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Research Article

Genetic Diversity among Nile Delta Isolates of *Rhizoctonia solani* Kühn Based on Pathogenicity, Compatibility, Isozyme Analysis and Total Protein Pattern

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Abstract: The present study obtained 12 isolates from *Rhizoctonia solani* Kühn isolated from *Cotton* L., *Trifolium* L. and *Vicia faba* L. from different localities in the Nile Delta of Egypt. All strains were pathogenic and caused seed rot, wilt, stunting, and pre-emergence and post-emergence damping-off. The isolated strains produced different forms of infection cushions that ensure the pathogenicity of these strains. SDS-PAGE of the 12 *R. solani* isolates showed that although the *R. solani* isolates were isolated from very divergent host plants, the total soluble protein patterns among them were similar. Isozyme analysis revealed higher polymorphism among *R. solani* isolates using the EST isozyme. The cluster analysis based on isozyme data showed that the *R. solani* isolates fell into 4 distinct groups, whereas the dendrogram resulting from cluster analysis based on the compatibility test and other morphological characteristics of pathogen infection cushion showed that all *R. solani* isolates were placed into 3 main distinct groups.

Key Words: Genetic diversity, *Rhizoctonia solani*, SDS-PAGE, isozymes, anastomoses

Introduction

Rhizoctonia solani Kühn pathogenicity is complex; it has heterogeneous strains and diversity in host range. It occurs throughout the world and can damage any part or all of a plant. It is known to cause seed decay, damping-off, seedling blight, root rot and crown rot, as well as soreshin and wirestem, hypocotyls cankers, bud rot, foliage blight and storage rots (Mubarak, 2003). *R. solani* generally attacks seedlings at the ground level (hypocotyls) and grows downwards into the roots. Meristimatic tissues of seedlings are susceptible to *R. solani*. As tissues mature they become increasingly resistant to *R. solani* due to the conversion of pectin to calcium pectate, which is unaffected by the polygalacturonase produced by the fungus (Bateman & Lumsden, 1995). Susceptibility to *R. solani* may be due to polygalacturonate trans-eliminase, which may partially degrade pectate (Ayers et al., 1999). *R. solani* penetrates plants in various ways: through the intact plant surface by means of complex infection structures (infection cushions), which are characteristic of different isolates, through natural openings and through wounds. *R. solani* may also penetrate the host mechanically or by means of enzymes or toxins (Dodman & Flentje, 1985). *R. solani* produces cutinolytic enzymes (Linskens & Haage, 1993), which could degrade the cuticle-like pectinases and cellulases, and then helps the pathogen to penetrate the host.

Induction of infection cushions by *R. solani* as a response to root exudates has been reported by many researchers (Doman et al., 1968; Kamara et al., 1980; El-Samra et al., 1981; Stockwell & Hanchey, 1984; El-Faham & Aboshosha, 1987; El-Farnawany, 1991). Kamara et al. (1980) suggested 5 types of infection cushions and a correlation was established between the types of infection cushions developed and host resistance (El-Samra et al., 1981).

Mycelia compatibility groups (MCGs) have been used to evaluate genetic variability in fungal plant pathogens, such as *Sclerotinia sclerotium* (Lid.) de Bary (Kohn et al., 1990), *Fusarium oxysporum* Schltdl (Harveson & Rush, 1997) and *Phomopsis subordinaria* (Desm.) Traverso

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(Meijer et al., 1994). In many species mycelia incompatibility results in the formation of macroscopic reaction lines (barrages) between fungal colonies, interpreted as an agonistic response resulting from the recognition of non-self antigens (allorecognition) (Leslie, 1993). In *R. solani*, the formation of the incompatible reaction line (evidenced by a dark line or a strip of thin mycelium and discontinuous sclerotia) between pairs of isolates has been observed, which indicated their failure to anastomose (Kohn et al., 1990).

In order to distinguish different anastomosis groups (AGs) in *R. solani*, total protein profiles have been used. Reynolds et al. (1983) used soluble protein patterns to differentiate between AG1 and AG5 in *R. solani*. Similarly, Liu & Ge (1988) described polymorphisms in soluble protein patterns among 11 AGs of *R. solani*. Nevertheless, isolates consisting of one particular AG or subgroup had a similar banding pattern, although they represented different geographical regions, host plants, or pathogenicity. Moreover, Lodwig et al. (1999) showed that although clonal lineages of *F. oxysporum* were not separated using total protein profiles analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), 2 isolates were distinguishable.

Genetic diversity studies using isozyme analysis have been used as a discriminative tool in AGs and within subgroups of *R. solani* (Micales et al., 1992). Liu et al. (1990) identified 3 groups in AG2 isolates of *R. solani* using different enzyme systems. Jin & Korpradiskul (1998) differentiated 23 isolates of *R. solani* of AG1 into 3 groups based on cluster analysis using data of 7 enzyme systems. Likewise, Laroche et al. (1992) used isozyme analysis to distinguish AGs 3 and 9 in *R. solani*. Moreover, AG11 isolates from Australia and Arkansas were differentiated using isozyme analysis (Kaufman & Rothrock, 1995). Mohammadi et al. (2003) used isozyme analysis and total soluble protein profiles to measure the genetic diversity of the Iranian *R. solani* isolate AG1. Banniza & Rutherford (2001) reported that *R. solani* isolates from various rice-growing countries, representing different AGs, showed very distinguishable patterns based on analysis of pectin enzymes, as well as RFLP analysis of A+T-rich DNA.

The main objectives of the present study were to characterise the genetic variability among 12 isolates of *R. solani* collected from different foliage blight-infected host plants from various localities in the Nile Delta by using isozymes and SDS protein profiles, and anastomosis within and among the isolates, to find out if there was a relationship between aggressiveness of the pathogen strains and any of these criteria.

Materials and Methods

Isolates

Rhizoctoni solani strains from different infected plants with damping-off, seedling blight, root rot and crown rot were isolated and identified on potato dextrose agar (PDA) medium. The identification of these isolated strains was confirmed with reference strains of this pathogen. We obtained 12 *Rhizoctoni solani* strains from different localities of the Nile Delta (Figure 1). These strains have been kindly provided by the Plant Pathology Research Institute, Agriculture Research Center, Al-Giza, Egypt.

Plant Host

Phaseolus vulgaris (bean seed cultivar Giza 6) was selected. It was kindly supplied by the Al-Gemmeza Agricultural Research Station (Al-Gharbia, Egypt) and was used as the host plant for the tested *R. solani* strains.

Pathogenicity Test

Each strain of *R. solani* was grown on barley grain medium, which is suitable for spore germination, mycelial growth and multiplication. Glass bottles (500 ml) each containing 200 g of a mixture of wheat bran:sand (1:3) was moistened with water and autoclaved for 30 min. The prepared bottles were inoculated separately with the pathogenic strains that had been grown on PDA and incubated at 37 °C for 14 days. Sterilised sandy-clay soil was compacted into 25-cm diameter plastic pots, each pot containing 3 kg of soil. Soil infestation was carried out 1 week before sowing the bean seeds (at the rate of 2% inoculum/100 g of soil). Soil was kept moist to allow spore germination and dispersion.

Twenty seeds of *Phaseolus vulgaris* Giza 6 were sown in each plastic pot for testing the pathogenicity of different *R. solani* strains and in order to determine their infection cushion type. All measurements carried out during this study were triplicated.

Both pre- and post-emergence damping-off were calculated for each treatment 15 and 45 days after sowing as follows:

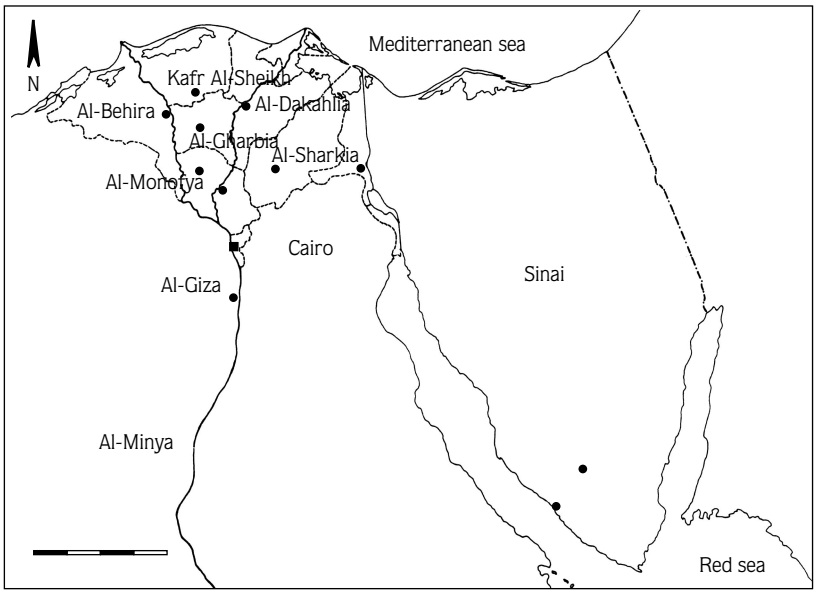

Figure 1. Different locations of *Rhizoctonia solani* strains used in the present study.

$$\frac{\text{Number of infected plants}}{\text{Total plant number}} \times 100$$

Percentage of survival plants (% survival) was calculated 45 days after sowing for each treatment as follows:

$$\frac{\text{Number of uninfected plants}}{\text{Total plant number}} \times 100$$

Infection Cushions by *R. solani* as a Response to Bean Seedling Infection

Study of the morphology of infection cushions was carried out according to El-Samra et al. (1981). Plastic pots, 8 cm in diameter, which contained autoclaved sandy soil, were infested with the tested fungi as previously described.

The bean seed samples (*Phaseolus vulgaris* Giza 6) were allowed to germinate for 1 week; then 8 groups of bean seedlings were wrapped in a cellophane sheet and each was cultivated under the soil surface in the infested pots. Seedlings were then irrigated every 2 days for 1 week. Cellophane sheets were then lifted and the outer surface facing the sandy soil of each sheet was gently cleaned using a soft brush. Areas of each sheet were cut at random, stained with Lactophenol Cotton Blue (2 x 4 cm), mounted in glycine on a glass slide, and examined microscopically for the incidence of infection cushions.

Infection cushions were categorised according to the complexity of branching hyphae and size, according to El-Samra et al. (1981). The frequency of each type of infection cushion was estimated as a mean percentage of cushions per microscopic field for each strain.

Mycelial Compatibility Groups

A representative sample of 12 isolates of *R. solani* originating from 12 sites in Egypt was selected. These isolates were then paired in all combinations on PDA to identify compatible strains. Mycelial compatibility was determined using the method of Earnshaw & Boland (1997). Inoculum plugs (5 mm in diameter) were placed 7 cm apart on PDA in 9-cm petri dishes and incubated at 28 °C in pairs of isolates, and were assessed 3 days later for compatible and incompatible reactions. Compatible reactions (+) were recorded if the 2 colonies merged without forming a dark line or a strip of thin mycelium. Incompatible reactions were recorded when a reaction line formed between the 2 colonies (–).

Protein Extraction

From each fungal isolate, 0.5 g of the fungal mycelium was collected from the fungal growth on solid medium. Using a mortar and pestle, the fungal mass was homogenised in 300 µl of cold extraction buffer (0.1 M Tris-HCl pH 7.5), which contained 0.001 M EDTA, 0.01 KCl, 0.01 M $MgCl_2$ and 4% PVP (polyvinyl pyrrolidone, MW 40,000). The homogenate was transferred into 1.5-ml Eppendorf tubes and centrifuged for 20 min at 15,000 x g. The centrifugation was performed at 4 °C using a Sigma laboratory centrifuge (Sigma 3k18, Biotech International, Germany). The supernatant was transferred into new Eppendorf tubes and kept at –80 °C until use.

SDS-PAGE Analysis

Denaturing polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to Laemmli (1970); 20 µl of fungal protein extract was mixed with 5 µl of sample buffer in Eppendorf tubes and then boiled in a water bath for 5 min. Denatured proteins were cooled at room temperature and quick spinning was performed before electrophoresis. A 25-µl protein sample was loaded in each well in stacking gel (5% w/v). The resolving gel was 15% (w/v). Protein fractionation was performed at a constant voltage of 100 V for 3 h, the gel was stained in 0.1% (w/v) Coomassie Brilliant Blue R250 and then the gel was destained in a destaining solution (20% methanol, 10% acetic acid, and 70% H_2O).

Isozyme Analysis

Isozyme analysis was carried out using a non-denaturing PAGE similar to the denaturing PAGE, but under non-denaturing conditions. After sample electrophoresis, a specific isozyme staining protocol was performed for each gel. In our study, 4 isozymes [esterase (EST), glutamate oxaloacetate transaminase (GOT), catalase (CAT), and peroxidase (PER)] were investigated, and only 3 isozymes worked well and gave reproducible isozyme patterns. For isozymes, electrophoresis was performed on 10% polyacrylamide gel, using a Tris/glycine buffer system (pH 8.3). As described for SDS-PAGE, electrophoresis was carried out at 100 V for 3 h. ESTs were stained using 0.15 g of Fast Blue RR and 0.02 g of a-naphthyl acetate as a substrate. GOT was assayed by incubating the gels for 15 min in 0.25 M Tris-HCl buffer (pH 7.2) containing 0.026 M L-aspartic acid and 0.007 M a-ketoglutaric acid. Gels were rinsed with distilled water and again incubated for 20 min in the same buffer containing 2% (w/v) Fast Blue BB. PERs were stained by incubating the gels in 0.05 M acetate buffer (pH 5.0) containing 65 mg of o-dianisidine dissolved in 5 ml of ethanol. CAT activity was assayed by incubating the gels in 50 ml of H_2O_2 for 5 min and then they were stained in a solution containing 0.5 g of ferric chloride and 0.5 g of potassium ferricyanide until bands became visible. The gels were then washed, fixed in 7% acetic acid and immediately photographed.

Data Analysis

The electrophoretic patterns were interpreted in terms of loci coding for EST, GOT, and PER. The presence or absence of a band, considered as a locus, was determined by calculating the Rf value, i.e. the ratio of the distance an enzyme migrated to the distance that the loading dye migrated (Bromophenol Blue). Zymogram patterns were transformed into a binary matrix: 1 for the presence of and 0 for the absence of a band with a particular Rf value. The data matrix was standardised using a standardisation program (STAND) in order to get the linear transformation of the variables in the data matrix, which were used to calculate the taxonomic distance (DIST) between each pair of the studied strains using the similarity of interval data program (SIMINT), in order to measure the dissimilarity between strains. Clustering was performed based on the UPGMA by using the sequential, agglomerative, hierarchal and nested clustering method (SAHN), as defined by Sneath & Sokal (1973), and Dunn & Everitt (1982). The output of the SAHN-clustering program was plotted in the form of a phenogram using the tree display graphic program (TREE PLOT).

The morphology of the infection cushion and the different symptoms observed in the pathogenicity test were used as characters to construct a dendrogram. For each isolate, the presence of cushion type or symptom and its absence were coded as 1 or 0, respectively. Based on these morphological characters, a data matrix was obtained. Then the data matrix was analysed, as described above, for electrophoretic data. Therefore, with the isozyme data and morphological character (shape of the infection cushion), pathogenicity test information (symptoms of infection, like presence or absence of seed rot, wilt, stunting, pre-emergence damping-off, post-emergence damping-off and survival percentage) and the use of computer software NTSYS-pc, cluster analysis was carried out according to the unweighted paired-group method with arithmetic averaging (UPGMA) method and dendrograms resulting from the dissimilarity matrix were drawn (Rohlf, 2001).

Results

Rhizoctonia solani isolates were collected from different host plants. Table 1 shows the location of the 12 *R. solani* isolates that were isolated from cotton, *Trifolium* and faba bean. *R. solani* isolates were obtained from different localities (Table 1) of the Nile Delta in Egypt.

The pathogenicity of the isolated *R. solani* strains on *Paseolus vulgaris* Giza 6 is shown in Table 2. Different disease symptoms, such as seed rot, wilt, stunting and pre- and post-emergence damping-off, were recorded for the host plants. All the isolated strains were pathogenic. The most pathogenic strains were nos. 2 and 3, while the least pathogenic strains were nos. 10 and 11. More than one symptom appeared on the same plant.

The isolated *R. solani* strains produce more than one type of infection cushion (Table 3). Strains 10 and 11 produced more simple types than complicated types of

Table 1. *Rhizoctonia solani* strains used in the present investigation.

Rhizoctonia solani strains	Host plants	Obtained from (locations)
Strain (1)	*Gossypium barbadensel* Giza 90	Al-Giza
Strain (2)	*Trifolium alexandrinum*	Al–Sharkia
Strain (3)	*Gossypium barbadensel* Giza 77	Al–Behira
Strain (4)	*Gossypium barbadensel* Giza 89	Al–Sharkia
Strain (5)	*Gossypium barbadensel* Giza 88	Al–Behira
Strain (6)	*Gossypium barbadensel* Giza 85	Al-Monofya
Strain (7)	*Gossypium barbadensel* Giza 86	Al–Gharbia
Strain (8)	*Gossypium barbadensel* Giza 86	Al–Gharbia
Strain (9)	*Gossypium barbadensel* Giza 86	Kafr Al–Sheikh
Strain (10)	*Gossypium barbadensel* Giza 86	Al–Dakahlia
Strain (11)	*Gossypium barbadensel* Giza 80	Al-Minya
Strain (12)	*Vicia faba*	Al-Monofya

Table 2. Pathogenicity of different strains of *Rhizoctonia solani*.

Strain\Symptoms	Seed rot	Wilt	Stunting	Pre- emergence damping-off	Post- emergence damping-off	Survival	Plant survival (%)
Rhizoctonia solani strain (1)	7	5	13	2	1	5	25
Rhizoctonia solani strain (2)	5	10	15	10	0	2	10
Rhizoctonia solani strain (3)	3	12	17	4	0	2	10
Rhizoctonia solani strain (4)	8	9	12	0	3	4	20
Rhizoctonia solani strain (5)	2	8	18	0	5	3	15
Rhizoctonia solani strain (6)	10	7	10	1	0	4	20
Rhizoctonia solani strain (7)	5	10	15	3	0	8	40
Rhizoctonia solani strain (8)	4	5	16	0	2	9	45
Rhizoctonia solani strain (9)	6	6	14	2	5	4	20
Rhizoctonia solani strain (10)	5	12	15	0	3	12	60
Rhizoctonia solani strain (11)	4	8	16	0	4	12	60
Rhizoctonia solani strain (12)	7	12	13	0	5	8	40
Control	0	0	0	0	0	20	100

Table 3. Types of infection cushions formed by different strains of *Rhizoctonia solani* in response to bean seedlings.

Types	Frequency of infection cushions (%)											
	Simple types							Complicated types				
Strains	Simple structure	Parallel structure	Sun structure	Tree structure	Dome structure	Broom structure	Total	Top fan structure	Parallel structure	Dome structure	Broom structure	Total
R. solani strain (1)	14.67	0	7	16.67	21.66	0	60	3.33	17.67	10.67	8.33	40
R. solani strain (2)	28.5	0	3.5	13.5	0	7	52.5	0	5	2.5	40	47.5
R. solani strain (3)	9.63	15.38	2.13	7.25	0	18.75	53.13	4.13	5.25	34.38	3.13	46.88
R. solani strain (4)	10.73	15.65	0	12.84	15.69	5.88	60.78	9.8	5.88	13.73	9.8	39.22
R. solani strain (5)	10.63	8.64	3.41	4.63	10.05	6.02	43.37	8.43	9.64	29.52	9.04	56.63
R. solani strain (6)	24.63	9.38	3.13	5.25	0	10.75	53.13	3.13	6.25	34.38	3.13	46.88
R. solani strain (7)	33.61	0	0	15.33	8.2	0	47.14	0	2.04	20.41	20.41	42.86
R. solani strain (8)	18.52	10.26	0	6.41	11.11	9.26	55.56	0	3.7	18.52	22.22	44.44
R. solani strain (9)	27.83	16.07	5.88	0	6.32	0	56.1	0	7.32	14.63	21.95	43.9
R. solani strain (10)	10.73	26.64	0	11.84	15.69	5.88	70.78	9.1	1.1	10.01	9.01	29.22
R. solani strain (11)	12.13	0	0	23.51	9.99	34.26	79.89	10.75	2.15	2.91	4.3	20.11
R. solani strain (12)	23.64	18.18	0	3.64	9.09	0	54.55	3.64	0	32.73	9.09	45.45
Control	0	0	0	0	0	0	0	0	0	0	0	0

cushions (70.78% and 79.89%, and 29.22%, and 20.11%, respectively). This means that the pathogenicity of these strains was not high. On the other hand, strains 2 and 3 produce the highest percentage of complicated infection cushions (52.5% and 53.13%, respectively) and the pathogenicity of these strains were very high. Table 4 presents the reactions of different isolates against each other for incompatible hyphal fusion, which was observed as a dark line between the 2 colonies (Figure 2), and the compatible reactions (Figure 3).

Table 4. Mycelial compatibility groups (MCGs) of different strains of *Rhizoctonia solani*.

Rhizoctonia solani strains	Strain (2)	Strain (3)	Strain (4)	Strain (5)	Strain (6)	Strain (7)	Strain (8)	Strain (9)	Strain (10)	Strain (11)	Strain (12)
Strain (1)	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve
Strain (2)		+ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
Strain (3)			+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Strain (4)				+ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve
Strain (5)					+ve	-ve	-ve	+ve	+ve	+ve	-ve
Strain (6)						+ve	+ve	+ve	+ve	+ve	-ve
Strain (7)							+ve	+ve	+ve	-ve	-ve
Strain (8)								+ve	-ve	+ve	+ve
Strain (9)									+ve	+ve	+ve
Strain (10)										+ve	+ve
Strain (11)											+ve

+ve: mycelia were compatible.
-ve: no mycelial compatibility occurred.

Figure 2. Incompatible strains 2 and 11.

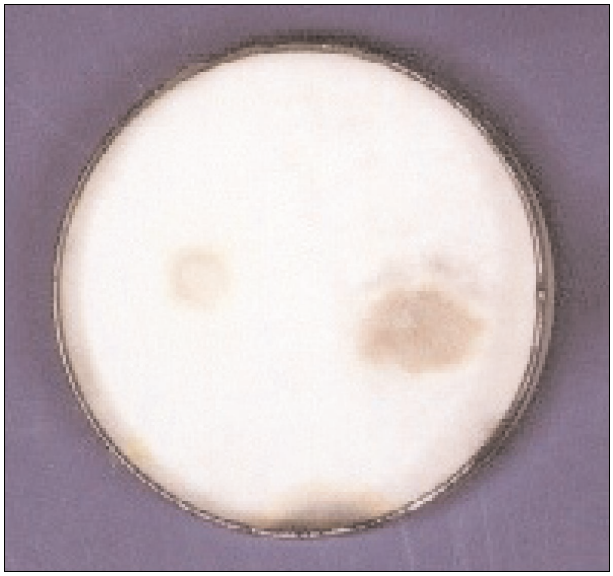

Figure 3. Compatible strains 2 and 6.

Soluble Protein Analysis

Although *R. solani* was isolated from very divergent host plants, the total soluble protein patterns among them were similar. However, the only observed difference was in 2 low MW protein bands (approximately 30 and 20.1 kD) between the mycelium sample collected from the compatibility region between *R. solani* isolates 8 (Al-Gharbia, Al-Santa) and 11 (Al-Minya), where they showed positive compatibility. There was the absence of one band in the low molecular weight region (approximately 20.1 kD), which was characteristic of the protein profiles of all the isolates, and the appearance of a new protein band slightly higher in molecular weight, which was 30 kD (data not shown). As expected, the compatibility between *R. solani* isolates 7 (Al-Gharbia, Tanta) and 11 (Al-Minya, Upper Egypt) showed similar SDS-PAGE banding patterns, as did all other isolates, as they were incompatible.

Isozyme Analysis

Isozyme analysis showed that only 3 enzymes, namely EST, GOT, and PER, produced pronounced enzyme activities, whereas others, such as CAT, gave unsatisfactory results.

A total of 7 electrophoretic bands were detected for EST. Among all isolates tested, a minimum of 1 and a maximum of 4 bands were observed. Higher polymorphism was observed among *R. solani* isolates using the EST isozyme; however, the *R. solani* isolates 1 (Al-Giza), 2 (Al-Sharkia), 3 (Al-Behira), 4 (Kafr Al-Dawar) and 5 (Al-Behira) showed similar isozyme patterns (Figure 4). The isolates 6 (Al Monofya), 8 (Al-Gharbia, Al-Santa) and 9 (Kafr Al-Sheikh, Sakha) exhibited different EST isozymes compared to the rest of the isolates selected from different locations. The positive and negative compatible samples showed variation in EST isozymes. Some of the EST isozymes showed high activity in some isolates. An arrow indicates the high activity observed in this particular isozyme band (Figure 4). Regarding the positive compatible samples (8 (Al-Gharbia, Al-Santa) and 11 (Al-Minya)), one unique low molecular weight isozyme was observed and is indicated with an arrow (Figure 4).

GOT isozyme analysis revealed 3 electrophoretic bands among the *R. solani* isolates examined, and a minimum of 1 and a maximum of 3 GOT bands. The isolates 5 and 7 showed only one GOT isozyme band, but with higher activity compared to other isolates. No polymorphism was found between the negative and positive compatible samples (7 (Al-Gharbia, Tanta), 11 (Al-Minya), 8 (Al-Gharbia, Al-Santa) and 11 (Al-Minya)) analysed.

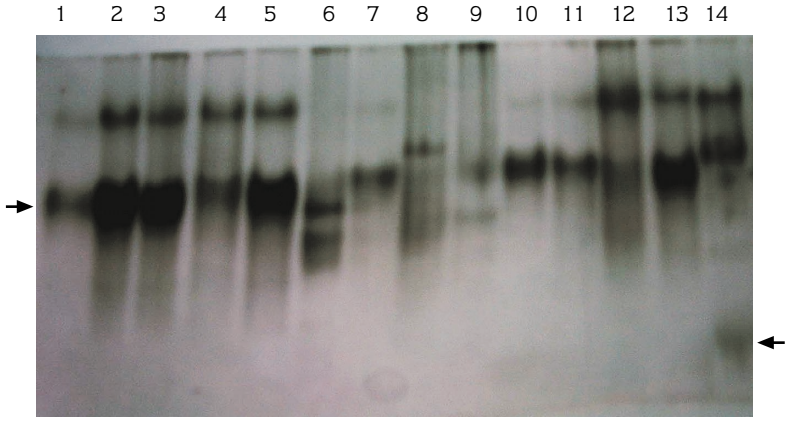

Figure 4. Electrophoretic banding pattern of the 12 different *R. solani* isolates (1-12), and negative and positive compatible 13 and 14, respectively, based on esterase isozyme analysis.

With respect to PER analysis, there were 4 electrophoretic bands among *R. solani* isolates tested, and a minimum of 1 and a maximum of 3 bands. The PER isozymes were polymorphic among most of the *R. solani* isolates and higher expression of PER isozymes was found for isolates 6 (Al-Monofya) and 8 (Al-Gharbia, Al-Santa). Although the compatibility regions between isolates 7 (Al-Gharbia, Tanta), 8 (Al-Gharbia, Al-Santa) and 11 (Al-Minya) showed similar PER isozymes, very high PER isozyme expression was observed for the compatibility region between isolates 7 (Al-Gharbia, Tanta) and 11 (Al-Minya) (showed negative compatibility test).

Figure 5 shows the dendrogram analysis based on the isozyme data of the 3 polymorphic enzymes tested. This cluster analysis revealed that the *R. solani* isolates fell into 4 distinct groups. Group I included isolates 1 (Al-Giza), 2 (Al-Sharkia), 3 (Al-Behira), 4 (Kafr Al-Dawar) and 5 (Al-Behira). Group II constituted isolates 7 (Al-Gharbia, Tanta), 10 (Al-Dakahlia), 11 (Al-Minya) and 12 (Al-Monofya). Group III contained 2 isolates, 6 (Al-Monofya) and 9 (Kafr Al-Sheikh). In group IV, there was only isolate 8 (Al-Gharbia, Al-Santa).

The dendrogram resulting from cluster analysis based on compatibility tests (Table 4) and other characteristics of the infection cushions (Table 3) showed that the 12 *R. solani* isolates were placed into 3 main distinct groups (Figure 6). Group I included isolates 1 (Cairo, Giza), 2 (Al-Sharkia), 3 (Al-Behira), 5 (Al-Behira), 6 (Al-Monofya) and 11 (Al-Minya), whereas group II was composed of 4 isolates (7, 8, 9, 10) isolated from 3 governorates of the Middle Delta: Al-Gharbia, Kafr Al-Sheikh, and Al-Dakahlia, respectively. Group III constituted only isolates 4 (Kafr Al-Dawar) and 12 (Al-Monofya).

Discussion

R. solani strains were collected from infected *Cotton, Trifolium* and *Vicia faba* plants in cultivated areas of the Nile Delta (Egypt). These strains were isolated. The pathogenicity of the isolated *R. solani* strains was tested in the greenhouse on *Phaseolus vulgaris* Giza 6. Infected plants show seed rot where the fungal mycelium invades the seed coat and enters the cotyledons of the seed; then the fungal hyphae grow between starch grains in the seed, disintegrate the cells and starch grains due to the secretion of amylases, and finally the component of the seed is changed into a dark brown liquid and the fungal hyphae form sclerotia and remain in the soil.

Infection by *R. solani* also causes wilt in *Phaseolus vulgaris* seedlings. Wilt or seedling blight is due to the browning and blocking of xylem vessels by fungal elements; this blocking prevents water from moving up through the plants (Mubarak, 2003).

Infection with *R. solani* leads to stunting of the seedling, which might be due to disturbance in cell division of the meristimatic cells in roots and the shoot apex (Mubarak, 2003). Merestimatic tissues are the tissues most affected by infection due to their thin walls; they are the first to be invaded by the fungus, followed

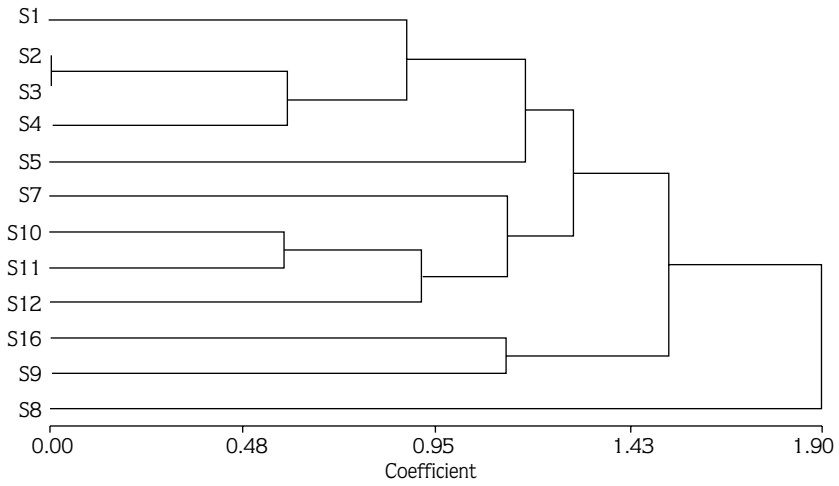

Figure 5. A UPGMA tree based on isozyme data of the 12 different *R. solani* isolates.

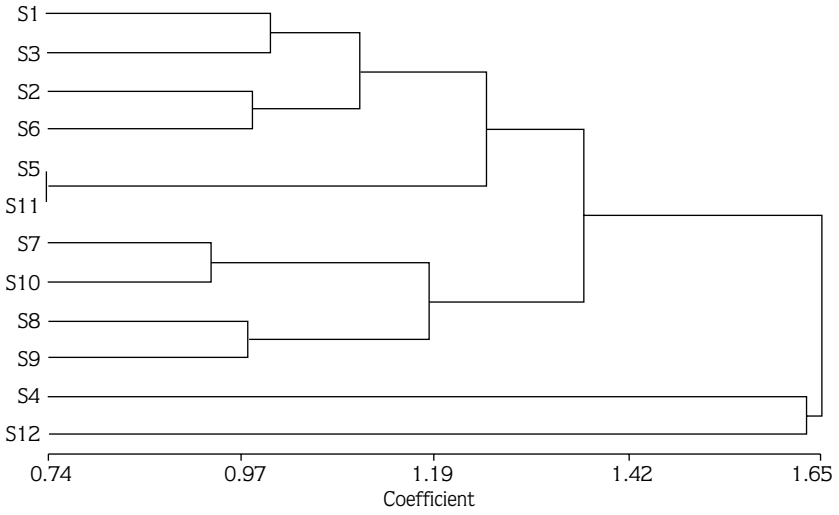

Figure 6. A UPGMA tree based on the pathogenicity and other morphological characteristics of 12 different *R. solani* isolates.

by the other tissues of the plant. Moreover, it invades the plant at the transition zone due to the hyphae invading the epidermis, cortex, phloem and xylem in this region, and form sclerotia between cortical cells. Then, the pathogen secrets pectinolytic enzymes that degrade the cell walls of this zone, which rot, and the plant falls to the ground (Mubarak, 2003).

R. solani strains produce different types of infection cushions, which can be either simple (simple-like structure, parallel-like structure, sun-like structure, tree-like structure, dome-like structure and broom-like structure) or complicated (top fan-like structure, complex parallel-like structure, complex dome-like structure and complex broom-like structure). The occurrence of more complicated cushion types than simple types of *R. solani* strains might have been related to its high pathogenicity in the present study. This result is similar to El-Samra et al.'s (1981). They found that simple infection cushions were more commonly formed on cotton cultivar susceptible to *R. solani*, whereas more complex forms predominated on resistant cultivars. It was determined that as susceptibility to a specific isolate of *R. solani*

increases, so does the induction of simple infection cushion types (El-Faham & Aboshosha, 1987; El-Farnawany, 1991).

The presented data suggest that there are no variations among the Egyptian *R. solani* isolates according to their protein patterns; however, the mycelia of the positive compatible region of isolates 8 (Al-Gharbia, Al-Santa) and 11 (Al-Minya) showed different protein profiles. Similarly, Mohammadi et al. (2003) found that the total soluble protein patterns among *R. solani* isolates within AG1 were similar in spite of the fact that they were isolated from diverse host plants. The only observed differences were in the low MW protein bands between the AG1A and AG1B subgroups (Mohammadi et al., 2003).

In contrast, Kuninaga (1986) showed that not only total soluble protein profiles are different among AGs, but distinct patterns can also be detected between homologous groups within one particular AG. Comparison of the soluble protein patterns of the 11 studied groups of *R. solani* revealed that AGs exhibited significant differences between various groups, but that there was slight variation among isolates from each group (Liu & Ge, 1988).

Biochemical and molecular markers are considered important tools for studying the genetic diversity of pathogens in which morphological characteristics are not adequate to properly distinguish different isolates (Sharma et al., 2005). Moreover, morphological characters are also influenced by environmental and cultural conditions. Consequently, problems related to studying different levels of genetic diversity in *R. solani* have been proposed to be best solved by the employment of molecular techniques (Toda et al., 1999). In the present study, 12 *R. solani* isolates were genetically separated and classified into different groups by using 3 polymorphic isozymes, namely EST, GOT, and PER. In the present study, some of the isolates were collected from the same host and location. Likewise, based on cluster analysis and similarity matrix of 6 isozyme analyses, the fungal isolates were grouped into 2 genetically distinct groups (I and II) consistent with the previously described AG1-IA and AG1-IB subgroups in AG1 (Mohammadi et al., 2003).

The results obtained from isozyme analysis in this study suggested that isozyme analysis could be useful in genetic diversity studies and identification of various *R. solani* isolates. Similar results were observed by Mohammadi et al. (2003, 2004), who used isozymes and total soluble protein in studying the genetic diversity of several isolates of *R. solani* and *Fusarium oxysporium* isolated from different locations in Iran. Toda et al. (1998) showed that a dendrogram constructed from RAPD analysis made one cluster of *R. solani* isolates of European pear web blight belonging to the anastomosis group AG1-BI. Upmanyu et al. (2003) also grouped 18 French bean *R. solani* isolates collected from different geographical regions of Himachal Pradesh into 2 anastomosis groups, i.e. AG-1 (AG1-AI and AG1-BI) and AG-4. In conclusion, although this study observed some grouping of isolates from the related locations (Figures 5 and 6), there was no clear relationship between pathogenicity, host plant and different locations. However, Singh et al. (2002) reported that pathogenic variation is related to the distribution of isolates in different climatic regions and that environment might influence pathogenic variability. Sharma et al. (2005) reported some relationship between genetic variation based on molecular markers and similar sclerotial characteristics among the isolates of *R. solani*.

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