Pathotyping and AFLP-based molecular characterization of *Macrophomina phaseolina* isolates from different plant hosts

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Abstract

Ninety-six isolates of *Macrophomina phaseolina*, from five different hosts, were tested for pathogenicity on seedlings of cotton (*Gossypuim barbadense* L.) cultivar Giza 89 under greenhouse conditions. Of the cotton isolates, 73 were pathogenic representing 98.65% of the cotton isolates, 76.04% of the total isolates, and 76.84% of the pathogenic isolates from all hosts. The pathogenic isolates of the other hosts ranged from four to seven. The percentage of isolates, which significantly affected post-emergence damping-off, was much greater than that of the isolates which significantly affected pre-emergence damping-off or plant height. Dry weight was not affected by any of the tested isolates. A highly significant negative correlation was observed between post-emergence damping-off and survival. Grouping the isolates by cluster analysis based on pathogenicity was neither related to their geographic origins nor to hosts. AFLP was used to evaluate the genetic diversity among the isolates. In this analysis, polymerase chain reaction was performed by using four AFLP primers. Grouping the isolates by cluster analysis based on AFLP banding patterns was also neither related to their geographic origins nor to hosts. These results may suggest that isolates of *M. phaseolina* from each geographic origin or host were a heterogeneous group of isolates.

Key words – AFLP markers – charcoal rot – *Macrophomina phaseolina* – pathogenicity

Introduction

Charcoal rot is caused by *Macrophomina phaseolina* (Tassi) Goid., which is considered one of the most destructive necrotrophic plant pathogens. It has a broad host range infecting about 500 plant species in more than 100 families throughout the world (Mihail & Taylor 1995, Su et al. 2001, Saleh et al. 2010). It can also infect many native plants and it is an adaptable pathogen of human beings (Tan et al. 2008). *M. phaseolina* has been recorded on cotton in south-eastern USA in Oklahoma and Texas (Watkins 1981), but is of little economic significance there compared to the Indian subcontinent, East and Central Africa, and elsewhere in the tropics and sub-tropics (Suriachandraselvan et al. 2005, Omar et al. 2007). The severity of charcoal rot on cotton in Pakistan, India, the Sudan, and Central Africa may be linked to soil moisture deficit and hot

weather (Watkins 1981). The importance of *M. phaseolina* as a cotton pathogen in Egypt is undervalued (Omar et al. 2007, Abd-Elsalam 2010, Asran-Amal 2012). This vision has come from the observation that during the last 50 years, *M. phaseolina* on cotton was almost absent from the literature of cotton diseases in Egypt. Thus, a handful of studies, most of them not dealing with *M. phaseolina per se,* has been published (Mostafa 1959, Mohamed 1962, Sabet & Khan 1969, Omar et al. 2007). This lack of concern is not justifiable because this fungal pathogen is of a widespread distribution in Egyptian soils and it is commonly isolated from cotton roots particularly late in the growing season. Pathogenicity of the fungus on commercial cotton cultivars in Egypt has been confirmed (Omar et al. 2007, Abd-Elsalam 2010).

Molecular tools have been used to examine the genetic variation of *M. phaseolina* isolates in order to categorize them into specific groups (Jana et al. 2005). Amplified fragment length polymorphism (AFLP) analysis is valuable in revealing genetic variability in the fungal populations and has been used to study genetic diversity in populations of *M. phaseolina* in several countries including Australia (Fuhlbohm 1997), Mexico (Vandemark et al. 2000, Mayék-Pérez et al. 2001), Brazil (Almeida et al. 2003), USA (Brooker et al. 2008) and China (Linhai et al. 2011). In Egypt, resistance to *M. phaseolina* is totally lacking in the commercial cotton cultivars (Omar et al. 2007). A clear understanding of the extent of genetic variation among *M. phaseolina* isolates would be helpful in developing resistant cotton cultivars. Therefore, the objectives of this investigation were (1) to assess pathogenicity of *M. phaseolina* isolates from different hosts on the seedlings of the commercial cotton cultivar Giza 89 under greenhouse conditions, and (2) to assess genetic diversity differences among these isolates by AFLP analysis.

Materials & Methods

Fungal isolates and inoculum production

Pathological studies and genetic variation in 96 isolates of *Macrophomina phaseolina* obtained from the fungal collection of the Cotton and Fiber Crops Diseases Research Section, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt were evaluated. Most isolates were originally isolated from cotton roots (Table 1).

Table 1 Geographic origins, hosts, cotton cultivars and variables used in testing pathogenicity of *Macrophomina phaseolina* isolates used in this study.

| Isolate no. | Geographic origin | Host | Cultivar | Pathogenicity variables | | | | |
				Pre-emergence damping-off[a] %	Post-emergence damping-off[a] %	Survival[a] %	Plant height cm/plant	Dry weight mg/plant
1	Giza	Cotton	Giza 75	40.61*	29.73*	40.61*	27.03	276.75
2	Minufiya	Cotton	Giza 75	28.22	34.56*	46.51*	22.91	241.00
3	Minufiya	Cotton	Giza 75	24.53	33.05*	49.61*	23.10	258.00
4	Daqahliya	Cotton	Giza 75	31.39	29.89*	45.00*	25.61	271.00
5	Daqahliya	Cotton	Giza 75	24.53	26.19*	49.39*	26.44	317.50
6	Minufiya	Cotton	Giza 75	34.56*	22.50*	42.12*	25.61	301.00
7	Giza	Cotton	Giza 75	26.19	38.89*	40.39*	24.32	280.50
8	Kafr El-sheikh	Cotton	Giza 86	34.72*	37.66*	34.72*	21.83	232.75
9	Beheira	Cotton	Giza 75	31.55	24.16*	46.38*	24.50	322.50
10	Gharbiya	Cotton	Giza 75	31.39	33.21*	46.51*	21.30	261.25
11	Kafr El-shaikh	Cotton	Giza 86	25.83	40.45*	35.94*	21.29	274.50
12	Daqahliya	Cotton	Giza 75	34.72*	33.97*	36.92*	18.66	255.00
13	Gharbiya	Cotton	Giza 75	36.92*	42.12*	24.16*	22.98	244.75
14	Minya	Cotton	Giza 80	37.66*	20.47*	45.00*	25.63	355.00
15	Kafr El-sheikh	Cotton	Giza 86	33.05*	33.21*	39.17*	22.42	226.25
16	Giza	Cotton	Giza 86	36.00*	29.89*	39.17*	23.17	233.25
17	Assiut	Cotton	Giza 83	24.53	31.55*	47.89*	21.38	352.50
18	Sharqiya	Cotton	Giza 85	28.22	39.17*	37.73*	23.96	232.50
19	Damietta	Cotton	Giza 45	31.55	28.22*	45.00*	22.76	204.00

Table 1 continued

Isolate no.	Geographic origin	Host	Cultivar	Pathogenicity variables				
				Pre-emergence damping-off[a] %	Post-emergence damping-off[a] %	Survival[a] %	Plant height cm/plant	Dry weight mg/plant
20	Minufiya	Cotton	Giza 86	29.89	34.72*	40.61*	21.98	287.75
21	Minufiya	Cotton	Giza 86	49.33*	22.50*	31.55*	24.33	280.50
22	Minufiya	Cotton	Giza 75	28.22	28.22*	47.89*	20.75	288.75
23	Beheira	Cotton	Giza 75	47.89*	33.21*	22.50*	24.04	221.00
24	Daqahliya	Cotton	Giza 84	28.22	36.17*	42.05*	27.18	237.00
25	Daqahliya	Cotton	Giza 84	28.22	37.73*	39.23*	22.69	239.50
26	Minufiya	Cotton	Giza 75	22.50	37.73*	43.56*	24.68	304.25
27	Gharbiya	Cotton	Giza 86	36.23*	45.00*	22.50*	23.99	285.75
28	Daqahliya	Cotton	Giza 75	43.56*	40.67*	31.39*	21.67	325.75
29	Daqahliya	Cotton	Giza 86	29.89	46.44*	15.86*	18.95	191.25
30	Minufiya	Cotton	Giza 75	43.56*	9.22	43.56*	24.91	365.50
31	Minya	Cotton	Giza 80	49.33*	29.89*	24.53*	23.00	229.75
32	Giza	Cotton	Giza 86	39.17*	42.05*	19.55*	16.43	291.75
33	Damietta	Cotton	Giza 45	47.89*	39.17*	9.22*	12.26*	154.00
34	Gharbiya	Cotton	Giza 75	49.33*	27.33*	29.89*	21.77	249.50
35	Qualyubiya	Cotton	Giza 85	43.50*	48.01*	20.47*	20.13	231.75
36	Daqahliya	Cotton	Giza 75	36.22	52.40*	33.21*	20.46	275.50
37	Sharqiya	Cotton	Giza 85	28.22	43.56*	13.28*	10.20*	138.50
38	Assiut	Cotton	Giza 83	22.50	40.61*	18.44*	21.75	212.50
39	Assiut	Cotton	Giza 83	19.92	39.23*	37.73*	24.35	279.00
40	Assiut	Cotton	Giza 83	9.22	42.75*	40.61*	22.70	290.25
41	Assiut	Cotton	Giza 83	33.90*	42.05*	47.89*	22.75	251.75
42	Assiut	Cotton	Giza 83	24.16	38.95*	17.30*	11.95*	122.75
43	Assiut	Cotton	Giza 83	29.36	24.53*	46.50*	23.46	180.50
44	Gharbiya	Cotton	Giza 75	24.16	42.59*	22.50*	18.00	197.75
45	Sohag	Cotton	Giza 83	39.11*	14.42*	40.39*	17.35	126.25
46	Assiut	Cotton	Giza 83	42.05*	25.67*	35.19*	22.19	254.75
47	Assiut	Cotton	Giza 83	4.61	22.50*	65.70	24.09	222.50
48	Assiut	Cotton	Giza 83	14.94	27.70*	54.53	23.40	233.25
49	Assiut	Cotton	Giza 83	23.25	52.56*	17.30*	11.81*	120.25
50	Assiut	Cotton	Giza 83	17.52	64.18*	11.25*	12.88*	100.75
51	Sohag	Cotton	Giza 83	15.86	33.91*	49.39*	24.63	333.00
52	Sohag	Cotton	Giza 83	20.47	25.67*	55.73*	22.05	330.75
53	Sohag	Cotton	Giza 83	24.53	51.05*	23.25	17.77	182.25
54	Sohag	Cotton	Giza 83	33.05	32.53*	33.05*	27.14	281.75
55	Sohag	Cotton	Giza 83	19.92	26.52*	52.93	24.51	226.25
56	Assiut	Cotton	Giza 83	27.86	39.80	31.50*	18.29	123.50
57	Assiut	Cotton	Giza 83	17.52	27.86*	54.53	23.50	214.25
58	Damietta	Cotton	Giza 75	19.55	21.22*	55.73	23.63	223.50
59	Assiut	Cotton	Giza 83	14.94	46.51*	36.00*	24.58	205.50
60	Sohag	Cotton	Giza 83	19.55	9.22	64.33	24.60	277.00
61	Assiut	Cotton	Giza 83	21.06	26.19*	52.56*	21.1	204.25
62	Minya	Cotton	Giza 80	25.67	36.00*	41.83*	23.15	200.75
63	Sohag	Cotton	Giza 83	24.53	35.41*	43.34*	24.00	178.75
64	Sohag	Cotton	Giza 83	37.44*	37.66*	29.36*	22.15	196.25
65	Assiut	Cotton	Giza 83	17.52	39.87*	42.64*	22.73	272.75
66	Sohag	Cotton	Giza 83	24.16	31.02*	51.05*	21.67	217.75
67	Assiut	Cotton	Giza 83	26.19	23.25*	50.90*	24.96	243.50
68	Assiut	Cotton	Giza 83	34.50*	31.02*	38.95*	24.27	221.00
69	Assiut	Cotton	Giza 83	9.22	27.33*	59.14*	23.45	220.00
70	Sohag	Cotton	Giza 83	22.50	26.19*	45.00*	22.68	239.25
71	Minufiya	Cotton	Giza 75	24.53	39.17*	40.61*	23.29	265.25
72	Sharqiya	Cotton	Giza 85	24.16	33.05*	46.51*	23.15	248.00
73	Sharqiya	Cotton	Giza 85	26.19	25.67*	54.00	22.88	223.75
74	Beheira	Cotton	Giza 75	17.52	47.89*	34.50*	21.99	263.75

Table 1 continued

Isolate no.	Geographic origin	Host	Cultivar	Pathogenicity variables				
				Pre-emergence damping-off[a] %	Post-emergence damping-off[a] %	Survival[a] %	Plant height cm/plant	Dry weight mg/plant
75	Netherlands	Flax	unknown	29.36	34.56*	39.17*	23.54	229.25
76	Giza	Flax	unknown	38.95*	34.61*	22.50*	10.12*	151.50
77	Beheira	Flax	unknown	25.67	19.54*	53.78	24.69	195.25
78	Sharqiya	Flax	unknown	17.89*	25.83*	55.51	22.63	256.75
79	Daqahliya	Flax	unknown	32.31	28.55*	38.20*	17.08	182.00
80	Netherlands	Flax	unknown	21.22	32.47*	46.51*	24.58	272.00
81	Giza	Sesame	unknown	25.67	30.22*	42.97*	18.75	130.25
82	Faiyum	Sesame	unknown	17.89	25.67*	41.48*	24.09	235.75
83	Giza	Sesame	unknown	24.53	23.09*	53.08	22.22	241.00
84	Faiyum	Sesame	unknown	24.53	29.89*	49.39*	24.92	221.00
85	Giza	Sesame	unknown	11.25	24.75*	57.47	23.79	217.25
86	Beheira	Sesame	unknown	33.05*	26.19*	41.91*	23.65	222.25
87	Giza	Sesame	unknown	27.70	22.50	52.50*	23.38	220.00
88	Giza	Sunflower	unknown	24.53	43.72*	32.25*	16.72	140.50
89	Giza	Sunflower	unknown	15.86	14.42	47.94*	24.83	206.75
90	Giza	Sunflower	unknown	19.55	35.94*	45.00*	23.90	208.50
91	Giza	Sunflower	unknown	24.16	37.44*	41.25*	24.47	231.50
92	Giza	Sunflower	unknown	22.50	34.50*	46.38*	21.92	256.25
93	Giza	Soybean	unknown	31.55	17.52*	44.94*	26.17	274.00
94	Giza	Soybean	unknown	24.16	29.36*	49.61*	23.65	281.00
95	Giza	Soybean	unknown	31.39	21.58*	50.83*	19.92	272.50
96	Giza	Soybean	unknown	27.33	34.28*	41.31*	23.25	220.50
Control				15.86	0.00	74.14	22.00	251.75
LSD(p≤ 0.05)				12.41	15.06	17.61	7.62	NS

[a]Precentage data were transferred into arcsine angles before carrying out analysis of variance to produce approximately constant variance. An asterisk denotes a significant difference from the control.

A substrate for growth of isolates was prepared in 500 ml glass bottles; each bottle contained 40 g of sorghum grains and 50 ml of tap water. Contents of each bottle were autoclaved for 30 min at 125°C. Isolate inoculum, taken from one-week-old cultures on potato dextrose agar (PDA) medium, was aseptically introduced into the bottle and allowed to colonize the substrate for 3 weeks at 25°C. Inoculum was air-dried and stored in plastic bags at 10°C. Inoculum level was 50 g of fungus/sorghum mixture/kg of soil.

Pathogenicity test of *M. phaseolina* isolates

Pathogenicity assay was carried out using autoclaved clay-based loam soil. Batches of soil were separately infested with inoculum of each isolate at a rate of 50 g/kg of soil. In the control treatment, autoclaved soil was infested with autoclaved sorghum grains at rate of 50 g/ kg of soil. Pots were planted with 10 seeds per pot for each of cv. Giza 89. Pots were randomly distributed on greenhouse benches under a temperature regime ranging from 19.5 ± 1.5 to 34 ± 4°C. There were three pots (replicates) for each isolate. Pre-emergence damping-off was recorded 15 days after planting, post-emergence damping-off, survival, plant height (cm), and dry weight (mg/plant) were recorded 45 days after planting. The trial was replicated twice with almost the same results.

Statistical analysis of the data

Randomized complete block experimental design with three replicates was subjected to analysis of variance (ANOVA) and correlation using MSTAT-C Statistical Package. The least significant difference (LSD) was used to compare isolate means. Percentage data were transformed into arc sine angles before carrying out the ANOVA to normalize data and stabilize variances

throughout the data range. Cluster analysis of *M. phaseolina* isolates was performed using software package SPSS 13.

DNA isolation

Hyphal tip cultures were transferred to PDA plates and incubated for 4 days and then transferred to a flask with 25 ml potato dextrose broth and grown on a shaker at 75 rpm for 5 days. The mycelia were harvested by filtration through a double layer of cheese cloth and freeze dried under vacuum. Frozen mycelium was ground in liquid nitrogen to a fine powder. Total genomic DNA was extracted as described by Abd-Elsalam et al. (2011).

AFLP analysis

AFLP analysis was accomplished as previously described by Abd-Elsalam et al. (2004). In short, DNA (50 ng) was digested with EcoRI and MseI and the resulting fragments were ligated to double-stranded adapters (EcoRI-adapter: 5' CTC GTA GAC TGC GTA CC 3' plus 3' CTG ACG CAT GGT TAA 5' and MseI-adapter: 5' GAC GAT GAG TCC TGA G 3' plus 3' TAC TCA GGA CTC AT 5', respectively). The digested and ligated DNA was then amplified by PCR (30 cycles) by using the EcoRI and MseI primers in a final volume of 50 µl (EcoRI primer: 5' GTA GAC TGC GTA CCA ATT C 3', MseI primer: 5' GAC GAT GAG TCC TGA GTA A 3'). The PCR cycling conditions included the cycling profile of 94°C for 30 s, 60°C for 30 s and 72°C for 60 s repeated for 30 cycles. Three microliter were used in subsequent selective amplifications with the primer combination of EcoRI-C (5' GTA GAC TGC GTA CCA ATT CC 3') and MseI-G (5' GAC GAT GAG TCC TGA GTA AG 3'). Selective amplifications were performed in 20 µl reaction mixtures using a cycling profile of 94°C for 30 s, 65°C for 30 s and 72°C for 1 min for one cycle and then lowering the annealing temperature by 1°C each cycle to 56°C (nine cycles), followed by additional 26 cycles at a 56°C annealing temperature.

Equal volumes of amplified product (3 µl) and loading buffer (95% formamide, 20 mmol/l EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol) were mixed, then heated at 95°C for 3 min and snap-cooled on ice. The amplified fragments were separated in a denaturing 6% polyacrylamide sequencing gel with 8 mol/l ultrapure urea (Roth, Germany). Electrophoresis was performed with a nucleic acid sequencing unit (Model Base Runner 100, Sigma-Aldrich, Germany) at 55 W with 1× Tris-Borate-EDTA (1× solution contains 89 mmol/l Tris, 89 mmol/l boric acid and 2.0 mmol/l EDTA) as a running buffer. After electrophoresis, AFLP fingerprints were detected by silver staining protocol described by Creste et al. (2001). DNA patterns obtained by AFLP were clustered by a gel documentation system (Uvitec, Cambride, UK).

Results

Pathogenicity test

Ninety-six isolates of *M. phaseolina* from different hosts were tested for pathogenicity on seedlings of cotton cultivar Giza 89 under greenhouse conditions (Table 1). In the present test, the isolate was considered pathogenic when it significantly increased percentage of pre-emergence or post-emergence damping-off compared with the control or when it significantly decreases survival, plant height, or dry weight. Of the cotton isolates, only isolate no. 60 was nonpathogenic while 73 isolates were pathogenic representing 98.65% of the cotton isolates, 76.04% of the total isolates, and 76.84% of the pathogenic isolates from all hosts. The pathogenic isolates of the other hosts ranged from four to seven. The distribution of pathogenic *M. phaseolina* isolates based on the five hosts is shown in Table 2. Correlations among variables used for evaluating pathogenicity of *M. phaseolina* isolates are shown in Table 3. The highest correlation was observed between post-emergence damping-off and survival.

Table 2 Distribution of pathogenic isolates of *Macrophomina phaseolina* based on the host used in isolation.

Host	No. of tested isolates	No. of pathogenic isolates	Percentage of isolates from host	Percentage of total isolates[a]	Percentage of pathogenic isolates
Cotton	74	73	98.65	76.04	76.84
Sesame	7	7	100	7.29	7.37
Sunflower	5	5	100	5.21	5.26
Soybean	4	4	100	4.17	4.21
Flax	6	6	100	6.25	6.31

[a]A total of 96 isolates from different hosts were tested for pathogenicity on seedlings of cotton cultivar Giza 89 under greenhouse conditions.

Table 3 Correlation[a] among variables used for evaluating pathogenicity of 96 *Macrophomina phaseolina* isolates on seedlings of cotton cultivar Giza 89 under greenhouse conditions.

	Variables			
Variables	1	2	3	4
1. Pre-emergence damping-off (%)				
2. Post-emergence damping-off (%)	-0.08			
3. Survival (%)	-0.54**	-0.69**		
4. Plant height (cm)	-0.10	-0.46**	0.52**	
5. Dry weight (mg/plant)	0.09	-0.30**	0.27**	0.60**

Liner correlation coefficient (r) is significant at $p \leq 0.01$ (**).

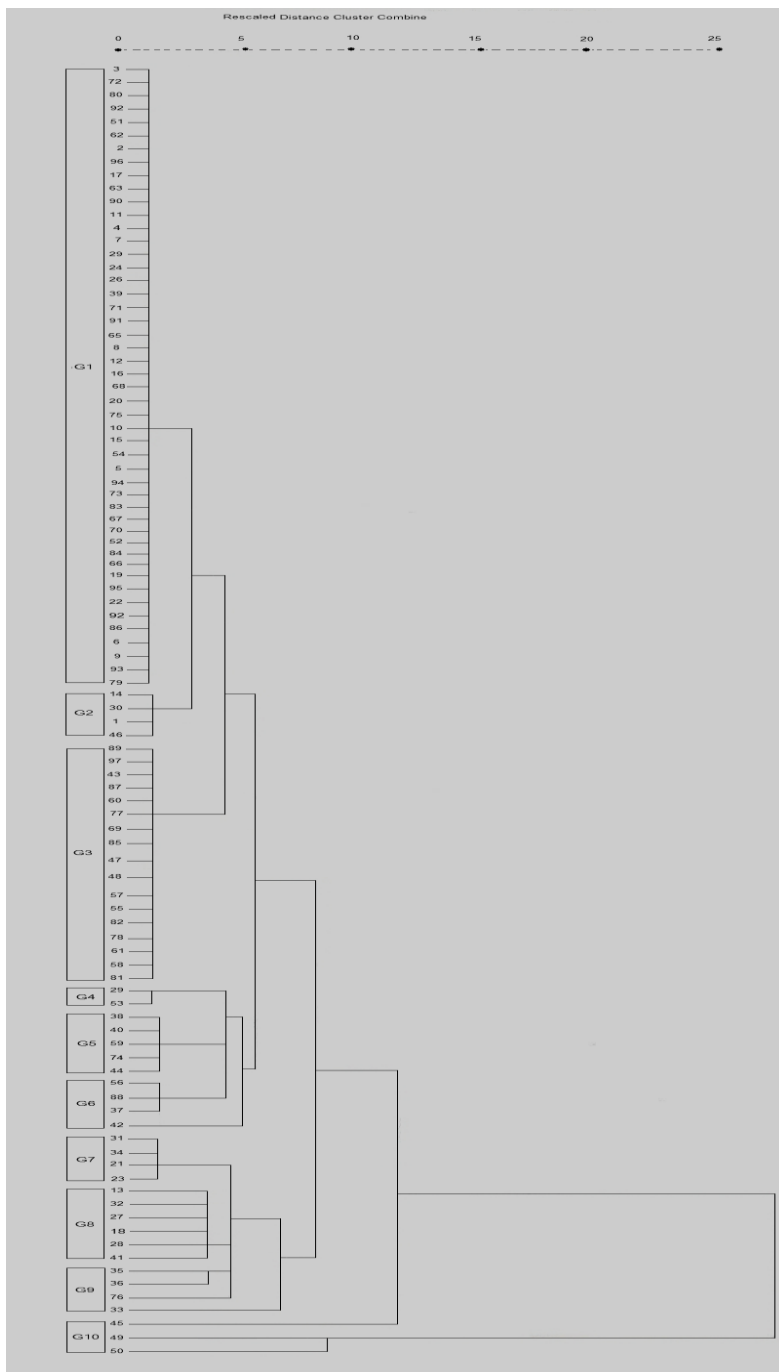## Cluster analysis of *M. phaseolina* isolates based on pathogenicity test

A phenogram based dissimilarily distance (DD) generated from cluster analysis of virulence patterns of *M. phasolina* isolates is shown in (Fig. 1).

The smaller the DD, the more closely the isolates were related in their virulence patterns. Ten groups (Gs) of similar isolates (from G1 through G10) were identified by cluster analysis. Isolates 42, 76, 33 and 45 showed unique virulent patterns quite different from those of the other isolates. Grouping the isolates by cluster analysis was neither related to their geographic origins nor to hosts.
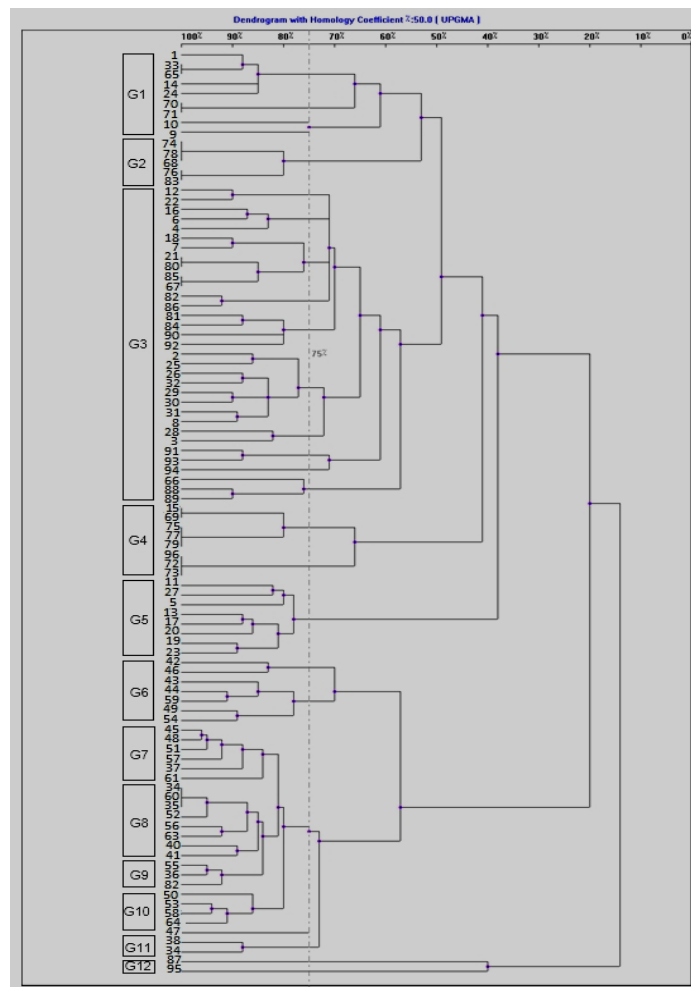
Cluster analysis of *M. phaseolina* isolates based on AFLP banding patterns

AFLP analysis was used to evaluate the genetic diversity of the isolates. In this analysis, polymerase chain reaction was performed by using four AFLP primers. Amplification of genomic DNA of all isolates produced large number of distinct amplicons, the size of amplicon was detected by comparing with a DNA ladder ranged approximately from 200 bp to 3500 bp. Very rarely, some amplicons were larger than 3500 bp. All the primers detected polymorphisms in all the tested isolates. A phenogram based on similarity level (SL) generated from cluster analysis of AFLP banding patterns of *M. phasolina* isolates is presented (Fig. 2).

The greater the SL, the more closely the isolates were related in their AFLP banding patterns. Twelve groups (from G1 through G12) of similar isolates were identified by cluster analysis. The overall SL among isolates was 20% while the intraspecific SLs ranged from 40 to 100%. The banding pattern of isolate 87 and 95 were quite different from those of the other isolates. Grouping the isolates by cluster analysis was neither related to their geographic origins nor to hosts.

Fig 1 – A phenogram based on average linkage cluster analysis of virulence of 96 isolates of *Macrophomina phaseolina* on seedlings of cotton cultivar Giza 89. Virulence of isolates was evaluated based on pre-emergence damping-off, post-emergence damping-off, survival, plant height, and dry weight. The isolates were divided into 10 groups (from G1 through G10).

Fig 2 – A phenogram based on cluster analysis of AFLP banding patterns of 96 isolates of *Macrophomina phaseolina* obtained by four primers and electrophoresed on polyacrylamide gel. The isolates were divided into 12 groups (from G1 through G12).

Discussion

Although *M. phaseolina* is considered plurivorous, some reports suggested the presence of a general trend to host specialization among *M. phaseolina* isolates (Saleh et al. 2010, Rayatpanah et al. 2012). For example, Pearson et al. (1986, 1987) used chlorate sensitivity to partition *M. phaseolina* into three physiologically distinct phenotypic groups. These groups were differentially capable of attacking different host plants. Diourte (1987) inoculated sorghum, groundnut, bean and cotton with isolates from each of these hosts. There was a general trend to host preference for the same host isolate. The results of pathogenicity test in the present study did not substantiate these reports because all the isolates, which were recovered from the other hosts (22 isolates) were pathogenic on seedlings of cotton cultivars Giza 89. Giza 75 and Giza 83 yielded 71.24% of the pathogenic isolates from cotton as they were the two most predominant cultivars in Egypt when the present study was carried out (A.A. Aly, *personal observation*). Giza 75 was the predominant cultivar in lower Egypt, while Giza 83 was the predominant cultivar in upper Egypt.

Significant correlations were observed between post-emergence damping-off and the variables used in assessing vigour of seedlings (plant height and dry weight). These correlations imply that the higher the disease pressure during the post-emergence stage, the less vigorous would be the surviving seedlings. That is, even the seedlings, which survived post-emergence damping-off suffered from a subtle weakness, which reduced both plant height and dry weight. Evidently, such seedlings would develop into unthrifty and less productive plants (Watkins 1981, Minton & Garber

1983). The significant positive correlation between survival and each of plant height and dry weight implies that the lower the disease pressure, the more vigorous would be the seedlings

Conflicting results have reported regarding the relationship between molecular grouping of *M. phaseolina* isolates and their host or their geographic origin. For example, Mayék-Pérez et al. (2001), Su et al. (2001) and Jane et al. (2003) found some tendencies of *M. phaseolina* isolates to form groups related to geographic origin. On the contrary, other workers reported molecular grouping of *M. phaseolina* isolates irrespective of host (Babu et al. 2010) and geographic origin (Csondes et al. 2011). In the present study, grouping the isolates by AFLP analysis was neither related to geographic origin nor to host. This result may suggest that *M. phaseolina* isolates from each host or geographic origin were heterogeneous group of isolates.

The present study included only 96 isolates of *M. phaseolina*. It is unlikely that this limited number of isolates represents the full range of variation within this fungus. Despite this limitation, the overall SL among the isolates was as low as 20%, that is, a high level of genetic variation was observed among the isolates. This was surprising because a low level of genetic variation is usually observed in populations of fungi that do not reproduce sexually as occurs with *M. phaseolina*. However, in retrospect, we speculate that parasexualism with fusion of cells from different hyphae may form hetrokaryons that contribute to the variation identified (Rydholm et al. 2006). Genetic variability in the pathogen population may also reflect the lack of resistance among the currently cultivated commercial cotton cultivars in Egypt (Almeida et al. 2003). The high SLs observed among isolates from different governorates is strong evidence suggesting that such isolates did not evolve independently from each other; therefore, they may be considered as part of the same ancestral population (Almeida et al. 2003). Presumably, the spread of this population from one location to another was by physical means such as seeds, contaminated equipment, soil infested with microsclerotia. The isolates of *M. phaseolina* used in the present study were recovered from five hosts belonging to five genera. Therefore, homogeneity of the AFLP data among isolates from different hosts suggests that selective pressure for host specificity in *M. phaseolina* in the sampled population had been minimal (Tuskan et al. 1990).

Acknowledgements

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