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Determination of the Anastomosis Grouping and Virulence of *Rhizoctonia* spp. Associated with Potato Tubers Grown in Lincoln, New Zealand

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Abstract: A total of 58 isolates of *Rhizoctonia* spp. (46 *R. solani* and 12 binucleate *Rhizoctonia*) were recovered from potato tubers showing black scurf disease symptom during the 2004 growing season in Lincoln, New Zealand. The isolates were assigned to 5 Anastomosis Groups (AG) of *R. solani* AG-3 (54.34%), AG-5 (28.26%), AG-8 (8.69%), AG-4 (6.52%) and AG-2-2 IIIB (2.17%) and six anastomosis groups of binucleate *Rhizoctonia*, AG-K (25%), AG-Bi (25%), AG-Ba (8.33%), AG-C (8.33%), AG-D (8.33%) and AG-E (8.33%). Two isolates of BNR did not anastomose with any of the tester strains and remain unidentified. In pathogenicity tests that were carried out on radish, carrot, lettuce, onion, tomato and hemp, it was found that all the isolates of both *R. solani* and binucleate *Rhizoctonia* to be virulent at varying degrees to these 6 plants species from different families. In these tests, isolates of AG-3 and AG-8 from *R. solani* population caused the highest and lowest disease severity on all 6 plant species, respectively. In population of binucleate *Rhizoctonia*, on the other hand, the highest and lowest disease severities were caused by the isolates of AG-D and AG-Ba on all test plants, respectively. When the results of the pathogenicity tests were examined in terms of the susceptibility levels of the plants, the most resistant plant was tomato against different AGs of *R. solani* and BNR. On the other hand, radish was the most susceptible plant species tested in this study against both *R. solani* and BNR isolates.

Key words: *Rhizoctonia*, black scurf, anastomosis groups, pathogenicity, Lincoln, New Zealand

INTRODUCTION

Rhizoctoniasis of potato (*Solanum tuberosum* L.) caused by the soil borne plant pathogen *Rhizoctonia solani* kühn is one of the most common potato disease world-wide. The pathogen attacks under ground parts of potato plants including roots, stolons and tubers. Typically, disease causes *Rhizoctonia* canker, named for the presence of characteristic lesions or cankers on infected sprouts, stems and stolons and black scurf which refers to the presence of sclerotia on tubers. Either or both disease may occur in an individual potato plant (Banville *et al*., 1996).

Population of *Rhizoctonia* is divided into 3 major groups- mono, bi and/or multinucleate *Rhizoctonia* based on the nuclear number in each cell of their young hyphae (Stalpers and Anderson, 1996).

R. solani that belongs to the multinucleate *Rhizoctonia*, is a heterogeneous species composed of a number of independent populations (Sneh *et al*., 1991). *R. solani* [teleomorph: *Thanatephorus cucumeris* (Frank) Donk.] and binucleate *Rhizoctonia* (teleomorph: *Ceratobasidium* Rogers) are divided into Anastomosis Groups (AG) based on hyphal anastomosis and cultural characteristics (Sneh *et al*., 1991).

The classification of *R. solani* and other *Rhizoctonia* species into AGs is widely accepted as the first way of subgrouping these heterogeneous species into more homogeneous subspecies groups (Carling, 1996). Some AG have been further divided into subsets according to diverse characteristics including pectic zymogram and DNA based molecular techniques (Balali *et al*., 2007). In *R. solani* so far, 13 anastomosis groups designated AG-1 through AG-13 and 21 subgroups designated AGs 1-IA, 1-IB, 1-IC, 1-ID, 2-1, 2-2-IIIB, 2-2-IV, 2-2-LD, 2-3, 2-4, 2-BI, 3-IIA, 3-IIB, 3-IIC, 3-TB, 4-HG-I, 4-HG-II, 6-GV, 6-HG-I, 9-TX, 9-TP have been described (Ogoshi, 1987; Naito and Kanematsu, 1994; Carling, 1996; Hyakumachi *et al*., 1998; Carling *et al*., 1999, 2002a, b; Kuniniga *et al*., 2000; Priyatmojo *et al*., 2001). Binucleate *Rhizoctonia* isolates are also grouped into different anastomosis groups designated AG-A to AG-S (Sneh *et al*., 1991).

Members of AG-3 are the principal cause of black scurf disease of potato (Bains and Bisht, 1995; Balali *et al*., 1995; Abdul Rauf *et al*., 2000; Truter and Wehner, 2004; Yanar *et al*., 2005), but there are reports indicating that other AGs including AG1-IA (Abdul Rauf *et al*., 2000), AG-2-1 (Abdul Rauf *et al*., 2000; Chand and Logan, 1983; Petkowski *et al*., 2003), AG-2-2 (Petkowski *et al*., 2003), AG-4 (Abdul Rauf *et al*., 2000;

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Balali *et al*., 1995; Petkowski *et al*., 2003), AG-5 (Abe and Tsuboki, 1978; Balali *et al*., 1995; Petkowski *et al*., 2003; Truter and Wehner, 2004), AG-6 (Abdul Rauf *et al*., 2000), AG-7 (Abdul Rauf *et al*., 2000; Carling *et al*., 1998) also could be involved.

Although, it seems that *R. solani* and binucleate *Rhizoctonia* are distributed in different parts of New Zealand and attack to different host crops, there is no official information about their distribution, host range, population diversity and diseases that are caused by these important soil borne plant pathogens.

In the absence of adequate information on different aspects of *Rhizoctonia* species in general and black scurf disease of potato specifically, this study was conducted to isolate and characterize *Rhizoctonia* spp. associated with potato tubers in Lincoln, New Zealand. A synopsis of the results has been published (Farrokhi-Nejad *et al*., 2006).

MATERIALS AND METHODS

Isolation, purification and storage of *Rhizoctonia* spp.: Thirteen potato tubers were collected from experimental fields at the New Zealand Institute for Crop and Food Research at Lincoln, New Zealand. Potato tubers were washed under running tap water; surface sterilized in 1% sodium hypochlorite for 30 sec and blotted dry on sterilized towel tissues. From each tuber several sclerotia were plated onto the Potato Dextrose Agar (PDA) plates. Plates were incubated in the dark at 23°C for 4-5 days. Cultures which exhibited *Rhizoctonia* like growth characteristics were transferred to the plates containing 2% Water Agar (WA) and placed in the dark at 23°C. After 2-3 days, isolates were purified using the hyphal tip method (Singleton *et al*., 1992). Plates were incubated in the dark at 23°C for 4-5 days, then stored at 4°C in refrigerator either in test tube slants of PDA or on sterilized barley grains.

Nuclear staining: Single 9 mm diameter disks of 2 to 3-day-old cultures of *Rhizoctonia* spp. growing on PDA were transferred on clean glass slides which had been dipped in 99.5% ethanol and flamed before use. Inoculated glass slides were incubated in a moist chamber at 25°C in the dark. A drop of safranin O and 3% KOH was placed directly on the mycelium of 1 to 2-day-old cultures (Kronland and Stanghellini, 1988). The cells of each isolate were examined for nuclei at ×400 magnification using bright field microscope. The nuclear numbers of the strains were counted in at least 20 cells of the young hyphae per each isolate.

AG determination: AG identities of the isolates was determined by using the glass-slide technique according to the procedures described by Herr and Roberts (1980). Single 7 mm-diameter disks were cut from the perimeter of a 2 to 3 day-old colony of each isolate on PDA and placed on a glass slide covered with 2% WA. Tester strains of multinucleate *Rhizoctonia* including AG-1-IA, AG-1-IB, AG-1-IC, AG-2-IB, AG-2-2-B, AG-2-2-IIIB, AG-3, AG-4, AG-5, AG-6, AG-8, AG-9 and AG-11 and for binucleate *Rhizoctonia*, AG-A, AG-Ba, AG-Bi, AG-C, AG-D, AG-E, AG-G, AG-I, AG-K and AG-R were used.

Tester isolates were placed 3 to 4 cm away from each tested isolate. Slides were transferred to a moist chamber and incubated at 25°C for 24 to 48 h in the dark. Excess moisture was wiped off from the bottom of the slides. When the hyphae from the two disks were overlapping, they were stained with safranin O and 3% KOH and examined microscopically to determine anastomosis reaction (Carling, 1996).

Pathogenicity tests: In order to determine the virulence of *Rhizoctonia* spp. recovered in this study 6 plant species from different families including radish (*Raphanus sativus*), tomato (*Lycopersicon esculentum*), carrot (*Daucus carota*), onion (*Allium cepa*), hemp (*Cannabis sativa*) and lettuce (*Lactuca sativa*) were selected as host. Agar-plates assay was used in the pathogenicity tests. From stored cultures, isolates were transferred to the PDA plates and incubated at 25°C in the dark for 7 days. After that, discs were excised from 7 day old agar cultures, centrally inoculated on agar 2% plates and incubated for 2 days at room temperature. Ten replicate plates were inoculated per strain. Five seeds of each test plant (disinfected in 1% sodium hypochlorite for 10 min) were placed around the periphery of each colony. Subsequent incubation was at room temperature for 10 days, following which disease severity was recorded on a scale of 0 to 5 based on the relative size of necrotic area on the roots as follows : 0 = no disease, 1 = 1-10%, 2 = 11-30%, 3 = 31-50%, 4 = 51-80% and 5 = entire root infected. Isolates causing a mean disease severity between 0 and 1 were considered non-pathogenic (Robinson and Deacon, 2002).

Experimental design and statistical analysis: The experimental design was a completely randomized design with ten replications and the experiment was repeated twice. Data were subjected to analysis of variance (ANOVA) and treatment means were separated by Duncan's multiple range test ($\alpha = 0.05$).

RESULTS

In this study 13 potato tubers were used and totally 58 isolates of *Rhizoctonia* spp. were obtained. The

number of *Rhizoctonia* spp. isolates recovered from each tuber varied from 2-12. Nuclear staining of the isolates revealed that 12 (20.7%) were binucleate and 46 (79.3%) were multinucleate (Table 1). The number of nuclei per each cell varied from 4 to 11 in isolates of multinucleate *Rhizoctonia* (*R. solani*). All isolates of binucleate *Rhizoctonia* contained only two nuclei per each of their hyphal cell.

AG determination: Results of this study indicated that both binucleate and multinucleate populations were present on a single potato tuber. These results determined that of the *R. solani* isolates, 25 (54.34%) were AG-3, 13 (28.26%) AG-5, 4 (8.69%) AG-8, 3 (6.52%) AG-4 and 1 (2.17%) AG-2-2 IIIB (Table 1). From the binucleate population, 3 (25%) belonged to AG-Bi,3 (25%) to AG-K, 1 (8.33%) to AG-Ba, 1 (8.33%) to AG-C, 1 (8.33%) to AG-D, 1 (8.33%) to AG-E and 2 (16.66%) did not anastomose with any of the tester strains and remain unidentified (Table 1).

Pathogenicity tests: Results of the pathogenicity tests indicated that in general all *R. solani* and binucleate *Rhizoctonia* isolates were pathogenic to all seedlings of plant species tested (Table 2). However, there was a difference among the virulence of the isolates of both *R. solani* and binucleate *Rhizoctnia* (Table 2).Virulence differences existed not only among the *R. solani* and binucleate *Rhizoctonia* isolates, but also, in some cases it was found among the isolates from the same anastomosis groups of both populations. This indicated that all isolates caused different levels of disease on seedlings tested. Disease severity means caused by different isolates from both populations were significantly different from that of control plants (Table 2). The overall effects of different AGs of *R. solani* on all plants tested were significantly different from each other (Fig. 1). For example, the highest disease severity mean on all plants caused by AG-3 isolates and this followed by disease severity means caused by AG-5, AG-4 and AG-2-2IIIB isolates, respectively. The least disease severity means on the other hand, caused by AG-8 isolates (Fig. 1). Similarly, the overall effects of different AGs of BNR on all plants tested were significantly different from each other. For instance, the highest disease severity mean on all plants caused by AG-D isolate and this followed by disease severity means caused by AG-Bi, AG-K, AG-C and AG-E isolates, respectively. On the other hand, disease severity mean caused by AG-Ba isolate was the least (Fig. 1). When comparison was made between disease severity means caused by all AGs (MNR and BNR) on all plant seedlings, significant differences were observed. Disease severity means caused by AG-D was the highest and this followed by the disease severity means caused by AGs, 3, Bi, K, 5, C, 4, 2-2IIIB, E and 8, respectively. Disease severity means caused by AG-Ba was the least (Fig. 1).

Similarly, susceptibility levels of the plants differed significantly from each other. When the effect of all isolates of *R. solani* were examined together, radish was found to be the most susceptible plant species tested, while susceptibility level of tomato plants was the least. Susceptibility levels of hemp, onion, lettuce and carrot did fall in between of radish and tomato, respectively (Fig. 2). Also, when the effects of all BNR isolates on plants susceptibility were determined, significant differences were found in plants susceptibility. According to these results, radish was found to be the most susceptible plant, whereas, the susceptibility of tomato plants was the least. Susceptibility levels of hemp, lettuce, carrot, onion did fall in between, respectively (Fig. 2).

When the effects of all isolates of both *R. solani* and BNR were examined together, no difference was observed

Table 1: No. of isolates of *R. solani* and BNR isolated from different potato tubers grown in Lincoln, New Zealand and their assigned AGs

		AGs											
		R. solani					BNR						
Potato No.	No. of isolates	3	4	5	8	2-2IIIB	Ba	Bi	C	D	E	K	Unknown
1	5	3	-	1	-	-	-	-	-	1	-	-	-
2	5	2	-	1	-	-	1	-	-	-	-	-	1
3	5	4	-	1	-	-	-	-	-	-	-	-	-
4	5	2	-	2	1	-	-	-	-	-	-	-	-
5	4	2	1	1	-	-	-	-	-	-	-	-	-
6	12	1	1	2	2	1	-	2	1	-	1	1	-
7	4	-	-	2	-	-	-	-	-	-	-	2	-
8	3	3	-	-	-	-	-	-	-	-	-	-	-
9	5	1	-	3	-	-	-	1	-	-	-	-	-
10	3	2	1	-	-	-	-	-	-	-	-	-	-
11	3	3	-	-	-	-	-	-	-	-	-	-	-
12	2	1	-	-	-	-	-	-	-	-	-	-	1
13	2	1	-	-	1	-	-	-	-	-	-	-	-
Total	58	25	3	13	4	1	1	3	1	1	1	3	2

Table 2: Disease severity means caused by different isolates of *Rhizoctonia* sp. obtained from potato tubers grown in Lincoln, New Zealand on roots of each and all plant seedlings

		Disease severity means[1]						
		Host plant						
AGs	Isolate No.	Carrot	Hemp	Lettuce	Onion	Radish	Tomato	All plants[2]
AGD	1	2.700	3.700	3.500	1.900	3.900	1.600	2.883 a
AG3	10	1.687	3.445	2.400	2.740	3.580	1.500	2.558 b
AG3	5	1.641	3.475	2.365	2.700	3.580	1.480	2.540 b
AG3	7	1.641	3.425	2.365	2.740	3.520	1.525	2.536 b
AG3	20	1.687	3.445	2.390	2.680	3.520	1.480	2.533 b
AG3	15	1.641	3.445	2.380	2.680	3.560	1.480	2.531 b
AG3	14	1.687	3.445	2.300	2.760	3.480	1.500	2.528 b
AG3	25	1.641	3.410	2.320	2.820	3.500	1.465	2.526 b
AG3	12	1.687	3.425	2.320	2.700	3.520	1.500	2.525 b
AG3	22	1.687	3.425	2.340	2.680	3.540	1.480	2.525 b
AG3	3	1.641	3.545	2.340	2.720	3.520	1.480	2.541 b
AG3	21	1.641	3.380	2.340	2.720	3.540	1.500	2.520 b
AG3	16	1.687	3.360	2.340	2.680	3.520	1.525	2.518 b
AG3	18	1.687	3.410	2.310	2.700	3.520	1.480	2.517 b
AG3	8	1.687	3.410	2.320	2.860	3.520	1.480	2.516 b
AG3	17	1.641	3.425	2.365	2.660	3.520	1.480	2.515 b
AG3	11	1.641	3.425	2.340	2.680	3.500	1.500	2.514 b
AG3	24	1.687	3.480	2.340	2.580	3.480	1.500	2.511 b
AG3	1	1.641	3.425	2.340	2.680	3.500	1.480	2.511 b
AG3	6	1.687	3.410	2.365	2.620	3.480	1.500	2.510 b
AG3	23	1.641	3.380	2.320	2.720	3.520	1.480	2.510 b
AG3	4	1.687	3.410	2.320	2.680	3.480	1.480	2.509 b
AG3	2	1.687	3.380	3.320	2.660	3.520	1.480	2.507 b
AG3	19	1.641	3.445	2.320	2.680	3.480	1.480	2.507 b
AG3	13	1.641	3.380	2.340	2.640	3.520	1.500	2.503 b
AGBi	2	2.060	3.780	2.640	1.440	3.600	1.500	2.503 b
AG3	9	1.641	3.410	2.320	2.660	3.480	1.500	2.501 b
AGBi	1	2.080	3.165	2.700	1.440	3.500	1.500	2.497 b
AGBi	3	2.060	3.000	2.660	1.520	3.500	1.500	2.373 c
AGK	3	2.640	2.780	2.340	2.016	2.830	1.340	2.324 cd
AGK	2	2.700	2.740	2.320	1.980	2.830	1.340	2.318 de
AGK	1	2.660	2.760	2.340	1.920	2.830	1.320	2.305 def
AG5	1	1.500	3.260	2.000	2.540	3.100	1.260	2.276 defg
AG5	3	1.520	3.120	2.000	2.580	3.140	1.240	2.266 efg
AG5	11	1.500	3.200	2.000	2.620	3.035	1.240	2.265 efg
AG5	10	1.520	3.120	2.000	2.550	3.140	1.260	2.265 efg
AG5	7	1.500	3.200	2.000	2.540	3.100	1.240	2.263 efg
AG5	13	1.520	3.120	2.000	2.540	3.120	1.260	2.260 efg
AG5	5	1.520	3.160	2.000	2.520	3.080	1.240	2.253 fg
AG5	4	1.480	3.225	2.000	2.540	3.020	1.240	2.250 fg
AG5	12	1.500	3.120	2.000	2.540	3.020	1.300	2.246 fg
AG5	6	1.500	3.060	2.000	2.580	3.080	1.260	2.246 fg
AG5	8	1.520	3.140	2.000	2.520	3.065	1.220	2.244 fg
AG5	9	1.480	3.100	2.000	2.560	3.040	1.250	2.238 g
AGC	1	2.200	2.300	2.800	1.600	3.100	1.400	2.233 g
AG5	2	1.500	3.060	2.000	2.520	3.035	1.260	2.229 g
AG4	2	1.438	2.860	2.165	2.420	2.780	1.380	2.173 h
AG4	1	1.440	2.840	2.100	2.480	2.760	1.420	2.173 h
AG4	3	1.422	2.800	2.150	2.400	2.760	1.400	2.155 hi
AG2-2IIIB	1	1.460	2.600	2.100	1.900	3.300	1.300	2.110 i
AGE	1	1.900	2.400	2.400	1.700	2.400	1.400	2.033 j
AG8	1	1.200	2.400	1.820	1.580	2.700	1.220	1.820 k
AG8	2	1.200	2.400	1.840	1.560	2.640	1.260	1.816 k
AG8	4	1.200	2.400	1.820	1.540	2.700	1.220	1.813 k
AG8	3	1.200	2.400	1.840	1.540	2.660	1.220	1.810 k
AGBa	1	1.800	2.100	1.800	1.200	1.900	1.100	1.650 l
Control	1	0.000	0.000	0.000	0.000	0.000	0.000	0.000 m

1: Means of 50 replicates. Disease severity was assigned to each plant on a scale of 0-5 after 10 day incubation. 0 = no disease; 1 = 1-10%; 2 = 11-30%; 3 = 31- 50%; 4 = 51- 80%; 5 = entire root infected. Means followed by the same letter are not significantly different from each other according to Duncan's Multiple Range Test ($\alpha = 0.05$), 2: Means of disease severity caused by each isolate is the mean of 300 observations

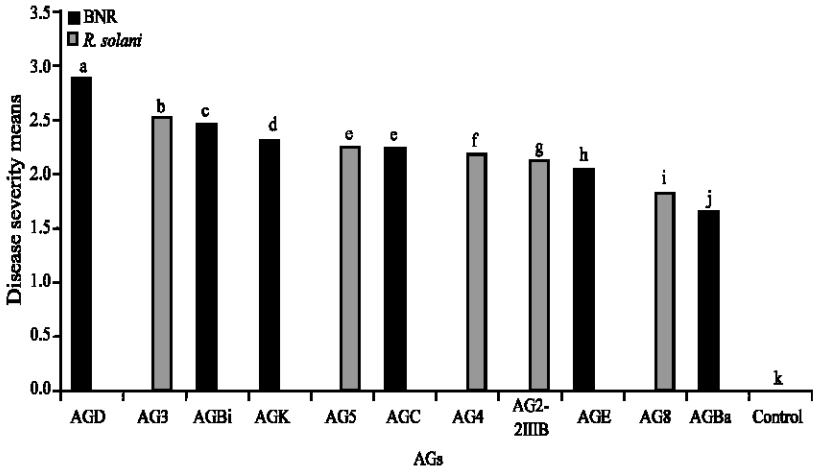

Fig. 1: Disease severity means caused by all isolates of different AGs of *R. solani* and BNR on roots of all seedlings. Bars with the same letter are not significantly different from each other according to Duncan's Multiple Range Test ($\alpha = 0.05$). Mean values of disease severity caused by different AGs on plant seedling roots is the mean of 300 observations per each isolate

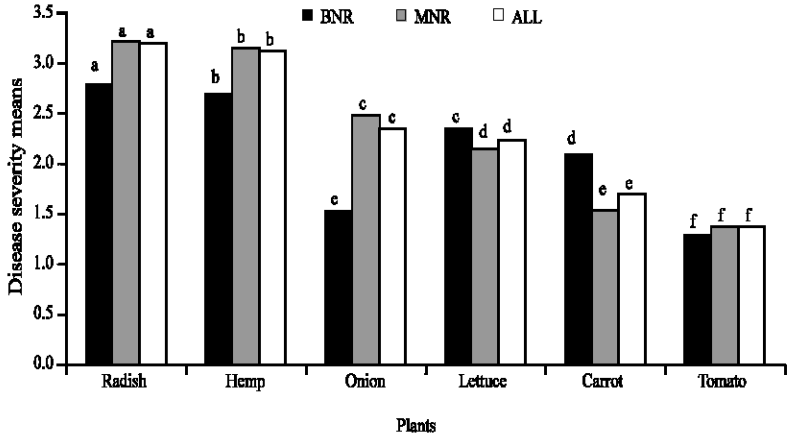

Fig. 2: Disease severity means on each plant species caused by all isolates of BNR, MNR and both populations of *Rhizoctonia*. For each class of *Rhizoctonia*, bars followed by different letter (s) are significantly different according to Duncan's Multiple Range Test ($\alpha = 0.05$). Disease severity means are the mean of 300 observations per each isolate

among the order of the plants susceptibility compared with the order of plants susceptibility against all isolates of *R. solani*. In other word, radish was the most susceptible plant and the susceptibility of tomato was the least (Fig. 2).

Disease symptoms on the host plants were pre and post emergence damping- off, root rot, seed rot and dark brown lesions in the end of the stem. Moreover, sclerotia were observed on the surface of the plants grown in petri plates.

DISCUSSION

Although, the size and number of sampling sites comprised only 3 experimental fields located at Crop and Food Research Institute based at Lincoln New Zealand and the number of potato tubers that used in this study was only 13, results revealed a high level of diversity in *Rhizoctonia* population associated with black scurf disease of potato in this part of the world.

Both *R. solani* and BNR were recovered from potato tubers showing black scurf disease symptom. *R. solani* comprised 79.3% of the isolated *Rhizoctonia* population and most of its isolates (54.34%) belonged to AG-3. This is in accordance with the majority of reports indicating that AG-3 is the main cause of the black scurf disease of potato (Balali *et al*., 1995; Abdul Rauf *et al*., 2000; Petkowski *et al*., 2003; Yanar *et al*., 2005). In this study besides AG-3, members of other *R. solani* AGs 4, 5, 8 and 2-2III B with different frequencies 6.52, 28.26, 8.69 and

2.17% were recovered, respectively (Table 1). From these AGs, members of AG-4 and AG-5 have previously been isolated from potato tubers bearing sclerotia of black scurf disease by (Abdul Rauf *et al*., 2000; Petkowski *et al*., 2003; Truter and Wehner, 2004), respectively, however, there is no any recorded information about the occurrence of AG-8 and AG-2-2IIIB on potato tubers. Therefore, it seems this is the first report indicating their association with black scurf disease of potato.

AG-8 isolates attack to different crops including cereals, legumes and are the causal agents of bare patch diseases in these crops. They also can cause severe hypocotyl and root rot in canola (Khangura *et al*., 1999), however, there is discrepancy about their pathogenicity to the potato plants in the literatures. For instance, Truter and Wehner (2004), did not recover AG-8 isolates nor from potato plants, nor from potato tubers, instead, they were able to isolate this AC,s member from the potato field soil. Results of their pathogenicity tests revealed that these isolates were not pathogenic on potato plants. In contrast, there are reports indicating that wheat and barley isolates of this AG produced severe root cankers and caused loss of feeder roots on inoculated potato plants (Carling and Leiner, 1990; Balali *et al*., 1995). In the present study all isolates of AG-8 in general were pathogenic to all plant seedlings but in compare with the other *R. solani* isolates caused the least disease severity mean on these plants (Table 2, Fig. 1).

AG-2-2IIIB isolates are the causal agents of severe diseases on different crops. They causes crown and root rot of sugar beet (Zenller *et al*., 2003) and have major hosts in graminae including maize and in fabaceae including soybean (Liu and Sinclair, 1991; Dorrance *et al*., 2003). In this study only one isolate of this AG was recovered (Table 1). Results of the pathogenicity test indicated that it was pathogenic to all plant seedlings tested (Table 2). Among them, radish was the most susceptible and susceptibility of tomato was the least (Fig. 1).

Among the *Rhizoctonia* spp. isolates recovered in the present study, 20.7% of them were binucleate, that assigned to 6 different AGs Ba, Bi, C, D, E and K (Table 1). To our knowledge, this is the first report indicating the presence of the BNR sclerotia along with the *R. solani* sclerotia on potato tubers showing black scurf disease symptoms.

AG-Ba, is the causal agent of the grey sclerotium disease of rice (Ogoshi *et al*., 1983). One isolate of this AG was recovered in current study that its pathogenicity to all seedlings was the least (Fig. 1 and Table 2). It has been reported that AGs C, D, E and K could be the cause of sugar beet seedlings damping off (Uchino *et al*., 1982), sharp eye spot of cereals (Lipps and Herr, 1982), root canker of radish (Burpee *et al*., 1980), damping off of several crops including radish, tomato, carrot and onion (Ichielevich-Auster *et al*., 1985), respectively. Isolates of the above AGs recovered in this study all were pathogenic to plant seedlings tested, but the levels of the pathogenicity of different AGs on all these seedlings were statistically different (Fig. 1, Table 2). In this study the highest level of pathogenicity on all seedlings caused by AG-D and this followed by AGs 3, Bi, K, 5, C, 4, 2-2IIIB, E and 8, respectively. Disease severity means caused by AG-Ba was the least (Fig. 1). Also, there was significant differences between the susceptibility of different plant species used in this study (Fig. 2). The reason that different groups of AG were obtained from potato tubers in this part of the world, may be related to differences in crop pattern, cultural practices, climate, etc.

In conclusion, five different AGs of *R. solani* and 6 different AGs of BNR were obtained in this study. From several of these AGs including 2-2IIIB, Ba, C, D and E, only one isolate per each AG is recovered. Moreover, the pathogenicity of these isolates was determined on 6 different plant species, but not on potato plants. Therefore, for giving any speculation about their involvement in black scurf disease of potato more investigation should to be done.

REFERENCES

Abdul Rauf, C., M. Ashraf and I. Ahmad, 2000. Anastomosis groups of *Rhizoctonia solani* isolates from potatoes in Pakistan. Abstracts of 3rd International Symposium on *Rhizoctonia*, Taiwan, pp: 36.

Abe, H. and K. Tsuboki, 1978. Anastomosis groups of isolates of *Rhizoctonia solani* Khun. from potatoes. Bull. Hokkaido Prefecture Agric. Exp. Station, 40: 61-70.

Bains, P.S. and V.S. Bisht, 1995. Anastomosis group identify and virulence of *Rhizoctonia solani* isolates collected from potato plants in Alberta, Canada. Plant Dis., 79: 241-242.

Balali, G.R., S.M. Neate, E.S. Scott, D.L. Whisson and T.J. Wicks, 1995. Anastomosis group and pathogenicity of isolates of *Rhizoctonia solani* from crops in South Australia. Plant Pathol., 44: 1050-1057.

Balali, G.R., S.M. Neate, A.M. Kasalkheh, B.J. Stodart, D.L. Melanson and E.S. Scott, 2007. Intraspecific variation of *Rhizoctonia solani* AG 3 isolates recovered from potato fields in Central Iran and South Australia. Mycopathologia, 163: 105-115.

Banville, G.J., D.E. Carling and B.E. Otrysko, 1996. *Rhizoctonia* Disease on Potato. In: *Rhizoctonia* Species, Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Sneh, B., S. Jabaji-Hare, S. Neate and G. Dijst (Eds.), Kluwer Academic Publisher, Dordrecht, Netherlands, pp: 321-330.

Burpee, L.L., P.L. Sanders, H.Jr. Cole and R.T. Sherwood, 1980. Anastomosis groups among isolates of *Ceratobasidium cornigerum* and related fungi. Mycologia, 72: 689-701.

Carling, D.E. and R.H. Leiner, 1990. Effect of temperature on virulence of *Rhizoctonia solani* and other *Rhizoctonia* on potato. Phytopathology, 80: 930-934.

Carling, D.E., 1996. Grouping in *Rhizoctonia solani* by Hyphal Anastomosis Reaction. In: *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Snesh, B., S. Jabaji-Hare, S. Neate and G. Dijst (Eds.), Kluwer Academic Publishers, Dordrecht, Netherlands, pp: 37-47.

Carling, D.E., K.A. Brainard, G. Virgen-Calleros and V.O. Portugal, 1998. First report of *Rhizoctonia solani* AG-7 on potato in Mexico. Plant Dis., 82: 127.

Carling, D.E., E.J. Pope, K.A. Brainard and D.A. Carter, 1999. Characterization of mycorrhizal isolates of *Rhizoctonia solani* from an orchid, including AG-12, a new anastomosis group. Phytopathology, 89: 942-946.

Carling, D.E., R.E. Baird, R.D. Gitaitis, K.A. Brainard and S. Kuninaga, 2002a. Characterization of AG-13 a newly reported anastomosis group of *Rhizoctonia solani*. Phytopathology, 92: 893-899.

Carling, D.E., S. Kuninaga and K.A. Brainard, 2002b. Hyphal anastomosis reactions, r-DNA-internal transcribed spacers sequences and virulence levels among subsets of *Rhizoctonia solani* anastomosis group 2 (AG-2) and AG-BI. Phytopathology, 92: 43-50.

Chand, T. and C. Logan, 1983. Cultural and pathogenic variation in potato isolates of *Rhizoctonia solani* in Northern Ireland. Trans. Bri. Mycol. Soc., 81: 585-589.

Dorrance, A.E., M.D. Klienhenz, S.A. McClure and N.T. Tuttle, 2003. Temperature, moisture and seed treatment effects on *Rhizoctonia solani* root rot of soybean. Plant Dis., 87: 533-538.

Farrokhi-Nejad, R., S. Azimi and M.G. Cromey, 2006. Diversity in population of *Rhizoctoni* spp. associated with potato tubers grown in Lincoln, New Zealand. Proceedings of the 4th Australian Soilborne Disease Symposium, 3-6 Sept. Queenstown, New Zealand, pp: 58-59.

Herr, L.J. and D.L. Roberts, 1980. Characterization of *Rhizoctonia solani* for populations obtained from sugar beet fields with different soil textures. Phytopathology, 70: 479-480.

Hyakumachi, M., T. Mushika, Y. Ogiso, T. Toda, K. Kageyama and T. Tsuge, 1998. Characterization of a new cultural type (LP) of *Rhizoctonia solani* AG-2-2 isolated from warm season turfgrasses and its genetic differentiation from other cultural types. Plant Pathol., 47: 1-9.

Ichielevich-Auster, M., B. Sneh, I. Barash and Y. Koltin, 1985. Suppression of damping-off caused by *Rhizoctonia* sp. by a nonpathogenic isolate of *R. solani*. Phytopathology, 75: 1080-1084.

Khangura, R.K., M.J. Barbetti and M.W. Sweetingham, 1999. Chracterization and pathogenicity of *Rhizoctonia* species on canola. Plant Dis., 83: 714-721.

Kronland, W.C. and M.E. Stanghellini, 1988. Clean slide technique for the observation of anastomosis and nuclear condition of *Rhizoctonia solani*. Phytopathology, 78: 820-822.

Kuniniga, S., D.E. Carling, T. Takeuchi and R. Yokosawa, 2000. Comparison of rDNA-ITS sequences between potato and tobacco strains in *Rhizoctonia solani* AG-3. J. Genet. Plant Pathol., 66: 2-11.

Lipps, P.E. and L.J. Herr, 1982. Etiology of *Rhizoctonia cerealis* in sharp eyespot of wheat. Phytopathology, 72: 1574-1577.

Liu, Z. and J.B. Sinclair, 1991. Isolates of *Rhizoctonia solani* anastomosis group 2-2 pathogenic to soybean. Plant Dis., 75: 682-687.

Naito, S. and S. Kanematsu, 1994. Characterization and pathogenicity of a new anastomosis subgroup AG 2-3 of *Rhizoctonia solani* Kuhn isolated from leaves of soybean. Ann. Phytopathol. Soc. Jpn., 60: 681-690.

Ogoshi, A., M. Oniki, T. Araki and T. Ui, 1983. Studies on the anastomosis groups of binucleate *Rhizoctonia* and their perfect states. J. Fac. Agric. Hokkaido Univ., 61: 244-260.

Ogoshi, A., 1987. Ecology and pathogenicity of anastomosis and intraspecific groups of *Rhizoctonia solani* Kuhn. Annu. Rev. Phytopathol., 25: 125-143.

Petkowski, J.E., B. Czerniakowski and R.F. deBoer, 2003. *Rhizoctonia solani* anastomosis groups associated with potatoes in Victoria, Australia. In: 8th International Congress of Plant Pathology, Vol. 2, Abstracts of Offered Papers, 27 Feb-2March Christchurch, New Zealand, pp: 127.

Priyatmojo, A., V.E. Escopalao, N.G. Tangonan, C.B. Pascual, H. Suga, K. Kageyama and M. Hyachumachi, 2001. Characterization of a new subgroup of *Rhizoctonia solani* anastomosis group 1(AG-1-ID) causal agent of a necrotic leaf spot on coffee. Phytopathology, 91: 1054-1061.

Robinson, H.L. and J.W. Deacon, 2002. Doble-stranded RNA elements in *Rhizoctonia solani* AG 3. Mycol. Res., 106: 12-22.

Singleton, L.L., J.D. Mihail and C.M. Rush, 1992. Methods for Research on Soilborne Phytopathogenic Fungi. APS Press, pp: 265.

Sneh, B., L. Burpee and A. Ogoshi, 1991. Identification of *Rhizoctonia* Species. APS Press Inc., Stpaul, Minnesota, USA., pp: 133.

Stalpers, J.A. and T.F. Andersen, 1996. Asynopsis of the Taxonomy of Teleomorphs Connected with *Rhizoctonia* S.L. In: *Rhizoctonia* species, Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Sneh, B., S. Jabaji-Hare, S. Neate and G. Djist (Eds.), Kluwer Academic Publisher, Dordrecht, Netherlands, pp: 49-63.

Truter, M. and F.C. Wehner, 2004. Anastomosis grouping of *Rhizoctonia* solani associated with black scurf and stem canker of potato in South Africa. Plant Dis., 88: 83.

Uchino, H., K. Kanzawa and A. Ogoshi, 1982. Studies on the damping off of sugar beet seedlings. Bull. Sugar Beet Res., 24: 170-176.

Yanar, Y., G. Yilmaz, I. Cesmeli and S. Coskun, 2005. Characterization of *Rhizoctonia solani* isolates from potatoes in Turkey and screening potato cultivars for resistance to AG-3 isolates. Phytoparasitica, 33: 370-376.

Zenller, M., A. Hugl, P. Buttner and K. Optiz, 2003. The incidence of *Rhizoctonia solani* in sugar beets, maize and weeds. Proceedings of the 8th International Congress of Plant. Pathology, 2: 328.