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Morphological and Molecular Characterization of Potato Black Scurf Disease (*Rhizoctonia solani*) in Egypt

Abdlla, M. E. [1] ; A. M. Tohmy[2]; I. A. Rashid[2] and Noha E. Ahmed[2]
[1]Plant pathology Department. Faculty of Agriculture Mansoura University
[2]Plant Pathology Research Institute, Agriculture Research Center (ARC), Giza, Egypt

ABSTRACT

Rhizoctonia solani is the biological causal agent of the potato black scurf disease. The symptoms were examined on vegetative and tuber potato plants in eight Egyptian governorates (Aswan, Behera, Dakahlyia, Gharbia, Giza, Ismailia, Menofyia, and New Valley) during 2012-13 and 2013-14. Representative infected samples of Diamant, Hermes, Lady Rosetta and Spunta cultivars were collected randomly from different conventional and organic farms. Field survey produced a total of 182 isolates. The highest disease severity occurred in Ismailia and Behera, while the lowest severity was in Menofyia. The cultivar Herms was resistant in greenhouse and field trials, while Lady Rosetta was the susceptible. Disease incidence and severity were higher in organic than conventional cultivation farms. The ١٨٢isolates were subdivided into 14 group based on the morphological examination. The anastomosis test on ٥٠ selected isolates produced four different interactive groups (C0-C3). Sequence analysis was performed on the internal transcribed spacer (ITS) 18s rDNA of 35 selected isolates and confirmed the identified groups. High molecular similarity and single nucleotide polymorphisms (SNP) were produced between the representing ITS sequences of the (AG3, AG4, AGA, and AGF) groups using the comparative analysis. The AG3 and AG4 groups included 25 and six isolates; respectively, and severely infected potato. The AGA and AGF groups included three and one isolates; respectively, and unregistered for potato invasion. These results of great applications at the level of potato black scurf disease characterization in Egypt.

Keywords: potato, black scurf, *Rhizoctonia solani,* anastomosis group, molecular characterization

INTRODUCTION

R. solani Khun (Teleomorph: *Thanatephorus cucumeris* (Frank) Donk) has long been recognized as a destructive pathogen on a wide variety of economically important agricultural crops (Dodman and Rentje, 1970, Weinhold *et al*., 1982). *R. solani* is an important soil-borne pathogen of potato (Solanum tuberosum L.), causing shoot/stolon canker on young plants and black scurf on tubers (Bienkowski *et al*., 2010). Scurf disease reduce the quality of both ware and seed potatoes and affect crop industry establishment. Several reports recognized *R. solani* as a complex anastomosis groups, which can be delimited on the basis of their hyphal fusion (Anderson, 1982; Ogoshi, 1987; Balali *et al*., 1995). Anastomosis groups of *Rhizoctonia* share the following characteristics: I) multinucleate cells and pale to dark brown pigment, II) formation of monilioid, barrel-shaped cells, and chains of aggregated cells producing vegetative resting structures, III) sclerotia, and IV) lack of conidia or sexual spores. The well-known symptom of black scurf disease is recognized by the appearance of sclerotia on the surface or inside the potato tubers. Sclerotia infections cause increase in the number, size, and distribution of malformed cracked tubers, and the production of aerial tubers (Otrysko and Banville, 1992). Sequence analysis of the rDNA region containing the internal transcribed spacer (ITS) was used to evaluate the genetic diversity of some isolates within and between anastomosis groups (AGs) in *R. solani* (Kuninaga *et al*., 1997). The AG-3 isolates had 56 to 91% sequence similarities in the ITS-1 region with *R. solani* isolates of the other AGs (Kuninaga *et al*., 2000). A biodiversity study between two AGs of *R. solani* at different Egyptian potato growing areas in 2012 reported that majority of all isolates were belonged to AG3 group and differed in their severity (Moussa *et al*., (2014). The objectives of the current study are set to enrich the information about the current status of black scurf disease at the morphological and molecular characterization levels and to optimize its management methods.

MATERIALS AND METHODS

Field monitoring and samples collection:

Survey was conducted to determine the prevalence of stem canker and black scurf disease of potato grown at conventional area (Behera, Dakahliya, Gharbia, Minofiya and Ismailia and organic farms (Behera, Giza, New Valley, Aswan and Ismailia) during seasons 2012/13 and 2013/14. Survey was conducted 3 times during growing season, after 6 weeks of planting, 30 days later and at harvesting time. From each field, 10 spots were selected diagonally, each spot was approximately 1 m2 area and 10 samples of potato plants/ tubers showing symptoms were taken for isolation depending on visible symptoms. Representative samples were collected randomly from 4 potato cultivars, i.e. Lady Rosetta, Diamant, Spunta and Hermes,. Infected tubers with black scurf (%) and disease severity (%) were calculated while stem canker was estimated during vegetative growth of potato crops, 45 days and 70 days plant age.

Isolation of black scurf causal pathogen:

Potato tubers with visible sclerotic symptoms were obtained from different locations as mentioned above. Tubers were washed thoroughly in tap water to remove adhering soil particles, then rinsed in sterilized water and left to dry for few hours. Small pieces of sclerotia were removed aseptically from the tuber using a flame- sterilized scalpel and placed onto streptomycin amended tap water agar (TWA: 15 g agar, 1000ml water sterilize) in 9cm.diam Petri-dishes containing 10 ml, then dishes were incubated at 25±2 °C until new mycelia growth developed. Transfers were made from the advanced edge of the fungal colonies and cultures were maintained on PDA slants in test tubes and kept for further studies. Purification of each isolated fungus carried out using the hyphal tip technique. Identification of the isolated fungi carried out according to their cultural and morphological characteristics as described by (Ogoshi, 1987). Disease frequency and incidence were calculated according to (Malik *et al*., 2014).

Identification was confirmed in Mycological Research and Disease Survey Department, Agricultural Research Center (ARC), Giza, Egypt. Further molecular identification of Rizoctonia isolates was confirmed using DNA sequencing.

Anastomosis test

Anastomosis test was performed according to parameter *et al*., (1969). The isolates were assigned with tester isolates belonging to AG-1, AG-2-2, AG-3 and AG-4 groups.

DNA extraction and PCR

Fresh mycelia of each isolate of R. solani were collected and genomic DNA was extracted according to Dellaporta *et al*., (1983). Internal transcribed spacer (ITS) was amplified using the universal primers of ITS-1 (TCCGTAGGTGAACCTGCGCAG) and ITS-4 (TCCTCCGCTTATTGATATGC) that amplify the flanking regions of the 18S and 28S rRNA genes (White *et al*., 1990). PCR reactions included 2.5 µl of dNTP (2.5 µM), 2.5 µl of 10X PCR buffer, 2.5µl of $MgCl_2$, 0.2 µl of Taq DNA polymerase enzyme, 1 µl of each primer (10 µM), 2 µl of DNA template (50 ng/µl), and total reaction volume was reached to 25 µl using nuclease free H_2O. Amplification was performed in a thermal cycler DNA Engine Opticon 2 (Biorad, Hercules, CA) using the following program: initial denaturation 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min and extension at 72°C for 2 min, and a final extension at 72°C for 7 min. The PCR products was cleaned using the QiaQuick spin column Kit (Qiagen, USA) according the manufactures manual.

DNA sequencing

Direct sequencing of the purified PCR products was carried out in both directions using the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Bio systems, Foster City, CA, USA) and the universal ITS-1 and ITS-4 primers following the manufacturing procedure. Sequence reads were performed using the ABI Prism 3730XL Sequencer (Applied Bio systems) according to the manufacturer's instructions.

Sequence analysis

All sequences were aligned using the MEGA v.6 (Tamura *et al*., 2013). Sequence alignment search was done using the (megablast) tool of GenBank database (www. ncbi. nlm. nih. gov / blastn). The molecular evolutionary analyses of aligned sequences was applied using the MegAlign module of DNASTAR software (http://www. dnastar.com/). Robustness of the phylogeny was assessed using ClustalW approach and bootstrap value of 1000 replications.

RESULTS

Field survey

Field survey revealed the spread of potato black scurf disease at all investigated areas of the eight Egyptian governorates. Before harvesting, the leaf rolling and dark brown discoloration symptoms were observed. After harvesting, sclerotia masses also were observed as on the surface of tubers. The highest number of infected samples were collected from Ismailia (50 isolates), while the least number form Menofyia (seven isolates).

Under conventional areas, the regions of Ismailia and Behera showed high frequencies of R. solani causal agent of black scurf potato tuber disease (45.46% and 45%, respectively), while the lowest frequencies were in Gharbia and Minofiya (40% and 42.86% respectively). On the other hand, Under Organic cultivation, the regions of Ismailia, Behera and New Valley showed high frequencies of R. solani isolates (47.09%, 44.44 and 43.48 % respectively), while the lowest frequencies were observed in Giza and Aswan (38.09 and 39.13 respectively). Regarding to the cultivars Lady Rosetta had a highest frequency of R. solani isolates in two cultivation areas (conventional and Organic) with (43.66% and 42.34 respectively), while Hermes and Diamant cultivar had lowest percentage of R. solani isolates (9.85 and 10.81%).

Table 1. Number and frequency of *R. solani* isolates obtained from diseased potato tubers of certain cultivar produced in different Governorates

Type of cultivated area	Cultivars	Governorate																Total frequency	
		Beheira		Dakahliya		Gharbia		Minofiya		Ismailia		Giza		New Valley		Aswan			
		*No.	**%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Common cultivating fields	Lady Rosetta	9	45	5	45.46	4	40	3	42.86	10	43.48	na	Na	na	na	na	na	31	43.66
	Diamant	2	10	1	9.09	2	20	1	14.28	3	13.04	na	Na	na	na	na	na	9	12.67
	Spunta	7	35	4	36.36	3	30	2	28.58	8	34.78	na	Na	na	na	na	na	24	33.80
	Hermes	2	10	1	9.09	1	10	1	14.28	2	8.70	na	Na	na	na	na	na	7	9.85
Total		20	100	11	100	10	100	7	100	23	100	-	-	-	-	-	-	71	71
Organic farm	Lady Rosetta	10	43.48	na	na	na	na	na	na	12	44.44	8	47.09	9	39.13	8	38.09	47	42.34
	Diamant	2	8.70	na	na	na	na	na	na	3	11.11	1	5.87	3	13.04	3	14.29	12	10.81
	Spunta	8	34.78	na	na	na	na	na	na	9	33.34	7	41.17	7	30.43	7	33.33	38	34.23
	Hermes	3	13.04	na	na	na	na	na	na	3	11.11	1	5.87	4	17.40	3	14.29	14	12.61
Total		23	100	-	-	-	-	-	-	27	100	17	100	23	100	21	100	111	

***: Number of obtained isolates from diseased potato tubers100 collected samples.**

****: Percentage of frequency of obtained isolates.**

na: variety was not available in mentioned governorates for survey during stud

The overall average percentages of black scurf disease incidence during the growing seasons of 2012-13 and 2013-14 were listed in Table 2. The highest disease incidence was observed in Ismailia and Beheira regions (38.75% and 32.5%; respectively), while the lowest disease incidence was in Dakahliya and Menofyia (21.25% and 22.75%; respectively). At the level of genotype assessment, Lady Rosetta cultivar showed highest disease incidence (37.5%) and Diamant was the lowest (19.6%).

Table 2. Disease Incidence of potato black scurf disease in conventional and organic farms among the eight Egyptian governorates.

Governorate (A)	Cultivars (B)				Mean (A)
	Lady Rosetta	Diamant	Spunta	Hermes	
Beheira (CCF)	40	17	30	15	25.50
Dakahliya (CCF)	30	11	27	17	21.25
Gharbia (CCF)	35	15	35	23	27.00
Menofyia (CCF)	28	13	33	17	22.75
Ismailia (CCF)	42	20	37	25	31.00
Beheira (OF)	45	25	35	25	32.50
Giza (OF)	35	20	30	20	26.25
New Valley (OF)	40	22	40	27	32.25
Aswan (OF)	30	23	37	23	28.25
Ismailia (OF)	50	30	42	33	38.75
Mean(B)	37.5	19.6	34.6	22.5	

CCF: Common cultivating fields OF: Organic farm, LSD at 0.05: (A)= 2.06 (B)= 1.84 (AB)= 5.03

The multinucleate isolates 1-16 were isolated from the conventional area and anastomosed by interaction with each other (Table 3A). Isolates 1, 3 and 10 showed no interaction with the other isolates. The hyphal wall contact was only appeared in the isolates number 2 and 6. While the isolates number 4, 7 and 9 had hyphal wall of membrane fusion death at fused and adjacent cells. On the other hand, the isolates 5, 8, 11, 12, 13, 14, 15 and 16 showed hyphal wall of membrane fusion with no death at fused and adjacent cells.

The multinucleate isolates 1-19 were isolated from the organic area and anastomosed by interaction with each other (Table 3B). Isolates number 4, 7, 10 and 11 showed no interaction with the other isolates, but the hyphal wall contact interaction were appeared in isolates number 3, 6 and 16. While Isolates number 2, 5, 9 and 14 had same interaction; hyphal wall of membrane fusion death at fused and adjacent cells. The isolates 1, 8, 12, 13, 15, 17, 18 and 19 showed that the hyphal wall of membrane fusion with no death at fused and adjacent cells.

Table 3A. Anastomoses group test of sixteen *R. solani* isolats conventional area.

Code of *R. solani* isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	C1	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0
2		C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1
3			C1	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0
4				C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2
5					C3	C3	C3	C3	C3	C3	C3	C3	C3	C3	C3	C3
6						C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1
7							C2	C2	C2	C2	C2	C2	C2	C2	C2	C2
8								C3	C3	C3	C3	C3	C3	C3	C3	C3
9									C2	C2	C2	C2	C2	C2	C2	C2
10										C1	C0	C0	C0	C0	C0	C0
11											C3	C3	C3	C3	C3	C3
12												C3	C3	C3	C3	C3
13													C3	C3	C3	C3
14														C3	C3	C3
15															C3	C3
16																C3

C0, No interaction (not related); C1, Hyphal wall contact; C2, Hyphal wall of membrane fusion death in fused and adjacent cells (related); C3, Hyphal wall and membrane fusion, no death in fused and adjacent cells (perfect fusion-closely related).

Table 3B. Anastomoses group test of sixteen *R. solani* isolats organic area.

Code no. of *Rh. solani* isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	C3	C3	C3	C3	C3	C3	C3	C3	C3	C3	C3	C3	C3	C3	C3	C3	C3	C3	C3
2		C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2
3			C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1
4				C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0
5					C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2
6						C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1	C1
7							C2	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0	C0
8								C3	C3	C3	C3	C3	C3	C3	C3	C3	C3	C3	C3
9									C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2
10										C2	C0	C0	C0	C0	C0	C0	C0	C0	C0
11											C1	C0	C0	C0	C0	C0	C0	C0	C0
12												C3	C3	C3	C3	C3	C3	C3	C3
13													C3	C3	C3	C3	C3	C3	C3
14														C2	C2	C2	C2	C2	C2
15															C3	C3	C3	C3	C3
16																C1	C1	C1	C1
17																	C3	C3	C3
18																		C3	C3
19																			C3

Sequence analysis of PCR fragments

The 35 sequences obtained from the amplified fragments of the selected isolates were highly similar and represented in four different sequences at about 621 bp (Fig.1). No single nucleotide polymorphisms (SNPs) were detected within the generated sequences of the same AG while many SNPs were detected between the different AGs. The BLAST results confirmed that AG3 included 25 isolates, AG4 included six isolates, AGA included three isolates, and AGF included one isolate.

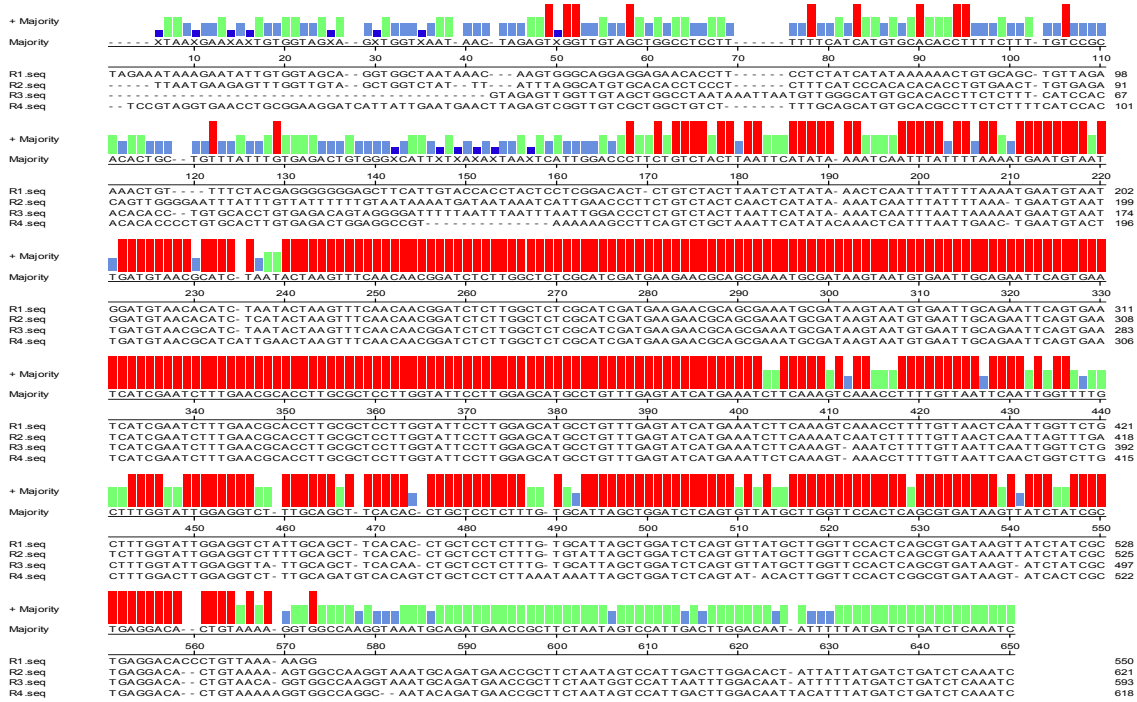

Fig. 1. Sequence comparison of 18s rDNA in *R. solani*.

R1, the isolates belong to AG4; R2, the isolates belong to AG3; R3, the isolates belong to AGF; and R4, the isolate belong to AGA. Red, Green, and Blue bars represent 100, 75 and 50% of sequence similarities, respectively. Dark-blue bars represents 75% of sequence dissimilarity. – symbol, represents deletion points.

The AG4 strain (denoted as R1, Fig. 1) had shown some allelic variation at the SNP level. Some SNP of transition substitutions at the positions of 46, 570, and 571bp of (G/A), and of (C/T) and (T/G) at 76 and 572bp; respectively, were detected. The AG3 strain (denoted as R2, Fig.1) had shown SNP of (A/T), (G/A), and (G/T) at 47, 46, and 49 bp, respectively. The AGF (denoted as R3, Fig. 1) had shown SNP of (G/A), (T/C), and (C/A) at the positions of 105, 104, and 474 bp, respectively. The strain AGA (denoted as R4, Fig. 1) had shown SNP of (T/A) at the positions of 161, 162, and 545 bp; while of SNP (G/A) at the positions of 163, 164, and 514 bp; and of SNP (C/A), (T/C), and (A/C) at 166; 513 and 546 bp, respectively. At the level of a single-base deletion, the strain R1 and R2 each had included eight deletions, while the strain R3 included 10 deletions, and the strain R4 included three deletions. At the level of two-base deletions, each of the strains R2 and R3 had included for once, while the strain R4 had included for twice and R1 did not have for any. At the level of three-base and six-base deletions, only R1 and R2 had shown scores. At the level of four-base deletion, only the strain R4 had scored. At the level of seven-base and 14-base segment deletions, only the strain R4 had scores. At the level of single-base insertion, the strain R4 showed the base (T) at two locations (103 and 475 bp), the base (C) at 191 bp, the base (A) at 235, 489 and 569 bp, and the base (G) at 468 bp. At the level of three- and seven-base segment insertion, the strain R3 showed (GTA) at 43-45 bp and (AAATTAA) at 70-76 bp (Fig. 1).

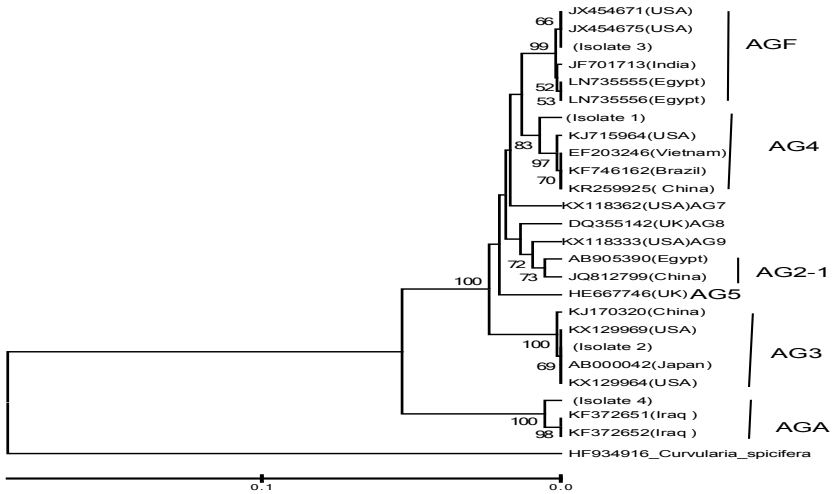

Figure 2. Phylogeny tree of the ITS sequences of the *R. solani*.

The phylogeny tree analysis involved 26 nucleotide sequences represented the four anastomosis groups. The isolate 1 fall into a single subclade and related to the isolates of AG4 group from USA (KJ715964), Vietnam (EF203246), Brazil (KF746162) and China (R259925) at BP value of 83%. The isolate 2 was clustered among the

isolates of AG3 group from USA (KX129969 and KX129964), Japan (AB000042) and China (KJ170320) at a strong BP value of 100%. The isolate 3 belonged to the (AGF) clade that highly supported with bootstrap (BP) value of 99%. It also showed similarity with the two isolates of *R. solani* from USA at low BP value of 66% and distantly sub-clustered from the two Egyptian isolates (LN735555 and LN735556) at BP value of 55%. The isolate 4 fall into a single subclade and closely related to the two isolates of AGA group from Iraq (KF372651 and KF372652) at a strong BP value of 100%.

DISCUSSION

In Egypt, Potato is produced either for seed or ware industry, which subject the plants to the infection and development of many fungal diseases. One of the most common fungal diseases in potato are those caused by Rhizoctonia (Stem canker and black scurf). Importance of potato production increases in Egypt due to its nutritional and economical values for exportation to the EU and Russia. Egypt ranked the biggest potato producer in Africa (FAO, 2014).

Survey of black scurf disease, on top cultivars for potato processing (Diamant, Hermes, Lady Rosetta and Spunta), was carried out on freshly harvested potato tubers. Symptoms surveyed for black scurf in eight locations showed that the highest infection rate was recorded in Ismailia and also in the organic cultivation. Meanwhile, symptoms in the Menofyia and conventional cultivation showed the least infection.

Generally, infection of black scurf was high in Lady Rosetta in Egypt (El-Aziz *et al*., 2013 and Hassan, 2016). The cultivars Spunta and Hermes showed varied susceptibility, which was emphasized by the varietal susceptibility test. However, the highest infection records for black scurf was detected in Lady Rosetta and Ismailia. This indicating that response of Lady Rosetta toward *R. solani* infection is affected by environmental conditions including the soil type and virulence of pathogenic isolates. A previous survey in two Egyptian governorates, El-Sharkiya (New El-Salhia region) and Ismailia (El-Shabab region), showed variation in disease resistance among planted cultivars. The highest infection for black scurf symptoms was recorded in Lady Rosetta cultivar during the growing seasons of 2013-2014 and 2014-2015 (Hassan, 2016). This due to the high susceptibility of Lady Rosetta to the infection caused by *R. Solani*. The variation in pathogenic reaction of Lady Rosetta could be due to the chemical composition of either tubers or plants (El-Nagger *et al*., 2013).

Rhizoctonia is divided into multinucleate and binucleate species that can further be sub-divided into different anastomosis groups (AGS). The multinucleate species of *R. solani* Kuhn (teleomorph: *Thanatephorus cucumeris* (Frank) Donk): includes the AG-1 – AG-10 and AG-B1 (Sneh *et al*., 1991), AG-11 (Carling *et al*., 1994), AG-12 (Carling *et al*., 1999), and AG-13 (Carling *et al*., 2002). The identified isolates in current study were placed to four anastomosis groups (AG3, AG4, AGF and AGA). The percentage of similarity was 83.1% between the strains R1 and R2. Both R1 and R2 strains were 81.7% similar to R3, while R4 was the most distant with 80.2% of similarity. Similar studies showed that some isolates were placed to three anastomosis groups and predominantly AG3PT with a small proportion of isolates to AG2-1 and AG5 (Woodhall *et al*., 2007).

Sequence-based identification has become an essential tool in plant pathology. The official sequence used for barcoding of fungi is the ITS (Selvaraj *et al*., 2015). In this study, two primers (ITS1 and ITS4) were used to amplify the ribosomal RNA gene in the Egyptian *R. solani* isolates. Sequence results revealed that the region of 5.8S ribosomal RNA gene is highly conserved. However, the ITS1 and ITS2 at the 5'-end region showed similarity only within the same AG, while variability between the different AG. This observation was supported in previous studies of AG4 isolates (Meinhardt *et al*., 2002). In addition, these universal primers had the ability to differentiate between the different anastomosis groups, and in most cases their respective subgroups based on online published ITS sequences. Similarly, part of the 5.8S rDNA and ITS regions have enabled for the identification of subgroups beloged to AG2 (Salazar *et al*., 2000). Further work is needed to design new specific primers based on the current polymorphic regions of our Egyptian AG. Other studies identified the percentage of similarity between the different AGs. The sequence homology in the ITS regions was above 96% for isolates of the same AG subgroup. It also was 66 – 100% of similarity for isolates of different subgroups within the same AG, while was 55 – 96% for isolates of different AGs (Kuninaga *et al*., 1997). The sequence similarity in the ITS1 region was high (96 – 100%) for isolates within each of the two populations. However, it was 91 – 92% of similarity for isolates from different populations. The AG-3 isolates had 66 – 91% of sequence similarity in the ITS1 region of *R. solani* (Kuninaga *et al*., 2000).

In conclusion, the identified isolates in current study can be used as new pathogenic testers for *R. solani* in Egypt. In organic cultivation, the recommended potato cultivar is the resistant Hermes and the susceptible Lady Rosetta cultivar should be avoided. In the conventional cultivation, the recommended potato cultivar is the resistant Diamant and the susceptible Lady Rosetta cultivar should be avoided. The molecular differences found in our study can be used as marker progenitors to characterize the Egyptian *R. solani* isolates.

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التوصيف المظهري والجزيئي لمرض القشره السوداء للبطاطس في مصر

محمد السيد عبد الله[١] ، محسن تهامى عبدالغنى[٢] ، اسماعيل عبداللطيف سليمان رشيد[٢] و نهى الدسوقى رمضان احمد[٢]

[١]قسم امراض النبات – كلية الزراعة – جامعة المنصورة

[٢]معهد امراض النباتات – مركز البحوث الزراعية - الجيزة

ريزكتونيا سولاني هي العامل المسبب لمرض القشره السوداء في البطاطس تم اختبار الأعراض علي درنات البطاطس وعلي المجموع الخضري في ثماني محافظات مصريه (اسوان, بحيره, دقهليه,الغربيه, الجيزه, الاسماعليه,المنوفيه, الوداي الجديد) خلال الموسم الزراعي ٢٠١٢-٢٠١٣ وايضا ٢٠١٣-٢٠١٤ تم تجميع لعينات الممثله لاصابه عشوائيا من الاصناف المنزرعه ديمونيت وهيرميس وليدي روزتا وهيرمس من المزراع التلقيديه والعضويه المختلفه تم حصر ١٨٢ عزله حقليا. اعلي شده اصابه تم الحصول عليه كانت في الاسماعليه والبحيره بينما اقل اصابه كانت في المنوفيه. اظهر الصنف الزراعي هيرميس مقاومه في الصوبه والحقل بينما الصنف ليدى روزتا الزراعي اظهر حساسيه. كانت شده الاصابه ونسبتها في المزراع العضويه اعلي منه في التقليديه. تم تقسيم ١٨٢ عزله الي ١٤ مجموعه بناءا علي الفحص المظهري . كما امكن تقسيم ٥٠ عزله المختاره الي ٤ مجاميع متفاعله بناء علي اختبار المجاميع التشابكيه (C0,C1,C2 and C3)اضافه الي ذلك تم عمل تحليل لتتابعات الحمض النووي الريبوزي المتضاعفة من الفاصل المستنخ الداخلي (ITS) لجين (18srDNA) في ٣٥ عزله مختاره والذي اكد بدوره المجاميع المعرفه عن طريق اختبار المجاميع المتشابكة. وقد اظهر التحليل المتقارن لهذه التتابعات نسبه تشابهات جزئيه واختلافات احاديه القواعد (SNP) بين المجاميع (AG3,AG4,AGA and AGF). وعلى ذلك فقد تضمنت المجموعتين AG3 و AG4 على ٢٥ و ستة عزلات, بالترتيب, والتى اظهرت شدة اصابة على البطاطس. بينما تضمنت المجموعتين AGA وAGF على ثلاث عزلات وعزلة, بالترتيب, وهى غير مسجلة علي البطاطس. تعد النتائج المتحصل عليها فى هذا اللبحث ذات تطبيقات مفيدة على مستوى توصيف مرض القشرة السوداء فى البطاطس مصر.