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Differential interaction between isolates of *Rhizoctonia solani* AG-3 and potato cultivars

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Twenty fungal isolates of *Rhizoctonia solani* AG-3 sclerotia were isolated from diseased potato tuber samples that were obtained from potato-producing areas in the Lower Egyptian governorates. The pathogenicity of the 20 isolates of *R. solani* AG-3 was tested on nine potato cultivars under greenhouse conditions. The black scurf disease incidence (BSDI) and stem canker index (SCI) were used as the criteria to evaluate the pathogenicity. An analysis of variance (ANOVA) showed that the isolate, cultivar and interaction between isolate and cultivar were very highly significant sources of variation for all the tested parameters. The statistical significance between the isolate and cultivar suggests that a physiological specialization exists among the *R. solani* AG-3 isolates that cause pathogenicity in potato. Additionally, the pathogenicity of the tested isolates was found to result from a combination of virulence and aggressiveness, and the isolates significantly differ for both types of pathogenicity. Similarly, the resistance of the tested cultivars was a mixture of both vertical and horizontal resistance, and there were significant differences among the cultivars for each type of resistance. A cluster analysis (isolates) of all of the tested parameters differentiated the isolates into four groups based on their virulence patterns in the nine potato cultivars, but there was no relationship among these groups and their geographical origin. The cluster analysis for the cultivars using all of the tested parameters differentiated the three groups based on the cultivar's reaction pattern to the twenty *R. solani* AG-3 isolates.

Key words: Potato, cultivars, *Rhizoctonia solani*, pathogenicity, cluster analysis.

INTRODUCTION

Rhizoctonia solani Kühn [(teleomorph: Thanatephorus cucumeris (Frank) Donk.) is an important fungal pathogen that causes stem canker and black scurf in potato (*Solanum tuberosum* L.). This fungus is widespread in all potato-growing areas of the world (Frank and Leach, 1980). The current classification within the *R. solani* species complex is largely based on the grouping of isolates into anastomosis groups (AGs) depending on their hyphal interactions. At least 13 AGs have been described within the *R. solani* species complex, including the AG-1 to AG-13 and AG-21 subgroups (Carling, 1996; Kuniniga et al., 2000; Priyatmojo et al., 2001). *R. solani* Kühn, particularly AG-3, is the principal cause of the black scurf and stem canker disease of potato (Truter and Wehner, 2004; Yanar et al., 2005; Mahmoud, 2010).

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Table 1. Geographic origin and sources of *R. solani* AG-3 isolates used in the study.

Isolate no.	Geographic origin	Region	Source	Anastomosis groups (AGs)
1-4	Beheira	West Delta	potato	AG-3
5-8	Daqahiliya	East Delta	potato	AG-3
9-12	Gharbiya	West Delta	potato	AG-3
13-15	Minufiya	Middle Delta	potato	AG-3
16-20	Sharqiya	East Delta	potato	AG-3

There are reports implicating other AGs, including AG-6 and AG-7 (Abdul Rauf et al., 2000), AG-4 (Petkowski et al., 2003) and AG-5 (Truter and Wehner, 2004). This disease is prevalent in South Africa, the United States, New Zealand, Pakistan, Finland, Turkey, Egypt and France (Du Plessis, 1999; Ceresini et al., 2002; Nejad et al., 2007; Naz et al., 2008; Lehtonen et al., 2008; Demirci et al., 2009; Mahmoud, 2010; Fiers et al., 2011). *R. solani* causes the inhibition of eye germination, death of underground sprouts, stem cankers and stolon cankers, resulting in a poor and uneven stand of weak plants and subsequent reductions in yield. The infected plants are generally smaller (< 3 cm in diameter) (Banville, 1989), with malformed progeny tubers (Carling et al., 1989) and comparatively small numbers of stems (Scholte, 1989). In some cases, the yield reductions induced by *R. solani* are often regarded as insignificant or not worth controlling. However, previous studies have indicated that a cultivar-dependent yield reduction of 7-64% (average of 35%) may result when the isolate has a high virulence (Carling et al., 1989). Both soil-borne and tuber-borne inocula are important in the development of the disease, and the soil-borne nature of the fungus makes the management of the disease challenging and expensive to control (Scholte, 1989). The current disease management integrates cultural practices, the use of agrochemicals and host resistance. Unfortunately, cultural management practices alone are currently inadequate to control this disease, and the effectiveness of agrochemicals against *R. solani* can vary, generating an inconsistent control that may be very limited (Wicks and Morgan, 1995; Virgen-Calleros et al., 2000). Thus, the key to controlling this disease is the identification and incorporation of genetic resistance into cultivars that have acceptable horticultural characteristics to provide more effective disease management (Gudmestad et al., 2007). In the present study, a biometrical approach was used to study the interactions among Egyptian potato cultivars and different isolates of *R. solani* collected from five potato-growing governorates in Egypt.

MATERIALS AND METHODS

Isolates collection

In this study, twenty isolates of *R. solani* were obtained from diseased potato samples collected from different governorates (Beheira, Daqahiliya, Gharbiya, Minufiya and Sharqiya) in Egypt (Table 1 and Figure 1).

Characterization of isolates

Characterization of these isolates was performed during the course of the Ph.D. project of the first author according to Papavizas and Davey (1959). Identification of the selected isolates was confirmed by the Fungal Taxonomy Department, Plant Pathology Research Institute, Agricultural Research Centre, Giza, Egypt. The collected *R. solani* isolates were maintained on potato-dextrose agar (PDA) slants in a refrigerator (5°C).

Anastomosis grouping

Isolates of *Rhizoctonia* were assigned to anastomosis group by pairing the isolates with tester strains and observing the hyphae for fusion. Each Isolate was paired with tester isolate of each AG on 2% water agar-coated slides with two replicates in Petri dishes (Windels and Nabben, 1989). Mycelial transfers from the growing margins of young colony on PDA were plated 2 - 3 cm apart in a slide in a 9 cm Petri dish and incubated at 24°C in the dark until advancing hyphae made contact and slightly overlapped.

Pathogenicity test

The growth substrate for the *R. solani* isolates was prepared in 500 ml glass bottles; each bottle contained 100 g sorghum grains, 50 g sand and 90 ml tap water. The contents of each bottle were autoclaved for 30 min. The isolate inoculum at 20°C, obtained from a one week old culture on PDA, was aseptically transferred into the bottle and allowed to colonize the sorghum for three weeks.

The potting mixture (clay, sand and farmyard manure, 1:1:1) was sterilized using 37% commercial formalin. One part of the formalin was diluted with nine parts of water. The potting mixture was placed over a cemented path in layers, and the mixture was then moistened with the formalin solution and covered with a polyethylene sheet for 48 h. The mixture was exposed to air for 4-5 days until the formalin vaporized or volatilized.

Clay put in the pots (50 cm in diameter), filled with sterilized potting mixture, were inoculated with each isolate at a rate of 20 g/kg of soil mixed to the depth of 5 cm and watered for 4 days prior to planting. Three whole sprouted tubers (35-50 mm in diameter) with 3-4 eyes / pot were planted for each of the tested cultivars: Draga, Kara, Diamond, Hermes, Nicola, Spunta, Mondial, Monalisa and Lady Rosetta (taken from the Brown rot of Potato Project., ARC, Giza, Egypt). The plants were watered weekly as required.

In the control treatments, no fungal inoculum added to autoclaved sorghum grains were thoroughly mixed with soil at a rate of 20 g/kg soil. The pots were randomly distributed in a

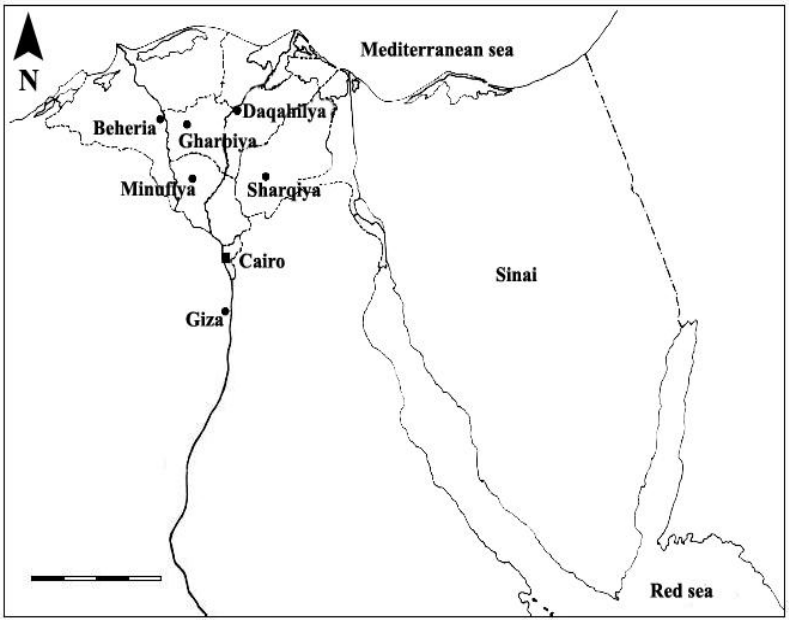

Figure 1. Egyptian governorates constituting the source of isolates used in this study.

greenhouse at 20°C, and three pots were used as the replicates for each treatment. The incidence of black scurf and stem canker was recorded at 90 days after planting.

Parameters studied

For each plant, the black scurf disease index (BSDI) and stem canker index (SCI) were assessed and compared to the non-inoculated control (check). The incidence and severity of black scurf disease were expressed as the BSD, which was calculated using the following formula:

BSDI (%)=[0(n1) + 1(n2) + 2(n3) + 3(n4) + 4(n5) + 5(n6)] / [N (Total number of tubers)] X100

Where, n1 is the number of tubers with a rating of 0; n2 is the number of tubers with a rating of 1; n3 is the number of tubers with a rating of 2; n4 is the number of tubers with a rating of 3; n5 is the number of tubers with a rating of 4 and n6 is the number of tubers with a rating of 5.

The severity of black scurf was assessed using a visual disease rating scale of 0-5 based on the percent of the tuber surface showing disease symptoms, as described by Ahmad et al. (1995): 0 = no symptoms on the potato tubers; 1 = less than 1% of the tuber area affected; 2 = 1-10% of the tuber area affected; 3 =11-20 % of the tuber area affected; 4 =21-51 % of the tuber area affected and 5= 51 % or more of the tuber area affected.

The stem canker incidence and severity were expressed collectively as the stem canker index (SCI). The severity was assessed on a 0-5 visual disease rating scale, as described by Frank et al. (1976) using the following formula:

SCI(%)=[Number of stems in each rating × rating] / [Total number of stems] X100

Where, 0 is no lesion; 1 is a single lesion of less than 25 mm; 2 is a single lesion of 25-50 mm (or a composite of small lesions totaling less than 50 mm); 3 is a single lesion >50 mm (or a composite of small lesions totaling > 50 mm but not girdling the stem; 4 is the lesion(s) less than 25 mm that are girdling the stem; and 5 is the lesion(s) more than 25 mm that are girdling the stem.

Statistical analysis:

A randomized complete block design with three replicates was used in this study. Duncan's multiple range test was used to compare the treatment means. The percentage data were subjected to the appropriate transformation before performing the ANOVA to produce approximately constant variances. The results were expressed as a phenogram (Joseph et al., 1992). Cluster analyses based on two pathological parameters, that is black scurf and stem cankers, were performed using a computer program.

RESULTS

Twenty isolates of *R. solani* AG-3 derived from five governorates in Egypt were tested for their levels of aggressiveness on two-month-old greenhouse-grown plants of nine Egyptian potato cultivars. The ANOVA showed very highly significant effects of the isolate upon the cultivar for the black scurf incidence. The ANOVA also showed highly significant effects of the cultivar, isolate and/or interaction of isolate × cultivar with the stem canker incidence (Table 2). The isolate was the most important factor in determining the variation in the black scurf and stem cankers (Table 3), whereas the cultivar and isolate × cultivar were marginal factors in determining the variation in the black scurf and stem

Table 2. analysis of variance of interaction between potato cultivars and isolates of *R. solani* AG-3 under greenhouse conditions.

Parameters and Source of variation [a]	D.F	M.S	F. value	P>F
Black scurf				
Replication	2	703.077	21.570	0.0000
Isolates (I)	20	5172.935	158.700	0.0000
Cultivar (C)	8	357.486	10.967	0.0000
I × C	160	21.015	0.6447	
Error	376	32.596		
Stem canker				
Replication	2	0.084	0.0059	
Isolates (I)	20	5896.393	413.885	0.0000
Cultivar (C)	8	377.110	23.662	0.0000
I × C	160	22.544	1.582	0.0000
Error	376	14.246		

[a] Replication is random, while each value of cultivar and isolate is fixed.

Table 3. Relative contribution of potato cultivars, *R. solani* AG-3 and their interaction to variation in black scurf and stem canker.

Source of variation	Relative contribution [a] to variation in	
	Black scurf	Stem canker
Isolates (I)	93.13	94.92
Cultivar (C)	2.57	2.17
I × C	3.03	2.90

[a] Calculated as percentage of sun squares of the explained (model) variation.

cankers. It was possible to assess the relative contribution of each of cultivar, isolate and cultivar × isolate interaction in the explained (model) variation (Table 3). The aggressiveness of the isolate accounted for 93.13 and 94.92% of the explained variation in the black scurf and stem cankers, respectively. In contrast, the horizontal resistance of the cultivars accounted for only 2.57 and 2.17% of the explained variation in the black scurf and stem cankers, with the vertical resistance of the cultivars or virulence of the isolates accounting for 3.03 and 2.90% of the explained variation in the black scurf and stem cankers. There was no significant effect of the isolate × cultivar interaction on black scurf (Table 4). Therefore, a least significant difference (LSD) analysis was used to compare the general mean of the isolates for all of the cultivars. These comparisons demonstrated the differences in the black scurf incidence between the isolates and the control, though each isolate or group of isolates had a single effect upon all of the cultivars. Thus, isolates 16, 17, 18, 19 and 20 were highly virulent, and isolates 2 to 12 were virulent. Isolates 1, 13 and 14 were moderately virulent, and isolate 15 had a weak virulence. Due to the highly significant effect of the isolate × cultivar interaction on the stem cankers, the least significant difference (LSD) was calculated to compare the isolate means for each cultivar (Table 5). These comparisons showed that the differences in the stem canker incidence between the isolates and the control were not the same for each cultivar, that is, the cultivars responded differently to the isolates. Isolates 16, 20, 19, 16, 17 and 20 were highly virulent to cultivars Draga, Kara, Diamond, Hermez, Nicola and Spounta, respectively. It was found that the magnitude of the differences between the isolates differed from one cultivar to another. For example, the difference between isolates 5 and 6 was highly significant for Kara, whereas it was insignificant for Draga. Similarly, the difference between isolates 9 and 11 was highly significant within cultivar Mondial, though it was insignificant for Nicola.

To represent the relationship between the isolates (black scurf), a cluster analysis was performed between four groups of similar isolates in a virulence pattern (isolates 18, 20, 19, 17, 16, 10, 9, 11, isolates 8 and 2, isolates 6, 12, 5, and 7, isolates 14 and 13; and isolates 1 and 4) were identified by cluster analysis (Figure 2). Based on the analysis of the virulence of the isolates,

Table 4. Effect of the interaction between potato cultivars and *R. solani* AG-3 isolates on black scurf of potato tuber under greenhouse condition.

Isolates	Cultivars																			
	Draga		Kara		Diamond		Hermes		Nicola		Spunta		Mondial		Monalisa		Lady Rosetta		Mean	
	%[a]	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T
1	36.70	37.29	40.00	39.23	43.30	40.98	33.30	35.10	40.00	39.23	43.30	40.98	33.70	37.29	40.00	39.23	40.00	39.23	38.92	38.73
2	33.30	35.10	43.30	40.98	40.00	39.23	43.30	40.98	43.30	40.98	40.00	39.23	40.00	39.23	40.00	39.23	50.00	45.00	41.47	39.99
3	36.70	37.29	43.30	40.98	50.00	45.00	43.30	40.98	43.30	40.98	36.70	37.29	36.70	37.29	50.00	45.00	36.70	37.29	41.86	40.23
4	46.70	43.28	43.30	40.98	50.00	45.00	43.30	40.98	43.30	40.98	40.00	39.23	40.00	39.23	43.30	40.98	40.00	39.23	43.32	41.10
5	46.70	43.28	46.70	43.28	46.70	43.28	46.70	43.28	40.00	39.23	53.30	46.72	56.70	49.02	53.30	46.72	56.70	49.02	49.64	44.87
6	46.70	43.28	43.30	40.98	53.30	46.72	46.70	43.28	43.30	40.98	53.30	46.72	50.00	45.00	53.30	46.72	56.70	49.02	49.62	44.74
7	43.30	40.98	43.30	40.98	53.30	46.72	40.00	39.23	46.70	43.28	50.00	45.00	56.70	49.02	56.70	49.02	50.00	45.00	48.89	44.36
8	46.70	43.28	40.00	39.23	46.70	43.28	46.70	43.28	53.30	46.72	43.30	40.98	53.30	46.72	53.30	46.72	53.30	46.72	48.51	44.10
9	46.70	43.28	50.00	45.00	46.70	43.28	40.00	39.23	50.00	45.00	40.00	39.23	53.30	46.72	50.00	45.00	60.00	50.77	48.52	44.17
10	46.70	43.28	46.70	43.28	40.00	39.23	46.70	43.28	46.70	43.28	43.30	40.98	53.30	46.72	56.70	49.10	56.70	49.10	48.53	44.25
11	46.70	43.28	43.30	40.98	46.70	43.28	46.70	43.28	46.70	43.28	40.00	39.23	46.70	43.28	53.30	46.72	56.70	49.10	47.42	43.60
12	43.30	40.98	40.00	39.23	43.30	40.98	46.70	43.28	40.00	39.23	46.70	43.28	53.30	46.72	56.70	49.10	60.00	50.77	47.78	43.73
13	36.70	37.29	33.30	35.10	33.30	35.10	40.00	39.23	33.30	35.10	33.30	35.10	43.30	40.98	50.00	45.00	50.00	45.00	39.24	38.66
14	36.70	37.29	33.30	35.10	33.30	35.10	30.00	33.21	36.70	37.29	36.70	37.29	30.00	33.21	43.30	40.98	50.00	45.00	36.67	37.16
15	26.70	31.31	26.70	31.30	23.30	28.66	26.70	31.31	23.30	28.66	26.70	31.31	23.30	28.66	40.00	39.23	40.00	39.23	28.52	32.19
16	73.30	58.69	76.70	61.34	76.70	61.34	73.30	58.69	70.00	56.79	76.70	61.34	73.30	58.69	86.70	68.86	90.00	71.56	77.41	61.92
17	76.70	61.34	73.30	58.69	73.30	58.69	76.70	61.34	73.30	58.69	73.30	58.69	80.00	63.44	80.00	63.44	80.00	63.44	76.28	60.86
18	76.70	61.37	70.00	56.79	70.00	56.79	76.70	61.34	76.70	61.34	76.70	61.34	76.70	61.34	86.70	68.87	86.70	68.87	77.43	62.01
19	73.30	58.69	70.00	56.79	80.00	63.44	73.30	58.69	76.70	61.34	76.70	61.34	73.30	58.69	86.70	68.87	90.00	71.56	77.78	62.16
20	73.30	58.69	73.30	58.69	76.70	61.34	76.70	61.34	76.70	56.79	76.70	61.34	80.00	63.44	83.30	65.65	80.00	63.44	77.41	61.19
Control[b]	00.00	00.00	00.00	00.00	0.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Mean	47.31	42.82	46.66	42.33	48.89	43.69	47.47	42.92	47.78	42.82	47.94	43.17	50.17	44.51	55.40	47.83	56.36	49.49		

T= Transformed value; LSD (transformed data for isolates x isolates interaction =9.17($p<0.05$ or 12.07 $p<0.01$). LSD (transformed data for isolates=3.06 ($p<0.05$ or 4.02 $p<0.01$). LSD (transformed data for cultivars=2.00 ($p<0.05$ or 2.63 $p<0.01$). [a] Percentage data were transformed into arc sine angles before carrying out the analysis of variance. [b] non-infested soil.

four isolates (100%) of high virulence were grouped into the same subcluster (isolates 18, 20, 19, and 17), two isolates (100%) of low virulence were grouped into the same subcluster (isolates 14 and 13) and the very low virulence isolate (15) was grouped into a separate cluster. These results demonstrate that the relationship between the isolates depends on their virulence. Based on the geographical origin, the isolates appear to be randomly distributed among the groups, and no associations were observed between the virulence of the isolates and their geographical origin. To represent the relationship between the isolates (stem canker), a cluster analysis was performed. The results indicated four groups of similar isolates in a virulence pattern (isolates 20, 18, 17, 16, 19, 6, 9, and 7, isolates 10, 11, and 8, isolates 5, 4, and 2, and isolates 14, 15, and 1) (Figure 3). Based on the virulence of the isolates, there were four isolates (100%) of high virulence in the same subcluster (isolates 20, 18, 17, 16, and 19) and two isolates (100%) of low virulence in the same subcluster (isolates 14 and 15). This result clearly demonstrates that the relationship

Table 5. Effect of the interaction between potato cultivars and *R. solani* AG-3 isolates on stem canker of potato stem under greenhouse condition.

Isolates	Cultivars																			
	Draga		Kara		Diamond		Hermes		Nicola		Spunta		Mondial		Monalisa		Lady Rosetta		Mean	
	%[a]	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T
1	33.30	35.10	43.30	40.98	46.70	43.28	43.30	40.98	43.30	40.98	36.7	37.29	30.00	33.21	43.30	40.98	36.70	37.29	39.62	39.00
2	40.00	39.23	43.30	40.98	43.30	40.98	30.00	33.21	36.70	37.29	40.00	39.23	30.00	33.21	40.00	39.23	40.00	39.23	38.14	38.14
3	40.00	39.23	40.00	39.23	33.30	35.10	40.00	39.23	46.70	43.28	43.30	40.98	36.70	37.29	40.00	39.23	40.00	39.23	40.00	39.23
4	36.70	37.29	40.00	39.23	40.00	39.23	43.30	40.28	40.00	39.23	33.30	35.10	33.30	35.10	33.30	35.10	46.70	43.28	38.51	38.35
5	46.70	43.28	50.00	45.00	53.30	46.72	40.00	39.23	46.70	43.28	46.70	43.28	56.70	49.02	46.70	43.28	50.00	45.00	48.35	44.05
6	46.70	43.28	40.00	39.23	46.70	43.28	46.70	43.28	46.70	43.28	53.30	46.72	50.00	45.00	56.70	49.02	53.33	46.72	48.90	44.36
7	43.30	40.98	40.00	39.23	46.70	43.28	46.70	43.28	46.70	43.28	46.70	43.28	60.00	50.77	56.70	49.02	56.70	49.02	49.28	44.59
8	46.70	43.28	43.30	40.98	53.30	46.72	43.30	40.98	43.30	40.98	53.30	46.72	50.00	45.00	56.70	49.02	56.70	49.02	49.62	44.78
9	50.00	45.00	50.00	45.00	43.30	40.98	40.00	39.23	50.00	45.00	40.00	39.23	50.00	45.00	70.00	56.79	60.00	50.77	50.37	45.21
10	43.30	40.98	46.70	43.28	40.00	39.23	43.30	40.98	46.70	43.28	43.30	40.98	56.70	49.10	50.00	45.00	56.70	49.10	47.41	43.52
11	46.70	43.28	46.70	46.28	46.70	46.28	46.70	43.28	50.00	45.00	40.00	39.23	43.30	40.98	50.00	45.00	56.70	49.10	47.42	43.69
12	46.70	43.28	43.30	40.98	43.30	40.98	46.70	43.28	40.00	39.23	46.70	43.28	53.30	46.72	60.00	50.77	60.00	50.77	48.89	44.36
13	46.70	43.28	46.70	43.28	40.00	39.23	40.00	39.23	46.70	43.28	43.30	40.98	43.30	40.98	56.70	49.10	60.00	40.77	47.07	43.32
14	16.70	24.35	13.30	21.13	16.70	24.35	13.30	21.13	13.30	21.13	16.70	24.35	10.00	18.44	26.70	31.31	30.00	33.21	17.41	24.66
15	16.70	24.35	13.30	21.13	16.70	24.35	16.70	24.35	13.30	21.13	13.30	21.13	16.70	24.35	26.70	31.31	30.00	33.21	18.15	25.22
16	76.70	61.34	73.30	58.69	76.70	61.34	80.00	63.44	70.00	56.79	70.00	56.79	73.30	63.44	90.00	71.56	90.00	71.56	78.52	62.39
17	76.70	61.34	73.30	58.69	73.30	58.69	73.30	58.69	76.70	61.34	76.70	61.34	73.30	58.69	83.30	65.65	86.70	68.87	77.03	61.36
18	73.30	58.69	70.00	56.79	76.70	61.34	76.70	61.34	73.30	58.69	76.70	61.34	76.70	61.34	86.70	68.87	90.00	71.56	77.79	61.88
19	70.00	56.79	73.30	58.69	80.00	63.44	70.00	56.79	73.30	58.69	73.30	58.69	80.00	63.44	76.70	61.34	76.70	61.34	74.81	59.92
20	73.30	58.69	73.30	58.69	73.30	58.69	73.30	58.69	73.30	58.69	80.00	63.44	76.70	61.34	80.00	63.44	86.70	68.87	76.66	61.21
Control [b]	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00	00.00
Mean	46.20	42.05	45.86	41.79	47.14	42.74	45.40	41.47	46.51	42.09	46.35	42.07	47.94	42.97	53.82	46.91	55.41	47.52		

T= Transformed value; LSD (transformed data for isolates x isolates interaction =6.06($p<0.05$ or 7.98 $p<0.01$). LSD (transformed data for isolates=2.02 ($p<0.05$ or 2.66 $p<0.01$). LSD (transformed data for cultivars=1.32 ($p<0.05$ or 1.74 $p<0.01$). [a] Percentage data were transformed into arc sine angles before carrying out the analysis of variance. [b] non-infested soil.

between the isolates depends on their virulence. Based on their geographical origin, the isolates appear to be randomly distributed among the groups. No associations were observed between the virulence of the isolates and their geographical origin.

A cluster analysis was performed to represent the relationship between the cultivars (black scurf) (Figure 4). The tested potato cultivars were placed into three clusters. The first cluster included cultivars Draga, Hermes, Nicola and Kara, the second cluster included Diamond, Spunta and Mondial, and the third cluster included Monalisa and Lady Rosetta. The infection percentages were 42.33-56.36%. In general, the cultivars in each cluster showed high levels of similarity in their reactions to the *R. solani* isolates. A cluster analysis was similarly performed to identify the relationship between cultivars (Figure 5), with the tested potato cultivars being placed into three clusters. The first cluster included cultivars Draga, Hermes, Kara, Nicola, and Diamond, the second cluster included Spunta, and the third cluster included Monalisa Lady Rosetta and Mondial.

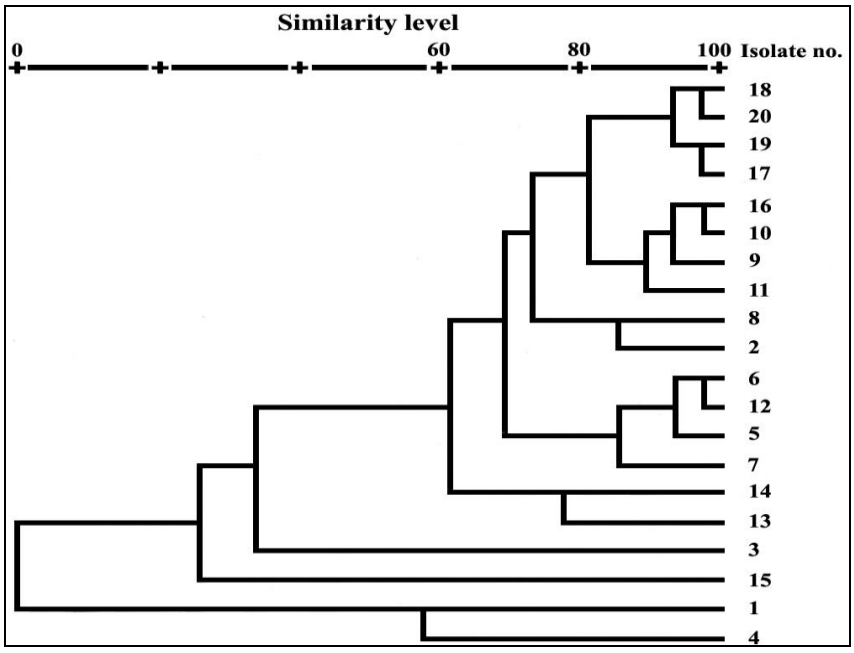

Figure 2. Phenogram based on average linkage cluster analysis of virulence of twenty isolates of *Rhizoctonia solani* (black scurf) on nine potato cultivars (Draga, Kara, Diamond, Hermes, Nicola, Spunta, Mondial, Monalisa and Lady Rosetta).

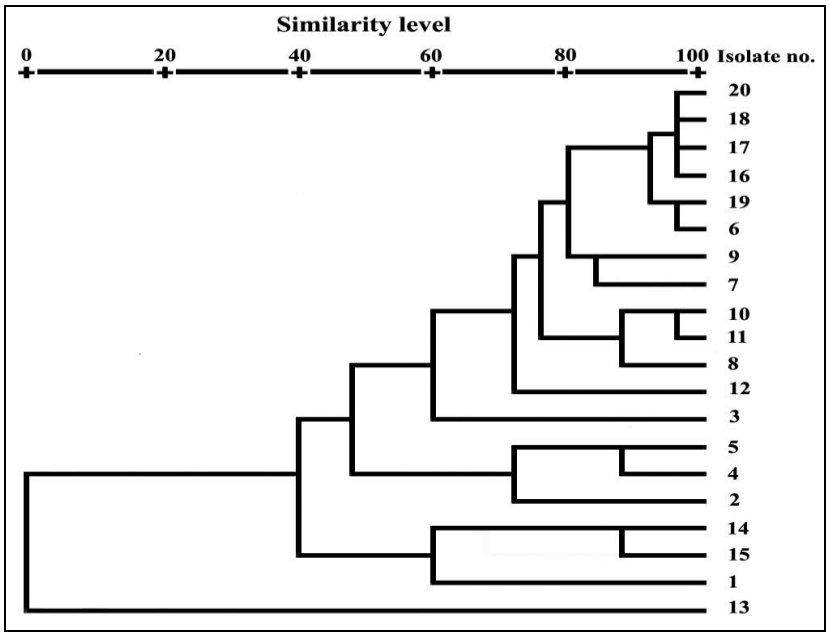

Figure 3. Phenogram based on average linkage cluster analysis of virulence of twenty isolates of *Rhizoctonia solani* (stem canker) on nine potato cultivars (Draga, Kara, Diamond, Hermes, Nicola, Spunta, Mondial, Monalisa and Lady Rosetta).

The infection percentages were 41.47-55.41%. In general, the cultivars within each cluster showed high levels of similarity in their reactions to *R. solani* AG-3 isolates.

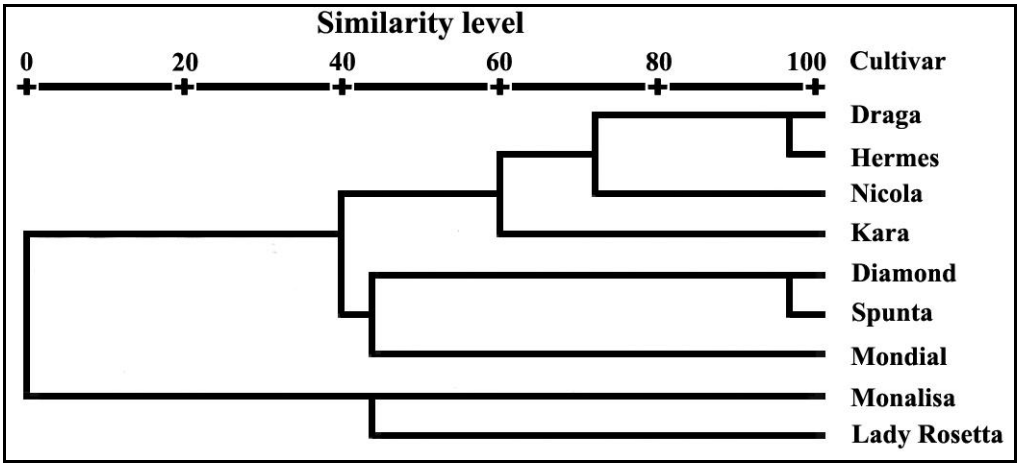

Figure 4. Phenogram based on average linkage cluster analysis of reaction of nine potato cultivar to twenty isolates of *R. solani* (black scurf).

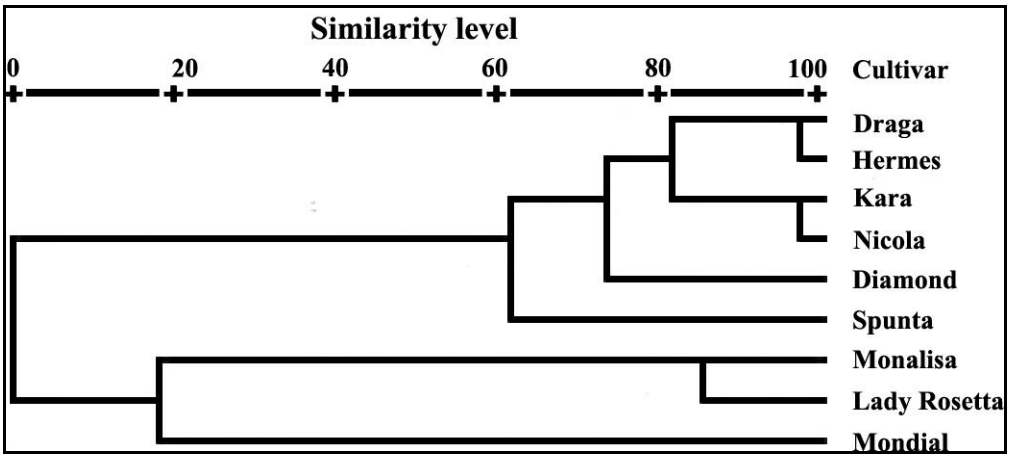

Figure 5. Phenogram based on average linkage cluster analysis of reaction of nine potato cultivar to twenty isolates of *R. solani* (stem canker).

DISCUSSION

The management of soil-borne diseases is a continuous challenge to growers. The structural, physical, and biological complexity of the soil environment in which pathogens interact with plant roots inherently limits the options available for disease control. For example, *Pythium* spp., *Fusarium* spp., *Sclerotinia sclerotiorum* and *Rhizoctonia solani* annually cause significant losses in the quantity and quality of many crop species (Banville, 1989; Martin and English, 1997). Moreover, population biology and genetics in *Rhizoctonia* has been confounded, as *R. solani* is actually a large species complex composed of many genetically distinct groups that have very diverse life histories (Ogoshi, 1987; Sneh et al., 1996). The current classification within the *R. solani* complex is largely based on the grouping of isolates into anastomosis groups (AGs). At least 12 AGs have been described within the R. solani complex. The considerations of mating compatibility systems (homothallic versus heterothallic) and how they may influence the population structures are also presented. The major reason for the limited success in disease management is our limited understanding of the genetic structure of pathogen populations. Indeed, the cultivation of resistant potato varieties is the most economical and environmentally safe method of black scurf and stem canker disease management (Yanar et al., 2005; Naz et al., 2008).

Specificity in host-pathogen relationships is often indicated by significant isolate × variety interactions using the analysis of variance (ANOVA) of an experiment in which a number of pathogen isolates are tested in all possible combinations on a set of host genotypes.

Conversely, non-specificity is identified by the lack of such an interaction (Vanderplank, 1984). It has been suggested that the presence of a significant cultivar × isolate interaction using analysis of variance is evidence for a differential (vertical) host-pathogen relationship (Vanderplank, 1984).

In contrast, the lack of a significant interaction is indicative of an association that is nondifferential (horizontal), implying that the differences in cultivar susceptibility are consistent in relation to one another, regardless of the pathogen isolates. In any host-pathogen relationship, the two types of resistance may act together in determining the outcome of the association between the host and the pathogen (Vanderplank, 1984).

In the present study, the ANOVA showed that the main effects of the cultivar, isolate or the interaction of cultivar × isolate were a highly significant source of variation for all of the tested parameters. Statistically, the significant interactions between the potato cultivars and isolates in this study suggest that a physiological specialization exists among the *R. solani* AG-3 isolates for pathogenicity in potato. Thus, potato cultivars should be tested by using as many isolates of *R. solani* AG-3 as possible, as this will improve the chances of identifying potato cultivars that have resistance to several isolates of *R. solani* AG-3. In addition, promising cultivars must be tested under different agroecological systems representing various potato-producing regions to ensure their susceptibility to many populations of *R. solani* AG-3.

The application of cluster analysis (isolates) has been previously suggested for assessing the similarity and/or dissimilarity in genes for host-parasite relationships (Lebeda and Jendrulek, 1987). This method was used to express the precise genetic similarity among 48 physiological races of *Bremia lactucae* (Lebeda and Jendrulek, 1987), 20 isolates of *Macrophomina phaseolina* (Omar, 2005), 52 isolates of *R. solani* (El-Samawaty et al., 2008), 20 isolates of *R. solani* AG-3 (Mahmoud, 2010) and 23 *R. solani* AG-4 (Mirmajlessi et al., 2012). In the present study, cluster analysis proved to be useful in determining the similarity of *R. solani* AG-3 isolates based on their virulence patterns in nine potato cultivars .

The gene pool is considered a valuable initial resource for plant breeding because it contains co-adapted gene complexes providing tolerance or adaptation to diseases and specific ecological conditions for many plant species (Harlan, 1975; Williams et al., 1991). Cluster analysis (cultivars) can be useful for plant breeders, and genetic resources must be characterized by morphological and agronomic traits (Martins et al., 2006). For this reason, cluster analysis was used based on standardized data to classify the potato genotypes under investigation (Ahmadizadeh and Felenji, 2011). The cluster analysis contained different genotypes based on their similarity and, thus, provided a hierarchical classification (Sozen and Bozoglu, 2007). Cluster analysis was used to express the precise genetic similarity among 8 potato cultivars in their variation for a resistance to early blight pathogens (El-Komy et al., 2012), 6 potato cultivars (susceptible, moderately resistant, and highly resistant) for their resistance to late blight caused by *Phytophthora infestans* (El-Komy, 2007) and the susceptibility of 43 commercially available potato cultivars to the dry rot caused by *Fusarium sulphureum*, *F. solani* and *F. oxysporum* (Esfahani, 2005).

Control strategies to prevent Rhizoctonia disease in potato crops have two strategies. The first strategy is short term, controlling an immediate disease problem (agrochemical). However, increased environmental regulations and the development of fungicide-resistant fungal isolates are restricting the availability of agrochemicals. The second strategy is long-term management (crop rotation, transgenic crops or plant breeding), which is the most efficient, cost effective and environmentally friendly (Human et al., 2000; Bains et al., 2002).

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