International Journal of Research in Agriculture and Forestry
Volume 6, Issue 6, 2019, PP 26-31
ISSN 2394-5907 (Print) & ISSN 2394-5915 (Online)

Rhizoctonia Species and Anastomosis Groups Isolated from Tomato and Cucumber in Erzincan, Turkey

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ABSTRACT

Seventy isolates of Rhizoctonia spp. were obtained from roots of tomato (Lycopersicon esculentum L.) and cucumber (Cucumis sativus L.) grown in Erzincan, Turkey during the period of 2007 and 2008. Majority of these isolates (84.3%) had multinucleate cells and were identified as Rhizoctonia solani. The remaining isolates were recognized as binucleate Rhizoctonia spp. Of the 59 isolates from tomato, the most prevalent was AG4 (39% of isolates), followed by AG-2 type1 (8.5%), AG-3 (5.1%) and 1.7% of the isolates were identified as AG5. AG-A (35.6%) and AG-K (10.2%) isolates of binucleate Rhizoctonia also were recovered from tomato roots. Of the 11 isolates from cucumber, 81.8% were AG-4 and 18.2% were AG-5. Pathogenicity experiments in vitro, differences in virulence level were observed between R. solani and binucleate Rhizoctonia isolates and among different AGs. Isolates of R. solani AG-2 type 1 and AG-4 were the most aggressive, binucleate Rhizoctonia isolates of AG A and AG-K were weakly aggressive. This is the first report of R. solani AG-2 type 1 and binucleate Rhizoctonia AG-K on tomato and R. solani AG-5 on cucumber in Turkey.

Keywords: *Rhizoctonia solani, binucleate Rhizoctonia, Lycopersicon esculentum, Cucumis sativus, pathogenicity*

INTRODUCTION

Tomato (*Lycopersicon esculentum Mill.*) and cucumber (*Cucumis sativus L.*) are important consumed food sources among all the vegetables throughout the world. Tomato and cucumber is also popular vegetable crop of Turkey and are a good source of vitamins.

Rhizoctonia comprises both multinucleate and binucleate species which are further divided into anastomosis groups (AGs). Currently, the multi nucleate isolates *R. solani Kühn* (teleomorph: *Thanatephorus cucumeris* (Frank) Donk.) are grouped by anastomosis between hyphae into AG-1 to AG-13 and a bridging isolate AG-BI (García et al., 2006). Binucleate *Rhizoctonia* (teleomorph: *Ceratobasidium* Rogers) isolates are also grouped into AG-A to S (Sneh et al., 1991; García et al., 2006; Sharon et al., 2008). The soilborne plant pathogen *R. solani* is a basidiomycete that occurs worldwide and causes economically important diseases to a large variety of vegetable and field crops, turf grasses, ornamentals and fruit and forest trees (Ogoshi, 1996). *Rhizoctonia solani* causes root rot, stem rot, fruit and seed decay, damping-off, foliar blight, stem canker and crown rot, in various crops (Tu et al., 1996).

Rhizoctonia solani AG 4 is the major AG worldwide, and it can cause several types of damage, including stem and fruit rot (Rahimian, 1988; Kuramae et al., 2003), root rot (Montealegre et al., 2003a,b) in the tomato. Isolates of *R. solani* AG-3 have been found that cause leaf blight of tomato (Date et al., 1988), foot rot (Misawa and Kuninaga, 2010).

Additionally, *R. solani* AG-1(Bolkan and Ribeiro, 1985) and AG 2 type 1 (Montealegre et al., 2003a, b; Misawa and Kuninaga, 2010) isolated from tomato have been reported. On the other hand, *R. solani* has been reported to be the major causal organism of damping-off in the tomato (Jiskani et al., 2007). *Rhizoctonia solani* AG-2 type 2 and AG-4 has been reported to be the major causal organism of damping-off in the cucumber (Villajuan-Abgona et al., 1996; Lucon et al., 2009). In Turkey, the isolates of AG-3, AG-5 (Demirci and Döken, 1995) and AG-4 (Tuncer and Erdiller, 1990; Demirci and Döken,

1995) of *R. solani* and binucleate *Rhizoctonia* (AG-A, AG-G) were determined on tomato (Demirci and Döken, 1995). *Rhizoctonia solani* AG-3 (Demirci and Döken, 1995), AG-4 (Demirci and Döken, 1995; Erşahin et al.2009) and binucleate *Rhizoctonia* AG-A, AG-G (Demirci and Döken, 1995) isolates were recovered from cucumber in Turkey. The objectives of this study were to identify anastomosis groups of *Rhizoctonia* isolates collected from tomato and cucumber in Erzincan, Turkey and to determine their pathogencity.

MATERIALS AND METHODS

Seventy isolates of *Rhizoctonia* spp. were recovered from tomato and cucumber plants. During year 2007 and 2008 from fields in two districts (Center and Üzümlü) of Erzincan (Table 1).Isolations were made from discolored or necrotic lesions on root and hypocotyls tissues. Affected plant tissues were washed under running tap water, surface disinfected in 0.5 % sodium hypochlorite for 1 min and placed on 1.5 % water agar containing 50 mg/l streptomycin sulfate (Demirci and Döken, 1995).

After 48-72 h incubation at 20-25°C, hyphae from the margin of each developing colony were placed on water agar or potato dextrose agar (PDA). All isolates were maintained on (PDA) medium at 10°C and transferred from time to time to new medium.

Table1. *Identity of 70 isolates of Rhizoctonia spp. isolated from tomato and cucumber in Erzincan, Turkey*

	Host Plant Species	
Species / Anastomosis		
Groups (AGs)	**Tomato**	**Cucumber**
Rhizoctonia solani		
AG-2 type 1	5	-
AG-3	3	-
AG-4	23	9
AG-5	1	2
Binucleate Rhizoctonia		
AG-A	21	-
AG-K	6	-
Total	59	11

Isolates of *Rhizoctonia* obtained in this manner were identified on the basis of characteristics of their vegetative hyphae (Ogoshi, 1975), nuclear condition (Bandoni, 1979), requirement for thiamine (Rovira et al., 1986) and hyphal anastomosis with known tester isolates of *R. solani* (Tester isolates included: AG-1, AG-2 type 1, AG-2-2, AG-3, AG-4, AG-5, AG-6, AG-7, AG-8, AG-9, AG-10, AG-11, AG-12, AG-13 and AG-BI, provided by Dr. A. Ogoshi, Hokkaido University, Japan. Dr. D.E. Carling, University of Alaska Fairbanks, USA, Dr. S. M. Neate, CSIRO, Division of Soils, Australia and Dr. D. A. Carter, University of Sydney, Australia) and binucleate *Rhizoctonia* (Tester isolates included: AG-A, AG-Ba, AG-Bb, AG-C, AG-D, AG-E, AG-F, AG-G, AG-H, AG-I, AG-K, AG-L, AG-N, AG-O,AG-P, AG-Q, AG-J, AG-R and AG-S provided by Dr. A. Ogoshi, Hokkaido University, Japan and Dr. M. Mazzola, Tree Fruit Research Laboratory, Western Ave., USA) by using standardized techniques for anastomosis group determination (Parmeter et al., 1969).The aggressiveness was studied using in vitro bio-assays. The aggressiveness of 70 isolates (59 isolates for tomato and 11 isolates for cucumber) representing six anastomosis groups (Table 1) was determined on tomato (cv. H-2274) and cucumber (cv. Beirt Alpha) seedlings.

For in vitro experiments, an agar plate assay was adapted from the method of Muyolo et al. (1993). Seeds were surface disinfested in 1.0 % NaOCI for 5 min, and air-dried before use. Six seeds of each host were placed on 10 ml of sterile 1.5 % water agar in 10-cm-diameter petri dishes. The center of each dish was subsequently inoculated with a 6-mm-diameter mycelial disk from a 2- to 3 day-old cultures of isolates on PDA. Cultures were incubated under continuous darkness for 4 days at 25 ± 1°C, after which they were placed on a laboratory bench under 12 h light and 12 h dark.

Disease severity was rated, 10 days after inoculation using a scale of 0-4, where 0= healthy, no lesions on the hypocotyls; 1= lesions covering <25% of the hypocotyls; 2= lesions covering 10% to 50% of the hypocotyls; 3= lesions covering 50% to 100% of the hypocotyls; and 4= seedlings is dead. Isolations of *Rhizoctonia* from lesions were successful in all pathogenicity tests. The resulting cultures

were paired with the original cultures and confirmed to anastomosis group. Pathogencity tests were carried out in a completely randomized design of four replicates. As all data showed normal distribution, they were directly analyzed by analysis of variance (ANOVA) with JMP Software (5.0.1.). Least significant differences (Fisher's protected LSD) were calculated following significant F tests.

RESULTS AND DISCUSSION

During 2007–2008, a total of 70 isolates of *Rhizoctonia* spp. were recovered from tomato and cucumber plants in Erzincan province, 43 were identified as *R. solani* and 27 were binucleate *Rhizoctonia* (Table 1).

The majority of isolates recovered (74.4% *R. solani*, 100% binucleate *Rhizoctonia*) were isolated from tomato. The majority (53.5%) of *R. solani* isolates from tomato were AG-4; the majority of binucleate *Rhizoctonia* (77.8%) were AG-A. Considerably lower numbers of isolates of *R. solani* were recovered from cucumber compared to tomato. Among the isolates of *Rhizoctonia* recovered from tomato, 54.2% were *R. solani* (AG-2 type 1, AG-3, AG-4 and AG-5) and 45.8% binucleate *Rhizoctonia* (AG-A and AG-K); whereas from cucumber, 100% of the isolates were *R. solani* (AG-4 and AG-5). Of the 59 isolates from tomato, the most prevalent was AG4 (39% of isolates), followed by AG-2 type1 (8.5%), AG-3 (5.1%) and 1.7% of the isolates were identified as AG5. AG-A (35.6%) and AG-K (10.2%) isolates of binucleate *Rhizoctonia* also were recovered from tomato roots. Of the 11 isolates from cucumber, 81.8% were AG-4 and 18.2% were AG-5.

The most common occurrence of *R. solani* AG 4 isolates on tomato and cucumber has also been reported from other countries of the world by many workers (Rahimian, 1988; Villajuan-Abgona et al., 1996; Kuramae et al., 2003; Montealegre et al., 2003a, b; Lucon et al., 2009). *Rhizoctonia solani*; AG-3, AG-4, AG-5 and binucleate *Rhizoctonia*; AG-A isolates were recovered from tomato in Turkey (Tuncer and Erdiller, 1990; Demirci and Döken, 1995). In a study carried out in Eastern Anatolia of Turkey, *R. solani*; AG-4 isolates were recovered from cucumber (Demirci and Döken, 1995). In the present study, AG-2 type 1of *R. solani* and AG-K of binucleate *Rhizoctonia* from tomato and AG-5of *R. solani* from cucumber were isolated for the first time in Turkey.

Isolates of *R. solani* and isolates representing different anastomosis groups of binucleate *Rhizoctonia* varied in virulence (Table 2, Table 3).As a result of in vitro pathogen city tests, it was found that the differences among the virulence of the *Rhizoctonia* isolates were statistically significant.

Table2. *In vitro pathogenicity of Rhizoctonia species on tomato (cv. H-2274)*

Species/ Anastomosis Groups (AGs)	**Isolate**	**Disease index[y]**
Rhizoctonia solani		
AG-2 type 1	T305	4.00a[z]
	T308	4.00a
	T221	4.00a
	T314	3.94a
	T140	3.89ab
AG-3	T198	2.67hn
	T185	2.50io
	T189	2.00np
AG-4	T151	4.00a
	T155	4.00a
	T343	4.00a
	T71	4.00a
	T122	4.00a
	T15	3.89ab
	T337	3.83ac
	T267	3.83ac
	T99	3.78ac
	T248	3.72ad
	T54	3.72ad
	T230	3.72ad
	T358	3.67ae

	T341	3.61af
	T342	3.61af
	T345	3.56af
	T334	3.50ag
	T373	3.50ag
	T333	3.44ah
	T165	3.44ah
	T378	3.28ai
	T338	2.94dl
	T286	2.67hn
AG-5	T106	2.50io
Binucleate *Rhizoctonia*		
AG-A	T67	3.28ai
	T127	3.22aj
	T85	3.22aj
	T61	3.11bk
	T370	3.06ck
	T376	2.94dl
	T313	2.89el
	T147	2.83fm
	T311	2.72gn
	T377	2.72gn
	T82	2.56io
	T371	2.44jo
	T277	2.38ko
	T77	2.39ko
	T83	2.22lp
	T269	2.17lp
	T366	2.17lp
	T62	2.06mp
	T66	1.83oq
	T310	1.56pr
	T364	1.44pr
AG-K	T193	2.67hn
	T273	2.67hn
	T203	2.50io
	T76	2.39ko
	T312	1.17qs
	T290	0.94rs
Control	-	0.50s
LSD		0.80

[Y] *Disease index 0-4; 0= healthy, no lesions on the hypocotyls; 1= lesions covering <25% of the hypocotyls; 2= lesions covering 10% to 50% of the hypocotyls; 3= lesions covering 50% to 100% of the hypocotyls; and 4= seedlings is dead.*

[Z] *Means compared with Fisher's protected least significant difference (LSD) (P=0.01).*

Table3. *In vitro pathogenicity of Rhizoctonia species on cucumber (cv. Beirt Alpha)*

Species/ Anastomosis		
Groups (AGs)	**Isolate**	**Disease index[y]**
Rhizoctonia solani		
AG-4	C117	4.00a[z]
	C150	4.00a
	C157	4.00a
	C160	4.00a
	C167	4.00a
	C186	3.83b
	C151	0.33c
	C179	0.28cd
	C173	0.11ef

AG-5	C175	3.78b
	C178	0.17de
Control	-	0.00f
LSD		0.14

[Y] *Disease index 0-4; 0= healthy, no lesions on the hypocotyls; 1= lesions covering <25% of the hypocotyls; 2= lesions covering 10% to 50% of the hypocotyls; 3= lesions covering 50% to 100% of the hypocotyls; and 4= seedlings is dead.*

[Z] *Means compared with Fisher's protected least significant difference (LSD) (P=0.01).*

All isolates of *R. solani* AG-2 type1and AG-4 were pathogenic to tomato (cv. H-2274) seedlings (Table 2). The AG-5 (T-106) isolate was found to be weakly pathogenic, whereas some isolates of binucleate *Rhizoctonia* (AG-A, AG-K) isolates were not pathogenic on the tomato cultivar tested. *Rhizoctonia solani* AG-4 and AG-5 isolates were found to be virulent, whereas some isolates of AG-4 (C151, C171 and C179) and AG-5 (C178) were not pathogenic on the cucumber (cv. Beirt Alpha) seedling (Table 3). Differences in virulence level were observed between *R. solani* and binucleate *Rhizoctonia* isolates and among different AGs. Isolates of AG-4 have been reported to damping-off on tomato and cucumber (Jiskani et al., 2007; Lucon et al., 2009).Some AG isolates of binucleate *Rhizoctonia* have been reported as pathogenic, avirulent or weakly pathogenic cultivated plants (Sumner, 1985; Eken and Demirci, 2003).Non-pathogenic or hypovirulent binucleate *Rhizoctonia* isolates have previously been reported to induce systemic resistance in bean and soybean against pathogenic *R. solani* isolates (Poromarto et al., 1998; Jabaji-Hare et al., 1999). In addition, other studies have shown that binucleate *Rhizoctonia* spp. could be effective for the biocontrol of *R. solani* and Pythium spp. diseases (Cubeta and Echandi, 1991; Villajuan-Abgona et al., 1996).

CONCLUSION

This study revealed the presence of various AGs and demonstrated the predominance of AG 4 isolates in tomato and cucumber fields in Erzincan, Turkey. Therefore, AG-4 isolates of *R. solani* may be used while breeding tomato and cucumber for resistance to hypocotyl rot and root rot.

ACKNOWLEDGEMENTS

The work was funded by Ministry of Food, Agriculture and Livestock, the General Directorate of Agricultural Research and Policies (TAGEM), Project No. TAGEM-BS-07/10-01/02-02 and the Scientific Research Fund of Atatürk University, Grant No. 2007/2

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Citation: *Cafer Eken, Serdar Tuncer, "Rhizoctonia Species and Anastomosis Groups Isolated from Tomato and Cucumber in Erzincan, Turkey", International Journal of Research in Agriculture and Forestry, 6(6), 2019, pp 26-31.*