

Anastomosis Groups, Pathogenicity, and Cellulase Production of *Rhizoctonia solani* from Cotton

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ABSTRACT

Fifty-two isolates of *Rhizoctonia solani* were isolated from cotton seedlings infected with postemergence damping-off disease, and obtained from cotton-producing areas in Upper Egypt governorates. Morphological characterization indicated that 13.46% of the isolates belonged to AG 2-2, 25% belonged to subgroup AG 4-HgI and 61.54% were AG 4-HgII. Fifty percent of *R. solani* isolates were effective in producing cellulase when they were grown on artificial medium. Pathogenicity assay of the 52 isolates of *R. solani* was conducted on four cotton cultivars under greenhouse conditions. Analysis of variance showed that isolates and interaction between isolates \times cultivars were highly significant source of variation in all the tested parameters. Statistically significant, isolates and the interaction between cotton cultivars and isolates suggest that physiologic specialization exists within *R. solani* isolates pathogenic on cotton. Also, pathogenicity of the tested isolates is a mixture of virulence and aggressiveness, and the isolates significantly differ in both types of pathogenicity. Correlation between seedling disease variables (pathogenicity parameters) and efficiency in cellulase production was affected by cotton cultivar and the anastomosis group (AG) of the fungus. Conversely, correlation among pathogenicity parameters was not affected by cultivar. Cluster analysis of isolates of *R. solani* was constructed based on their virulence on four cotton cultivars. Grouping the isolates was not related to their geographic origin or AG type.

Keywords: AG-4, enzymes, *Gossypium, Thanatephorus cucumeris*, virulence **Abbreviations:** AG, anastomosis group; **ANOVA**, analysis of variance; **LSD**, least significant difference

INTRODUCTION

Rhizoctonia solani Kühn (telemorph, Thanatephorus cucumeris (Frank) Donk) is a destructive plant pathogen and can cause damage worldwide on more than 142 plant species, including many agricultural and horticultural crops (Ogoshi 1996). R. solani is composed of genetically isolated groups (Adams 1988). The identification and classification of these groups are based on the anastomosis behavior (Anderson 1982 and Ogoshi 1976). To date, 14 anastomosis groups (AGs) have been recognized (Ogoshi 1996; Carling et al. 1999, 2002). Many of these AGs have been subdivided on the basis of host range, cultural morphology and biochemical reaction (Ogoshi 1987). R. solani exists in its vegetative form in nearly all agricultural soils. In this non-spore-producing phase, the fungus lives saprophytically on dead plant remains, but it can become vigorously parasitic when roots or other parts of a susceptible host penetrate the infested zone (Watkins 1981). R. solani was the primary agent in cotton seedling damping-off and was associated with severe necrosis on the root and/or hypoctyl (Malero-Vara and Jimenez-Diaz 1990). Thirteen isolates of R. solani exhibited variation in virulence on cotton 'DS-1' seedlings (Monga and Sheo-Raj 1994). Rush et al. (1994) reported that some isolates of R. solani AG2-2, AG4 and AG5 reduced emergence and caused root discoloration on seedlings of maize, cotton and sorghum during pathogenicity studies. *R. solani* AG4 and AG5 were among pathogenic fungi

R. solani AG4 and AG5 were among pathogenic fungi isolated from diseased cotton plants in Tifton, Georgia, USA. The pathogenicity test of the fungi was demonstrated and they were re-isolated from lesion tissue (Baird *et al.* 1995). The pathogenicity of 39 isolates of *R. solani* AG4 and one isolate belong to AG2-2 were evaluated under greenhouse conditions on cotton 'Giza 75'. Most of the virulent isolates

exhibited preemergence damping-off (El-Akkad 1997). Most damage by *R. solani* was observed on cotton 'ST453' and 'ST907' during evaluation of the radicle assay to screen germplasm for principal pathogens of cotton seedling diseases (Aqil and Batson 1999).

Seventeen isolates of *R. solani* AG4 and one isolate of binuclate *Rhizoctonia* were tested for pathogenicity on seedlings of cotton 'Giza 83'. All isolates were so virulent that they caused 100% mortality in the preemergence stage (El-Samawaty 1999). *R. solani* AG4 was the major causal agent of postemergence cotton seedling disease in Missouri (Wrather *et al.* 2002). Eighteen *R. solani* isolates belonging to AG4 were tested for pathogenicity on seedlings of cotton 'Giza 83' and 'Giza 86'. Ten isolates were so virulent that they caused more than 95% mortality at the preemergence stage (Asran-Amal 2001). Pathogenicity of 12 isolates of *R. solani* varied when these isolates were tested on *Gossypium arboretum* variety RG-8 (Monga *et al.* 2004).

In the current research, we identified AGs of *R. solani* involved in cotton damping-off and assayed their pathogenicity on some cotton cultivars under greenhouse conditions. We also evaluated the relationship between virulence of *R. solani* isolates and the *in vitro* cellulase production of the isolates.

MATERIALS AND METHODS

Isolation, purification and identification of *Rhizoctonia solani* isolates

Isolates of *R. solani* used in present study were isolated from samples of cotton seedlings infected with postemergence damping-off disease and obtained from cotton-producing areas in Upper Egypt governorates (mainly Sohag and Assiut). Pure cultures of isolates

were obtained using the hyphal tip technique described by Dhingra and Sinclair (1985). Individual isolates were examined microscopically to verify the branching near the distal septum of cells as well as the constriction of the branch near the point of origin (Ogoshi 1987).

Nuclear number

Determination of the number of nuclei of hyphal cells, is the first step to identify *Rhizoctonia* isolates into binucleate AGs (*Rhizoctonia* spp.), or multinucleate AGs (*R. solani*). Nuclei were counted in young vegetative hyphae taken from the outer edge of a colony grown on PDA for 7-14 days and placed in center of a 9-cm Petri dish having only 10 ml of 2% water agar medium. Determination of the number of nuclei was usually counted by staining the vegetative hyphae with Aniline Blue. Aliquots of acidified (HCl) 1% Aniline Blue in 50% glycerine were placed directly on young hyphae grown on water agar medium. Stained hyphae were then covered with a cover slip and inspected directly in Petri dishes using a 400X magnification.

Rhizoctonia anastomosis group typing

Anastomosis tests were done in 2 stages. In the first stage, it was conducted between the obtained isolates and each others i.e. without testers. In the second stage, the tested isolates were assigned with 3 tester isolates, which included AG2-2, AG4-HgI and AG4-HgII.

The methods for the observation of hyphal anastomosis and determination of anastomosis groups were carried out as described by Herr and Robert (1980) and Kronland and Stanghellini (1988), by using clean slides and cover slip techniques. A 5-mm diameter discs from the growing margins of young colonies on PDA from tester and unknown isolates were placed 2-3 cm apart on a clean glass slide. Slides were placed on glass bars in 15-cm diameter Petri dishes lined with wet filter paper to maintain high relative humidity and incubated for 24-48 h at 25°C. When the hyphae from the two isolates overlapped, slides were removed and the excess moisture was retracted from the bottom of the slide. Overlapping hyphae were stained with Safranin O or Trypan Blue, covered with a cover slip and examined microscopically at 400X magnification. All anastomosis (hyphal fusion) were traced back to avoid false positives (self anastomosis).

Cellulase activity in vitro

Measurement of cellulase activity was done by the method of Garrett (1966). Groups of five circles of Whatman No. 3 filter paper (7-cm diam.) were weighed and placed in 250 ml conical flasks with 12 ml mineral salts broth (5 g NaNO₃, 1 g K₂HPO₄, 2 g MgSO₄·7 H₂O, 10 µg biotin, 100 µg thiamine, 1 mg FeCl₃, 0.9 mg ZnSO₄ in 1 L of water). The flasks were autoclaved and inoculated with an agar block of *R. solani*, 8 mm in diameter and incubated at 25°C for 4 weeks. After incubation, the flasks' contents were removed and oven dried at 80°C overnight. Uninoculated controls were treated similarly. From comparison of the weight change of uninoculated and inoculated filter paper, it was possible to determine the amount of loss of cellulose caused by the fungus.

Pathogenicity test of R. solani on cotton cultivars

Substrate for growth of each isolate was prepared in 500-ml glass bottles, each containing 50 g of sorghum grains (cv. 'Balady') and 40 ml of tap water. Contents of the 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 sorghum for 3 weeks. The present test was carried out by using autoclaved clay loam soil. Batches of soil were infested separately with inoculum of each isolate at a rate of 1 g/kg of soil. Infested soil was dispended in 15-cm-diameter clay pots and these were planted with 10 seeds per pot for each of the tested cultivars ('Giza 80', 'Giza 83', 'Giza 90' and 'Giza 91'). In the control treatment, sterilized sorghum grains (cv. 'Balady') were mixed thoroughly with soil at the rate of 1 g/kg of soil. Pots were randomly distributed on a greenhouse bench under a tempe-

rature regime that ranged from 20 ± 7 to $29 \pm 9^{\circ}$ C. Preemergence damping-off was recorded 15 days after planting. Postemergence damping-off, survivals and dry weight (mg/plant) were recorded 45 days after planting.

Statistical analysis of data

Pathogenicity test was carried out in a randomized complete block design with three replicates. Analysis of variance (ANOVA) of the data was performed with the MSTAT-C Statistical Package (Michigan State Univ., USA). Duncan's multiple range test or least significant difference (LSD) were used to compare treatment means. Some percentage data were transformed into arc sine angles before

 Table 1 Production of cellulose, nuclear number and anastomosis groups of *Rhizoctonia solani* isolates.

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Sample	Dry wt. of	Nuclei	AG	AG 4 Hal	AG 4 Hall
1		JN2 4	2-2	4-Hgi	4-ng11
1	367.3 a-d	4	-	+	-
2	303.3 fg*	5	-	+	-
3	378.3 a-d	4	-	+	-
4	263.3 g-n*	3	+	-	-
5	392.3 a-c	6	-	-	+
6	243.7 l-o*	6	-	-	+
7	369.0 a-d	4	-	-	+
8	360.7 b-d	3	-	-	+
9	407.0 a	5	-	+	-
10	289.3 f-j*	3	-	-	+
11	360.7 b-d	4	-	-	+
12	397.0 ab	3	-	-	+
13	260.0 h-n*	5	-	+	-
14	391.3 a-c	3	-	-	+
15	410.7 a	3	-	-	+
16	362.0 b-d	4	-	-	+
17	383.0 a-d	4	+	-	-
18	294.0 f-i*	5	-	-	+
19	385 3 a-d	3	-	-	+
20	394 3 ah	4	_	+	_
20	286.3 f-k*	3	_	_	+
21 77	200.5 T k 396 7 ab	3	_	_	+
22	301 7 f-h*	4		+	_
23	285.2 o.d		-		-
24	303.5 a-u 274 7 f 1*	5	-	Ŧ	-
25	2/4.71.0*	3	Ŧ	-	-
20	240.7 K-0 ⁻	4	-	Ŧ	-
27	242.7 1-0*	3	-	-	+
28	349.7 c-e	3	-	-	+
29	391.3 a-c	5	-	-	+
30	265.3 g-n*	4	+	-	-
31	391.0 a-c	4	+	-	-
32	235.7 l-o*	3	+	-	-
33	396.7 ab	3	-	+	-
34	314.3 ef*	5	-	-	+
35	273.7 f-l*	5	-	-	+
36	269.3 g-m*	3	-	-	+
37	357.0 b-d	5	-	-	+
38	384.7 a-d	6	-	-	+
39	375.0 a-d	5	-	-	+
40	225.0 no*	3	-	+	-
41	345.7 de*	3	-	-	+
42	300.0 f-h*	5	-	+	-
43	244.3 k-o*	3	-	-	+
44	378.3 a-d	4	-	-	+
45	254.0 i-n*	3	-	-	+
46	314.0 ef*	3	-	-	+
47	249.0 i-n*	3	+	-	_
48	394 0 ab	3	_	-	+
19	207.3 0*	3	_	_	+
50	207.50 354 3 h-d	6	-	-	+
51	206.3 c*	3	-	-	+
52	200.50°	5	-	-	'
54 Cant	202.2	5	-	Ŧ	-

Cont. 393.3 a-c

Values in a column followed by the same letter(s) are not significantly different (p<0.05) according to Duncan's multiple range test. An asterisk denotes a significant difference from the control.



Isolate number

Fig. 1 Effect of interaction between cotton cultivars and *Rhizoctonia solani* isolates on preemergence damping-off of cotton seedlings under greenhouse conditions.



Fig. 2 Effect of interaction between cotton cultivars and *Rhizoctonia solani* isolates on postemergence damping-off of cotton seedlings under greenhouse conditions.

carrying out ANOVA to produce approximately constant variance. Correlation and cluster analyses were performed with computerized programs.

RESULTS

Cultural characteristics of R. solani isolates

Fifty-two R. solani isolates were confirmed to the genus

level by examining their microscopic characteristics. The main characteristics observed were the presence of septa, the constriction of the hyphae, and the formation of septa at a short distance from the point of origin of branches.

Nuclear number

Determination of the number of nuclei in hyphae cells is an important criterion in the identification of *Rhizoctonia* iso-

 Table 2 Efficiency in producing cellulose of Rhizoctonia solani (AGs).

(AG) groups	№ of isolates	Efficiency in producing cellulose %
AG 2-2	7	25.08 A
AG 4-HgI	13	18.73 A
AG 4-HgII	32	16.01 A

Values in a column followed by the same letter are not significantly different (p<0.05) according to Duncan's multiple range test.

 Table 3 Analysis of variance of the interaction between cotton cultivars and isolates of *Rhizoctonia* spp. under greenhouse conditions.

Parameter and source	D.F.	M.S.	F-value	P>F
of variation				
Preemergence damping	g-off			
Replication	2	133.330	0.717	
Cultivar (C)	3	340.580	1.831	0.141
Isolate (I)	52	1270.305	6.828	0.000
$C \times I$	156	839.928	4.515	0.000
Error	422	186.040		
Postemergence dampin	g-off			
Replication	2	0.375	2.265	0.105
Cultivar (C)	3	0.609	3.679	0.012
Isolate (I)	52	0.765	4.625	0.000
$C \times I$	156	0.440	2.662	0.000
Error	422	0.165		
Survival				
Replication	2	68.465	0.296	
Cultivar (C)	3	834.772	3.611	0.013
Isolate (I)	52	1634.270	7.069	0.000
$\mathbf{C} \times \mathbf{I}$	156	523.589	2.264	0.000
Error	422	231.179		
Dry weight				
Replication	2	5884.307	0.172	
Cultivar (C)	3	26762.819	0.782	0.000
Isolate (I)	52	117606.547	3.437	0.000
$\mathbf{C} \times \mathbf{I}$	156	62272.780	1.820	
Error	422	34221 939		

^a Replication is random, while each of cultivar and isolate is fixed.

Table 4 Relative contribution of cotton cultivars, *Rhizoctonia solani* isolates, and their interaction to variation in preemergence damping-off and postemergence damping-off and survival and dry weight of cotton seed-lings.

Source of	Rela	n		
variation	Preemergence damping-off	Postemergence damping-off	Survival	Dry weight
Cultivar (C)	0.51	1.64	1.48	0.50
Isolate (I)	33.30	35.82	50.21	38.41
$C \times I$	66.05	61.86	48.23	61.01

^a Calculated as percentage of sum squares explained.

lates to the species level. The number of nuclei per cell was then determined by staining the mycelium with Aniline Blue and examining them microscopically. All isolates examined were multinucleate *R. solani* (Table 1).

Determination of anastomosis groups of *R. solani* isolates

The proposed isolates of *R. solani* were identified into anastomosis groups (AGs) by using cultures of known AG testers. All isolates of *R. solani* were tested for their ability to anastomose with a tester belonged to AG2-2, AG4-HgI and AG4-HgII. Seven (13.46%), 13 (25%) and 32 (61.54%) isolates of *R. solani* belonged to AG 2-2, AG 4-HgI and AG 4-HgII, respectively (**Table 1**).

Cellulase-producing isolates

The results in **Table 1** show that 50% of the tested isolates were efficient in producing cellulase compared with the control. Of these isolates, 19.23% belonged to AG 2-2, 26.93% belonged to AG 4-HgI and 53.85% belonged to AG 4-HgII, however, the three AGs did not significantly differ in effici-

ency of cellulase production (**Table 2**). Efficiency of each isolate in producing cellulase was calculated based on the data shown in **Table 1** according to the formula described by Garrett (1966); WC-WS \div WC ×100, where WC is the dry weight of filter paper of the control, where WS is the dry weight of filter paper of the designated isolate.

Pathogenicity assay

ANOVA (**Table 3**) showed that cultivar was a significant source of variation in each of postemegrence damping-off and survival, but it was a non-significant source of variation in preemergence damping-off and dry weight. Isolates and cultivar \times isolate interaction were very highly significant sources of variation in all the tested parameters.

Cultivar \times isolate interaction was the first in importance as a source of variation in preemergence damping-off, postemergence damping-off and dry weight, while isolates were the second in importance (**Table 4**). Isolate and cultivar \times isolate interaction showed almost the same relative contribution to variation in survival.

Due to the very highly significant effect of cultivar \times isolate interaction on preemergence damping-off, a Duncan's multiple range test was calculated to compare isolate mean within each cultivar (data not shown). 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, isolates 23, 38, 39, 49, 50, and 51 were highly pathogenic on 'Giza 91' and non-pathogenic on the other cultivars. Isolates 3, 7, and 26, in contrast, were highly pathogenic on 'Giza 83', 'Giza 90', and 'Giza 91', but were nonpathogenic on 'Giza 80' (Fig. 1).

Also, in the postemergence stage, the effect of cultivar \times isolate interaction was highly significant source of variation. Cultivars responded differently to isolates, for example, 'Giza 80' was affected only by isolates number 6, 11, 13, and 22, while 'Giza 90' was affected only by 9, 12, 18, 21, and 48 isolates (**Fig. 2**).

All cultivars were susceptible to isolates number 7, 22, 26, 28, 33, 36, and 46 during the survival stage (Fig. 3). Moreover, 'Giza 80' was the only cultivar that showed susceptibility to isolates number 17, 20, 41, and 44. 'Giza 83', however, was the only susceptible cultivar to isolates number 2, 6, 40, and 52. The differences between effects of isolates on dry weight of seedlings differed from one cultivar to another. Thus, the difference between the effects of isolates nos. 27, and 29 on dry weight of seedlings was highly significant on 'Giza 80', while it was non-significant on 'Giza 83'. Another example was the highly significant difference between isolates nos. 4, and 16 on 'Giza 90, but non-significant on 'Giza 91' (Fig. 4). Correlation among variables used for evaluating pathogenicity of R. solani isolates and efficiency in producing cellulase for same isolates are shown in Table 5. A highly significant negative correlation was found between preemergence damping-off and each of survival and postemergence damping-off of all cultivars. Also, a highly significant positive correlation was found between survival and dry weight. When isolates of R. solani were classified into AGs, a significant positive correlation was found between survival and dry weight of 'Giza 80' and 'Giza 83', and between preemergence damping-off and efficiency in producing cellulase on 'Giza 90' (Table 6)

R. solani AG 4-HgI (**Table 7**), a highly significant negative correlation was found between preemergence dampingoff and survival on 'Giza 90' and 'Giza 91', while a negative correlation was found between efficiency in producing cellulase and postemergence damping-off of 'Giza 90'. Within *R. solani*, AG 4-HgII (**Table 8**), a negative correlation was observed between preemergence damping-off and efficiency in producing cellulase on 'Giza 80', while a positive correlation was observed between efficiency in producing cellulase and survival. A highly significant negative correlation was found between preemergence damping-off



Isolate number

Fig. 3 Effect of interaction between cotton cultivars and Rhizoctonia solani isolates on survival of cotton seedlings under greenhouse conditions.



Fig. 4 Effect of interaction between cotton cultivars and *Rhizoctonia solani* isolates on dry weight of cotton seedlings under greenhouse conditions.

and each of postemergnce damping-off and survival on all of the tested cultivars. In general, correlation among seedling disease variables was not affected by cultivar, while correlation between efficiency in cellulase production and seedling disease variables was affected by cultivar and AG.

A cluster analysis (**Fig. 5**) of 52 isolates of *R. solani* was constructed based on virulence of these isolates on four cultivars. Grouping the isolates based on their virulence patterns was not related to their geographic origins or AG.

DISCUSSION

Fifty-two *R. solani* isolates were obtained from cotton-producing areas in Upper Egypt governorates. These isolates belonged to AG 2-2, AG 4-HgI, and AG 4-HgII. This result is consistent with the findings of Rush *et al.* (1994), El-Akkad (1994) and El-Samawaty (1999), who reported similar trends on Egyptian cottons. Of these isolates, 50% showed cellulytic activity.

Virulence of *R. solani* isolates on seedlings of cotton cultivars was variable during the pathogenicity test. Similar results were reported by Monga and Sheo-Raj (1994), Aqil and Batson (1999), and Asran-Amal (2001). Specificity in host-pathogen relationship is often indicated by a significant isolate \times variety interaction in the ANOVA of an experiment where the number of pathogen isolates is tested in all possible combinations on a set of host genotypes. Non-specificity is identified by a lack of such interaction (Vanderplank 1982, 1984).

The ANOVA in the present work showed that the main



Fig. 5 Dendrogram based on average linkage cluster analysis of virulence of 52 isolates of *R. solani* on cotton cultivars 'Giza 80', 'Giza 93', 'Giza 90' and 'Giza 91'.

effects of both isolate (p=0.0000) and cultivar \times isolate interaction (p=0.0000) were very highly significant sources of variation in all the tested parameters, while cultivar was a significant source of variation in postemergence damping-off and survival. A statistically significant interaction bet-

ween cotton cultivars and isolates in this study suggests that physiologic specialization exists within R. solani isolates pathogenic on cotton. Therefore, results of screening tests for cotton seedling disease resistance could change considerably depending on the isolates used. Thus, cotton cultivars should be tested by using as many isolates of R. solani as possible, as this will improve the chance of identifying cotton cultivars effective against several isolates of R. solani. It has also been suggested that the presence of a significant cultivar sorshin isolate interaction in the ANOVA is an 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 other, 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 pathogenicity of the tested isolates is a mixture of virulence and aggressiveness, and the isolates signifcantly differ in both types of pathogenicity. Furthermore, the resistance of the tested cultivars is a mixture of both vertical and horizontal in the postemergence stage, while, it is horizontal in the preemergence stage.

Significant positive and negative correlations were observed between efficiency in producing cellulase and some variables used for evaluating the pathogenicity of the isolates. This result is in agreement with that of Walton (1994) and Novo *et al.* (2006) who indicated that cellulase may not be a determining factor for aggressiveness and consequently the cause of symptoms, although it may play an important role in the penetration process. Robert *et al.* (1986) also reported that the correlation between *in vitro* production of cell wall-degrading enzymes and pathogenesis by fungi is not necessarily strong.

The application of cluster analysis has been suggested previously for assessing similarity and/or dissimilarity in gene-for-gene host-parasite relationships (Priestley *et al.* 1984; Lebeda and Jendrulek 1987). The method was used to express exactly the genetic similarity among 48 physiological races of *Bremia lactucae* Regel (Lebeda and Jendrulek 1987), 41 isolates of *Ascochyta rabiei* (Pass.) Labrousse (Porta-Puglia *et al.* 1996) and 20 isolates of *Macrophomina phaseolina* (Tassi) Goid. (Omar 2005).

In this study, cluster analysis divided the isolates into groups based on their virulence patterns on four cotton cultivars; however, grouping the isolates was not related to their geographic origin or AG.

Cultivar	Variable	-	Variable					
		1	2	3	4	5		
Giza 80	1. Efficiency in producing cellulase (%)		0.152	0.032	- 0.103	- 0.267		
	2. Preemergence damping-off (%)			- 0.495**	- 0.464**	- 0.214		
	3. Postemergence damping-off (%)				- 0.412**	- 0.033		
	4. Survival (%)					0.345*		
	5. Dry weight (mg/plant)							
Giza 83	1. Efficiency in producing cellulase (%)		0.240	- 0.229	- 0.072	- 0.075		
	2. Preemergence damping-off (%)			- 0.687**	- 0.575**	- 0.324*		
	3. Postemergence damping-off (%)				- 0.198	- 0.073		
	4. Survival (%)					0.523**		
	5. Dry weight (mg/plant)							
Giza 90	1. Efficiency in producing cellulase (%)		0.089	- 0.236	0.065	0.049		
	2. Preemergence damping-off (%)			- 0.620**	- 0.809**	- 0.758**		
	3. Postemergence damping-off (%)				0.041	0.508**		
	4. Survival (%)					0.581**		
	5. Dry weight (mg/plant)							
Giza 91	1. Efficiency in producing cellulase (%)		0.014	0.077	- 0.086	- 0.190		
	2. Preemergence damping-off (%)			- 0.576**	- 0.674**	- 0.276*		
	3. Postemergence damping-off (%)				- 0.215	- 0.043		
	4. Survival (%)					0.369**		
	5. Dry weight (mg/plant)							

 Table 5 Relationship among seedling disease variables and efficiency of *Rhizoctonia* isolates in producing cellulase.

Pearson's correlation coefficient (r) is significant at p < 0.01 (**) or p < 0.05 (*).

Cultivar	Variable	Variable					
		1	2	3	4	5	
Giza 80	1. Efficiency in producing cellulase (%)		0.287	0.472	- 0.693	- 0.619	
	2. Preemergence damping-off (%)			- 0.458	- 0.676	- 0.693	
	3. Postemergence damping-off (%)				- 0.346	- 0.225	
	4. Survival (%)					0.916**	
	5. Dry weight (mg/plant)						
Giza 83	1. Efficiency in producing cellulase (%)		0.456	- 0.412	- 0.294	- 0.094	
	2. Preemergence damping-off (%)			- 0.744	- 0.789*	- 0.676	
	3. Postemergence damping-off (%)				0.176	0.162	
	4. Survival (%)					0.848*	
	5. Dry weight (mg/plant)						
Giza 90	1. Efficiency in producing cellulase (%)		0.818*	- 0.541	- 0.880**	- 0.675	
	2. Preemergence damping-off (%)			- 0.810*	- 0.838*	- 0.837*	
	3. Postemergence damping-off (%)				0.383	0.866*	
	4. Survival (%)					0.580	
	5. Dry weight (mg/plant)						
Giza 91	1. Efficiency in producing cellulase (%)		0.404	- 0.007	- 0.380	0.065	
	2. Preemergence damping-off (%)			- 0.488	- 0.461	- 0.116	
	3. Postemergence damping-off (%)				- 0.550	- 0.452	
	4. Survival (%)					0.570	
	5. Dry weight (mg/plant)						

Pearson's correlation coefficient (r) is significant at p < 0.01 (**) or p < 0.05 (*).

Table 7 Relationship amon	g seedling disease van	riables and efficiency of	of Rhizoctonia (AG	G 4-HgI) isolates in	n producing cellulase.
	8				

Cultivar	Variable			Variable		
		1	2	3	4	5
Giza 80	1. Efficiency in producing cellulase (%)		0.066	0.104	- 0.074	0.021
	2. Preemergence damping-off (%)			- 0.388	- 0.583*	- 0.639*
	3. Postemergence damping-off (%)				- 0.507	- 0.132
	4. Survival (%)					0.667*
	5. Dry weight (mg/plant)					
Giza 83	1. Efficiency in producing cellulase (%)		0.395	- 0.187	- 0.405	- 0.075
	2. Preemergence damping-off (%)			- 0.839**	- 0.380	- 0.237
	3. Postemergence damping-off (%)				- 0.181	- 0.089
	4. Survival (%)					0.586*
	5. Dry weight (mg/plant)					
Giza 90	1. Efficiency in producing cellulase (%)		0.094	- 0.572*	0.194	0.158
	2. Preemergence damping-off (%)			- 0.340	- 0.869**	- 0.479
	3. Postemergence damping-off (%)				- 0.170	0.095
	4. Survival (%)					0.445
	5. Dry weight (mg/plant)					
Giza 91	1. Efficiency in producing cellulase (%)		- 0.032	0.025	0.019	- 0.149
	2. Preemergence damping-off (%)			- 0.539	- 0.755**	- 0.082
	3. Postemergence damping-off (%)				- 0.146	- 0.028
	4. Survival (%)					0.117
	5. Dry weight (mg/plant)					

Pearson's correlation coefficient (r) is significant at p < 0.01 (**) or p < 0.05 (*).

Table 8 Relationship	among seedling disease variables and efficiency of	of Rhizoctonia (AG 4-HgII) isolates in	producing cellulase.
Cultiver	Variable	Va	riahla

Cultivar	Variable	Variable					
		1	2	3	4	5	
Giza 80	1. Efficiency in producing cellulase (%)		- 0.351*	0.030	0.363*	0.235	
	2. Preemergence damping-off (%)			- 0.536**	- 0.591**	- 0.372*	
	3. Postemergence damping-off (%)				- 0.359*	- 0.075	
	4. Survival (%)					0.485**	
	5. Dry weight (mg/plant)						
Giza 83	1. Efficiency in producing cellulase (%)		0.060	- 0.187	0.144	- 0.057	
	2. Preemergence damping-off (%)			- 0.672**	- 0.571**	- 0.187	
	3. Postemergence damping-off (%)				- 0.004	- 0.169	
	4. Survival (%)					0.255	
	5. Dry weight (mg/plant)						
Giza 90	1. Efficiency in producing cellulase (%)		- 0.126	- 0.130	0.279	0.177	
	2. Preemergence damping-off (%)			- 0.697**	- 0.769**	- 0.847**	
	3. Postemergence damping-off (%)				0.079	0.519**	
	4. Survival (%)					0.722**	
	5. Dry weight (mg/plant)						
Giza 91	1. Efficiency in producing cellulase (%)		0.026	0.015	- 0.050	- 0.085	
	2. Preemergence damping-off (%)			- 0.637**	- 0.680**	- 0.475**	
	3. Postemergence damping-off (%)				- 0.130	0.144	
	4. Survival (%)					0.477**	
	5. Dry weight (mg/plant)						

Pearson's correlation coefficient (r) is significant at p < 0.01 (**) or p < 0.05 (*).

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REFERENCES

- Adams GC Jr. (1988) Thanatephorus cucumeris (Rhizoctonia solani), a specific complex of wide host range. In: Sdihu GS (Ed) Advances in Plant Pathology (Vol 6) Genetics of Plant Pathogenic Fungi, Academic Press, New York, pp 353-550
- Anderson NA (1982) The genetics and pathology of *Rhizoctonia solani*. Annual Review of Phytopathology 20, 329-347
- Asran-Amal A (2001) Studies on cotton rhizosphere microorganisms and their role as biocontrol agents for root rot diseases. PhD thesis, Cairo University, 156 pp
- Aqil T, Batson EW (1999) Evaluation of radical assay for screening cotton genotypes for resistance to the pathogens of seedling disease complex. *Pakistan Journal of Phytopathology* 11, 11-16
- Baird RE, Brenneman BT, Bell KD (1995) First report of *Rhizoctonia* sp. CAG-5 on cotton in Georgia. *Plant Disease* **79**, 320
- Carling DE, Pope EJ, Brainard KA, Carter DA (1999) Characterization of mycorrihizal isolates of *Rhizoctonia solani* from orchid, including AG-12, a new anastomosis group. *Phytopathology* 89, 942-946
- Carling DE, Baird RE, Gitaitis RD, Brainard KA, Kuninaga S (2002) Characterization of AG-13, a new reported anastomosis group of *Rhizoctonia* solani. *Phytopathology* 92, 893-899
- Dhingra OD, Sinclair JB (1985) Basic Plant Pathology Methods, CRC Press, Inc., Florida, USA, 355 pp
- El-Akkad-Salwa AF (1997) Studies on anastomosis group of *Rhizoctonia* solani. PhD thesis, Cairo University, 143 pp
- El-Samawaty AMA (1999) Studies on cotton root rot disease. MSc thesis, Faculty of Agriculture, Assiut University, 105 pp
- Garrett SD (1966) Cellulose decomposing ability of some cereal foot-rot fungi in relation to their saprophytic survival. *Transactions British Mycological Society* 49, 57-68
- Herr LJ, Robert DL (1980) Characterization of *Rhizoctonia* population obtained from sugar beet fields with differing soil textures. *Phytopathology* 70, 476-780
- Kronland WC, Stanghellini ME (1988) Clean slide technique for the observation of anastomosis and nuclear condition of *Rhizoctonia solani*. *Phytopathology* 78, 820-822
- Lebeda A, Jendrulek T (1987) Application of cluster analysis for establishment of genetic similarity in gene-for-gene host-parasite relationships. *Journal of Phytopathology* 119, 131-141
- Malero-Vara JM, Jimenez-Diaz RM (1990) Etiology, incidence and distribu-

tion of cotton seedling damping-off in Southern Spain. Plant Disease 74, 597-600

- Monga D, Sheo-Raj (1994) Cultural and pathogenic variations in the isolates of *Rhizoctonia* species causing root rot of cotton. *Indian of Phytopathology* 47, 403-407
- Monga D, Rathere SS, Mayee DC, Sharma RT (2004) Differentiation of isolates of cotton root rot pathogens *Rhizoctonia solani* and *R. bataticola* using pathogenicity and RAPD markers. *Journal of Plant Biochemistry and Biotechnology* 13, 135-139
- Novo M, Pomar F, Gayoso C, Merino F (2006) Cellulase activity in isolates of Verticillium dahliae, differing and aggressiveness. Plant Disease 90, 155-160
- Ogoshi A (1976) Studies on the grouping of *Rhizoctonia solani* Kühn with hyphal anastomosis and on the perfect stage of groups. *Bulletin of the National Institute of Agricultural Science (Japan)* 30, 1-63
- Ogoshi A (1987) Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kühn. *Annul Review of Phytopathology* 25, 125-143
- **Ogoshi A** (1996) The genus *Rhizoctonia*. In: Sneh B, Jabaji-Hare S, Neate S, Dijst G (Eds) *Rhizoctonia Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control*, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 1-9
- Omar MR (2005) Pathological and biochemical studies on Macrophomina phaseolina pathogenic on cotton. PhD thesis, Seuz Canal University, Ismailia, Egypt, 178 pp
- Porta-Puglia A, Crino P, Mosconi C (1996) Variability in virulence to chickpea of an Italian population of Ascochyta rabiei. Plant Disease 80, 39-41
- Priestley RH, Bayles RA, Ryall J (1984) Identification of specific resistance against *Puccinia striformis* (yellow rust) in winter wheat varieties. II. Use of cluster analysis. *Journal of the National Institute of Agricultural Botany* 16, 477-485
- Robert NG, Kiraly Z, Wood KR (1986) The Biochemistry and Physiology of Plant Disease, University of Missouri Press, Columbia, Missouri, USA, 433 pp
- Rush CM, Carling ED, Harveson MR, Mathieson TJ (1994) Prevalence and pathogenicity of anastomosis groups of *Rhizoctonia solani* from wheat and sugar beet in Texas. *Plant Disease* **78**, 349-352
- Vanderplank JE (1982) Host-Pathogen Interactions in Plant Disease, Academic Press, New York, 207 pp
- Vanderplank JE (1984) Disease Resistance in Plants (2nd Edn), Academic Press, Orlando, Florida, 194 pp
- Walton JD (1994) Deconstructing the cell wall. *Plant Physiology* 104, 1113-1118
- Watkins GM (Ed) (1981) Compendium of Cotton Diseases, The American Phytopathological Society, St. Paul., Minnesota, 87 pp
- Wrather JA, Phipps B, Rothrock CS (2002) Fungi associated with post-emergence cotton seedling disease in Missouri. *Plant Health Progress* July, 1-4