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Differential Interaction between Isolates of *Macrophomina phaseolina* and Egyptian Cotton Cultivars

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ABSTRACT

Pathogeniciy of 20 isolates of *Macrophomina phaseolina* was tested on six cotton cultivars under greenhouse conditions. Preemergence damping-off, postemergence damping-off, survival, plant height, and dry weight were used as criteria to evaluate pathogenicity. Analysis of variance showed that the main effects of both cultivars (p=0.0001) and isolates (p=0.0001) were very highly significant sources of variation in all the tested parameters as was cultivar × isolate interaction (p=0.0001). Statistically significant cultivars, isolates, these significant main effects and interactions suggest that physiologic specialization exists within *M. phaseolina* isolates pathogenic on cotton. It also implies that the resistance of the tested cultivars is a mixture of both vertical and horizontal resistance and there are significant differences among cultivars in both types of resistance. Similarly, pathogenicity of the tested isolates is also mixture of virulence and aggressiveness, and the isolates significantly differ in both types of pathogenicity. Cluster analysis differentiated the isolates into 4 pathotypes based on their virulence on the 6 cotton cultivars.

Keywords: cotton, cultivars, *Macrophomina*, pathogenicity
Abbreviations: CRR, charcoal root rot; **LSD**, least significant difference; **PDA**, potato dextrose agar

INTRODUCTION

Charcoal root rot (CRR), caused by *Macrophomina phaseolina*, can cause charcoal rot (ashy stem) on cotton, and is a soil- and seed-borne pathogen with a wide distribution and a wide host range (Dhingra and Sinclair 1978). When *M. phaseolina* invades roots or stems of cotton, colonization of internal tissues proceeds rapidly and plants die due to destruction of vascular tissues. Examination of parasitized tissue reveals disintegration of parenchyma with many small, black sclerotia attached to the vascular bundles (Watkins 1981). A negative correlation (r=-0.85, p<0.01) was found between disease incidence and yield (Turini *et al*. 2000).

M. phaseolina is widely distributed in Egyptian soils, and it is easily and frequently isolated from cotton roots late in the growing season, which extends from March to October. Thus, when Aly *et al*. (1996) collected and assayed 88 samples of infected cotton roots from 12 Egyptian governorates; *M. phaseolina* was isolated from 37.5% of samples examined.

Although initial infection of cotton by *M. phaseolina* occurs at the seedling stage, infections usually remain latent until plants approach maturity (Dhingra and Sinclair 1978). However, *M. phaseolina* appears to affect some cotton cultivars less severely than others, which suggests the existence of potential genetic resistance to *M. phaseolina.* Watkins (1981) mentioned that the Greek cotton variety Dadiotico suffered losses from charcoal rot (5-40% dead plants), while the American varieties were not affected. The difference was attributed to an exceptionally thin bark (phellem) developed on the roots of Dadiotico, which cracked rapidly and permitted easy entry by *M. phaseolina*. Lee *et al*. (1986) reported variation in resistance levels in cotton infected with a range of *M. phaseolina* isolates. However, it was not clear whether this variation represented usable resistance in a breeding program. Monga and Raj (1996) screened 18 cotton varieties commonly grown in northern India against root rot (*M. phaseolina*) in infected fields. The results revealed that desi cotton (*Gossypium arboreum*) varieties were more susceptible (43.9-63.1% disease incidence) than American cotton (*G. hirsutum*) varieties (19.4-47.0% disease incidence). Among desi cotton varieties, the minimum disease incidence was noted in G-1 (43.9%) followed by LD 327 (52%), and RG-8 (69.2%). The maximum seed cotton yield was also recorded in variety G-1 followed by G-27, and DS-5. Monga and Raj (2000) screened forty lines of cotton (*G. hirsutum*) with a control (H-777) against root rot (*M. phaseolina*) in a root rot sick field at Haryana, India. The lines B-1371, A-72-62, Arkansas green and FS-128 had a relatively lower root rot incidence compared with the control. Furthermore, these lines had higher seed cotton yield and span length compared with the control. The ginning outturn percentage and micronaire values of FS-128, Arkansas green and B-1371 were higher than those of the control. These lines can be used in root rot endemic areas due to their tolerance to the disease and also as donors in hybridization programmes for the development of root rot resistant cultivars. Turini *et al.* (2001) evaluated 10 cotton (*G. hirsutum*) cultivars or breeding-lines for relative susceptibility to charcoal rot.

In Egypt, resistance to *M. phaseolina* is completely lacking in commercial cotton (*Gossypium barbadense* L.) (Aly *et al*. 2006); therefore, more research is needed to identify charcoal rot resistant genotypes. A clear understanding of the extent of variation in virulence among *M. phaseolina* isolates would be helpful in devolving cotton cultivars with effective resistance. Therefore, this investigation was undertaken to evaluate the pathogenic variability among isolates of *M. phaseolina* originating from different regions in Egypt and their interactions with cotton cultivars.

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MATERIALS AND METHODS

Fungal isolates and inoculum production

Isolates of *M. phaseolina* used in the present study were obtained from the fungal collection of the Cotton Disease Research Section, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. Nineteen isolates originated from cotton roots and one originated from sesame. A substrate for growth of isolates was prepared in 500-ml glass bottles; each bottle contained 50 g of sorghum grains and 40 ml of tap water. Contents of each bottle were autoclaved for 30 min. Isolate inoculum, taken from one-week-old culture on Potato Dextrose Agar PDA, was aseptically introduced into the bottle and allowed to colonize the substrate for three weeks.

Interaction between cotton cultivars and isolates of *M. phaseolina*

Twenty isolates of *M. phaseolina*, obtained from different locations (**Table 1**) were used in this study. Batches of autoclaved clay loam soil were separately infested with inoculum of each isolate at a rate of 50/kg of soil. The infestation process was carried out by thoroughly mixing the inoculum with soil,so the inoculum evenly spread in soil. Infested soil was dispensed in 10-cm-diameter clay pots and these were planted with 10 seeds per pot for each of the tested cultivars ('Giza77', 'Giza80', 'Giza 83', 'Giza 85', 'Giza 86', and 'Giza 89'). In the control treatments, autoclaved sorghum (cv. 'Balady') grains were thoroughly mixed with soil at a rate of 50/kg of soil. Pots were distributed on greenhouse benches under a temperature regime that ranged from 30.5 ± 3.5 to 40 ± 4°C. There were five pots (replicates) for each treatment. Preemergence damping-off was recorded 15 days after planting. Postemergence damping-off, numbers of surviving plants, plant height (cm), and dry weight (mg/plant) were recorded 45 days after planting. This experiment was replicated three times.

Statistical analysis of the data

The experimental design of the present study was a randomized complete block with five replicates. Analysis of variance (ANOVA) of the data and correlations were performed with the MSTAT-C Statistical Package. Least significant difference (LSD) was used to compare between isolate means within cultivars. Percentage data were transformed into arcsine angles before carrying out the ANOVA to produce approximately constant variance. Cluster analysis of *M. phaseolina* isolates was performed with the software package SPSS 6.0.

Table 1 Geographic origins and sources of *M. phaseolina* isolates used in studying the interaction between the isolates and cultivars of cotton.

Isolate №	Geographic origin	Source
1	Manzala, Daqagliya	Cotton
2	Sakha, Kafr El-Sheikh	Cotton
3	Minouf, Minufiya	Cotton
4	Santa, Gharbiya	Cotton
5	Damanhoor, Beheira	Cotton
6	El-Riyad, Kafr El-Sheikh	Cotton
7	Giza, Giza	Cotton
8	Faiyoum	Sesame
9	Sohag, Sohag	Cotton
10	Manfaloot, Assiute	Cotton
11	Manfaloot, Assiute	Cotton
12	Abou-Korkas, Minya	Cotton
13	El-Minya, Minya	Cotton
14	Sohag, Sohag	Cotton
15	Sohag, Sohag	Cotton
16	Shandaweel, Sohag	Cotton
17	Meet Ghamr, Daqahilya	Cotton
18	Abou-Kibeer, Sharqiya	Cotton
19	Sirs El-Lian, Minufia	Cotton
20	Damanhoor, Beheira	Cotton

RESULTS

ANOVA (**Table 2**) showed very highly significant (p=0.0001) effects of cultivar, isolate, and cultivar × isolate interactions for the tested parameters. Cultivar × isolate interactions were the most important factor in determining the variation in preemergence damping-off, survival, plant height, and dry weight (**Table 3**). Isolate and cultivar × isolate interaction were almost equally important in determining variation in postemergence damping-off. Cultivar was almost as important as isolate in determining variation in plant height.

Due to significant cultivar × isolate interactions for preemergence damping-off, a least significant difference (LSD) was calculated to compare isolate means within each cultivar (**Table 4**). These comparisons showed that the differences in preemergence damping-off between isolates and the control were not the same for each cultivar-that is, cultivars responded differently to the isolates. Thus, 'Giza 77', 'Giza 80', 'Giza 83', 'Giza 85', 'Giza 86', and 'Giza 89' were susceptible to 15, 16, 7, 15, 6, and 4 isolates, respectively. It was also found that the magnitude of differences between isolates differed from one cultivar to another. For example, the difference between isolates 1 and 2 was highly significant on 'Giza 77', while it was non-significant on 'Giza 83'. None of the isolates was pathogenic on all the cultivars. With the exception of the resistance of 'Giza 77' to isolate 10 in the postemergence stage (**Table 5**) and the resistance of 'Giza 89' to isolates 6, 10, and 15 in terms of surviving seedlings (**Table 6**), all cultivars were susceptible to all isolates and thus were considered universally susceptible (**Tables 5, 6**). For any cultivar, the number of isolates, which significantly reduced plant height (**Table 7**), was much greater than that of the isolates, which significantly reduced dry weight (**Table 8**).

Correlations among variables used for evaluating pathogenicity of *M. phaseolina* isolate are shown in **Table 9**. In all cultivars, a non-significant correlation was observed bet-

Table 2 Analysis of variance of the interaction between cotton cultivars and isolates of *M. phaseolina* under greenhouse conditions.

Parameter and source of variation [a]	D.F.	M.S.	F. value	$P > F$
Preemergence damping-off				
Replication	4	143.132	2.996	0.0184
Cultivar (C)	5	957.476	20.040	0.0000
Isolate (I)	20	645.819	13.517	0.0000
C × I	100	358.146	7.496	0.0000
Error	500	47.777		
Postemergence damping-off				
Replication	4	58.182	0.811	
Cultivar (C)	5	4305.551	59.992	0.0000
Isolate (I)	20	1514.438	21.102	0.0000
C × I	100	295.677	4.120	0.0000
Error	500	71.769		
Survival				
Replication	4	31.754	0.697	
Cultivar (C)	5	5893.428	129.318	0.0000
Isolate (I)	20	1702.789	37.364	0.0000
C × I	100	394.063	8.647	0.0000
Error	500	45.573		
Plant height				
Replication	4	39.833	2.070	0.0836
Cultivar (C)	5	367.699	19.104	0.0000
Isolate (I)	20	97.753	5.079	0.0000
C × I	100	56.441	2.932	0.0000
Error	500	19.248		
Dry weight				
Replication	4	6770.542	1.583	0.1774
Cultivar (C)	5	176732.09	41.330	0.0000
Isolate (I)	20	75552.050	17.668	0.0000
C × I	100	30463.156	7.124	0.0000
Error	500	4276.149		

[a] Replication is random, while each value of cultivar and isolate is fixed.

Table 3 Relative contribution of cotton cultivars, *M. phaseolina* isolates, and their interaction to variation in preemergence damping-off, postemergence damping-off, survival, plant height, and dry weight of cotton seedlings.

Source of variation	Relative contribution [a] to variation in				
	Preemergence damping-off	Postemergence damping-off	Survival	Plant height	Dry weight
Cultivar (C)	8.85	26.38	28.59	19.16	16.16
Isolate (I)	23.88	37.11	33.05	20.37	27.63
C x I	66.21	36.23	38.24	58.81	55.71

[a] Calculated as percentage of sum squares of the explained (model) variation.

Table 4 Effect of the interaction between cotton cultivars and *M. phaseolina* isolates on preemergence damping-off of cotton seedlings under greenhouse conditions.

Isolate	Cultivars													
	Giza 77		Giza 80		Giza 83		Giza 85		Giza 86		Giza 89		Mean	
	%	T	%	T	%	T	%	T	%	T	%	T	%	T
1	26.00	30.13	10.00	16.38	42.00	40.33	4.00	7.38	28.00	31.88	28.00	31.88	23.00	26.33
2	46.00	42.69	16.00	23.31	40.00	39.18	26.00	30.55	24.00	29.22	40.00	39.18	32.00	34.02
3	14.00	21.69	20.00	26.27	54.00	47.31	18.00	24.94	16.00	23.31	24.00	29.22	24.33	28.79
4	52.00	46.15	36.00	36.82	20.00	26.27	24.00	27.44	32.00	34.41	44.00	41.54	34.67	35.44
5	24.00	29.22	16.00	23.31	36.00	36.82	6.00	6.64	46.00	42.69	26.00	30.55	25.67	28.21
6	16.00	23.02	36.00	36.82	50.00	45.00	34.00	35.62	16.00	23.31	14.00	21.69	27.67	30.91
7	26.00	30.00	36.00	36.82	24.00	28.80	34.00	35.62	26.00	30.55	28.00	31.75	29.00	32.26
8	6.00	11.06	30.00	33.21	36.00	36.77	24.00	29.22	46.00	42.69	16.00	23.31	26.33	29.38
9	22.00	27.18	18.00	24.64	18.00	24.94	10.00	16.38	24.00	29.22	46.00	42.69	23.00	27.51
10	32.00	34.29	16.00	20.95	4.00	5.31	18.00	24.64	20.00	26.27	24.00	28.51	19.00	23.33
11	22.00	27.60	34.00	35.62	14.00	21.69	16.00	23.31	36.00	36.82	46.00	42.69	28.00	31.28
12	14.00	19.33	22.00	27.60	18.00	24.94	6.00	11.06	20.00	26.56	16.00	23.31	16.00	22.13
13	40.00	39.00	32.00	33.87	18.00	24.64	4.00	7.38	18.00	24.64	14.00	21.69	21.00	25.20
14	18.00	24.64	16.00	23.02	18.00	24.64	12.00	18.00	16.00	21.25	24.00	29.22	17.33	23.46
15	10.00	14.31	6.00	11.06	40.00	39.18	16.00	23.31	14.00	21.69	24.00	28.93	18.33	23.08
16	32.00	34.29	8.00	10.62	12.00	15.22	22.00	28.93	16.00	23.31	22.00	27.89	18.67	23.38
17	14.00	19.33	36.00	36.82	30.00	33.08	12.00	20.06	34.00	35.49	30.00	33.21	26.00	29.67
18	8.00	12.69	28.00	31.75	22.00	27.60	44.00	41.54	36.00	36.82	30.00	32.96	28.00	30.56
19	36.00	36.82	38.00	37.98	28.00	31.88	36.00	36.82	18.00	24.64	24.00	29.22	30.00	32.89
20	18.00	24.35	14.00	19.33	14.00	21.69	8.00	12.69	22.00	27.60	18.00	24.64	15.67	21.72
Control [b]	8.00	12.69	6.00	11.06	18.00	24.94	4.00	7.38	16.00	23.31	24.00	29.22	12.67	18.10
Mean	23.05	26.69	22.57	26.54	26.48	29.53	18.00	22.33	24.95	29.32	26.76	30.63	22.32	27.51

T = transformed value; LSD (transformed data for cultivar × isolates interaction = 8.59 (p<0.05 or 11.30 (p<0.01).
[a] Percentage data were transformed into arc sine angles before carrying out the analysis of variance.
[b] Non-infested soil.

Table 5 Effect of the interaction between cotton cultivars and *M. phaseolina* isolates on postemergence damping-off of cotton seedlings under greenhouse conditions.

Isolate	Cultivars													
	Giza 77		Giza 80		Giza 83		Giza 85		Giza 86		Giza 89		Mean	
	%	T	%	T	%	T	%	T	%	T	%	T	%	T
1	18.00	22.28	24.00	28.93	56.00	48.51	30.00	33.08	14.00	19.62	12.00	20.06	25.67	28.75
2	32.00	33.87	26.00	30.55	56.00	48.46	18.00	24.64	20.00	25.97	8.00	14.75	26.67	29.71
3	36.00	36.65	38.00	38.03	40.00	39.13	38.00	37.85	26.00	30.55	16.00	23.02	32.33	34.20
4	30.00	33.21	46.00	42.64	70.00	57.09	32.00	34.16	12.00	18.00	6.00	11.06	32.67	32.70
5	38.00	38.03	30.00	32.91	46.00	42.64	28.00	31.46	14.00	16.97	18.00	24.94	29.00	31.16
6	28.00	31.75	20.00	26.56	30.00	32.61	8.00	12.69	14.00	19.62	18.00	24.35	19.67	24.60
7	16.00	23.02	30.00	32.66	60.00	50.87	32.00	33.82	34.00	35.49	18.00	22.58	31.67	33.07
8	30.00	32.96	18.00	24.64	32.00	33.87	18.00	22.28	20.00	25.55	26.00	30.42	24.00	28.29
9	20.00	17.91	24.00	28.80	56.00	48.46	18.00	24.22	28.00	31.88	44.00	41.31	31.67	32.10
10	6.00	9.07	20.00	25.55	66.00	55.84	22.00	25.11	30.00	32.96	8.00	14.75	25.33	27.21
11	28.00	25.75	16.00	23.31	64.00	54.51	28.00	31.75	28.00	31.46	8.00	14.75	28.67	30.26
12	16.00	19.31	18.00	22.16	54.00	46.15	26.00	30.13	34.00	35.62	40.00	39.13	31.33	32.11
13	26.00	24.01	20.00	23.49	48.00	43.81	42.00	40.28	26.00	30.55	26.00	30.55	31.33	32.11
14	30.00	26.61	18.00	22.16	54.00	47.31	16.00	23.31	20.00	23.91	14.00	19.33	25.33	27.11
15	26.00	26.55	36.00	36.82	36.00	36.82	14.00	19.33	26.00	30.42	8.00	14.75	24.33	27.45
16	38.00	29.98	30.00	32.96	62.00	52.07	14.00	19.62	22.00	27.60	44.00	41.26	35.00	33.92
17	30.00	32.44	24.00	26.44	44.00	41.44	28.00	28.97	30.00	33.08	14.00	21.70	28.33	30.68
18	36.00	36.77	38.00	37.93	42.00	40.28	28.00	31.75	24.00	28.80	16.00	23.02	30.67	33.09
19	18.00	21.86	34.00	35.02	18.00	24.64	28.00	31.33	18.00	24.94	20.00	26.56	22.67	27.39
20	42.00	40.16	28.00	31.75	18.00	24.64	18.00	24.94	26.00	30.42	22.00	27.60	25.67	29.92
Control [b]	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mean	27.91	26.77	25.62	28.73	45.33	41.39	23.14	26.70	22.19	26.35	18.38	23.14	26.76	30.27

T = transformed value; LSD (transformed data) for cultivar × isolates interaction = 10.53 (p<0.05) or 13.85 (p<0.01).
[a] Percentage data were transformed into arc sine angles before carrying out the analysis of variance.
[b] Non-infested soil.

ween preemergence damping-off and postemergence damping-off. A significant or a highly significant negative correlation was found between survival and each of preemergence damping-off and postemergence damping-off of 'Giza 77', 'Giza 85', 'Giza 83', 'Giza 80', and 'Giza 89'. On 'Giza 86', a highly significant negative correlation was

Table 6 Effect of the interaction between cotton cultivars and *M. phaseolina* isolates on survival of cotton seedlings under greenhouse conditions.

Isolate	Cultivars													
	Giza 77		Giza 80		Giza 83		Giza 85		Giza 86		Giza 89		Mean	
	%	T	%	T	%	T	%	T	%	T	%	T	%	T
1	56.00	48.56	66.00	54.93	2.00	3.69	66.00	54.51	58.00	49.67	60.00	50.82	51.33	43.70
2	22.00	27.00	58.00	49.62	4.00	7.38	56.00	48.46	56.00	48.46	52.00	46.15	41.33	37.85
3	50.00	45.05	42.00	40.38	6.00	9.00	44.00	41.54	58.00	49.62	60.00	50.82	43.33	39.40
4	18.00	24.64	18.00	19.80	10.00	14.02	54.00	47.30	56.00	48.46	50.00	45.00	34.33	33.21
5	38.00	37.98	54.00	47.31	18.00	24.64	66.00	57.34	40.00	39.13	56.00	48.46	45.33	42.48
6	56.00	48.56	44.00	41.54	20.00	23.49	58.00	40.67	70.00	56.92	68.00	55.71	52.67	45.98
7	58.00	49.72	34.00	35.44	16.00	23.31	34.00	35.44	40.00	39.13	54.00	47.31	39.33	38.39
8	64.00	53.40	52.00	46.15	32.00	34.41	58.00	49.67	34.00	35.49	58.00	49.65	49.67	44.79
9	58.00	49.62	58.00	49.67	26.00	30.55	72.00	58.72	48.00	43.85	10.00	14.02	45.33	41.07
10	62.00	52.15	64.00	53.23	28.00	31.63	60.00	50.99	50.00	45.00	68.00	55.89	55.33	48.15
11	50.00	45.05	50.00	45.00	22.00	27.89	56.00	48.56	36.00	36.77	46.00	42.69	43.33	40.99
12	70.00	56.02	60.00	50.82	28.00	31.75	68.00	56.18	46.00	42.69	44.00	41.54	52.67	46.65
13	34.00	35.26	48.00	43.80	34.00	35.62	54.00	47.41	56.00	48.46	60.00	50.77	47.67	43.55
14	52.00	46.15	66.00	54.98	28.00	31.88	72.00	58.54	64.00	53.17	62.00	52.02	57.33	49.46
15	64.00	53.18	58.00	49.67	24.00	29.22	70.00	57.04	60.00	42.87	68.00	55.71	57.33	47.95
16	30.00	33.08	62.00	51.97	26.00	30.55	64.00	53.18	62.00	51.97	34.00	35.27	46.33	42.67
17	56.00	48.46	40.00	38.95	26.00	30.42	60.00	50.99	36.00	36.82	56.00	48.46	45.67	42.35
18	56.00	48.46	34.00	35.44	36.00	36.82	28.00	31.75	40.00	39.18	54.00	47.36	41.33	39.84
19	46.00	42.59	28.00	31.46	54.00	47.31	36.00	36.65	64.00	53.18	56.00	48.46	47.33	43.27
20	40.00	39.13	58.00	49.67	68.00	55.59	74.00	59.45	52.00	46.15	60.00	50.77	58.67	50.13
Control [b]	92.00	77.31	94.00	78.94	82.00	65.06	96.00	82.62	84.00	66.69	76.00	60.78	87.33	71.90
Mean	51.05	45.82	51.81	46.13	28.10	29.73	59.33	51.24	52.82	46.37	54.86	47.51	49.67	44.67

T = transformed value; LSD (transformed data) for cultivar × isolates interaction = 8.39 ($p<0.05$) or 11.04 ($p<0.01$).
[a] Percentage data were transformed into arc sine angles before carrying out the analysis of variance.
[b] Non-infested soil.

Table 7 Effect of interaction between cotton cultivars and *M. phaseolina* isolates on plant height and dry weight of cotton seedlings under greenhouse conditions.

Isolate	Plant height (cm)						
	Giza77	Giza 80	Giza 83	Giza 85	Giza86	Giza 89	Mean
1	26.06	27.63	8.10	28.02	33.83	24.91	25.82
2	26.50	27.92	16.00	27.63	30.16	26.35	29.87
3	26.77	22.92	15.05	30.85	26.05	25.33	30.35
4	27.17	16.37	23.57	33.85	24.67	25.46	27.11
5	29.14	28.33	29.98	28.86	23.17	28.43	31.12
6	27.81	24.14	19.72	30.40	24.07	25.26	25.41
7	26.68	27.86	26.09	29.24	29.52	28.04	28.88
8	26.98	26.20	27.23	31.30	32.32	28.57	27.40
9	28.43	29.74	31.49	31.21	28.36	27.92	18.26
10	28.80	28.20	30.72	30.22	30.36	29.91	31.18
11	27.63	30.72	25.69	28.44	33.09	29.70	32.63
12	28.14	27.01	31.30	29.66	28.25	29.03	29.80
13	27.97	29.56	26.44	28.83	30.08	29.20	32.31
14	29.42	25.04	30.81	29.53	32.16	29.36	29.21
15	25.59	24.02	24.10	26.12	31.36	27.16	31.78
16	23.83	27.56	28.07	26.94	31.24	27.65	28.25
17	22.51	24.20	24.59	27.54	28.82	26.07	28.76
18	21.10	25.74	21.62	26.04	30.30	25.47	27.99
19	23.18	25.22	24.85	26.32	27.18	25.94	28.86
20	25.02	28.42	27.73	30.48	31.34	28.50	28.02
Control [a]	26.64	30.18	30.18	31.73	33.26	30.93	33.60
Mean	26.45	26.52	24.92	29.20	29.51	28.89	27.58

LSD for cultivar × isolate interaction = 5.45 ($p<0.05$) or 7.18 ($p<0.01$).
[a] Non-infested soil.

observed only between survival and preemergence damping-off. Plant height was negatively correlated with each of preemergence damping-off of 'Giza 83', postemergence damping-off of 'Giza 80' and 'Giza 89', while it was positively correlated with survival of 'Giza 83', 'Giza 80', and 'Giza 89'. Dry weight and plant height were positively correlated on 'Giza 77', 'Giza 86', 'Giza 83', and 'Giza 89'.

Four groups of similar isolates (isolates 10, 11, 13, 18, 15, 12, 14, 16, isolates 2, 3, 1; isolates 4, 5, 6; and isolates 17, 20, 19, 7, respectively) were identified by cluster analysis (**Fig. 1**). All the isolates of the first group, except isolate 18, were collected from Upper Egypt governorates. Of the 10 isolates that were collected from Upper Egypt governorates, 7 (70%) were contained in this cluster. Isolates of the other 3 groups, except isolate 7, were collected from Lower Egypt governorates. No associations were observed between virulence pattern of Lower Egypt isolates and their geographic origins. For example, Kafr El-Sheikh isolates 2 and 6 differed from each other, as each was associated with a different group. Similarly, each of Menofiya isolates 3 and 19 was included in a different group. The virulence pattern of Faiyoum isolate 8 was quite different from the others, as was the virulence patterns of Sohag isolate 9.

DISCUSSION

The main objective of our previous study (Aly *et al.* 2006) was to evaluate the reactions of commercial Egyptian cot-

Table 8 Effect of interaction between cotton cultivars and *M. phaseolina* isolates on dry weight of cotton seedlings under greenhouse conditions.

Isolate	Dry weight (mg/plant)						
	Giza 77	Giza 80	Giza 83	Giza 85	Giza 86	Giza 89	Mean
1	420.80	528.20	491.80	444.80	93.00	462.20	504.80
2	430.53	507.20	516.20	407.00	218.00	446.60	487.80
3	421.77	452.00	446.60	442.00	207.20	517.80	465.00
4	344.53	361.20	340.20	318.60	269.20	277.00	501.00
5	406.50	438.40	311.40	371.40	346.40	414.00	557.40
6	349.47	308.00	313.00	369.00	307.80	250.80	548.00
7	422.50	424.60	420.60	386.60	403.80	474.40	425.00
8	416.63	415.40	535.40	417.40	436.80	255.80	439.00
9	316.57	189.20	308.60	444.40	312.20	340.00	305.00
10	472.40	456.00	424.60	521.00	412.20	538.80	481.80
11	438.93	430.40	433.40	423.60	368.80	479.00	498.40
12	419.50	362.60	522.60	360.00	313.40	453.20	505.20
13	412.93	418.80	482.20	425.00	345.80	356.00	449.80
14	402.97	417.80	437.60	304.60	319.60	428.20	510.00
15	429.93	468.00	470.80	522.00	361.60	336.20	421.00
16	421.80	502.00	419.80	334.20	316.80	552.80	405.20
17	386.67	495.20	362.40	415.20	346.60	433.40	268.40
18	406.37	481.60	428.20	419.80	315.20	370.40	430.00
19	340.57	431.60	318.20	514.60	363.20	237.40	178.40
20	435.93	492.40	373.20	536.00	412.40	433.00	368.60
Control [a]	556.40	579.00	564.00	593.40	500.20	527.00	574.80
Mean	444.4	408.76	331.93	426.84	424.80	436.17	411.65

LSD for cultivar × isolate interaction 81.26 (p<0.05) or 106.90 (p<0.01).
[a] Non-infested soil.

Table 9 Correlation‡ among variables used for evaluating pathogenicity of *M. phaseolina* isolates on seedlings of cotton cultivars under greenhouse conditions.

Cultivar	Variable	2	3	4	5
Giza 77	1. Preemergence damping-off (%)	-0.159	-0.778**	0.154	0.021
	2. Postemergence damping-off (%)		-0.497*	-0.287	0.094
	3. Survival (%)			0.047	-0.078
	4. Plant height (cm)				0.605**
	5. Dry weight (mg/plant)				
Giza 86	1. Preemergence damping-off (%)	-0.198	-0.769**	-0.039	-0.040
	2. Postemergence damping-off (%)		-0.435	0.305	0.275
	3. Survival (%)			-0.153	-0.133
	4. Plant height (cm)				0.635**
	5. Dry weight (mg/plant)				
Giza 85	1. Preemergence damping-off (%)	-.0154	-0.769**	-0.194	-0.057
	2. Postemergence damping-off (%)		-0.491*	0.060	0.012
	3. Survival (%)			0.228	-0.017
	4. Plant height (cm)				-0.245
	5. Dry weight (mg/plant)				
Giza 83	1. Preemergence damping-off (%)	-0.399	-0.469*	-0.694**	-0.481*
	2. Postemergence damping-off (%)		-0.622**	0.079	-0.255
	3. Survival (%)			0.517*	0.652**
	4. Plant height (cm)				0.777**
	5. Dry weight (mg/plant)				
Giza 80	1. Preemergence damping-off (%)	-0.006	-0.783**	-0.250	-0.477*
	2. Postemergence damping-off (%)		-0.616**	-0.637**	-0.159
	3. Survival (%)			0.585**	0.477*
	4. Plant height (cm)				0.390
	5. Dry weight (mg/plant)				
Giza 89	1. Preemergence damping-off (%)	-0.260	-0.532*	-0.265	-0.187
	2. Postemergence damping-off (%)		-0.679**	-0.453*	-0.424
	3. Survival (%)			0.599**	0.514*
	4. Plant height (cm)				0.609**
	5. Dry weight (mg/plant)				

[a] Linear correlation coefficient ‡ is significant at p<0.05 (*) or p<0.01 (**).

tons to infection by *M. phaseolina*. Agronomic traits of the tested cultivars were compared in the soil infested or uninfested with a highly pathogenic isolate of the fungus. Most agronomic traits of the tested cultivars severely deteriorated in infested soil. However, could not draw any conclusions regarding specificity of *M. phaseolina* isolates because the study was conducted by using only one isolate. On the other hand, the main aim of the current study was to evaluate specificity of *M. phaseolina* isolates on cotton cultivars because the concept of specificity in host-pathogen interaction has both theoretical and applied relevance to the understanding and control of many plant diseases. True specificity implies that genetic variation in the host and the pathogen are correlated and may affect the durability of host resistance to the pathogen (Kulkarni and Chopra 1982). Physiologic specialization, i.e. differential adaptation of pathogen isolates to certain host genotypes, could also complicate screening strategies in the development of disease-resistant host varieties. This is certainly important in host-pathogen systems where resistance is governed by major genes and

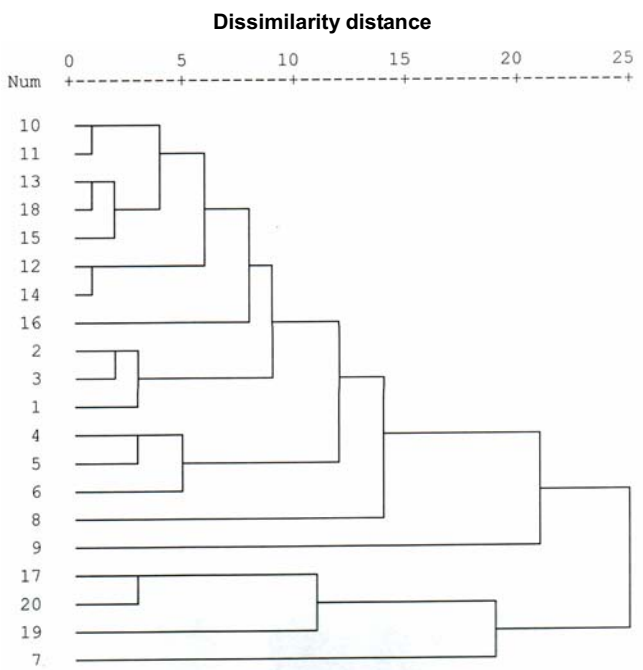

Fig. 1 Phenogram based on average linkage cluster analysis of virulence of 20 isolates of *M. phaseolina* on six cotton cultivars ('Giza 77', 'Giza 80', 'Giza 83', 'Giza 85', 'Giza 86', 'Giza 89').

distinct physiologic races can be identified. However, isolate-cultivar specificity should also be a consideration in quantitative host-pathogen systems, where physiologic specialization may be less obvious and based on quantitative differences in disease expression (Schilder and Bergstrom 1990).

Specificity in host-pathogen relationships is often indicated by significant isolate × variety interaction in the analysis of variance (ANOVA) of an experiment where a number of pathogen isolates are tested in all possible combinations on a set of host genotypes. Non-specificity is identified by a lack of such an interaction (Vanderplank 1982, 1984).

The ANOVA in the present work showed that the main effects of both cultivars (p=0.0001) and isolates (p=0.0001) were very highly significant sources of variation in all the tested parameters as was cultivar × isolate interaction (p= 0.0001).

Statistically significant interaction between cotton cultivars and isolates in this study suggests that physiologic specialization exists within *M. phaseolina* isolates pathogenic on cotton. Therefore, results of cotton screening tests for charcoal rot resistance could change considerably depending on the isolate(s) used. Thus, cotton cultivars should be tested by using as many isolates of *M. phaseolina* as possible, as this will improve the chance of identifying cotton cultivars effective against several isolates of *M. phaseolina*.

It has also been suggested that the presence of a significant cultivar × isolate interaction in the ANOVA is evidence for a differential (vertical) host-pathogen relationship (Vanderplank 1984). Lack of a significant interaction is taken to indicate that association is non-differential (horizontal), implying that differences in cultivar susceptibility are consistent relative to one another, regardless of pathogen isolates. In any host-pathogen relationship the two types of resistance may act together in determining the outcome of the association between the host and the pathogen (Vanderplank 1984).

Accordingly, the ANOVA in the present work implies that the resistance of the tested cultivars is a mixture of both vertical and horizontal resistance and there are significant differences among cultivars in both types of resistance. Similarly, pathogenicity of the tested isolates is also a mixture of virulence and aggressiveness, and the isolates significantly differ in both types of pathogenicity.

The application of cluster analysis has been suggested previously for assessing similarity and/or dissimilarity in gene-for-gene host-parasite relationships (Lebeda and Jendrulek 1987; Priestley *et al*. 1984). The method was used to express exactly the genetic similarity among 48 physiological races of *Bremia lactucae* Regel (Lebeda and Jendrulek 1987), 17 isolates of *Pyrenophora tritici-repentis* (Died.) Drech. (Schilder and Bergstrom 1990), and 41 isolates of *Ascochyta rabiei* (Pass.) Labrousse (Porta-Puglia *et al*. 1996).

In this study, cluster analysis proved to be useful in determining the similarity of *M. phaseolina* isolates, based on their virulence on six cotton cultivars. The set of selected cultivars differentiated the isolates reasonably well and grouped them into four pathotypes. However, virulence patterns of two isolates were quite different from the others.

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