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USE OF CLUSTER ANALYSIS TO DIFFERENTIATE AMONG ISOLATES OF *Rhizoctonia solani* FROM COTTON BASED ON VARIATION IN VIRULENCE AND IN SENSITIVITY TO PESTICIDES

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ABSTRACT

Pathogenicity of 20 isolates of *Rhizoctonia solani* was evaluated on cotton cultivar Giza 75 under greenhouse conditions. The *in vitro* sensitivity of the isolates to three fungicides (Rizolex T, Vitavax Captan, and Monceren Combi) and an insecticide (Gaucho) was also evaluates. Cluster analysis was used to differentiate among the isolates based on variation in virulence and in sensitivity to pesticides. No obvious relationship was observed between grouping the isolates by cluster analysis and their geographic origins. Thus, isolates collected from different governorates had a high level of genetic similarity. On the other hand, genetic diversity was identified among isolates from the same governorate.

INTRODUCTION

Rhizoctonia solani Kühn (telemorph: *Thanatephorus cucumelis* (Frank) Donk), is one of the more primitive basidiomycetes. *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). Brown and McCarter (1976) reported that *R. solani* was a major cause of cotton damping-off throughout much of the Cotton Belt in the USA. Plants that survived attack might exhibit various degrees of root rot or hypocotyls lesions near the soil line, the latter condition sometimes is being referred to as sorshin. Huisman (1988) found that colony density for *Rhizoctonia* spp. was higher on cotton roots with severe tissue damage than on roots exhibiting litter or without tissue damage. In Spain, Melero-Vara and Jiménez-Diaz (1990) found that *R. solani* was the primary agent in cotton seedling damping-off and was associated with severe necrosis on the root and/or hypoctyl. When Aly *et al.* (1996) conducted a survey encompassed 88 samples of infected cotton roots from different governorates of Egypt, *R. solani* was isolated from 76.1% of the samples examined. El-Akkad (1997) evaluated the pathogenicity of 52 isolates of *Rhizoctonia* spp. on cotton cultivar Giza 75 in both laboratory and greenhouse. In laboratory, virulent isolates caused lesions and root rot, while moderately pathogenic ones showed only root discoloration. In the greenhouse test, most of the virulent isolates exhibited preemergence damping-off.

The identification of *R. solani* isolates is based on the ability of their hyphae to anastomose when grown on solid media, 11 anastomosis groups are now recognized (Laroche *et al.*, 1992).

Most of the available reports on cotton seedling disease indicate that isolates of *R. solani* implicated in the disease belong to the anastomosis group (AG)4 (Anderson, 1982; Melero-Vara and Jiménez-Diaz, 1990; Davis *et al.*, 1997, and El-Akkad, 1997).

Differences in virulence have been widely used as phenotypic and genotypic markers to study pathogen diversity (Al-Kherb *et al.*, 1987 and Linde *et al.*, 1990). Knowledge of the variation within and between fungal populations is important for a better understanding of diseases epidemics and forecasting of disease development (Huff *et al.*, 1994). In this study, we differentiated among 20 isolates of *R. solani* from cotton, using variations in virulence and in sensitivity to some pesticides.

MATERIALS AND METHODS

Isolation, purification and identification of isolates

Cotton seedlings showing typical symptoms of damping-off were collected from different locations. Small pieces of the infected tissues were surface sterilized with 10% Clorox solution for 2 minutes, and washed several times with sterilized water. The surface sterilized pieces were then dried on sterilized filter papers and plated on potato dextrose agar (PDA) medium amended with streptomycin sulphate and Rose Bengal to eliminate bacterial contamination. The plates were incubated at 26 °C ± 3°C for 3 days when the developing colonies were isolated in pure culture. Isolates were identified to species level according to El-Akkad (1997).

Pathogenicity test of isolates

Substrate for growth of each isolate was prepared in 15-cm test tube, each tube contained 2 gm of sorghum grains and 3 ml of tap water. Contents of tubes were autoclaved for 30 minutes. Fungal inoculum, taken from one-week-old culture on PDA, was aseptically introduced into the tubes and allowed to colonize sorghum for two weeks. The sorghum-fungus mixture of each isolate was used to infest autoclaved soil at a rate of 1 g/kg of soil. In the control treatment, autoclaved sorghum grains were mixed with autoclaved soil at the same rate. Infested soil was dispended in 10-cm-diameter clay pots and these were planted with 10 seeds/pot of cotton cultivars Giza 75. Pots were randomly distributed on greenhouse bench. The greenhouse ranged from 23.5 °C ± 2.5°C to 38 °C ± 3°C. percentage of infected seedlings were recorded 45 days after planting.

***In vitro* evaluation of isolate sensitivity to pesticides**

Effects of three fungicides and an insecticide (Table 1), at the concentrations of 0.01, 0.1, 1, 10 and 100 ppm on linear growth of *R. solani* isolates were studied *in vitro*. Concentrations were obtained by adding the appropriate amount of stock suspension of each of the tested pesticides to autoclaved PDA medium cooled to about 45 °C. PDA medium without pesticides was served as control. For each treatment, approximately 45 ml of PDA were poured into each of three 9-cm plates. After solidification of agar, each plate was inoculated with 5-mm-diameter disk of the fungal growth. After 2 days of incubation at 28 °C, percentage of toxicity was calculated

according to the following formula (Topps and Wain, 1957): Toxicity = [(A-B)/A] x 100 where A = growth diameter of control and B = growth diameter of treatment.

Table 1. Pesticides used in the present study and their active ingredients.

Pesticides[a]	Classification	Active ingredient	Formulation[b]
Monceren combi	Fungicide	20% pencycuront + 50% captan	DS
Rizolex T	Fungicide	20% tolclofos-methyl + 30% thiram	WP
Vitavax 300	Fungicide	37.5% carboxin + 37.5% captan	WP
Gaucho	Insecticide	70% imidacloprid	WS

[a] Trade name.
[b] Powder for Seed treatment (DS), Wettable Powder (WP), and Water Soluble (WS).

Statistical analysis of data

The experimental design of the greenhouse study was a randomized complete block with five replicates. Analysis of variance (ANOVA) of the data was performed with the MSTAT-C Statistical Package. Duncan's multiple range was used to compare between isolate means. Percentage data were transformed into arc sine angles before carrying out the ANOVA to produce approximately constant variance. Cluster analysis of isolates was performed with the software package SPSS 6.0.

RESULTS AND DISCUSSION

Pathogenicity of 20 isolates of *R. solani* (Table 2) showed that 16 isolates (80%) significantly reduced the percentage of surviving seedlings. Eleven (68.8%) of these pathogenic isolates showed their pathogenicity in the preemergence stage (Table 3).

The tested isolates showed high level of variability in their response to each of the tested pesticides (Table 4). The majority of the isolates (60%) were extremely sensitive to Rizolex T (IC_{50} < 0.01 ppm), while isolates no_s. 1, 2, 3,10, 11, 12, 13, and 20 (40%) were the least sensitive ones. This high efficiency of Rizolex T against most of *R. solani* isolates is in conformity with the results of other workers (Kesavan, 1984; Kataria and Verma, 1990, and Kataria *et al.*, 1991).

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 and Priestley *et al.*, 1984). The method was used to express exactly the genetic similarity among 48 physiological races of *Bremia lactucae* Regel (Lebeda and Jendrulek, 1987), 17 isolates of *Pyrenophora tritici-repentis* (Died.) Drech. (Schilder and Bergstrom, 1990), 41 isolates of *Ascochyta rabiei* (Pass.) Labrousse (Porta-Puglia *et al.*, 1996), and 29 isolates of *Fusarium solani* (Mart.) Sacc. (Abd-Elsalam *et al.*, 2007).

Table 2. Geographical origins of *Rhizoctonia solani* isolates used in the present study.

Isolate no.	Geographic origin	Region[a]
1	Daqahliya – Dikirnis	ED
2	Daqahliya – Mansoura	ED
3	Sharkiya – Ibrahemiya	ED
4	Sharkiya – Hihya	ED
5	Sharkiya – Kinayat	ED
6	Kafr El-Sheikh – Sidi Salem	MD
7	Kafr El-Sheikh – Sidi Salem	MD
8	Kafr El-Sheikh – Sakha	MD
9	Sharkiya – Hihya	ED
10	Gharbiya – Zifta	MD
11	Giza	ME
12	Fayoum	ME
13	Gharbiya – Kutour	MD
14	El-Minya	ME
15	Kafr El-Sheikh	MD
16	Fayoum - Tamiya	ME
17	Gharbiya – El-Mahalla	MD
18	Gharbiya – El-Mahalla	MD
19	Gharbiya – El-Santa	MD
20	El-Minya	ME

[a] Regions were East Delta (ED), Middle Delta (MD), and Middle Egypt (ME).

Table 3. Pathogenicity[a] of *Rhizoctonia solani* isolates used in the present study.

Isolate no.	Preemergence damping-off (%)	Postemergence damping-off (%)	Survival (%)
1	12[b] E	16 FE	72 AB
2	56 D	40 B-E	4 E
3	10 E	42 B-E	48 BC
4	100 A	0 F	0 E
5	86 C	4 F	10 E
6	8 E	68 AB	24 C-E
7	4 E	76 A	20 DE
8	100 A	0 F	0 E
9	88 CB	0 F	12 E
10	6 EC	36 B-E	58 AB
11	10 E	44 B-D	46 B-D
12	100 A	0 F	0 E
13	6 E	46 B-D	48 B-D
14	98 AB	2 F	0 E
15	80 C	6 F	14 E
16	10 E	24 C-F	66 AB
17	8 E	18 C-F	74 AB
18	100 A	0 F	0 E
19	100 A	0 F	0 E
20	100 A	0 F	0 E
Control	6 E	12 D-F	82 A

[a] all isolates were tested on cotton cultivar Giza 75.

[b] mean of five replications. Percentage data were transformed into arc sine angles carrying out the analysis of variance. Means within column followed by the same letter(s) are not significantly different ($P < 0.05$) according to Duncan's multiple range test.

The phenogram of Fig. 1 was constructed based on rescaled distances generated from cluster analysis of correlation similarity coefficients shown in

Table 5. In this phenogram, the smaller the distance, the more closely the isolates were related. Two unrelated groups of isolates were identified by cluster analysis based on variation in virulence patterns and in sensitivity to pesticides. The first group included isolates no_s. 18, 19, 20, 14, 4, 8, 5, 15, 12, 2, and 9, while the second group included isolates no_s. 1, 16, 3, 11, 6, 7, 10, 13, and 17. Within each group, isolates were associated strongly and positively, whereas between groups, isolates were associated weakly or negatively (Table 5). Of the 11 isolates that were found in the first group, 4 (36.4%), 4 (36.4%), and 3 (27.3%) were collected from East Delta, Middle Delta, and Middle Egypt, respectively. Of the 9 isolates that were found in the second group, 2 (22.2%), 5 (55.6%), and 2 (22.2%) were recovered from East Delta, Middle Delta, and Middle Egypt, respectively.

Table 4. IC_{50} values and slopes of pesticides dosage-linear growth response curves for *Rhizoctonia solani* isolates in pesticide-amended PDA after 48 hr. of growth at 28 °C.

Isolate	Pesticide							
	Rizoles T		Vitavax/captan		Monceren Combi		Gaucho	
	IC_{50}	Slope	IC_{50}	Slope	IC_{50}	Slope	IC_{50}	Slope
1	0.09	0.69	0.02	0.55	0.4	0.33	>100	1.18
2	0.2	0.12	0.5	0.26	10.0	0.37	>100	1.01
3	0.08	1.02	0.05	0.55	3.0	0.73	30.0	0.56
4	<0.01	--	<0.01	--	5.0	0.39	4.5	0.42
5	<0.01	--	<0.01	--	1.0	0.37	10.0	0.38
6	<0.01	--	<0.01	--	0.09	0.51	2.0	0.23
7	<0.01	--	<0.01	--	10.0	0.36	30.0	0.31
8	<0.01	--	<0.01	--	<0.01	--	10.0	0.19
9	<0.01	--	<0.01	--	0.01	0.55	100.0	0.34
10	0.08	--	<0.01	--	0.01	0.31	1.0	0.28
11	1.0	0.74	0.05	0.64	8.0	0.53	50.0	1.14
12	0.03	0.59	0.05	0.61	0.8	0.65	40.0	0.24
13	0.1	0.61	1.0	0.81	5.0	0.57	2.5	0.55
14	<0.01	--	<0.01	--	0.8	0.65	5.0	0.31
15	<0.01	--	<0.01	--	4.0	0.43	5.0	0.31
16	<0.01	--	0.06	0.60	8.0	0.62	>100	1.33
17	<0.01	--	<0.01	--	<0.01	--	<0.01	0.20
18	<0.01	--	<0.01	--	<0.01	--	<0.01	--
19	<0.01	--	<0.01	--	<0.01	--	0.1	0.14
20	0.1	0.75	<0.01	--	<0.01	--	1.0	0.22

IC_{50} values (ppm a.i.) and slopes were determined by extrapolation from log-dosage probit-inhibition relative growth plots for each isolate-pesticide combinations.

Isolates from East Delta and Middle Egypt tended to group in the first cluster, which included 66.7 and 60% of these isolates, respectively. On the other hand, isolates of Middle Delta tended to group in the second cluster, which included 55.6% of these isolates. No obvious relationship was observed between grouping the isolates and their geographic origin. For instance, isolates no_s. 19 and 20 showed a very high level of similarity in their virulence patterns and in their sensitivity to pesticides although they were recovered from Gharbiya in Middle Delta and Menya in Middle Egypt, respectively. Gharbiya isolates no_s. 18 and 17 strikingly differed from each other as they were included in unrelated subclusters.

The present study included only 20 isolates of *R. solani*. It is unlikely that this limited number of isolates represents the full range of variation within the fungus. Despite this limitation, a high level of genetic variation was observed

fungus. Despite this limitation, a high level of genetic variation was observed among the isolates. At first, this finding was surprising because a low level of genetic variation is usually observed in fungal populations that do not reproduce sexually like *R. solani*; however, in retrospect, this variation may be explained by the occurrence of parasexuality through fusion of cells from different hyphae belonging to the same AG, which may form heterokaryons, thus contributing to genetic variation. Genetic variation in the pathogen population may also reflect the lack of resistance among the currently cultivated commercial cotton cultivars (Almeida *et al.*, 2003).

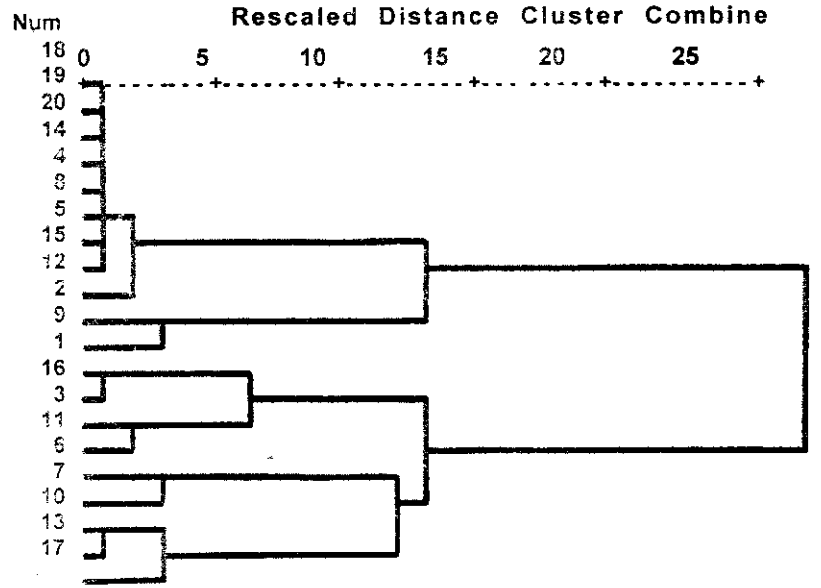

Fig. 1. **Phenogram based on average linkage cluster analysis of variation in virulence and sensitivity to pesticides for 20 isolates of *Rhizoctonia solani* from cotton.**

The genetic variation obtained within groups did not depend on geographic origin. Thus, isolates collected from different governorates had a high level of similarity, and were cognate to each other, proposing that these isolates may not emerge independently from each other (Purkayastha *et al.*, 2006). For example, isolates no_s. 19, 20, and 4, originating from different governorates (Gharbiya, Menya, and Sharkiya), were identical. They may be part of a similar familial population and may perhaps have drifted to different regions following genotype exchange and importation of contaminated seeds and equipments, and of soil infested with sclerotia (Purkayastha *et al.*, 2006). On the other hand, genetic diversity was identified among isolates from the same governorate. For example, isolates 18 and 17 were collected from Gharbiya but they showed a very high level of dissimilarity.

Table 5. Correlation similarity coefficient matrix among 20 isolates of *Rhizoctonia solani* from cotton.

Isolate no.	Isolate no.																		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1																			
2	.609																		
3	.687	.313																	
4	-.164	.333	-.203																
5	-.048	.380	-.078	.990															
6	.031	.069	.712	-.141	-.085														
7	.258	.365	.736	-.266	-.210	.908													
8	-.105	.390	-.172	.997	.994	-.141	-.248												
9	.566	.880	.117	.608	.654	-.255	-.056	.659											
10	.359	-.178	.858	-.188	-.081	.691	.511	-.188	-.249										
11	.829	.544	.945	-.241	-.122	.571	.732	-.196	.307	.652									
12	.125	.622	-.101	.934	.945	-.204	-.195	.956	.850	-.262	-.037								
13	.268	-.123	.869	-.220	-.127	.845	.693	-.223	-.300	.966	.675	-.299							
14	-.149	.350	-.176	.999	.993	-.111	-.238	.998	.617	-.169	-.216	.938	-.199						
15	-.080	.324	-.050	.985	.996	-.048	-.198	.983	.601	-.019	-.117	.915	-.065	.986					
16	.993	.643	.702	-.205	-.094	.080	.336	-.147	.550	.344	.861	.093	.288	-.190	-.116				
17	.431	.252	.740	-.126	-.017	.392	.199	-.127	-.172	.935	.540	-.195	.816	-.116	.045	.386			
18	-.181	.303	-.193	.998	.990	-.116	-.261	.995	.582	.935	-.247	.922	-.194	.998	.986	-.226	-.102		
19	-.181	.304	-.193	.998	.990	-.116	-.260	.995	.583	-.161	-.246	.922	-.195	.998	.986	-.225	-.102	1.000	
20	-.174	.312	-.192	.998	.991	-.119	-.260	.996	.590	-.164	-.242	.925	-.198	.999	.986	-.218	-.105	1.000	1.000

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إستعمال التحليل العنقودى للتفرقة بين عزلات فطر ريزوكتونيا سولانى المعزولة من القطن بناءً على ما بينها من تباين فى القدرة المرضية وفى الحساسية لمبيدات الآفات

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قيمت القدرة المرضية لعشرين عزلة من فطر ريزوكتونيا سولانى على صنف القطن جيزة 75 ، وذلك تحت ظروف الصوبة ، كما إختبرت حساسية العزلات لثلاثة مبيدات فطرية (ريزولكس تى وفيتافاكس كابتان ومونسرين كومبى) ومبيد حشرى (جاوشو) ، وذلك تحت ظروف المعمل . إستعمل التحليل العنقودى لتصنيف هذه العزلات إلى مجموعات ، بناءً على ما بينها من تباين فى القدرة المرضية وفى الحساسية لمبيدات الآفات. لم يلاحظ إرتباط واضح بين المجموعات التى انقسمت إليها العزلات وأصولها الجغرافية . وعلى ذلك ، فإن العزلات المتحصل عليها من محافظات متباعدة جغرافيا أظهرت درجة عالية من التشابه الوراثى، أما العزلات المتحصل عليها من نفس المحافظة فقد أظهرت درجة عالية من التباين الوراثى.