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Anastomosis grouping and phylogenetic analysis of Rhizoctonia isolates on wheat in Türkiye

Türkiye'de buğdaydaki Rhizoctonia izolatlarının anastomosis gruplandırması ve filogenetik analizi

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ABSTRACT

This study aims to determine the species and evaluate the genetic diversity of the pathogenic and nonpathogenic *Rhizoctonia* spp. and anastomosis groups (AG) from wheat plants and rhizosphere soils in Turkey. *Rhizoctonia* species were isolated from plants and rhizosphere soils in wheat fields in 5 provinces in the Central Anatolian Region of Türkiye. As a result of the isolations, a total of 88 multinucleate (MN) and binucleate (BN) *Rhizoctonia* isolates were obtained. Identifications of the isolates were determined by rDNA-ITS sequence analyses. The identified isolates belonged to MN Waitea *circinata* var. *zeae*, *W. circinata* var. *oryzae*, *W. circinata* var. *circinata*, MN *Rhizoctonia solani* AG 2-1, AG 2-2, AG 3, AG 4-HGII, AG 4-HGIII, AG 5, AG 8, AG 11 and BN AG A, AG DI, AG E, AG G, AG H, AG I, AG I-like and AG K. The most isolated group was *W. circinata* var. *circinata*. In the pathogenicity studies, the most virulent group was determined as *R. solani* AG 4. Among the binucleate isolates, groups other than *R. cerealis* AG DI were not found to be pathogenic. Neighbor-joining phylogenetic trees of isolates were constructed from rDNA-ITS sequences. As a result of this study, the regional distribution of MN and BN *Rhizoctonia* AG isolates in important wheat production areas in the Central Anatolia Region, Türkiye was determined. In addition, this study is the first comprehensive study in which the genetic diversity of *Rhizoctonia* AGs isolates obtained from wheat and rhizosphere soils in the region was evaluated with a molecular approach.

INTRODUCTION

Wheat is one of the most used crop plants in human nutrition in the world and is one of the main nutrients in the world. It has an important place not only in terms of its use in the flour and bakery products industry (flour, bread, bulgur, semolina, pasta, biscuits, starch, etc.), which is the sub-branch of the food industry but also in terms of its use in the livestock sector such as bran and straw. According to strategists, wheat is the most important geoeconomic power of the 21st century (Koca 1999). *Rhizoctonia* genus includes many species with highly pathogenic, weakly pathogenic, endophyte, saprophytic, and mycorrhizal characters (González et al. 2006). It is one of the main causes of root rot

disease, which is a problem in wheat fields in Türkiye. The species in this genus are divided into many groupwith the number of nuclei in the hyphae cells [multinucleate (MN), binucleate (BN), uninucleate (UN)] and the anastomosis fusions they form together. *Rhizoctonia solani* Kühn is divided into 13 anastomosis groups (AGs) designated as AG 1-13, as the AG BI group has been integrated into AG 2 (Carling et al. 2002), and 20 subgroups (AG 1IA, AG 1IB, AG 1IC, AG 1ID, AG 1IE, AG 2-1, AG 2-2IIB, AG 2-2IV, AG 2-2LP, AG 2-3, AG 3PT, AG 3TB, AG 3TM, AG 4-HGI, AG 4-HGII, AG 4-HGIII, AG 6-HGI, AG-6GV, AG 9TP and AG 9TX) (Priyatmojo et al. 2001). Anastomosis groups can be diagnosed by anastomosis reactions between hyphae and molecular methods, while molecular diagnosis is required for further subgroups. *Waitea circinata* var. *oryzae* and *W. circinata* var. *zeae* have also anastomosis groups called WAG O and WAG Z, respectively (Sneh et al. 1996). Okubara et al. (2008) reported that three different genotypes of *W. circinata* var. *oryzae* (*R. oryzae* genotype I, II, and III). Binucleate *Rhizoctonia* species are divided into 19 different anastomosis groups (AG A, AG B, AG C, AG D, AG E, AG F, AG G, AG H, AG I, AG K, AG L, AG O, AG P, AG Q, AG R, AG S, AG U, AG V, AG W) (Dong et al. 2017, Erper et al. 2021, Hyakumachi et al. 2005, Misawa and Kurose 2018, Ogoshi et al. 1983, Sharon et al. 2008, Yang et al. 2015, Zhao et al. 2019).

In previous studies in the world, *Rhizoctonia solani* AG 1IB, AG 2-1, AG 2-2, AG 3, AG 4, AG 5, AG 6, AG 8, AG 11, *W. circinata* var. *circinata*, *W. circinata* var. *zeae*, *W. circinata* var. *oryzae* and *R. cerealis* AG D were determined to cause disease in wheat (Meyer et al. 1997, Roberts and Sivasithamparam 1986, Sneh et al. 1996, Tomaso-Peterson and Trevathan 2007). Of these groups, *W. circinata* var. *circinat*a and *W. circinata* var. *zeae*, AG 2-1, AG 3, AG 4-HGII, AG 5, AG 8, AG 11, BN AG I and AG K have been reported on wheat in Türkiye in some previous studies (Demirci 1998, Ünal and Dolar 2012, Ünal et al. 2015).

The classical identification of *Rhizoctonia* AGs is based on the number of nuclei in hyphae cells and the ability of the hyphae to anastomose with known tester isolates (Sneh et al. 1996). Although the anastomosis method is an accurate, valid, and still used method, it is not sufficient for the detection of advanced subgroups. Molecular identification is required for the detection of advanced subgroups. When the studies conducted in the world are examined, various molecular markers have been used for the characterization and grouping of *Rhizoctonia species*. The genetic diversity of *Rhizoctonia* isolates has been studied using RAPD-PCR, SSR-PCR, rDNA-RFLP, rDNA-ITS sequence analysis, universally primed-,PCR, and rep-PCR (Sharon et al. 2006). Currently, the rDNA-ITS sequence analysis seems to be the most appropriate method for the classification of *Rhizoctonia* spp. and sequence analysis of the ITS-5.8S rDNA region has been used as a suitable molecular tool the for identification of *R. solani* subgroups (Carling et al. 2002, Hyakumachi et al. 1998, Priyatmojo et al. 2001, Salazar et al. 2000a, 2000b, Toda et al. 2000). Similarly, the rDNA-ITS sequence analysis most accurately divided subgroups within AG 1 (Kuninaga et al. 1996, Toda et al. 2004), AG 3 (Kuninaga et al. 2000), AG 4 (Boysen et al. 1996), and AGs 6 (Pope and Carter 2001). Fewer studies were reported on rDNA-ITS sequence analyses of Binucleat isolates than on Multinucleate isolates (González et al. 2002, Hyakumachi et al. 2005, Ma et al. 2003, Otero et al. 2002, Sharon et al. 2007, 2008).

This study aims to determine the pathogenic and non-pathogenic *Rhizoctonia* anastomosis groups and subgroups in wheat roots and rhizosphere soils in Türkiye and to reveal the genetic diversity among them.

MATERIALS AND METHODS

Collection of plants and soils and isolation of Rhizoctonia spp.

Wheat fields in 58 districts within the borders of Ankara, Konya, Yozgat, Eskişehir, and Kırıkkale provinces in the Central Anatolian Region of Türkiye were examined and 330 wheat roots and 330 rhizosphere soils were collected. In the isolations from the plants, tissue pieces of the diseased root and root collar were dried on sterile blotting paper after 1-minute surface disinfection in 1% sodium hypochlorite. Then, it was placed on acidic water agar prepared by adding 3 ml of lactic acid (10%) per liter to 1.5% water agar medium. After 3-4 days, the hyphae tips of Rhizoctonia-like fungi were removed with a sterile loop and transferred to Potato Dextrose Agar (PDA; Merck, Germany). For the isolation of *Rhizoctonia* species from the soil, wheat straws sterilized by autoclaving in heat-resistant bottles were used. Soil samples were filled into pots in a greenhouse and watered until the field capacity. Sterile wheat stalks, approximately 4 cm long, were placed vertically in the soil, 4 per pot, and covered with a clean, opaque nylon bag for 3 days and left uncovered for 4 days. Then, wheat stalks were taken from the pots, washed, and transferred to Petri plates containing acidic water agar (Ogoshi et al. 1990).

Determination of anastomosis groups

The hyphae of the isolates obtained as a result of isolations from wheat and rhizosphere soil were first stained with Safranin O solution and the number of nuclei in each hyphae septa was determined (Bandoni 1979). All isolates were grouped by considering colony morphology, color compared with tester isolates, and number of nuclei. Anastomosis group determination studies were performed according to Kronland and Stanghellini (1988) using tester isolates.

Tester isolates were obtained from Türkiye (MN and BN *Rhizoctonia* spp.), Italy (*R. solani*), Japan (*MN Rhizoctonia* spp.), Poland (*Rhizoctonia* AG DI, II, III) and USA (*W. circinata* var. *oryzae* genotype I, II, III).

Pathogenicity tests

Pathogenicity tests were performed in pots using the Kate A-1 wheat variety, which is known to be susceptible to the disease (Arslan and Baykal 2002). The trials were done in a greenhouse (12 hours photoperiod at 24 ± 2 °C and 55-65% relative humidity) with plastic pots of 10 cm diameter. Inoculums were prepared by inoculating each fungal isolate into moistened sterile wheat grains in heat-resistant glass bottles. Eight wheat grains infested fungi were placed on the soil surface filling the 40 cm^3 of vermiculite and 30 cm^3 of silt loam in the pots. A clean nylon cover was covered over the pots and incubated for 4 days. Controls were created from inoculum-free pots. Trials consisted of 5 replications. At the end of the 4th day, eight Kate A-1 wheat seeds were sown in the soil, covered with 12 cm^3 of sterile soil, and irrigated with 15 ml of distilled water (Paulitz et al. 2003). After 20 days, the plant roots were washed and evaluated according to a 0 to 5 scale: 0= no disease, 1= 1-10%, 2= 11-30%, 3= 31-50%, 4= 51-80%, 5= the entire hypocotyl infected (Ichielevich-Auster et al. 1985). The scale rates were transformed into disease severity rates using the Townsend and Heuberger (1943) formula. At the end of the study, reisolation of fungi from plants was carried out.

Molecular identification and genetic diversity

DNA was isolated from the Qiagen DNeasy ® Plant Mini Kit. PCR studies were performed using ITS 1 and ITS 4 primers (White et al. 1990) according to Cobos and Martin (2008). PCR products were sequenced by a private biotechnology company. Nucleotide sequences were performed by BLAST analysis and compared with the other sequences in GenBank. Sequences in this study were registered with their accession numbers to GenBank at NCBI. Phylogenetic trees were constructed using ClustalW alignments (Thompson et al. 1994), The Tamura 3 Parameter model for MN isolates, and the Kimura 2-parameter model (Kimura 1980) for BN isolates in the Mega 7 Program (Kumar et al. 2016). Bootstrap analysis was performed with 500 copies.

RESULTS

The isolates obtained as a result of isolations from 330 wheat root and 330 rhizosphere soil samples were classically identified with the help of the number of nuclei in each hyphae septum and hyphal anastomosis reaction tests with known test isolates. As a consequence of the classical identification studies total of 88 *Rhizoctonia* isolates were identified including 36 *R. solani*, 30 *W. circinata*, and 22 BN *Rhizoctonia* spp. All isolates were also diagnosed molecularly by rDNA-ITS sequencing analysis to support anastomosis-based diagnoses andidentify advanced subgroups. The resulting sequences were checked to the sequences,in GenBank, and species, anastomosis groups and subgroups of 66 MN and 22 BN *Rhizoctonia* isolates were determined. MN *Rhizoctonia* spp. was determined as *R. solani* AG 2-1, AG 2-2, AG 3, AG 4-HGII, AG 4-HGIII, AG 5, AG 8, AG 11, *W. circinata* var. *circinata*, *W. circinata* var. *zeae* and *W. circinata* var. *oryzae*, BN *Rhizoctonia* spp. as AG A, AG DI, AG E, AG G, AG H, AG I, AG I-like and AG K (Table 1).

Table 1. Number of isolates belonging to *Rhizoctonia* species and anastomosis groups.

Anastomosis groups	Provinces					Total
	Ankara	Konya	Yozgat	Eskişehir	Kırıkkale	
AG 2	-	5	-	-	-	5
AG 3	3	-	-	-	-	3
AG 4-HGII	5	2	1	2	-	10
AG 4-HGIII	1	-	-	-	1	2
AG 5	3	5	3	-	1	12
AG 8	1	1	-	1	-	3
AG 11	1	-	-	-	-	1
W. circinata var. circinata	5	1	8	-	-	14
W. circinata var. zeae	4	1	2	1	-	8
W. circinata var. oryzae	3	-	1	2	2	8
AG A	-	1	-	-	-	1
AG DI	3	3	1	-	-	7
AG E	1	-	-	-	-	1
AG G	1	-	-	-	-	1
AG H	1	-	-	-	-	1
AG I	1	-	-	-	-	1
AG I-like	2	1	-	1	5	9
AG K	1	-	-	-	-	1
Total	36	20	16	7	9	88

All isolates generated amplicons at ≈650 bp during amplification with primers ITS1 and ITS4. Sequences were registered with GenBank at NCBI and accession numbers were got. The majority of the MN *Rhizoctonia* isolates had the highest (96-100%) ITS sequence identity with *Rhizoctonia* isolates in GenBank. Isolates 0612, 6651, 20105, 26102, 7121, and 0663 showed 96-98% similarity to DQ356414 (*R. oryzae* genotype I) from the USA (Okubara et al. 2008). In this study, it was observed that colony morphologies of 0612, 6651, 26105, 7121 and 0663 isolates different from the other *Waitea* isolates and the other *Rhizoctonia oryzae* pathogens in wheat. It was detected that they were not pathogen on wheat (Table 2).

Isolates 0612, 6651, 26105, 26102, 7121 and 0663 showed 96-98% similarity to DQ356414 (*R. oryzae* genotype I) from USA (Okubara et al. 2008). In this study, it was observed that colony morphologies of 0612, 6651, 26105, 7121 and 0663 isolates different from the other *Waitea* isolates and the other *R. oryzae* (WAG O) pathogens in wheat. It was detected that they were not pathogen on wheat (Table 3).

The majority of the BN *Rhizoctonia* isolates had the highest (83-100%) ITS sequence identity with *Rhizoctonia* isolates in GenBank. The phylogenetic neighboor-joining tree belonging to BN *Rhizoctonia* isolates consisted of seven small clusters which composed AG A, AG DI, AG E, AG G, AG H, AG I and AG K (Figure 3). Isolates 7107, 7118,

Table 2. Anastomosis group, geographic origin, source of isolation, percentage of sequence similarity with Genbank isolates and disease severity of *Rhizoctonia* solani isolates used in this study

Isolate number	Subgroup	Origin	Source of isolation	Disease severity [a] (%)	Accesion number		Similarity (%)
4246	AG 2-1	Konya	Plant	Non-pathogen	KC590548	JQ676880	99
4278		Konya	Plant	Non-pathogen	KC590570	EU730809	99
4248	AG 2-2	Konya	Plant	Non-pathogen	KC590550	EU730809	99
4269		Konya	Soil	Non-pathogen	KC590564	EU730809	99
2636		Eskişehir	Soil	Non-pathogen	KC590538	EU730809	100
0601		Ankara	Soil	Non-pathogen	KC590579	MW999160	99
0642	AG 3	Ankara	Soil	Non-pathogen	KC590544	MW999167	100
0676		Ankara	Plant	Non-pathogen	KC590568	MW999167	96
0689		Ankara	Plant	90	KC590607	MZ379606	100
6684		Yozgat-	Plant	98	KC590602	MZ379607	99
2666		Eskişehir	Plant	84	KC590595	MZ379606	99
2633		Eskişehir	Plant	90	KC590535	MZ379606	99
4230	AG 4HGII	Konya	Soil	58	KC590533	MZ379606	99
4274		Konya	Soil	78	KC590566	MZ379606	99
0617		Ankara	Soil	59	KC590589	MZ379598	100
0667		Ankara	Soil	75	KC590563	MZ379606	100
0682		Ankara	Soil	88	KC590571	MZ379606	99
0687		Ankara	Soil	86	KC590605	MZ379602	99
0640	AG 4 HGIII	Ankara	Plant	87	KC590542	KR006070	100
7108		Kırıkkale	Plant	100	KC590521	KR006070	100
0690		Ankara	Plant	Non-pathogen	KC590609	AF478452	98
4275		Konya	Plant	58	KC590567	AF478452	97
4250		Konya	Plant	Non-pathogen	KC590552	AF478452	99
4234		Konya	Plant	Non-pathogen	KC590536	AF478452	99
0639		Ankara	Plant	Non-pathogen	KC590541	AF478452	98
4268		Konya	Soil	58	KC590596	AF478452	98
4235	AG 5	Konya	Soil	56	KC590537	AF478452	97
0665		Ankara	Soil	70	KC590594	AF478452	98
6643		Yozgat	Soil	Non-pathogen	KC590545	JX162013	98
6649		Yozgat	Soil	Non-pathogen	KC590551	AF478452	99
6658		Yozgat	Plant	Non-pathogen	KC590558	AF478452	98
7155		Kırıkkale	Soil	Non-pathogen	KC590608	AF478452	99
0610		Ankara	Plant	76	KC590583	AB000011	97
26111	AG 8	Eskişehir	Plant	73	KC590576	AB000011	99
4254		Konya	Soil	72	KC590555	AB000011	99
0673	AG 11	Ankara	Soil	Non-pathogen	KC590598	AF153802	98
Control				0			

[a] Roots and hypocotyl symptoms were evaluated on the following scale: 0=no disease, 1= 1-10%, 2= 11-30%, 3= 31-50%, 4= 51-80%, 5= the entire hypocotyl infected.

Table 3. Anastomosis group, geographic origin, source of isolation, percentage of sequence similarity with genbank isolates and disease severity of *Waitea circinata* isolates used in this study

Isolate number	Subgroup	Origin	Source of isolation	Disease severity a (%)	Accesion number	The highest similar isolate in the Gen Bank	Similarity (%)
6656	*W. cir. var. circinata*	Yozgat	Plant	68	KC590556	HM807352	100
6657		Yozgat	Plant	75	KC590557	HM807352	99
0641		Ankara	Soil	86	KC590543	HQ166066	100
0611		Ankara	Soil	Non-pathogen	KC590584	FJ755887	99
0637		Ankara	Soil	50	KC590539	HM807352	100
0681		Ankara	Soil	76	KC590600	FJ154894	99
6659		Yozgat	Soil	70	KC590559	HM807352	100
6677		Yozgat	Soil	75	KC590569	HM807352	100
6629		Yozgat	Soil	95	KC590532	HM807352	100
0638		Ankara	Plant	81	KC590540	HM807352	100
6685		Yozgat	Soil	Non-pathogen	KC590603	FJ755887	100
6688		Yozgat	Soil	Non-pathogen	KC590606	FJ154894	99
4225		Konya	Soil	99	KC590530	FJ755878	97
6686		Yozgat	Soil	83	KC590604	JX631228	89
0670	*W. cir. var zeae*	Ankara	Soil	Non-pathogen	KC590565	JX631228.1	97
4226		Konya	Plant	98	KC590515	JQ350856	97
06115		Ankara	Plant	79	KC590518	JQ350862	95
0631		Ankara	Soil	78	KC590517	KC620582	96
0614		Ankara	Soil	72	KC590587	KJ623715	96
6628		Yozgat	Soil	75	KC590514	JQ350860	96
6622		Yozgat	Plant	88	KC590516	KC620580	97
26110		Eskişehir	Soil	84	KC590513	KC709579	96
0612	*W. cir. var. oryzae*	Ankara	Soil	Non-pathogen	KC590585	DQ356414	98
6651		Yozgat	Soil	Non-pathogen	KC590553	DQ356414	96
26105		Eskişehir	Soil	Non-pathogen	KC590575	DQ356414	96
26102		Eskişehir	Soil	Non-pathogen	KC590574	DQ356414	96
7121		Kırıkkale	Plant	Non-pathogen	KC590527	DQ356414	96
0662		Ankara	Soil	Non-pathogen	KC590562	KX468809	97
0663		Ankara	Soil	Non-pathogen	KC590592	DQ356414	98
7103		Kırıkkale	Soil	Non-pathogen	KC590580	EU693449.1	99
Control				0			

[a] Roots and hypocotyl symptoms were evaluated on the following scale: 0=no disease, 1= 1-10%, 2= 11-30%, 3= 31-50%, 4= 51-80%, 5= the entire hypocotyl infected.

7120, 7105, 0661, 2671, 4264 and 7106 were named as AG I-like, because as a result of the blast analysis, these isolates matched with the DQ356409.1, DQ356407 and JQ247570 Accession numbers AG I-like isolates (Okubara et al. 2008, Schroeder and Paulitz 2012) at the highest rate in Genbank (Table 4).

Three phylogenetic trees were constructed by bootstrap neighbor-joining analysis of nucleotide sequences to evaluate genetic variability among isolates belonging to *R. solani*, *Waitea* spp. and BN *Rhizoctonia* spp. The phylogenetic neighboor-joining tree belonging to MN *R. solani* isolates clearly demonstrated that the isolates were grouped into eight distinct clusters (Figure 1). It was observed that this eight clusters constituted small clusters between each other including different AGs. when the eight clusters examined, it was observed that small clusters which different anastomosis groups generated between each other. The small clusters which in the tree of *R. solani* isolates belonged to AGs 2-1, AG 2-2, AG 3, AG 4-HGII, AG 4-HGIII, AG 5, AG 8 and AG 11. The small clusters which in the tree of *W. circinata* isolates belonged to *W. circinata* var. *circinata*, *W. circinata* var. zeae (*R. zeae*) and *W. circinata* var. *oryzae* (Figure 2).

In pathogenicity studies, AG 4 was determined as the most virulent group with disease severity values that vary between 58-100% (Figure 4). The most virulent isolate was 7108 (AG 4-HGIII) with 100% diseases severity value. While the majority of MN *Rhizoctonia* isolates were pathogenic, the majority of BN *Rhizoctonia* isolates were found to be nonpathogenic. Among binucleate isolates; the groups other than *R. cerealis* AG DI were not found to be pathogen. While some isolates belonging to *R. solani* AG 5 was found as weak pathogen, some of them were not found as a pathogen. It was observed that MN *R. solani* AG 2, AG 3, AG 11, *W. circinata* var. *oryzae* genotype I was not a pathogen. Although isolates

Table 4. Anastomosis group, geographic origin, source of isolation, percentage of sequence similarity with genbank isolates and disease severity of binucleate *Rhizoctonia* isolates used in this study

Isolate number	Subgroup	Origin	Source of isolation	Disease severity a (%)	Accesion number	The highest similar isolate in the Gen Bank	Similarity (%)
4252	AG A	Konya	Plant	Non-pathogen	KC590554	MF070679.1	100
4224		Konya	Plant	80	KC590529	MZ569567.1	99
4227		Konya	Plant	62	KC590531	KJ012010	88
0645		Ankara	Plant	61	KC590547	MZ569568.1	92
0653	AG DI	Ankara	Plant	83	KC590591	M Z569567.1	83
0623		Ankara	Plant	70	KC590528	KJ012006.1	99
6632		Yozgat	Plant	50	KC590534	MZ569498.1	99
4247		Konya	Soil	65	KC590549	KY379507.1	97
06100	AG E	Ankara	Soil	Non-pathogen	KC590572	KX831960.1	99
0615	AG G	Ankara	Soil	Non-pathogen	KC590522	AB196658.1	98
0660	AG H	Ankara	Soil	Non-pathogen	KC590560	MZ396073.1	95
0616	AG I	Ankara	Soil	Non-pathogen	KC590588	AB196650.1	100
06101		Ankara	Soil	Non-pathogen	KC590573	JQ247570	96
7107		Kırıkkale	Plant	Non-pathogen	KC590525	DQ356409.1	96
7118		Kırıkkale	Plant	Non-pathogen	KC590523	MT487892.1	85
7120		Kırıkkale	Plant	Non-pathogen	KC590526	AJ242882.1	96
7105	AG I-like	Kırıkkale	Plant	Non-pathogen	KC590524	AJ242884.1	94
0661		Ankara	Soil	Non-pathogen	KC590561	JQ247570	95
2671		Eskişehir	Soil	Non-pathogen	KC590611	DQ356407	97
4264		Konya	Plant	Non-pathogen	KC590593	KC989057.1	97
7106		Kırıkkale	Plant	Non-pathogen	KC590581	MN898129	92
0680	AG K	Ankara	Soil	Non-pathogen	KC590599	MN160708.1	90
Control				0			

[a] Roots and hypocotyl symptoms were evaluated on the following scale: 0=no disease, 1= 1-10%, 2= 11-30%, 3= 31-50%, 4= 51-80%, 5= the entire hypocotyl infected

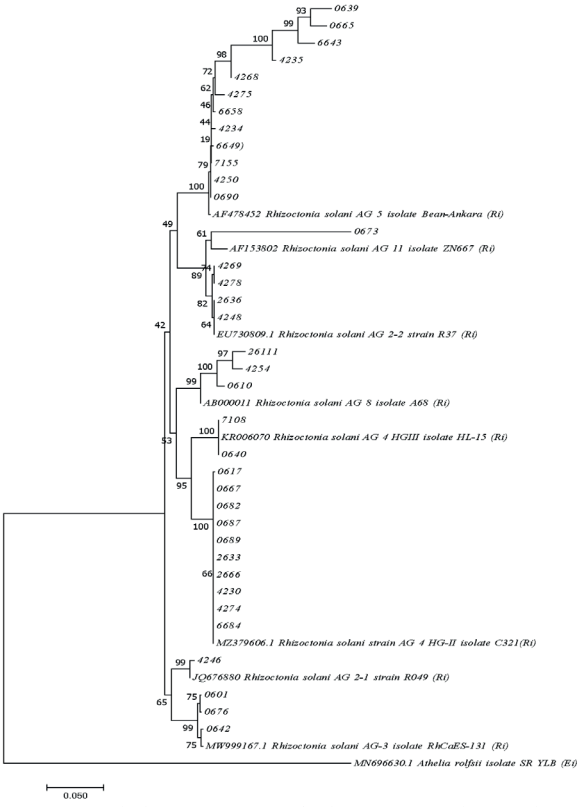

Figure 1. Phylogenetic tree of Rhizoctonia solani isolates AGs based on neighbour-joining method using MEGA 7

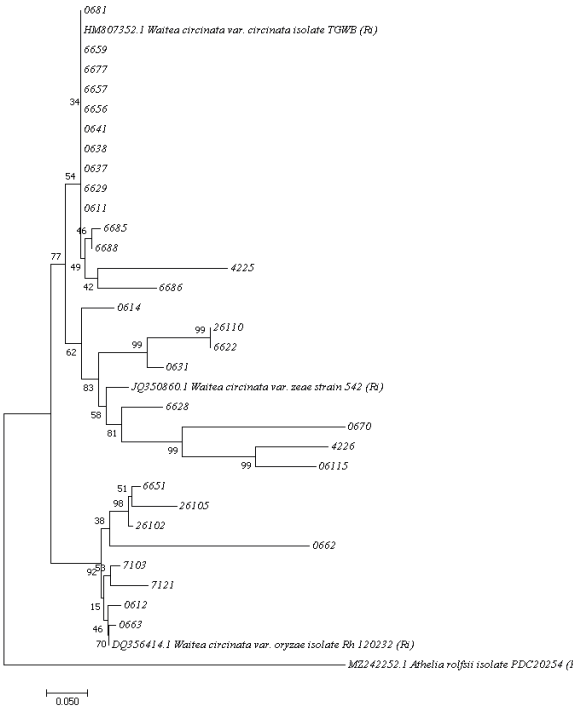

Figure 2. Phylogenetic tree of Waitea circinata isolates based on neighbour-joining method using MEGA 7

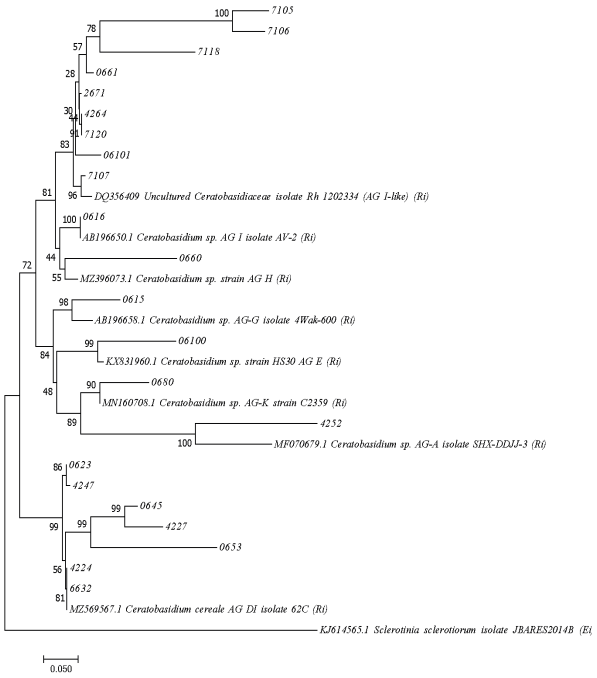

Figure 4. Phylogenetic tree of binucleate Rhizoctonia spp. isolates AGs based on neighbour-joining method using MEGA 7

Figure 3. Brown lesions caused by *Rhizoctonia solani* AG 4 anastomosis group on wheat root and crown root

belonging to pathogen *R. solani* and *R. cerealis* AG D isolates caused brown lesions at different severity in wheat root and hypocotyls as a result of pathogenicity studies, the isolates in AG groups belonging to *Waitea* spp. Caused besides light brown lesions they mostly caused symptoms such as a decrease and shortening in root formation, weak germination, or damping-off.

DISCUSSION AND CONCLUSION

In this study that was performed including different regions of Türkiye, it was determined that different anastomosis groups were causing and not causing disease on wheat. While BN isolates that were isolated from the plants consisted of AG A and DI groups, BN isolates that were isolated from the soil consisted of AG DI, AG I, AG E, AG G, AG K, and AG H. Isolates belonging to AG I-like group were isolated from both plant and soil. While MN *R. solani* AGs were not very different in terms of the place (plant or soil) where the groups were isolated, *Waitea* species were generally isolated from the soil, BN *Rhizoctonia* AGs were generally isolated from the plants. But, in some studies carried out around the world, BN *Rhizoctonia* species were mostly isolated from soil (Chen and Chuang 1997, Juan-Abgona et al. 1996). Previously, several studies were carried out in Türkiye for determining anastomosis groups on wheat, *R. solani* AG 2-1, AG 3, AG 4, AG 5, AG 8, AG 11, W. c. var. *circinata* and binucleate AG I and AG K were determined (Demirci 1998, Ünal and Dolar 2012). In this study, all isolates belonging to *R. solani* AG 4 group that was isolated from five different provinces constituted the most virulent group by causing dark brown and severe lesions in the root and hypocotyls. When examining the studies that were carried out on wheat in the world, AG 4 was the most virulent group in parallel with our study (Sneh et al. 1996). It was observed that there were differences in virulence between different AG groups belonging to the same species in this study. For example, some of the AG 5 isolates were found to be pathogenic while others were non-pathogenic. It was observed that this situation corresponded to the studies that were carried out on this subject in the world. While the rate of disease severity of twelve AG 5 isolates obtained as a result of pathogenicity tests that were made was 0% in 6 isolates, this rate changed between 56-70% in the others. When examining the studies performed in the world, while the isolates belonging to AG 5 group were reasonably virulent in some studies, it is seen that they are not pathogen or have mycorrhizal characteristic in some studies. In the study performed by Tomaso-Peterson and Trevathan (2007) and Xia and Li (1989), while they determined AG 5 as a pathogen in wheat, Demirci (1998) found it as reasonably virulent.

Waitea circinata var. *circinata* isolates that were determined

in this study showed different pathogenic characteristics as in AG 5. While 3 of the isolates that were isolated were not pathogen, it was determined that one of them (0637) was a weak pathogen, and the others were found virulent. They were also determined as a pathogen in wheat and barley. In the study performed on wheat by Demirci (1998) in Türkiye, *W. circinata* var. *circinata* was found reasonably virulent on wheat. In this study, nonpathogen and different levels of virulent *W. circinata* var. *circinata* isolates were obtained. All of the non pathogen isolates were isolated from the soil. *W. circinata* var. zeae species have been determined to significantly affect wheat emergence in wheat fields in the USA and Iran (Kuznia and Windels 1994, Telmadarrehei et al. 2011). In this study, as a result of pathogenicity studies, similar to the results of Kuznia and Windels, a decrease in the germination of *W. circinata* var. zeae isolates in wheat, stunting in plants, a decrease in the number of seminal roots, and superficial discoloration of hypocotyls and roots were observed. Due to the severe symptoms, it should be considered a potential threat to wheat cultivation in Türkiye. Okubara et al. (2008), *R. oryzae* isolates were divided into three genotypes based on their morphology, colony development, and genetic structure, and they were named *R. oryzae* genotype I, *R. oryzae* genotype II and *R. oryzae* genotype III. Okubara et al. (2008) have also shown differences between these genotypes in their study. *R. oryzae* genotype III is a species of *R. oryzae* (*W. circinata* var. *oryzae*) that has been widely known for years and is known as AG WAG O and it was determined as pathogen in many products including wheat in many studies that were performed in the world (Mazzola et al. 1996, Paulitz et al. 2003). Okubara et al. (2008) stated that genotype III is pathogen in wheat and barley but they did not give any information about the pathogenity of genotype I. In our study, six *W. circinata* var. *oryzae* isolates detected in this study took place in the same group with *R. oryzae* genotype I isolates of Okubara et al. (2008). Seven out of eight isolates of *W. circinata* var. *oryzae* isolates were isolated from the soil and none of them was found as pathogen on wheat. The results obtained in this study support the studies of Okubara et al. (2008). In the present study, all binucleate isolates except for *R. cerealis* AG DI were not found to be pathogen. These groups use in the studies of biological control in the world (Cardoso and Echandi 1987, Gutierrez and Torres 1990).

With the present study, *Rhizoctonia* species, AGs, and subgroups in wheat fields in the Central Anatolia Region of Türkiye were matched with isolates from the same group from other countries in Genbank, and their virulence status was determined. Afterward, the relationship status of these groups was revealed. With future studies, it is necessary to focus on the development of pathogenic and non-pathogenic *Rhizoctonia* species and anastomosis groups, and resistant lines and varieties against pathogenic groups in other wheat production areas of Türkiye. Studies to be carried out on biological control with pathogenic groups will provide great benefits to the producer in this field.

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ÖZET

Bu çalışmanın amacı, Türkiye'deki buğday bitkileri ve rizosfer topraklarından patojenik ve patojenik olmayan *Rhizoctonia* tür ve anastomosis gruplarının (AG) türlerini belirlemek ve genetik çeşitliliklerini değerlendirmektir. Türkiye'nin Orta Anadolu Bölgesi'ndeki 5 ildeki buğday tarlalarının bitki ve rizosfer topraklarından *Rhizoctonia* türleri izole edilmiştir. İzolasyonlar sonucunda, toplam 88 adet çok çekirdekli (MN) ve iki çekirdekli (BN) *Rhizoctonia* izolatı elde edilmiştir. İzolatların teşhislerinde rDNA-ITS dizi analizi yöntemi kullanılmıştır ve MN Waitea circinata var. zeae, *W. circinata* var. *oryzae*, *W. circinata* var. *circinata*, MN *Rhizoctonia solani* AG 2-1, AG 2-2, AG 3, AG 4-HGII, AG 4-HGIII, AG 5, AG 8, AG 11 ve BN AG A, AG DI, AG E, AG G, AG H, AG I, AG I-benzeri ve AG K'ya ait oldukları belirlenmiştir. En çok izole edilen grup *W. circinata* var. *circinata* olmuştur. Patojenite çalışmalarında, en virulent grubun *R. solani* AG 4 olduğu saptanmıştır. BN izolatlar arasında, *R. cerealis* AG DI dışındaki diğer grupların patojen olmadığı tespit edilmiştir. İzolatların rDNA-ITS dizilerinden neighbor-joining filogenetik ağaçları oluşturulmuştur. Bu çalışmanın sonucunda, Türkiye'nin Orta Anadolu Bölgesi'ndeki önemli buğday üretim alanlarında MN ve BN *Rhizoctonia* AG izolatlarının bölgedeki dağılımı belirlenmiştir. Ayrıca, bu çalışma, bölgeden elde edilen *Rhizoctonia* AG izolatlarının genetik çeşitliliğinin moleküler bir yaklaşımla değerlendirildiği ilk kapsamlı çalışmadır.

Anahtar kelimeler: akrabalık ilişkileri, anastomosis grup, buğday, *Rhizoctonia*, toprak

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