IJAFLS
e-ISSN: 2602-4381
International Journal of Agriculture, Forestry and Life Sciences
Original Article | Open access | Int J Agric For Life Sci (2023) 7(2): 56-59

Identification and Genetic Diversity of Hypovirulent Binucleate Rhizoctonia spp. Isolated from Turfgrass and Soil

Filiz Ünal[1]* **Aysun Çavuşoğlu[2]**

[1]Eskişehir Osmangazi University, Faculty of Agriculture, Department of Plant Protection, Eskişehir, Türkiye
[2]Kocaeli University, Faculty of Agriculture and Natural Sciences, Department of Plant Protection, Kocaeli, Turkey
***Correspondence:** fucar06@yahoo.com

Citation
Unal, F., Çavuşoğlu, A. (2023). Identification and Genetic Diversity of Hypovirulent Binucleate Rhizoctonia spp. Isolated from Turfgrass and Soil. Int J Agric For Life Sci (2023) 7(2): 56-59.

Received	:2 October 2023
Accepted	:19 December 2023
Published Online	:22 December 2023
Year:	:2023
Volume	:7
Issue	:2 (December)
Pages	:56-59

Available online https://dergipark.org.tr/ijafls

Abstract
The aim of this study was to obtain hypovirulent binucleate Rhizoctonia anastomosis groups from turfgrass roots and rhizosphere soils in Eskişehir, Ankara and Kocaeli. In 2022, 153 turfgrass and 78 rhizosphere soil were taken from turfgrass areas. Isolations were made from the roots and rhizosphere soils. Isolations from soils were performed by colonization of trap plant tissues. Detection of anastomosis groups of the isolates were done according to the hyphal nucleus staining, colony apparences and rDNA-ITS sequences analysis. The ITS regions of the isolates were amplified by polymerase chain reaction (PCR) using rDNA primer pair ITS 1 and ITS2. Pathogenicity tests of all isolates were conducted with pot assays in greenhouse conditions. As a result of the study, 17 BNR isolates belonging to 7 different anastomosis groups were obtained. Two AG D isolates were found pathogenic. As a result, 15 hypovirulent binucleate Rhizoctonia isolates belonging to AG A, AG B(o), AG Fa, AG Fb, AG G and AG K groups were obtained. The phylogenetic neighbor-joining tree of 17 Binucleate isolates clearly shows that the isolates are grouped into seven distinct clusters. AG Fa and AG Fb appeared on the tree as genetically close isolates. In future, studies should be conducted to investigate the effect levels of the hypovirulent strains detected in this study on the control of pathogenic fungi that cause diseases in cultivated plants.

Key words
Anastomosis group, Turfgrass, Soil, Phylogeny.

Introduction
Rhizoctonia is a huge genus with more than three hundred different host ranges. This genus includes different groups that are pathogenic and non-pathogenic to plants. The genus *Rhizoctonia* is divided into subgroups called 'anastomosis groups', taking into account hyphal union reactions. According to the number of nuclei in the hyphal compartments, they are divided into 3 groups as uninucleate (UNR), binucleate (BNR) or multinucleate *Rhizoctonia* (MNR) (Sharon et al., 2007). Binucleate *Rhizoctonia* species are divided into 19 different anastomosis groups (AG A-L, AG O - S and AG U, V, W) (Dong et al. 2017, Hyakumachi et al. 2005, Misawa and Kurose 2018, Sharon et al. others 2008, Yang et al. 2015, Zhao et al. 2019). Binuclear *Rhizoctonia* species live both freely in the rhizosphere soil and colonized in plant roots. Therefore, in the studies carried out, they can be isolated both from the soil and from the roots of the plants they host. Some of them live in the soil as saprophytes, some of them have a symbiotic relationship with plant roots. Another group, *Rhizoctonia*, has parasitic relationships with plant roots and has the ability to cause disease (Sneh et al., 1996; Herr, 1995). Nonpathogenic *Rhizoctonia* species prevent many plant pathogens from infecting their hosts through the hypovirulence mechanism (Cardoso and Echandi 1987a, b; Roberts and Sivasithamparam 1986; Gutierrez and Torres 1990; Sneh et al. 1996, Herr 1995). For this reason, Non-pathogenic *Rhizoctonia* anastomosis groups are important in terms of biological control as they are used successfully in the control against pathogenic fungi. The majority of nonpathogenic groups within the genus *Rhizoctonia* are binucleates. But, in recent years, several studies revealed that some BNR *Rhizoctonia* AGs were pathogenic on some agricultural plants (Oniki et al., 1986; Sneh et al., 1996; Yang et al., 2015; Alaei et al., 2017; Dong et al., 2017; Türkkan et al., 2018; Türkölmez et al., 2019; Basbagci and Dolar, 2020). In wheat, only BNR AG D is pathogenic. When the studies conducted in the world on turfgrass are examined, among BNR isolates; subgroups I, II and III of AG D, AG Q and AG P were not found to be pathogenic on turfgrass (Oniki et al., 1986; Sneh et al., 1996; Ünal and Cavusoglu, 2023). The genetic diversity of *Rhizoctonia* isolates has been studied using RAPD-PCR, SSR-PCR, rDNA-RFLP, rDNA-ITS sequence analysis, with universally primers, PCR, and rep-PCR (Sharon et al. 2006). Currently, the rDNA-ITS sequence analysis is 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 *Rhizoctonia* subgroups (Carling et al., 2002, Sharon et al., 2006, 2007, 2008).
While some BNR *Rhizoctonia* species have been reported as non-pathogen or pathogen on various hosts, there is no a comprehensive data describing their behavior and fungus-host relationships on turfgrass. The aim of this study is identification, pathogenicity and phylogenetic evaluation of binucleate *Rhizoctonia* isolates isolated from turfgrass and rhizophere soil in Eskişehir, Ankara and Kocaeli provinces.

Material and Methods
Collection of Samples and Isolation of Fungi
In the spring of 2022, turfgrass samples and rhizosphere soils were taken from large parks, recreation areas, picnic areas and feruges in Eskişehir, Ankara and Kocaeli provinces, and were put in paper bags and brought to the laboratory. Symptomatic and asymptomatic root surfaces were sterilized in 1.5% sodium hypochlorite for approximately one minute. Then, the root pieces were rinsed by soaking in sterile pure water for 30 seconds, was kept and allowed to dry on sterile filter papers, and placed on PDA to which 100 µg/ml streptomycin sulfate was added. In isolations from soil, sterile wheat stalks were used as trap plants. Soil samples taken from the surveyed grass areas were transferred to pots in the greenhouse. After the soil in the pots was watered, sterilized wheat stalks, approximately 6-7 cm long, were placed vertically in each pot and covered with a nylon bag. After waiting for 4 days, the stems were washed, dried and placed on water agar (WA) to which 10% (v/v) lactic acid was added. After 7 days, the developing similar *Rhizoctonia* hyphae were transferred to potato dextrose agar (PDA).
Pathogenicity Assays
To obtain fungal inoculums, first 500 g of wheat seeds were filled into 1- liter bottles and 120 ml of water was added. These bottles were autoclaved twice at 100°C for one hours, with a one-day break. Then, 12 pieces of agar with a diameter of 3-4 mm containing hyphae of different *Rhizoctonia* isolates were put to each of these bottles and waited in incubator at 24°C for three weeks. The inoculum obtained after incubation was dried on sterile papers and chopped in a blender. Then, this inoculum was mixed into a 5% sterilized mixture of sand, soil and fertilizer (1:2:1) (twice for 45 minutes at 121 ºC with a one-day break). Three pots (12x12 cm) were used for each application. No inoculum was added to the control pots. Then, the pots were covered with nylon cover and left to incubate for three days. Three days later, 25 *Festuca arundinaceae* variety seeds (sensitive to *Rhizoctonia* spp.) were sowed in each pot and covered with approximately soil. Then, 20 ml of pure water was dropped to each pot. The experiments were carried out in a greenhouse with 12 h daylight 60 % RH, 23±3°C. The evaluations were done one month later. Disease assessments were made using Ichielevich-Auster et al. (1985)'s 0-5 scale. Using the values of the scale, disease severity values were calculated using the Townsend and Heuberger formula.

Determination of Nucleus Numbers of Isolates
The fungi hyphae obtained from the cultures were transferred to lamella water agar medium. Then, the coverslips, sterilized by burning with alcohol, were immersed in soft PDA medium containing 0.5% agar and placed in water agar

medium. The petri dishes prepared in this way kept in the incubator on 24°C for one day. After incubation, the samples were examined under a coverslip microscope. For examination, Safranin O dye (0.5%) was dropped on a slide. Then, the coverslip taken from the water agar medium was placed on the solution on the slide (Bandoni, 1979). After the coverslips were prepared in this way, they were examined under the microscope and the number of nuclei in the hyphal septa were determined. Nucleus numbers in at least 15 septa were examined (x100 and x400). Three petri dishes were used for each sample (Ogoshi et al., 1990, Carling et al., 2002, Karaca et al., 2002).

DNA isolations, PCR studies and phylogenetic analysis of isolates

DNA isolations of fungal isolates were done using the QIAGEN (Blood and Tissue Kit) DNA isolation kit. PCR studies were carried out with ITS 1/2 primers (White et al., 1990). In the PCR analyses, the final mixture was 50 µl. For this; 25 µl GoTaq® Hot Start Green Master mix (2x), 2 µl of 5 µM each primers, 13 µl in a 2 ml ependroph tube double distilled water and 4 µl BSA were mixed. Then, the DNAs were added to 4 µl of each PCR mixture distributed in different PCR tubes. The cycle PCR protocol consists of an initial denaturation of 4 minutes at 94°C, followed by 30 cycles of 45 seconds at 94°C, 45 seconds at 55°C, and 2 minutes at 72°C, and finally at 72°C. It consisted of a 10-minute overtime. Direct sanger sequencing analyzes of PCR products were performed in a special R&D Laboratory. Sequence results were compared with isolate sequences in GenBank by BLAST analysis at NCBI. Sequences were edited and aligned (Thompson et al., 1994). The phylogenetic tree was constructed using ITS sequences of fungi using the neighbor-joining method using MEGA ver 7.0 software (Kumar et al., 2016), with sequence distance calculated by the Kimura 2-parameter model (Kimura, 1980). Bootstrap analysis was performed with 1,000 replicates to determine support for each class.

Results

As a result of the survey studies carried out in Eskişehir, Ankara and Kocaeli provinces in 2022, 153 turfgrass and 78 rhizosphere soils were taken and isolation studies of fungi were carried out from all plant and soil samples. As a result of the isolations, nuclei staining was done to display the nuclei numbers of the isolates detected at the genus level. As a result of dyeing, the nucleus number that was found in each hypha cell was two, and width of the main runner hyphae was less than 7 µm (Figure 1). As a result of mycelial staining, *Rhizoctonia* isolates with two nuclei in each hyphal compartment and a hypha width of less than 7 µm were identified (Figure 1). Considering the hypha width and the number of nuclei in the hypha compartments, colony structures and colors, 17 isolates were determined as binucleate *Rhizoctonia*. As a result of the molecular diagnostic studies conducted with these isolates, it was determined that the isolates belonged to the *Rhizoctonia* AG A, AG B(o), AG D I, AG Fa, AG Fb, AG G and AG K groups (Figure 1, Table 1).

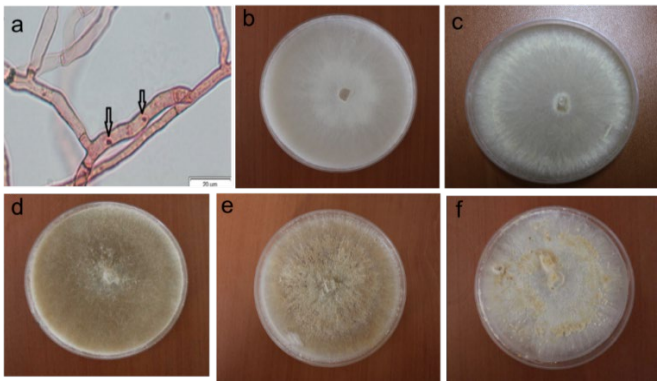

Figure 1. Binucleate *Rhizoctonia* AGs isolated from in this study: a: Binucleate hyphae, b-f: Colony appearance of some binucleate *Rhizoctonia* isolates on potato dextrose agar; (b) AG A, (c) AG D, (d) AG F, (e) AG G, (f) AG K.

When the growth of the BNR isolates obtained on PDA medium was examined, the morphological feature of the isolates matched the 'features of binucleate isolates' described in Sneh et al. (1994). While BNR AG A, D and K isolates grown on PDA were initially white and then turned beige, AG D and G isolates initially turned light yellow and turned buff within 3 weeks as they aged. Sclerotia formation was rarely observed in isolates other than AG D and G in the developing colonies. In the isolates with sclerotia development, sclerotia of 0.5-1.3 mm in diameter, close to a sphere, formed singly or in clusters, and yellow (AG G) or light to dark brown (AG D) were observed (Figure 1).

As a result of the isolations made from plant and soil samples, the most isolated group was the AG A anastomosis group. This was followed by AG B(o) and AG G with 3 isolates each. 9 isolates were isolated from soil and 8 isolates from plant roots. The most isolates were isolated from Ankara province. As a result of pathogenicity tests, two AG D I isolates were found to be pathogenic. It was determined that all isolates and groups except these two isolates were no pathogens in turfgrass. The disease severity values of the two AG D I isolates were determined as 82.50% and 88.12%.

Table 1. Anastomosis group, geographic origin, source of isolation and pathogenicity value of binucleate *Rhizoctonia* isolates used in this study

Isolate Number	Anastomosis group	Origin	Source of isolation	Pathogenicity (%)
BNR204	AG A	Ankara	Plant	Non-pathogen
BNR932	AG A	Kocaeli	Soil	Non-pathogen
BNR141	AG A	Ankara	Soil	Non-pathogen
BNR1341	AG A	Eskişehir	Plant	Non-pathogen
BNR939	AG A	Kocaeli	Plant	Non-pathogen
BNR1844	AG A	Ankara	Soil	Non-pathogen
BNR314	AG B(o)	Ankara	Plant	Non-pathogen
BNR354	AG B(o)	Eskişehir	Soil	Non-pathogen
BNR497	AG B(o)	Eskişehir	Soil	Non-pathogen
BNR98	AG DI	Ankara	Plant	82.50
BNR1484	AG DI	Kocaeli	Soil	88.12
BNR2013	AGFa	Ankara	Soil	Non-pathogen
BNR316	AGFb	Eskişehir	Plant	Non-pathogen
BNR1325	AG G	Kocaeli	Soil	Non-pathogen
BNR1552	AG G	Ankara	Plant	Non-pathogen
BNR1415	AG G	Ankara	Soil	Non-pathogen
BNR1577	AG K	Eskişehir	Plant	Non-pathogen

Rhizoctonia AG A, AG DI, DII, DIII, G and K were previously detected in wheat in Türkiye. Only AG DI, DII, DIII groups pathogens were found in wheat (Demirci 1998; Ünal and Dollar 2023). In this study, AG D was found to be a pathogen in turfgrass. All the other isolates were found to be non-pathogenic. In previous studies conducted on turfgrass, *Rhizoctonia* AG D (subgroups I, II, III), AG Q and AG P were found to be pathogen in turfgrass (Oniki et al., 1986; Sneh et al., 1996; Ünal and Çavuşoğlu, 2023). Ünal and Çavuşoğlu (2023) reported that AG P causes damping off in turfgrass. Although binucleate *Rhizoctonia* spp. has been reported to be pathogenic in some hosts, these species generally live saprophytically in soil and dead plant residues. Many studies have shown that low-virulent or non-virulent species have hypovirulent properties (Sneh et al., 1996; Tewoldemedhin et al., 2006). There are studies showing that these strains, which generally consist of binucleate species, can be used successfully in biological control studies against pathogens (Cardoso and Echandi, 1987 a,b; Roberts and Sivasithamparam 1986; Gutierrez and Torres, 1990; Sneh et al., 1996; Herr, 1995).

Phylogenetic tree was constructed by bootstrap neighbor-joining analysis of nucleotide sequences to evaluate genetic differences among isolates belonging to 17 binucleate *Rhizoctonia* anastomosis groups. The phylogenetic neighboor-joining tree belonging to BNR isolates clearly demonstrated that the isolates were grouped into 7 distinct clusters (Figure 2). It was observed that AG Fa and AG Fb subgroups formed their own small groups within the same group on the tree. This situation showed that these two species were genetically closely related species. Even though AG A isolates were in the same group, a slight intragroup genetic difference was observed between them. Isolates of belonged the other 4 groups were grouped within themselves and formed groups in different places on the tree than the groups of other species (Figure 2).

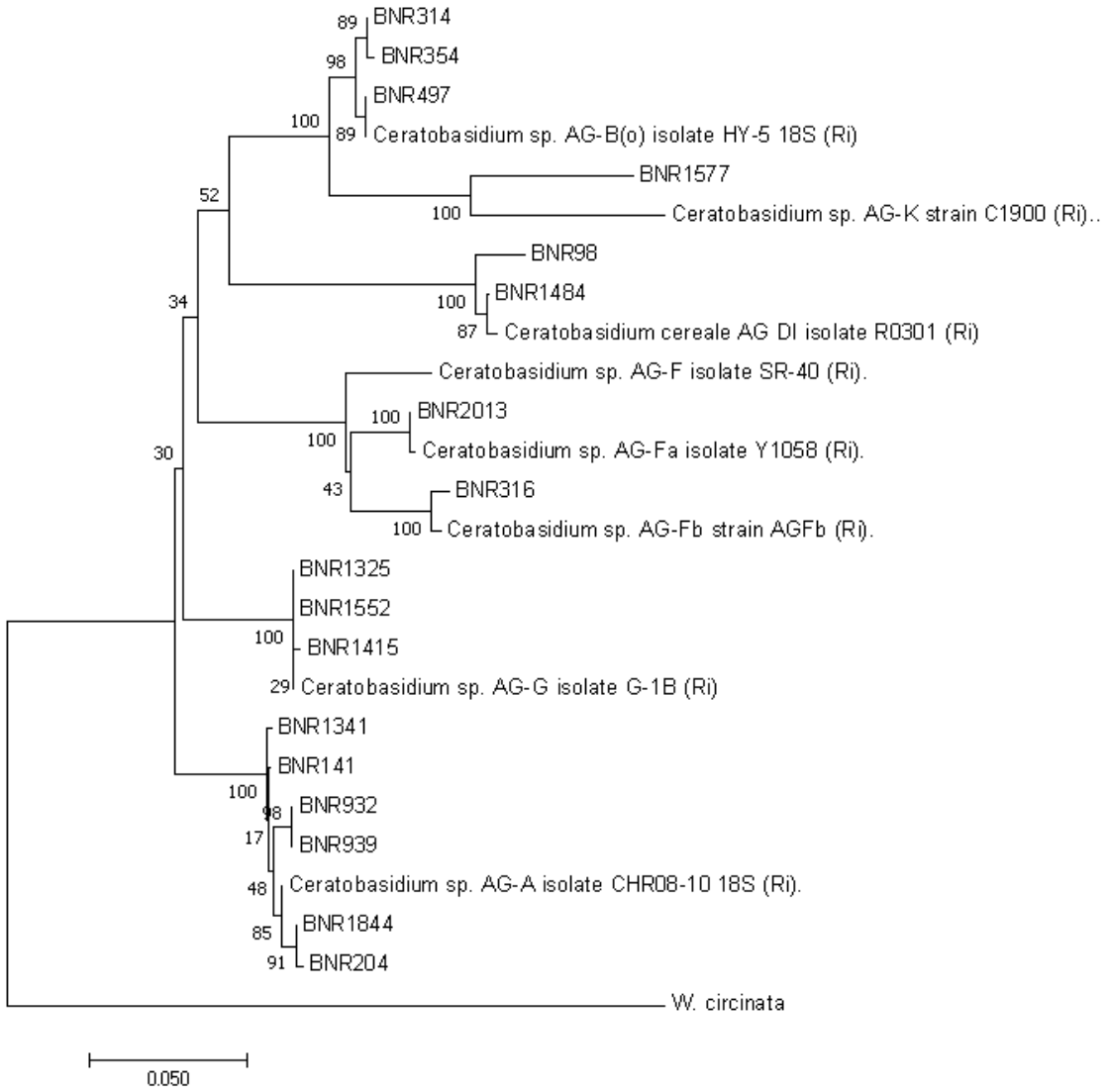

Figure 2. Neighbour-Joining phylogenetic tree of BNR isolates obtained with 1000 bootstrap replicates.

Discussions

Non-pathogenic species of *Rhizoctonia* are important as biological control agents of other pathogenic fungi. In recent studies, non-pathogenic multinucleate and binucleate *Rhizoctonia* species have been used as hypovirulent strains in the fight against pathogenic species (Sneh et al. 1996). Herr (1995) states that in the studies conducted on biological control since the 1930s, the biocontrol of diseases caused by *Rhizoctonia solani* does not have any economic commercial value, but in new approaches to this subject, new agents, especially in field crops, are identified and used, and an applicable biocontrol management system is needed. Studies conducted since 1989 have shown that isolates from different groups of binucleate *Rhizoctonia* spp. and hypovirulent *R. solani* isolates will be effective in the biocontrol of diseases caused by *R. solani* in different hosts. These are several different binucleate *Rhizoctonia* anastomosis group (AG) and hypovirulent *R.solani* AG isolates, and the effects of none of these isolates on *R. solani* are through mycoparasitism and antibiosis, but through systemic promotion of host resistance or competition for nutrients or habitat. The tested binucleate *Rhizoctonia* spp. It was also reported by the researcher that the effects of hypovirulent *R. solani* isolates on the diseases caused by *R. solani* were different, and that isolates that colonized the plant surface were effective as biocontrol agents, while non-colonized isolates were ineffective. Ichielevich-Auster et al. (1985) isolated 107 *Rhizoctonia* spp. from soil samples taken from 26 different locations in Israel. They found that 32 of the isolates were not pathogenic in 11 different hosts. Cardoso and Echandi (1987a) investigated the effect of binucleate *Rhizoctonia* against *R. solani*, which causes root rot in bean seedlings, under laboratory and greenhouse conditions, and showed that BNR isolates suppressed *R. solani* at the infection site by promoting the metabolic response in bean seedlings. The same researchers investigated the effects of 11 BNR, 1 *R. zeae*, 1 *Trichoderma hamatum* and 1 *T. harzianum* isolate as a biological control agent against *R. solani*, which causes root rot in bean seedlings. In their other study, they investigated the disease severity of BNR isolates under greenhouse conditions. They found that they reduced the in field trials, they also observed that some of the BNR isolates were more effective against root rot than *Trichoderma* isolates (Cardoso and Echandi, 1987b). Herr (1988) reported that 7 of 10 BNR isolates obtained from sugar beet cultivation areas in Ohio prevented root and crown rot caused by *R. solani* anastomosis group 2, type 2 in sugar beet. Bandy and Tavantzis (1990) recorded a 56% decrease in the infected stem tissues of potato plants inoculated with the virulent AG 3 (Rhs 27) isolate of *R. solani* and the Rhs1A1 isolate from the hypovirulent anastomosis group (AG). When potato plants were inoculated with only the Rhs1A1 isolate, a 4-fold increase in stolon dry weight and a 1.7-fold increase in stem dry weight was observed compared to control plants. Haris et al. (1994) investigated the effect of 9 BNR isolates against the collapse caused by AG 4 and AG 8 isolates of *R. solani*. All isolates inhibited the collapse caused by AG 4 in cucumber, and most of the isolates increased shoot length in plants infected with AG 4 and AG 8. Two of the binucleate isolates were also found effective against *Pythium* spp. Potvin et al. (1999) determined that binucleate nonpathogenic AG-G isolates provided effective protection against root rot caused by AG-4 in bean seedlings. Villajuan-Abgona et al. (1996) in their study in which they investigated the effect of 3 binucleate *Rhizoctonia* (BNR) against the collapse caused by AG 2-2 and AG 4 groups of *R. solani* in cucumber plant, found that BNR isolates L2 (AG Ba), W1 and W7 (AG A) were affected by the virulent isolate C4 (AG 4). They found that it provided 58-71% protection against another virulent isolate, RH 65 (AG 2-2), was between 64-75%. W7, one of the BNR isolates, was not only effective in protecting against disease, but also caused a significant increase in plant fresh weight. Muslim et al. (2003a) in their study investigating the effect of 4 hypovirulent binucleate *Rhizoctonia* isolates isolated from soil in the Gifu region of Japan on *Fusarium oxysporum* f.sp *spinaciae* (Fos)- induced wilt disease in spinach, they found that HBNR isolates reduced the severity of the disease by 56-100% and the severity of discoloration by 52-100%. In addition, they also observed that extracts obtained from roots with HBNR significantly inhibited the germination and grass tube development of Fos conidia. The same researchers determined that the hypovirulent binucleate 4 isolate of *Rhizoctonia* (G1, L2, W1 and W7) significantly reduced the disease severity values, including leaf symptoms and

color change in the stem, caused by *Fusarium oxysporum* f.sp. *lycopersici* (Fol) in tomatoes under field conditions, and Fol colonization in the roots and stems of tomato plants. The reduction in disease severity varied depending on the HBNR isolates and method of administration. Among these isolates, G1, W1 and W7 also caused an increase in fresh weight. Researchers have reported that HBNRs can be used as biocontrol agents against *Fusarium* wilt (Muslim et al. 2003 b).

Conclusions

The success of microorganisms used in biological control studies is generally more successful in the soil and climatic conditions to which they are isolated and adapted. Therefore, it is important to use local isolates rather than imported ones in these studies. It is necessary to conduct studies on the possibilities of using the domestic hypovirulent binucleate strains obtained in this study in the fight against diseases caused by pathogenic fungi. It is also necessary to focus on bioformulation studies of isolates that are found to be effective.

Statement of Conflict of Interest

The authors declares no conflict of interest

Author's Contributions

FÜ contributed to the survey, isolation, identification, pathogenicity, phylogenetic studies of BN *Rhizoctonia* isolates and writing of the manuscript, AÇ contributed to the survey, isolation and pathogenicity. All authors read and approved the final manuscript.

References

Alaei, H., Molaei, S., Mahmoodi, S.B., Saberi-Riseh, R., (2017). New anastomosis group F (AG F) of binucleate Rhizoctonia causing root and stem rot of *Pistacia vera*. Journal Crop Protection, 6(1): 1-13.

Bandoni R.J., (1979). Safranin O as a rapid stain for fungi. Mycologia, 71 (4), 873–874.

Bandy, B.R. Tavantzis, S.M., (1990). Effect of hypovirulent *Rhizoctonia solani* on *Rhizoctonia* disease, growth, and development of potato plants, American Journal of Potato Research, 67(3), 189-199.

Basbagci, G., Dolar, F.S. (2020). First report of binucleate *Rhizoctonia* AG-K causing root rot on chickpea. Archives of Phytopathology and Plant Protection, 53(13-14), 640-652.

Cardoso, J.E. Echandi, E. (1987a). Biological control of *Rhizoctonia* root rot of snap bean with binucleate Rhizoctonia-like fungi. Plant Disease, 71:167-170.

Cardoso, J.E Echandi *Rhizoctonia*, E. (1987b). Nature of protection of bean seedlings from *Rhizoctonia* root rot by a binucleate -like fungus, Phytopathology, 77, 1548-1551.

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.

Demirci, E. (1998). *Rhizoctonia* species and anastomosis groups isolated from barley and wheat in Erzurum, Turkey. Plant Pathology, 47 (1), 10-15.

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.

Gutierrez, P. Torres, H., (1990). Biological control of *Rhizoctonia solani* with binucleated *Rhizoctonia*. Fitopatología, 25 (2), 45-50 pp.

Haris, A.R., Schisler, D.A., Neate, S.M. Ryder, M.H. (1994). Suppression of damping-off caused by *Rhizoctonia solani*, and growth promotion, in bedding plants by binucleate *Rhizoctonia* spp., Soil Biology and Biochemistry, 26, 263-268.

Herr, L.J. (1988). Biocontrol of Rhizoctonia crown and root rot of sugar beet by binucleate Rhizoctonia spp. and Laetisaria arvalis, Annuals of Applied Biology, 113,107-118.

Herr, L.J. (1995). Biological control of Rhizoctonia solani by binucleate Rhizoctonia spp. and hypovirulent R. solani agents. Crop Protection, Volume 14 Number 3, 179-186.

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.

Ichielevich-Auster, M., Sneh, B., Koltin, Y. Barash, I. (1985). Suppression of damping-off caused by *Rhizoctonia* species by a nonpathogenic isolate of *R. solani*. Phytopathology 75: 1080-1084.

Karaca, G.H., Özkoç, İ. Erper, İ. (2002). Determination of Anastomosis grouping of *Rhizoctonia solani* Kühn isolates associated with bean plants grown in Samsun, Turkey. Pakistan Journal of Biological Sciences, 5(4): 434-437,

Kimura, M.A. (1980). simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16: 111–120.

Kumar, S., G. Stecher K. Tamura. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33: 1870–1874.

Misawa, T. and Kurose, D. (2018). First report of binucleate *Rhizoctonia* AG U causing black scurf on potato tubers in Japan. New Disease Reports , 38, (1), 24-24.

Muslim, A., Horinouchi, H., Hyakumachi, M. (2003a). Suppression of *Fusarium* wilt of spinach with hypovirulent binucleate *Rhizoctonia*, J. Gen. Plant.Pathol. 69, 143-150.

Muslim, A., Horinouchi, H., Hyakumachi, M. (2003b). Biological control of *Fusarium* wilt of tomato with hypovirulent binucleate *Rhizoctonia* in greenhouse conditions, Mycoscience, 44, 77-84.

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.

Oniki, M., Kobayashi, K., Araki, T., Ogoshi, A. (1986). A New disease of turfgrass caused by binucleate *Rhizoctonia* AG Q. Japanese Journal of Phytopathology, 52(5), 850-853.

Potvin, C.L., Balmas, V., Charest, P.M., Hare, S.J., (1999). Development of reliable molecular markers to detect non-pathogenic binucleate *Rhizoctonia* isolates (AG-G) using PCR, Mycological Research, 103, 1165-1172.

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. Europan Journal of Plant Pathology, 92(5): 185-195.

Sharon, M., Kuninaga S., Hyakumachi M. Sneh B. (2006). The advancing ıdentification and classification of *Rhizoctonia* spp. using molecular and biotechnological methods compared with the classical anastomosis grouping. Mycoscience 47: 299-316.

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.

Tewoldemedhin, Y.T., Lamprecht, S.C., McLeod, A. Mazzola, M. (2006). Characterization of *Rhizoctonia* spp. Recovered from crop plants used in rotational cropping systems in the Western Cape Province of South Africa. Plant Disease, 90(11):1399-1406.

Thompson, J.D., D.G Higgins 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 Res. 22: 4673–4680.

Türkkan, M., Erper, I., Kılıçoğlu, M.Ç., Yazıcıoğlu, E., Özcan, M. (2018). Characterization and pathogenicity of *Rhizoctonia* spp. isolated from kiwifruit in the Middle and Eastern Black Sea region of Turkey. J Phytopathol.;166:761–774.

Türkölmez, Ş., Çiftçi, O., Derviş, S., Ulubaş Serçe, Ç. (2019). First report of binucleate *Rhizoctonia* AG-F Causing stalk and root rot of corn (*Zea mays*) in Turkey. Plant Diseases, 103(8).

Ünal F. Cavusoglu A. (2023). Characterization And Pathogenicity of *Rhizoctonia* AG P Causing Damping-off on Turfgrass. 5th Internatıonal Balkan Agriculture Congress, Edirne, Türkiye, 20- 23 September 2023, p.146.

Ünal F. Dolar F. S. (2023). Anastomosis grouping and phylogenetic analysis of *Rhizoctonia* isolates on wheat in Türkiye. Plant Protection Bulletin, 63 (2), 5-15.

Villajuan-Abgona, R., Kageyama, K. Hyakumachi, M. (1996). Biocontrol of *Rhizoctonia* damping off of cucumber by non-pathogenic binucleate Rhizoctonia. European Journal of Plant Pathology, 102(3), pp 227-235.

White, T.J., T. Bruns, S. Lee J. Taylor. (1990). Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. In PCR Protocols; Elsevier: Amsterdam, The Netherlands, pp. 315–322.

Yang, Y. G., Zhao, C., Guo, Z. J., Wu, X. H. (2015). Characterisation of a new anastomosis group (AG-W) of binucleate *Rhizoctonia,* causal agent for potato stem canker. Plant Disease, 99, 1757–1763.

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.