

CHARACTERIZATION AND PATHOGENICITY OF *Rhizoctonia* SPP. ISOLATED FROM VEGETABLE CROPS GROWN IN GREENHOUSES IN SAMSUN PROVINCE, TURKEY

CARACTERIZAÇÃO E PATOGENICIDADE DE *Rhizoctonia* SPP. ISOLADA A PARTIR DE CULTURAS VEGETAIS CRESCIDAS EM ESTUFAS NA PROVÍNCIA DE SAMSUN, TURQUIA

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ABSTRACT: A total of one hundred and five isolates of *Rhizoctonia* belonging to 7 anastomosis groups (AGs) were obtained from the diseased roots and rhizosphere soils of bean, cucumber, eggplant, pepper and tomato plants grown in greenhouses in Samsun province (Black Sea region, Turkey) during the period 2011–2012. The isolates of *Rhizoctonia* spp. were examined for their cultural characteristics, anastomosis groups and pathogenicity. Of these, 83.8% were multinucleate *Rhizoctonia solani* (AG-2, AG-4, AG-5 and AG-6) and 16.2% were binucleate *Rhizoctonia* (AG-A, AG-E and AG-F). Sixty five of the isolates belonged to AG-4 which was the most frequent group (61.9%) in all greenhouses surveyed. Numbers of the isolates belonging to AG-2 (7.6%), AG-5 (6.7%) and AG-6 (7.6%) were 8, 7 and 8, respectively. Seventeen isolates recovered from greenhouses surveyed were identified as binucleate *Rhizoctonia* AG-A (1.9%), AG-E (6.7%) and AG-F (7.6%). All isolates of *Rhizoctonia* spp. tested for growth rates grew at temperatures of 10, 15, 20, 25 and 30°C, whereas they were completely inhibited at 5°C. The results of pathogenicity tests showed that the differences in virulence among isolates of *Rhizoctonia* spp. were statistically significant ($P < 0.001$). The tests on bean seedlings showed that the highest disease severity was caused by AG-4 isolates. The disease severity index (DSI) of the *R. solani* AG-4 isolates ranged from 3.2 to 3.8. In addition, the isolates of three AGs belonging to binucleate *Rhizoctonia* spp. were generally found to be moderately virulent (DSI 2.0–2.4).

KEYWORDS: Anastomosis group. *Rhizoctonia* spp. Root rot. Vegetables. Virulence.

INTRODUCTION

Turkey is the fourth largest vegetable producer in the world after China, India and the USA (FAO, 2012). Vegetables are cultivated well in tropical and subtropical regions and also grown in greenhouses or under plastic structure in cooler areas. Turkey's total vegetable production in greenhouses reaches a cultivation area of 647 594 da and yields about 6,3 million tonnes. Samsun province, located in the Black Sea region of Turkey, has the largest greenhouse production area in the region with a total cultivation area of 27 678 da and production of 130 696 tonnes/year (ANONYMOUS, 2015). The most common vegetable species grown in greenhouses in Samsun province are bean (*Phaseolus vulgaris*), cucumber (*Cucumis sativus*), eggplant (*Solanum melongena*), pepper (*Capsicum annuum*) and tomato (*Solanum lycopersicum*).

Rhizoctonia species are soilborne fungi with a wide host range, which occur commonly worldwide. There are three main groups of the *Rhizoctonia* form genus in the anamorphic classification: multinucleate (MN) *Rhizoctonia* (teleomorphs: *Thanatephorus* and *Waitea*), binucleate (BN) *Rhizoctonia* (teleomorphs:

Ceratobasidium and *Tulasnella*), and uninucleate (UN) *Rhizoctonia* (teleomorph: *Ceratobasidium*) (SHARON et al., 2008). They exist as groups whose cultural characteristics, hosts and virulence differ (ERPER et al., 2006). *Rhizoctonia solani* Kühn [teleomorph: *Thanatephorus cucumeris* (Frank) Donk] is a common soilborne fungus. The fungus is considered as a very destructive soilborne plant pathogen, with a broad host range, and causes diseases in a great variety of crops, including agronomical, ornamental and forestry species (GONZALEZ GARCIA et al., 2006; JAISWAL et al., 2014).

Isolates of *R. solani* and BN *Rhizoctonia* spp. are classified into anastomosis groups (AGs) based on their hyphal anastomosis interactions. So far, 14 AGs of *R. solani*, AG-1 to AG-13, including AG-BI as a subset of AG-2 and 16 AGs of BN *Rhizoctonia* (AG-A-S) have been reported (SHARON et al., 2008). The classical hyphal fusion method for the identification and classification of *Rhizoctonia* spp. into AGs has widely been used.

Root rot disease caused by soilborne pathogens cause significant losses on different vegetable plants. *R. solani* is found on bean, cucumber, eggplant, pepper and tomato, which are

important vegetable crops. In addition, *R. solani* can cause several types of damage, including damping-off, root rot and web blight on bean (MUYOLO et al., 1993; KARACA et al., 2002; EKEN; DEMIRCI, 2004; ERPER et al., 2011), damping-off and root rot on cucumber (ERPER et al., 2002; HARATIANA et al., 2013; JAISWAL et al., 2014), brown spot on eggplant (SNEH et al., 1991), damping-off, hypocotyl rot and root rot on pepper (KARACA et al., 2002, VELÁSQUEZ; VICTORIANO, 2007; LOPEZ et al., 2009; TUNCER; EKEN, 2013) and damping-off, foot and root rot on tomato (KARACA et al., 2002; MISAWA; KUNINAGA, 2010; SOLANKI et al., 2012).

Five AGs of *R. solani* (AG-1, AG-4, AG-5, AG-6 and AG-7) and 3 AGs of BN *Rhizoctonia* spp. (AG-A, AG-B and AG-K) had been recovered from plants of bean and their rhizosphere soils in Samsun province (ERPER et al., 2011). Additionally, in Turkey, isolates of AG-1, AG-2 type 1, AG-3, AG-4 and AG-6 of *R. solani*, and the binucleate *Rhizoctonia* spp. (AG-A, AG-G and AG-K) had been determined on pepper (DEMIRCI; DOKEN, 1995; TUNCER; EKEN, 2013). Similarly, BN *Rhizoctonia* AG-A, AG-E and AG-F have been reported from bean, cucumber and tomato (SNEH et al., 1991).

Although pathogen-free soilless mix and fungicide seed treatments are used in greenhouse culture, *R. solani* may be introduced on seed, in contaminated potting mix, or by residues in the greenhouse bench (LEWIS; LUMSDEN, 2001). The pathogen may then induce post emergence damping-off, or after transplanting to a pathogen-infested field, root rot, foliar blight or fruit rot may occur. In the previous studies, researchers reported that *Rhizoctonia* group fungi were detected on different vegetable crops grown in greenhouses, including cucumber, eggplant, lettuce, tomato and pepper worldwide (LEWIS; LUMSDEN, 2001; ERPER et al., 2002). Mitidieri and Mitidieri (1994) detected *R. solani* on tomato and pepper plants grown in greenhouses in Argentina. In another study, Erper et al., (2002) isolated forty seven isolates of *R. solani* AG-4 and one isolate of BN *Rhizoctonia* AG-Ba from the cucumber plants with root rot symptoms in greenhouses in Samsun province.

Although *Rhizoctonia* spp. have been considered as very important pathogens causing high yield losses on different vegetable crops in Turkey, there is no detailed study describing the AGs of *Rhizoctonia* spp. occurring on some important vegetable crops grown in greenhouses in Samsun province. Thus, the aim of this study was to determine the AGs of the isolates of *Rhizoctonia*

spp. obtained from the five plants (bean, cucumber, eggplant, pepper and tomato) showing symptoms of root rot and from their rhizosphere soils in greenhouses in Samsun province (Black Sea region). We also examined the colony morphology and virulence of the isolates by inoculation tests.

MATERIAL AND METHODS

Collection and isolation of *Rhizoctonia*

The surveys were conducted at 70 locations in eight towns (Alaçam, Atakum, Bafra, Çarşamba, Ondokuzmayıs, Salıpazarı, Tekkeköy and Terme) belonging to Samsun province in the vegetation period of 2011–2012 years. Root samples showing necrotic lesions and rhizosphere soils of five vegetable crops (bean, cucumber, eggplant, pepper and tomato) were randomly collected from greenhouses in the eight towns. Two to three diseased plants were uprooted per greenhouses and necrotic root sections were cut from affected areas. Infected plant tissues were washed under tap water and they were cut into small pieces, placed in 1% NaOCl for 2 min, rinsed twice with sterile-distilled water (SDW) and blotted dry on sterile paper towels. Small pieces of discoloured root tissues were placed on 2% water agar (WA) amended with 3 mL 10% lactic acid in 9-cm-diameter Petri plates (ERPER et al., 2011). After incubation for two days at 25°C in the dark, hyphae were examined under a light microscope (100X), and fungal colonies possessing the general characteristics of *Rhizoctonia* spp. were transferred to the plates containing potato dextrose agar (PDA) (Oxoid Ltd, UK). In addition, soil samples were placed in two parallel small plastic pots and watered to saturation with SDW. Sterile oat stem pieces of 2–3 cm long were embedded into soil and pots were kept at room temperature for 5 days under the plastic covering. Oat stem pieces were then washed with tap water, surface-disinfected and placed on acidified WA plates. Similarly, after incubation for two days at 25°C in the dark, hyphae were examined, and fungal colonies possessing the general characteristics of *Rhizoctonia* spp. were transferred to the plates containing PDA. One set of isolates was kept on PDA slants at 4°C in the dark and another set was stored as colonized sterile oat seeds in test tubes under similar conditions.

Staining of nuclei

To distinguish isolates of MN *R. solani* and BN *Rhizoctonia* spp., they were transferred to PDA and incubated at 25°C in the dark. Developing young hyphae of the isolates were stained with

Safranin O and 3% KOH and the number of nuclei were observed for at least 20 different cells, by using an Olympus CX31 compound microscope (Olympus America Inc., USA) at 400X magnification (MISAWA; KUNINAGA, 2010).

Hyphal anastomosis

Anastomosis groups were determined by pairing the isolates with known AG tester isolates. The isolates of *Rhizoctonia* and testers were transferred to PDA, and activated at 25°C. Sterile coverslips were dipped in PDA containing 0.5% agar and placed on 1.5% WA plates. Five millimeter diameter agar discs of the isolates cut from the margins of the growing colonies were placed 1 cm apart on opposite sides of the coverslips on WA. After 24–48 h incubation at 25°C, hyphal anastomosis was determined by transferring the coverslip onto a microscope slide and staining with Safranin O and 3% KOH using the Olympus CX31 compound microscope at 400X magnification (ERPER et al., 2006). All tester isolates used in this study were provided by Dr. Erkol Demirci, Karadeniz Technical University.

Morphological characterization of *Rhizoctonia* isolates

The colour of colony and size, shape and colour of sclerotia of the twenty-two isolates of randomly selected *Rhizoctonia* spp., representing all anastomosis groups were evaluated for colony morphology (Table 2). The isolates were grown on PDA in the dark at 25°C for 3 weeks. The size, shape and colour of sclerotia and colour of colony on the reverse of the plates of the isolates grown were described using the colour chart of the Royal Horticultural Society of London (ERPER et al., 2013). Five replicates were used per isolate and the experiment was conducted once.

Determination of the number of nuclei and hyphal diameter

The twenty-two isolates of *Rhizoctonia* spp. were grown on PDA plates at 25°C in the dark (Table 2). Sterile coverslips were dipped in PDA containing 0.5% agar and placed on 1.5% WA plates. Five mm diameter agar disks from the edge of *Rhizoctonia* colonies growing on PDA were placed 1 cm near the coverslips on WA, and coverslips were transferred onto microscope slides after 24–48 h incubation at 25°C. The number of nuclei was counted after staining with one drop of Safranin O solution and one drop of 3% KOH. Nuclei were counted in 20 hyphal cells of actively growing cultures per isolate. Besides, hyphal

diameter was determined for each isolate by measuring 20 cells at right angles to the longitudinal cell wall by using the Olympus CX31 compound microscope at 400X magnification (ERPER et al., 2006).

Radial growth of isolates of *Rhizoctonia*

Seven isolates of randomly selected *Rhizoctonia* spp., representing each anastomosis group (Figure 1) were tested for growth rate at seven temperatures: 5, 10, 15, 20, 25, 30 and 35 °C. Five mm diameter agar disks from the edge of 4-day-old colonies growing on PDA at 25°C were placed in the centre of 9-cm-diameter Petri plates containing PDA, which were then incubated in the dark at seven temperatures above (ERPER et al., 2006). The radius of each colony was measured at two different points after 24 and 48 h. Radial growth rates in millimeters per day were calculated (ERPER et al., 2013). Five replicates were used for each isolate at each temperature. The experiment was conducted once.

Pathogenicity assays

The pathogenicity test was performed using *in vitro* bioassays (agar-plate) and *in vivo* experiments. The pathogenicity and level of virulence of 105 isolates of *Rhizoctonia* spp. belonged to seven AGs (Table 1) was determined on bean cv. Gina seedlings. For *in vitro* experiments, an agar plate assay was used the method of Tuncer and Eken (2013). Isolates were transferred to PDA and incubated at 25°C for 2–3 days. Five mm diameter agar plugs with mycelium from the growing edge of the cultures were then transferred to 9-cm-diameter Petri plates including 2% water agar and incubated under same conditions for two and more days. Bean seeds were surface disinfested in 1% NaOCl for 3 minutes, rinsed with sterile-distilled water and blotted dry. Five seeds were placed on the plates adjacent to the margin of the growing mycelia. After 10 days incubation at 25°C, disease severity index (DSI) was assessed by evaluating seedling/root symptoms using the following scale modified from Muyolo et al. (1993): 0 = healthy seedling; 1 = very little superficial lesions on the roots or on the hypocotyls; 2 = deep and large lesions on the roots or on the hypocotyl; 3 = severe root rot, lesions surrounding hypocotyl, partially restricted root length and 4 = complete root rot.

According to *in vitro* pathogenicity test results, twenty-one isolates belonging to different AGs that showed the highest virulence *in vitro* were chosen for *in vivo* experiments. Pathogenicity of the

selected isolates was determined on bean (cv. Gina) seedlings *in vivo*. Inoculum was prepared on the moistened sterile oat grains in test tubes, which were autoclaved twice at 24 h intervals, inoculated with plugs of mycelium from cultures of the isolates grown on PDA and incubated at 25°C for 21 days.

For seedling tests, two bean seeds were planted in 16-cm-diam plastic pots (1 L) containing sterilized mixture of sandy loam soil:composted manure:washed sand (2:2:1,v/v/v), in a greenhouse at 17–25°C. Two-week-old seedlings were inoculated by gently removing the soil mixture from one side of the stem, placing 15 colonized oat grains in contact with each seedling and covering the inoculum with the soil mixture (ERPER et al., 2011). In control treatments, sterile oat grains were used. Pathogenicity tests were carried out in a completely randomized design with five replicates for each isolate. Twenty-one days after inoculation, plants were gently uprooted and washed free of soil. DSI was assessed by evaluating seedling/root symptoms using the same scale used *in vitro* bioassays. In addition, plant height, shoot and root dry weights and root length were also recorded for each plant. Symptomatic roots were aseptically plated on PDA to complete Koch's postulates. The experiments were conducted once.

Statistical analysis

To analyze the data of *in vivo* test, Kolmogorov-Smirnov one sample test was performed to examine the normality, pathogenicity test results showed that all data have not distributed normally. Levene variance homogeneity test was performed to obtain the information about homoscedasticity, and according to the results, data for disease severity and plant growth data (plant height, shoot and root dry weights and root length) had not similar variances. Then, non-parametric permutation tests were applied to the data. To compare the means, posterior pairwise permutation tests were used. For disease severity and plant growth data 999999 permutation was done. Data analyses were utilized with software *NPMANOVA* written by Anderson (ONDER, 2007).

RESULTS

Rhizoctonia species and anastomosis groups

A total of one hundred and five isolates of *Rhizoctonia* belonging to 7 AGs were obtained from their diseased roots and rhizosphere soils of bean,

cucumber, eggplant, pepper and tomato plants grown in greenhouses in Samsun. Out of those, 88 isolates were identified as multinucleate *R. solani* (83.8%) and 17 isolates as binucleate *Rhizoctonia* (16.2%) by nucleus staining. Four anastomosis groups were determined among the multinucleate isolates. Sixty-five of the isolates belonged to AG-4 which was the most frequent group (61.9%) in all greenhouses surveyed. Numbers of the isolates belonging to AG-2 (7.6%), AG-5 (6.7%) and AG-6 (7.6%) were 8, 7 and 8, respectively. Seventeen isolates recovered from the greenhouses surveyed were identified as binucleate *Rhizoctonia* AG-A (1.9%), AG-E (6.7%) and AG-F (7.6%). As a result of the study, of 37 cucumber isolates, 19 were obtained from root samples and the remaining 18 were from rhizosphere soils. Numbers of the isolates of obtained from eggplant and pepper were less than the number of isolates obtained from three other plants. Additionally, a result of distribution of isolates of *Rhizoctonia* spp. belonging to 7 AGs obtained from the roots of five plants and from their rhizosphere soils were given in Table 1.

Cultural characteristics

Colony morphology

After 3 weeks of incubation in the dark at 25°C, all colonies of *R. solani* AG-2 and AG-4 became brown, whereas AG-5 and AG-6 colonies became grey-brown with age. Additionally, the isolates belonging to four AGs of *R. solani* also produced sclerotia on PDA, which were variable in size and often aggregated into compound sclerotia. For instance, the diameters of sclerotia of isolates of AG-4 varied from 0.3 to 1.10 mm. The sclerotia were generally grey-brown coloured at first, then became dark brown with age. The isolates of BN *Rhizoctonia* AG-A had white-orange mycelia and formed superficial sclerotia (ranged from 0.1 to 0.35 mm) that were scattered on the plate surface. Additionally, the colonies of BN *Rhizoctonia* AG-E became grey-brown, whereas AG-F colonies became greyed-yellow with age. Nuclear numbers, hyphae and sclerotia diameters of the isolates of *Rhizoctonia* obtained from five plants were given in Table 2.

Table 1. Anastomosis groups (AGs) and isolate numbers of *Rhizoctonia* spp. recovered from roots and rhizosphere soils of bean, cucumber, eggplant, pepper and tomato plants grown in greenhouses in Samsun province.

Species/ Anastomosis Groups (AGs)	Origin of the isolates										Total
	Cucumber		Bean		Tomato		Eggplant		Pepper		
	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	
<i>Rhizoctonia solani</i>											
AG-2	-	-	-	-	1	7	-	-	-	-	8
AG-4	15	11	10	8	9	3	2	3	2	2	65
AG-5	2	-	-	2	1	-	-	-	-	2	7
AG-6	2	1	2	-	3	-	-	-	-	-	8
Binucleate <i>Rhizoctonia</i>											
AG-A	-	2	-	-	-	-	-	-	-	-	2
AG-E	-	1	2	2	-	-	-	-	-	3	8
AG-F	-	2	-	5	-	-	-	-	-	-	7
Total	19	17	14	17	14	10	2	3	2	7	105

Table 2. Colour of colony, number of nuclei in mature hyphal cells, hyphal diameter and diameter of sclerotia of isolates of *Rhizoctonia* spp. obtained from roots and rhizosphere soils of bean, cucumber, eggplant, pepper and tomato, grown on potato dextrose agar (PDA).

Species/Anastomosis groups (AGs)	Number of isolates studied	Colony colour ^a	Number of nuclei per cell ^b	Hyphal diameter ^b (µm)	Sclerotia diameter ^b (mm)
<i>Rhizoctonia solani</i>					
AG-2	3	Brown	(3-) 5.5 (-8) ^c	(4.3-) 6.9 (-9.5)	(0.15-) 0.60 (-1.05)
AG-4	5	Brown	(2-) 5.5 (-9)	(4.5-) 7.0 (-9.5)	(0.30-) 0.70 (-1.10)
AG-5	3	Grey-brown	(3-) 6.0 (-9)	(4.9-) 7.7 (-10.5)	(0.28-) 0.67 (-1.07)
AG-6	3	Grey-brown	(3-) 6.5 (-10)	(4.7-) 6.8 (-8.9)	(0.25-) 0.68 (-1.12)
BN <i>Rhizoctonia</i>					
AG-A	2	White-orange	(1-) 1.7 (-2)	(3.0-) 3.8 (-4.5)	(0.10-) 0.22 (-0.35)
AG-E	3	Grey-brown	(1-) 1.5 (-2)	(3.4-) 4.7 (-6.0)	(0.15-) 0.40 (-0.65)
AG-F	3	Greyed-yellow	(1-) 1.5 (-2)	(3.2-) 4.7 (-6.1)	(0.10-) 0.19 (-0.28)

^a – colony colours were determined on PDA by using the color chart of the Royal Horticultural Society of London; ^b – twenty observations per isolate; ^c – mean and range values

Radial growth

All isolates of *Rhizoctonia* spp. tested for growth rates grew at temperatures of 10, 15, 20, 25 and 30 °C, whereas they were completely inhibited at 5°C. However, four isolates (Rs-7, Rs-70, Rs-86 and R-40) grew at 35°C on PDA. Growth rates of the isolates of *R. solani* AG-2 (Rs-7), AG-4 (Rs-70) and AG-5 (Rs-86), and isolates of BN *Rhizoctonia* AG-A (R-40), AG-E (R-23) and AG-F (R-15) increased as temperature increased up to 25°C, and decreased above this temperature. However, the growth of AG-6 isolate (Rs-64) increased up to 30°C, then decreased (Figure 1).

Pathogenicity of the *Rhizoctonia* isolates

As a result of the pathogenicity test, it was found that the differences among the pathogenicity and levels of virulence of isolates of MN *R. solani* and BN *Rhizoctonia* were statistically significant ($P < 0.001$). The tests on bean seedlings showed that the highest disease severity was caused by AG-4 isolates. The DSI of the *R. solani* AG-4 isolates ranged from 3.2 to 3.8. Symptoms developed by one week after inoculation in some treatments, and were damping-off, namely rotting of stem and root tissues at and below the soil surface. However, nine isolates belonging to *R. solani* AG-2, 5 and 6 were found to be moderately virulent (DSI ranged from 2.4 to 2.8), with the exception of Rs-42 isolate belonging to AG-2.

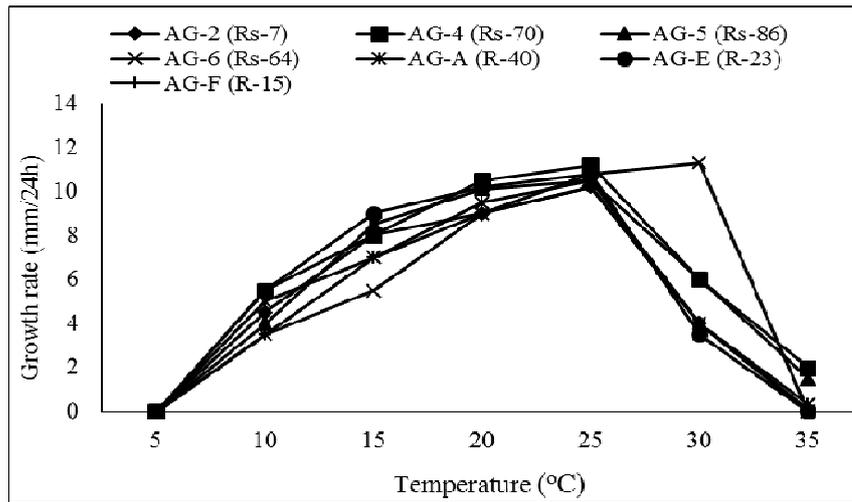


Figure 1. Radial growth rates of *Rhizoctonia solani* AG-2 (Rs-7), AG-4 (Rs-70), AG-5 (Rs-86) and AG-6 (Rs-64), and BN *Rhizoctonia* AG-A (R-40), AG-E (R-23) and AG-F (R-15) isolates at 5–35°C.

The four isolates of three AGs belonging to binucleate *Rhizoctonia* spp. were found to be moderately virulent (DSI 2.0–2.4). However, the virulence of isolates of BN *Rhizoctonia* AG-E (R-23) and AG-F (R-8) were found to be low, namely, DSI of these isolates were 1.8 and 1.6, respectively. In addition, most of the isolates of *Rhizoctonia* spp.

reduced plant growth parameters (plant height, shoot and root dry weights and root length) when compared to the uninoculated controls (Table 3). All the isolates used in the pathogenicity test were reisolated from the affected plants on PDA as described above, confirming Koch’s postulates.

Table 3. Pathogenicity of isolates of *Rhizoctonia* obtained from the roots and rhizosphere soils of bean, cucumber, eggplant, pepper and tomato plants grown in greenhouses on seedlings of the cv. Gina twenty-one days after inoculation.

Species/ Anastomosis Groups (AGs)	Isolate number	Root			Shoot dry weight (g)	Plant height (cm)
		Disease severity index ^a	Dry weight (g)	Length (cm)		
<i>Rhizoctonia solani</i>						
AG-2	Rs-7	2.4 ^b ±0.24 ^{c-f}	0.026±0.01 ^b	3.50±0.50 ^{de}	0.40±0.06 ^{c-g}	21.30±1.81 ^{cd}
	Rs-42	1.8±0.37 ^{ef}	0.018±0.00 ^{bc}	6.00±0.94 ^{bc}	0.48±0.08 ^{cd}	19.70±1.81 ^{cde}
	Rs-58	2.4±0.24 ^{c-f}	0.018±0.00 ^{bc}	4.30±0.83 ^{cde}	0.25±0.03 ^{ghi}	16.50±1.24 ^{ef}
AG-4	Rs-10	3.8±0.20 ^a	0.010±0.00 ^c	0.46±0.26 ^f	0.15±0.02 ^{ij}	7.50±0.79 ^{gf}
	Rs-44	3.6±0.24 ^{ab}	0.010±0.00 ^c	0.42±0.16 ^f	0.16±0.02 ^{hij}	9.70±0.98 ^g
	Rs-57	3.8±0.20 ^a	0.010±0.00 ^c	0.94±0.26 ^f	0.02±0.00 ^j	4.50±1.02 ^h
	Rs-70	3.6±0.24 ^{ab}	0.010±0.00 ^c	0.62±0.27 ^f	0.12±0.03 ^{ij}	5.90±0.75 ^{gh}
AG-5	Rs-80	3.2±0.37 ^{abc}	0.010±0.00 ^c	0.94±0.26 ^f	0.05±0.01 ^j	5.80±0.68 ^{gh}
	Rs-94	3.6±0.24 ^{ab}	0.010±0.00 ^c	0.82±0.39 ^f	0.14±0.02 ^{ij}	8.20±1.66 ^{gh}
AG-5	Rs-9	2.4±0.24 ^{c-f}	0.020±0.00 ^{bc}	3.10±0.51 ^e	0.45±0.04 ^{c-f}	19.80±0.98 ^{cde}

	Rs-26	2.4±0.24 c-f	0.020±0.00 bc	5.1±1.10 b-e	0.58±0.09 bc	21.70±1.45 c
	Rs-86	2.8±0.20 bcd	0.020±0.00 bc	6.50±0.63 b	0.48±0.07 cd	21.40±1.73 bc
AG-6	Rs-36	2.4±0.24 c-f	0.014±0.00 c	5.10±0.76 b-e	0.28±0.09 e- i	15.10±1.79 f
	Rs-64	2.4±0.24 c-f	0.018±0.00 bc	3.40±0.43 de	0.38±0.08 d- g	20.00±0.61 cde
	Rs-93	2.6±0.24 cde	0.018±0.00 bc	5.60±0.58 bc	0.43±0.08 c- g	18.80±1.54 c-f
Binucleate <i>Rhizoctonia</i>						
AG-A	R-40	2.4±0.24 c-f	0.018±0.00	5.60±0.29	0.68±0.07 b	27.20±1.27 b
	R-41	2.4±0.24 c-f	0.014±0.00 c	4.80±0.51 b-e	0.46±0.08 cde	22.60±0.70 c
AG-E	R-23	1.8±0.37 ef	0.018±0.00 bc	5.40±0.53 bcd	0.38±0.05 d- g	21.80±0.51 c
	R-98	2.0±0.32 def	0.016±0.00 bc	4.70±0.25 b-e	0.39±0.04 d- g	20.10±0.83 cde
AG-F	R-8	1.6±0.24 f	0.014±0.00 c	4.00±0.35 cde	0.32±0.01 d- h	19.70±0.98 cde
	R-82	2.0±0.32 def	0.016±0.00 bc	4.20±0.25 cde	0.27±0.02 f- i	17.40±0.62 def
	Control	0.0±0.0 g	0.068±0.01 a	24.30±1.47 a	0.91±0.05 a	47.60±2.38 a
P value		<0.001	<0.001	<0.001	<0.001	<0.001

^a – Roots symptoms were evaluated on the following scale: 0 = healthy seedling, 1 = very little superficial lesions on roots or on the hypocotyls, 2 = deep and large lesions on the roots or on the hypocotyl, 3 = severe root rot, lesions surrounding hypocotyl, partially restricted root length and 4 = complete root rot. ^b – values represent the mean of five replications for each isolate; ^c – mean values followed by standard error of the mean; ^d – means in a column followed by the same letter are not significantly different according to Pairwise permutation test ($P < 0.001$).

DISCUSSION

This is the first detailed study on various isolates of *Rhizoctonia* spp. belonging to different AGs obtained from diseased roots and rhizosphere soils of bean, cucumber, eggplant, pepper and tomato plants grown in greenhouses in Samsun province, which has the largest greenhouse production area of Black Sea region. Of all the *Rhizoctonia* isolates obtained from the five plants, 83.8% were *R. solani* (AG-2, AG-4, AG-5 and AG-6), and 16.2% were binucleate *Rhizoctonia* (AG-A, AG-E and AG-F).

In the previous studies, researchers reported that *R. solani* and BN *Rhizoctonia* spp. were detected on different vegetable crops, including bean, cucumber, eggplant, melon, onion, pepper, pumpkin, tomato, watermelon and winter squash worldwide (SNEH et al., 1991; ERPER et al., 2002; 2006; 2011; 2015; MIRMAJLESSI et al., 2012; MISAWA; KUNINAGA, 2010).

Karaca et al. (2002) isolated 229 isolates belonging to *R. solani* AG-2-2, AG-4 and AG-5 from roots and rhizosphere soils of the bean plants

in Samsun province. In a similar study, a total of 434 isolates of *Rhizoctonia* spp. belonging to 9 anastomosis groups (MN *R. solani* AG-1, AG-4, AG-5, AG-6, AG-7, and BN *Rhizoctonia* AG-A, AG-B, AG-E and AG-K) were obtained from the roots and rhizosphere soils of bean and soybean plants grown in Samsun province, and AG-4 was found to be the most common group on bean and soybean plants (ERPER et al., 2011). In another study, isolates of *R. solani* AG-4 were recovered from infected winter squash plants grown in the Black Sea region (ERPER et al., 2015). However, in Turkey, isolates of AG-1, AG-2 type 1, AG-3, AG-4, AG-5, AG-6, AG-9, AG-10 and AG-11 of *R. solani* and AG-A, AG-E, AG-F, AG-G, AG-I and AG-K of BN *Rhizoctonia* were previously detected on bean and pepper plants in different regions of Turkey (DEMIRCI; DOKEN, 1995; EKEN; DEMIRCI, 2004; TUNCER; EKEN, 2013). In addition, Misawa and Kuninaga (2010) identified foot rot caused by *R. solani* AG-2-1 and AG-3 on mature tomato plants in Hokkaido, Japan.

Similarly, other researchers reported that *Rhizoctonia* group fungi were detected on

cucumber, eggplant, lettuce, tomato and pepper grown in greenhouses worldwide (MITIDIERI; MITIDIERI, 1994; LEWIS; LUMSDEN, 2001; ERPER et al., 2002). Mitidieri and Mitidieri (1994) detected *R. solani* on tomato and pepper plants grown in greenhouses in Argentina. In the present study, 65 of the isolates belonged to *R. solani* AG-4 which was the most frequent group (61.9%) in all greenhouses surveyed. Similar findings were reported by previous researchers. Erper et al. (2002) found that a total of 48 *Rhizoctonia* isolates were recovered from the roots of cucumber plants, which were with root rot symptoms in greenhouses in Samsun. As a result of the study, *R. solani* AG-4 was found as the most prevalent anastomosis group (97.9%). Additionally, Tuncer and Eken (2013) found that more than three quarters of the 81 *R. solani* isolates obtained from peppers grown in Erzincan province, Turkey belonged to AG-4. Alike, the most common occurrence of *R. solani* AG-4 isolates on cucumber, pepper and snap bean plants has also been reported from other countries by several workers (MIRMAJLESSI et al., 2012; HARATIANA et al., 2013).

Colony characteristics of isolates belonging to *R. solani* AG-4 were found to be similar to those of other studies (SNEH et al., 1991; ERPER et al., 2006). After 3 weeks of incubation, all colonies of *R. solani* AG-4 became brown colour, and their sclerotia were generally grey-brown coloured at first, then became dark brown with age. The results are compatible with those of Erper et al. (2006), who reported that AG-4 isolates produced brown coloured colonies on PDA after 2 weeks incubation and their sclerotia were approximately 0.4 mm diameter, light brown, becoming dark brown with age. We found that the optimum growth temperature of isolates of *R. solani* AG-2 (Rs-7), AG-4 (Rs-70) and AG-5 (Rs-86), and isolates of BN *Rhizoctonia* AG-A (R-40), AG-E (R-23) and AG-F (R-15) was 25°C. The growth rates of the isolates decreased above this temperature. The temperature of 25°C has previously been reported to be optimal for *R. solani* AG-4, BN *Rhizoctonia* AG-A and AG-K isolates obtained from different plants (DEMIRCI, 1998; ERPER et al., 2006; MISAWA; KUNINAGA, 2010). Demirci (1998) found that *R. solani* AG-4 (WH1) and AG-K (BO2) isolates from wheat and barley grew best at 25°C. In another study, optimum growth of *R. solani* AG-4 isolates (TM-40 and TY-19) obtained from onion were between 25–30°C (ERPER et al., 2006). Likewise, Misawa and Kuninaga (2010) found that *R. solani*

AG-2-1 (N1 and N2) isolates obtained from tomato grew optimum at 25°C, and their growth rate at this temperature was 15.7 mm/24 h. On the contrary, in the present study, the isolate of AG-6 (Rs-64) grew best at 30°C (Table 2).

In our study, among all the isolates of *Rhizoctonia* spp. examined, the isolates of *R. solani* AG-4 had the highest virulence (DSI 3.2–3.8) on the bean plants. In addition, BN *Rhizoctonia* AG-A, AG-E and AG-F isolates were moderately virulent (DSI 2.0–2.4), whereas the virulence of R-23 (AG-E) and R-8 (AG-F) isolates belonging to BN *Rhizoctonia* were low. The results of previous studies are in agreement with the finding that isolates of AG-4 were more aggressive than those of other BN *Rhizoctonia* anastomosis groups on different host plants (ERPER et al., 2002; 2011). Erper et al. (2006) found that the isolates of *R. solani* AG-4 had the highest virulence on onion plants, whereas the isolate TA-18 of BN *Rhizoctonia* AG-B had low virulence.

CONCLUSIONS

The results have showed that root rot on the five plants is predominantly caused by *R. solani* AG-4 isolates, and that the virulence of the isolates of other AGs belonging to *R. solani* and BN *Rhizoctonia* on these crops was moderate or low.

Soilborne fungal pathogens can cause economic yield losses on different plants in the countries, where vegetable cultivation is common. Therefore, suitable cultural practices, such as the use of resistant cultivars and the improvement of soil conditions, will be useful.

In addition, it is known that chemical control is not so efficient against *Rhizoctonia* spp. Therefore, it will be useful to determine biocontrol agents that could be effective against *R. solani* AG-4, and to evaluate their efficiency by *in vivo* and *in vitro* trials.

This work provided a better understanding of the *Rhizoctonia* spp. associated with bean, cucumber, eggplant, pepper and tomato plants and their rhizosphere soils in greenhouses in Samsun, the Black Sea region.

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RESUMO: Um total de cento e cinco isolados de *Rhizoctonia* pertencentes a 7 grupos de anastomose (AGs) foram obtidos a partir de raízes doentes e solos rizosféricos de plantas de feijão, pepino, berinjela, pimenta e tomate cultivados em estufas na província de Samsun (região do Mar Negro, Turquia) durante o período 2011-2012. Os isolados de *Rhizoctonia* spp. foram examinados por suas características culturais, grupos de anastomose e patogenicidade. Destes, 83,8% eram *Rhizoctonia solani* multinucleadas (AG-2, AG-4, AG-5 and AG-6) e 16,2% era *Rhizoctonia* binucleadas (AG-A, AG-E and AG-F). Sessenta e cinco dos isolados pertenciam ao AG-4, que foi o grupo mais freqüente (61,9%) em todas as estufas pesquisadas. O número de isolados pertencentes a AG-2 (7,6%), AG-5 (6,7%) e AG-6 (7,6%) foi de 8, 7 e 8, respectivamente. Dezesete isolados recuperados de estufas pesquisadas foram identificados como *Rhizoctonia* binucleada AG-A (1,9%), AG-E (6,7%) e AG-F (7,6%). Todos os isolados de *Rhizoctonia* spp. testados para taxas de crescimento cresceram a temperaturas de 10, 15, 20, 25 e 30°C, enquanto que foram completamente inibidos a 5°C. Os resultados dos testes de patogenicidade mostraram que as diferenças de virulência entre os isolados de *Rhizoctonia* spp. foram estatisticamente significativas ($P < 0,001$). Os testes em mudas de feijão mostraram que a maior severidade da doença foi causada por isolados AG-4. O índice de gravidade da doença (do inglês, disease severity index - DSI) dos isolados de *R. solani* AG-4 variou de 3,2 a 3,8. Além disso, os isolados de três AGs pertencentes à *Rhizoctonia* spp. binucleadas foram geralmente encontrados como moderadamente virulentos (DSI 2,0-2,4).

PALAVRAS-CHAVE: Grupo de anastomose. *Rhizoctonia* spp. Podridão radicular. Legumes. Virulência.

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