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Research article

Rhizoctonia solani AG-3PT is the major pathogen associated with potato stem canker and black scurf in Jordan

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Abstract: *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*) is a global soil-borne pathogen that severely harms potato crops, leading to significant product losses. Black scurf and stem canker are two manifestations caused by this pathogen, with variable intensity based on the distinctive anastomosis group endemic to the region. During the growing season of 2017 (March and April), 57 different fungus isolates were collected from potato crops farmed in the Jordan Valley. The identity of all the isolates was confirmed by sequencing the internal transcribed spacer (ITS) of the ribosomal DNA gene, and the hyphal interactions were also performed with *R. solani* isolates. The sequences were deposited in GenBank, where accession numbers were obtained. 21 of the isolates were AG-3PT *R. solani*, with 98–99% identity to reference strains. Somatic compatibility was determined by hyphal interactions, which showed pairing compatibility among the isolates. Around 86.7% of the pairings were somatically incompatible, indicating a high level of genetic diversity among the isolates, while only 13.3% of the pairings were somatically compatible. Testing for pathogenicity revealed that AG-3PT affected the stems of solanaceous plants, including potatoes, and the roots of other plant species. Based on the findings of this study, *R. solani* AG-3PT was the primary pathogen associated with potato stem canker and black scurf diseases in Jordan. To our knowledge, this is the first report on this pathogen's isolation and identification in Jordan.

Keywords: black scurf; pathogenicity; *Rhizoctonia solani*; stem canker; *Thanatephorus cucumeris*

1. Introduction

Rhizoctonia solani is a soil-borne fungus that belongs to the phylum Basidiomycota [1,2]. This fungus severely impairs the quality and quantity of potato production, resulting in significant economic losses [3,4]. However, a variety of mild symptoms, such as misshaped tubers, growth cracks, netted scab, dry core, and tuber greening, which manifest more frequently than any other symptoms, contribute to yield losses [5,6]. Observed moderate symptoms include sunken lesions on stems (cankers), necrotic lesions on stolons and roots, as well as tuber sclerotia (irregularly shaped masses of fungal mycelium also known as black scurf), which cause seedling mortality and reduced tuber output. In severe infections, the fungus can girdle and kill the stems, damaging the entire plant [1,7]. Other fungi, such as *Fusarium* spp. and *Alternaria* spp. also cause problems in potato crops and inflict various damages but to a lesser extent than *Rhizoctonia* spp. which is the more prominent potato pathogen [8,9]. Tuber quality is often reduced by the development of sclerotia on progeny tubers, and thus, the product's marketability is adversely affected, particularly for pre-packaged potatoes [5]. In some cases, marketable yield losses approaching 30% have been reported [10]. The potato (*Solanum tuberosum* L.) is one of the most important non-grain food crops in the world [11]. In Jordan, potatoes are widely grown in a wide variety of environments. The potato crop, as with many other agricultural and horticultural crops, is subjected to attacks by species of *Rhizoctonia*, a globally ubiquitous fungus genus [1,3]. Due to its moderate climate, the Jordan Valley is a major producer of potatoes in the autumn and winter. In contrast, the rain-fed regions (the Highlands) are mainly cultivated in the spring. To cultivate their crops, farmers use a variety of imported potato tubers most of them came from the Netherlands, France, and Germany without assurances that these tubers are free of *R. solani*. According to Abu El Samen and Al Bodor [12], *R. solani* was present in 42.5% of the tested 109 samples of imported potato tubers from Europe.

Rhizoctonia solani is a complex species composed of 13 anastomosis groups (AG1-AG13, with AG B1 being a subset of AG-2 B1) [13]. Traditionally, the AG grouping is assigned phenotypically based on hyphal interactions, where hyphae of isolates belonging to the same AG are able to anastomose [1]. However, such a traditional method does not offer reliable results, particularly for designating group subsets [3]. Other parameters, including pathogenicity, biochemical traits, and modern genetic markers, have been used to more reliably and definitively assign the AG groupings [14]. Molecular approaches for accurately identifying *Rhizoctonia* subgroups have been increasingly used to assign each isolate to its AG group. Polymerase chain reaction (PCR)-based techniques for amplifying the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) are frequently used in conjunction with phylogenetic analysis to understand the genetic relationship among isolates [3,15]. This method is thought to be superior in terms of assigning anastomosis AG grouping to hyphal interactions. There is relatively little data given about the biology of the disease and the relative importance of the various AGs in the etiology of *Rhizoctonia* diseases in potatoes in Jordan as well as other countries. Knowing this information is crucial for assessing the use of particular fungicides that may work effectively against specific AGs. Furthermore, collecting such information will help predict the disease's severity once it appears and the correct approach to diagnose and treat it [14]. According to studies, different AGs have different hosts. Hence, they are designated by

an abbreviation. For example, the AGs for potato (AG3-TP), tobacco (AG3-TB), and tomato are (AG3-TM). Each is consequently associated with various disease symptoms.

There are no projections of the impact of *Rhizoctonia* diseases on tuber quality and yield in Jordan, and farmers are frequently unaware of the underlying causes of their potato crop damage. The objective of this study therefore, was to isolate and identify all causative agents affecting the potato crops in the Jordan Valley using molecular techniques and to study the pathogenicity of specific *R. solani* AGs to potato and other vegetable plants.

2. Materials and methods

2.1. Sample collection and fungal isolation

One hundred fifty potato plants and tubers with typical *R. solani* symptoms (black scurf and canker on stems, stolons, and roots) were obtained during March and April of 2017 from 15 transects within fields from three geographically different locations in the Jordan Valley. These locations represent the most important potato-growing areas in the Jordan Valley (Table 1).

The collected samples were brought to the plant pathology laboratory in the faculty of agriculture at the Jordan University of Science and Technology to isolate the pathogen and examine its phenotypic and pathogenic traits. Infected plant materials with obvious disease symptoms were rinsed in running water and dried for four hours in a laminar flow cabinet in order to separate *Rhizoctonia* and other pathogens. Small pieces of infected tissue, 4 mm in diameter and 5 mm deep, were excised using a sterile scalpel blade and plated onto petri plates containing 1.5% water agar (WA) supplemented with streptomycin sulfate (Sigma-Aldrich) at 50 mg/liter. The plates were incubated at 25 ℃ for 48 hours. The colonies of each isolate that was determined to be a *Rhizoctonia* spp. were examined under a microscope, and fungal hyphal tips were collected. Following the procedures outlined by Carling and Leiner [16], tissue samples were placed onto potato dextrose agar (PDA; Biolab, Hungary), and were incubated for three days at 22 ℃.

2.2. Molecular identification

DNA was extracted from 57 pure fungal samples that were phenotypically identified as *Rhizoctonia* spp. according to the method described by Liu et al. [17]. The quantity and quality of DNA samples were detected using NanoDrop (Thermo Fisher scientific cat ID: ND-2000) and gel electrophoresis, respectively. Working DNA solutions of 20 ng/μL were prepared, and both the stock and working solution were stored at −20 ℃.

Table 1. The location and climatic conditions of the infected potato populations analyzed in this study.

Jordan Valley Areas	Municipality	Field	Location and climatic conditions					Potato species or cultivar
			Average temperature (℃)		Average rainfall (mm)	Average air humidity (%)	Altitude (M.o.s.l.)	
			Min	Max				
North	Krima, Shikh Hussien, Abo Habil, Abo Sido, North Shuna, Alezba	70	15.0	31.2	342.0	40.7–73.8	−100 to −200	Nicola, Kenebec, Diamant, Spunta, Draga.
Middle	Khazma, Tharar, Maadi, Alramel	50	15.8	29.0	281.0	32.2–65.3	−200 to −300	Nicola, Kenebec, Diamant, Spunta, Draga.
South	Alsafi, Almamora, Alslemani, Khanzira	30	18.2	30.8	72.6	27.5–64.0	−300 to −425	Nicola, Kenebec, Diamant, Spunta, Draga.

The ITS region for each isolate was amplified using the universal primers; ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') [18] and ITS-4 (5'-TCCTCCGCTTATTGATATGC-3') [19] that anneal to the flanking 18S and 28S rDNA genes. PCR reactions were conducted under the following conditions; 95 ℃ initial denaturation for 5 minutes, 35 cycles of 1 min 95 ℃ denaturation, 30 sec 45 ℃ annealing, 1 min 72 ℃ extension, 72 ℃ final extension, and holding at 4 ℃. Each primer (1 μL) was added to the reaction from a 10 μM working solution alongside with 2xPCR master mix solution (I-MAX II) (cat ID 25266), 20 ng template DNA, and an appropriate amount of nuclease free water to reach 20 μL final volume. A negative control sample was used containing all the components except the DNA template was used to check for any contamination.

PCR amplifications were analyzed using agarose gel electrophoresis stained with ethidium bromide. Prior to sequencing, PCR products were cut from the agarose gels and purified using Zymoclean Gel DNA Recovery Kits (cat IDD4007).

Sequencing reactions were carried out using BigDye™ Terminator v3.1 Cycle Sequencing Kit (cat ID 4337455) and analyzed using the genetic analyzer 3100 DNA sequencer located in the Princess Haya Biotechnology Center, Jordan University of Science and Technology (JUST).

NCBI BLASTn database was used to check the identity of each sequence and to find the closest match based on maximal percentage identity. Confirmed sequences were deposited in GenBank and were assigned accession numbers.

Table 2. The identity of the isolated mold species from potato samples studied in this project based on the ITS-rDNA sequencing.

Group	Group of the isolated mold	No of isolates	% Isolates
1	*Alternaria* spp.	4	7
2	*Aspergillus* spp.	15	26.3
3	*Botryotrichum* spp.	1	1.8
4	*Cylindrocarpon* spp.	1	1.8
5	*Fusarium* spp.	9	15.7
6	*Gymnosascus* spp.	1	1.8
7	*Rhizoctonia* spp.	21	36.8
8	*Rhizopus* spp.	1	1.8
9	*Trichoderma* spp.	4	7
Total		57	100

2.3. Phylogenetic analysis

Two phylogenetic trees were constructed, the first of which (Figure 1) displayed the phylogenetic relatedness between all 57 isolates, while the second of which (Figure 2) displayed only the 21 *Rhizoctonia* isolates (Figure 2). Both trees were constructed with 5 *Rhizoctonia* sequences representing out groups: an out group belonging to a different AG group (AB054845.1,(AG-2-1), an AG3 group infecting tobacco (AB000004.1 AG-3-TB), an AG3 group infecting tomato (AB000023.1(AG-3-TM), and two isolates of an AG3 group infecting potatoes isolated from other parts of the world (KX631235.1) AG-3-PT, and KX631236.1 (AG-3-PT) [20].

Phylogenetic trees were constructed using the Neighbor-Joining method [21]. The optimal tree with a sum of branch lengths of 0.22 mm is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches [22]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura 3-parameter method [23] and are in units of the number of base substitutions per site. The phylogenetic trees were constructed using Molecular Evolutionary Genetics Analysis [MEGA X] software [24].

2.4. Determination of somatic compatibility grouping

All the confirmed *Rhizoctonia* isolates as AG-3PT by the ITS rDNA sequencing were used in this study. Slides with a thin covering of water agar used to detect microscopic somatic compatibility reactions (WA Oxoid) were made for the pairings. The interaction was evaluated under a light microscope using safranin O staining [25]. According to the method of Carling [26], we determined the number of fields in each interaction category (C0, C1, C2, and C3) in 15 visual fields. The fusion frequency has been determined as %FF = (A*100)/B. According to MacNish et al. [27], the macroscopic somatic reactions were defined as merge and tuft according to predefined categories after the plates were incubated at 15 ℃ for 21 days and assayed.

2.5. Pathogenicity tests

Twelve plant species were tested as potential hosts for three isolates confirmed as AG-3PT, which were selected randomly from each of the three regions to be tested for their pathogenicity. These were seedlings of carrot (*Daucus carota* L.), bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum* L.), soybean (*Glycine max*), corn (Zea mays L.), Wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), tomato (*Solanum lycoper*sicum L.), pepper (*Capsicum annuum* L.), okra (*Abelmoschus esculentus*), cauliflower (*Brassica oleracea* L.) and potatoes (*Solanum tuberosum* L.). The seeds were germinated in peat. The seedlings were planted in pots containing sterile sand. After five days, each plant was inoculated with a piece of a *Rhizoctonia* growth with a diameter of 5 mm from the fungal colonies' margins. This part of the mycelium was placed one cm deep next to the seedling's stem and covered with sterile quartz sand to prevent drying. As per Carling and Leiner [28], potato plants were used as a positive control. Plants were incubated at 20 °C for a 12 hour photoperiod in a growth chamber, irrigated with distilled water every 2–3 days, and fertilized with a nutrient solution weekly [29]. The symptoms on the stems and roots were evaluated after 15 days of inoculation. On the stems, the symptoms were classified as small superficial lesions (SS L = lesions size < 5 mm) or cankers (C). On the roots, disease incidence, and lesion categories were characterized as on the stems. Re-isolation was carried out on infected and healthy plant tissues to confirm the presence of fungi in the tested plants.

2.6. Statistical analysis

The experimental design for the pathogenicity test was a complete randomized block design with three replications. The data were subjected to an analysis of variance, and the treatment means were separated by Duncan's multiple range test ($\alpha = 0.05$). The data were analyzed using GENSTAT 14th Edition (VSN International).

3. Results

3.1. Identification of Potato infecting species by ribosomal DNA sequencing

For primary identification, morphological characterization was performed on all 57 isolates. Phenotypically, all conformed to *Rhizoctonia*. However, their identity was determined by sequencing the internal transcribed spacer (ITS-rDNA). Surprisingly, only 21 isolates were confirmed as *R. solani*, while the rest were distributed among different fungi species described in Table 2. It was noticed that *Aspergillus* spp. and *Fusarium* spp. were the most predominant after *Rhizoctonia* spp.

The obtained rDNA sequences were deposited in the GenBank, and accession numbers were assigned (Table 3). All the *Rhizoctonia* isolates (100%) were of anastomosis group 3 (AG-3PT). Among these, 12 isolates were isolated from stem cankers, while the other nine isolates were isolated from sclerotia on the mother tubers.

Table 3. The isolates with their names and ITS sequence ID and the GenBank accession numbers.

Isolate	Isolate identity	Accession numbers
1	*Alternaria_chlamydosporigena*_R18	MN258559
2	*Alternaria_chlamydosporigena*_R21_1	MN258560
3	*Alternaria_chlamydosporigena*_R30	MN258561
4	*Alternaria_chlamydosporigena*_R8	MN258562
5	*Aspergillus_fumigatus*_11.6-R34_06-35-29	MN258563
6	*Aspergillus_fumigatus*_11.6-R48_07-36-02	MN258564
7	*Aspergillus_fumigatus*_R17	MN258565
8	*Aspergillus_fumigatus*_R22--	MN258566
9	*Aspergillus_fumigatus*_R29	MN258567
10	*Aspergillus_fumigatus*_R39	MN258568
11	*Aspergillus_fumigatus*_R47	MN258569
12	*Aspergillus_niger*_R1	MN258570
13	*Aspergillus_niger*_R20_	MN258571
14	*Aspergillus_niger*_R3	MN258572
15	*Aspergillus_niger*_R8_1.phd	MN258573
16	*Aspergillus_ochraceus*_R29.2	MN258574
17	*Aspergillus_ruber*_R9	MN258575
18	*Aspergillus_rugulosus*_R53-DOWN	MN258576
19	*Aspergillus_ustus*_R23_1	MN258577
20	*Botryotrichum_piluliferum*_R50	MN258578
21	*Cylindrocarpon_olidum*_R36	MN258579
22	*Fusarium_equiseti*_11.6-R7_07-36-02	MN258580
23	*Fusarium_equiseti*_R11D	MN258581
24	*Fusarium_equiseti*_R16	MN258582
25	*Fusarium_equiseti*_R17_e	MN258583
26	*Fusarium_equiseti*_R22	MN258584
27	*Fusarium_redolens*_R10	MN258585
28	*Fusarium_solani*_R37	MN258587
29	*Fusarium_solani*_R45	MN258588
30	*Fusarium_solani*_R49-DOWN	MN258589
31	*Gymnoascus_reesii*_R54	MN258590
32	*Rhizoctonia_solani*_AG-3_R11	MN258591
33	*Rhizoctonia_solani*_AG-3_R24	MN258592
34	*Rhizoctonia_solani*_AG-3_R25	MN258593
35	*Rhizoctonia_solani*_AG-3_R26	MN258594
36	*Rhizoctonia_solani*_AG-3_R28-UP	MN258595
37	*Rhizoctonia_solani*_AG-3_R3_r	MN258596
38	*Rhizoctonia_solani*_AG-3_R32	MN258597
39	*Rhizoctonia_solani*_AG-3_R38	MN258598
40	*Rhizoctonia_solani*_AG-3_R40	MN258599
41	*Rhizoctonia_solani*_AG-3_R41-UP	MN258600

Continued on the next page

Isolate	Isolate identity	Accession numbers
42	*Rhizoctonia_solani*_AG-3_R43	MN258601
43	*Rhizoctonia_solani*_AG-3_R44	MN258602
44	*Rhizoctonia_solani*_AG-3_R46	MN258603
45	*Rhizoctonia_solani*_AG-3_R49-UP	MN258604
46	*Rhizoctonia_solani*_AG-3_R51_1	MN258605
47	*Rhizoctonia_solani*_AG-3_R53-UP	MN258606
48	*Rhizoctonia_solani*_AG-3_R57	MN258607
49	*Rhizoctonia_solani*_AG-3_R59	MN258608
50	*Rhizoctonia_solani*_AG-3_R6	MN258609
51	*Rhizoctonia_solani*_AG-3_R9_r	MN258610
52	*Rhizopus_oryzae*_R21	MN258611
53	*Trichoderma_guizhouense*_R4	MN258612
54	*Trichoderma_harzianum*_R2	MN258613
55	*Trichoderma_harzianum*_R21_th	MN258614
56	*Trichoderma_harzianum*_R52	MN258615
57	*Fusarium_solani*_R35	MN258586

3.2. Phylogenetic analysis

The phylogenetic tree was constructed for all 57 isolates (Figure 1) to study the clonal relationship among all the fungi that grow and cause diseases in potatoes. As expected, all strains of *R. solani* clustered together along with the out group isolates that infect potatoes. Indeed, the phylogenetic tree separated each species into a single cluster. Except for a few isolates that appeared to cluster with isolates from different species, this was true for the majority of fungal species. The other tree was constructed for the 21 *Rhizoctonia* isolates (Figure 2) and five other isolates obtained from published sequences. The bootstrap values on all nodes showed no significant differences between our isolates, which reflect their common source with the two AG3 isolates from China [30] that appeared within the Jordan AG3 isolates. However, the other three outer isolates, the AG3 isolates (TB and TM) and the out group AG-2-1 isolates, appeared distant from the potato AG3 group isolated in this study.

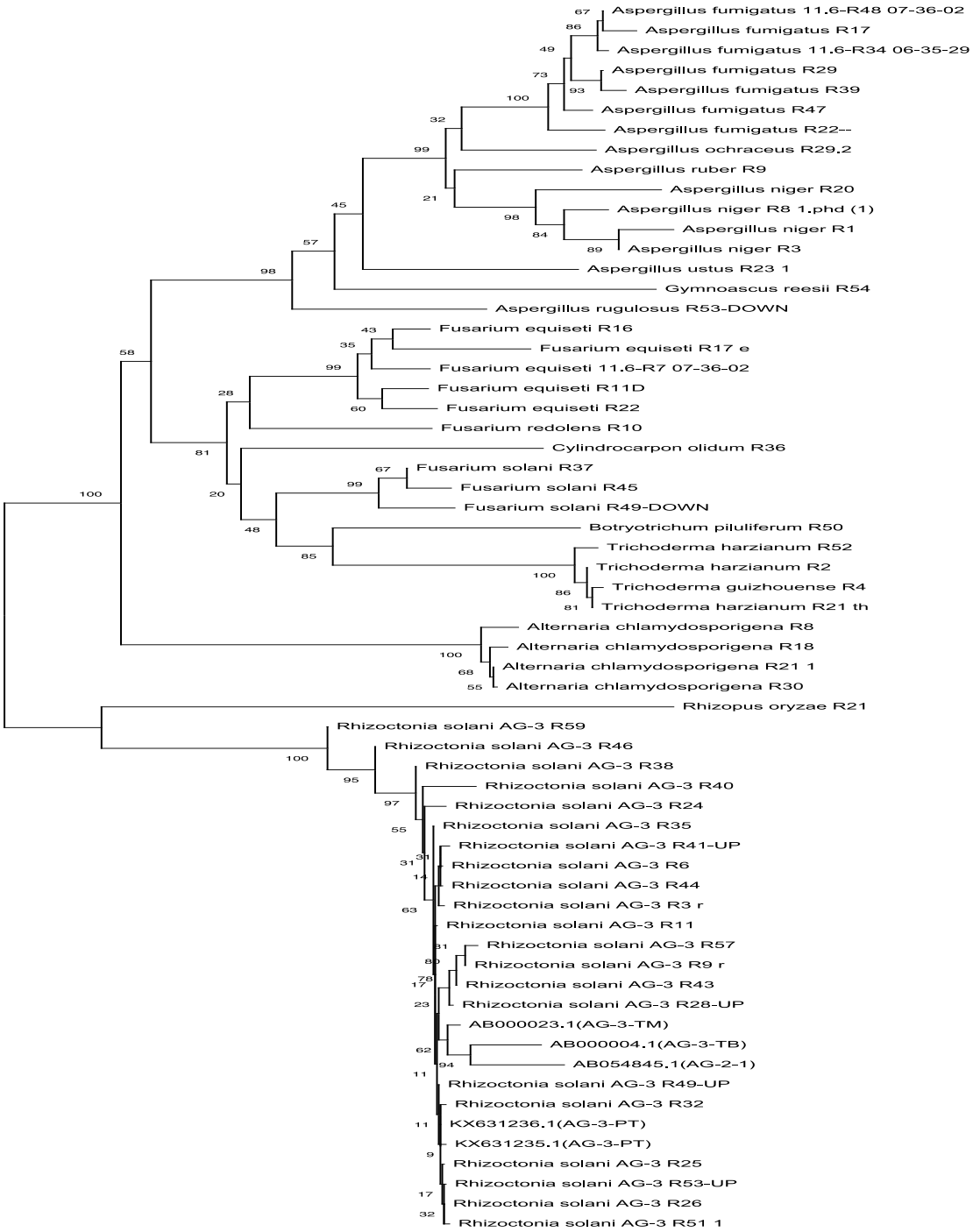

Figure 1. Evolutionary relationships of taxa; the evolutionary history was inferred using the Neighbor-Joining method [21]. The optimal tree with the sum of branch length = 2.89804503 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches [22]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura 3-parameter method [23] and are in the units of the number of base substitutions per site. This analysis involved 62 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pair wise deletion option). There were a total of 1045 positions in the final dataset. Evolutionary analyses were conducted using MEGA X software [24].

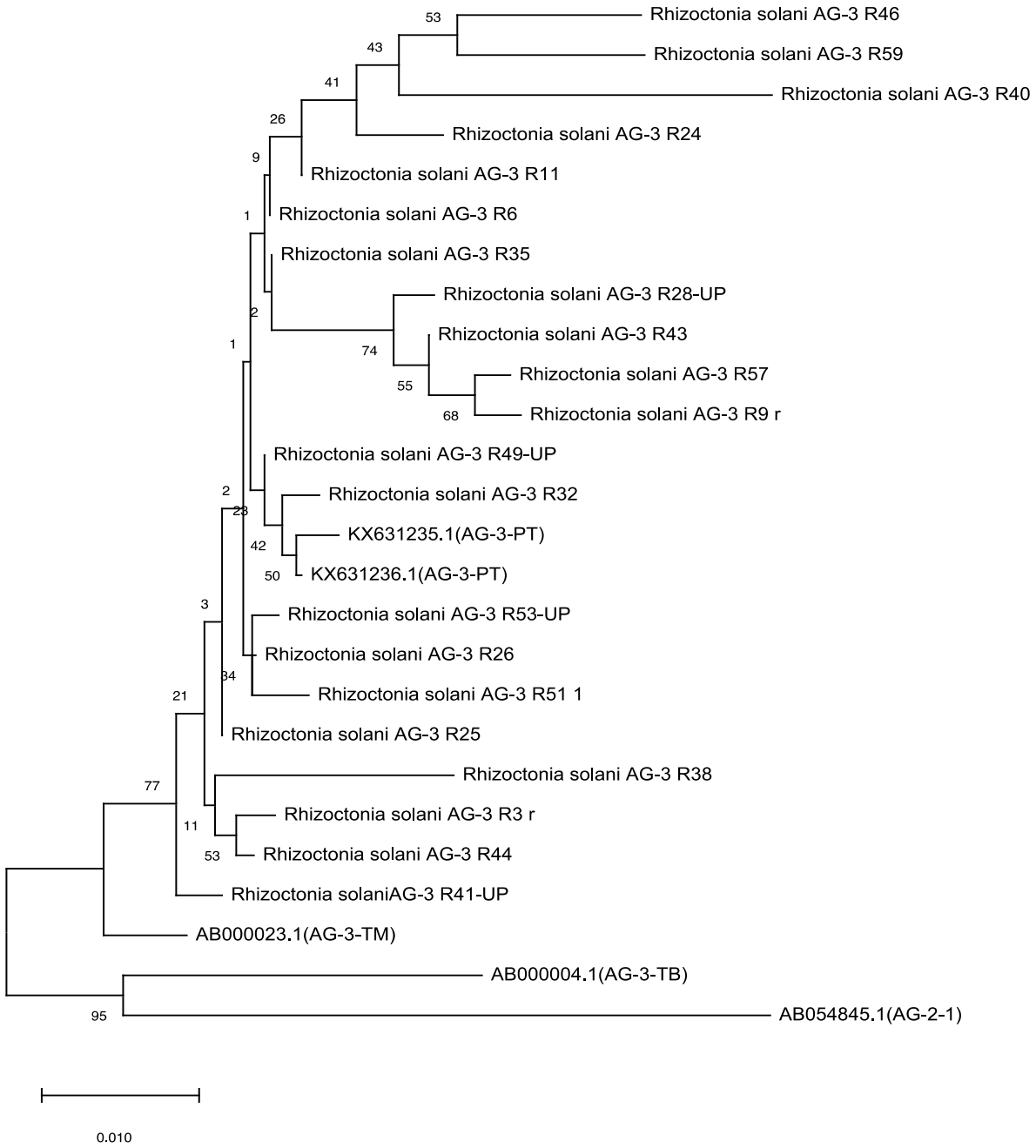

Figure 2. Evolutionary relationships of taxa; the evolutionary history was inferred using the Neighbor-Joining method [21]. The optimal tree with the sum of branch length = 0.21783581 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [22]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura 3-parameter method [23] and are in the units of the number of base substitutions per site. This analysis involved 26 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pair wise deletion option). There were a total of 757 positions in the final dataset. Evolutionary analyses were conducted using MEGA X software [24].

3.3. Hyphal interactions

The hyphal fusion ratio of 15 isolates belonging to AG3 ranged from 45 to 96%. In addition, a high percentage of isolates have frequencies between 47 and 74%, which indicates a lack of harmony between individuals indicating that the anastomosis reaction was positive and the frequency of hyphal fusion exceeded 50%. About 86.7% of isolates pairs were evaluated, and the formation of tufts was common in the macroscopic somatic assay indicating a somatic incompatibility between the isolates. However, the perfect fusion occurred in only 13.3% of the pairs tested, indicating their compatibility (Figure 3). Table 4 presents the results of the hyphal interaction.

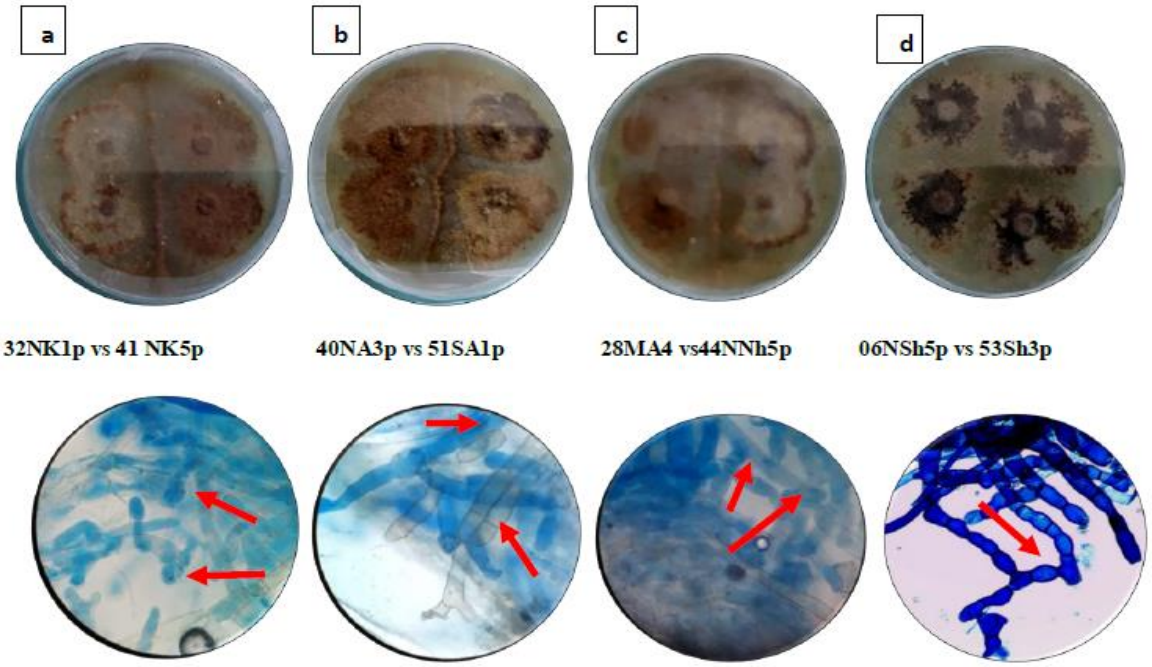

Figure 3. The macroscopic anastomosis reaction between *Rhizoctonia solani* AG-3PT isolates. (a) Strong tuft reaction between isolates collected in the same field, (b) Strong tuft reaction between isolates collected in the different fields, (c) Mild tuft reaction between isolates collected in the different fields, (d) Merge tuft reaction between isolates collected in the different fields. Numbers underneath each figure are codes for isolate pairs, depending on the geographic source of the isolates. The microscopic images below show different degrees of tuft reaction between isolates.

3.4. Pathogenicity tests

Isolates of *R. solani* AG3-PT caused large lesions (>10 mm) on the stems of potato, tomato, pepper, and okra, while their roots did not show any symptoms. As for the roots of pea, bean, soybean, wheat, barley, corn, and carrot plants, the AG3-PT isolates caused numerous small lesions. Furthermore, the AG3-PT isolates caused lesions on the stems of soybean, barley, corn, and carrot plants while not causing any lesions on pea, bean, or wheat. The cauliflower showed no signs of disease (Table 5 and Figure 4).

Table 4. Results of pairings in hyphal interaction assays performed on 15 isolates with tester isolates of *Rhizoctonia solani* (C0, no interaction; C1, hyphal contact; C2, killing reaction; C3, fusion with no cell death).

AG/3 Isolate	R 06	R 09	R 11	R 25	R 28	R 32	R 38	R 40	R 41	R 44	R 46	R 51	R 53	R 57	R 59
R 06	C3	C1	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C3	C2	C2
R 09		C3	C2	C2	C1	C2	C2	C2	C1	C2	C2	C2	C2	C2	C2
R 11			C3	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2	C2
R 25				C3	C2	C2	C2	C2	C2	C3	C2	C2	C2	C2	C2
R 28					C3	C2	C1	C2	C2	C2	C2	C2	C2	C2	C1
R 32						C3	C2	C2	C2	C2	C2	C2	C1	C2	C2
R 38							C3	C2	C1	C2	C2	C2	C2	C2	C2
R 40								C3	C3	C2	C2	C2	C2	C2	C2
R 41									C3	C2	C2	C2	C2	C1	C2
R 44										C3	C2	C2	C2	C2	C2
R 46											C3	C2	C2	C2	C1
R 51												C3	C2	C2	C2
R 53													C3	C2	C2
R 57														C3	C2
R 59															C3

Table 5. Host range and pathogenicity of *Rhizoctonia solani* AG-3 isolates obtained from commercial potato fields in the Jordan Valley.

Host Plants		Stem lesion	Size(cm)	Root lesion	Diseased roots (%)
Daucus carota	Carrots	NI*	0.0^{a}	NI/C	52.8^{b}
Brassica oleracea	Cauliflower	NI	0.0^{a}	NI	0.0^{a}
Pisum sativum	Peas	NI	0.0^{a}	SSL/C	21.4^{c}
Phaseolus vulgaris	Bean	NI	0.0^{a}	SSL	38.7^{d}
Glycine max	Soybean	SSL/C	0.32^{b}	SSL/C	62.3^{e}
Zea mays	Corn	SSL	0.48^{c}	SSL	33.9^{d}
Triticum aestivum	Wheat	NI	0.00^{a}	SSL	0.07^{a}
Hordeum vulgare	Barely	SSL	0.21^{d}	SSL	0.22^{c}
Solanum lycopersicum	Tomato	NI/C	1.04^{e}	NI	0.0^{a}
Capsicum annuum	Pepper	C	1.40^{f}	NI	0.0^{a}
Abelmoschus esculentus	Okra	C	1.02^{e}	NI	0.0^{a}
Solanum tuberosum	Potato	C	1.50^{f}	NI	0.0^{a}

*Stem and root lesions were categorized as follows: NI (non-infected); SS L (superficial small lesion) and C (canker). The size of the lesions is the average of 15 plants. Means followed by the same letter within a column are not significantly different from each other according to Duncan's Multiple Range Test ($\alpha = 0.05$).

Figure 4. Symptoms of infection by *Rhizoctonia solani* AG-3PT isolates on different Hosts. (a) on *Phaseolus vulgaris*, (b) on *Pisum sativum*, (c) on *Glycine max*, (d) on *Solanum lycopersicum*, (e) on *Capsicum annuum*, (f) on *Triticum aestivum*, (g) on *Hordeum vulgare*, (h) on *Zea mays*, (i) on *Brassica oleracea*, (j) on *Daucus carota*, (k) on *Abelmoschus esculentus*, (l) on *Solanum tuberosum*.

4. Discussion

This is the first study in Jordan to describe and characterize the disease-causing pathogens of potato crops and the identification of these pathogens both at the phenotypic and genotypic levels. Despite the fact that *R. solani* is generally thought to be the major pathogen infecting potatoes, several other fungi species were also isolated from infected potatoes grown in the Jordan Valley. The types of these pathogenic fungi and their distribution are presented in Table 2. Many of these fungi are known

plant pathogens and could inflect various degrees of damage to potato plants as well as to other crop plants [31]. The most prevalent species among these isolated pathogens was *R. solani* (35%). All of the isolates' species identities were determined by ITS rDNA sequencing (Table 2). As inferred from the ITS rDNA sequences, *R. solani* isolates found in Jordan that were recovered from various potato diseases were all AG3-PT (100%). This result confirms previous reports about the predominance of AG3-PT on potato crops [32–37]. Furthermore, the phylogenetic analysis revealed that the isolates of *R. solani* are associated with each other with a low degree of heterogeneity which could be due to the differences in the locations of samples collection (north, central, and south of Jordan Valley). Interestingly, AG3-PT isolates from China clustered with the AG3-PT isolates from Jordan. In contrast, the cluster analysis showed that AG3-TB and AG3-TM did not cluster with the AG3-PT isolates, indicating the low heterogeneity among the AG3-PT and the vast difference between the AG3-PT and the AG3-TB or AG3-TM as inferred from the phylogenetic analysis. In addition to its high virulence on potatoes, one of the reasons AG3-PT is so common in potatoes is its ability to form sclerotia (black scurf) on tubers, inflicting severe damage to the corp. The results are in accordance with the observations of Lehtonen et al. [38] who postulated that AG3-PT is much more efficient in producing sclerotia on tubers than other AG subgroups, reflecting the highly specialized nature of AG3-PT in infecting potatoes and thus being more aggressive. Nonetheless, other AG groups inflict severe damage to other plants, such as tomatoes and tobacco [39,40].

The correlation between microscopic killing anastomosis (C2 reaction) and perfect fusion (C3 reaction) with the corresponding macroscopic somatic interactions “tuft” and “merge” has been reported for some *R. solani* groups, such as AG8 [27] and AG3-TB from tobacco [41], as well as *R. solani* AG3-PT from potatoes [3]. Nevertheless, the macroscopic somatic interactions were not good predictors for the microscopic anastomosis reactions between isolates of the soybean-infecting pathogen *R. solani* AG1-IA [42]. Similar to previous reports on the soybean-infecting pathogen, perfect fusion and killing anastomosis reactions at the microscopic level were not distinguished in this study. Thus, a correlation between macroscopic and microscopic for *R solani* AG3-PT was not established. The results of this study indicated incompatibility between the tested isolates, as some fusions appeared in large quantities of aerial mycelia (tuft) (Fig 3a, b) indicating a high level of genetic diversity among the isolates (Figure 3a, b). Somatic compatibility existed in only 13.3% of the pairs. Further, the obtained results indicated that *R. solani* AG3 is capable of infecting different plant species and inflicting severe damage, which is consistent with previous reports that even specialized mold pathogens have shown some levels of damage in hosts distinct from their own original host [3,16,39,43–46]. Indeed, these results have shown the need to adopt crop rotation strategy and the use of specific fungicides to control *R. solani* from accumulating in the soil and infecting other plants that showed readiness to be infected by this pathogen. In addition, even though the fungal inoculums could remain viable on plant debris and hidden roots for a long time, planting cauliflower as a non-host to interrupt the pathogen's life cycle can be a part of the fighting strategy against this mold [31].

This work has provided knowledge about the *R. solani* groups in different locations in Jordan. Further, it has confirmed the predominance of AG3-PT associated with potato disease in Jordan. The significance of determining the AG group for a location could be used as a guide for selecting an effective disease management regimen. This belief stems from the idea that genetic differences exist among the different AG groups, which might be reflected in susceptibility/resistance to a certain fungicide [34,40]. Further, understanding the host range for the different AG groups is of prime importance for fighting strategies [44].

5. Conclusions

The internal transcribed spacer (ITS) sequencing and the phylogenetic analysis of the *Rhizoctonia solani* isolates confirmed the identity of the isolates in which AG-PT3 was the major pathogen affecting the potato crop in Jordan.

The obtained results for the different AG groups can be used to implement strategies for combating this fungus. Crop rotation is one of the regimens that can break the fungi's life cycle by growing non-host-specific crops and choosing the most appropriate fungicide for a particular AG group.

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Conflict of interest

The authors declare that they have no conflict of interest.

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