

Characterization of *Rhizoctonia solani* Isolates from Potatoes in Turkey and Screening Potato Cultivars for Resistance to AG-3 Isolates

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A total of 304 *Rhizoctonia solani* isolates and 60 binucleate *Rhizoctonia*-like fungi were recovered from stems and tubers of infected potato plants over a 2-yr period in northeast Turkey. *R. solani* isolates were identified to 11 anastomosis groups (AGs): AG-1 (0.66%), AG-2-1 (5.6%), AG-2-2 (0.99%), AG-3 (83.9%), AG-5 (4.6%), AG-6 (0.66%), AG-8 (1.32%), AG-9 (0.33%), AG-10 (1.32%), AG-12 (0.33%), and AG-13 (0.33%). In the greenhouse tests, most of the AG-3 isolates were significantly more virulent than isolates belonging to other AGs on potato cv. Batum. Isolates of other anastomosis groups differed in their virulence. Results indicated that AG-3 is an important pathogen on potatoes grown in the study area. Five of 22 commercial and local potato cultivars evaluated for their reaction to *R. solani* AG-3 isolates (TP-2) under greenhouse conditions were highly resistant; the remaining cultivars exhibited different levels of susceptibility to the pathogen isolate.

KEY WORDS: Potato; *Rhizoctonia solani*; anastomosis; resistance.

INTRODUCTION

Stem canker and black scurf caused by *Rhizoctonia solani* Kühn. (telemorph *Thanatephorus cucumeris* (A.B. Frank) Donk is a serious disease of potato (*Solanum tuberosum* L.) grown in cooler regions of the world. *R. solani* has a wide host range (6,11,21) and the pathogen is also widely distributed in the potato fields of central and northern Anatolia of Turkey (14,28). *R. solani* comprises a collection of non-interbreeding populations and is recognized through the anastomosis group concept (1). The virulence and host range of these groups are different. Therefore, data on anastomosis group affiliation of an isolate involved in a particular disease are very useful for the determination of crop rotation sequence in a particular area (1,5,21). Currently, 13 AGs have been described (AG-1 through 13) (20,27).

Members of AG-3 are the major causal agents of stem canker of potato (4,8,9). Members of other AGs (AG-1, AG-2,1, AG-4, AG-5, AG-9) also have been reported to cause stem canker, as well as black scurf disease of potato (2,4,8,10,13). Although AG-3 was reported as a major causal agent of stem canker and black scurf disease of potato in the central and eastern Anatolia regions of Turkey (14,28), AG-2-1, AG-2-2, AG-4 and AG-5 were also isolated from stems and tubers of potatoes with stem canker from eastern Turkey (14). Despite the reports on *R. solani* AG groups causing the disease on potato from different regions of Turkey, there is an absence of adequate information on the distribution

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and virulence of *R. solani* AG groups in northeast Turkey, where crop pattern, climate and the altitude differ from the eastern and central Anatolia regions of Turkey.

Potato is one of the major agricultural products grown in the northeast region of Turkey. In the region of study, a total of 104,000 tons of potatoes were harvested from 5,105 ha of land cropped to potato during 2002 (government statistics). Most of the potatoes go to the fresh market. *R. solani* causes a considerable amount of yield loss each year and reports of losses attributed to this pathogen have varied from 5% to 34% in different parts of the world (6,15,17). Chemical control of soilborne fungi like *Rhizoctonia* is not desirable, since it causes environmental pollution. Therefore, plant resistance, combined with crop rotation, offers the most practical and feasible way to control the disease (25).

The purpose of this study was to (i) determine the anastomosis groupings for isolates of *R. solani* recovered from potato plants grown in the northeast region of Turkey, (ii) determine pathogenicity of these isolates and (iii) evaluate the susceptibility of potato cultivars to AG-3 isolates of *R. solani*.

MATERIALS AND METHODS

Collection, isolation and identification of isolates Potato plants at the beginning of the growth stage with symptoms of stem canker were collected from 100 fields in five counties located in the primary potato production region of northeast Turkey (Central district, Niksar, Erbaa, Basciftlik and Artova) in 2000 and 2002. Roots and basal stem pieces with lesions were washed under running tap water, surface-disinfested with 1% NaOCl for 2 min, rinsed in sterile distilled water, and then placed on water agar (WA) containing streptomycin sulfate (120 mg l^{-1}). The culture was incubated at 22–24°C for 2 to 4 days. Cultures which exhibited *Rhizoctonia*-like growth characteristics were hyphal-tipped from water agar to potato-dextrose agar (PDA). All cultures were examined ($400\times$) for hyphal branching, septal pore and number of nuclei per hyphal cell after 2 to 4 days of growth on WA followed by staining with safranin O (29).

TABLE 1. Anastomosis group (AG) identity of *Rhizoctonia solani* isolates obtained from potatoes grown in northeastern Turkey during 2000–2002

Location	AGs										
	AG-1	AG-2-1	AG-2-2	AG-3	AG-5	AG-6	AG-8	AG-9	AG-10	AG-12	AG-13
Central district	1	2		11							
Artova		6		44	5					1	
Basciftlik		4	2	117	7		2		3		
Erbaa		1	1	10		1					
Niksar	1	4		73	2	1	2	1	1		1
Total	2	17	3	255	14	2	4	1	4	1	1

All cultures were paired with known AG tester cultures of *R. solani* (AG-1, AG-2-1, AG-2-2-4, AG-3, AG-4, AG-5, AG-6, AG-8, AG-9, AG-10, AG-12 and AG13) provided by Dr. Erkol Demirci (Dept. of Plant Protection, Ataturk University, Erzurum, Turkey). Pairing was done on 2% WA according to the procedures described by Parmeter *et al.* (22). A 5-mm-diam disk from the edge of a 3-day-old colony of an unknown isolate was placed on 2% WA in a 9-cm-diam glass petri dish and a mycelial disk from similarly grown

AG tester culture was placed in the petri dish at a distance of 2 cm from the first disk, and incubated at 22°C for 48 h. When mycelia of two isolates slightly overlapped, the anastomosis points were stained with a mixture of 0.5% safranin O and 3% KOH and examined under a compound microscope (400x). Anastomosis group was determined on the basis of hyphal fusion between two isolates. Hyphal fusion at a minimum of five points was considered to be a positive indication that both isolates belonged to the same anastomosis group. This procedure was replicated three times for each unknown isolate.

Pathogenicity testing Fungal inocula for pathogenicity tests were produced by growing isolates in a 2000-ml flask containing 600 g of oat seeds in a modified procedure of Mazzola (18). Oat seeds were soaked overnight in tap water prior to autoclaving. The flasks were autoclaved two times at 121°C for 60 min, inoculated with half a plate of agar plug transfers of a specific isolate, and incubated at 22°C. The flasks were shaken at 3-day intervals to eliminate the formation of large, sticky clumps of oat and fungus. After 3 weeks of growth at 22°C, oat seeds completely colonized by the fungus were used as inoculum.

Thirty-eight isolates of *R. solani* representing 11 AGs were used for pathogenicity tests (Table 1). Pathogenicity of the isolates was determined on potato (local cv. 'Batum') plants. The tubers were surface-disinfested with 2% NaOCl and kept at room temperature for sprouting. After sprouting was initiated, one tuber was planted at a depth of approximately 5 cm in a 30-cm plastic pot containing sterilized soil mixture (soil/sand/cow manure, 1:1:0.5 v/v). Each tuber was inoculated by adding 10 g of oat seed inoculum above it (0.2% wt/wt), and covered with the soil mixture. The same amount of uninfested autoclaved oat seeds was added to the control. The plants were grown in a greenhouse at 20–25°C. The plants were harvested 6 weeks after planting (at the tuber initiation stage) and washed to determine the presence of cankers on stems and stolons. Disease severity index (DSI) was rated as 0–4, where 0=no disease; 1=few scattered cankers on stem or stolon; 2=cankers coalescing; 3=cankers girdling stem and stolons; and 4=dead plant as based on the modified scale of Carling and Leiner (9). Data were subjected to analysis of variance (ANOVA) and grouped by using Fisher's least significance test. The experimental design was a randomized complete block with four replications, and the experiment was repeated twice. The isolates were recovered from infected plants for each treatment and paired with tester isolates again for confirmation.

Susceptibility of potato cultivars to a pathogenic isolate of *R. solani* AG-3 (TP-2) Susceptibility of 22 commercial and local potato cultivars (Table 2) against the highly virulent isolate *R. solani* TP-2 (AG-3) was determined under greenhouse conditions. The isolate TP-2 (AG-3) was chosen based on the pathogenicity tests we conducted. Planting, inoculation, evaluation and greenhouse conditions were the same as described for the pathogenicity test.

RESULTS

Determination of anastomosis groups All together 304 isolates from 100 potato fields met the specific descriptions of *R. solani*. Of these, 255 belonged to AG-3, one each to AG-9, AG-12 and AG-13, two each to AG-1 and AG-6, four each to AG-8 and AG-10, 14 to AG-5, 17 to AG-2-1 and three to AG-2-2 (Table 1). In addition, 60 binucleate *Rhizoctonia*-like fungi were also isolated from the infected potato plants.

TABLE 2. Pathogenicity of *Rhizoctonia solani* isolates on potato cv. Batum

Isolate ^z	DSI ^y	Isolate	DSI
AG-3 (TP-2)	4.00	AG-2-1 (TP-58)	1.67
AG-3 (TP-50)	3.66	AG-2-1 (TP-54)	1.67
AG-3 (TP-30)	3.67	AG-2-1 (TP-53)	1.67
AG-3 (TP-10)	3.67	AG-10 (TP-61)	1.67
AG-3 (TP-47)	3.33	AG-2-1 (TP-57)	1.67
AG-3 (TP-12)	3.33	AG-10 (TP-63)	1.33
AG-3 (TP-40)	3.00	AG-1 (TP-81)	1.33
AG-3 (TP-4)	3.00	AG-1 (TP-82)	1.33
AG-8 (TP-90)	2.33	AG-10 (TP-62)	1.33
AG-5 (TP-21)	2.33	AG-2-2 (TP-97)	1.33
AG-8 (TP-92)	2.33	AG-2-2 (TP-96)	1.33
AG-5 (TP-22)	2.00	AG-2-2 (TP-95)	1.00
AG-5 (TP-27)	2.00	AG-3 (TP-17)	1.00
AG-8 (TP-91)	2.00	AG-3 (TP-42)	1.00
AG-9 (TP-60)	2.00	AG-13 (TP-80)	0.67
AG-5 (TP-23)	2.00	AG-6 (TP-93)	0.67
AG-5 (TP-28)	2.00	AG-6 (TP-94)	0.33
AG-2-1 (TP-52)	1.67	AG-12 (TP-70)	0.00
Control	0.00		
LSD	0.97 ^x		

^zAnastomosis group (AG) and isolate number listed for each isolate.

^yDisease severity index (DSI) determined 6 weeks after planting and based on a 0 to 4 scale, where 0=no disease and 4=dead plant.

^xMeans compared with Fisher's protected least significant difference (LSD) ($P=0.05$).

TABLE 3. Reactions of potato cultivars to *Rhizoctonia solani* AG-3 isolate TP-2 under greenhouse conditions

Cultivar	DSI ^z	Cultivar	DSI
Batum	4.00	Konsul	2.77
Carlita	4.00	Tomensa	2.57
Gurgentepe-sarisi	4.00	Gurgentepe-beyazi	1.93
Liseta	4.00	Alleddian-beyazi	1.57
Rus-beyazi	4.00	Kadioglu	1.43
Jaerla	4.00	Resadie	1.33
Aybasti-beyazi	3.67	Alleddian-sarisi	0.77
Basciftlik	3.33	Victoria	0.67
Van Gogh	3.13	Aybasti-sarisi	0.67
Ausonia	3.07	Romanya-beyazi	0.67
Cosmos	3.00	Golkoy	0.33
		LSD	1.24 ^y

^zDisease severity index (DSI) determined 6 weeks after planting and based on a 0 to 4 scale, where 0=no disease and 4=dead plant.

^yMeans compared with Fisher's protected least significant difference (LSD) ($P=0.05$).

Pathogenicity on potatoes Data from the two pathogenicity experiments indicated that isolates of AG-1, AG-2-1, AG-2-2, AG-3, AG-5, AG-8, AG-9 and AG-10 were pathogenic on susceptible cultivar Batum, whereas AG-6, AG-12 and AG-13 were not pathogenic (Table 2). Isolates causing DSI of <1.0 were considered non-pathogenic. Most of the AG-3 isolates were significantly more virulent than the isolates of other AG groups tested. AG-5, AG-9 and AG-8 isolates resulted in higher disease severity rating than isolates of AG-1 or AG-2-2. Lesions caused by AG-1, AG-10, AG-2-2, AG-2-1 and two of the AG-3

isolates appeared to be more superficial than lesions caused by other isolates. There were significant differences ($P=0.05$) in disease severity among isolates of AG-3 (ranged from 1.00 to 4.00). TP-17 and TP-42 isolates of AG-3 demonstrated lower virulence as compared to those of AG-3 isolates. The most virulent isolate of AG-3 was isolate TP-2 (Table 2).

Cultivar reactions Based on the pathogenicity test results, the most virulent isolate of AG-3 (TP-2) was used for cultivar reaction tests. Twenty-two local and commercial potato cultivars were tested under greenhouse conditions. Reactions of 21 potato cultivars to *R. solani*, together with the susceptible control Batum, are listed in Table 3. Canker severity induced by TP-2 varied significantly among cultivars tested. The susceptible cultivar, Batum, showed the same susceptibility as that of Carlita, Gurgentepe-beyazi, Liseta, Rus-beyazi, and Jaerla. Susceptible cultivars exhibited post-emergence stem death, whereas resistant cultivars (Alleddian-sarisi, Victoria, Aybasti-beyazi, Romanya-beyazi and Golkoy) showed small superficial lesions scattered on stems and stolons. Cultivars Golkoy, Romanya-beyazi, Aybasti-sarisi, Victoria, Alleddian-sarisi, Resadie, Kadioglu and Alleddian-beyazi had significantly lower disease severity than 11 cultivars, e.g. Batum, Carlita, Gurgentepe-beyazi and Liseta. Cultivars Aybasti-beyazi, Basciftlik, Van Gogh, Ausonia and Cosmos showed levels of susceptibility similar to those of Batum, Carlita, Gurgentepe-beyazi, Liseta, Rus-beyazi and Jaerla.

DISCUSSION

The majority of *R. solani* isolates recovered in this study from infected potato plants and tubers belonged to AG-3 (83.88%). With the exception of AG-3, isolates belonging to all AGs were obtained at low frequency (0.33–5.59%) from infected potato plants and tubers. This is the first report of the association of AG-1, AG-6, AG-8, AG-9, AG-10, AG-12 and AG-13 isolates of *R. solani* with stem canker disease of potato in Turkey. Distribution of AG-2-1 and AG-3 was not restricted to any particular location in the region studied; however, isolates identified as AG-1, AG-2-2, AG-5, AG-6, AG-8, AG-9, AG-10, AG-12 and AG-13 were restricted to a particular location or district. Demirci and Doken (14) reported five anastomosis groups (AG-2-1, AG-2-2, AG-3, AG-4 and AG-5) among *R. solani* isolates obtained from infected potato plants and tubers in eastern Turkey. AG-4 was not recovered from potato in our study area. In a study carried out in central Anatolia of Turkey, only *R. solani* AG-3 isolates were recovered from infected potatoes (28). The reason that different groups were obtained from potatoes in this region may be related to differences in the crop pattern, climate and the altitude in the study area and the eastern and central Anatolia regions. *R. solani* other than AG-3, including AG-1, AG-2-1, AG-4, AG-5 and AG-9, have been reported in association with potato in different parts of the world (2,4,7,9,10,13). The evidence of the extensive occurrence of AG-3 isolates in northeastern Turkey implies that members of AG-3, which is reported to be the principal cause of Rhizoctonia disease of potatoes in different parts of the world (2,4,8), are also the primary causal agent of Rhizoctonia disease of potato in the study area.

There was significant variability in virulence among AG-3 isolates. In pathogenicity tests, isolates of *R. solani* AG-3 were the most virulent on potato, except for two isolates (TP-17 and TP-42) which exhibited lower virulence. However, AG-3 isolates with lower stem canker severity may cause severe black scurf symptoms on progeny tubers under field conditions. Other AGs recovered from infected potato plants had relatively low virulence, which typically was markedly lower than that expressed by AG-3 isolates, indicating that

the AGs of *R. solani* other than AG-3 have minor impact on potatoes in northeast Turkey. The results obtained in this study were in good agreement with the results of previous studies (4,7,8,13,14). Similar results were obtained from the previous studies conducted with AG-3 on potatoes (14,16). The variation obtained in this study emphasizes the importance of testing a range of *R. solani* AG-3 isolates for pathogenicity before choosing the isolates to determine the host resistance. Isolates causing DSI<1.0 were considered non-pathogenic in this study. Therefore, isolates of AG-6, AG-12 and AG-13 recovered from infected potato stems were not pathogenic on potato cv. Batum. This is not surprising for AG-12, because many AG-12 isolates have been reported to be mycorrhizal in the field with the Australian orchid (12).

Results from *in vitro* cultivar reaction tests revealed that the potato cultivars evaluated in this study differed in their reaction to *R. solani*. The data indicated that some of the cultivars tested were highly susceptible to Rhizoctonia disease of potato, but some cultivars had higher levels of resistance than the local susceptible cv. Batum. Cultivars Jaerla and Ausonia previously were reported to have low and medium levels of resistance to *R. solani* AG-3 (3,23). However, both cultivars were found highly susceptible to Rhizoctonia stem canker in our study.

There may be a correlation between stem canker severity and yield of progeny tubers. However, this is not always the case. Simons and Gilligan (26) reported that stem and stolon canker severity had no effect on total yields of potato, but did alter the size distributions. They observed that yield of main-sized tubers decreased, while the yields of over-sized and baker-sized tubers increased. Others (24) reported a decrease in tuber initiation sites due to severe stem and stolon infections resulting in fewer progeny tuber initiation sites. Therefore, over-sized tubers could form on noninfected stolons. It was suggested that severe stem canker alone in the growing period of potato plants may not affect the yield of progeny tubers (24). Research is needed to determine the effects of the results of pathogenicity tests and the cultivar reaction, on the quantity and marketed quality of potato yields. Therefore, further studies will be conducted to determine the effects of these factors under field conditions on the yield quantity and quality of potatoes.

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