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INCIDENCE, ANASTOMOSIS GROUPING AND GENETIC VARIABILITY OF *Rhizoctonia solani* INFECTING POTATO IN EGYPT.

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ABSTRACT

Potato (*Solanum tuberosum* L.) is the second vegetable crop in Egypt after tomato. Incidence percentage of stem canker and black scurf diseases of potato were monitored 2005/2006 and 2006/2007 in different locations at Behera, Dakahlia, Gharbia, Menofia and Sharkia Governorates. The two diseases were found in all examined locations. The highest percentage of infection was recorded at Sharkia Governorate during the two seasons of summer and winter. Three hundreds isolates of *Rhizoctonia solani* were obtained from the infected samples. These isolates were identified by classic method, and all isolates were determined as AG-3 by using the different AG- testers.

Pathogenicity tests of selected 20 *R.solani* isolates, according to difference in morphological characters of colony, were evaluated on potato (cv. Monalisa) plants under greenhouse conditions. The highest percentages of stem canker and black scurf were obtained by two Sharkia's isolates (code 18 and 17).

Genetic variability among 20 isolates of *R.solani* was determined using RAPD-PCR technique. The banding patterns generated from those isolates by five primers showed high levels of genetic similarity among the isolates from different geographical locations where the genetic similarity was more than 90%.This technique showed that there was no relation between the geographical locations and genetic content of the tested isolates.

Keywords: Potato, *Rhizoctonia solani*, Stem canker, Black scurf, Anastomosis group, AG-3, RAPD.

INTRODUCTION

Rhizoctonia solani was reported as the causal agent of Rhizoctoniosis of potato in Belarus, is composed of anastomosis groups AG-1, AG-3 and AG-4. These anastomosis groups differed in their tolerance to environmental factors, competitive ability and virulence (Ivanyuk *et al.* 2002). Also, Yanar *et al.* (2005) collected 304 *R. solani* isolates and 60 binucleate *Rhizoctonia*-like fungi which were recovered from stems and tubers of infected potato plants in Turkey. *R. solani* isolates were identified to 11 anastomosis groups (AGs): AG-1 (0.66%), AG-2-1 (5.6%), AG-2-2 (0.99%), AG-3 (83.9%), AG-5 (4.6%), AG-6 (0.66%), AG-8 (1.32%), AG-9 (0.33%), AG-10 (1.32%), AG-12 (0.33%), and AG-13 (0.33%). In the greenhouse tests, most of the AG-3 isolates were significantly more virulent than isolates belonging to other AGs on potato cv. Batum.

R. solani, the causal agent of stem canker and black scurf disease on potato, survives as sclerotia on tubers, in soil, in plant residues and can be considered it the main inoculum source for disease development. Also, the infested seed tubers and soil are important in disease development (Tsror *et al.*, 2005). All *R. solani* isolates belonged to AG-3 are multinucleate and the

teleomorphs of the pathogen is accepted as *Thanatephorus cucumeris* (Sneh *et al.*, 1991).

Bernat (2005) reported that during 1998-2000 in Bonin (northwestern Poland), the effects of meteorological conditions on the occurrence of black scurf (*R. solani*) on the tubers of 11 potato cultivars (Bard, Lord, Denar, Vineta, Alicja, Wigry, Wiking, Kuba, Danusia, Wawrzyn and Wolfram) were studied. The mean air temperature in July-September had significant effects on tuber infection. The following cultivars appeared to be the most susceptible to black scurf: Wolfram, Viking and Alicja. The lowest occurrence of *R. solani* sclerotia was recorded in cultivars Danusia, Vineta, Wawrzyn and Kuba.

A method based on restriction analysis of polymerase chain reaction (PCR)-amplified ribosomal DNA was developed for the rapid characterization of large populations of *R. solani* at the anastomosis group (AG) level. The restriction maps of the internal transcribed spacers (ITS) sequences were compared for 219 isolates of *R. solani* belonging to AG-1 to AG-12. The efficiency of the typing method was confirmed by analyzing PCR-amplified ITS sequences of 30 reference strains. Furthermore, the PCR-RFLP method was used to characterize at the AG level for 307 isolates of *R. solani* originating from ten sugar beet fields exhibiting patches of diseased plants in France. The PCR-based procedure described in this paper provides a rapid method for AG typing of *R. solani* in relation to geographical locations in Egypt and variability of genetic contents. The aim of the present work was to study the occurrence of stem canker and black scurf disease during two successive seasons in different locations in Egypt, and determine the anastomosis grouping and genetic variability for 300 *R.solani* isolates from potato.

MATERIALS AND METHODS

1. Incidence of stem canker and black scurf disease of potato:

Infection percentage of stem canker and black scurf disease of potato was estimated during 2 successive years of 2005/2006 and 2006/2007 in different locations at Behera, Dakahlia, Gharbia, Menofia, and Sharkia Governorates. Infection percentage was determined according to Johnston *et al*. (1994).

2. Isolation, purification and identification:

Three hundreds isolates of *R. solani* were isolated and purified following the method described by Papavizas and Davey (1959). Potato plants and tubers (90 days-old) exhibiting symptoms of stem canker and black scurf were cut into small fragments. Twenty isolates of *R. solani* were selected from the collective isolates which showed differences in morphological characters of the colonies and then were identified according to the description of Gilman (1959) and Sneh *et al*. (1991).

The hyphal anastomosis of *R. solani* isolates were examined on water agar medium based on hyphal magnification. The twenty isolates of *R. solani* were paired with AG-1, AG-2-2. AG-3 and AG-4 tester isolates and were paired with each other for all possible combination. Hyphal fusion at minimum

of five points was considered to be positive indication according to Parameter *et al*. (1969).

3. Pathogenicity test:

In this experiment pathogenicity of 20 *R.solani* isolates selected from five governorates (4 isolates from Behera , 4 isolates from Dakahlia , 4 isolates from Gharbia , 3 isolates from Monofia and 5 isolates from Sharkia) were evaluated on potato.Soil infestation was conducted with inoculum of each tested isolate on corn-meal sand medium (10 days-old). Loamy soil was sterilized with formalin solution 5 %, and after two weeks soil infestation was carried out carried out at the level of 5 % (w/w) inoculum. Potato tubers of cultivar Monalisa were surface sterilized by immersion in 0.2 % Sodium hypoclorite for 2 min. and putting in pots 50-cm. diam. (5tubers/pot) in infested soil with 4 replicates for each treatment under the greenhouse conditions. Disease assessment was carried out after one hundred days according to Barrett and Horsfall (1945).

4. Molecular Studies:

Possible genetic variations among *R. solani* isolates were determined using (RAPD-PCR) fingerprinting.

4.1. DNA extraction:

Discs (5mm diam.), was taken from 10 days-old *R. solani* culture grown on potato-dextrose agar (PDA) medium, floated on 100 ml of broth PDA media in 250 ml conical flasks, then incubated with shaking at 125 rpm using an orbital shaker (Gallenkamp), at 23°C for 4 days. Growing mycelia were harvested by filtration through sterile cloth, washed with sterile deionized water, frozen in liquid nitrogen freeze-dried and were stored at −20°C.

DNA was extracted from 50 mg of mycelial growth using Qiagen Kit. Extracted DNA was dissolved in 100µl of elution buffer. The concentration and purity of the obtained DNA was determined by using “Gen qunta” system, Pharmacia Biotec for all DNA samples. The concentration was adjusted to 6 ng/µl using TE buffer (pH. 8.0); meanwhile, the purity reached 90-97% with ratios between 1.7-1.8.

4.2. Random amplified polymorphic DNA technique (RAPD):

For each tested *R. solani* isolate, 30 ng of the extracted DNA was used for the amplification reaction. The polymerase chain reaction (PCR) mixture contained PCR beads tablet (manufactured by Amessham Pharmacia Biotech), which containing all the necessary reagents except the primer and the DNA which were added to the tablet. Code, nucleotide sequence and G+C percentage of tested primers used in these RAPD reactions are shown in Table (1).

Of each tested primer, 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 amplification protocol using PCR (Thermocycler T1, Biometra, Germany) was carried out as follows: a) Denaturation at 95ºC for 5 min (one cycle); b) 45 cycles, each consists of the following steps: 1- Denaturation at 95ºC for 1 min., 2- Annealing at 36ºC for 1.0 min., 3- Extension at 72ºC for 2 min.; c) Final extension at 72ºC for 5 min (one cycle) and d) Hold at 4ºC.

Table 1: Code, nucleotide sequence and G+C (%) of primers used in the random amplified polymorphic DNA (RAPD) reactions.

Primer	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

4.3. DNA electrophoresis:

For all samples, the amplified DNA (15 µl) was electrophoresed using electrophoresis unit (WIDE mini-sub-cell GT Bio-RAD) on 2% agarose containing ethedium bromide (0.5 µg/ml), at a constant 75 volt and 60 mA, and visualized with UV trans-illuminator.

4.4. Gel analysis:

DNA gel was scanned for band R_f using gel documentation system (AAB Advanced American Biotechnology 1166 E. Valencia Dr. Unit 6 C, Fullerton, CA 92631). The different molecular weights of bands were determined against a DNA standard (kb DNA ladder, Stratagene, Canada) with molecular weights 250, 500, 750, 1.000, 1.500, 2.000, 3.000, 4.000, 5.000, 6.000, 7.000, 8.000, 9.000, 10.000, and 12.000 bp. The similarity level was determined by un-weighted pair group method based on arithmetic mean (UPGMA).

5. Statistical analysis:

The statistical analysis was computed using analysis of variance procedure described by Sendecor and Cochran (1980), the significant mean differences between treatment means were separated by Duncan·s Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

1. Occurrence of the stem canker and black scurf in potato:

Table (2) indicate that, the highest percentage of infection of stem canker disease (31.1%) and (33.8%) was recorded in summer season at Sharkia Governorate in 2005/2006 and 2006/2007, respectively. While, the highest infection percentage of black scurf (19.6%) and (16.3%) was recorded at the same governorate in winter season.

Examination of potato fields during the two successive years of 2005/2006, and 2006/2007 in 5 different governorates of Egypt, showed that black scurf and stem canker diseases of potato were found in all examined locations. Several researchers had reported that stem canker and black scurf disease is usually found in everywhere of potato cultivation (Abd-El-Haleem, 1972; Bandy *et al.* 1988; and Anguize and Martin, 1989). However, there were similar fluctuations of the estimated occurrence of the disease in the surveyed sites that gave an impression that variation in disease incidence during the years of examination may be attributed to the geographical localities and plantation time or climates. Disease occurrence differed

significantly from plantation time to another and from location to the other. The fluctuation of the disease occurrence from year to another and differences within plantation seasons may be attributed probably to the meteorological factors (Hide *et al.* 1973; Hill, 1980 and Anderson, 1982).

Table 2: Occurrence of potato stem canker and black scurf at the different surveyed governorates during 2005/2007 potato plantations.

Governorate	Season	Percentage of infection (Mean ± SE)			
		Stem canker		Black scurf	
		2005 / 2006	2006 / 2007	2005 / 2006	2006 / 2007
Beheira	Summer	16.3 ± 0.55 ^{ef}	16.6 ± 0.55 ^{ef}	10.6 ± 0.7 ^{b}	12.3 ± 0.70 ^{b}
	Nili	12.3 ± 0.67 ^{g}	15.0 ± 0.67 ^{g}	7.0 ± 0.47 ^{c}	14.4 ± 0.47 ^{c}
	Winter	23.3 ± 0.73 ^{c}	24.7 ± 0.73 ^{c}	12.3 ± 0.75 ^{b}	16.5 ± 0.75 ^{b}
Dakahlia	Summer	19.3 ± 0.55 ^{d}	16.4 ± 0.55 ^{d}	12.6 ± 0.65 ^{b}	11.7 ± 0.65 ^{b}
	Nili	7.3 ± 0.70 ^{i}	5.7 ± 0.7 ^{i}	3.6 ± 0.47 ^{d}	3.0 ± 0.47 ^{d}
	Winter	16.0 ± 0.64 ^{ef}	12.6 ± 0.64 ^{ef}	10.3 ± 0.67 ^{b}	8.3 ± 0.67 ^{b}
Gharbia	Summer	16.6 ± 0.48 ^{ef}	15.7 ± 0.48 ^{ef}	11.6 ± 0.67 ^{b}	9.3 ± 0.67 ^{b}
	Nili	7.0 ± 0.41 ^{i}	6.3 ± 0.41 ^{i}	3.0 ± 0.42 ^{d}	2.3 ± 0.46 ^{d}
	Winter	17.3 ± 1.11 ^{e}	15.6 ± 1.11 ^{e}	10.6 ± 0.53 ^{b}	8.0 ± 0.53 ^{b}
Menofia	Summer	14.6 ± 0.81 ^{f}	12.7 ± 0.81 ^{f}	7.6 ± 0.62 ^{c}	10.8 ± 0.62 ^{c}
	Nili	9.8 ± 0.64 ^{h}	6.3 ± 0.64 ^{h}	3.3 ± 0.73 ^{d}	6.0 ± 0.73 ^{d}
	Winter	19.6 ± 0.54 ^{d}	20.0 ± 0.54 ^{d}	10.6 ± 0.70 ^{b}	11.3 ± 0.70 ^{b}
Sharkia	Summer	31.1 ± 0.68 ^{a}	33.8 ± 0.68 ^{a}	19.6 ± 0.57 ^{a}	16.3 ± 0.57 ^{a}
	Nili	12.3 ± 0.67 ^{g}	12.6 ± 0.67 ^{g}	7.6 ± 0.81 ^{c}	8.6 ± 0.81 ^{c}
	Winter	28.1 ± 0.68 ^{b}	26.0 ± 0.68 ^{b}	12.6 ± 0.65 ^{b}	12.5 ± 0.65 ^{b}
LSD (0.05)		1.938	1.938	1.834	1.834

- Each value represents the mean ± S.E (Standard Error) and mean of 100 replicates.
- Values in the same column with the same letter are not significantly different at ($p \leq 0.05$).

2. Isolation and purification of the causal pathogen of stem canker and black scurf diseases:

Data presented in Table (3) showed that 300 fungal isolates belonging to *R. solani* were identified according to the morphological and microscopical characters, as described by Parmeter *et al.* (1969) and Parmeter and Whitney (1970).

Table 3: Total isolates of *R. solani* from each governorate

Governorate	Total of isolates
Behera	60
Dakahlia	60
Gharbia	60
Menofia	45
Sharkia	75

3. Pathogenicity test:

Figure (1) show pathogenicity symptoms of some selected *R.solani* isolates, according to the difference in morphological characters of colony, evaluated on potato (cv.Monalisa) tuber under greenhouse conditions.

Data presented in Table (4) show significant differences of stem canker and black scurf disease were variable among the 20 tested isolates of *R. solani*. The highest percentage of stem canker was obtained by two Sharkia's isolates with code number (18 and 17) which recorded 31.6 and 28.4%, respectively. On the other hand, the same trend was observed in relation to black scurf symptom of the disease, the same isolates recorded 16.7 and 13.9, respectively.

Table 4: Pathogenicity test of 20 isolates of *R. solani* on potato under green house conditions.

Governorate	Isolate code No.	Percentages of infection (Mean ± SE)	
		Stem canker	Black scurf
Behera	1	21.3 ± 0.30 ^{d}	13.1 ± 0.219 ^{d}
	2	20.5 ± 0.29 ^{de}	13.9 ± 0.319 ^{b}
	3	16.6 ± 0.58 ^{g}	11.0 ± 0.580 ^{de}
	4	11.7 ± 0.32 ^{j}	8.9 ± 0.349 ^{f}
Dakahlia	5	18.8 ± 0.41 ^{f}	12.1 ± 0.174 ^{c}
	6	23.8 ± 0.26 ^{c}	8.4 ± 0.261 ^{f}
	7	17.0 ± 0.17 ^{g}	7.3 ± 0.203 ^{g}
	8	20.0 ± 0.23 ^{e}	10.6 ± 0.348 ^{de}
Gharbia	9	16.6 ± 0.35 ^{g}	8.6 ± 0.177 ^{f}
	10	11.3 ± 0.30 ^{j}	7.1 ± 0.21 ^{g}
	11	13.1 ± 0.15 ^{i}	5.2 ± 0.29 ^{h}
	12	20.8 ± 0.47 ^{de}	13.3 ± 0.20 ^{b}
Menofia	13	13.8 ± 0.27 ^{i}	7.1 ± 0.23 ^{g}
	14	15.4 ± 0.26 ^{h}	8.8 ± 0.27 ^{f}
	15	16.9 ± 0.19 ^{g}	10.2 ± 0.18 ^{e}
Sharkia	16	16.9 ± 0.12 ^{g}	10.2 ± 0.20 ^{e}
	17	28.4 ± 0.26 ^{b}	13.9 ± 0.38 ^{b}
	18	31.6 ± 0.35 ^{a}	16.7 ± 0.27 ^{a}
	19	23.7 ± 0.29 ^{c}	11.3 ± 0.18 ^{cd}
	20	18.4 ± 0.30 ^{f}	7.0 ± 0.17 ^{g}
L.S.D. $_{0.05}$		0.89	0.79

- Each value represents the mean ± S.E (Standard Error) and mean of 4 replicates.
- Values in the same column with the same letter are not significantly different at ($p \leq 0.05$).

Fig. 1: Symptoms of black scurf potato tuber (cv.Monalisa) induced after the artificial inoculation with *R.solani* (pathogenicity test).

The obtained results are in harmony with those by Rahman *et al.* (1996) and Khan and Javed (2001), the differences between the tested isolates may be attributed to the ability of secretion the proteolytic and cellulolytic enzymes and/or the variation in the genetically character of the tested isolates. Also, these differences may be due to the difference in the ability to secrete toxic materials which help the fungi to invade and colonize (Manoharachary *et al.,* 1989).

4. Anastomosis grouping:

Identification of 20 *R. solani* isolates from several governorates into anastomosis group AGs using AGs testers (AG-1, AG-2, AG-3 and AG-4). Data presented in Table (5) show twenty isolates of *R.solani* belonged to AG-3 group. The anastomosis processes occurred between any possible combinations by H shape procedures the compatibility through direct hyphal contact. The present work confirmed that *R. solani* isolated from potato tubers and plants showing the typical symptoms of black scurf and stem canker are belonging mostly to AG-3. The obtained results are in harmony with Demirci and Doken (1993).

Pathogenic isolates of AG-3 cause black scurf of potatoes (Hooker, 1981 and Anguize & Martin, 1989). However, further studies are required to identify the natural distributions of the pathogen under the Egyptian climate and conditions.

Table 5: Anastomosis group of 20 isolates of *R. solani* with four testers of AGs.

Governorate	Isolate code No.	Anastomosis group			
		AG-1	AG-2-2	AG-3	AG-4
Behera	1	-	-	+	-
	2	-	-	+	-
	3	-	-	+	-
	4	-	-	+	-
Dakahlia	5	-	-	+	-
	6	-	-	+	-
	7	-	-	+	-
	8	-	-	+	-
Gharbia	9	-	-	+	-
	10	-	-	+	-
	11	-	-	+	-
	12	-	-	+	-
Menofia	13	-	-	+	-
	14	-	-	+	-
	15	-	-	+	-
Sharkia	16	-	-	+	-
	17	-	-	+	-
	18	-	-	+	-
	19	-	-	+	-
	20	-	-	+	-

(+) anastomosis, (-) No anastomosis

5. Genetic variability of *R. solani* AG-3:

Genetic variability among 20 isolates of *R. solani* was determined using PCR-based techniques. Twenty isolates were recovered from five governorates in Egypt, all these isolates are AG-3 group.

5.1. Random amplified polymorphic DNA (RAPD) profiles:

Amount of genetic variation was evaluated by polymerase chain reaction amplification with 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.

5.2.1. RAPD profiles of *R. solani* isolates obtained using primer 1:

A UPGMA dendrogram analysis clustered the isolates into two main clusters based on the banding pattern (Fig. 2).

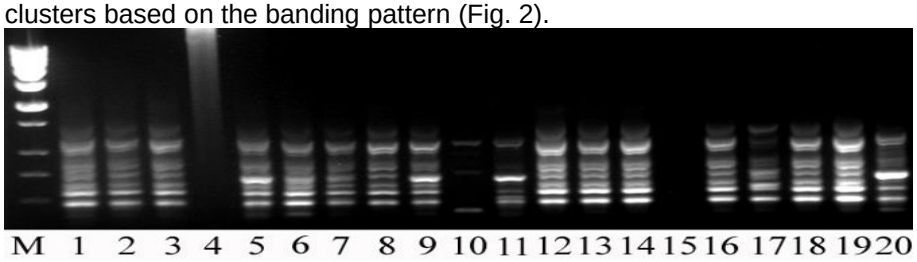

Fig. 2: Random amplified polymorphic DNA of 20 isolates of *R. solani* by using primer 1.

The genetic similarities (GS) among the tested isolates ranged from 71.31 - 99.79% (Fig. 3). There was no clear-cut kinship between clustering in this RAPD dendrogram and geographic origin of tested isolates. Most of the isolates collected from widely different regions were gathered in the same groups i.e., the isolates Sharkia 17 and Dakahlia 6 showed high genetic similarity of 98.89%. On the other hand, isolate 10 from Gharbia cave in a separate clustering group with 61.23%similarity with the rest of isolates.

Fig. 3: Cluster analysis and dendrogram derived from RAPD analysis of 20 *R. solani* isolates, using primer 1.

5. 2.2. RAPD profiles of obtained *R. solani* isolates using primer 2:

The 20 isolates of *R. solani* were clustered into two main clusters with genetic similarity range of 55.04-98.59% (Figs. 4 and 5). The first main cluster included two groups at 69.28% GS between two groups. No clear-cut kinship between clustering in this RAPD dendrogram and geographic origin of tested isolates, the isolates Dakahlia 8 and Menofia 13 showed high genetic similarity of 98.80%. The isolates Menofia 15 showed low genetic similarity of 55.04% with other isolates (Fig. 5).

5.2.3. RAPD profiles of obtained *R. solani* isolates using primer 3:

The 20 isolates of *R. solani* were clustered into two main clusters with genetic similarity range of 60.47-98.52% (Fig.6 and 7). The first main cluster included two groups at 67.77% GS between two groups. The second main cluster included two groups at 85.24% GS between two groups. In the first main cluster, no clear-cut kinship between clustering in this RAPD dendrogram and geographic origin of tested isolates. The isolates collected from widely different regions were gathered in the same groups. For example, the isolates Beheria 2 and Dakahlia 6 showed high genetic similarity of 95.99%. In the second main cluster no clear-cut kinship between clustering in this RAPD dendrogram and geographic origin of tested isolates. For example the isolates Gharbia 12 and Dakahlia 7 showed high genetic similarity of 97.20%. (Fig. 7).

5.2.4. RAPD profiles of obtained *R. solani* isolates using primer 4:

The 20 isolates of *R. solani* were clustered into two main clusters with genetic similarity range of 74.43-99.84% (Fig. 8 and 9). Only one isolate Menofia 15 separated in the first main cluster with 75.65% GS with the remaining isolates. The second main cluster divided to two sub-cluster at 94.03% GS between two groups. No clear-cut kinship between clustering in this RAPD dendrogram and geographic origin of tested isolates. The isolates collected from widely different regions were gathered in the same groups i.e., the isolates Gharbia 9 and Dakahlia 6 showed high genetic similarity of 99.45%.

5.2.5. RAPD profiles of obtained *R. solani* isolates using primer 5:

The 20 isolates of *R. solani* were clustered into two main clusters with genetic similarity range of 89.30-99.98% (Fig. 10 and 11). Only one isolate Gharbia 9 separated in the first main cluster, with 94.71% GS with the remaining isolates. No clear-cut kinship between clustering in this RAPD dendrogram and geographic origin of tested isolates. The isolates collected from widely different regions were gathered in the same groups, for example, the isolates Dakahlia 7 and Sharkia 18 showed high genetic similarity of 99.07%. (Fig. 10).Genetic variability among 20 isolates of *R. solani* was determined, using the random amplified polymorphic DNA (RAPD) technique, to analyze the genetic relationship between 20 isolates of *R. solani* and ascertain if the genetic content and geographical locations of *R. solani* isolates are correlated. The banding patterns generated from those isolates by five primers showed high levels of genetic similarity among and within the isolates from different geographical locations. The genetic similarity was more than 90% in view of the cluster analysis of the RAPD data that showed a high genetic similarity between 20 isolates of *R. solani.*

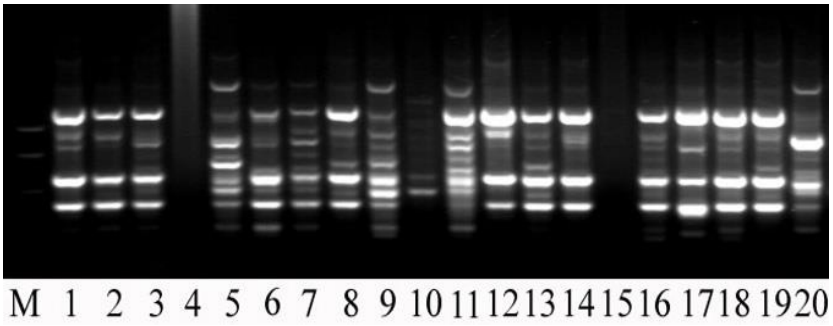

Fig 4: Random amplified polymorphic DNA of 20 isolates *R.solani* by using primer 2.

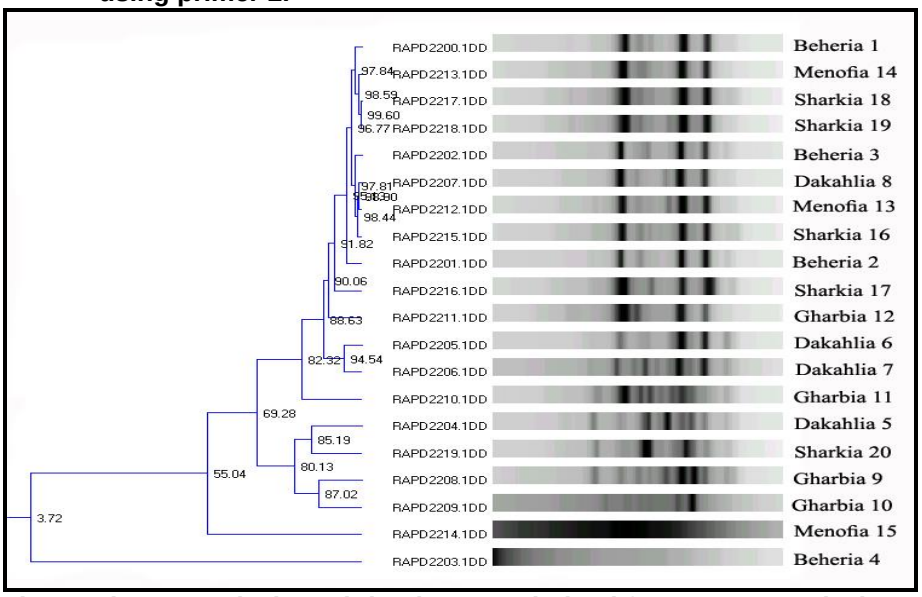

Fig. 5: Cluster analysis and dendrogram derived from RAPD analysis 20 isolates *R. solani* using primer 2.

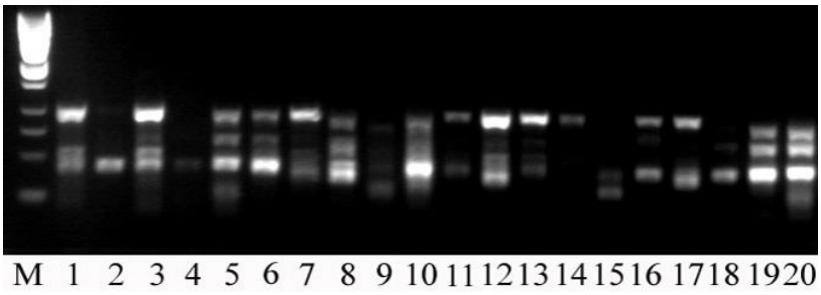

Fig. 6: Random amplified polymorphic DNA of 20 isolates of *R. solani* using primer 3.

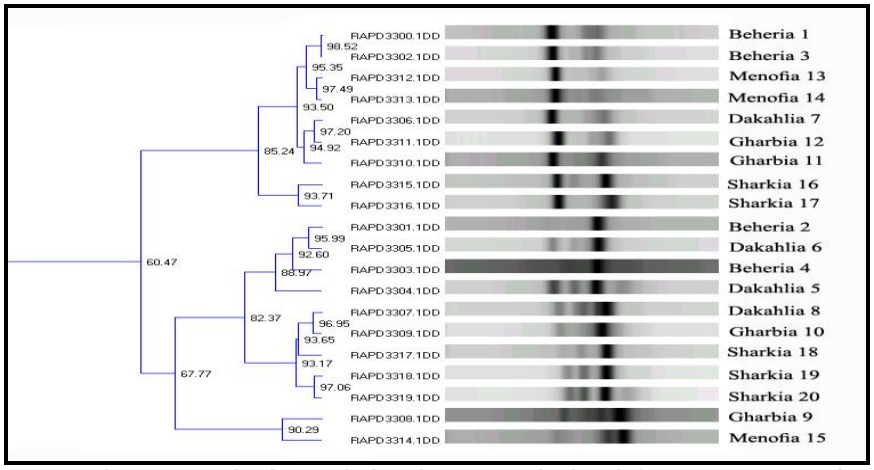

Fig. 7: Cluster analysis and dendrogram derived from RAPD analysis 20 isolates *R. solani* using primer 3.

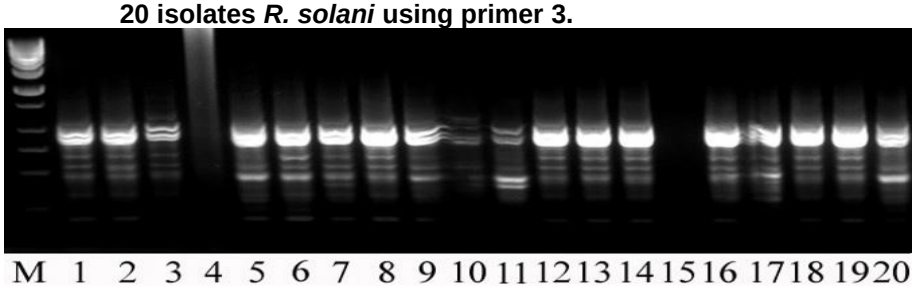

Fig. 8: Random amplified polymorphic DNA of 20 isolates *R. solani* by using primer4.

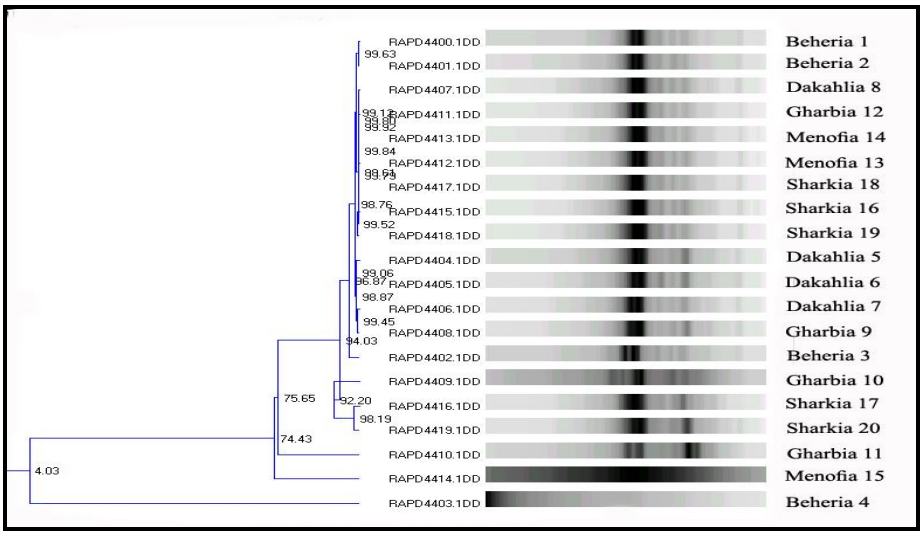

Fig. 9: Cluster analysis and dendrogram derived from RAPD analysis 20 isolates *R. solani* by using primer4.

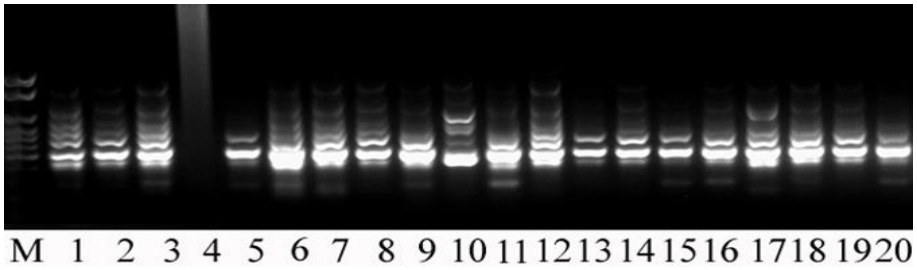

Fig. 10: Random amplified polymorphic DNA of 20 isolates *R. solani* using primer 5.

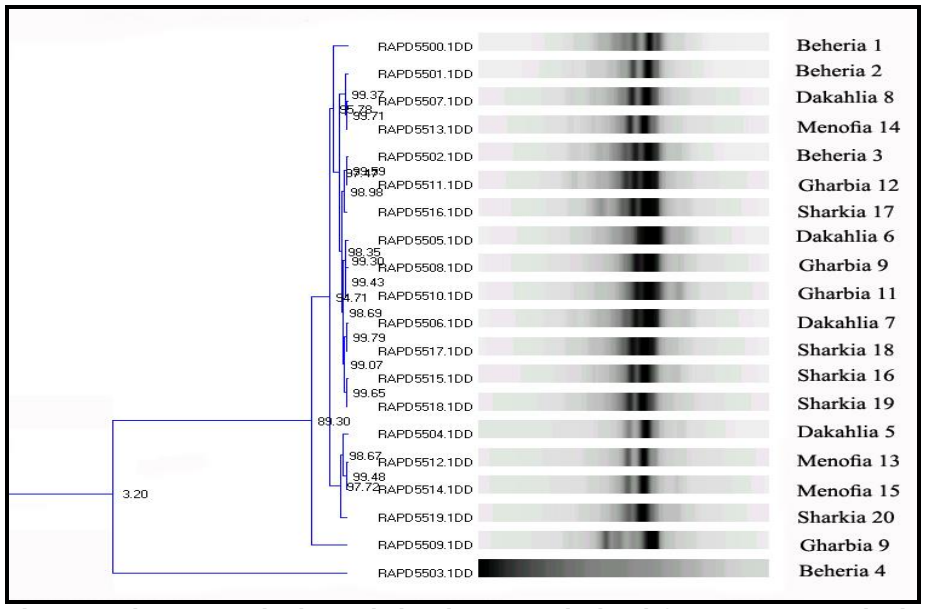

Fig. 11: Cluster analysis and dendrogram derived from RAPD analysis 20 isolates *R. solani* by using primer 5.

Results showed that the genetic similarity among *R. solani* isolates isolated from different geographical location indicated that there was no relation between the geographical locations and genetic content when using any primer of RAPD technique. This finding is also confirmed when using primer 4 the isolate from Sharkia and Menofia showed high genetic similarity of 99.83% regardless to their different sources. These results could be attributed to the fact that *R. solani* is not a sporulating fungus under the common condition. The non sporulation and the lack of sexual reproduction are considered as a major factor of the genetic stability of microorganisms. These results are in harmony with previous ones by Mohamed, 2004.

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حـــدوث المـــرض ومجموعـــات الالتحـــام الجســـماني والاختلافـــات الوراثيـــة للفطر *Rhizoctonia solani* الذي يصيب البطاطس في مصر .

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يعتبر محصول االبطاطس محصول الخضر الثانى في مصر بعد الطماطم . و يصاب بالعديد من الامراض الفطرية التى من اهمها مرض تقرح الساق والقشرة السوداء المسبب لها الفطر *Rhizoctonia solani* و لقد وجد المرض في موسميين متتالين هما ٢٠٠٥/٢٠٠٦ و ٢٠٠٦/٢٠٠٧ في العديد من المحافظات المنتشر زراعة البطاطس فيها؛ البحيرة و الدقهلية و الغربية و المنوفية و الشرقية. لقد كانت اعلى نسبة اصابة في محافظة الشرقية في العروتين الصيفى و الشتوى و تم الحصول على ٣٠٠ عزلة من مختلف المحافظات .و تم اختيار ٢٠ عزلة لتعريفها بواسطة الطرق العادية بواسطة اختبار مجاميع الالتحام الجسمانى وتم التعريف على انها تنتمى الى AG-3. عند اجراء اختبار العدوى الصناعية تحت ظروف الصوبة كانت اقوى العزلات عزلتان من محافظة الشرقية. و قد تم تقدير التنوع الوراثى بين ٢٠ عزلة بواسطة تكنيك PCR-RAPD باستخدام ٥ بادئات اعطت جميعها درجة عالية من التشابه تصل الى اكثر من ٩٠%. و قد اوضح استخدام هذا التكنيك انه لا توجد علاقة بين محتوى العزلة الوراثى و المكان الذى تم عزله منها.