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Molecular characterization of *Rhizoctonia* anastomosis groups from wheat

Buğdaydan elde edilen *Rhizoctonia* anastomosis gruplarının moleküler karakterizasyonu

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ABSTRACT

Rhizoctonia isolates isolated from plant and soil samples collected from Central Anatolian wheat fields were subjected to PCR-RFLP and DNA sequence analysis using *MseI, Ava II/HincII, MunI, MspI (HapII), HinfI, EcoRI, HaeIII, HinfI* and *HhaI* enzymes. As a result of the analysis, *Rhizoctonia* isolates were determined to be multinucleate *Rhizoctonia solani* AG 2-1, AG 2 BI, AG 3, AG 4 HG II, AG 5, AG 8, and *Waitea circinata* var. *circinata*, *Waitea circinata* var. zeae, *Waitea circinata* var. *oryzae* group I, binucleate Rhizoctonia AG DI, AG E, AG G, AG I and AG K. Isolates in the same group and subgroup in phylogenetic trees were in the same group. Isolates from different groups and subgroups were located in various branches, revealing the genetic differences between the groups and subgroups.

INTRODUCTION

Wheat is one of the most widely used crop plants in human nutrition worldwide. The *Rhizoctonia* genus encompasses a wide range of species, including those with pathogenic, weakly pathogenic, and endophytic characteristics (González et al. 2002). It is one of the main causes of root rot disease, which affects wheat fields. The species in this genus are divided into three groups as multinucleate, binucleate, and uninucleate by the number of nuclei in the hyphae cells. *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). There are 20 different subgroups within these 13 anastomosis groups (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 also have anastomosis groups called WAG O and WAG Z, respectively (Sneh et al. 1996). Okubara et al. (2008) reported that there are three different genotypes of *W. circinata* var. *oryzae* (*R. oryzae* genotypes I, II, and III). Binucleate *Rhizoctonia* species are divided into 19 different anastomosis groups (AG A, B, C, D, E, F, G, H, I, K, L, O, P, Q, R, S, U, V, 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).

Rhizoctonia solani AG 1IB, 2-1, 2-2, 3, 4, 5, 6, 8, 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

et al. 2007). Of these groups, *W. circinata* var. *circinata* and *W. circinata* var. *zeae*, AG 2-1, 3, 4 HGII, 5, 8, 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).

Although the identification of anastomosis groups using classical methods remains valid and widely practised today, these methods have certain limitations. Isolates belonging to a particular anastomosis group may combine with more than one anastomosis group or may not match any anastomosis group. In addition, although some anastomosis groups are divided into different subgroups based on features such as pathogenicity test, isoenzyme analysis and fatty acid profiles, they can form anastomosis groups with each other (Carling et al. 2002, Cubeta and Vilgalys 1997, Sneh et al. 1996). For these reasons, molecular methods based on DNA polymorphisms have become widely used today to determine anastomosis groups and subgroups of *Rhizoctonia* isolates (Ceresini et al. 2002, Vigalys and Cubeta 1994). Studies have shown that sequence differences in the 28S and ITS (internal transcribed spacer) regions of ribosomal DNA provide important polymorphisms in detecting anastomosis groups (Gonzales et al. 2002). The PCR-RFLP method is very useful, especially in large populations and in the discrimination of multinucleate *Rhizoctonia* isolates (Guillemaut et al. 2003, Pascual et al. 2000).

When other molecular studies are examined, various molecular markers have been used for characterization and grouping *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. 2007). Currently, the rDNA-ITS sequence analysis seems to be the most appropriate method for classification of *Rhizoctonia* spp., and sequence analysis of the ITS-5.8S rDNA region has been used as a suitable molecular tool for identification of *R. solani* subgroups (Carling et al. 2002, Hyakumachi et al. 2005, Priyatmojo et al. 2001, Salazar et al. 2000a, 2000b). 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 AG 6 (Pope and Carter 2001). Fewer studies were reported on rDNA-ITS sequence analyses of binucleate 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 anastomosis groups and subgroups of *Rhizoctonia* isolates obtained from wheat roots and rhizosphere soils using PCR-RFLP and DNA sequence methods, and to reveal the genetic diversity among them.

MATERIALS AND METHODS

Molecular characterization of anastomosis groups of Rhizoctonia and Waitea isolates

DNA isolation

Rhizoctonia isolates were collected from wheat plants and soil grown in the provinces of Konya, Ankara, Yozgat, Eskişehir, Kayseri, Nevşehir, Aksaray, Kırşehir and Kırıkkale within the scope of another study (Ünal et al. 2014, 2015). DNA isolation of *Rhizoctonia* and *Waitea* isolates obtained from wheat fields was realized according to the method of Lee and Taylor (1990). For this purpose, all isolates were grown on PDA (Merck) medium for 7 days at 23 °C, scraped with a sterile spatula, and then ground in liquid nitrogen. Then, 500 µl of extraction buffer (50 mM Tris-HCl pH 8, 150 mM NaCl, 100 mM EDTA, 2% SDS) was added to each tube and mixed until well suspended. Then, the obtained extract was incubated at 65 °C for 1 hour to break down nucleic acids. After the incubation period, an equal volume of phenol: chloroform (25:24 v/v) was added to the eppendorf tubes and centrifuged at 13,000 g for 15 minutes. 400 µl of the collected liquid was transferred to new eppendorf tubes and incubated by adding RNase A to it. After incubation, 250 µl of chloroform: isoamyl alcohol (24:1 v/v) was added to the eppendorf tubes and centrifuged again for 15 minutes. The liquid collected at the top was taken back into clean eppendorf tubes, an equal volume of isopropanol was added, and then incubated for 1 hour at -20 °C. It was centrifuged for 5 minutes, and collected at the bottom of the tube. Then, the obtained DNA pellet was washed with 80% ethanol, dried, suspended in 25-50 µl of sterile bi-distilled water and stored at -20 °C. DNA concentrations were determined using a spectrophotometer (NanoDrop-1000, Thermo Scientific) by measuring at 260 nm.

PCR-RFLP analysis of Rhizoctonia and Waitea isolates

Anastomosis groups were detected by PCR-RFLP analysis of multinucleated *Rhizoctonia solani* isolates according to Guillemaut et al. (2003). Primers RS1 (5′-CCTGTGCACCTGTGAGACAG-3′) and RS4 (5′-TGTCCAAGTCAATGGACTAT-3′) were used for amplification of the ITS1-5.8S-ITS2 regions. PCR reactions were performed in 50 µl volumes containing 200 µM dNTPs, 0.2 µM primer, 10X reaction buffer, 1.5 U Taq DNA polymerase (Thermo Scientific), 1.5 mM $MgCl_2$, and 30 ng fungal DNA. The PCR cycling protocol included an initial denaturation step at 94 °C for 5 minutes, 35 cycles of one minute at 94 °C, one minute at 56 oC, one minute at 72 °C, and finally five minutes extension at 72 °C. Determination of anastomosis groups of binucleate *Rhizoctonia* and *Waetia* isolates was carried out by White et al. (1990) using ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-

TCC TCC GCT TAT TGA TAT GC-3') primers. In PCR amplifications performed with ITS-1/4 primers, primer annealing and extension times were set to 30 seconds. Then, the obtained PCR products were observed by electrophoretic separation in 1xTAE buffer at 100V on a 1% agarose gel for control purposes (Sambrook et al. 1989).

PCR-RFLP analyzes of the isolates, whose ribosomal DNA ITS regions were amplified with different primers, were performed according to the manufacturer's protocol using distinct restriction enzymes specifically specified for the separation of anastomosis groups. For this purpose, approximately 10 µl of PCR product was incubated with 5 U of enzyme at appropriate temperatures. Then, the restriction fragments were separated electrophoretically in a 2-3% agarose gel.

In PCR-RFLP analyzes of multinucleated *Rhizoctonia solani* isolates, MseI, Ava II/*Hinc*II (double cut) and *Mun*I restriction enzymes were compared with the enzyme profiles reported by Guillemaut et al. (2003). *Msp*I (HapII) and *Hinf*I enzymes were used in PCR-RFLP studies of *Waitea* spp. isolates as reported by Toda et al. (2005).

In studies conducted to determine the AG of binucleate *Rhizoctonia* isolates, the obtained PCR products were cut with *Eco*RI, *Hae*III, *Hinf*I and *Hha*I restriction enzymes and the band sizes were compared with the cutting profiles reported by Toda et al. (1999). In addition, the ITS region of the isolates representing each identified AG group was sequenced and examined by BLAST and *in silico* analysis.

Analysis and evaluation of data

By using a 100 bp DNA ladder (Gene Ruler 100 bp DNA ladder, Thermo Scientific) and Syngene Gene Tools bioimaging (Cambridge, England) system, the sizes of the band profiles generated by enzyme cutting were evaluated, and anastomosis groups were determined by comparing them with the enzyme sizes and profiles reported by other researchers. The enzyme profiles of the isolates examined were also confirmed by *in silico* analysis using the program DNAstar version 7.1 (Madison, WI). In addition, using the Mega7 (Kumar et al. 2016) genetic program, the ITS sequences of the isolates examined in sequence analysis and the reference isolates obtained from GenBank were analyzed by UPGMA analysis, and dendrograms were created to illustrate the genetic relationship between the isolates.

RESULTS AND DISCUSSION

Molecular characterization of the anastomosis groups of Rhizoctonia and Waitea isolates

A PCR RFLP and DNA sequence analysis were used to confirm the anastomosis groups of *Rhizoctonia* and *Waieta* isolates in wheat production areas. The result of the PCR-RFLP analysis revealed that, although the various AGs tested generally showed similar enzyme profiles within the same group, depending on the enzymes used, there was a high degree of genetic polymorphism between the various AGs and subgroups. Detection of anastomosis groups of multinucleated *Rhizoctonia solani* isolates was performed according to Guillemaut et al. (2003). The PCR products obtained after amplification with RS1/4 primers were cut with MseI, Ava II/*Hinc*II (double cutting) and *Mun*I restriction enzymes and compared to the enzyme profiles reported by Guillemaut et al. (2003). Table 1 displays the band sizes and profiles that were produced by enzyme cutting.

Table 1. Enzyme profiles of multinucleated *Rhizoctonia solani* isolates obtained through PCR-RFLP analysis

AG	ITS region (bp)	*Mse*I	*Ava*II *Hinc*II	*Mun*I
AG-3	520	220,190,110	330,190	520
AG-4 HGII	524	217,138,82,62,25	474,50	330,194
AG-5	512	319,193	450,62	274,187,51
AG-8	495	216,195,84	451,44	306,189
AG2-1	522	216,196,89.21	452,70	332,190
AG2-BI	545	217,143,87,61,26	545	336, 209

The assessments revealed that the AG-3 isolates discovered by Guillemaut et al. (2003) produced an enzyme profile known as BNAN (Figure 1). This group was isolated from Kırıkkale-Karakeçili and Kırşehir. After cutting the isolates with MseI, three bands with a size of 220, 190, and 110 bp were formed. While Ava II/*Hinc*II enzyme cutting resulted in bands of 330, 190 bp in size, it was observed that the *Mun*I enzyme did not have a cutting site in these isolates.

The PCR-RFLP analysis of AG-4 isolates revealed that they belonged to the AG-4 HGII subgroup and formed the

enzyme profile specified as IENA (Figure 2). After cutting the isolates with *Mse*I, an enzyme profile of 217, 138, 82, 62, and 25 bp was formed. As a result of the *Ava*II/*Hinc*II enzyme cutting, two bands with a size of 474 and 50 bp were obtained, while the *Mun*I enzyme created two profiles with a size of 330 and 194 bp.

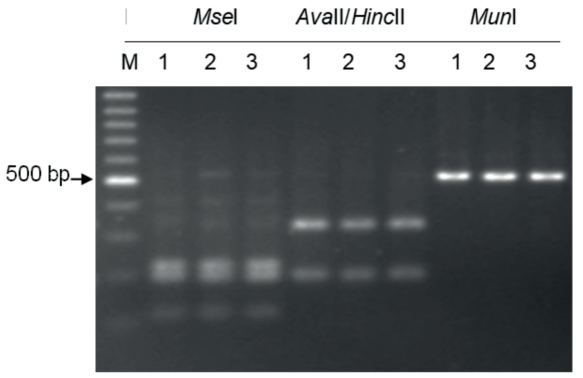


Figure 1. Band profiles obtained by cutting AG-3 isolates with *Mse*I, *Ava*II/*Hinc*II and *Mun*I restriction enzymes (M: Marker Gene Ruler 100 bp DNA ladder, Thermo Scientific; 1: ED-84 (Tester isolate AG-3), 2: Isolate Karakeçili, 3:Isolate Krş-22)

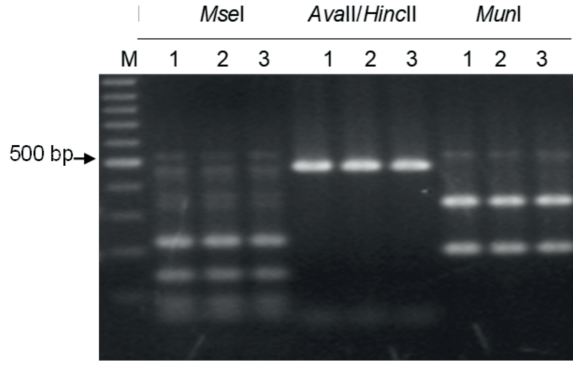


Figure 2. Band profiles obtained by cutting AG-4 isolates with *Mse*I, *Ava*II/*Hinc*II and *Mun*I restriction enzymes (M: Marker Gene Ruler 100 bp DNA ladder, Thermo Scientific; 1: Rh-74 (Tester isolate AG-4 HGII), 2: Isolate KÇ-5/8, 3: Isolate 1070)

In classical anastomosis tests, AG-5 isolates were found to be related to the reference isolate, and Guillemaut et al. (2003) reported different enzyme profiles from HAAC (Figure 3). After the isolates were cut with *Mse*I, two bands of 319, 193 bp were formed. Two bands of 450, 62 bp in size were obtained as a result of *Ava*II/*Hinc*II enzyme cleavage, while *Mun*I enzyme formed an enzyme profile of 274, 187 and 51 bp. Additionally, AG-5 isolates were examined by sequence analysis using ITS1/4 primers.

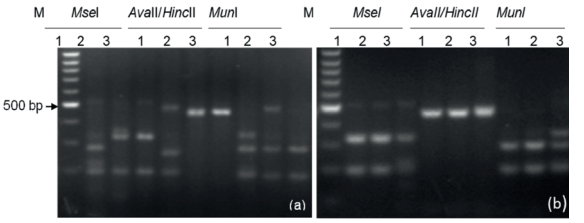


Figure 3. Band profiles obtained by cutting AG-5 isolates with *Mse*I, *Ava*II/*Hinc*II and *Mun*I restriction enzymes (M: Marker Gene Ruler 100 bp DNA ladder, Thermo Scientific; a) 1:GM-10 (Tester isolate AG-5), 2: Isolate 1033, Isolate 3: 1035; b) 1: Isolate 1022, 2: Isolate EA-7, 3: Isolate 1009

According to BLAST analysis, some sequences were listed in GenBank as AG-5 (DQ278975), Guillemaut et al. (2003) showed a different enzyme profile from the enzyme profile in the form of HAAC. An *in silico* analysis revealed a T base in the GTYRAC region (323 bp), which should be the *Hinc*II enzyme cutting site, resulting in the expected enzyme profile not being achieved. However, it was determined that the tested isolates showed 99-96% genetic similarity with the AG-5 isolates in GenBank. Sharon et al. (2007) stated that the similarity rate between AG-5 isolates varied between 96-100%.

It was observed that AG-8 isolates formed an enzyme profile similar to that reported by Guillemaut et al. (2003) in the form of DENA (Figure 4). A cut with *Mse*I resulted in three bands of 216, 195, and 84 bp. A cut with MseI resulted in three bands of 216, 195, and 84 bp. Two bands of 451, 44 bp in size were obtained through enzyme cleavage by *Ava*II/*Hinc*II, while *Mun*I enzyme created two profiles with sizes of 306 and 189 bp.

As reported by Guillemaut et al. (2003), AG 2-1 isolates formed an enzyme profile in the form of DANA after enzyme digestion (Figure 5). Following *Mse*I cutting, an enzyme profile with a size of 216, 196, 89, 21 bp was formed. 452, 70 bp in size with *Ava*II/*Hinc*II; An enzyme profile of 332, 190 bp in size was created with the *Mun*I enzyme. Additionally, it was observed that some isolates classified as AG 2-1 exhibited distinct enzyme profiles. However, in the sequence analysis, it was determined that these isolates showed 99% genetic similarity with the AG 2-1 isolates in the GenBank.

In the KÇ-6/6 isolate, which was classified as AG 2-BI, enzyme fractionation in the form of INNA was detected (Figure 6). Using *Mse*I, a profile of 217, 143, 87, 61, and 26 bp was formed. *Ava*II/*Hinc*II, on the other hand, does not have any cut-off regions in this subgroup. Following the *Mun*I enzyme cut, two profiles of 336 and 209 bp were formed.

In the UPGMA analysis performed using the MEGA7 program of the multinucleate *Rhizoctonia* isolates examined

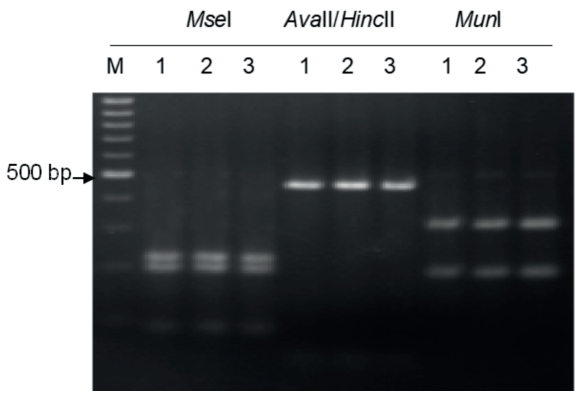


Figure 4. Band profiles obtained by cutting AG-8 isolates with *Mse*I, *Ava*II/*Hinc*II and *Mun*I restriction enzymes (M: Marker Gene Ruler 100 bp DNA ladder, Thermo Scientific; 1: JW-92 (Tester isolate AG-8), 2: Isolate 1037, Isolate 3:1056)

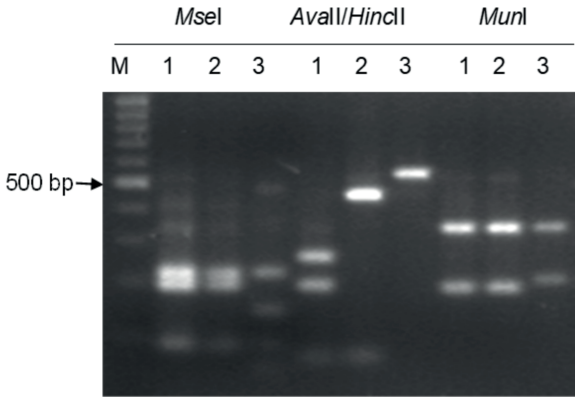


Figure 5. Band profiles obtained by cutting AG-2 isolates with *Mse*I, *Ava*II/*Hinc*II and *Mun*I restriction enzymes (Marker Gene Ruler 100 bp DNA ladder MBI Fermantase; 1: F56L (Tester isolate AG 2-1), 2: Isolate 1026 (AG 2-1), 3: Isolate KÇ-6/6 (AG-BI)

by sequence analysis within the scope of the study, the isolates belonging to different AGs were grouped with the reference isolates obtained from GenBank (Figure 6). Apart from AG 2-1 isolates, all other isolates were found to belong to the same group. The bootstrap values show it is in the same group as the reference isolates. AG 2-1 isolates were included in two subgroups, along with the reference isolates. Similarly, Zhou et al. (2009), in their study characterizing *Rhizoctonia* isolates obtained from lupine, stated that sequence analysis of the ITS region was useful in distinguishing AG-4 isolates but insufficient in distinguishing AG 2-1 and 2-2 isolates.

With ITS1/4 primers, approximately 645, 626, and 630 bp of PCR products were amplified from the tested *Waitea circinata* var. *circinata*, *oryzae* and *zeae* isolates, respectively, to determine subgroups of *Waitea* isolates. PCR RFLP studies using *Msp*I (*Hap*II) and *Hinf*I enzymes were performed by Toda et al. (2005) (Table 2). *Msp*I enzyme digestion of isolates classified as *W. circinata* var. *circinata*

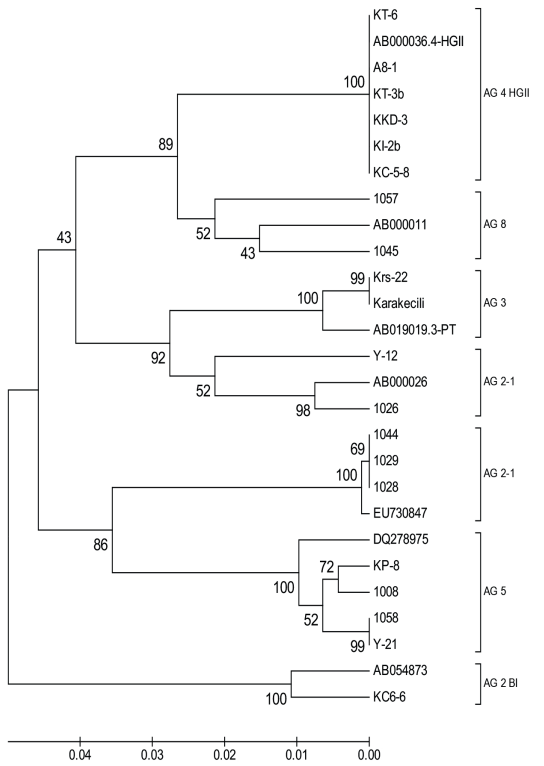


Figure 6. BUPGMA dendrogram obtained by sequence analysis of the ITS region of multinucleated *Rhizoctonia solani* isolates and showing the genetic relationship between the isolates

produced two fragments of 475 and 170 bp in size, DNA profiles of 330, 90, and 80 bp were obtained, while *Hinf*I enzyme produced three DNA profiles of 330, 90, and 80 bp (Figure 7a). Nevertheless, following *Msp*I enzyme cutting, it was observed that certain isolates had an uncut 645 bp band (Figure 7b). Similarly, Chen et al. (2009) reported that a similar 645 bp band was found in some isolates in enzyme cleavage with *Msp*I of *W. circinata* var. *circinata* isolates. In the sequence analysis, they reported that these isolates were heterozygous for the *Msp*I enzyme cleavage region and that this ITS region in some isolates was not cut due to the presence of 4 additional sequence positions in the ITS region.

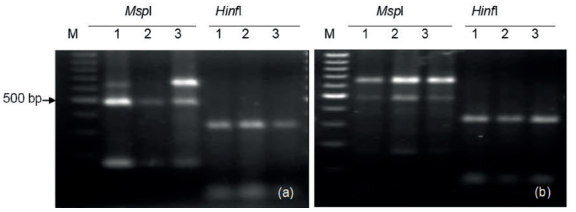


Figure 7. Band profiles obtained by cutting *Waitea circinata* var. *circinata* isolates with *Msp*I(HapII) and *Hinf*I restriction enzymes (M: Marker Gene Ruler 100 bp DNA ladder, Thermo Scientific; (a): 1: W616 (Tester isolate Wcc), 2: Isolate A-10, Isolate 3: KBÜ-3; (b): 1: Isolate K-15, 2: Isolate KP-5, 3: Isolate N-18

Table 2 . Enzyme profiles derived from PCR-RFLP analysis of *Waitea* spp. isolates

***Waitea* spp.**	**ITS bp**	***MspI***	***HinfI***
circinata	645	645/475,170	330,90,80
oryzae	626	355,271	313,117,65,62,61
zeae	630	630	330,90,80

The isolates classified as *W. circinata* var. zeae had similar enzyme profiles to those reported by Toda et al. (2005), and they did not possess the *Msp*I enzyme cutting site. Using the *Hinf*I enzyme, DNA profiles of 330, 90, and 80 bp in size were obtained (Figure 8).

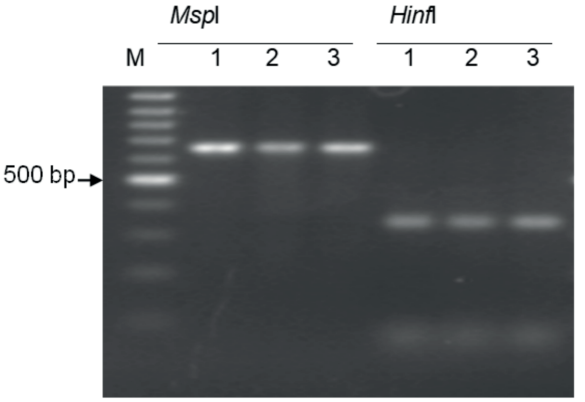


Figure 8. Band profiles obtained by cutting *Waitea circinata* var. *zeae* isolates with *Msp*I *Msp*I(*Hap*II) and *Hinf*I restriction enzymes (M: Marker Gene Ruler 100 bp DNA ladder Thermo Scientific; 1: Rz590 (Tester isolate Wcz), 2: Isolate AB-7, Isolate 3: 1010

It was determined that the isolates classified as Waitea cir. var. oryzae matched with *Waitea cir.* var. *oryzae* group I tester isolate (Rh120232) in classical mating tests. However, in PCR RFLP analysis, it was determined that the tester isolate obtained from wheat showed a different enzyme profile than the *Msp*I (660 bp) and *Hinf*I (200, 130, 90, 70 bp) enzyme profiles stated by Toda et al. (2005). Based on the ITS sequence of the tester isolate in the GenBank, an *in silico* analysis revealed 99% similarity to the isolate obtained from wheat. *Waitea cir.* var. *oryzae* group II isolates were also found to have the enzyme profile described by Toda et al. (2005). It was determined that the isolates obtained from wheat formed two bands of 355 and 271 bp in size with *Msp*I, while they formed an enzyme profile of 313, 117, 65, 62, and 61 bp in size with *Hinf*I enzyme (Figure 9).

UPGMA analysis of the sequence data of the ITS region of *Waitea* spp. isolates confirmed the subgroup distribution, and all isolates were included in the same group in the dendrogram with the reference isolates. The high bootstrap values obtained also supported the grouping of these isolates in the dendrogram (Figure 10).

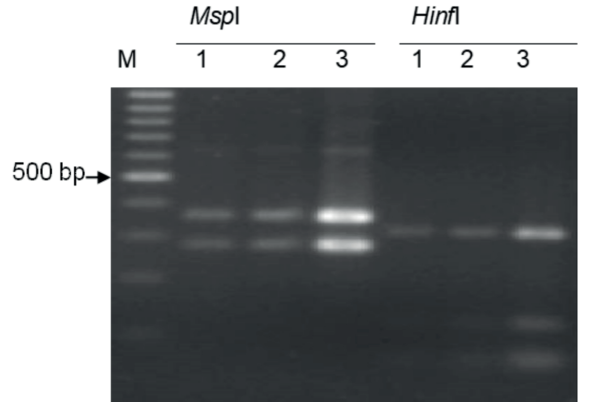


Figure 9. Band profiles obtained by cutting with *Waitea circinata* var. *oryzae Msp*I(*Hap*II) and *Hinf*I restriction enzymes (M: Marker Gene Ruler 100 bp DNA ladder, Thermo Scientific; 1: Rh120232 (Tester isolate Wco), 2: Isolate Y-1/d, Isolate 3: 1049

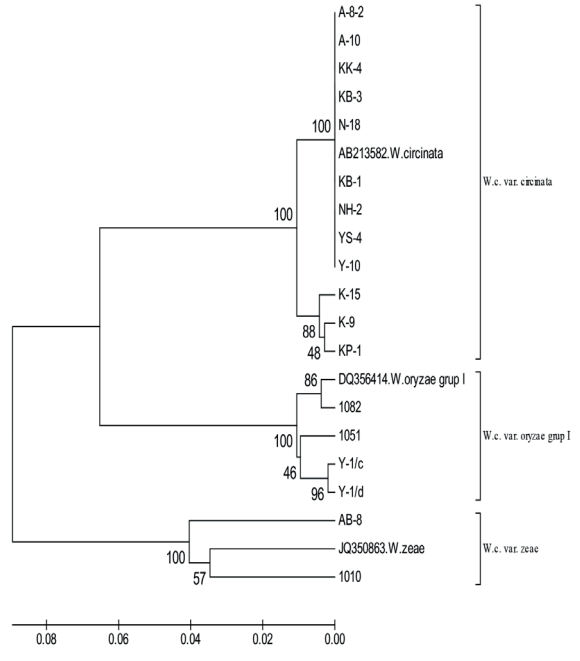


Figure 10. Dendrogram obtained by UPGMA analysis of the ITS region of *Waitea* spp. isolates

To determine the AG of the binucleate *Rhizoctonia* isolates obtained in the studies, ITS1/4 primers were used, and PCR products were cut with *Eco*RI, *Hae*III, *Hinf*I and *Hha*I restriction enzymes (Table 3). These cutting profiles

were compared with those reported by Toda et al. (1999). In addition, the ITS region of the isolates representing each identified AG group was sequenced and examined by BLAST and *in silico* analysis.

Figure 11 a, b.

Isolates classified as binucleate *Rhizoctonia* AG-K, similar to the tester isolate, created enzyme profiles of 373, 303 bp; and 439, 122, 115 bp sizes, respectively, as a result of cutting with

Table 3. PCR-RFLP enzyme profiles of Binucleate *Rhizoctonia* isolates.

AG	ITS bp	*Eco*RI	*Hae*III	*Hinf*I	*Hha*I
AG-I	655	359,296	478,117,60	305,217,125	334,321
	690	363,327	573,117	336,177,125,44	365,325
AG-K	676	373,303	439,122,115	356,267,45	341,335
AG-D	689	368,321	404,120,88,77	283,127,116,108,47	359,330
AG-G	656	367,289	476,120,60	350,298	327,324
AG-E	680	370,320	380,120	350,330	340,340

After enzyme digestion of the binucleate *Rhizoctonia* AG-I isolates, two different groups were observed (Figure 11). The enzyme profile of some AG-I isolates was similar to that of the tester AG-I isolate, but other isolates formed a different profile than the tester isolate. When the isolates that displayed the same profile as the Tester AG-I isolate were cut with the *Eco*RI enzyme, fragments of 359 bp and 296 bp were produced. In contrast, when using the *Hae*III enzyme, the resulting enzyme profile consisted of fragments measuring 478 bp, 117 bp, and 60 bp. As a result of cutting with *Hinf*I and *Hha*I enzymes, band profiles with sizes of 305, 217, 125 and 334, 321 bp were obtained, respectively (Figure 11a). In the enzyme digestion of other AG-I isolates, *Eco*RI: 363, 327 bp; *Hae*III: 573, 117 bp; enzyme profiles with sizes of *Hinf*I: 336, 177, 125, 44 bp and HhaI: 365, 325 bp were obtained (Figure 11b). Similarly, Sharon et al. (2008) reported that there were two groups among binucleate AG-I isolates, and the genetic similarity rate between isolates varied between 94-100%.

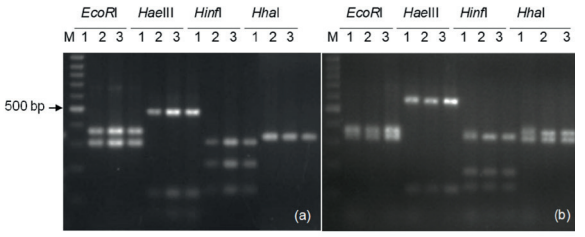


Figure 11. Band profiles obtained by cutting AG-I isolates with *Eco*RI, *Hae*III, *Hinf*I and *Hha*I restriction enzymes (M: Marker Gene Ruler 100 bp DNA ladder, Thermo Scientific; (a): 1: AV-2 (Tester isolate AG-I), 2: Isolate 1081, 3: Isolate 1092; (b): Isolate 1: Kırş-2: Isolate Aç-10, Isolate 3: 1048)

*Eco*RI, *Hae*III enzymes (Figure 12). After cutting with *Hinf*I and *Hha*I enzymes, band profiles with sizes of 356, 267, 45 and 341, 335 bp were obtained, respectively.

Molecular analysis revealed that the isolates classified as binucleate Rhizoctonia AG-D belonged to the AG-D (I) group and exhibited a distinct enzyme profile compared to the TAK-14-K-T isolate (AG-D (II)) used as a reference. However, using BLAST and in silico analyses, it was determined that these isolates belonged to the AG-D (I) group and showed 99% genetic similarity with the isolates in the gene bank. Cutting with *Eco*RI and *Hae*III enzymes produced enzyme profiles of 368, 321 bp and 404, 120, 88, 77 bp (Figure 13). With *Hinf*I and *Hha*I enzymes, band profiles of 283, 127, 116, 108, 47 and 359, 330 bp sizes were obtained, respectively. In addition, it was observed that some isolates formed 330 (283+47), 127, 116, 108 bp-sized enzyme profile due to a mutation in the *Hinf*I cutting region.

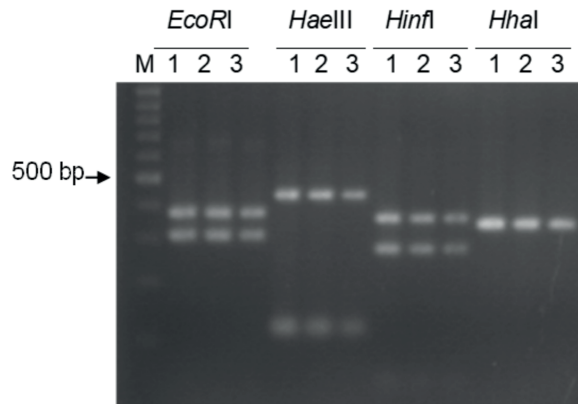


Figure 12. Band profiles obtained by cutting AG-K isolates with *Eco*RI, *Hae*III, *Hinf*I and *Hha*I restriction enzymes (M: Marker Gene Ruler 100 bp DNA ladder, Thermo Scientific; 1: B-145 (Tester isolate AG-K), 2: İzolat AP-Kargalıyolu, İzolat 3: KK-2)

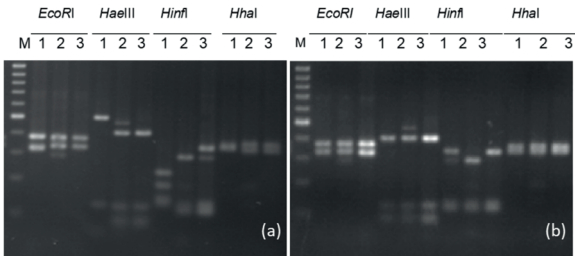


Figure 13. Band profiles obtained by cutting AG-D(I) isolates with *Eco*RI, *Hae*III, *Hinf*I and *Hha*I restriction enzymes (M: Marker Gene Ruler 100 bp DNA ladder, Thermo Scientific; (a): 1: TAK-14-K-T (Tester isolate AG-D(II), 2: Isolate 1012, Isolate 3: 1036; (b): Isolate 1: 1061: Isolate 1012, Isolate 3: 1038)

Isolates classified as binucleate *Rhizoctonia* AG-G showed similar enzyme profiles to the tester isolate (Figure 14). As a result of digestion with *Eco*RI and *Hae*III enzymes, enzyme profiles of 367, 289 bp and 476,120, 60 bp sizes were obtained, respectively. As a result of cutting with *Hinf*I and *Hha*I enzymes, band profiles of 350, 298 and 327, 324 bp in size were obtained, respectively.

The isolate classified as binucleate *Rhizoctonia* AG-E is similar to the tester isolate, as a result of cutting with *Eco*RI and *Hae*III enzymes. The sizes of the enzyme profiles were 370, 320 bp, and 380, 120 bp, respectively (Figure 15). Cutting with *Hinf*I and *Hha*I enzymes produced band profiles that were 350, 330 and 340, 340 bp in size, respectively.

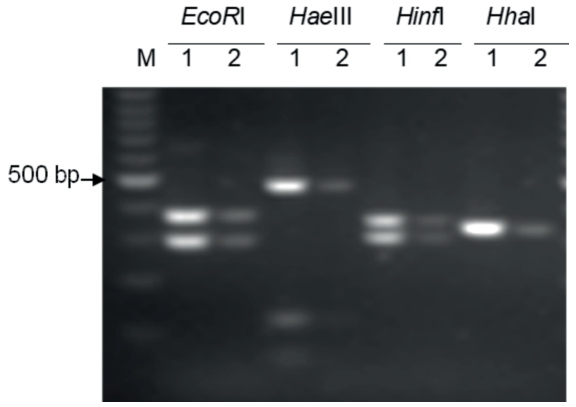


Figure 14. Band profiles obtained by cutting AG-G isolates with *Eco*RI, *Hae*III, *Hinf*I and *Hha*I restriction enzymes (M: Marker Gene Ruler 100 bp DNA ladder MBI Fermantase; 1: AH-9 (Tester isolate AG-G), 2: Isolate 1096)

UPGMA analysis of the ITS region sequence data also confirmed the grouping of binucleated *Rhizoctonia* isolates, as in other isolates, and the isolates were included in the same group in the dendrogram with the reference isolates (Figure 16).

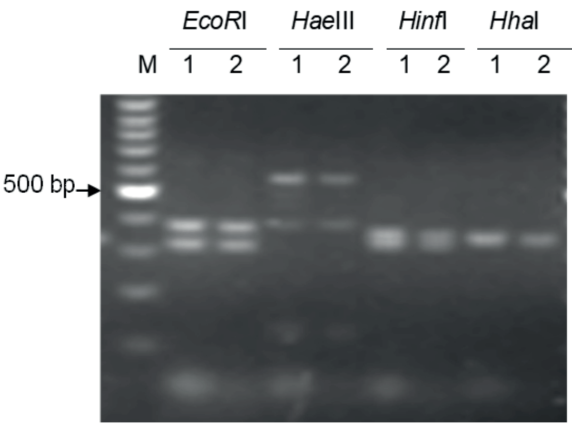


Figure 15. Band profiles obtained by cutting AG-E isolates with *Eco*RI, *Hae*III, *Hinf*I and *Hha*I restriction enzymes (M: Marker Gene Ruler 100 bp DNA ladder, Thermo Scientific; 1: F-18 (Tester isolate AG-E), 2: Isolate 1093)

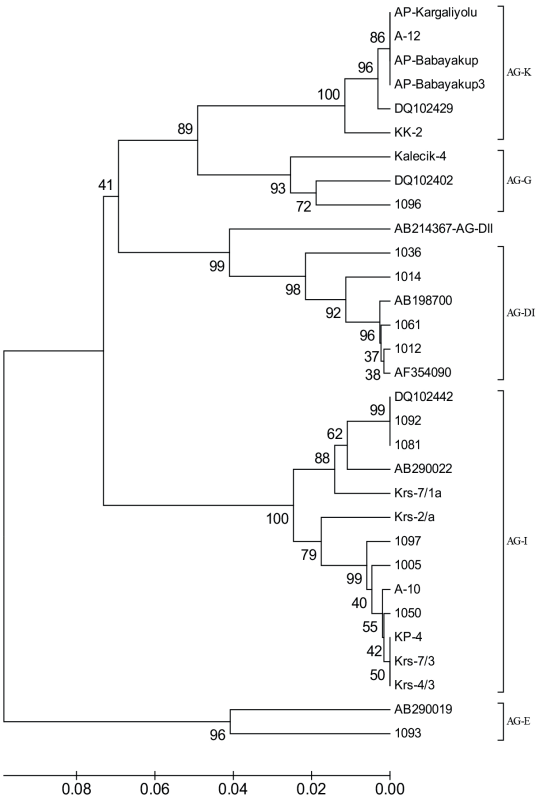


Figure 16. Dendrogram obtained by UPGMA analysis of the ITS region of binucleate *Rhizoctonia* spp. isolates

Rhizoctonia species and anastomosis groups colonizing wheat fields in different geographical and climatic regions should be identified using various molecular techniques and primers. Genetic differences between the anastomosis groups and the new groups should be revealed. Determining both the distribution and genetic differences of *Rhizoctonia* anastomosis groups isolated from different provinces will contribute to both pathogen control and rotation plant

selection, as *Rhizoctonia* anastomosis groups are host-specific. It is also important to compare with studies on anastomosis groups in other hosts.

Author's Contributions

Authors declare that each author's contribution is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

ÖZET

Orta Anadolu buğday tarlalarından toplanan bitki ve toprak örneklerinden izole edilen *Rhizoctonia* izolatları *Mse*I, *Ava* II/*Hinc*II, *Mun*I, *Msp*I (*Hap*II), *Hinf*I, *Eco*RI, *Hae*III, *Hinf*I ve *Hha*I enzimleri kullanılarak PCR-RFLP ve DNA sekans analizine tabi tutulmuştur. Analiz sonucunda *Rhizoctonia* izolatlarının çok çekirdekli *Rhizoctonia solani* AG 2-1, AG 2 BI, AG 3, AG 4 HG II, AG 5, AG 8 ve *Waitea circinata* var. *circinata*, *Waitea circinata* var. *zeae*, *Waitea circinata* var. *oryzae* grup I, çift çekirdekli *Rhizoctonia* AG DI, AG E, AG G, AG I ve AG K olduğu belirlenmiştir. Filogenetik ağaçlarda aynı grup ve altgrupta yer alan izolatlar aynı grupta yer almışlardır. Farklı grup ve alt gruptaki izolatlar ise farklı dallarda yer alarak grup ve altgruplar arasındaki genetik farklılığı ortaya koymuştur.

Anahtar kelimeler: *Rhizoctonia*, anastomosis grup, PCR, RFLP, buğday

REFERENCES

Boysen M., Borja M., del Moral C., Salazar O., Rubio V., 1996. Identification at strain level of *Rhizoctonia solani* AG 4 direct isolates by sequence of asymmetric PCR products of the ITS regions. Current Genetics, 29 (2), 174–181. doi: 10.1007/BF02221582

Carling D.E., Kuninaga S., Brainard K.A., 2002. Hyphal anastomosis reactions, rDNA-internal transcribed spacer sequences, and virulence levels among subsets of *Rhizoctonia solani* anastomosis group-2 (AG-2) and AG-BI. Phytopathology, 92 (1), 43–50.

Ceresini P.G., Shew H.D., Vilgalys R.J., Cubeta M.A., 2002. Genetic diversity of *Rhizoctonia solani* AG 3 from potato and tobacco in North Carolina. Mycologia, 94 (3), 437-449.

Chen C., Cerda K.A., Kaminski J.E., Douhan G.W., Wong F.P., 2009. Geographic distribution and rDNA-ITS region sequence diversity of *Waitea circinata* var. *circinata* isolated from annual bluegrass in the United States. Plant Disease, 93 (9), 906-911. doi: 10.1094/PDIS-93-9-0906

Cubeta M.A., Vilgalys R., 1997. Population Biology of the *Rhizoctonia solani* complex. Phytopathology, 87 (4), 480-484. https://doi.org/10.1094/PHYTO.1997.87.4.480

Demirci E., 1998. *Rhizoctonia* species and anastomosis groups isolated from barley and wheat in Erzurum, Turkey. Plant Pathology, 47 (1), 10-15. https://doi.org/10.1046/j.1365-3059.1998.00214.x

Dong W., Li Y., Duan C., Li X., Naito S., Conner R.L., Yang G., Li C., 2017. Identification of AG-V, a new anastomosis group of binucleate *Rhizoctonia* spp. from taro and ginger in Yunnan province. European Journal of Plant Pathology, 148 (4), 839-851.

Erper I., Ozer G., Kalendar R., Avci S., Yildirim E., Alkan M., Turkkan M., 2021. Genetic diversity and pathogenicity of *Rhizoctonia* spp. isolates associated with red cabbage in Samsun (Turkey). Journal of Fungi, 7 (3), 234. doi: 10.3390/jof7030234

González V., Salazar O., Julián M.C., Acero J., Portal M.A., Muñóz R., López-Córcoles H., Gómez-Acebo E., López-Fuster P., Rubio V., 2002. *Ceratobasidium albasitensis*. A new Rhizoctonia-like fungus isolated in Spain. Persoonia, Molecular Phylogeny and Evolution of Fungi, 17 (4), 601614.

Guillemaut C., Edel-Hermann V., Camporota P., Alabouvette C., Richard-Molard M., Steinberg C., 2003. Typing of anastomosis groups of *Rhizoctonia solani* by restriction analysis of ribosomal DNA, Canadian Journal of Microbiology, 49 (9), 556–68. doi: 10.1139/w03-066

Hyakumachi M., Priyatmojo A., Kubota M., Fukui H., 2005. New anastomosis groups, AG-T and AG-U, of binucleate *Rhizoctonia* causing root and stem rot of cut-flower and miniature roses. Phytopathology, 95 (7), 784–792. doi: 10.1094/PHYTO-95-0784

Kumar S., Stecher G., Tamura K., 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evoluation, 33 (7), 1870-1874. doi: 10.1093/molbev/msw054

Kuninaga S., 1996. DNA base sequence complementary analyses. In: *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology and disease control. Sneh B., Jabaji-Hare S.H., Neate S., Dijst G. (Eds.). Kluwer, Dordrecht, pp 73–80.

Kuninaga S., Carling D.E., Takenuichi T., Yokosawa R., 2000. Comparison of rDNA-ITS sequences between potato and tobacco strains in *Rhizoctonia solani* AG-3. Journal of General Plant Pathology, 66 (1), 2-11. https://doi.org/10.1007/PL00012917

Lee S.B., Taylor J.W., 1990. Isolation of DNA from fungal mycelia and single spores. In: PCR protocols: a guide to methods and applications. . Innis M.A., Gelfand D.H., Sninsky J.J., White T.J., (Eds.). Academic Press, New York, 282-287 pp.

Ma M., Tan T.K., Wong S.M., 2003. Identification and molecular phylogeny of Epulorhiza isolates from tropical orchids. Mycological Research, 107 (9), 1041–1049. doi: 10.1017/s0953756203008281

Meyer L., Nel L.H., Carling D.E., 1997. Anastomosis grouping and molecular genetic typing of *Rhizoctonia solani* causing crater disease of wheat in South Africa. Diagnosis and identification of plant pathogens. Proceedings 4th International Symposium of the European Foundation for Plant Pathology, 9-12 September 1996, 53-57, Bonn, Germany.

Misawa T., Kurose D., 2018. First report of binucleate *Rhizoctonia* AG U causing black scurf on potato tubers in Japan. New Disease Reports, 38, 24.

Ogoshi A., Cook R.J., Bassett E.N., 1990. *Rhizoctonia* species and anastomosis groups causing root rot of wheat and barley in the Pacific Northwest. Phytopathology, 80 (9), 785-788.

Okubara P.A., Schroeder K.L., Patilitz T.C., 2008. Identification and quantification of *Rhizoctonia solani* and *R. oryzae* using real-time polymerase chain reaction. Phytopathology, 98 (7), 837-847.

Otero J.T., Ackerman J.D., Bayman P., 2002. Diversity and host specificity of endophytic *Rhizoctonia*-like fungi from tropical orchids. American Journal of Botany, 89 (11),18521858. doi: 10.3732/ajb.89.11.1852

Pascual C.B., Toda T., Raymondo A.D., Hyakumachi M., 2000. Characterization by conventional techniques and PCR of *Rhizoctonia solani* isolates causing banded leaf sheath blight in maize, Plant Pathology, 49 (1), 108–118. https://doi.org/10.1046/j.1365-3059.2000.00429.x

Pope E.J., Carter D.A., 2001. Phylogenetic placement and host specificity of mycorrhizal isolates belonging to AG-6 and AG-12 in the *Rhizoctonia solani* species complex. Mycologia, 93 (4), 712-719. https://doi.org/10.1080/00275514.2001.12063202

Priyatmojo A., Escopalao V.E., Tangonan N.G., Pascual C.B., Suga H., Kageyama K., Hyakumachi M., 2001. Characterization of a new subgroup of *Rhizoctonia solani* anastomosis group 1 (AG-1-ID), causal agent of a necrotic leaf spot on coffee. Phytopathology, 91 (11), 1054-1061.

Roberts F.A., Sivasithamparam K., 1986. Identity and pathogenicity of *Rhizoctonia* spp. associated with bare patch diseases of cereals at field site in Western Australia. Netherlands Journal of Plant Pathology, 92 (5), 185-195. https://doi.org/10.1007/BF01977685

Salazar O., Julian M.C., Hyakumachi M., Rubio V., 2000a. Phylogenetic grouping of cultural types of *Rhizoctonia solani* AG 2-2 based on ribosomal ITS sequences. Mycologia, 92 (3), 505–509. doi:10.1080/00275514.2000.12061186

Salazar O., Julian M.C., Rubio V., 2000b. Primer based on specific rDNA-ITS sequences for PCR detection of *Rhizoctonia solani*, *R. solani* AG 2 subgroups and ecological types, and binucleate *Rhizoctonia.* Mycological Research, 104 (3), 281–285. doi:10.1017/S0953756299001355

Sambrook J., Fritsch E.F., Maniatis T., 1989. Molecular cloning: a Laboratory manual (3 volume), 2nd Ed. Cold Spring Harbor Laboratory Press, New York.

Sharon M., Freeman S., Kuninaga S., Sneh B., 2007. Genetic diversity, anastomosis groups, and pathogenicity of *Rhizoctonia* spp. isolates from strawberry. European Journal of Plant Pathology, 117 (3), 247–265.

Sharon M., Kuninaga S., Hyakumachi M., Naito S., Sneh B., 2008. Classification of *Rhizoctonia* spp. using rDNA-ITS sequence analysis supports the genetic basis of the classical anastomosis grouping. Mycoscience, 49 (2), 93–114.

Sneh B., Jabaji-Hare S., Neate S., Dijst G., 1996. *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology and diseases control (1st Ed.), Kluwer Academic Publishers, Dordrecht, 1-559.

Toda T., Hyakumachi M., Suga H., Kageyama K., Tanaka A., Tani T., 1999. Differentiation of *Rhizoctonia* AG-D isolates from turfgrass into subgroups I and II based on rDNA and RAPD analysis. European Journal of Plant Pathology, 105, 835–846. https://doi.org/10.1023/A:1008736404263

Toda T., Mushika T., Hyakumachi M., 2004. Development of specific PCR primers for the detection of *Rhizoctonia solani* AG 2-2 LP from the leaf sheaths exhibiting large-patch symptom on zoysia grass. FEMS Microbiology Letters, 232 (1), 67–74. doi: 10.1016/S0378-1097(04)00016-3

Toda T., Mushika T., Hayakawa T., Tanaka A., Tani T., Hyakumachi M., 2005. Brown ring patch: a new disease on bentgrass caused by *Waitea circinata* var. *circinata*. Plant Disease, 89 (6), 536-542. doi: 10.1094/PD-89-0536

Tomaso-Peterson M., Trevathan L.E., 2007. Characterization of *Rhizoctonia*-like fungi isolated from agronomic crops and turfgrasses in Mississippi. Plant Disease, 91 (3), 260-265. doi: 10.1094/PDIS-91-3-0260

Ünal F., Dolar F.S., 2012. First report of *Rhizoctonia solani* AG 8 in Turkey. Journal of Phytopathology, 160 (1), 52–54. https://doi.org/10.1111/j.1439-0434.2011.01856.x

Ünal F., Dolar F.S., Yıldırım A.F., Demirci E., 2014. Isolation and Identification of binucleat *Rhizoctonia* spp. from wheat field soils in the Central Anatolia Region, Turkey. Turkish

Journal of Agricultural and Natural Sciences, 1 (2), 1933-1938.

Ünal F., Bayraktar H., Yıldırım A.F., Akan K., Dolar F.S., 2015. Kayseri, Kırşehir, Nevşehir ve Aksaray illeri buğday ekim alanlarındaki *Rhizoctonia* tür ve anastomosis gruplarının belirlenmesi. Bitki Koruma Bülteni (Plant Protection Bulletin), 55 (2), 107-122.

White T.J., Bruns T., Lee S., Taylor J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR protocols: a guide to methods and applications, Innis M.A., Gelfand D.H., Sninsky J.J., White T.J. (Eds.). Academic Press, Inc., New York. pp. 315-322.

Yang Y.G., Zhao C., Guo Z.J., Wu X.H., 2015. Characterization of a new anastomosis group (AG-W) of binucleate *Rhizoctonia*, causal agent for potato stem canker. Plant Disease, 99 (12), 1757-1763. doi: 10.1094/PDIS-01-15-0036-RE.

Zhao C., Li Y., Liu H., Li S., Han C., Wu X.A., 2019. Binucleate *Rhizoctonia* anastomosis group (AG-W) is the causal agent of sugar beet seedling damping-off disease in China. European Journal of Plant Pathology, 155 (1), 53–69. doi:10.1007/s10658-019-01749-4

Zhou Q.X., Chang K.F., Hwang S.F., Strelkov S.E., Gossen B.D., Chen Y.Y., 2009. Pathogenicity and genetic diversity of *Rhizoctonia solani* isolates from lupin and other crops in Alberta, Canada. Canadian Journal of Plant Pathology, 31 (3), 340–347. https://doi.org/10.1080/07060660909507608

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