

**Journal of Animal & Plant Sciences, 32(3): 2022, Page: 721-728**

ISSN (print): 1018-7081; ISSN (online): 2309-8694

<http://doi.org/10.36899/JAPS.2022.3.0473>

## **RHIZOCTONIA SPECIES FROM STRAWBERRY PLANTS IN ERZINCAN, TURKEY: ANASTOMOSIS GROUPS AND PATHOGENICITY**

T. G. Kesimci<sup>1</sup>, E. D. Durak<sup>2</sup> and E. Demirci<sup>3</sup>

<sup>1</sup>Department of Plant Protection, Faculty of Agriculture, Iğdir University, Iğdir, TURKEY

<sup>2</sup>Department of Plant Protection, Faculty of Agriculture, Yuzuncu Yil University, Van, TURKEY

<sup>3</sup>Department of Molecular Biology and Genetics, Faculty of Science, Karadeniz Technical University, Trabzon, TURKEY

Corresponding author's email: tubagenc25@hotmail.com

### **ABSTRACT**

This study was carried out to determine anastomosis groups and pathogenicity of 166 *Rhizoctonia* isolates obtained from strawberry plant samples in Erzincan province, Turkey during 2009 and 2010. *Rhizoctonia* species and anastomosis groups (AGs) of all the isolates were determined using classical techniques. Molecular characterizations of the selected *Rhizoctonia* isolates were performed by sequence analysis of the internal transcribed spacer (ITS) regions of the ribosomal DNA (rDNA). Of these, 155 isolates were identified as binucleate (BN) *Rhizoctonia* spp., and 11 isolates were as multinucleate (MN) *Rhizoctonia solani*. Three BN *Rhizoctonia* isolates were identified as *Ceratobasidium albasitensis*, the remaining BN *Rhizoctonia* isolates were assigned to AG-A (54 isolates), AG-E (11 isolates), AG-G (37 isolates), AG-H (6 isolates), AG-K (44 isolates). *Rhizoctonia solani* isolates were classified as AG-2-1 (4 isolates), AG-4 HGII (3 isolates) and AG-5 (4 isolates). Pathogenicity test was conducted on strawberry plants (cv. Fern), and AG-G isolates were found to constitute the highest disease severity than other species or AGs of *Rhizoctonia* obtained in this study. BN *Rhizoctonia* AG-E, *R. solani* AG-5 and *C. albasitensis* were first time reported on strawberry plants in Turkey.

**Keywords:** Anastomosis group, black root rot, *Ceratobasidium albasitensis*, rDNA-ITS region, *Rhizoctonia*, strawberry.

Published first online October 20. 2021

Published final May 30. 2022

### **INTRODUCTION**

The cultivated strawberry (*Fragaria x ananassa* Duchesne) is one of the most important berry crops and has increasingly become economically important in the world (Fang *et al.*, 2013). Turkey was the fourth largest strawberry producer after China, United States of America and Mexico, respectively in 2018 (FAO, 2020). Strawberry yields and fruit quality are influenced by diseases, pests and unsuitable environmental factors. Fungal diseases have caused economic losses and decreased fruit quality in strawberry cultivation (Mirmajlessi *et al.*, 2018). Black root rot is considered to be a complex disease, caused by the interaction of several pathogens (*Pythium* spp., *Fusarium* spp., *Cylindrocarpon* spp. and *Rhizoctonia* spp.), environmental factors and nematodes on strawberry (Botha *et al.*, 2003; Manici *et al.*, 2005; Matsumoto and Yoshida, 2006). Typical symptoms of black root rot are shorter root system, presence of darker areas on the root, extensive death of feeder rootlets and death of some parts or all of the main roots (Yılmaz, 2006). The pathogens also infect other parts of strawberry plants,

causing stunting, wilting, necrosis and death of plant (Vico, 1994; Sharon *et al.*, 2007). The genus *Rhizoctonia* is one of the most important pathogens of strawberry black root rot disease (Martin, 1988; Sneh *et al.*, 1991; Manici and Bonora, 2007; Sharon *et al.*, 2007).

In several studies, *Fusarium* spp. (Demirer-Durak and Demirci, 2014; Sarigül Ertek *et al.*, 2018), *Verticillium dahliae* (Genc Kesimci and Demirci, 2020), *Rhizoctonia* spp., (Dinler, 2014; Demirer-Durak and Demirci, 2018; Dinler *et al.*, 2018; Sarigül Ertek *et al.*, 2018), *Alternaria* spp., *Macrophomina phaseolina* and *Phytophthora* spp. (Sarigül Ertek *et al.*, 2018) were isolated from underground parts of strawberry plants in Turkey.

*Rhizoctonia* species were divided into three main groups including multinucleate (MN) *Rhizoctonia*, binucleate (BN) *Rhizoctonia* and uninucleate (UN) *Rhizoctonia* (Stalpers and Andersen, 1996). Multinucleate *Rhizoctonia solani* (teleomorph: *Thanatephorus cucumeris*) isolates have been divided into 13 anastomosis groups (AG-1 to AG-13) (Sneh *et al.*, 1991; Carling *et al.*, 1994; Ogoshi, 1996; Carling *et al.*, 1996; 1999; 2002; Sharon *et al.*, 2006). According to the results of the latest publications, BN *Rhizoctonia* isolates (teleomorph:

*Ceratobasidium* spp.) have been designated to be 19 anastomosis groups (AG-A to AG-I, AG-K, AG-L, AG-O to AG-S, AG-U to AG-W) (Sharon *et al.*, 2008; Yang *et al.*, 2015; Dong *et al.*, 2017; Misawa and Kurose, 2019). Furthermore, some AGs of *R. solani* and BN *Rhizoctonia* are categorized into different subgroups (Sharon *et al.*, 2006; 2008). *Ceratobasidium albasitensis* (teleomorph; there is no anamorphic name) was isolated and described as a new species on *Crocus sativus* and *Pinus halepensis* (Gonzalez *et al.*, 2002).

Although BN *Rhizoctonia* AG-A, AG-G, AG-I and/or AG-K are frequently isolated from strawberry plants, AG-B, AG-C and AG-F are infrequently isolated. At the same time, *R. solani* AG 4 HGI, AG-5 and AG-6 were obtained from strawberry plants in various studies (Martin 1988, 2000; Botha *et al.*, 2003; Manici and Bonora, 2007; Sharon *et al.*, 2007; Fang *et al.*, 2013). In Turkey, BN *Rhizoctonia* AG-A, AG-G, AG-H, AG-K and *R. solani* AG-2-1, AG-3, AG-4 were isolated from strawberry plants or seedlings (Dinler, 2014; Demirer-Durak and Demirci, 2018; Dinler *et al.*, 2018).

Anastomosis grouping is frequently used for identification and classification of *R. solani* and BN *Rhizoctonia* isolates. Conventionally, anastomosis grouping using test isolates is principally the most helpful method to identify *Rhizoctonia* AGs, although some subgroups of AGs are not distinguishable on the basis of hyphal anastomosis reactions from other members of their AGs (Carling, 1996). Therefore, the classical method is supported by molecular techniques (Botha *et al.*, 2003; Sharon *et al.*, 2007; 2008). Internal transcribed spacer (ITS) regions of the ribosomal DNA (rDNA) are widely used for molecular identification of species, AGs and subgroups of *Rhizoctonia* isolates (Gonzalez *et al.*, 2001; Sharon *et al.*, 2006; 2008).

There are very few studies about AGs and pathogenicity of *Rhizoctonia* spp. on strawberry in Turkey. We aimed to investigate *Rhizoctonia* isolates obtained from strawberry in Erzincan province. This study was conducted to determine the species and/or AGs of *Rhizoctonia* isolates using the classical and/or molecular techniques, and to determine the pathogenicity of the selected isolates from each species and/or AGs on strawberry. In addition, another research question was whether the results had similarities or differences with those obtained from studies conducted in other countries.

## MATERIALS AND METHODS

**Isolation and Identification of *Rhizoctonia* species:** Diseased strawberry plants were collected from 16 fields of three districts (Table 1) in Erzincan province during 2009-2010. Depending on the growth area, 10 to 40 diseased plant samples were taken from one field. Collected plants were taken in polyethylene bags, brought

to the laboratory in an ice box cooler and kept at 4°C until isolation.

Before isolation, plant samples were washed using tap water to remove soil particles. Plant segments (approximately 1.5 cm long) were cut from basal stems. The segments were surface-disinfected for 1 min in 1% sodium hypochlorite (NaOCl), rinsed with steril water, and dried on steril filter paper. Superficially disinfected segments were placed on 1.5% Water Agar (WA) containing 50 mg/l streptomycin sulfate in Petri plates. The plates were incubated at 25 °C in the dark for 2-3 days. After the period, cultures were purified from colony growth of *Rhizoctonia* hyphae and transferred to plates WA and Potato Dextrose Agar (PDA). Purified isolates were stored at 5 °C on PDA slant in the laboratory (Demirci and Döken, 1993).

*Rhizoctonia* isolates obtained from strawberry plants were characterized on the basis of characteristics of vegetative hyphae under the light microscope (Ogoshi, 1975). Anastomosis groups of *Rhizoctonia* isolates were determined using classical and/or molecular method. In the classical method, AGs of the isolates were determined by using standardized technique (Demirci and Döken, 1995). *Rhizoctonia* isolates from strawberry plants were compared with *R. solani* and BN *Rhizoctonia* tester isolates in our culture collection (Eken and Demirci, 2004). Tester isolates and unknown isolate were placed 2-4 cm apart from each other in Petri plates including %1.5 WA. Then, the plates were incubated at 25 °C for 2-3 days, and hyphal interaction between the isolates was investigated under the light microscope. If hyphal anastomosis was observed, the isolates were identified as the same AG.

In the molecular method, a total of fifteen isolates, at least one or more isolates representing each *Rhizoctonia* species and AG, were subjected to molecular diagnosis using PCR and sequence analysis. To obtain mycelia to be used in DNA isolation, *Rhizoctonia* isolates were grown on the PDA Petri plate at 25 °C for 48-72 hours, and transferred to flask containing 100 ml of Potato Dextrose Broth (PDB) media, and incubated for 4-7 days at 25 °C in the dark without shaking. Fungal mycelium was harvested onto sterile ependorf tubes. Total genomic DNA isolation, PCR amplification and sequence analysis of the rDNA-ITS regions (ITS1, 5.8, ITS2) of *Rhizoctonia* isolates were performed by REFGEN (Ankara University Technopolis, Ankara, Turkey). Universal primers ITS1 and ITS4 were used to amplify the rDNA-ITS regions. The sequences obtained from ITS regions of *Rhizoctonia* isolates were queried against the National Center for Biotechnology Information (NCBI) sequence database using the online BLAST (Basic Local Alignment Search Tool) analysis to determine sequence identity and find the closest match based on maximal percent identity. Only sequence homologies of 97 to 100% were considered in assigning a species, AG, and/or subgroup to each

*Rhizoctonia* isolate. All sequences were edited using BioEdit software, version 7 (Hall, 1999), and aligned using the Clustal W algorithm (Thompson *et al.*, 1994). A phylogenetic tree was inferred using the neighbor-joining method (Saitou and Nei, 1987) implemented in MEGA software, version 6 (Tamura *et al.*, 2013) and 1000 bootstrap replicates. The sequences of 15 *Rhizoctonia* isolates were deposited in NCBI database as accession numbers of MT380166 to MT380180).

**Pathogenicity test:** Pathogenicity test of 59 *Rhizoctonia* isolates was carried out on strawberry seedlings *cv.* Fern. (Table 3). The isolates were grown on PDA in 9 cm diam Petri dish for 7 days at 25 °C in the dark. Wheat grains were soaked and boiled with steril water (Bandy *et al.*, 1984). The bottles containing wheat grains were autoclaved at 121 °C for 1 h on two consecutive days. For each isolate, PDA plugs with *Rhizoctonia* mycelium were transferred to bottle including steril grains (Martin, 1988), and bottles were incubated for 1 month at 22-24 °C. Sterile PDA plugs were used for control treatment.

Roots of strawberry seedlings were cut in length of 5 cm before planting (Sharon *et al.*, 2007). Subsequently, the seedlings were transplanted into plastic pots where containing soil, manure and perlite at 1:1:1 (v:v:v). The mix were autoclaved at 121 °C for 60 min for two consecutive days. Fifteen infected wheat grain were added to each potting soil (Ichielevich-Auster *et al.*, 1985; Botha *et al.*, 2003; Sharon *et al.*, 2007). Control pots were inoculated with sterile wheat grains. Four strawberry seedlings were inoculated for each isolate, and each inoculation test was replicated twice. All inoculated plants were maintained for 75 days at 24 °C with a 12 h:12 h L:D photoperiod. At the end of this period, plant roots were removed and washed. The diseases severity was evaluated

according to modified 0-4 descriptive scale (Muyolo *et al.* 1993), where: 0= healthy plant; 1 = localized tissue discoloration without necrosis; 2 = nearly complete root necrosis, partially restricted root length; 3 =root rot, root length severely restricted and 4=dead plant. Re-isolations were made from plant roots as described previously, and each recovered isolate was confirmed with anastomosis test by original isolate to complete Koch's postulates.

Root lengths (LaMondia and Martin, 1989) and the fresh weights of plants (Porrás *et al.*, 2007; Sharon *et al.*, 2007) were measured. The plants were dried at 60 °C for 72 h, and their dry weights were weighed (Botha *et al.*, 2003; Sharon *et al.*, 2007). The data obtained from the experiment results were analyzed by Duncan's multiple comparison test using SPSS (P<0.05).

## RESULTS AND DISCUSSION

***Rhizoctonia* species and anastomosis groups:** Isolation was performed from 438 plant samples, and 166 *Rhizoctonia* isolates (Table 1) were successfully obtained from basal stems of strawberry plants in Erzincan province. Initially, the isolates were examined for their characteristic feature such as typical hyphal branching, dolipore septum, and no clamp connection (Ogoshi, 1975). According to classical technique (hyphal anastomosis reactions), *R. solani* and BN *Rhizoctonia* isolates were separated into species and AGs. With hyphal anastomosis tests, *R. solani* and BN *Rhizoctonia* isolates grouped to three AGs (AG-2, AG-4 and AG-5) and five AGs (AG-A, AG-E, AG-G, AG-H and AG-K), respectively. Three *Rhizoctonia* isolates that hyphal fusion with each other failed to anastomose with all *R. solani* and BN *Rhizoctonia* tester isolates.

**Table 1. Number of isolates of *Rhizoctonia* species and anastomosis groups (AGs) determined by classical and molecular techniques.**

Species	AGs	Locations			Total
		Center	Üzümlü	Refahiye	
<i>Rhizoctonia solani</i>	2-1	-	1	3	4
	4 HGII	-	1	2	3
	5	1	1	2	4
Binucleate <i>Rhizoctonia</i>	A	14	24	16	54
	E	-	-	11	11
	G	12	2	23	37
	H	-	-	6	6
	K	1	3	40	44
<i>Ceratobasidium albasitensis</i>	-	-	1	2	3
<b>Total</b>		<b>28</b>	<b>33</b>	<b>105</b>	<b>166</b>

Species and/or AGs of selected 15 *Rhizoctonia* isolates (Table 2) in this study were determined according to analysis of the rDNA-ITS sequences using BLAST. Phylogenetic identification of these isolates against known

sequences from GenBank corresponded consistently to the results of AG tester isolates. Finally, *R. solani* isolates were identified as AG-2-1, AG-4 HGII and AG-5, BN *Rhizoctonia* isolates were identified as AG-A, AG-E, AG-

G, AG-H, AG-K and *C. albasitensis* (Table 2). A neighbor-joining phylogenetic tree that was produced from the rDNA-ITS sequence data and percent sequence similarity of 15 *Rhizoctonia* isolates are given in Fig.1. The subgroups of the two AGs (AG-2 and AG-4) of *R. solani* and three isolates of *C. albasitensis* were only determined using the molecular technique.

As a results, *R. solani* isolates were classified as AG-2-1 (2.41%), AG-4 HGII (1.81%) and AG-5 (2.41%), BN *Rhizoctonia* isolates were as AG-A (32.53%), AG-E (6.63%), AG-G (22.29%), AG-H (3.60%), AG-K (26.51%) and *C. albasitensis* (1.81%). Binucleate *Rhizoctonia* AG-A (54 isolates), AG-G (37 isolates) and AG-K (44 isolates) were obtained from all locations in this research, and the isolate numbers (135/166 isolates, 81.33%) of these three AGs are very high when compared to the total number of isolates (Table 1). However, the isolation frequency of *R. solani* AG-2-1, AG-4 HGII, AG-5, BN *Rhizoctonia* AG-E, AG-H and *C. albasitensis* were found relatively close to each other.

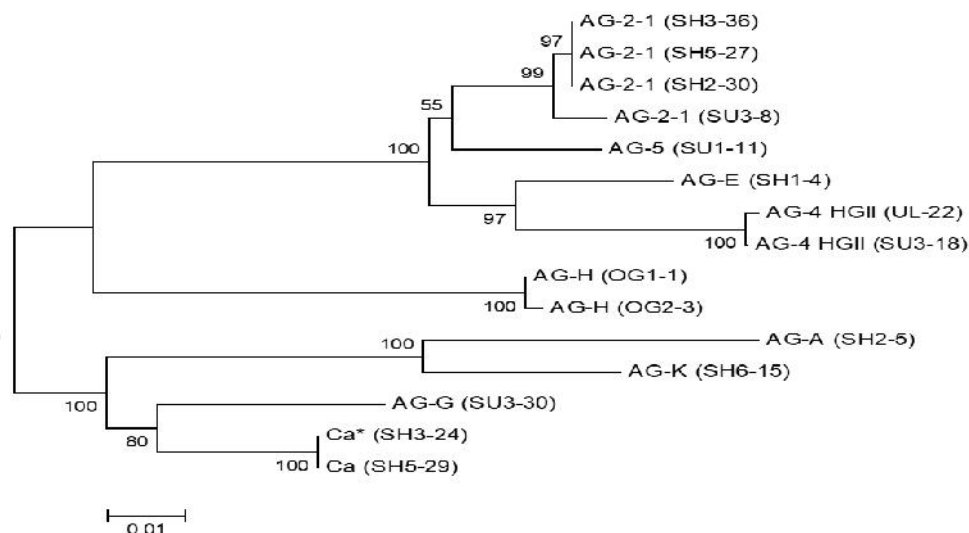
The results obtained from this study showed that BN *Rhizoctonia* isolates from strawberry plants were frequently isolated than *R. solani* isolates. Similarly, BN *Rhizoctonia* isolates were more commonly reported from strawberry plants in many studies as AG-A, AG-G and AG-I in Connecticut and California (Martin, 1988; Martin, 2000), AG-A, AG-G and AG-I in South Africa (Botha *et al.*, 2003), AG-A, AG-F, AG-G and AG-K in Israel (Sharon *et al.*, 2007), AG-A, AG-G, AG-I and AG-F in Italy (Manici and Bonora, 2007), AG-A, AG-B, AG-C, AG-G, AG-I and AG-K in Australia (Fang *et al.*, 2013), AG-A, AG-G, AG-K and AG-H in Turkey (Dinler, 2014; Demirer-Durak and Demirci, 2018; Dinler *et al.*, 2018). In previous studies, *R. solani* AG-2-1, AG-3, AG-4, AG-4 HGI, AG-5 and AG-6 have also been reported on strawberry plants (Martin, 1988; Botha *et al.*, 2003; Sharon *et al.*, 2007; Dinler, 2014; Demirer-Durak and Demirci, 2018). Therefore, the findings of us and other researchers supports that BN *Rhizoctonia* AGs are the major pathogen related to black root rot of strawberry plants in the world.

**Table 2. Species, anastomosis groups (AGs), subgroups, isolate code, sampling location, sampling date and GenBank accession number of selected 15 *Rhizoctonia* isolates used to molecular diagnosis.**

Species	AGs	Subgroup	Isolate	Location	Date	GenBank accession number
<i>Rhizoctonia solani</i>	2	1	SU3-8	Üzümlü	2009	MT380166
	2	1	SH2-30	Refahiye	2010	MT380167
	2	1	SH3-36	Refahiye	2010	MT380168
	2	1	SH5-27	Refahiye	2010	MT380169
	4	HGII	UL-22	Refahiye	2010	MT380170
	4	HGII	SU3-18	Üzümlü	2009	MT380171
	5		SU1-11	Üzümlü	2009	MT380172
Binucleate <i>Rhizoctonia</i>	A		SH2-5	Refahiye	2010	MT380173
	E		SH1-4	Refahiye	2010	MT380174
	G		SU3-30	Üzümlü	2009	MT380175
	H		OG1-1	Refahiye	2010	MT380176
	H		OG2-3	Refahiye	2010	MT380177
	K		SH6-15	Refahiye	2010	MT380178
<i>Ceratobasidium albasitensis</i>			SH3-24	Refahiye	2010	MT380179
			SH5-29	Refahiye	2010	MT380180

Binucleate *Rhizoctonia* AG-H and AG-E isolates were isolated only from strawberry plants in Refahiye, but AG-A, AG-G and AG-K obtained from three locations (Center, Üzümlü and Refahiye) in this study (Table 1). Binucleate *Rhizoctonia* AG-A, AG-G and/or AG-K have been identified on strawberry plants in many previous studies (Martin, 1988, 2000; Botha *et al.*, 2003; Manici and Bonora, 2007; Sharon *et al.*, 2007; Fang *et al.*, 2013; Demirer-Durak and Demirci, 2018; Dinler *et al.*, 2018). The diversity of species or AGs of *Rhizoctonia* obtained from a cultivated plant can be affected by the mainly host, also geographical location, environmental conditions as temperature and moisture, or previously grown plant species in the same location (Manici and Bonora, 2007;

Demirer-Durak and Demirci, 2018). For example, AG-I was found more virulent than AG-A or AG-G at 10 or 15 °C, whereas AG-G was more virulent than AG-A or AG-I at 24 °C; AG-G isolates were isolated more frequently than AG-A or AG-I in spring samples, whereas AG-I isolates were isolated more frequently than AG-A or AG-G in fall samples (Martin, 1988; LaMondia and Martin, 1989). On the other hand, *R. solani* AG-3 has been reported as the major group causing disease on potato plants (Bandy *et al.*, 1988; Demirci and Döken, 1993; Woodhall *et al.*, 2007). Demirer-Durak and Demirci (2018) reported that *R. solani* AG-3 widely isolated from strawberry plants and suggested that this could be related to potato grown before strawberry.



**Fig 1. A neighbor-joining tree based on rDNA-ITS sequence data for 15 isolates of *Rhizoctonia* spp. Bootstrap values were based on 1000 replicates. \* Ca: *Ceratobasidium albasitensis*.**

**Pathogenicity test:** All *R. solani* isolates and randomly selected 48 BN *Rhizoctonia* isolates (Table 3) were used to in the pathogenicity test on strawberry cv. Fern seedlings. The inoculated strawberry plants showed symptoms including brown discoloration or necrosis on the roots, the presence of short roots or dead plants. No symptoms were observed in the control plants.

As a result of pathogenicity test was found virulence differences among the species or AGs of *Rhizoctonia* (Table 3). Moreover, there were also differences in virulence of isolates belonging to the same AGs (data not shown), and similar results were observed by Martin (2000). In this study, AG-G isolates which caused moderate disease severity (2.17 according to 0-4 scale) had the highest disease severity among the tested *Rhizoctonia* species or AGs. Whereas isolates of BN

*Rhizoctonia* AG-A, AG-H, AG-K, *R. solani* AG-2-1, AG-4, and *C. albasitensis* were weakly pathogenic. On the other hand, BN *Rhizoctonia* AG-E and *R. solani* AG-5 were not pathogenic on strawberry seedlings when compared to control plants. Binucleate *Rhizoctonia* isolates obtained frequently from strawberry plants are generally more pathogenic on strawberry seedlings in Turkey and other countries (Martin, 1988; 2000; Botha *et al.*, 2003; Manici and Bonora, 2007; Sharon *et al.*, 2007; Fang *et al.*, 2013; Demirer-Durak and Demirci, 2018; Dinler *et al.*, 2018). It has been reported that *R. solani* AG-3, AG-4 and AG-6 are also virulent on strawberry seedlings (Botha *et al.*, 2003; Sharon *et al.*, 2007; Demirer-Durak and Demirci, 2018). Fang *et al* (2013) were demonstrated that the BN *Rhizoctonia* isolates showed a wide variation in virulence on strawberry plants.

**Table 3. Pathogenicity of *Rhizoctonia* isolates on strawberry plants.**

Species	AGs	Number of isolates	Fresh weight (g)	Dry weight (g)	Root length (cm)	Disease severity*
<i>Rhizoctonia solani</i>	2-1	4	11.19±2.27 <sup>bc**</sup>	2.98±0.75 <sup>c</sup>	12.42±2.11 <sup>c</sup>	0.93±0.75 <sup>cd</sup>
	4 HGII	3	10.26±1.68 <sup>cd</sup>	3.06±0.74 <sup>c</sup>	12.35±2.08 <sup>c</sup>	1.50±0.97 <sup>b</sup>
	5	4	11.68±2.00 <sup>b</sup>	3.73±0.62 <sup>b</sup>	13.84±2.14 <sup>b</sup>	0.43±0.56 <sup>c</sup>
Binucleate <i>Rhizoctonia</i>	A	10	8.53±2.09 <sup>f</sup>	2.76±0.77 <sup>cd</sup>	9.11±2.77 <sup>c</sup>	1.67±1.04 <sup>b</sup>
	E	10	11.48±2.18 <sup>b</sup>	3.84±0.73 <sup>b</sup>	12.68±2.24 <sup>bc</sup>	0.47±0.71 <sup>de</sup>
	G	10	9.66±1.53 <sup>ed</sup>	2.95±0.75 <sup>c</sup>	9.12±1.97 <sup>c</sup>	2.17±0.97 <sup>a</sup>
	H	6	11.21±2.26 <sup>bc</sup>	3.56±0.75 <sup>b</sup>	12.76±2.60 <sup>bc</sup>	1.16±1.17 <sup>bc</sup>
	K	10	10.13±1.71 <sup>cde</sup>	3.01±0.66 <sup>c</sup>	10.51±2.08 <sup>d</sup>	1.35±0.99 <sup>bc</sup>
<i>Ceratobasidium albasitensis</i>		2	9.16±1.75 <sup>ef</sup>	2.50±0.64 <sup>e</sup>	9.53±1.92 <sup>cd</sup>	1.31±0.79 <sup>bc</sup>
Control			13.99±2.23 <sup>a</sup>	4.39±0.61 <sup>a</sup>	15.60±1.68 <sup>a</sup>	0.00±0.00 <sup>e</sup>

\*: Disease severity was on a scale of 0-4; 0= healthy plant; 1 = localized tissue discoloration without necrosis; 2 = nearly complete root necrosis, partially restricted root length; 3 = root rot, root length severely restricted and 4=dead plant.

\*\* : Mean±Standard Deviation. There are differences between the averages indicated by different letters within columns according to Duncan's multiple range test (P<0,05).

In the pathogenicity test, it was observed that all isolates of *R. solani* and BN *Rhizoctonia* led to significantly reduced fresh weight, dry weight and root length of strawberry plants when compared with the control plants. Similar results were previously reported by other researchers (Demirer-Durak and Demirci, 2018; Borrero *et al.*, 2019). Isolates of *Rhizoctonia* were recovered from all inoculated plant roots to complete Koch's postulate and each isolate was confirmed with original isolate. *Rhizoctonia* was never obtained from control plants.

**Conclusion:** This study provided a basis for increasing our knowledge about species or AGs and pathogenicity of BN *Rhizoctonia* and *R. solani* on strawberry plants. The data obtained from this study showed the diversity and significance of *Rhizoctonia* species and/or AGs on strawberry. In this study, it has been determined that the main problem in strawberry root rot is caused by BN *Rhizoctonia*. Thus, it has been confirmed that BN *Rhizoctonia* is one of the important causes of black root rot disease of strawberry. Understanding genetic diversity of *Rhizoctonia* may help to understand and will also facilitate the control of black root rot disease.

**Acknowledgments:** A part of this article was published at the 1st International Conference on Food Agriculture and Animal Sciences (ICOFAAS 2018) in Erzincan, Turkey.

## REFERENCES

- Bandy, B.P., S.S. Leach, and S.M. Tavantzis (1988). Anastomosis group 3 is the major cause of *Rhizoctonia* disease of potato in Maine. *Plant Dis.* 72(7): 596-598.
- Bandy, B.P., D.H. Zanzinger, and S.M. Tavantzis (1984). Isolation of anastomosis group 5 of *Rhizoctonia solani* from potato field soils in Maine. *Phytopathology.* 74(10): 1220-1224.
- Borrero, C., I. Avilés-García, N. López, and M. Aviles (2019). First report of root rot on strawberry caused by Binucleate *Rhizoctonia* AG-K in Spain. *Plant Dis.* 103(2): 376.
- Botha, A, S. Denman, S.C. Lamprecht, M. Mazzola, and P.W. Crous (2003). Characterisation and pathogenicity of *Rhizoctonia* isolated associated with black root rot strawberries in the Western Cape province, South Africa. *Austral Plant Pathol.* 32(2): 195-201.
- Carling, D.E., R.E. Baird, R.D. Gitaitis, K.A. Brainard, and S. Kunita (2002). Characterization of AG-13, a newly reported anastomosis group of *Rhizoctonia solani*. *Phytopathology.* 92(8): 893-899.
- 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(10): 942-946.
- Carling, D.E. (1996). *Rhizoctonia* Species: Taxonomy, molecular biology, ecology, pathology and disease control. In: Sneh B, Jabaji-Hare S, Neate S, Dijst G (Eds) *Grouping in Rhizoctonia solani* by Hyphal Anastomosis Reaction, Kluwer Academic Publishers, Dordrecht, Netherlands, pp 37-47.
- Carling, D.E., C.S. Rothrock, G.C. MacNish, M.W. Sweetingham, K.A. Brainard, and S.W. Winters (1994). Characterization of anastomosis group 11 (AG-11) of *Rhizoctonia solani*. *Phytopathology.* 84(12): 1387-1393.
- Demirci, E. and M.T. Döken (1995). Anastomosis groups of *Rhizoctonia solani* Kühn and binucleate *Rhizoctonia* isolates from various crops in Türkiye. *J Turk Phytopathol.* 24(2): 57-62.
- Demirci, E. and M.T. Döken (1993). Anastomosis groups and pathogenicity of *Rhizoctonia solani* Kühn isolates from potatoes in Erzurum-Türkiye. *J Turk Phytopathol.* 22(2-3): 95-102.
- Demirer-Durak, E. (2018). Anastomosis groups, pathogenicity and biological control of *Rhizoctonia* species isolated from pepper (*Capsicum annum* L.) plants in lake Van Basin. *Fresenius Environ Bull.* 27(6): 4198-4205.
- Demirer-Durak, E. and E. Demirci (2018). Anastomosis groups and pathogenicity of *Rhizoctonia* species from strawberry plants in Erzurum province, Turkey. *Fresenius Environ Bull.* 27(6): 4206-4211.
- Demirer-Durak, E. and E. Demirci (2014). Pathogenicity of *Fusarium* species isolated from strawberry plants in Erzurum province. *Plant Protection Bull.* 54(3): 247-253.
- Dinler, H, S. Benlioglu, and K. Benlioglu (2018). *Rhizoctonia fragariae* causes black root rot on strawberry seedlings in Turkey. *Australas Plant Dis Notes.* 13(1): 23.
- Dinler, H. (2014). Studies on the determination of soil-borne fungal pathogens in strawberry seedlings. M.Sc. thesis (unpublished). University of Adnan Menderes, Institute of Naturel and Applied Sciences, Aydın.
- Dong, W., Y. Li, C. Duan, X. Li, S. Naito, R.L. Conner, G. Yang, and C. Li (2017). Identification of AG-V, a new anastomosis group of binucleate *Rhizoctonia* spp. from taro and ginger in Yunnan province. *Eur J Plant Pathol.* 148(4): 895-906.
- Eken, C. and E. Demirci (2004). Anastomosis groups and pathogenicity of *Rhizoctonia solani* and binucleate *Rhizoctonia* isolates from bean in Erzurum, Turkey. *J Plant Pathol.* 86 (1): 49-52.
- FAO. (2020). Statistical Database of the Food and Agriculture Organization of the United

- Nations. <http://www.fao.org>. (Accessed: 06.05.2020).
- Fang, X., M.P. Finnegan, and M.J. Barbetti (2013). Wide variation in virulence and genetic diversity of binucleate *Rhizoctonia* isolates associated with root rot of strawberry in Western Australia. *PLoS ONE* 8(2): e55877.
- Genç Kesimci, T. and E. Demirci (2020). Vegetative compatibility groups and pathogenicity of *Verticillium dahliae* isolates from strawberry plants in Erzurum and Erzincan provinces, Turkey. *Fresen Environ Bull.* 29 (1): 454-462.
- Gonzalez, V., O. Salazar, M.C. Julian, J. Acero, M.A. Portal, R. Munoz, H. Lopez-Corcoles, E. Gomez-Acebo, P. Lopez-Fuster, and V. Rubio (2002). *Ceratobasidium albasitensis*-A new *Rhizoctonia*-like fungus isolated in Spain. *Persoonia.* 17 (4): 601-614.
- Gonzalez, D, D.E. Carling, S. Kuninaga, R. Vilgalys, and M.A. Cubeta (2001). Ribosomal DNA systematics of *Ceratobasidium* and *Thanatephorus* with *Rhizoctonia* anamorphs. *Mycologia.* 93(6): 1138-1150.
- Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT, pp. 95-98.
- Ichielevich-Auster, M., B. Sneh, Y. Koltin, and I. Barash (1985). Pathogenicity host specificity and anastomosis groups of *Rhizoctonia* spp. isolated from soils in Israel. *Phytoparasitica.* 13(2): 103-112.
- LaMondia, J.A. and S.B. Martin (1989). The influence of *Pratylenchous penetrans* and temperature on black root rot of strawberry by binucleate *Rhizoctonia* spp. *Plant Dis.* 73(2): 107-110.
- Manici, L.M. and P. Bonora (2007). Molecular genetic variability of Italian binucleate *Rhizoctonia* spp. isolates from strawberry. *Eur J Plant Pathol.* 118(1): 31-42.
- Manici, L.M., F. Caputo, and G. Baruzzi (2005). Additional experiences to elucidate the microbial component of soil suppressiveness towards strawberry black root rot complex. *Ann App Biol.* 146(4): 421-431.
- Martin, F.N. (2000). *Rhizoctonia* spp. recovered from strawberry roots in Central Coastal California. *Phytopathology.* 90(4): 345-353.
- Martin, S.B. (1988). Identification, isolation frequency, and pathogenicity of anastomosis groups of binucleate *Rhizoctonia* spp. from strawberry roots. *Phytopathology.* 78(4):379-384.
- Matsumoto, M. and T. Yoshida (2006). Characterization of isolates of binucleate *Rhizoctonia* spp. associated with strawberry black root rot complex using fatty acid methyl ester (FAME) profiles. *J Gen Plant Pathol.* 72(5): 318-322.
- Mirmajlessi, S.M., M. Bahram, M. Mänd, N. Najdabbasi, S. Mansouripour, and E. Loita (2018). Survey of soil fungal communities in strawberry fields by illumina amplicon sequencing. *Eurasian Soil Sci.* 51(6): 682-691.
- Misawa, T. and D. Kurose (2019). Anastomosis group and subgroup identification of *Rhizoctonia solani* strains deposited in NARO Genebank, Japan. *J Gen Plant Pathol.* 85(4): 282-294.
- Muyolo, N.G., P.E. Lipps, and A.F. Schmitthenner (1993). Reactions of dry bean, lima bean, and soybean cultivars to *Rhizoctonia* root and hypocotyl rot and web blight. *Plant Dis.* 77(3): 234-238.
- Ogoshi, A. (1996). Introduction the genus *Rhizoctonia*. In: Sneh B., Jabaji-Hare S., Neate S., Dijst G. (eds). *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology and disease control, Kluwer Academic Publishers; Dordrecht (Netherlands). pp. 1-9.
- Ogoshi, A. (1975). Grouping of *Rhizoctonia solani* Kühn and their perfect stages. *Rev Plant Protect Res.* 8: 93-103.
- Porras, M., C. Barrau, F.T. Arroyo, B. Santos, C. Blanco, and F. Romero (2007). Reduction of *Phytophthora cactorum* in strawberry fields by *Trichoderma* spp. and soil solarization. *Plant Dis.* 91(2): 142-146.
- Saitou, N. and M. Nei (1987). The Neighbor-Joining method - a new method for reconstructing phylogenetic trees. *Mol Biol Evol.* 4(4): 406-425.
- Sarıgül Ertek, T., Y.Z. Katircioğlu, and S. Maden (2018). Fungal diseases of strawberry grown in Düzce province of Turkey. *Plant Protection Bull.* 58(3): 123-129.
- Sharon, M., S. Kuninaga, M. Hyakumachi, S. Naito, and B. Sneh (2008). Classification of *Rhizoctonia* spp. using rDNA-ITS sequence analysis supports the genetic basis of the classical anastomosis grouping. *Mycoscience.* 49(2): 93-114.
- Sharon, M., S. Freeman, S. Kuninaga, and B. Sneh (2007). Genetic diversity, anastomosis groups and virulence of *Rhizoctonia* spp. from strawberry. *Eur J Plant Pathol.* 117(3): 247-265.
- Sharon, M., S. Kuninaga, M. Hyakumachi, and B. Sneh (2006). The advancing identification and classification of *Rhizoctonia* spp. using molecular and biotechnological methods compared with the classical anastomosis grouping. *Mycoscience.* 47(6): 299-316.
- Sneh, B., L. Burpee, and A. Ogoshi (1991). Identification of *Rhizoctonia* Species. APS Press, St. Paul, Minnesota (USA). 133 p.
- Stalpers, J.A. and F. Andersen (1996) A synopsis of the taxonomy of teleomorphs connected with *Rhizoctonia* S.L.. In: Sneh B., Jabaji-Hare S., Neate S., Dijst G. (Eds). *Rhizoctonia* species:

- taxonomy, molecular biology, ecology, pathology and disease control, Kluwer Academic Publishers, Dordrecht (Netherlands). 49-63 p.
- Tamura, K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol.* 30(12): 2725-2729.
- Thompson, J.D., D.G. Higgins, and T.J. Gibson (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22(22): 4673-4680.
- Vico, I. (1994). Investigation of anastomosis groups of binucleate *Rhizoctonia* spp. isolated from strawberries. *Phytopathol Mediter.* 33(2): 165-167.
- Woodhall, J.W., A.K. Lees, S.G. Edwards, and P. Jenkinson (2007). Characterization of *Rhizoctonia solani* from potato in Great Britain. *Plant Pathol.* 56(2): 286-295.
- Yang, Y.G., C. Zhao, Z.J. Guo, and X.H. Wu (2015). Characterization of a new anastomosis group (AG-W) of binucleate *Rhizoctonia*, causal agent for potato stem canker. *Plant Dis.* 99(12): 1757-1763.
- Yılmaz, H. (2006). Strawberry Diseases. University of Yuzuncu Yil, Faculty of Agriculture Department of Horticulture publications, Van (Turkey). 65 p.