Egypt. J. Phytopathol., Vol. 37, No. 1, pp. 21-33 (2009)

Pathogenicity and Protein Electrophoresis of Different Cotton *Rhizoctonia solani* Isolates

M.S. Mikhail*; K.K. Sabet*; M.R. Omar; E.M. Hussein** and Kh.K. Kasem*****

* *Plant Pathol. Dept., Fac. of Agric., Cairo Univ., Egypt.*

** *Plant Pathol. Res. Inst., Agric. Res. Centre, Giza, Egypt.*

*** *Agric. Res. Centre in Hamah, General Commission for Scientific Agric. Res., Syria.*

Twenty one isolates of *Rhizoctonia solani* were categorized according to anastomosis groups into AG-4-HG-I (8 isolates), AG-2-2 (9 isolates) and AG-5 (4 isolates). Their pathogenic abilities were tested on cotton cultivar Giza 86. Variable levels of pre-emergence damping-off resulted in response to the different isolates, however, no significant differences were found as to the levels of post-emergence damping-off. Fourteen isolates led to significant reduction in the survival. Soluble proteins of the fungal isolates were electrophoresed using PAGE and SDS-PAGE. Cluster analysis of the protein banding patterns by the UWPGM of arithmetic means placed the fungal isolates in distant groups in case of PAGE and SDS-PAGE; however, isolates differed dispending on the method of protein separation. Similarity coefficients matrix (SCM) was calculated for both. Results revealed no relationship between anastomosis grouping, level of virulence or geographic origin.

Keywords: Anastomosis groups, cotton, PAGE, pathogenicity, SDS-PAGE and *Rhizoctonia solani*.

Rhizoctonia solani Kühn the anamorphic of *Thanatephorus cucumeris* (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 cushions, from these cushions, the fungus penetrates the epidermis and destroys plant cells (Watkins, 1981). *R. solani* is composed of genetically isolated groups (Adams, 1988). The identification and classification of these groups is primarily based on anastomosis behaviour (Ogoshi, 1972). To date, 14 anastomosis groups (AGs) have been recognized (Carling *et al.*, 2002; El-Samawaty, 2008). Some isolates of *R. solani* AG-2-2, AG-4 and AG-5 reduced emergence of maize, cotton and sorghum seedlings (Rush *et al.*, 1994). The pathogenicity of 39 isolates of *R. solani* AG-4 and one isolate of AG-2-2 were evaluated under greenhouse conditions on cotton cultivar Giza 75; most of the virulent isolates exhibited pre-emergence damping-off (El-Akkad, 1997).

Gel electrophoresis of proteins has been widely used for studying variation in fungal populations. For instance, Zuber and Manibushanrao (1982) found that polyacrylamide gel electrophoresis (PAGE) patterns of protein reflected marked variation among five differentially virulent isolates of *R. solani*. El-Akkad (1997)

found heterogeneity in protein banding patterns among AG-4 isolates of *R. solani*. Hussein *et al.* (2000) used cluster analysis to compare protein banding patterns obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) from 17 isolates of multinucleate *R. solani* (AG-4) and one isolate of binucleate *Rhizoctonia*. The analysis provided clear-cut differentiating features between binucleate and multinucleate *Rhizoctonia* spp. The binucleate isolate of *Rhizoctonia* was placed in a separate cluster remote from that which included all the multinucleate isolates of AG-4. However, the latter cluster was divided into several sub-clusters. The observed differences in protein profiles of the isolates were not related to virulence, geographic origin, or source (cultivar used in isolation). These results suggest that AG-4 of *R. solani* is a heterogeneous group of isolates. Mohammadi *et al.* (2003) studied the genetic variation among 20 isolates of *R. solani* AG-1 subgroups (AG-1-IA and AG-1-IB) collected from Mâzandaran province, Iran, and standard isolates of these subgroups, by total soluble protein profile. The soluble protein patterns were similar among the *R. solani* isolates examined; however, minor differences in banding pattern were observed between the two subgroups. Based on cluster analysis and similarity matrix, the fungal isolates were divided into two distinct groups of I and II consistent with the previously reported AG-1-IA and AG-1-IB subgroups in AG1. The present investigation was initiated to determine whether *R. solani* isolates can be distinguished by their pathogenicity on cotton, in conjunction with their electrophoretic protein patterns.

Materials and Methods

Isolates collection:

Twenty one isolates of *R. solani* (Table 1), originated from cotton seedlings, were obtained from the fungal collection of the Cotton Dis. Res. Sect., Plant Pathol. Res. Inst., Agric. Res. Centre, Giza, Egypt.

Pathogenicity test:

A substrate for growth of isolates was prepared in 500-ml glass bottles; each bottle contained 50g of sorghum grains and 40ml 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 test was carried out by using autoclaved clay loam soil. Batches of soil were infested separately with each isolate at the rate of 1g/kg soil. Infested soil was dispensed in 15-cm-diam. clay pots and these were planted with 10 seeds per pot cultivar Giza 86 (5 replicates). In the control treatment, autoclaved sorghum was added to the autoclaved soil. Pots were randomly distributed on a greenhouse bench under a temperature 24±3°C. Pre-emergence damping-off was recorded 15 days after planting, while post-emergence damping-off, survival plant, height (cm) and dry weight (mg/plant) were recorded 45 days after planting.

Extraction of proteins from R. solani isolates:

Proteins were prepared according to the methods described by Guseva and Gromova (1982). The grown mycelium for 8 days at 20-30°C on liquid Czapeck's medium was harvested by filtration through cheesecloth, washed with distilled water several times, and freeze-dried. This frozen mycelium was suspended in phosphate buffer pH 8.3 (1-3 ml/g mycelium), mixed thoroughly with glass beads and ground in liquid nitrogen to a fine powder. The ground mycelium was centrifuged at 19,000rpm for 30 min. at 0°C. The protein content in supernatant was estimated according to Bradford (1976) by using bovine serum albumin as a standard protein.

Electrophoresis of native protein (PAGE):

Protein extract supernatant was mixed with an equal volume of a solution containing 20% glycerol (v/v) and 0.1ml bromophenol blue in 0.15M Tris-HCl, pH6.8. Twenty microlitres of the resulting suspension (40 to 60µg of protein) were subjected to electrophoresis in 25mM Tris buffer containing 192mM glycine at pH8.3. Electrophoresis was conducted at 10°C for 4hrs. in a 7.5% polyacrylamide gel with a 3.5% stacking gel, at 15 and 30mA, respectively, until the dye band reached the bottom of the separating gel (Laemmli, 1970). Electrophoresis was performed in a vertical slab mould (16x18x0.15cm). Gels were stained with silver nitrate for the detection of protein bands (Sammons *et al.*, 1981).

Electrophoresis of dissociated protein (SDS-PAGE):

Each supernatant was mixed with an equal volume of a solution consisting of (by volume) 64% buffer (0.15 M Tris-HCl, pH6.8), 20% glycerol; 6% SDS; 10% 2-mercaptoethanol and 0.1% bromophenol blue, before boiling in a water bath for 3min. Twenty-microlitre samples (40µg of protein) were subjected to electrophoresis in a 7.5% polyacrylamide gel prepared in 0.1% SDS with a 3.5% stacking gel (Laemmli, 1970). Electrophoresis and staining of gels were carried out as previously mentioned in PAGE.

Gel analysis:

Protein patterns obtained by PAGE and SDS-PAGE were clustered by gel documentation system (Uvitec, Cambridge, UK) by the unweighted pair group method of arithmetic means (UPGMA) according to Sneath and Sokal (1973). Similarity coefficient matrix among protein banding patterns was calculated based on the number of shared bands (Nei and Li, 1979).

Statistical analysis of pathogenicity test:

Pathogenicity test was carried out in a completely randomized design of five replicates. Percentage data were transformed into $\sqrt{x + 0.5}$ or arcsine 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 was carried out by MSTAT-C statistical package.

Results and Discussion

Pathogenicity test:

Table (1) show the *R. solani* isolates used in this investigation, originated from 8 governorates of lower Egypt, were categorized into 3 anastomosis groups,

i.e. AG-2-2, AG-4-HG-I and AG-5. It is obvious that different AGs were found in the same governorate, except the two isolates from Gharbiya which belonged to AG-4-HG-I and one from Qualubiya which belonged to AG-2-2.

Pathogenicity of 21 *R. solani* isolates was evaluated on cotton cultivar Giza 86 under greenhouse conditions (Table 2). Eleven isolates significantly increased pre-emergence damping-off, 14 isolates decreased survival significantly. Plant height and dry weight were significantly affected by 6 and 5 isolates respectively. Isolates no$_s$. 28, 32, 39, 43, and 45 were the most pathogenic because they significantly affected all parameters except post-emergence damping-off and dry weight for isolate 45. None of the tested isolates caused significant post-emergence damping-off infection, while 52.38% of the isolates caused significant pre-emergence damping-off (Table 3). The most pathogenic isolates were isolated from Beheira, Sharqiya, Minufiya, Gharbiya, and Damietta governorates representing 21.43% of the total pathogenic isolates (Table 4). It is obvious that different isolates behaved differently with respect to disease-causing ability in the pre-emergence stage, some isolates affected plant height as well as dry weight. Similar results were reported by Monga and Sheo-Raj (1994), Aqil and Batson (1999) and Asran (2001). The above mentioned isolates resulted in level of pre-emergence damping-off amounting to 88% and more. These isolates except no. 43 belonged to AG-2-2. Considering to the rest of the data in Table 2 reveals that there was no general trend correlating AGs with virulence.

Table 1. Anastomosis Groups, governorates and region of *Rhizoctonia solani* used in pathogenicity test

Isolate No.	Anastomosis Group (AG)	Governorate	Region
26	4-HG-I	Daquahlyia	East Delta
27	5	Daquahlyia	East Delta
28	2-2	Minufiya	Mid-Delta
29	4-HG-I	Minufiya	Mid-Delta
30	4-HG-I	Minufiya	Mid-Delta
31	2-2	Minufiya	Mid-Delta
32	2-2	Sharqiya	East Delta
33	2-2	Sharqiya	East Delta
34	4-HG-I	Sharqiya	East Delta
35	5	Sharqiya	East Delta
36	5	Kafr El-Sheikh	North Delta
37	4-HG-I	Kafr El-Sheikh	North Delta
39	2-2	Beheira	West Delta
40	2-2	Beheira	West Delta
41	5	Beheira	West Delta
42	4-HG-I	Gharbiya	Mid-Delta
43	4-HG-I	Gharbiya	Mid-Delta
45	2-2	Damietta	East Delta
46	2-2	Damietta	East Delta
47	4-HG-I	Damietta	East Delta
50	2-2	Qualubiya	South Delta

Table 2. Pathogenicity of *Rhizoctonia solani* isolates on cotton seedlings (cultivar Giza 86) under greenhouse conditions

Isolate No.	Pre-emergence damping-off (%) [a]	Post-emergence damping-off (%) [b]	Survival (%) [a]	Plant height (cm)	Dry weight (mg/plant)
26	58 c-f*	2 ab	40 f-k*	15.50 a-d	293 f-i
27	22 e-j	2 b	76 a-f	17.67 a-c	506 a-f
28	88 a*	4 b	8 m*	3.00 g*	106 ij*
29	16 j	0 ab	84 ab	19.13 a-c	435 b-f
30	22 g-j	2 b	76 a-d	18.73 a-c	431 b-f
31	44 c-i	4 b	52 c-k*	15.07 b-d	337 d-h
32	100 a*	0 b	0 m*	0.00 g*	0 j*
33	68 bc*	2 b	30 i-k*	16.57 a-d	494 a-f
34	58 c-f*	4 ab	38 g-k*	15.33 a-d	306 e-h
35	42 c-j	2 b	56 b-k	19.40 a-c	571 a-c
36	48 c-i	4 ab	48 c-k*	17.90 a-c	690 a*
37	60 b-d*	6 ab	34 jk*	13.63 cd	371 b-h
39	90 a*	0 b	10 m*	2.33 g*	67 j*
40	56 c-e*	4 ab	40 h-k*	11.47 de	353 c-h
41	60 bc*	6 ab	34 kl*	8.50 ef*	195 g-j
42	20 g-j	10 ab	70 a-h	15.47 a-d	487 a-f
43	88 a*	4 ab	8 lm*	3.73 fg*	72 j*
45	88 Ab*	2 b	10 lm*	5.17 fg*	168 h-j
46	32 c-j	2 b	66 a-j	17.73 a-c	498 a-f
47	22 e-j	0 b	78 a-e	17.73 a-c	573 a-c
50	40 c-j	6 ab	54 c-k*	13.67 cd	326 d-h
Control	14 ij	0 b	86 a-b	a-d	507 a-f

[a] Percentage data were transformed into arcsine angles before carrying out the analysis of variance to produce approximately constant variance.

[b] Percentage data were transformed into $\sqrt{x + 0.5}$ angles before carrying out the analysis of variance to produce approximately constant variance.

[c] 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 3. Distribution of *Rhizoctonia solani* isolates based on their effects on cotton seedlings (cultivar Giza 86)

Total number of tested isolates	Percentage of isolates, which significantly affected [a]				
	Pre-emergence damping-off (%)	Post-mergence damping-off (%)	Survival (%)	Plant height (cm)	Dry weight (mg/plant)
21	52.38	0.00	66.67	28.57	23.81

[a] Tested isolates significantly increased pre-emergence damping-off, while they significantly decreased survival, plant height and dry weight.

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

Geographic origin	Total number of tested isolates	Number of pathogenic isolates	Pathogenic isolates as percentage of		
			governorate	total isolates	pathogenic isolates
Beheira	3	3	100.00	14.29	21.43
Minufiya	4	2	50.00	9.52	14.29
Daquahlyia	2	1	50.00	4.76	7.14
Qualubiya	1	1	100.00	4.76	7.14
Gharbiya	2	1	50.00	4.76	7.14
Sharqiya	4	3	75.00	14.29	21.43
Kafr El-Sheikh	2	2	100.00	9.52	14.29
Damietta	3	1	33.33	4.76	7.14

Differentiation among Rhizoctonia solani isolates based on their protein banding patterns obtained by PAGE:

Native proteins of the tested isolates were electrophoresed by PAGE. Electrophoretically fractionated proteins (Fig. 1) were used to calculate the similarity coefficients matrix (SCM) shown in Table (5). A phenogram (Fig. 2) was constructed based on similarity levels (SLs) generated from cluster analysis of SCM values. In this phenogram, the isolates appear to segregate into four groups (clusters). The first included 10 isolates with an overall similarity level 41%; however, there were pairs of isolates representing subclusters having high similarity levels, such as isolates 26 and 37; 45 and 46; 32 and 33; T4 and 39. Similarity levels of these subclusters ranged between 64% as in 45-46 and 52% as in T4-39. These four pairs showed an overall similarity with the pair 27-41 of 41%. The second group included four isolates in two 50-43 and 40-42 with SLs 33% and 52% respectively, with an overall SL of 29%. The second cluster showed SL with the first of 23% or 77% dissimilarity. The third cluster included seven isolates (36, T2, 28, 29, 30, 31, and 34). Similarity level in pairs ranged between 71% (isolates 30 and 31) and 37% (isolates 36 and T2). Member of this cluster exhibited overall SL 22% and showed an SL with the above two of 18%. The fourth cluster included only three isolates (34, T5, and 50) with SL 32% and which was connected with the above three clusters at a similarity level 12% only.

It is obvious from the above and referring to data in Tables 1 and 2 that each cluster encompasses isolates showing different geographic origin, levels of virulence as well as different anastomosis groups. Therefore, grouping isolates of *R. solani* based on their protein pattern as clarified by PAGE is not related to their virulence, AGs or geographic origin, this confirming previous result repeated by Hussein *et al.* (2000).

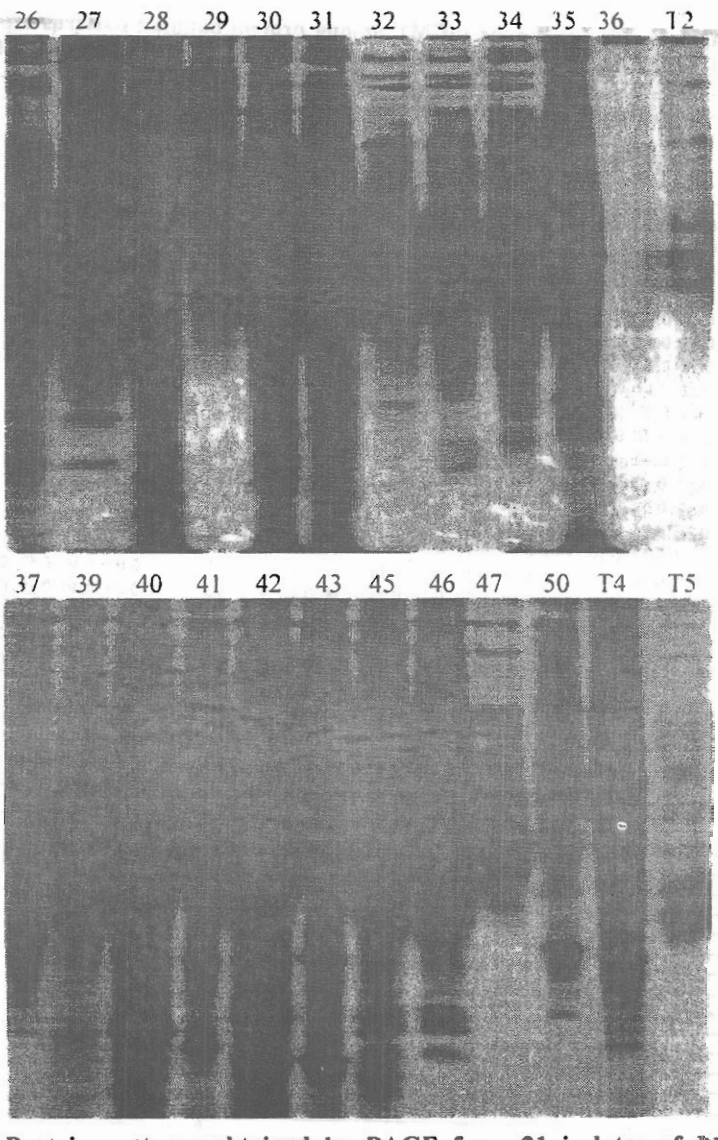

Fig. 1. Protein patterns obtained by PAGE from 21 isolates of *Rhizoctonia solani* from cotton and three tester isolates. (T2: Tester of AG-2-2, T4: Tester of AG-4-HG-I, T5: Tester of AG-5).

Table 5. Similarity coefficient matrix (SCM)* among protein banding patterns separated by PAGE for 21 isolates of *Rhizoctonia solani* from cotton and three tester isolates

Isolate	26	35	36	T2	27	28	29	30	31	32	33	34	37	50	T4	T5	39	40	41	42	43	45	46	47
26																								
35	0.08																							
36	0.22	0.17																						
T2-2[a]	0.14	0.17	0.37																					
27	0.41	0.32	0.21	0.21																				
28	0.25	0.20	0.35	0.25	0.16																			
29	0.14	0.25	0.15	0.21	0.21	0.42																		
30	0.18	0.11	0.19	0.18	0.00	0.33	0.18																	
31	0.26	0.21	0.18	0.17	0.17	0.42	0.43	0.71																
32	0.42	0.28	0.19	0.18	0.35	0.21	0.30	0.22	0.21															
33	0.47	0.23	0.21	0.33	0.39	0.23	0.20	0.25	0.32	0.63														
34	0.21	0.25	0.22	0.14	0.07	0.25	0.21	0.45	0.35	0.30	0.47													
37	0.62	0.16	0.14	0.07	0.40	0.24	0.34	0.09	0.17	0.41	0.45	0.14												
50	0.15	0.17	0.15	0.07	0.36	0.17	0.30	0.19	0.09	0.31	0.21	0.22	0.29											
T4[b]	0.43	0.11	0.09	0.00	0.33	0.32	0.35	0.24	0.22	0.29	0.32	0.35	0.33	0.45										
T5[c]	0.25	0.40	0.17	0.17	0.00	0.20	0.25	0.22	0.21	0.07	0.08	0.08	0.24	0.17	0.11									
39	0.42	0.10	0.17	0.08	0.24	0.40	0.08	0.22	0.21	0.28	0.41	0.33	0.48	0.35	0.53	0.20								
40	0.32	0.10	0.25	0.16	0.15	0.29	0.08	0.21	0.10	0.33	0.22	0.24	0.38	0.25	0.30	0.19	0.48							
41	0.42	0.20	0.09	0.17	0.16	0.10	0.17	0.11	0.11	0.34	0.31	0.33	0.16	0.26	0.11	0.30	0.30	0.29						
42	0.23	0.27	0.24	0.15	0.30	0.18	0.00	0.10	0.00	0.19	0.36	0.31	0.37	0.32	0.19	0.00	0.36	0.52	0.18					
43	0.25	0.20	0.26	0.33	0.32	0.20	0.25	0.22	0.21	0.34	0.38	0.33	0.32	0.35	0.32	0.20	0.40	0.19	0.20	0.27				
45	0.50	0.17	0.15	0.21	0.34	0.25	0.21	0.18	0.17	0.30	0.47	0.29	0.41	0.15	0.35	0.08	0.42	0.40	0.17	0.46	0.25			
46	0.45	0.22	0.20	0.19	0.35	0.37	0.32	0.32	0.38	0.33	0.48	0.45	0.38	0.20	0.23	0.15	0.30	0.36	0.22	0.34	0.30	0.65		
47	0.09	0.32	0.18	0.17	0.17	0.32	0.09	0.12	0.11	0.21	0.16	0.09	0.25	0.27	0.00	0.11	0.11	0.20	0.11	0.29	0.11	0.17	0.23	

* SCM was calculated based on the number of shared bands (Nei and Li 1979).

[a] Tester of AG-2-2, [b] tester of AG-4-HG-I and [c] tester of AG-5.

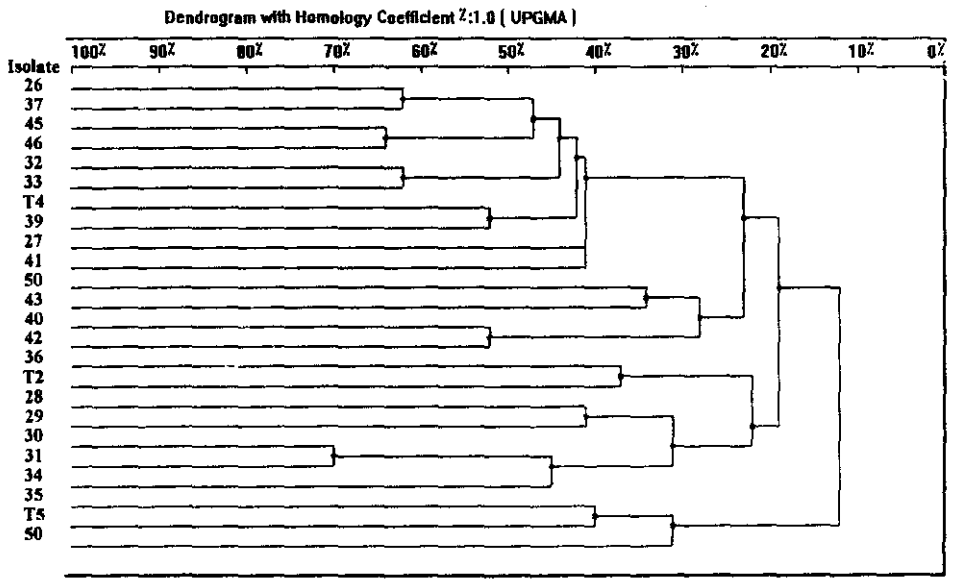

Fig. 2. Phenogram based on average linkage cluster analysis of electrophoretic protein patterns obtained by PAGE from 21 isolates of *R. solani* from cotton and three tester isolates. (T2: Tester of AG-2-2, T4: tester of AG-4-HG-I, T5: tester of AG-5).

Differentiation among Rhizoctonia solani isolates based on their protein banding patterns obtained by SDS-PAGE:

Considering data generated from SDS-PAGE (Fig. 3) and based on SCM shown in Table (6) and phenogram (Fig. 4); it is apparent that isolates separated into large number of clusters. The first cluster included 5 isolates (34, 35, 33, 30 and T2) with an overall similarity level of 34%. Isolates 35 and 33 showed SL of 57%; both from Sharqiya and resulted in 68% and 42% pre-emergence damping-off, respectively. These two isolates belonged to different AGs (2-2 and 5). Isolate 34 (AG-4-HG-I) showed SL with the isolates 33 and 35 of 48% and generated also from Sharqiya, resulting in 58% pre-emergence damping-off. The absence a given trend is obvious with respect to the possible relationship between clustering, virulence, AGs or geographic origin. In addition to the first cluster data may point to the presence of 6 more clusters, each containing sub-clusters, except (36-T5) and (26-39). Considering the overall similarity among all clusters, we found a low similarity of about 10%, in other words they showed a 90% dissimilarity indicating a very high variability among the tested isolates, where is reflected by their heterogeneity as to origin, AGs and virulence. In conclusion; protein profiling failed to correlate with the different parameters tested or estimated. Therefore, grouping isolates of *R. solani* based on their protein pattern as clarified by SDS-PAGE is not related to their virulence, AGs or geographic origin, this confirming previous result repeated by El-Akkad (1997) for *R. solani* and Abdel-Sattar *et al.* (2008) for *Macrophomina phaseolina* isolated from cotton.

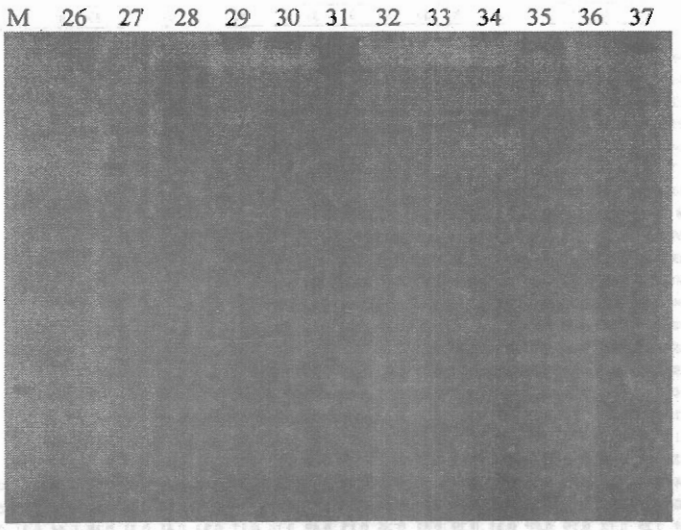

Fig. 3. Protein patterns obtained by SDS-PAGE from 21 isolates of *Rhizoctonia solani* from cotton and three tester isolates. (T2: Tester of AG-2-2, T4: tester of AG-4-HG-I, T5: tester of AG-5).

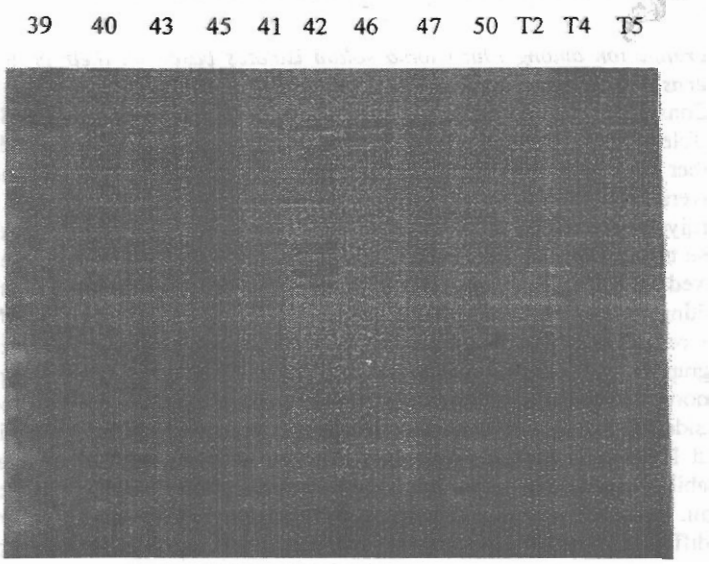

Fig. 3. Continued.

Table 6. Similarity coefficient matrix (SCM)* among protein banding patterns separated by SDS-PAGE for 21 isolates of *Rhizoctonia solani* from cotton and three tester isolates

Isolate	34	35	36	37	26	27	28	29	30	31	32	33	50	T2	T4	T5	39	40	43	45	41	42	46	47
34																								
35	0.48																							
36	0.35	0.37																						
37	0.32	0.29	0.28																					
26	0.07	0.15	0.10	0.12																				
27	0.24	0.30	0.29	0.32	0.00																			
28	0.17	0.31	0.26	0.17	0.00	0.10																		
29	0.30	0.32	0.06	0.41	0.20	0.18	0.21																	
30	038	0.23	0.14	0.31	0.12	0.19	0.22	0.34																
31	0.26	0.32	0.37	0.23	0.23	0.30	0.18	0.47	0.17															
32	0.23	0.24	0.29	0.13	0.09	0.32	0.19	0.17	0.31	0.44														
33	0.50	0.57	0.39	0.42	0.08	0.21	0.19	0.39	0.18	0.38	0.21													
50	0.34	0.31	0.36	0.20	0.10	0.29	0.35	0.06	0.20	0.31	0.33	0.22												
T2-2	0.34	0.24	0.00	0.08	0.13	0.28	0.24	0.30	0.17	0.24	0.20	0.32	0.21											
T4	0.25	0.16	0.06	0.00	0.20	0.24	0.26	0.25	0.21	0.16	0.06	0.17	0.18	0.37										
T5	0.18	0.19	0.46	0.09	0.29	0.14	0.12	0.23	0.00	0.19	0.28	0.13	0.15	0.10	0.38									
39	0.14	0.15	0.00	0.24	0.50	0.09	0.07	0.20	0.12	0.08	0.00	0.17	0.00	0.13	0.20	0.29								
40	0.32	0.22	0.20	0.15	0.22	0.13	0.27	0.20	0.22	0.22	0.18	0.24	0.19	0.32	0.47	0.08	0.11							
43	0.11	0.22	0.20	0.37	0.11	0.06	0.38	0.13	0.37	0.11	0.12	0.06	0.26	0.16	0.13	0.25	0.33	0.29						
45	0.22	0.23	0.14	0.15	0.12	0.26	0.22	0.21	0.23	0.29	0.44	0.18	0.27	0.42	0.28	0.09	0.24	0.37	0.30					
41	0.24	0.35	0.24	0.00	0.00	0.28	0.39	0.18	0.32	0.40	0.22	0.37	0.23	0.28	0.35	0.00	0.00	0.56	0.25	0.26				
42	0.20	0.26	0.25	0.14	0.10	0.24	0.31	0.19	0.34	0.26	0.17	0.33	0.18	0.30	0.25	0.08	0.10	0.60	0.13	0.28	0.65			
46	0.29	0.25	0.12	0.19	0.09	0.33	0.29	0.41	0.26	0.15	0.49	0.16	0.17	0.34	0.41	0.23	0.18	0.44	0.31	0.52	0.17	0.18		
47	0.29	0.30	0.15	0.08	0.00	0.07	0.35	0.15	0.17	0.18	0.27	0.26	0.43	0.18	0.37	0.19	0.00	0.40	0.16	0.33	0.28	0.37	0.28	

* SCM was calculated based on the number of shared bands (Nei and Li 1979).
[a] Tester of AG-2-2. [b] tester of AG-4-HG-I, and [c] Tester of AG-5.

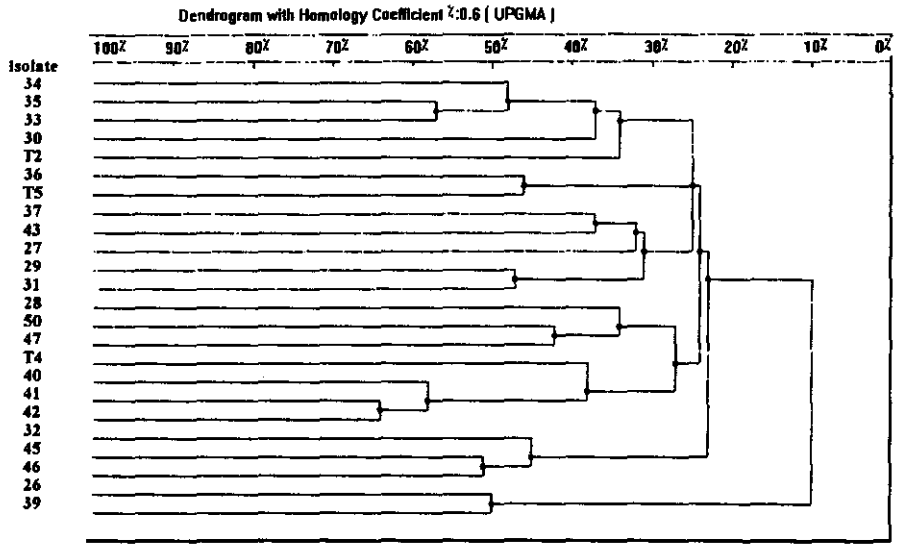

Fig. 4. Phenogram based on average linkage cluster analysis of electrophoretic protein patterns obtained by SDS-PAGE from 21 isolates of *R. solani* from cotton and three tester isolates. (T2: Tester of AG-2-2, T4: tester of AG-4-HG-I, T5: tester of AG-5).

References

Abdel-Sattar, M.A.; Aly, A.A. and Omar, M.R. 2008. Use of pathogenicity and protein electrophoresis to distinguish isolates of *Macrophomina phaseolina* pathogenic on cotton. *J Agric. Sci., Mansoura Univ.*, **33** (1): 207-217.

Adams, G.C. 1988. *Thanatephorus cucumeris* (*Rhizoctonia solani*), a species complex of wide host range. *Advanced Plant Pathology*, 6: 535–552.

Aqil, T. and Batson, E.W. 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.

Asran, Amal A. 2001. Studies on cotton rhizosphere microorganisms and their role as bio-control agents for root rot diseases. PhD thesis, Cairo University, 156pp.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**: 248-254.

Carling, D.E.; Baird, R.E.; Gitaitis, R.D.; Brainard, K.A. and Kuninaga, S. 2002. Characterization of AG-13, a newly reported anastomosis group of *Rhizoctonia solani*. *Disease Control and Pest Management*, **92** (8): 893-899.

El-Akkad, Salwa A.F. 1997. Studies on anastomosis groups of *Rhizoctonia solani*. Ph.D. Thesis, Cairo Univ., 132pp.

El-Samawaty, A.M.A.; Asran, Amal A.; Omar, M.R. and Abd-Elsalam K.A. 2008. Anastomosis groups, pathogenicity, and cellulase production of *Rhizoctonia solani* from cotton. *Pest Technology*, **1** (2): 117-124.

Fulton, N.D.; Awaddle, B. and Thomas, J.A. 1956. Influence of planting date on fungi isolated from diseased cotton seedlings. *Plant Dis. Reptr.*, **40**: 556- 558.

Guseva, N.N. and Gromova, B.B. 1982.Chemical and Biochemical Methods for Studying Plant Immunity. (In Russian). All Union Institute of Plant Protection, Leningrad, U.S.S.R.

Hussein, E.M.; Allam, A.D.A.; Aly, A.A.; Amein, A.M. and El-Samawaty, A.M.A. 2000. Separation by protein electrophoresis of *Rhizoctonia* spp. isolated from cotton seedlings. *J. Agric. Sci., Mansoura Univ.*, **25**: 4035-4046.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**: 680-685.

Mohammadi, M.; Banihashemi, M.; Hedjaroude, G.A. and Rahimian, H. 2003. Genetic diversity among Iranian isolates of *Rhizoctonia solani* Kühn anastomosis group1 subgroups based on isozyme analysis and total soluble protein pattern. *Phytopathology*, **93**: 162-170.

Monga, D. and Sheo-Raj 1994. Cultural and pathogenic variations in the isolates of *Rhizoctonia* species causing root rot of cotton. *Indian Phytopathology*, **47**: 403-407.

Nei, M. and Li, W. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA*, **79**: 5269-5273.

Ogoshi, A. 1972. Grouping of *Rhizoctonia solani* Kühn with hyphal anastomosis. *Ann. Phytopathol. Soc. of Japan*, **38**: 117–122.

Rush, C.M.; Carling, E.D.; Harveson, M.R. and Mathieson, T.J. 1994. Prevalence and pathogenicity of anastomosis groups of *Rhizoctonia solani* from wheat and sugar beet in Texas. *Plant Disease*, **78**: 349-352.

Sammons, D.W.; Adams, L.D. and Nishizawa, E.E. 1981. Ultrasensitive silver based colour staining of polypeptides in polyacrylamide gels. *Electrophoresis*, **2**: 135.

Sneath, P.H.A. and Sokal, R.R. 1973. Numerical Taxonomy. Freeman, San Francisco.

Watkins, G.M. 1981. Compendium of Cotton Diseases. Amer. Phytopathol. Soc., St. Paul, Minnesota, 87pp.

Zuber, M. and Manibhushanrao, K. 1982. Studies on comparative gel electrophoresis patterns of proteins and enzymes from isolates of *Rhizoctonia solani* sheath blight disease in rice. *Can. J. Microbiol.*, **28**: 762-771.

(Received 12/02/2009;
in revised form 15/04/2009)

القدرة المرضية والتفريد الكهربي لعزلات مختلفة للفطر رايزكتونيا سولاني من القطن

موريس صبري ميخائيل*، كامل كمال ثابت*، معوض رجب عمر**، عزت محمد حسين**، خالد قاسم قاسم***.

* قسم أمراض النبات ، كلية الزراعة ، جامعة القاهرة.

** معهد بحوث أمراض النبات ، مركز البحوث الزراعية، الجيزة ، مصر.

*** الهيئة العامة للبحوث العلمية الزراعية، مركز البحوث الزراعية في حماه، سورية.

صنفت ٢١ عزلة من الفطر رايزكتونيا سولاني حسب المجاميع الالتحامية إلى AG-2-2 (تسع عزلات) و AG-4-HG-I (ثماني عزلات) و AG-5 (أربع عزلات). ثم اختبرت قدرتها على إصابة بادرات القطن صنف جيزة ٨٦ وذلك تحت ظروف الصوبة. أظهرت النتائج مستويات مختلفة من موت البادرات في مرحلة ما قبل الانبثاق تبعاً لاختلاف العزلات، بينما لم توجد فروق معنوية في مرحلة ما بعد الانبثاق، كما أدت أربع عشرة عزلة إلى انخفاض معنوي في نسبة النباتات المتبقية مقارنة بالكنترول. أجريت دراسة مقارنة لأنواع البروتينات المستخلصة من العزلات باستخدام تقنية التفريد الكهربي للبروتين الخام PAGE والبروتين المفكك باستعمال مادة صوديوم دوديسيل سلفات SDS-PAGE. تم حساب درجة التشابه بين العزلات والتفرقة ما بينها باستخدام التحليل العنقودي وأظهرت النتائج إمكانية التفريق بين العزلات في كلتا الحالتين PAGE و SDS-PAGE مع عدم وجود علاقة بين أنماط البروتين وكل من المجاميع الالتحامية أو القدرة المرضية أو المصدر الجغرافي للعزلات.