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Molecular characterization of the pathogenic plant fungus *Rhizoctonia solani* (Ceratobasidiaceae) isolated from Egypt based on protein and PCR-RAPD profiles

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ABSTRACT. Twenty-one isolates of *Rhizoctonia solani* were categorized into three anastomosis groups consisting of AG-4-HG-I (eight isolates), AG-2-2 (nine isolates) and AG-5 (four isolates). Their pathogenic capacities were tested on cotton cultivar Giza 86. Pre-emergence damping-off varied in response to the different isolates; however, the differences were not significant. Soluble proteins of the fungal isolates were electrophoresed using SDS-PAGE and gel electrophoreses. A dendrogram of the protein banding patterns by the UPGMA of arithmetic means placed the fungal isolates into distinct groups. There was no evidence of a relationship between protein dendrogram, anastomosis grouping or level of virulence or geographic origin. The dendrogram generated from these isolates based on

PCR analysis with five RAPD-PCR primers showed high levels of genetic similarity among the isolates from the same geographical locations. There was partially relationship between the genetic similarity and AGs or level of virulence or geographic origin based on RAPD dendrogram. These results demonstrate that RAPD technique is a useful tool in determining the genetic characterization among isolates of *R. solani*.

Key words: *Rhizoctonia solani*; Cotton; Anastomosis groups; Pathogenicity; SDS-PAGE; RAPD-PCR

INTRODUCTION

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). The *R. solani* complex represents an economically important group of soil-borne pathogens that occur on many plant species throughout the world (Sneh et al., 1996). *R. solani* is composed of genetically isolated groups (Adams, 1988). The identification and classification of these groups is primarily based on anastomosis behavior (Ogoshi, 1972). *R. solani* is a heterogeneous species that has been divided into 14 anastomosis groups (AGs), designated as AG-1-13 and a bridging isolate group, AG-BI (Carling et al., 2002; El-Samawaty, 2008). Some isolates of *R. solani* AG-2-2, AG-4 and AG-5 reduce 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 on the cotton cultivar Giza 75 under greenhouse conditions; 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. El-Akkad (1997) found heterogeneity in protein banding patterns among the 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). A clear-cut relationship of protein profiles of the isolates with virulence, geographic origin, or source (cultivar used in isolation) was weak. These results suggested 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 the Mâzandaran Province, Iran, and standard isolates of these subgroups by total soluble protein profile. The soluble protein patterns were similar between 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, I and II, consistent with the previously reported AG-1-IA and AG-1-IB subgroups in AG1 (Mohammadi et al., 2003). Random amplified polymorphic DNA (RAPD) markers allow the identification of species or isolates, and the construction of dendograms from the computed distances (Williams et al., 1990). Genetic variation in Australian isolates of *R. solani* was analyzed by RAPD. All of the anastomosis groups (including subgroups) tested could be distinguished. For some groups, there was considerable variation in the fingerprint patterns between isolates. This variation was more marked between isolates from different geographic locations. Other groups showed very little variation between isolates.

RAPD-PCR (polymerase chain reaction) analysis is a very useful alternative in anastomosis grouping for identification of isolates of *R. solani* (Duncan et al., 1993). Twelve isolates of *R. solani*

and fifteen isolates of *R. bataticola* causing root rot of cotton were studied for their pathogenicity and genetic diversity using RAPD markers. The similarity values of RAPD profiles in *R. solani* isolates ranged from 0.35 to 1.00 with an average of 0.63 among all the isolates. Eighteen primers were used to fingerprint the individual isolates. Cluster analysis using unweighted pair-group method with arithmetic average (UPGMA) could distinguish *R. solani* and *R. bataticola* isolates into two separate fingerprint groups. Molecular markers are useful tools for detecting genetic variation in the population of *R. solani* (Monga et al., 2004). A method based on restriction analysis of PCR amplified ribosomal DNA was developed for the rapid characterization of large populations of *R. solani* at the anastomosis group (AG) level. The PCR-based procedure described in this paper provides a rapid method for AG typing of *R. solani* in relation to geographic locations in Egypt and variability of genetic contents.

The present investigation was initiated to determine whether *R. solani* isolates can be distinguished by their different locations in Egypt, pathogenicity to cotton, and the anastomosis grouping in conjunction with their protein and DNA patterns.

MATERIAL AND METHODS

Isolate collection

Twenty-one isolates of *R. solani*, originating from cotton seedlings, were obtained from the fungal collection of the Cotton Disease Research Section, Plant Pathology Research Institute, Agriculture Research Centre, Giza, Egypt.

Pathogenicity test

A substrate for the growth of isolates was prepared in 500-mL glass bottles; each bottle contained 50 g sorghum grains and 40 mL tap water. The bottles were autoclaved for 30 min. Isolate inoculum, taken from 1-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 1 g/kg soil. Infested soil was dispensed in 15-cm diameter clay pots and these were planted with 10 seeds per pot of 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 at a temperature of 24° ± 3°C. Pre-emergence damping-off was recorded 15 days after planting, while post-emergence damping-off, survival of plant, height (cm) and dry weight (mg/plant) were recorded 45 days after planting.

Molecular studies

Possible genetic variations among *R. solani* isolates were determined using by SDS-PAGE protein fingerprinting and RAPD-PCR analysis.

Extraction of proteins from *R. solani* isolates

Proteins were prepared according to the methods described by Guseva and Gromova (1982). The mycelium grown for 8 days at 20°-25°C in 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 19,000 rpm for 30 min at 0°C. The protein content in supernatant was estimated according to Bradford (1976) using bovine serum albumin as the protein standard.

Electrophoresis of soluble 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, pH 6.8), 20% glycerol; 6% SDS; 10% 2-mercaptoethanol and 0.1% bromophenol blue, before boiling in a water bath for 3 min. Twenty-microliter samples (40 µg protein) were subjected to electrophoresis on a 7.5% polyacrylamide gel prepared in 0.1% SDS with a 3.5% stacking gel (Laemmli, 1970). Electrophoresis was performed in a vertical slab gel (16 x 18 x 0.15 cm). Gels were stained with silver nitrate for the detection of protein bands (Sammons et al., 1981).

Gel analysis for protein

Protein patterns obtained by SDS-PAGE were clustered using a gel documentation system (Uvitec, Cambridge, UK) by UPGMA according to Sneath and Sokal (1973).

Extraction of DNA from *R. solani* isolates

R. solani cultures were grown in a 250-mL Erlenmeyer flasks containing 50 mL potato dextrose broth at 23°C for 4 days. Cultures were filtered through microglass filters, and the mycelia were frozen in liquid nitrogen and ground to a fine powder in a mortar. DNA was extracted from 50 mg fresh mat according to Guo et al. (2005).

RAPD technique

For each *R. solani* isolate tested, 30 ng extracted DNA were used for the amplification reaction. The PCR mixture contained PCR beads (Amersham Pharmacia Biotech), which contained all the necessary reagents except the primer and DNA, which were added separately. Primer, nucleotide sequence and G+C percentage of test primers used in these RAPD reactions are shown in Table 1. For each primer tested, 1.0 µL was added to the mixture containing DNA extract. The total volume was adjusted to 25 µL by adding sterile distilled water. The PCR amplification protocol (Thermocycler T1, Biometra, Germany) was carried out as follows:

Table1. Code, nucleotide sequence and G+C (%) of primers used in the random amplified polymorphic DNA reactions.

Primer No.	Sequence	G+C (%)
1	5'-GGTGCGGGAA-3'	70
2	5'-GTTTCGCTCC-3'	60
3	5'-GTAGACCCGT-3'	60
4	5'-AAGAGCCCGT-3'	60
5	5'-AACGCGCAAC-3'	60

a) denaturation at 95°C for 5 min (one cycle); b) 45 cycles, each consisting of denaturation at 95°C for 1 min, annealing at 36°C for 1.0 min, and extension at 72°C for 2 min; c) a final extension at 72°C for 5 min (one cycle), and d) hold at 4°C.

DNA electrophoresis

For all samples, 15 µL amplified DNA was electrophoresed using a Bio-Rad Wide Mini-Sub Cell GT electrophoresis unit on 2% agarose gels containing 0.5 µg/mL ethidium bromide, at constant 75 V and 60 mA, and visualized with a UV trans-illuminator.

Gel analysis for DNA

The DNA gel was scanned for band Rf using a gel documentation system (AAB Advanced American Biotechnology, Fullerton, CA, USA). The different molecular weights of bands were determined against a DNA standard (kb DNA ladder, Stratagene, Canada) with molecular weights of 250, 500, 750, 1000, 1500, 2000, 3000, 4000, 5000, 6000, 7000, 8000, 9000, 10,000, and 12,000 bp. The similarity level was determined by UPGMA.

Statistical analysis of pathogenicity test

The pathogenicity test was carried out using a randomized complete block design with five replicates. The percentage data were transformed into $\sqrt{x + 0.5}$ or arcsine values to obtain approximately constant variance before carrying out analysis of variance (ANOVA). The Duncan multiple range test was used to compare isolate means. ANOVA was carried out using the MSTAT-C statistical package.

RESULTS

Anastomosis typing of *Rhizoctonia* spp isolates

Table 2 shows the *R. solani* isolates used in this investigation, originating from 8 governorates of lower Egypt, where they were categorized into 3 anastomosis groups, namely 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 test

The pathogenicity test of 21 *R. solani* isolates was evaluated on cotton cultivar Giza 86 under greenhouse conditions (Table 3). 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 Nos. 28, 32, 39, 43, 26, 33, 41, 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 isolates tested caused significant post-emergence damping-off infection, while 52.38% of the isolates caused

significant pre-emergence damping-off (Table 4). The most pathogenic isolates were those isolated from Beheira, Sharqiya, Minufiya, Gharbiya, and Damietta governorates, representing 21.43% of the total pathogenic isolates (Table 5).

Table 2. 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 3. 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	$58^{c\text{-}f*}$	2^{ab}	$40^{f\text{-}k*}$	$15.50^{a\text{-}d}$	$293^{f\text{-}i}$
27	$22^{c\text{-}j}$	2^{b}	$76^{a\text{-}f}$	$17.67^{a\text{-}c}$	$506^{a\text{-}f}$
28	88^{a*}	4^{b}	8^{m*}	3.00^{g*}	106^{ij*}
29	16^{j}	0^{ab}	84^{ab}	$19.13^{a\text{-}c}$	$435^{b\text{-}f}$
30	$22^{g\text{-}j}$	2^{b}	$76^{a\text{-}d}$	$18.73^{a\text{-}c}$	$431^{b\text{-}f}$
31	$44^{c\text{-}i}$	4^{b}	$52^{c\text{-}k*}$	$15.07^{b\text{-}d}$	$337^{d\text{-}h}$
32	100^{a*}	0^{b}	0^{m*}	0.00^{g*}	0^{j*}
33	68^{bc*}	2^{b}	$30^{j\text{-}k*}$	$16.57^{a\text{-}d}$	$494^{a\text{-}f}$
34	$58^{c\text{-}f*}$	4^{ab}	$38^{g\text{-}k*}$	$15.33^{a\text{-}d}$	$306^{e\text{-}h}$
35	$42^{c\text{-}j}$	2^{b}	$56^{b\text{-}k}$	$19.40^{a\text{-}c}$	$571^{a\text{-}c}$
36	$48^{c\text{-}i}$	4^{ab}	$48^{c\text{-}k*}$	$17.90^{a\text{-}c}$	690^{a*}
37	$60^{b\text{-}d*}$	6^{ab}	34^{jk*}	13.63^{cd}	$371^{b\text{-}h}$
39	90^{a*}	0^{b}	10^{m*}	2.33^{g*}	67^{j*}
40	$56^{c\text{-}e*}$	4^{ab}	$40^{b\text{-}k*}$	11.47^{de}	$353^{c\text{-}h}$
41	60^{bc*}	6^{ab}	34^{kl*}	8.50^{ef*}	$195^{g\text{-}j}$
42	$20^{g\text{-}j}$	10^{ab}	$70^{a\text{-}h}$	$15.47^{a\text{-}d}$	$487^{a\text{-}f}$
43	88^{a*}	4^{ab}	8^{lm*}	3.73^{fg*}	72^{j*}
45	88^{Ab*}	2^{b}	10^{lm*}	5.17^{fg*}	$168^{b\text{-}j}$
46	$32^{c\text{-}j}$	2^{b}	$66^{a\text{-}j}$	$17.73^{a\text{-}c}$	$498^{a\text{-}f}$
47	$22^{c\text{-}j}$	0^{b}	$78^{a\text{-}e}$	$17.73^{a\text{-}c}$	$573^{a\text{-}c}$
50	$40^{c\text{-}j}$	6^{ab}	$54^{c\text{-}k*}$	13.67^{cd}	$326^{d\text{-}h}$
Control	14^{ij}	0^{b}	$86^{a\text{-}b}$	$16.47^{a\text{-}d}$	$507^{a\text{-}f}$

[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 superscript letter(s) are not significantly different according to the Duncan multiple range test (P = 0.05); asterisks denote a significant difference from the control.

Table 4. Distribution of *Rhizoctonia solani* isolates based on their effects on cotton seedlings (cultivar Giza 86).

Total No. of isolates tested	Percentage of isolates, which are 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]Isolates significantly increased pre-emergence damping-off, while they significantly decreased survival, plant height and dry weight.

Table 5. Distribution of *Rhizoctonia solani* isolates based on geographic origin.

Geographic origin	Total No. of isolates tested	No. 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 *R. solani* isolates based on their protein banding patterns obtained by SDS-PAGE

Considering data generated from SDS-PAGE (Figure 1) and phenogram (Figure 2), it is apparent that isolates separated into a large number of clusters. The first cluster included 4 isolates (34, 35, 33, and 30) with an overall similarity level (SL) of 34%. For the anastomosis groups, isolates 34 and 30, belonging to AG-4-HG-I, showed SL of 64%. For geographic regions, isolates 34, 35 and 33 from the East Delta region showed an SL of 48%.

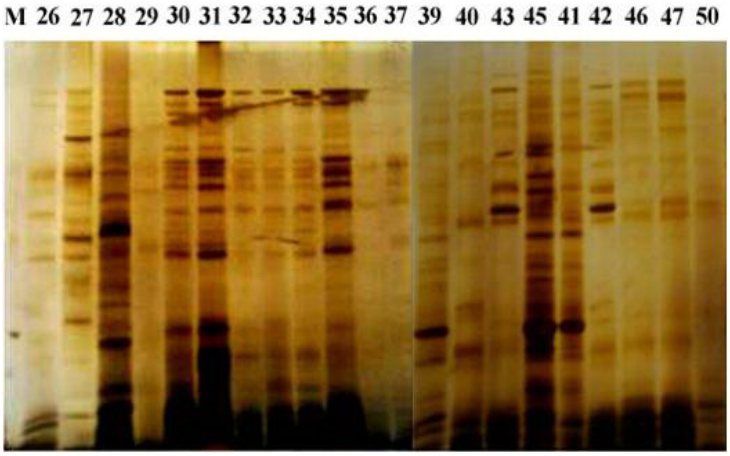

Figure 1. Protein patterns obtained by SDS-PAGE from 21 isolates of *Rhizoctonia solani*. *Lane M* = molecular weight.

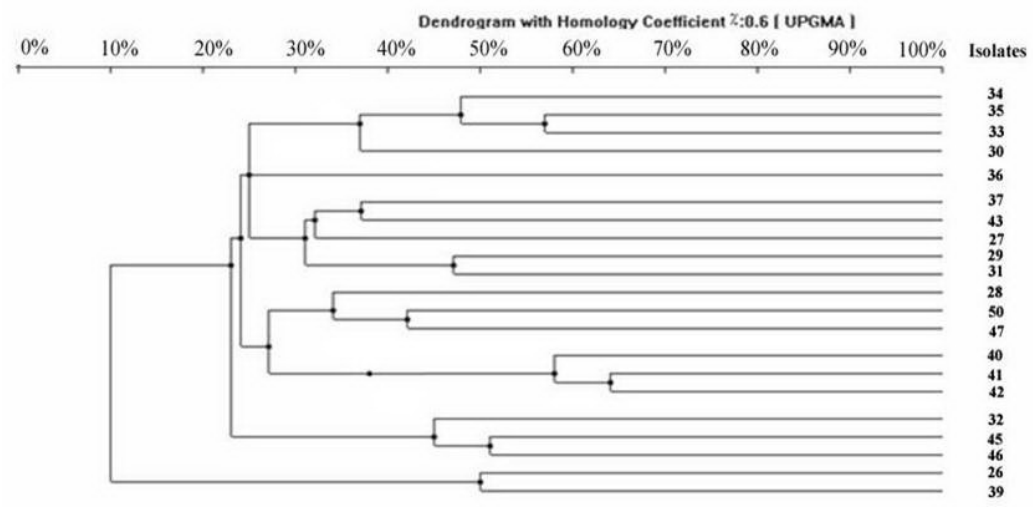

Figure 2. Dendrogram of electrophoretic protein patterns obtained by SDS-PAGE from 21 isolates of *Rhizoctonia solani*.

The second cluster included 1 isolate (36). The third cluster included 5 isolates (37, 43, 27, 29, and 31) with an SL of 31%. For the anastomosis groups, isolates 37 and 43, belonging to AG-4-HG-I, showed an SL of 38%. For geographic regions, isolates 29 and 31 from the Mid-Delta region showed an SL of 47%.

The fourth cluster included 3 isolates (28, 50 and 31) with an SL of 32%. The fifth cluster included 3 isolates (40, 41 and 42) with an SL of 57%.

The sixth cluster included 3 isolates (32, 45 and 46) with an SL of 45%. All of these isolates from the East Delta region showed an SL of 45%. The seventh cluster included 2 isolates (26 and 39) with an SL of 50%. All the isolates were the most pathogenic because they significantly affected all parameters.

Genetic characterization of *R. solani* isolates

Genetic variations were evaluated by PCR amplification using a set of 5 random 10-mer primers. The amplification products were analyzed for polymorphisms by gel electrophoresis to determine whether pathotypes could be distinguished at the molecular level.

RAPD profiles of *R. solani* isolates obtained using the 5 primers

RAPD profile of 21 R. solani isolates obtained with primer 1

Dendrogram analysis grouped the isolates into two main clusters at a genetic similarity level (GSL) of 93.96% based on the banding pattern (Figure 3). GSL between the isolates tested ranged from 75.23 to 99.49% (Figure 3). The first cluster consisted of many subclusters, where the main subcluster included 15 isolates (26, 28, 27, 33, 35, 41, 30, 47, 31, 50, 43, 32, 40, 36, and 39).

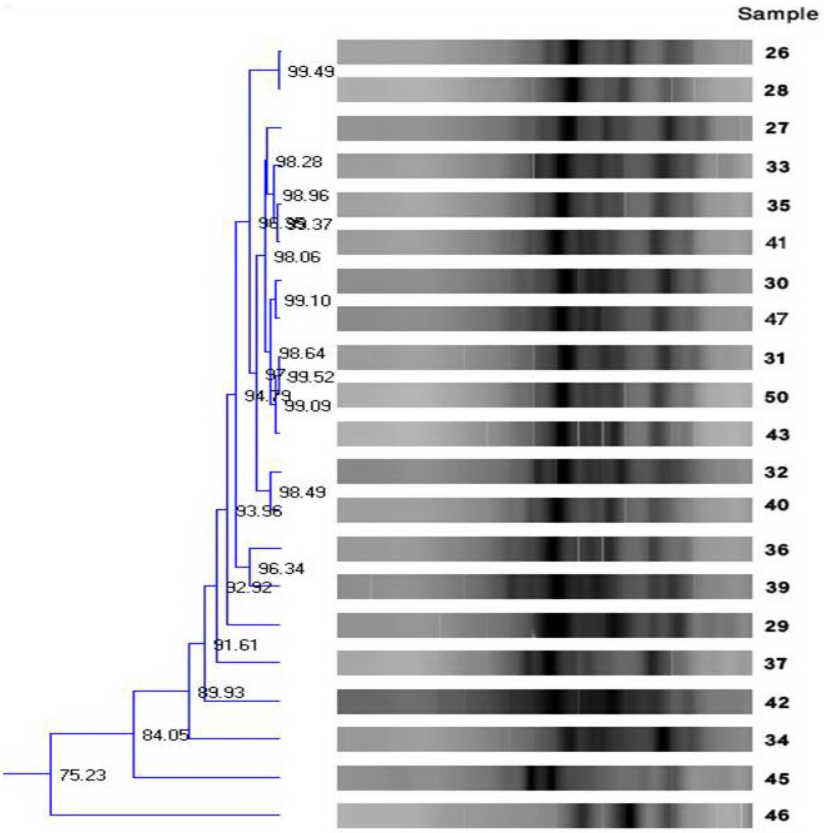

Figure 3. Dendrogram showing the genetic relationships among 21 *Rhizoctonia solani* isolates based on RAPD analysis, using primer 1.

For geographic regions, isolates 26, 27, 33, 35, 47, 31, and 32 from the East Delta overlapped with isolates 28, 30, 31, and 43 from the Mid-Delta and other isolates from other regions.

For the anastomosis groups, isolates 30 and 47, belonging to AG-4-HG-I, showed a high GSL of 99.10%. Isolates Nos. 27, 35 and 41, belonging to AG-5, showed a high GSL of 98.28%. Isolates 31 and 50, belonging to AG-5 showed a high GSL of 99.52%, and isolates 32 and 40, belonging to AG-2-2, showed a high GSL of 98.49%.

For the pathogenicity test, isolates 26, 28, 32, 33, 39, 41, 43, and 45 were the most pathogenic because they significantly affected all stages of plant infection (pre- and post-emergence damping-off and survival) and plant height and dry weight. These isolates showed a GSL of 89.05%. Isolates 26 and 28 showed a high GSL of 99.49%, and isolates 33 and 41 showed a high GSL of 96.35%.

RAPD profile of 21 *R. solani* isolates obtained with primer 2

Dendrogram analysis grouped the isolates into two main clusters with a GSL of 80.80% based on the banding pattern (Figure 4).

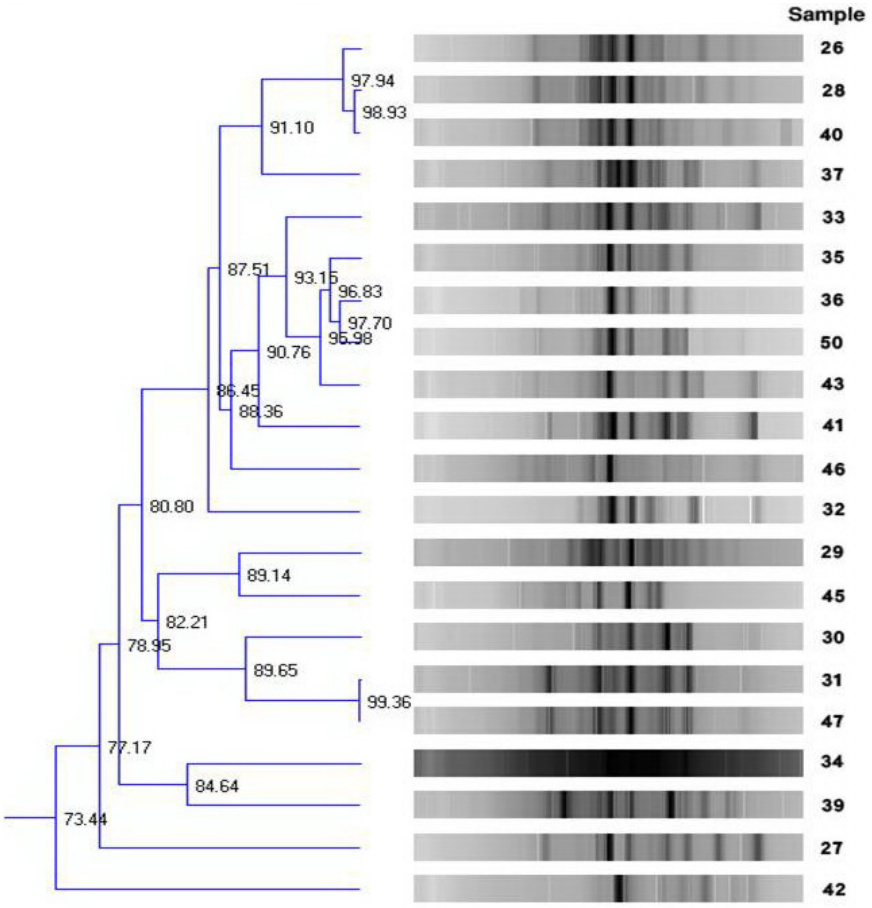

Figure 4. Dendrogram showing the genetic relationships among 21 *Rhizoctonia solani* isolates based on RAPD analysis, using primer 2.

The GSL between the isolates tested ranged from 74.44 to 99.36% (Figure 4). The first cluster consisted of many subclusters, including seventeen isolates (26, 28, 40, 37, 33, 35, 36, 50, 43, 41, 46, 32, 29, 45, 30, 31, and 47).

For geographic regions, isolates 26, 33, 35, 32, 45, and 47 from the East Delta overlapped with isolates 28, 30, 31, 43, and 29 from the Mid-Delta and other isolates from other regions.

For the anastomosis groups, isolates 26 and 37, belonging to AG-4-HG-I, showed a GSL of 91.10%. Isolates 29, 30 and 47, belonging to AG-4-HG-I, showed a GSL of 82.21%. Isolates 35 and 36, belonging to AG-5, showed a high GSL of 97.70%. Isolates 31 and 50, belonging to AG-2-2, showed high GSL of 99.52%, and isolates 32 and 40, belonging to AG-2-2, showed a high GSL of 98.49%.

The pathogenicity test indicated that the isolates 26, 28, 32, 33, 39, 41, 43, and 45 were the most pathogenic. Isolates 26 and 28 showed a high GSL of 97.94%. Isolates 33 and 43 showed a high GSL of 95.98%, and isolates 33, 43 and 41 showed a GSL of 93.15%.

RAPD profile of 21 *R. solani* isolates obtained with primer 3

Dendrogram analysis grouped the isolates into two main clusters with a GSL of 89.40% based on the banding pattern (Figure 5). The GSL between the isolates tested ranged from 71.24 to 99.26% (Figure 5). The first cluster consisted of many subclusters, including 17 isolates (26, 28, 27, 31, 33, 34, 37, 43, 41, 46, 47, 50, 36, 39, 42, 40, and 45).

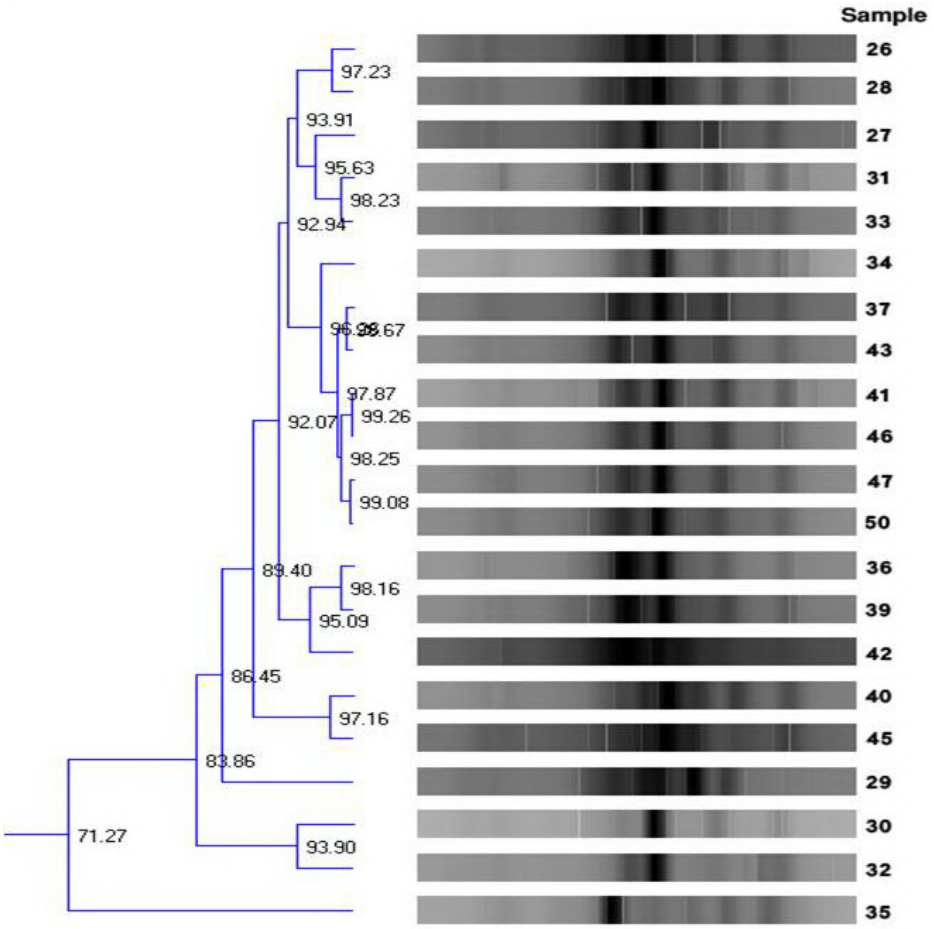

Figure 5. Dendrogram showing the genetic relationships among 21 *Rhizoctonia solani* isolates based on RAPD analysis, using primer 3.

For geographic regions, isolates 26, 27, 33, 34, 45, 46, and 47 from the East Delta overlapped with isolates 28, 31, 43, and 42 from the Mid-Delta and other isolates from other regions.

For the anastomosis groups, isolates 37 and 43, belonging to AG-4-HG-I, showed a GSL of 98.67%, and isolates 34, 37 and 43, also belonging to AG-4-HG-I, showed a high GSL of 97.87%. Isolates 31 and 33, belonging to AG-2-2, showed a high GSL of 98.23%. Isolates

40 and 45, belonging to AG-2-2, showed a high GSL of 97.16%, and isolates 46 and 50, belonging to AG-2-2 showed a high GSL of 98.25%.

The pathogenicity test showed that isolates 26, 28, 32, 33, 39, 41, 43, and 45 were the most pathogenic. Isolates 26 and 28 showed a high GSL of 97.23%, and isolates 43 and 41 showed a high GSL of 98.25%.

RAPD profile of 21 *R. solani* isolates obtained with primer 4

Dendrogram analysis grouped the isolates into two main clusters with a GSL of 82.72% based on the banding pattern (Figure 6). GSL between the isolates tested ranged from 65.19 to 99.19% (Figure 6). The first cluster consisted of many subclusters, including 20 isolates (26, 28, 40, 29, 45, 34, 42, 37, 39, 27, 36, 41, 31, 33, 43, 35, 50, 30, 47 and 46).

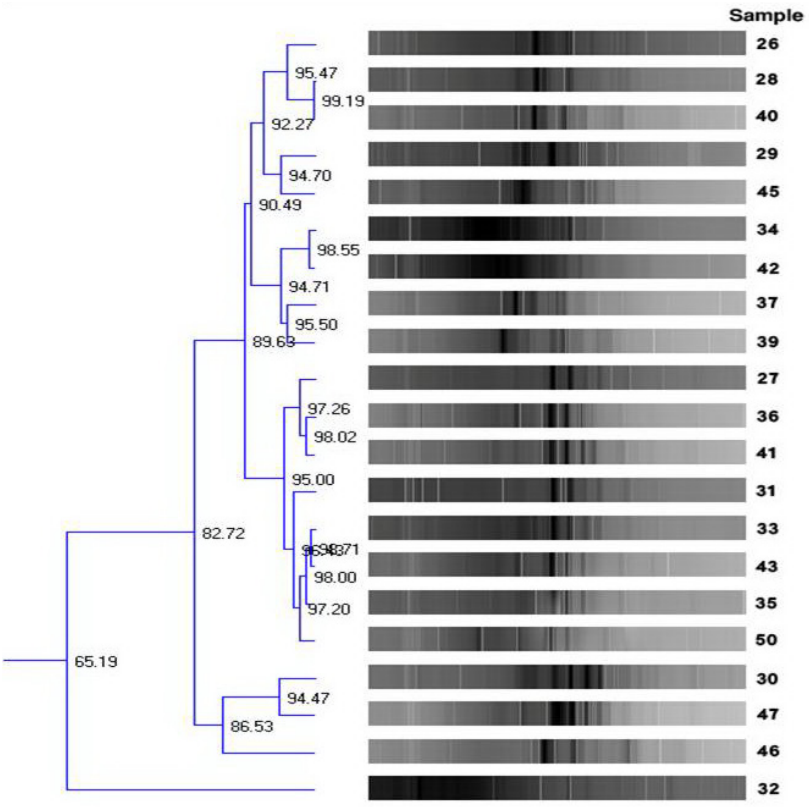

Figure 6. Dendrogram showing the genetic relationships among 21 *Rhizoctonia solani* isolates based on RAPD analysis, using primer 4.

For geographic regions, the first cluster included isolates 26, 27, 33, 34, 35, and 45 from the East Delta, overlapping with isolates 28, 29, 31, 42, and 43 from the Mid-Delta and other isolates from other regions.

For the anastomosis groups, the first cluster included isolates 26 and 29, belonging to AG-4-HG-I, which showed a GSL of 92.27%, and isolates 34 and 42, also belonging to AG-4-HG-I, which showed a high GSL of 98.55%. Isolates 34, 42 and 37, belonging to the same anastomosis group, showed a high GSL of 94.71%. Isolates 27, 36 and 41, belonging to AG-5, showed a high GSL of 97.26%. Isolates 28 and 42, belonging to AG-2-2, showed a high GSL of 99.19%, and isolates 31, 33 and 50, belonging to the same anastomosis group, showed a high GSL of 96.13%.

The pathogenicity test revealed that isolates 26, 28, 32, 33, 39, 41, 43, and 45 were the most pathogenic. Isolates 26 and 28 showed a high GSL of 95.47%, and isolates 33 and 43 showed a high GSL of 98.71%.

RAPD profile of 21 *R. solani* isolates obtained with primer 5

Dendrogram analysis grouped the isolates into two main clusters with a GSL of 80.93% based on the banding pattern (Figure 7). GSL between the isolates tested ranged from 75.38 to 99.00% (Figure 7). The main subcluster included 17 isolates (27, 39, 36, 37, 40, 29, 30, 32, 33, 43, 50, 47, 41, 42, 31, 34, and 35).

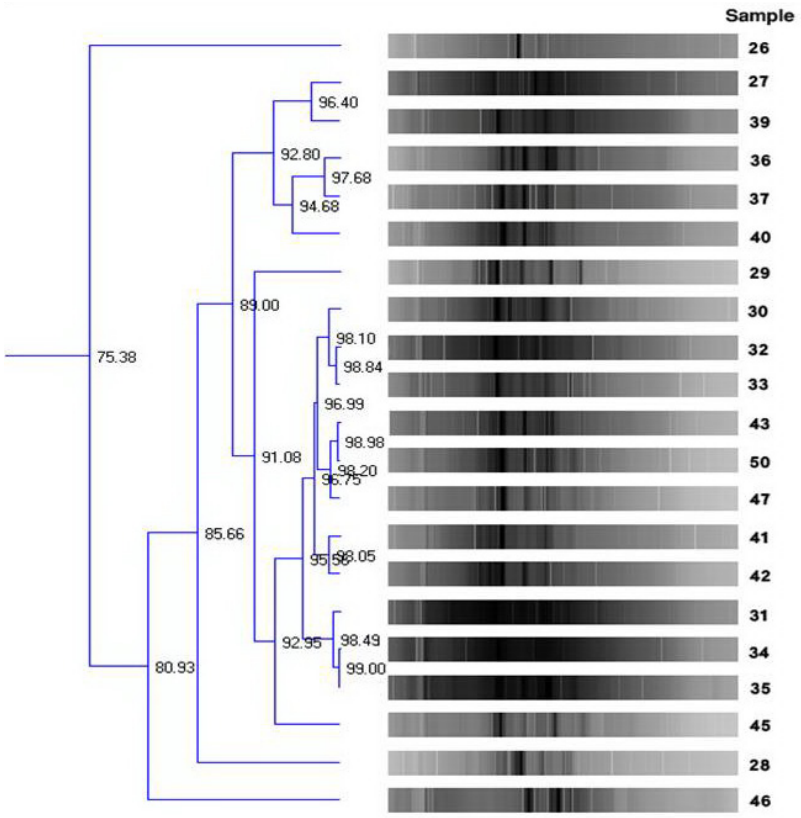

Figure 7. Dendrogram showing the genetic relationships among 21 *Rhizoctonia solani* isolates based on RAPD analysis, using primer 5

For geographic regions, the main subcluster included isolates 7, 32, 33, 47, 34, and 35 from the East Delta overlapping with isolates 29, 30, 31, 42, and 43 from the Mid-Delta and other isolates from other regions.

For the anastomosis groups, the main subcluster included isolates 43 and 47, belonging to AG-4-HG-I, which showed a high GSL of 98.20%. Isolates 32 and 33, belonging to AG-2-2, showed a high GSL of 98.84%. Isolates 27 and 36, belonging to AG-5, showed a GSL of 92.80%.

The pathogenicity test indicated that isolates 26, 28, 32, 33, 39, 41, 43, and 45 were the most pathogenic. Isolates 32 and 33 showed a high GSL of 98.84%, and isolates 33 and 43 showed a high GSL of 98.71%.

DISCUSSION

It is obvious that different isolates behaved differently with respect to disease-causing ability in the pre-emergence stage, and that 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 a level of pre-emergence damping-off amounting to 88% or more. These isolates, except No. 43 belonged to AG-2-2. Considering the rest of the data, Table 3 reveals that there was no general trend correlating AGs with virulence. SDS-PAGE is a widely used technique in biochemistry, forensics, genetics, and molecular biology to separate proteins according to their electrophoretic mobility. SDS gel electrophoresis of samples allows the separation of molecules with identical charge per unit mass due to binding of SDS, resulting in fractionation by size. Protein profiling failed to correlate with the different parameters tested or estimated.

The results showed that protein profiling failed to correlate with the different parameters tested or estimated. Therefore, grouping isolates of *R. solani* based on their protein pattern obtained by SDS-PAGE is not related to their virulence, AGs or geographic origin, confirming previous results reported by El-Akkad (1997) for *R. solani* and Abdel-Sattar et al. (2008) for *Macrophomina phaseolina* isolated from cotton.

Molecular techniques, such as RAPD-PCR, use a small sample of the subject's DNA, which is then selectively amplified a million times, thus producing a large amount of concentrated DNAs (PCR products). PCR products can be separated into bands of different molecular weight DNAs by agarose gel electrophoresis. Banding patterns from an individual or population can then be used as a "fingerprint" that may distinguish it from other genetic types (Ruiz et al., 2000).

RAPD-PCR uses low specificity primers that are aligned at random with sequences in the genome. The standardized RAPD-PCR yields reproducible amplification patterns that are characteristic of the strains. RAPD-PCR yields different information, since it analyzes different sequences and detects different types of variations in DNA. RAPD-PCR detects differences along the entire genome, not only in particular sequences. Thus, this system is helpful in characterizing fungal isolates over long periods (Ortiz-Herrera et al., 2004).

Genetic variability among 21 isolates and geographic locations of *R. solani* was determined, using the RAPD technique. The banding patterns generated from these isolates by five primers showed high levels of genetic similarity between the isolates from the same AG or level of virulence or geographic origin.

The results showed that the genetic similarity between *R. solani* isolates obtained from the same geographic location indicated that there was a partial relationship between

RAPD dendrogram and anastomosis grouping or level of virulence or geographic origin. The results demonstrate that RAPD technique is a useful marker system in determining the genetic characterization of isolates of *R. solani*.

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