

Full Length Research Paper

# Current *Rhizoctonia solani* anastomosis groups in Egypt and their pathogenic relation to cotton seedlings

Maurice S. Mikhail<sup>1</sup>, Kamel K. Sabet<sup>1</sup>, Moawad R. Omar<sup>2</sup>, Amal A. Asran<sup>3</sup> and Khaled K. Kasem<sup>1,4\*</sup>

<sup>1</sup>Plant Pathology Department, Cairo University, Egypt.

<sup>2</sup>Plant Pathology Research Institute, Agricultural Research Centre, Giza, Egypt.

<sup>3</sup>Botany and Microbiology Department, College of Science, King Saud University, Saudi, P. O. Box 2455, Riyadh 1145, Saudi Arabia.

<sup>4</sup>Agricultural Research Centre, Hamah, General Commission for Scientific Agricultural Research, Syria.

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Twenty eight isolates of *Rhizoctonia solani* were obtained from cotton seedlings and twenty three isolates from other hosts; eight from peanut, five from chickpea, two from each of flax, tomato and watermelon and one from each of potato, cantaloupe, pepper and lupine. Microscopic examination revealed that 17 isolates (33.33%) each belonged to AG-2-2, 17 and AG-4 HG-I, while 7 isolates (13.73%) belonged to AG-4 HG-II and 10 isolates (19.61%) belonged to AG-5. Pathogenicity test on cotton cultivar Giza 86, under greenhouse conditions, showed that 19 isolates significantly induced pre- and post-emergence damping-off, while they significantly decreased survival, plant height and dry weight. However, the pathogenic isolates of AG-2-2 represented 19.61% of the total isolates as well as the highest percentage of the pathogenic isolates (52.63%). There were no significant differences between effects of different AGs on the cotton seedling variables. Cluster analysis suggested that grouping the isolates based on their virulence patterns was not related to their geographic origins, AG or host.

**Key words:** *Rhizoctonia solani*, anastomosis groups, cotton, pathogenicity.

## INTRODUCTION

Cotton (*Gossypium barbadense* L.) is one of the strategic farm crops which is widely cultivated and traded across the world and one of the most important export crops of Egypt. Cotton seedling diseases are a worldwide problem; they are caused by a complex of soil-borne organisms. These organisms are found in all cotton producing areas in Egypt and include *Rhizoctonia solani* and *Fusarium* spp. (Asran et al., 2005). *R. solani* Kuhn, the anamorph of *Thanatephorus cucumeri* (Frank.) Donk, causes seedling blight, pre- or post-emergence damping-off, sore shin and root rot of cotton seedlings (Fulton et al., 1956). *R. solani* colonizes soft tissues and forms infection cautions. From these cautions, the fungus penetrates the epidermis and destroys plant cells (Watkins, 1981). Severe damping-off occurred by increasing sowing depth of seed during early cool conditions (Moubasher, 1958). *R. solani* is an ubiquitous soil-borne

fungus comprising plant parasites and saprophytes. The species *R. solani* affects many agricultural and horticultural crops (Ogoshi, 1987) and is composed of genetically isolated groups (Adams, 1988). The identification and classification of these groups are primarily based on anastomosis behaviour (Ogoshi, 1972). To date, 14 anastomosis groups (AGs) have been recognized (Carling et al., 2002; El-Samawaty, 2008). Isolates of *R. solani* from different AGs generally do not anastomose with each other (Carling, 1996). Many of these AGs have been subdivided on the basis of host range, cultural morphology and biochemical or molecular characteristics (Ogoshi, 1987). A certain degree of host specificity may occur amongst AGs (Grosch et al., 2004).

In Egypt, Moustafa et al. (1995) isolated several soil-borne pathogens from diseased cotton seedlings collected from different fields but they stated that *R. solani* AG-4 was the most frequently isolated causal pathogen of the disease.

Some isolates of *R. solani* AG-2-2, AG-4 and AG-5 reduced emergence and caused root discoloration on

\*Corresponding author. E-mail: kaldkas5@hotmail.com.

maize, cotton and sorghum seedlings during pathogenicity studies (Rush et al., 1994). Also, *R. solani* AG-4 and AG-5 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 tissues (Baird et al., 1995). The pathogenicity of 39 isolates of *R. solani* AG-4 and one isolate belonging to AG-2-2 were evaluated under greenhouse conditions on cotton (Giza 75); most of the virulent isolates exhibited pre-emergence damping-off (El-Akkad, 1997). The objective of this study is to classify *R. solani* isolates into AGs and study potential pathogenicity on cotton (cultivar Giza 86).

## MATERIALS AND METHODS

### Isolates collection

Twenty eight isolates of *R. solani* which originated from cotton seedlings were obtained from the fungal collection of the Cotton Disease Research Section, Plant Pathology Research Institute, Agricultural Research Centre, Giza, Egypt, and twenty three isolates from other hosts were obtained from the same institute; eight from peanut, five from chickpea, two from each of flax, tomato and watermelon and one from each of potato, cantaloupe, pepper and lupine (Table 1 and Figure 1).

### Identification of the anastomosis groups (AGs)

#### Staining nuclei

Aliquots of acidified (HCl) 0.5% trypan blue in lactophenol (Burse et al., 1978) were placed directly on young hyphae growing on agar media. Stained hyphae were covered with a slip and observed (400x) in a Petri dish, or a piece of agar infested with stained hyphae can be placed on a microscope slide. Nuclei are stained dark blue to purple.

#### Hyphal fusion (anastomosis)

Isolates of *Rhizoctonia* were assigned to anastomosis group by pairing the isolates with tester strains and observing the hyphae for fusion. Each isolate was paired with tester isolate of each AG on 2% water agar-coated slides with two replicates in Petri dishes (Windels and Nabben, 1989). Mycelial transfers from the growing margins of young colony on PDA were plated 2 - 3 cm apart in a slide in a 9 cm Petri dish and incubated at 24°C in the dark until advancing hyphae made contact and slightly overlapped.

#### Pathogenicity test

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. The bottles were autoclaved for 30 min. Isolate inoculum, taken from one-week-old culture on PDA was aseptically introduced into the bottle and allowed to colonize the substrate for three weeks.

The present test was carried out by using autoclaved clay loam soil. Batches of soil were infested separately with each isolate inoculum at the rate of 1 g/kg soil. Infested soil was dispensed in 15 cm diameter clay pots and these were planted with 10 seeds per

pot (cultivar Giza 86). In the control treatment, no fungal inoculum was added to the autoclaved soil. Pots were randomly distributed on a greenhouse bench under a temperature of  $24 \pm 3^\circ\text{C}$ ; pre-emergence damping-off was recorded 15 day after planting, while post-emergence damping-off, survival plant, height (cm) and dry weight (mg/plant) were recorded 45 days after planting.

### Statistical analysis of data

Pathogenicity test was carried out in a completely randomized design of five replicates. Percentage data were transformed into or arc sine angles to produce approximately constant variance before carrying out the analysis of variance (ANOVA). Duncan's multiple range test was used to compare between isolate means. ANOVA and correlation analyses were carried out by MSTAT statistical package. Cluster analysis of *R. solani* isolates was performed with the software package SPSS 6.0.

## RESULTS

### Number of nuclei

Determination of the number of nuclei in vegetative hyphal cell is an important process in the identification of *R. solani* (Parmeter and Whitney, 1970). Colonies of *R. solani* were stained with trypan blue and observed to determine numbers of nuclei in individual cells. All the isolates were multinucleate contain from 3 to 8 nuclei per cell (Figure 2).

### Hyphal anastomosis

Microscopic examination showed that all tested isolates were divided into next anastomosis groups. 17 isolates (33.33%) belonged to AG-2-2, 17 and AG-4-HG-I each, 7 isolates (13.73%) to AG-4-HG-II while 10 isolates (19.61%) belonged to AG-5 (Table 1). Perfect fusion between tester isolate and tested isolate is shown in Figure 3.

### Distribution of *R. solani* AGs based on their geographic origin

There are clear differences between AGs regarding their isolation frequencies from different regions. Most distribution of all AGs in different regions concentrated in East (16 isolate) and West Delta (14 isolate), but less distribution was in the North Delta (3 isolates) as shown in Tables 2 and 3. Thus, the isolate frequency of AG-2-2 was 37.5% from West Delta; for AGs-4, both HG-I and HG-II were 35.29 and 42.86% respectively from East Delta while AG-5 was 30% from West and East Deltas. As to South Delta region, the isolation frequency was 75% for AG-2-2, while AG-4-HG-I was 50% in Middle Delta and 42.86% in Middle Egypt. Most pathogenic isolates originated from Beheira but some isolates from

**Table 1.** Anastomosis groups (AGs), hosts, governorates and regions of *Rhizoctonia solani* used in pathogenicity test.

Isolate	Host	Governorate	Region	Anastomosis groups (AGs)
Pe-1	Peanut	Ismalia	East Delta	4-HG-II
Pe-2	Peanut	Ismalia	East Delta	4-HG-I
Pe-3	Peanut	Ismalia	East Delta	4-HG-I
Pe-4	Peanut	Ismalia	East Delta	4-HG-II
Pe-5	Peanut	Ismalia	East Delta	4-HG-II
Pe-6	Peanut	Beheira	West Delta	2-2
Pe-7	Peanut	Beheira	West Delta	2-2
Pe-8	Peanut	Beheira	West Delta	2-2
Ch-9	Chickpea	Beheira	West Delta	5
Ch-10	Chickpea	Beheira	West Delta	4-HG-I
Ch-11	Chickpea	Minya	Middle Egypt	4-HG-I
Ch-12	Chickpea	Minya	Middle Egypt	4-HG-I
Ch-13	Chickpea	Minya	Middle Egypt	2-2
Po-14	Potato	Giza	Middle Egypt	4-HG-II
Fl-15	Flax	Kafr El-Sheikh	North Delta	4-HG-II
Fl-16	Flax	Gharbiya	West Delta	4-HG-II
To-17	Tomato	New Valley	New Valley	2-2
To-18	Tomato	Beheira	West Delta	5
Wa-19	Watermelon	Qualyubiya	South Delta	5
Wa-20	Watermelon	Minufiya	Middle Delta	5
Ca-21	Cantaloupe	Minufiya	Middle Delta	4-HG-I
Pe-22	Pepper	Giza	Middle Egypt	4-HG-I
Lu-23	Lupine	Bani-Sweef	Middle Egypt	4-HG-II
Co-24	Cotton	Daqahlyia	East Delta	5
Co-25	Cotton	Daqahlyia	East Delta	4-HG-I
Co-26	Cotton	Daqahlyia	East Delta	4-HG-I
Co-27	Cotton	Daqahlyia	East Delta	5
Co-28	Cotton	Minufiya	Middle Delta	2-2
Co-29	Cotton	Minufiya	Middle Delta	4-HG-I
Co-30	Cotton	Minufiya	Middle Delta	4-HG-I
Co-31	Cotton	Minufiya	Middle Delta	2-2
Co-32	Cotton	Sharqiya	East Delta	2-2
Co-33	Cotton	Sharqiya	East Delta	2-2
Co-34	Cotton	Sharqiya	East Delta	4-HG-I
Co-35	Cotton	Sharqiya	East Delta	5
Co-36	Cotton	Kafr El-Sheikh	North Delta	5
Co-37	Cotton	Kafr El-Sheikh	North Delta	4-HG-I
Co-38	Cotton	Beheira	West Delta	2-2
Co-39	Cotton	Beheira	West Delta	2-2
Co-40	Cotton	Beheira	West Delta	2-2
Co-41	Cotton	Beheira	West Delta	5
Co-42	Cotton	Gharbiya	West Delta	4-HG-I
Co-43	Cotton	Gharbiya	West Delta	4-HG-I
Co-44	Cotton	Gharbiya	West Delta	4-HG-I
Co-45	Cotton	Damietta	East Delta	2-2
Co-46	Cotton	Damietta	East Delta	2-2
Co-47	Cotton	Damietta	East Delta	4-HG-I
Co-48	Cotton	Qualyubiya	South Delta	2-2
Co-49	Cotton	Qualyubiya	South Delta	2-2
Co-50	Cotton	Qualyubiya	South Delta	2-2
Co-51	Cotton	Faiyoun	Middle Egypt	5



Figure 1. Egyptian governorates constituting the source of isolates used in this study.

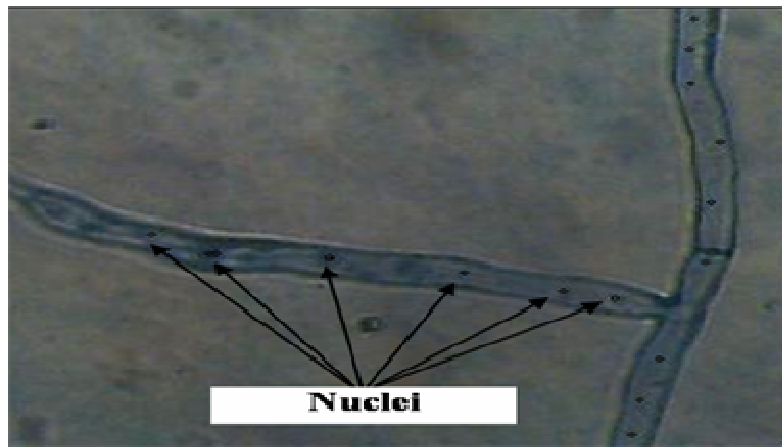


Figure 2. Staining nuclei in vegetative hypha of *R. solani*.

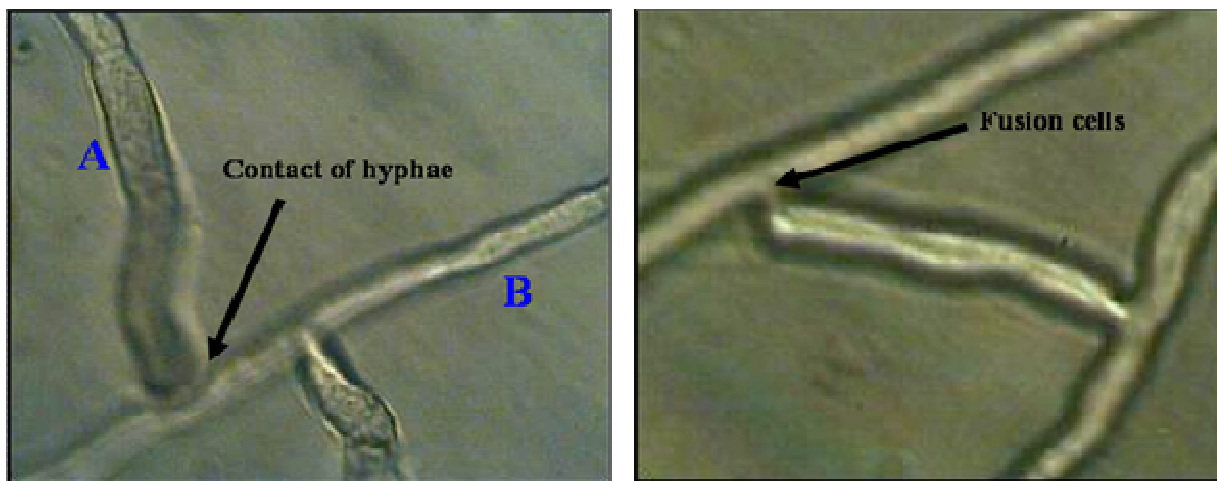


Figure 3. Perfect fusion between *R. solani* hyphae, where A is the teaser and B the isolate. Note lack of plasmolysis of fused cells.

**Table 2.** Distribution of *R. solani* anastomosis groups over different regions.

AG	Region											Total	
	South Delta		Middle Delta		North Delta		East Delta		West Delta		Middle Egypt		
2-2	3 <sup>a</sup>	(18.75) <sup>b</sup>	2	(12.50)	0	(0.00)	4	(25.00)	6	(37.50)	1	(6.25)	16
4-HG-I	0	(0.00)	3	(17.65)	1	(5.88)	6	(35.29)	4	(23.53)	3	(17.65)	17
4-HG-II	0	(0.00)	0	(0.00)	1	(14.29)	3	(42.86)	1	(14.29)	2	(28.57)	7
5	1	(10.00)	1	(10.00)	1	(10.00)	3	(30.00)	3	(30.00)	1	(10.00)	10

<sup>a</sup>Number of isolates from region; <sup>b</sup>number of isolates was expressed as percentage of the total.

**Table 3.** Distribution of *R. solani* anastomosis groups within different regions.

AG	Region											
	South Delta		Middle Delta		North Delta		East Delta		West Delta		Middle Egypt	
2-2	3 <sup>a</sup>	(75.00) <sup>b</sup>	2	(33.33)	0	(0.00)	4	(25.00)	6	(42.86)	1	(14.29)
4-HG-I	0	(0.00)	3	(50.00)	1	(33.33)	6	(37.50)	4	(28.57)	3	(42.86)
4-HG-II	0	(0.00)	0	(0.00)	1	(33.33)	3	(18.75)	1	(7.14)	2	(28.57)
5	1	(25.00)	1	(16.66)	1	(33.33)	3	(18.75)	3	(21.43)	1	(14.29)
<b>Total</b>	4		6		3		16		14		7	

<sup>a</sup>Number of isolates from region; <sup>b</sup>number of isolates was expressed as percentage of the total.

**Table 4.** Distribution of *R. solani* isolates based on geographic origin.

Geographic origin	Number of isolates	Number of cotton pathogenic isolates	Percentage of cotton pathogenic isolates		
			From each governorate	Total isolates <sup>a</sup>	To total pathogenic isolates <sup>b</sup>
Ismalia	5	0	0.00	0.00	0.00
Daqahlyia	4	1	25.00	1.96	5.26
Sharqiya	4	3	75.00	5.88	15.79
Damietta	3	1	33.33	1.96	5.26
Beheira	10	5	50.00	9.80	26.32
Gharbiya	4	2	50.00	3.92	10.53
Minya	3	0	0.00	0.00	0.00
Giza	2	1	50.00	1.96	5.26
Bani-Sweef	1	0	0.00	0.00	0.00
Faiyoum	1	0	0.00	0.00	0.00
Minufiya	6	2	33.33	3.92	10.53
Qalyubiya	4	1	25.00	1.96	5.26
Kefr El-Sheikh	3	3	100.00	5.88	15.79
New Valley	1	0	0.00	0.00	0.00

<sup>a</sup> A total of 51 isolates from different hosts were tested for pathogenicity on cotton seedling (cultivar Giza 86). <sup>b</sup> A total of 19 isolates from different hosts were pathogenic on cotton seedlings (cultivar Giza 86).

some governorates such as Ismalia, New Valley, Bani-Sweef, Faiyoum and Minya were not pathogenic (Table 4).

#### Distribution of *R. solani* AGs based on their different hosts

There were three anastomosis groups from cotton; AG-2-

2, AG-4-HG-I and AG-5, but from other hosts, AG-4-HG-II appeared. The isolate frequency of AG-2-2 was 42.86% within cotton, but each of HG-I and HG-II were 30.43% within other hosts (Table 5).

#### Pathogenicity test

Pathogenicity of 51 isolates of *R. solani* was evaluated on

**Table 5.** Distribution of *R. solani* anastomosis groups over and within different hosts.

AG	Host						Total
	Cotton			Other			
	Number of isolates	Over	Within	Number of isolates	Over	Within	
2-2	12 <sup>a</sup>	(70.59) <sup>b</sup>	(42.86) <sup>b</sup>	5	(29.41)	(21.74)	17
4-HG-I	10	(58.82)	(35.71)	7	(41.18)	(30.43)	17
4-HG-II	0	(0.00)	(0.00)	7	(100.00)	(30.43)	7
5	6	(60.00)	(21.43)	4	(40.00)	(17.39)	10
Total	28			23			

<sup>a</sup>Number of isolates from host; <sup>b</sup>number of isolates was expressed as percentage of the total.

**Table 6.** Pathogenicity of *R. solani* isolates on cotton seedlings (cultivar Giza 86) under greenhouse conditions.

Isolate no.	Pre-emergence damping-off (%) <sup>a</sup>	Post-emergence damping-off (%) <sup>b</sup>	Survival (%) <sup>a</sup>	Plant height (cm)	Dry weight (mg)
Pe-1	28	4	68	19.67	518
Pe-2	32	4	64	20.33	476
Pe-3	28	4	68	20.33	535
Pe-4	30	0	70	19.27	360
Pe-5	24	0	76	18.07	363
Pe-6	54	2	44	16.83	396
Pe-7	38	4	58	18.07	426
Pe-8	46	16	38	18.10	438
Ch-9	34	6	60	17.53	358
Ch-10	32	6	62	17.73	372
Ch-11	16	0	84	18.33	418
Ch-12	34	2	64	19.33	409
Ch-13	36	0	64	17.60	495
Po-14	30	4	66	16.90	483
Fl-15	94	6	0	0.00	0
Fl-16	24	0	76	18.87	427
To-17	28	2	70	19.40	377
To-18	40	2	58	19.33	389
Wa-19	30	2	68	20.80	468
Wa-20	20	2	78	21.33	448
Ca-21	20	4	76	19.47	412
Pe-22	100	0	0	0.00	0
Lu-23	22	4	74	18.20	381
Co-24	36	4	60	16.07	300
Co-25	36	6	58	17.60	494
Co-26	58	2	40	15.50	293
Co-27	22	2	76	17.67	506
Co-28	88	4	8	3.00	106
Co-29	16	0	84	19.13	435
Co-30	22	2	76	18.73	431
Co-31	44	4	52	15.07	337
Co-32	100	0	0	0.00	0
Co-33	68	2	30	16.57	494
Co-34	58	4	38	15.33	306
Co-35	42	2	56	19.40	571
Co-36	48	4	48	17.90	690
Co-37	60	6	34	13.63	371

Table 6. Contd.

Co-38	24	e-j	0	b	76	a-f	18.07	a-c	586	ab
Co-39	90	a*	0	b	10	m*	2.33	g*	67	j*
Co-40	56	c-e*	4	ab	40	h-k*	11.47	de	353	c-h
Co-41	60	bc*	6	ab	34	kl*	8.50	ef*	195	g-j
Co-42	20	g-j	10	ab	70	a-h	15.47	a-d	487	a-f
Co-43	88	a*	4	ab	8	lm*	3.73	fg*	72	j*
Co-44	52	c-h*	6	ab	42	e-k*	15.00	b-d	362	b-h
Co-45	88	Ab*	2	b	10	lm*	5.17	fg*	168	h-j
Co-46	32	c-j	2	b	66	a-j	17.73	a-c	498	a-f
Co-47	22	e-j	0	b	78	a-e	17.73	a-c	573	a-c
Co-48	18	h-j	2	b	80	a-c	19.00	a-c	526	a-e
Co-49	30	c-j	2	b	68	a-h	17.13	a-d	365	b-h
Co-50	40	c-j	6	ab	54	c-k*	13.67	cd	326	d-h
Co-51	26	e-j	2	b	72	a-f	16.13	a-d	441	b-f
Control	14	ij	0	b	86	a-b	16.47	a-d	507	a-f

<sup>a</sup> Percentage data were transformed into arc sine angles before carrying out the analysis of variance to produce approximately constant variance. <sup>b</sup> Percentage data were transformed into  $\sqrt{x+0.5}$  angles before carrying out the analysis of variance to produce approximately constant variance. <sup>c</sup> Means in a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (P= 0.05). An asterisk (\*) denotes a significant difference from the control.

Table 7. Distribution of *R. solani* isolates based on their effects on cotton seedlings (cultivar Giza 86).

Total number of tested isolates	Percentage of isolates, which significantly affected <sup>a</sup>				
	Pre-emergence damping-off (%)	Post-emergence damping-off (%)	Survival (%)	Plant height (cm/plant)	Dry Weight (mg/plant)
51	29.41	1.97	37.25	15.69	13.73

<sup>a</sup> The tested isolates (19 isolate) significantly increased Pre- and post-emergence damping-off, while they significantly decreased survival, plant height and dry weight.

cotton cultivar Giza 86 under greenhouse condition (Table 6). Isolates Pe-22, Co-28, Co-32, Co-39 and Co-43 were more pathogenic which significantly affected all parameters expect post-emergence damping-off. The tested isolates significantly increased pre- and post-emergence damping-off, 15 isolates significantly affected pre-emergence damping-off, but one isolate affected post-emergence damping-off, while they significantly decreased survival, plant height and dry weight. The highly significant effect of pathogenic isolates was in the survival and pre-emergence stages which represented 37.25 and 29.41 % respectively (Table 7).

Twenty eight isolates of *R. solani* which originated from cotton were variable in their pathogenic potential, 15 isolates were pathogenic to cotton cultivar Giza 86 and other were less pathogenic and caused no significant effect (Table 9). Also, two isolates from peanut and one isolate from flax and pepper showed pathogenic potential and caused significant effect on cotton seedling.

Correlation among variables used for evaluating pathogenicity of *R. solani* isolates on cotton seedlings are shown in Table 10 ( $p < 0.01$ ). Significant negative correlation was observed between pre-emergence

damping off and each of survival, plant height and dry weight.

Correlation between post-emergence and other variables was non-significant but highly significant positive correlation was found between survival and each of plant height and dry weight.

Effects of *R. solani* anastomosis groups on cotton seedling disease variables were compared on cultivar Giza 86 under greenhouse condition (Table 11). There are no significant differences between AGs in effects on the cotton seedling variables.

A cluster analysis (Figure 4) of 51 *R. solani* isolates was constructed based on virulence of these isolates on cotton seedlings (cultivar Giza 86). Three groups of similar isolates were identified by cluster analysis. Grouping the isolates based on their virulence patterns was not related to their geographic origins or AG or host.

## DISCUSSION

Twenty eight isolates of *R. solani* were obtained from cotton seedlings and twenty three isolates from other

**Table 8.** Distribution of *R. solani* isolates based on the anastomosis group.

AG	Number of isolates	Number of cotton pathogenic isolates	Percentage of cotton pathogenic isolates		
			From each AG	To total isolates <sup>a</sup>	To total pathogenic isolates <sup>b</sup>
2-2	17	10	58.28	19.61	52.63
4-HG-I	17	6	35.29	11.76	31.58
4-HG-II	7	1	14.29	1.96	5.26
5	10	2	20	3.92	10.53

<sup>a</sup> A total of 51 isolates from different hosts were tested for pathogenicity on cotton seedlings (cultivar Giza 86); <sup>b</sup> a total of 19 isolates from different hosts were pathogenic on cotton seedlings (cultivar Giza 86).

**Table 9.** Distribution of *R. solani* isolates based on the hosts used in isolation.

Host	Number of isolates	Number of cotton pathogenic isolates	Percentage of cotton pathogenic isolates		
			From each host	To total isolates <sup>a</sup>	To total pathogenic isolates <sup>b</sup>
Cotton	28	15	53.57	29.41	78.94
Peanut	8	2	25.00	3.92	10.53
Chickpea	5	0	0.00	0.00	0.00
Flax	2	1	50.00	1.96	5.26
Tomato	2	0	0.00	0.00	0.00
Watermelon	2	0	0.00	0.00	0.00
Cantaloupe	1	0	0.00	0.00	0.00
Pepper	1	1	100.00	1.96	5.26
Lupine	1	0	0.00	0.00	0.00
Potato	1	0	00.00	0.00	0.00

<sup>a</sup>A total of 51 isolates from different hosts were tested for pathogenicity on cotton seedlings (cultivar Giza 86); <sup>b</sup> a total of 19 isolates from different hosts were pathogenic on cotton seedlings (cultivar Giza 86).

**Table 10.** Correlation among variables used for evaluating pathogenicity of *R. solani* isolates on cotton seedlings (cultivar Giza 86).

Variable	Variables			
	2	3	4	5
Pre-emergence damping-off (%)	0.041	-0.993 <sup>a**</sup>	-0.811 <sup>**</sup>	-0.784 <sup>**</sup>
Post-emergence damping-off (%)		-0.160	0.022	0.042
Survival (%)			0.799 <sup>**</sup>	0.769 <sup>**</sup>
Plant height (cm/plant)				0.788 <sup>**</sup>
Dry weight (mg/plant)				

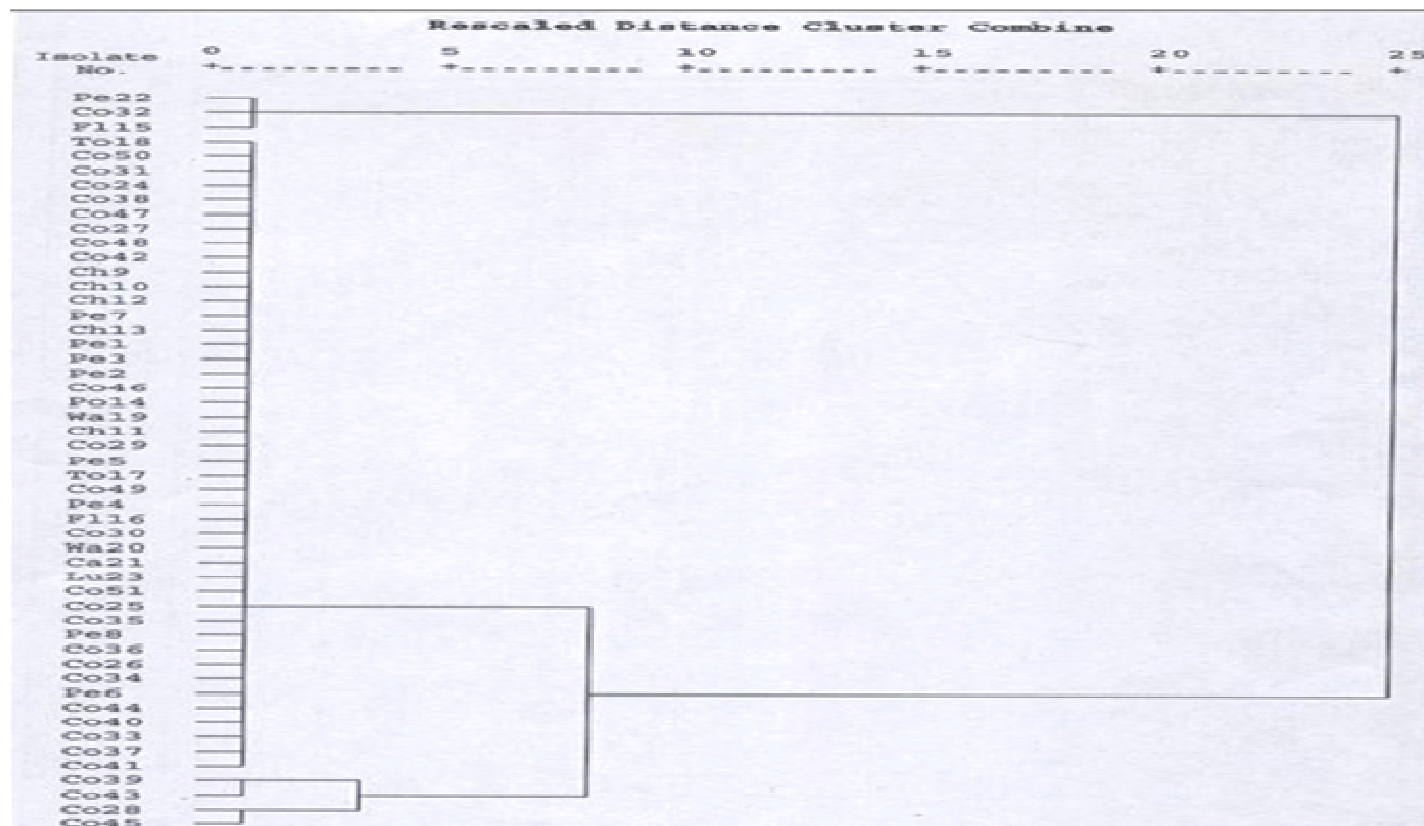
<sup>a</sup> Liner correlation coefficient is significant at  $P < 0.01$  (\*\*).

hosts. All the isolates were multinucleate containing 3 to 8 nuclei per cell. These isolates belonged to AG-2-2, AG-4-HG-I, AG-4-HG-II and AG-5. The first three AG are consistent with the findings of Rush et al. (1994), El-Akkad (1997) and El-Samawaty (2008), who reported similar trends on Egyptian cottons, but the fourth AG (AG-5) was a new record from Egyptian cottons.

Virulence of different *R. solani* isolates was variable during pathogenicity test. Similar results were reported

by Monga and Sheo-Raj (1994), Aqil and Batson (1999) and Asran-Amal (2001). The most pathogenic isolates belonged to AG-2-2 and AG-4-HG-I which represented 52.63 and 31.58% respectively from total pathogenic isolates (Table 8). Significant positive and negative correlations were observed between variables used for evaluating the pathogenicity of the isolates. Correlation between post-emergence and other variables was non-significant but highly significant positive correlation was





**Figure 4.** Phenogram based on average linkage cluster analysis for virulence of 51 *Rhizoctonia solani* isolates on cotton seedlings (cultivar Giza 86).

**Table 11.** Comparison among anastomosis groups of *Rhizoctonia solani* as to their effects on cotton seedling disease variables under greenhouse conditions (Cultivar Giza 86).

AG	Number of isolates	Variable <sup>a</sup>				
		Pre-emergence damping-off (%)	Post-emergence damping-off (%)	Survival (%)	Plant height (cm)	Dry weight (mg/plant)
2-2	17	51.76 <sup>a</sup>	3.06 <sup>a</sup>	45.18 <sup>a</sup>	13.48 <sup>a</sup>	350.47 <sup>a</sup>
4-HG-I	17	40.71 <sup>a</sup>	3.53 <sup>a</sup>	55.76 <sup>a</sup>	15.73 <sup>a</sup>	387.18 <sup>a</sup>
4-HG-II	7	36.00 <sup>a</sup>	2.57 <sup>a</sup>	61.43 <sup>a</sup>	15.85 <sup>a</sup>	361.71 <sup>a</sup>
5	10	35.80 <sup>a</sup>	3.20 <sup>a</sup>	61.00 <sup>a</sup>	17.47 <sup>a</sup>	436.60 <sup>a</sup>

<sup>a</sup>Means in a column followed by the same letter are not significantly different according to Duncans multiple range test (P = 0.05).

found between survival and each of plant height and dry weight. This result is in agreement with that of El-Samawaty (2008) who found highly significant positive correlation between survival and dry weight of cotton seedlings.

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). 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* (Porta-Puglia et al., 1996),

20 isolates of *Macrophomina phaseolina* (Omar, 2005) and 52 isolates of *R. solani* (El-samawaty, 2008). In this study, cluster analysis divided the isolates into groups based on their virulence patterns on cotton cultivar Giza 86; however, grouping the isolates was not related to their geographic origins, AG or host.

#### REFERENCES

- Adams GC (1988). Thanatephorus cucumeris (*Rhizoctonia solani*), a species complex of wide host range. *Adv. Plant Pathol.* 6: 535–552.  
Aqil T, Batson EW (1999). Evaluation of radical assay for screening

- cotton genotypes for resistance to the pathogens of seedling disease complex. Pakistan. J. Phytopathol. 11: 11-16.
- Asran, Amal A (2001). Studies on cotton rhizosphere microorganisms and their role as bio-control agents for root rot diseases. PhD thesis, Cairo University, p.156.
- Asran, Amal A, Abd-Elsalam KA, Omar MR, Aly AA (2005). Antagonistic potential of *Trichoderma* spp. against *Rhizoctonia solani* and use of M13 microsatellite-primed PCR to evaluate the antagonist genetic variation. J. Plant Dis. Prot. 112: 6, 550–561.
- Baird RE, Brenneman BT, Bell KD (1995). First report of *Rhizoctonia* sp. CAG-5 on cotton in Georgia. Plant Dis. 79: 320.
- Burpe LL, Sanders PL, Cole HJ, Kim SH (1978). A staining technique for nuclei of *Rhizoctonia solani* and related fungi. Mycologia 70: 1281-1283.
- Carling DE, Sneh B, Jabaji-Hare S, Neate SM, Dijkstra G (1996). Grouping in *Rhizoctonia solani* by hyphal anastomosis reaction. In: (eds) *Rhizoctonia* species: Taxonomy, Molecular Biology, Ecology; Pathology and Disease Control Kluwer, Dordrecht. pp. 37–43
- Carling DE, Baird RE, Gitaitis RD, Brained KA, Kuninaga S (2002). Characterization of AG-13, a Newly Reported Anastomosis Group of *Rhizoctonia solani*. Dis. Control Pest Manage. 92(8): 893-899.
- El-Akkad, Salwa AF (1997). Studies on anastomosis group of *Rhizoctonia solani*. PhD. thesis, Cairo University, p. 143.
- El-Samawaty AMA, Amal A, Asran MR, Omar, Abd-Elsalam KA (2008). Anastomosis Groups, Pathogenicity, and Cellulase Production of *Rhizoctonia solani* from Cotton. Pest technol. 1(2): 117-124.
- Fulton ND, Awadde B, Thomas JA (1956). Influence of planting date on fungi isolated from diseased cotton seedlings. Plant Dis. Rep. 40: 556- 558.
- Grosch R, Schneider JHM, Kofeet A (2004). Characterization of *Rhizoctonia solani* anastomosis groups causing bottom rot in field-grown lettuce in Germany. Eur. J. Plant Pathol. 110: 53–62.
- Lebeda A, Jendrulek T (1987). Application of cluster analysis for establishment of genetic similarity in gene-for-gene host-parasite relationships. J. Phytopathol. 119: 131-141.
- Monga D, Sheo-Raj (1994). Cultural and pathogenic variations in the isolates of *Rhizoctonia* species causing root rot of cotton. Indian of Phytopathol. 47: 403-407.
- Moubasher AH (1958). Studies on the damping-off disease of cotton in Egypt with a note on the effect of origin of *Rhizoctonia* isolate on its pathogenicity. Ph. D. thesis, Cairo University, p. 233.
- Moustafa SM, Mona M, Ragab DR, Sumner M, M Ragab (1995). Biological control of *Rhizoctonia solani* (AG-4) in cotton seedlings. Egypt. J. Agric. Res. 73: 561–573.
- Ogoshi A (1972). Grouping of *Rhizoctonia solani* Kuhn with hyphal anastomosis. Anal. Phytopathol. Soc. Jpn. 38: 117–122.
- Ogoshi A (1987). Ecology and pathogenicity of anastomosis and intra-specific groups of *Rhizoctonia solani* Kuhn. Annu. Rev. Phytopathol. 25: 125–143.
- Omar MR (2005). Pathological and biochemical studies on *Macrophomina phaseolina* pathogenic on cotton. PhD. thesis, Seuz Canal University, Ismailia, Egypt, p. 178.
- Parmeter JR, Whitney HS (1970). Taxonomy and nomenclature of the imperfect state. Pages 7-19 in: JR Parmeter Jr., ed. Biology and pathology of *Rhizoctonia solani*. University of California Press, Berkeley p. 255.
- Porta-Puglia A, Crino P, Mosconi C (1996). Variability in virulence to chickpea of an Italian population of *Ascochyta rabiei*. Plant Dis. 80: 39-41.
- 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 Dis. 78: 349-352.
- Watkins GM (1981). Compendium of Cotton Diseases. The American Phytopathol. Society. St. Paul, Minnesota, p. 87.
- Windels CE, Nabben DJ (1989). Characterization and pathogenicity of anastomosis Groups of *Rhizoctonia Solani* Isolated from *Beta vulgaris*. Phytopathology 79: 83-88.