FACULTY OF AGRICULTURE

Minia J. of Agric. Res. & Develop.
Vol. (37), No. 1, pp. 113-128, 2017

RHIZOCTONIA SOLANI ANASTOMOSIS GROUP AG3 - PT AND AG3-TB AS ROOT ROTTING TO TOMATO PLANTS GROWING AT EL-MINIA GOVERNORATE, EGYPT

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Received:2 April. (2017) Accepted: 18 April. (2017)

ABSTRACT

Ten *Rhizoctonia solani* isolates were isolated from rotted tomato plant roots cv. Super Jackal growing in El-Minia Governorate during 2012 growing season. Isolates were tested for their pathogenic ability on tomato plants cv. Super Jackal under greenhouse conditions. All isolates were pathogenic and varied in their pathogenicity to infect tomato plants. The most aggressive isolates to incite root rot were *R. solani* isolate (R1), isolate (R2) and (R3) as they covered the highest area under root rot progress curve (AURRPC). Isolates R1 (caused 92.5% DS), R2 (caused 86.5% DS) and R3 (caused 77.5% DS). Molecular biology-based *Rhizoctonia* AG group discrimination protocol revealed that *R. solani* isolates were identified to AG3 and to subgroups AG3-PT (isolates R1 and R2) while, isolate R3 was identified as AG3-TB. Reaction of tomato cultivars and hybrids to infection with *R. solani* anastomosis subgroups AG3 showed that some tomato cultivars (Nema Star, Marwa, Zaman and Super Red) and hybrids (010-65) are highly resistant while, others are very susceptible (Super Jackal cv. and 777 hybrid) or susceptible (Basha cv. and Nema 1400 hybrid).

Keywords: Tomato root rot, PCR-based markers, *Rhizoctonia solani*, anastomosis group, AG3-PT and AG3-TB

INTRODUCTION

Due to the increase area of cultivated tomato and repeated planting; tomato plants are affected by several diseases. One of the most

important soilborne disease is root rot that usually cause yield loss in the Middle Egypt. Among vegetable crops, tomato (*Solanum lycopersicum* L.) is one of the most important worldwide. Tomato is a member of the solanaceae family that includes several other economically important crops such as potato, pepper, and eggplant (Naika *et al*., 2005). The crop was introduced to cultivation in the Middle East around the end of the 18th century. In 2008 the country ranked 5th in the world with 9.2 MT of tomatoes produced. The crop is now by far the largest vegetable crop in Egypt. Tomatoes are grown mainly in three seasons; winter, summer and autumn on about 3 % of Egypt's total planted area (FAO, 2013). Nevertheless, lower yield of tomato is due to a number of factors e.g. 1) lack of improved well-performing varieties; 2) poor fruit setting due to excessively high temperatures, (which limit pollination) more specifically fecundation plus pollen viability and 3) insects and diseases (Lyons *et al*., 1999). In Egypt, various pathogens (fungi, bacteria, viruses and nematode) are partly constraints in tomato production.

Infection of root rot disease caused by *Rhizoctonia solani* is obviously spread under Minia Governorate conditions and recently led to severe reduction of tomato yield (Haggag, 2008). To date, *Rhizoctonia solani* is subdivided into 14 anastomosis groups (AGs) designated as AG 1 through 13 and bridging isolate (BI) group. Several AGs of *R. solani* such as AG 2-1, AG 3, (Misawa and Kuninaga, 2010) and AG 4 HG I (Taheri and Tarighi, 2012) have been shown to be pathogenic on tomato, the most frequently reported being AG 3. Knowledge about the prevalence and distribution of different AGs is important, since sensitivity to chemical control treatments and probably to other control strategies is varying among AGs. Molecular markers would accelerate pathogen identification (Lievens *et al*., 2008). Thus, the objectives of the present study were to: 1) isolate *R. solani* associated with root rot tomato plants and carry out the pathogenicity test of the obtained *Rhizoctonia* isolates, 2) identify of *R. solani* anastomosis groups (AGs) through PCR based DNA markers and 3) test the reaction of some tomato cultivars and hybrids to infection by *R. solani* AGs.

MATERIALS AND METHODS

1. Isolation and culture maintenance of tomato root rot-causing *Rhizoctonia solani*

Samples of tomato plants (cv. Super Jackal) displaying root rot symptoms (Fig.1) were collected from tomato-growing fields of different locations at El-Minia Governorate, Egypt. Morphological and Microscopical examinations were used to select *R. solani* isolates for this study (Barnett and Hunter, 1987).

The isolates of *R. solani* used in this study were recovered from tomato plants showing typical symptoms of root rot. Plant tissues of tomato were

washed thoroughly with tap water to remove adhering soil particles. To isolate the fungus, infected plant tissues were first treated with a surface disinfection with 1.0% sodium hypochloride for 2–3 min. Disinfected tissues of about 3–4 mm in length were placed on Petri dishes including 2.0% potato dextrose agar (PDA) and incubated at 25 °C for 5 days.

(A)

(B)

Fig. 1 Root rot symptoms on tomato plants cv. Super Jackal growing under natural (A), magnification of root rot symptoms is shown in (B).

2. Identification the causal pathogens based on morphological features

Identification based on the Morphological and Microscopical characters of isolates was conducted and cultures were examined for hyphal characteristics typical of *R. solani*. The

isolates were then stored at 4 °C for further studies (Barnett and Hunter, 1987)

3. Pathogenicity tests

3.1. Inoculum preparation

Inocula of 10 *R. solani* isolates were grown separately on barley grain medium in conical flasks for 7-10 days to be used as a source of inoculum. Inocula of these tested fungi were applied separately at the rate of 2.5% of the soil weight (Anderson, 1982). Mixed thoroughly with the soil then irrigated and left 7 days for establishment. Soil was sterilized by formalin (5% v/w) then left 2 weeks, aerated several times and distributed into plastic pots (200g soil/pot)

3.2. Inoculation procedure

Tomato transplants, cv. Super Jackal (40 day-old of 3-5 fully compound leaves) were removed from individual cells of the trays using a gentle pulling motion. Care was taken to prevent breaking the stems. After the seedlings were removed from the cell, excess vermiculite above the roots was cleaned by gentle shaking. The plants were replanted into 5-cm-diameter plastic pots (one plant/each) contained infested soil (clay/sand) which was used as the growing medium **(**Ignjatov *et al*., 2015).

3.3. Post-inoculation care

Inoculated plants were watered daily for 3 days after transplanting with sufficient water to keep the soil wet, but not as wet as to cause leaching. After the initial 3 days, seedlings were watered as needed. The experiment was done on Randomized Complete Block design with 4 replications (each 20 plants) and the experiment was repeated 3 times.

3.4. Disease assessments

To assay root rot severity, mortality percentages of tomato plants was estimated at 10 days intervals from 15 days after inoculation. Data converted to area under root rot progress curve (AURRPC). The mean value of (AURRPC) for each replicate was calculated as suggested by **Pandey *et al*. (1989)**. Re-isolation of *R. solani* from artificially inculcated tomato plants was conducted to achieve Koch`s postulates.

4. Identification based on molecular biology technique

In this experiment, selected three *R. solani* (R1, R2 and R3) isolates, which were identified according to the morphological, microscopical and pathological characteristics, were used.

4.1. Molecular biology study

Identification of *R. solani* isolates originating from tomato growing in different locations at El-Minia Governorate using morphological, microscopical and PCR methods. Identification of *R. solani* anastomosis groups (AGs) and subgroups based on PCR was done at the Lab of Plant Breeding, Faculty of Agriculture, Iwate University, Morioka, Iwate, Japan.

4.1. 1. DNA extraction

A CTAB DNA extraction protocol was used to acquire DNA

from the fungal isolates. Fungal isolates were grown in 20 ml of liquid medium (4 g l -1 of malt extract broth (Merck) or ¼ MYEDP medium (4.75 g malt extract, 0.5 g yeast extract, 0.75 g dextrose, 0.45 g peptone, 500 mM thiamine in a final volume of 1 l, pH 5.6). Five to six 0.5 cm^2 agar discs of each isolate were used to inoculate liquid medium. The cultures were incubated for four to five days at 28 °C. The mycelia were centrifuged and washed once in sterile water and twice in 500 mM $NaCl_2$ and 50 mM EDTA pH 8.0. The mycelia were blotted dry and macerated in liquid nitrogen with 2800 µl of DNA extraction buffer [1.0% CTAB, 0.5 M $NaCl_2$, 69 mM EDTA pH 8.0, 34 mM Tris pH 8.0, 0.05% N-lauryl sarcosine, 1% SDS, and 0.009% β- mercaptoethanol]. Twenty µl of proteinase K (20 mg l-1) was added and the solution was incubated at 55 °C for 2 h. After incubation, the solution was centrifuged for 10 min at 12000xg and phenol/chloroform extracted. DNA was precipitated in 0.8 volumes of isopropanol, centrifuged, and washed with 70% ethanol, dried, resuspended in 100 µl of sterile water and stored at 4 °C (Ausubel *et al*., 1994).

4.1.2. Polymerase Chain Reaction (PCR) detection

PCR-based *Rhizoctonia* AG group discrimination protocol was employed to clarify isolates of *R. solani* AG1 obtained from tomato plants. Specific primer sets and PCR condition for identification of various *Rhizoctonia* groups based on anastomosis grouping (AGs) are shown in **Table 1** as described by **(Kuninaga, 2003)**. The PCR was done using PCR Thermal Cycler (Takara, Japan) and the PCR products were fractionated in a 4% NuSieve 3:1 Agarose (Lonza, Rockland, ME, USA) by using gel electrophoresis apparatus (EIDD, Japan). After electrophoresis, the gel was stained with ethidium bromide and viewed with a UV illuminator.

4.1.3. Sequence analysis

The PCR products were sequenced with a DNA sequencing kit and an ABI 310 DNA sequencer (Applied Biosystems, Lincoln Centre Drive, Foster City, CA, USA). The sequences were used in a homology search of the NCBI (http://www.ncbi.nlm.nih.gov) databases using the BLAST program. Sequences that showed 93% identity or more over at least 100 bp were recorded.

5. Reaction of certain tomato cultivars and hybrids to infection by *R. solani* anastomosis groups (AGs)

Seven cultivars of tomato plants e.g. Super Jackal, Basha, Marwa, Nema Star, Zaman, Super Red, and Rawan plus three hybrids e.g. 777, Nema 1400 and 010-65 were subjected to infection by *R. solani*, AG3-PT and AG3-TB. Inoculum preparation, inoculation and disease severity were essentially done as above mentioned. The experiment was repeated 3 times (4 replicates, 20 plants /each replicate).

Table (1) Specific primer sets of PCR analyses for identification of various *Rhizoctonia* groups based on anastomosis grouping (AGs).

Primer name	Sequence 5'-3' (F)	Sequence 5'-3' (R)	1	2	3	4	2∼4 cycle	5
AG-1 IA F	CCTTAATTTGGCAGGAGGG	GACTATTAGAAGCGGTTCA	94°C,2min	94°C,40sec	58°C,1min	72°C,1min	30	72°C,5min
AG-1 IB F	TGTAGCTGGCCTTTTAAC	GGACTATTAGAAGCGGTTCG	94°C,2min	94°C,40sec	58°C,1min	72°C,1min	30	72°C,5min
AG-1 IC F	GAGTTGTTGCTGGCCTCTGG	CCAAGTCAATGGACTATTG	94°C,2min	94°C,40sec	58°C,1min	72°C,1min	30	72°C,5min
AG-1 ID F	TGGAGTTTGGGCAAGTG	GGACTATTAGAAGCGGTTCG	94°C,2min	94°C,40sec	58°C,1min	72°C,1min	30	72°C,5min
AG-2-1 F	CAAAGGCAATAGGTTATTGGAC	CCTGATTTGAGATCAGATCATAAAG	94°C,2min	94°C,40sec	60°C,1min	72°C,1min	30	72°C,5min
AG-2-2 IIIB F	AGGCAGAGACATGGATGGGAG	ACCTTGGCCAACCTTTTTATC	94°C,2min	94°C,40sec	62°C,1min	72°C,1min	30	72°C,5min
AG-2-2 IV F	AGGCAGAGACATGGATGGGAA	CTTGGCCACCC(A/C)TTTTTTAC	94°C,2min	94°C,40sec	62°C,1min	72°C,1min	30	72°C,5min
AG-2-2 LP F	AGGCAGAGAAACATGGATGGGC	CCTCCAATACCAAAGTGAAACCAAATC	94°C,2min	94°C,40sec	62°C,1min	72°C,1min	30	72°C,5min
AG-2-3 F	GTAGCTGGCTCATCGTTCTT	CATTTCCCTTGGCCACCTTTG	94°C,2min	94°C,40sec	60°C,1min	72°C,1min	30	72°C,5min
AG-2-4 F	GGGGAATTTATTTGTTGTTTTTTGTAATA	CAATGGACTATTAGAAGCA	94°C,2min	94°C,40sec	55°C,1min	72°C,1min	30	72°C,5min
AG-2BI F	GAATGAAGCAATCAGGGAACC	GATCATAAAAATATTGTCCAAGCT	94°C,2min	94°C,40sec	55°C,1min	72°C,1min	30	72°C,5min
AG-3 PT F	GTTTGGTTGTAGCTGGTCT	CTGAGATCCAGCTAATAC	94°C,2min	94°C,40sec	65°C,1min	72°C,1min	30	72°C,5min
AG-3 TB F	GTTTGGTTGTAGCTGGCCC	CTGAGATCCAGCTAATGT	94°C,2min	94°C,40sec	65°C,1min	72°C,1min	30	72°C,5min
AG-4 HG-I F	GGACCTACTCTCCTTGG	ACAGGGTGTCCTCAGCGA	94°C,2min	94°C,40sec	55°C,1min	72°C,1min	30	72°C,5min
AG-4 HG-II F	GGACCTTCTACTCCCCCT	ACAGGGTGTCCTCAGCGA	94°C,2min	94°C,40sec	55°C,1min	72°C,1min	30	72°C,5min
AG-4 HG-III F	GTTGTAGCTGGCATTTCC	CCACCCCTCCCAAACTCT	94°C,2min	94°C,40sec	58°C,1min	72°C,1min	30	72°C,5min

(Kuninaga, 2003)

6. Statistical analysis

All recorded data were subjected to the analysis of variance procedures and treatment means were compared using t Standard Deviation (SD) as described by **Gomez and Gomez (1984)**.

RESULTS

Isolation, purification and identification of the *R. solani* associated with root-rotted tomato plants

Ten *R. solani* isolates were isolated from root rotted tomato plants collected from different locations in the Middle Egypt mainly at El-Minia Governorate. Hyphal tip cultures of grown fungi were maintained on PDA medium. All fungi were purified using hyphal tip technique cultures, then they were identified. Results indicated that all fungal isolates which identified are belonging to *Rhizoctonia solani* as described by (Ogoshi, 1996).

Pathogenicity tests

The *R. solani* isolates obtained from naturally infected plants (Fig. 1), were tested for their pathogenic ability on tomato plants cv. Super Jackal under greenhouse conditions. The tested fungal isolates significantly varied in their ability to cause root rot infection of tomato plants (Table 2). Area under root rot progress curve (AURRPC) and disease severity (DS) after 15, 25, 35 and 45 days was calculated. The most aggressive isolates were *R. solani* isolate R1 followed by isolate R2 and R3 as they exhibited 2270, 2095 and 1760 AURRPC, respectively. On the other hand, *R. solani* isolate R9 and isolate R10 caused least potentiality of infection in tomato plants, 250 and 240 AURRPC, respectively. The highest disease severity was caused by isolate R1 (92.5% after 45 days) followed by R2 (86% after 45 days) and R3 (77.5% after 45 days) while, isolate R10 gave the lowest disease severity (7.5%).

Table (2) Root rot severity and AURRPC as influenced by different isolates of *Rhizoctonia solani* isolated from Super Jackal cv. in some locations of El-Minia Governorate, Egypt.

R. solani isolates	Locations	Disease severity (%)				AURRPC*
		After 15 days	After 25 days	After 35 days	After 45 days	
R1	Samalot	24.50±0.25	42.25±0.14	55.00±0.18	92.50±0.25	2270±0.29
R2	Samalot	22.75±0.29	39.25±0.14	46.25±0.29	86.50±0.25	2095±0.29
R3	Bani Mazar	18.50±0.29	38.50±0.14	67.75±0.14	77.50±0.14	1760±0.14
R4	Matai	11.50±0.29	14.25±0.14	17.50±0.29	27.50±0.29	805±0.14
R5	Samalot	8.75±0.29	11.50±0.29	16.25±0.14	25.50±0.29	850±0.14
R6	Minia	7.50±0.29	6.50±0.29	14.50±0.29	29.50±0.29	685±0.20
R7	Minia	5.50±0.41	7.25±0.20	12.50±0.29	25.50±0.14	470±0.25
R8	Minia	5.25±0.25	5.50±0.29	11.50±0.14	26.50±0.14	665±0.25
R9	Minia	4.50±0.25	5.50±0.29	8.50±0.20	19.50±0.14	250±0.14
R10	Minia	3.50±0.25	4.25±0.50	4.50±0.25	7.50±0.29	240±0.14

Data are means of 3 experiments (4 replicates, 20 plants/each) ±SD.

*AURRPC= area under root rot progress curve was calculated as described in **MATERIALS AND METHODS** confirmed the presence of *R. solani* and the subgroup. Isolates for which specific fragments did not amplify, the marker was considered to be absent.

Identification of *Rhizoctonia solani* anastomosis groups and subgroup based on molecular biology

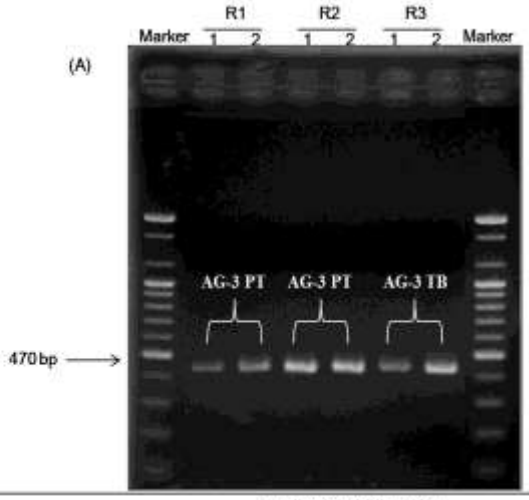
These studies have been conducted using the most virulent isolates of *R. solani*; R1, R2 and R3. The three isolates were checked by 17 DNA specific markers to identify their anastomosis group (AGs) and subgroups. These isolates were analysed with primer sets shown in **(**Table **1)**,

The electrophoresis of PCR products obtained for isolates belonging to Samalot (R1 and R2) and Bani Mazar (R3) where intensive tomato growing is practiced are given in **Figure 2**. The PCR analyses of the R1 and R2 isolates gave fragments (470 bp) with specific primer sets for AG-3 subgroup PT. The tomato isolate R3 was observed to amplify fragments (470 bp) with specific primer sets for AG-3 subgroup TB **(**Fig. 2A and B).

The DNA sequence analysis of the ribosomal RNA genes and in particular the internal transcribed spacer regions (ITS) of the ribosomal DNA (rDNA) sequence has also been used to identify the anastomosis group (AGs) and subgroups of the three isolates R1, R2 and R3 as shown in figures 5, 6 and 7, respectively. Characterizing of *R. solani* isolates was done by using rDNA ITS which considered to be an appropriate and advanced molecular identification. BLAST searches of the NCBI showed high similarity AG-3 PT for R1 and R2 with 93% **(**Fig. 3 and 4) identities and AG-3 TB for R3 with 96% (Fig.5) identities.

Reaction of different tomato cultivars and hybrids to infection by *R. solani* AG3-PT and AG3-TB

Data in **Table (3)** significantly expressed variation in response of tomato cultivars and hybrids to root rot-causing *R. solani*. Four cultivars; Zaman cv., Super Red cv., Nema Star cv. and Marwa cv. gave high resist reaction type to anastomosis groups AG3-PT (R1 and R2) and AG3-TB (R3) and the lowest disease severity percentage. Hybrid 010-65 showed resist reaction to AG3-PT (R1 and R2) and high resist reaction type to AG3-TB (R3), DS% recorded 23%, 25% and 15%, respectively. While, Super Jackal cv. was very susceptible for both AGs; AG3-PT and AG3-TB with 92, 89 and 80 DS% for R1, R2 and R3, respectively, hybrid 777 was high susceptible for both AGs; AG3-PT and AG3-TB with 87, 84 and 76 DS% for R1, R2 and R3, respectively The highest percentage DS in case of both tested AGs (92% and 89%) for AG3-PT (R1 and R2). Meanwhile, *R. solani* anastomosis groups R1 (AG3-PT), R2 (AG3-PT) and isolate R3 (AG3-TB) were virulent towards all tested cultivars.



(B)

Isolate	Anastomosis group (AG)																
	AG-1 IA	AG-1IB	AG-1IC	AG-1 ID	AG-2-1 F	AG-2-1	AG-2-2 IIIB	AG-2-2 IV	AG-2-2 LP	AG-2-3	AG-2-4	AG-2BI	AG-3 PT	AG-3 TB	AG-4 HG-I	AG-4 HG-II	AG-4 HG-III
R1	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
R2	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
R3	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-

Fig. 2 Identification of *Rhizoctonia solani* anastomosis groups (AG) by using DNA specific markers. **(A)** Agarose gel electrophoresis PCR products using specific primer sets to detect *R. solani* AG-3PT for R1 and R2 and AG-3TB for R3. **(B)** The isolates which were analyzed with these primer sets, confirmed the presence of *AG*. Isolates for which specific fragments did not amplify, the marker was considered to be absent. All PCR reaction products were electrophoresed in a 4% agarose gel, stained with ethidium bromide and visualized under UV light.

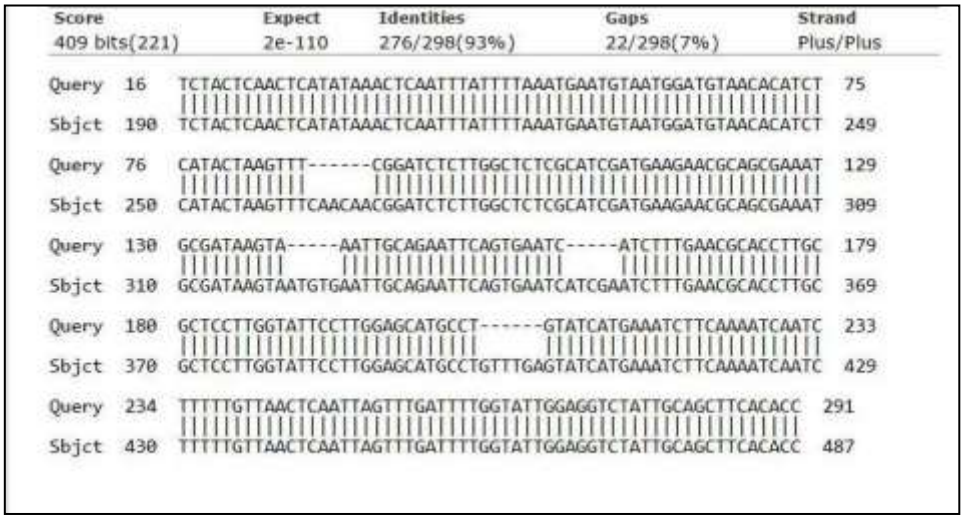


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Fig.3 *Rhizoctonia solani* (R1) AG-3 PT isolate NX-5 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence. Sequence ID: gb|KJ170329.1|Length: 701Number of Matches: 1.

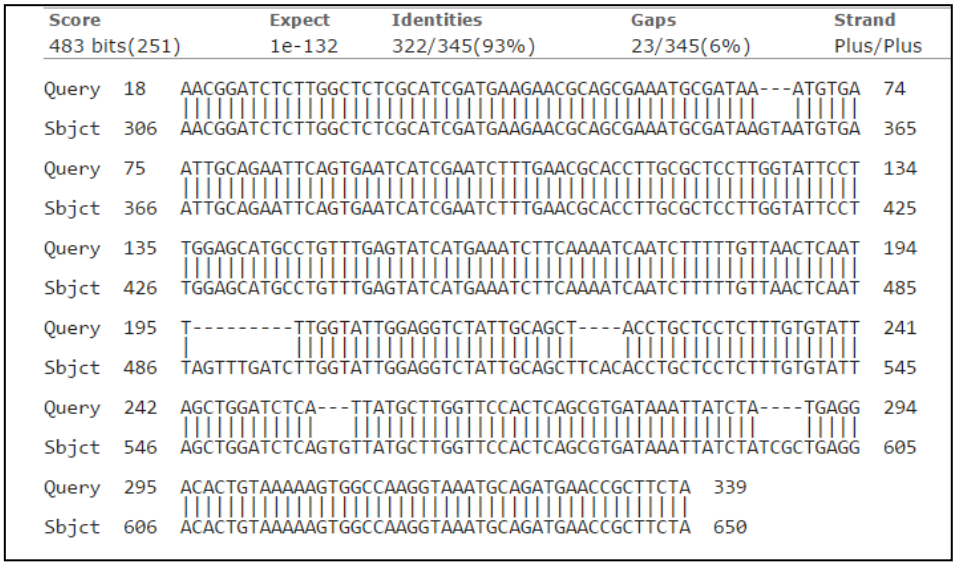

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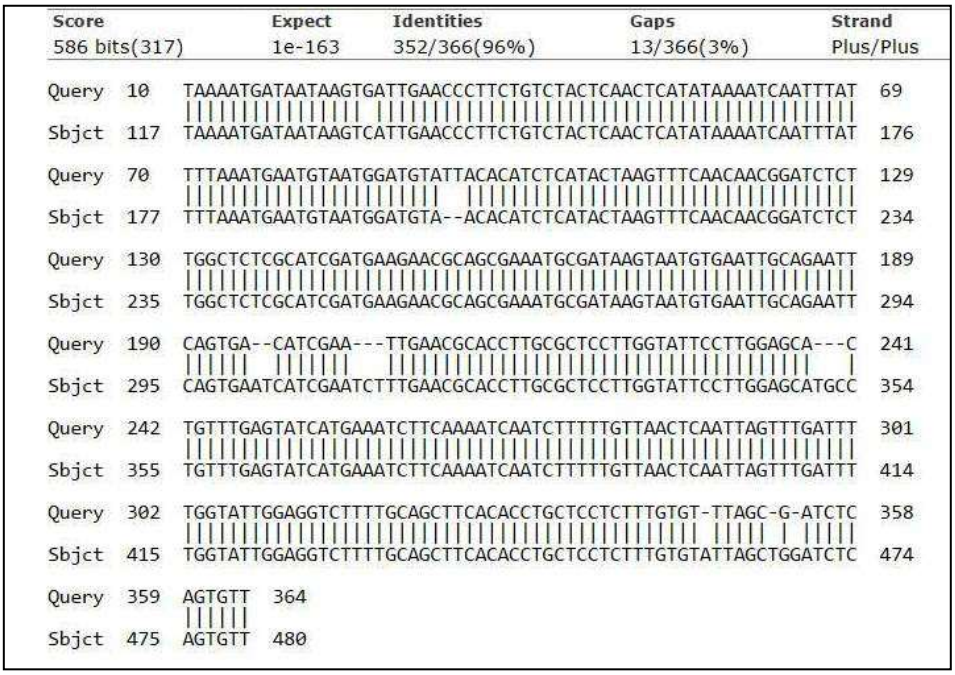
Fig. 4 *Rhizoctonia solani* (R2) AG-3 PT isolate NX-5 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA

gene, partial sequence. Sequence ID: gb|KJ170329.1|Length: 701Number of Matches: 1.



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            |||||||||||||||||||||||  ||||||||||||||||||||||||||||||||||||
Sbjct  177  TTTAAATGAATGTAATGGATGTA--ACACATCTCATACTAAGTTTCAACAACGGATCTCT  234

Query  130  TGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATT  189
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  235  TGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATT  294

Query  190  CAGTGA--CATCGAA---TTGAACGCACCTTGCGCTCCTTGGTATTCCTTGGAGCA---C  241
            ||||||  |||||||   ||||||||||||||||||||||||||||||||||||   |
Sbjct  295  CAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCTTGGAGCATGCC  354

Query  242  TGTTTGAGTATCATGAAATCTTCAAAATCAATCTTTTTGTTAACTCAATTAGTTTGATTT  301
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  355  TGTTTGAGTATCATGAAATCTTCAAAATCAATCTTTTTGTTAACTCAATTAGTTTGATTT  414

Query  302  TGGTATTGGAGGTCTTTTGCAGCTTCACACCTGCTCCTCTTTGTGT-TTAGC-G-ATCTC  358
            |||||||||||||||||||||||||||||||||||||||||||||| ||||| | |||||
Sbjct  415  TGGTATTGGAGGTCTTTTGCAGCTTCACACCTGCTCCTCTTTGTGTATTAGCTGGATCTC  474

Query  359  AGTGTT  364
            ||||||
Sbjct  475  AGTGTT  480
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Fig. 5 *Rhizoctonia solani* isolate (R3) AG-3 TB CR 5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence.
ID: gb|KT362071.1|Length: 604Number of Matches: 1.

Table (3) Reaction of ten tomato cultivars which are the most grown at El-Minia. Data expressed as disease severity (DS) percentage and reaction type of the selected isolates of *Rhizoctonia solani* AG3-PT and AG3-TB.

Tomato plants	*Rhizoctonia solani* (AGs)					
	AG3-PT (R1)		AG3-PT (R2)		AG3-TB (R3)	
	DS%	Reaction Type*	DS%	Reaction Type	DS%	Reaction Type
Cultivars						
Super Jackal	92±0.74	VS	89±1.05	VS	80±1.00	VS
Basha	85±0.95	VS	80±1.00	VS	75±1.00	MS
Marwa	12±1.00	HR	15±1.09	HR	5±1.06	HR
Nema Star	12±1.00	HR	10±1.00	HR	6±1.00	HR
Zaman	6±1.06	HR	3±100	HR	2±0.00	HR
Super Red	12±1.00	HR	13±1.00	HR	6±1.00	HR
Rawan	79±1.05	VS	76±0.06	VS	55±0.06	S
Hybrids						
777	87±1.05	VS	84±1.00	VS	76±0.06	VS
Nema 1400	77±0.88	VS	74±0.52	S	55±0.06	S
010-65	23±1.15	R	25±1.16	R	15±1.09	HR

Data are means of 3 experiments (4 replicates, 20 plant/each)±SD.
*Reaction Type; Very Susceptible (VS) 76~100%, Moderate Susceptible (MS) 61~75%, Susceptible (S) 46~60%, Moderate resistant (MR) 31~45%, Resistant (R) 16~30% and High Resistant (HR) 1~19%. Disease severity (DS) was monitored 45 days after inoculation and replanting time.

DISCUSSION

Several anastomosis groups of *Rhizoctonia solani* such as AG2-1, AG-3 and AG-4HG1 that have shown to be pathogenic to tomatoes were identified. Therefore, knowledge about the prevalence and distribution of *R. solani* isolates of different AGs is an important aspect in the integrated control of the problems related to *R. solani* **(**Misawa and Kuninaga, 2010 and Taheri and Tarighi 2012). Likewise, Rhizoctonia root rot is accompanied with tomato low plantation in Minia governorate. Thus, identification of *Rhizoctonia solani* anastomosis groups (AGs) became one of successful main targets of this study before further research to make an appropriate method for better Rhizoctonia root rot management and to help tomato breeders in Egypt to produce new resistant tomato hybrids.

R. solani, all 10 isolates exhibited root rot symptoms. Isolates R1, R2 and R3 which appeared highest infection were selected for accurate identification based on internal transcribed spacer regions (ITS) of the ribosomal DNA (rDNA) sequence. Molecular identification revealed that *R. solani* isolates were belong to one anastomosis group AG3. However, isolates R1 and R2 were identified to subgroup (AG3-PT), while R3 was identified as (AG3-TB). Data confirmed that *R. solani* AG3 is the most common *R. solani* AG as a

causal agent of Rhizoctonia root rot on tomato (Charlton *et al*., 2008 and Misawa and Kuninaga, 2010).

For tomato root rot and Rhizoctonia anastomosis groups, the AG-3 tomato isolates resembled the AG-3 PT potato isolates, the pathogen of black scurf disease, in pathogenicity and DNA sequence. Historically, isolates of *R. solani* AG-3 were associated with black scurf disease of potato. More recently, however, additional AG-3 isolates have been found to cause brown spot of eggplant, root rot of tomato. Sequences of rDNA-ITS regions have often been used for molecular identification of *R. solani* isolates and for the subdivision into AG and subgroups. *R. solani* AG-3 was divided into two subgroups, PT and TB based on sequence comparison of the rDNA ITS regions (Kodama *et al*., 1982, Kuninaga *et al*., 1997, Kuninaga *et al*., 2000 and Moussa, 2002).

The obtained results revealed that four tomato cultivars (Nema Star, Marwa, Zaman and Super Red) and hybrid (010-65) are highly resistant while, others are very susceptible (Super Jackal cv. and 777 hybrid) or susceptible (Basha cv. and Nema 1400 hybrid). Dealing with the root rot disease caused by *R. solani* could be managed through developing resistant varieties of tomato and improving disinfection practices of the soil. Moreover, if irrigation, fertilization, and similar cultivation practices are improved, the disease could be kept within tolerable limits (Takahashi *et al*., 2005 and Colak and Bicici, 2013). However, as mentioned in the present study, since the *R. solani* AG3-PT and AG3-TB are identified in the region, growing the varieties of tomato that are resistant to these *R. solani* AG will particularly decrease the rate of infection.

Meanwhile, the development of resistant tomato cultivars to *R. solani* AG identified will be more appropriate to be planted in this area. Nowadays, breeding programs are mostly focused on developing resistant varieties, since this method is the most effective in dealing with the diseases that cause huge economic losses (Colak and Bicici, 2013). Consequently, the identification of *R. solani* anastomosis group will help to promote the breeding programs of resistance to tomato root rot caused by *R. solani* AG3-PT and AG3-TB especially under El-Minia environmental conditions.

ACKNOWLEDGMENT

Authors are grateful to the Laboratory of Plant Breeding, Fac. of Agriculture, Iwate University, Iwate, Japan members for their help to run PCR protocol to complete this study.

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الملخص العربي

تحت المجموعات التلاحمية AG3 -PT و AG3 –TB للفطر *Rhizoctonia solani* كمسببات لعفن جذور الطماطم في محافظة المنيا

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نظرا لزيادة المساحة المنزرعة بمحصول الطماطم وتكرار زراعة هذا المحصول الهام، تأثرت نباتات الطماطم بالعديد من الأمراض خاصة تلك التي تنتشر عن طريق التربة ومن أهمها مرض عفن الجذور الناتج عن الاصابة بفطر الريزوكتونيا سولاني *Rhizoctonia solani* حيث ينتج عنه خسائر مباشرة في المحصول. تم عزل 10 عزلات من فطر *R. solani* أظهرت جميعها قدرة مرضية متباينة في شدة الاصابة وأظهرت عزلات R1، R2،R3 أعلي قدرة مرضية علي احداث عفن الجذور والتي تمثلت في تسجيل اعلي نسبة مئوية لشدة المرض العزلة R1 (92.5٪)، العزلة R2 (86.5٪) و العزلة R3 (77.5 ٪) علي الترتيب. تم تعريف عزلات *R. solani* باستخدام البيولوجيا الجزئية للمجموعة التلاحمية AG3 وتحت المجموعة AG3-PT (العزلة R1 والعزلة R2) في حين كانتAG3-TB هي المجموعة الفرعية للعزلة R3. أظهرت استجابة أصناف وهجن الطماطم للعدوى بالمجموعات الفرعية من AG3 *R. solani* أن اربعة أصناف للطماطم ذات مقاومة عالية (نيما ستار، مروة، زمان، سوبر ريد) والهجين (65-010) في حين أن البعض الآخر ذات قابلية للاصابة (صنف باشا و الهجين نيما 1400) أو ذات قابلية عالية للإصابة (صنف السوبر جاكال و الهجين 777).