaseolina* from different host or geographical origins were found in this work (*i.e.* low frequencies of

anastomosis among isolates), we suggest that the pathogenic and morphological differentiation among isolates could be produced for some reproductive isolation based on geographical origins. Thus, although we found a high conservation on endoglucanase genes among *M. phaseolina* isolates, we suggest that other virulence genes must be involved on pathogenic ability of *Macrophomina* populations, such as those that code others cell wall degrading enzymes like polygalacturonases, galactosidase, pectin methyl esterase, or cellulases (Dhingra and Sinclair, 1978). Further work is required to clarify the role of those DNA fragments detected by Southern analysis. Thus, we suggest a relation between the pathogenic differentiation of *M. phaseolina* and geographic origin of isolates.

Table 4. Growth, virulence index and presence/absence of endoglucanase genes in 30 isolates of *M. phaseolina* from Mexico and from other countries.

Isolate	Relative Growth Rate of colony at 24 h (mm d ⁻¹) ^a	Virulence index	Endoglucanase genes ^b	
			<i>egl1</i>	<i>egl2</i>
México				
MEX01	1.3 ± 0.1	4.8 ± 0.3	+	+
MEX02	1.0 ± 0.1	4.5 ± 0.3	+	+
MEX03	1.2 ± 0.2	4.4 ± 0.4	+	+
MEX04	1.2 ± 0.1	4.9 ± 0.2	+	+
MEX05	1.4 ± 0.3	4.8 ± 0.3	+	+
MEX06	1.0 ± 0.1	3.7 ± 0.2	+	-
MEX07	1.4 ± 0.2	4.5 ± 0.4	+	+
MEX08	0.9 ± 0.1	4.2 ± 0.2	-	-
MEX09	0.9 ± 0.2	4.0 ± 0.2	+	-
MEX10	1.5 ± 0.2	4.5 ± 0.3	+	+
MEX11	1.3 ± 0.1	3.9 ± 0.3	+	-
MEX12	1.4 ± 0.2	4.8 ± 0.3	+	+
MEX13	1.4 ± 0.1	4.2 ± 0.4	+	-
MEX14	1.0 ± 0.1	4.1 ± 0.5	+	-
MEX15	1.4 ± 0.1	4.7 ± 0.3	+	+
Mean	1.2 ± 0.2	4.4 ± 0.4		
Other Countries				
AUS01	1.2 ± 0.2	2.6 ± 0.3	+	-
AUS02	1.4 ± 0.3	2.9 ± 0.2	+	-
AUS03	1.1 ± 0.2	3.8 ± 0.4	+	-
AUS04	1.2 ± 0.3	2.5 ± 0.5	+	-
AUS05	0.9 ± 0.1	2.5 ± 0.2	+	+
ARG01	1.3 ± 0.2	2.4 ± 0.3	+	+
ARG02	1.7 ± 0.2	2.7 ± 0.3	+	+
BRA01	1.2 ± 0.1	1.9 ± 0.4	+	+
ITA01	1.1 ± 0.1	2.4 ± 0.3	+	+
ITA02	1.5 ± 0.3	2.1 ± 0.4	+	+
EUA01	0.9 ± 0.2	1.5 ± 0.2	+	+
EUA02	0.7 ± 0.2	2.2 ± 0.4	+	+
EUA03	0.7 ± 0.2	1.9 ± 0.3	+	+
EUA04	0.9 ± 0.1	2.7 ± 0.5	+	+
EUA05	1.4 ± 0.2	2.3 ± 0.2	+	+
Mean	1.1 ± 0.3	2.4 ± 0.5		

^a Mean ± standard error.

^b + = present; - = absent.

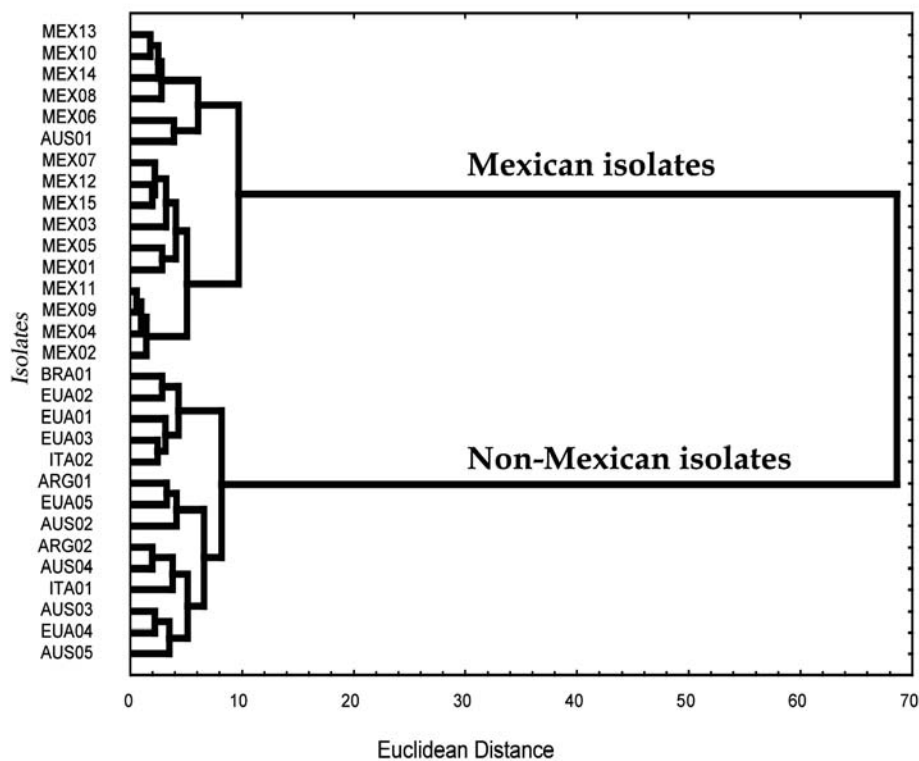


Fig. 2. Dendrogram of *M. phaseolina* isolates based on virulence index.

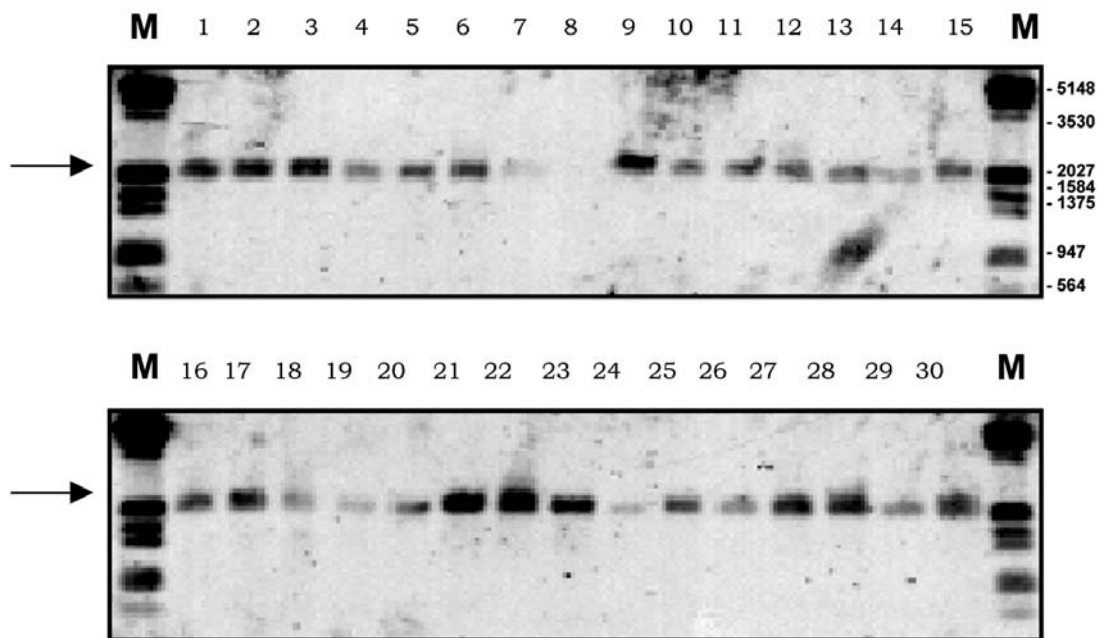


Fig. 3. Southern analysis of *egI1* gene on 30 *M. phaseolina* isolates (M=Molecular weight ladder; lanes 1 to 15 = Mexican isolates and lanes 16 to 30 = Isolates from other countries). Numbers indicate molecular weight on bp and arrows indicate the bands corresponding to the *egI1* gene (about 2 kb).

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