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Anastomosis Groups and Pathogenicity of *Rhizoctonia solani* Kühn Isolates Obtained from Pistachio (*Pistacia vera* L.) Saplings in Siirt Province, Turkey

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Abstract: Pathogenicity and anastomosis groups of seven *Rhizoctonia*-like isolates obtained from infected pistachio (*Pistacia vera* L.) seedlings were determined, in the central district of Siirt province at Southeast Anatolia Region in Turkey. Browning on the crown root of the collected saplings and drying of plants were observed. In the pathogenicity test, the most virulent isolate was Rs2 with a disease severity of 93.75%. The anastomosis group of this isolate, which caused the saplings to dry completely was determined as *Rhizoctonia solani* AG-4. The moderately virulent Rs3 and Rs1 isolates were determined as the binucleate AG-F group. These two isolates caused yellowing of the seedling leaves and browning of the roots, however, the plants did not die and preserved their vitality to a certain extent. The R9 and R14 isolates identified as anastomosis group AG-F did not cause any symptoms on inoculated plants and on the negative control. The Rs7 and Rs10 isolates were identified as the anastomosis group AG-4 and were determined to be pathogenic in saplings. The results of this study are the first record for the virulence and anastomosis groups of *Rhizoctonia* species that cause root and crown root diseases in Siirt pistachio.

Keywords: Pistachio, *Rhizoctonia*, pathogenicity, anastomosis group, Polymerase Chain Reaction

1. Introduction

The homeland of Pistachio (*Pistacia vera* L.) which was first cultivated in the Southeastern Anatolia by Etiler civilization, is known as the uplands of Small-Asia, Caucasus Iran, and Turkmenistan. Pistachio is grown as wild or semi-wild in Afghanistan, Northwest India, Iran, Turkey, Syria, and other Near East and North African countries for a long time. The most important producers of pistachio are Iran, the United States of America, Turkey, and Syria. The number of total pistachio trees in Turkey is 59.762.816 and annual production is 240.000 tons. The number of pistachio trees in Siirt province is 7.810.595 and the production is 11.301 tons. Siirt province has 13.06% of the pistachio trees in Turkey and produces 4.70% of the total pistachio production (Anonymous, 2018).

Due to the special climate requirements, pistachio cannot be grown everywhere economically (Arpacı, 2001). So the Southeastern Anatolia Region is the only region for pistachio production economically in Turkey. The pistachio variety cultivated in Siirt province has an important role the region. The pistachio variety cultivated in Siirt has the advantages of being large, having a large cracking space, and a white shell. Plant protection problems that negatively affect yield and quality have recently increased in parallel with the increase in planting areas. Especially drying was reported in orchards and nurseries (Aydın, 2019a). Aydın (2019b) stated that soil pathogens such as *R. solani* are the main reason for the dryings.

Rhizoctonia solani Kühn, a soil-borne fungus [Telemorph: *Thaatephorus cucumeris* (FR) Donk]

causes disease in many plants and can survive in the soil for a long time due to its specific sclerotic structures (Boosalis and Scharen, 1959). The pathogen, which generally causes various diseases such as root and crown root rot, leaf and stem burn, and can be found everywhere of the plants (Clarkson and Cook, 1983; Carling et al., 1994; Mohammadi et al., 2003). The main hosts are annual plants such as barley, pepper, wheat, tomato, bean, carrot, clove, cauliflower, chickpea, potato, sugar beet, soybean, tobacco, alfalfa, and also many fruits and forest trees such as pistachio.

The teleomorph of *Rhizoctonia* isolates belong to one of the three genera of Basidiomycota, which are *Thanathephorus* (anamorph= *R. Solani*), Ceratobasidium (anamorph = binucleate *Rhizoctonia*), and Waitea (anamorph= *R. zeae, R. oryzae*). *Rhizoctonia* is classified by the number of nuclei found in hyphae cells and has uninucleate, binucleate, and multinucleate varieties (Singleton et al., 1992; Sneh et al., 1998; Sharon et al., 2007). The two nuclei *Rhizoctonia* are called binucleate *Rhizoctonia*, and those with multinucleate are called *R. solani* and *Waitea* spp. The relationship between *R. solani* and binucleate *Rhizoctonia* (BNR) species are characterized by hyphal fusion conditions (Ogoshi, 1975, 1987). The groups having hyphae fuse are called the "anastomosis group (AG)". Currently, fourteen (AG 1-13 and BI) anastomosis groups of *R. solani* and eighteen anastomosis groups of binucleate *Rhizoctonia* have been identified (Ogoshi et al., 1983; Sneh et al., 1998; Yang et al., 2015; Dong et al., 2017).

Anastomosis groups can cause disease in different hosts (Carling et al., 2002). The AG-3 type was reported to cause severe disease in potatoes, while AG-4 causes moderately severe disease (Demirci and Döken, 1993; Campion et al., 2003; Aydın and Turhan, 2013). The *Rhizoctonia* solani AG-4 causes damping-off, root and stem rot in pistachio orchards and particularly in nurseries and leads to severe economic losses (Ashkan and Abusaidi, 1995; Ilkhan et al., 2011). *R. solani* AGs are generally considered as the important pathogens in plants. Binucleate *Rhizoctonia* species are known mostly non-pathogenic fungi or play a role in disease control as a biocontrol agent. They are commonly found in soil organic matter or plant debris (Andersen and Rasmussen, 1996). However, many anastomosis groups known as BNR species are pathogen, which causes damping-off, root and stem rot, leaf burn, and fruit rot in many important plants (Tanaka et al., 1994). Alaei et al. (2017) reported the first record that binucleate *Rhizoctania* AG-F causes root and stem rot in pistachio saplings in Iran (Alaei et al., 2017). The BNR types are divided into 18 anastomosis groups, from AG-A to AG-W (Yang et al., 2015; Dong et al., 2017). The disease caused by the fungus was recorded in pistachio, especially during the sapling period and in the first years of transferring to the field. The optimal conditions for disease development are cool and moist soils and the optimum temperature is 18 °C (Alaei et al., 2017). Cancer lesions with red-brown stains occur on the stems and roots of infected plants.

The aim of this study was to determine the virulence and anastomosis groups of some *Rhizoctonia* species isolated from pistachio saplings by comparing test isolates and molecular methods.

2. Materials and Methods

2.1. Collection, isolation, and maintenance of fungal cultures

One or two-year-old infected saplings with chlorotic lesions on the leaves, dry brown rot, and necrotic spots on the root and crown root were collected from some commercial nurseries located in the central district of Siirt province in Southeast Anatolia Region of Turkey in summer 2019. The roots of infected saplings were thoroughly washed under tap water, then they were cut into 0.5-1 cm pieces. The tissue pieces were kept in 1% sodium hypochlorite (NaOCl) for 1.5 minutes and their surfaces were disinfected. The root pieces were then washed twice in sterile distilled water and dried between filter papers. To prevent bacterial growth, 150 mg of L^{-1} streptomycin sulfate was added to the water-agar (WA) medium and incubated for 3 days at 24 ºC in the dark (Carling et al., 2002). Growing colonies were examined under a microscope and those showing typical *Rhizoctonia* symptoms (split thick hyphae and formation of 90° angle) were transferred to potato dextrose agar (PDA), (Merck, Germany). The pure cultures were then stored at +4 °C after transferring into slant agar tubes.

2.2. Pathogenicity test

Isolates were grouped by their morphological characteristics (colony color, structure, sclerotic structures), and Rs1, Rs2, Rs3, Rs7, Rs9, Rs10, and Rs14 isolates were used in the study. The pathogenicity test was performed as follows.

2.2.1. Inoculum preparation

The wheat seed wrapping method described by Sneh et al. (1991) was used to prepare the inoculum used in the experiments. Wheat grains were boiled and incubated in pure water containing 1 mg ml^{-1} chloramphenicol and kept in this solution overnight. After the seeds were drained,

they were placed into capped glass tubes and sterilized in an autoclave for 1 hour at 121 °C for two consecutive days. Then, 5 mm diameter agar discs taken from the hyphae tips of *Rhizoctonia* isolates previously grown in PDA medium for 5-6 days were placed in these tubes and incubated for 15-20 days at 24±1 °C and the hyphae were expected to wrap the wheat seeds.

2.2.2. Experimental design

One or two - year - old saplings of Siirt variety collected from commercial nurseries were used in this study. Twenty-five g of *Rhizoctonia* inoculum wrapped on the wheat seeds was added to the soil around the roots of the potted saplings. Uncontaminated wheat seeds were added as the negative control, and then the soil was mixed and irrigated. The experiment was established in the laboratory under room conditions, with 4 replications for each sapling. The saplings were removed 8 weeks after inoculation, the symptoms on the roots and crown roots were examined and the disease severity was determined according to the 0-8 scale (Ruppel et al., 1979). (0): No lesions, plants are healthy, (1): visible *Rhizoctonia* lesions on 1% of the root surface, (2): visible lesions on 1–5% of the root surfaces, (3): 5-10% cancer dry rot symptoms on the root surface, (4): dry-rot cancer lesions on >5–10% of the root surface, (5): 25–50% dry-rot cancer lesions on the root surface, (6): 50-75% dry-rot cancer lesions on the root surface, (7): >75% dry-rot cancer lesions on the root surface, (8): sapling is completely dried due to the root rot.

The results were evaluated by rating the severity of disease symptoms for each replicate. The percent value of disease severity was calculated as follows in Equation 1 (Towsend and Heuberger, 1943).

$$\text{Severity of disease} = \text{Total}\,(n \times V) \times 100 / xZN \quad (1)$$

According to the formula above, n is the number of plants in different damage groups, V is damage degrees divided into groups, N is the total number of plants subjected to control and Z is the highest scale value.

2.2.3. Statistical analysis

The variance analysis of the data was performed using JMP® (Version 7, SAS Institute Inc., Cary, NC, 1989-2019) statistical software, and the LSD test was used to determine the significance of the differences between the treatments.

2.3. Identification of *Rhizoctonia* isolates

Identification of isolates obtained from the root and crown root of the infected saplings was primarily performed based on some morphological and microscopic observations (Ogoshi, 1975). Isolates taken from slant agar tubes were incubated in PDA (potato dextrose agar) and WA (water agar) media at 22-24 °C in the dark for 5-7 days, and both the developments of isolates and the morphological characteristics and sclerotic conditions of isolates were examined macroscopically. Then, 90° angle and connection conditions of the hyphae were examined under a microscope. Two methods were used to determine the anastomosis groups of the isolates identified as *Rhizoctonia*. The first method was hyphal anastomosis reaction tests using the test isolates obtained from abroad. The isolates obtained from pistachio saplings (Rs1, Rs2, Rs3, Rs7, Rs9, Rs10, Rs14) and the test isolates AG-1 (isolate no: CS-KA), AG 2-1 (isolate no: PS 4), AG-3 (isolate no: ST-11-6), AG-4 isolate no: AH-1), AG-5 (isolate no: GM-10), AG-E (isolate no: F-18), and AG-K (isolate no: B 145) were incubated in PDA medium at 22-24 °C for one week, and 5 mm discs cut with a cork borer were placed opposite side of each other in WA medium at equal distance. The Petri dishes were incubated at 24 °C for 48 hours, then, the fusion or overlapping status of hyphae were examined under a light microscope (Kronland and Stanghellini, 1988). The anastomosis groups of the *Rhizoctonia* isolates were determined according to the fusion status of hyphal cells between the two isolates. Following the rDNA-ITS sequence, the analysis method was used as the second method to determine the anastomosis groups of the *Rhizoctonia* isolates.

Plant DNA extraction kit (QIAGEN Inc. Valencia, CA) was used in fungal DNA isolation according to manufacturer's instructions. The polymerase chain reaction (PCR) was performed using primers ITS-1 (5 'TCC GTA GGT GAA CCT GCGG 3') and ITS-4 (5 'TCC TCC GCT TAT TGA TATGC 3' in a 50 µl reaction mixture containing 25 µl GoTaq® Hot Start Green Master mix (2×) (Promega, USA), 2 µl forward primer (10 mM), 2 µl reverse primer (10 mM), 13 µl sterile double-distilled water, 4 µl BSA and 4 µl template DNA (White et al., 1990). The initial denaturation was carried out at 94 ºC for 3 min followed by 30 cycles at 94 ºC for 1 min, 49 ºC for 2 min, and 72 ºC for 3 min, and a final elongation step was at 72 ºC for 7 min (Kilicoglu and Ozkoç, 2010) in the ABI Veriti (Applied Biosystem) thermal cycler. The PCR products were sequenced in a private

Research and Development Laboratory (BM Gene Research and Biotechnology Company, Ankara, Turkey). Sequence electropherograms were compared to determine the isolate sequences in Gen Bank after BLAST screening in National Center for Biotechnology Information (NCBI).

The Phylogenetic tree was constructed using ClustalW alignments (Thompson et al., 1994), and the Maximum likelihood method tree was constructed using the Tamura-Nei model (Tamura and Nei, 1993) in MEGA 7 software (Kumar et al., 2016).

3. Results

3.1. Sample collection, preservation of cultures, and macroscopic and microscopic identification

The isolates were obtained from 1-2-year-old saplings with dry brown rot and some necrotic lesions on the root and crown root. The shoots and leaves of saplings showing symptoms of chlorosis turned to yellow and necrosis were formed. The saplings dried completely over time. *Rhizoctonia*-like isolates were selected among the fungi isolated from the root and crown roots of these saplings, and their morphological properties were examined after growing in the suitable media. After five days, the colony covered the entire petri dish, and the isolates, which were initially light cream tones, turned into dark brown and yellowish-brown (Boosalis and Scharen, 1959). During this period, the cream-colored isolates formed brown sclerotic in the medium. The microscope examinations revealed that thick compartmentalized hyphae branched to form a 90-degree angle.

3.2. Pathogenicity of isolates

The study was conducted under room conditions using a 1-2-year-old Siirt pistachio variety. In the evaluation stage, all the saplings were removed and the symptoms on the root and crown root were evaluated using the 0-8 scale. The results of disease severity determined as a percentage were given in Table 1.

Statistically significant differences ($p<0.01$) were obtained between the isolates. In pathogenicity studies, the highest disease severity was obtained in the R2 isolate with 93.75%, and the lowest disease severity was obtained from the R9 and R14 isolates which were in the same group with the control. The results indicated that R9 and R14 isolates were not pathogens. The disease severity values of the Rs3, Rs7, Rs10, and Rs1 isolates were 40.63, 28.13, 28.13, and 12.50%, respectively. *R. solani* Rs2 isolate caused the sapling deaths, while the Rs9 and Rs14 isolates did not cause any symptoms on the saplings. The Rs3, Rs7, and Rs10 isolates led to the occurrence of moderate disease symptoms. Symptoms in saplings were observed in the fourth week after infection. The first symptoms occurred in the upper parts of the plants as growth retardation, leaf necrosis, defoliation, and drying (Figure 1).

Table 1. Disease severity of *Rhizoctonia* isolates

Rhizoctonia isolates	Value of disease severity (%)*
Rhizoctonia Rs1	12.50 d
Rhizoctonia Rs2	93.75 a
Rhizoctonia Rs3	40.63 b
Rhizoctonia Rs7	28.13 c
Rhizoctonia Rs9	0.00 e
Rhizoctonia Rs10	28.13 c
Rhizoctonia Rs14	0.00 e
Control (-)	0.00 e

*: Values in the same column followed by the same letter are not significantly different at $p<0.01$, CV (Coefficient of variation): 10.41%

The infected saplings showed the following symptoms; rotted roots, brown lesions, destruction of all lateral roots, and dry rot in the main root (Figure 2). The *Rhizoctonia* was re-isolated from the infected saplings. No symptoms were observed in the negative control.

3.3. Anastomosis groups of *Rhizoctonia* isolates

The fusion status of *Rhizoctonia* isolates and test isolates in the water-agar medium were examined under a light microscope. The isolates were considered in the same anastomosis group if a fusion occurred. The DNA-based molecular identifications were carried out as stated in the method section. The data obtained with two methods were given in Table 2.

Under in-vitro conditions, the fusion occurred between the Rs2, Rs7, and Rs10 isolates and the test isolate (*R. solani* AG-4), and the anastomosis group was identified as *R. solani* AG-4. No fusion occurred between Rs1, Rs3, Rs9, and Rs14 isolates and the test isolates; thus, they were not included in any anastomosis group. The DNA-based molecular identification study revealed that Rs1, Rs3, Rs9 and Rs14, isolates were in the binucleate AG-F group. The results for Rs2, Rs7, and Rs10 isolates, were in agreement with the anastomosis reaction test result which confirmed that they were in the *R. solani* AG-4 group. The molecular identification studies showed that the Rs2 and Rs10, identified as *R. solani* AG-4, were in HGI which is considered as the advanced subgroup.

The phylogenetic tree was constructed from the bootstrap Maximum Likelihood analysis of nucleotide sequences to assess the genetic variability and group within seven *Rhizoctonia*

Figure 1. Symptoms caused by *Rhizoctonia* Rs2 isolate on the upper part of the pistachio saplings

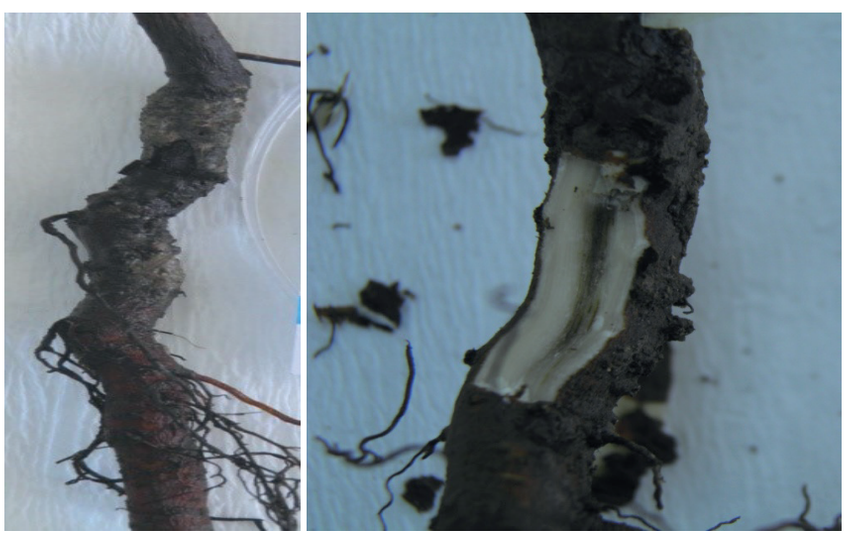

Figure 2. Symptoms caused by *Rhizoctonia* Rs2 isolate on the root zone of the pistachio saplings

Table 2. *Rhizoctonia* isolates identified by classical and molecular methods

Rhizoctonia isolates	Test isolates	Anastomosis reaction test	Molecular identificaion	Similarity ratio with isolates from GenBank (%)
Rs1	AG 1(-), AG 3(-), AG 4(-), AG 5(-),AG 2-1(-), AG E(-), AG K(-)	(-)*	AG F	99.68
Rs2	AG 1(-), AG 3(-), AG 4(+), AG 5(-), AG 2-1(-), AG E(-), AG K(-)	AG 4(+)**	AG 4-HGI	96.59
Rs3	AG 1(-), AG 3(-), AG 4(-), AG 5(-),AG 2-1(-), AG E (-), AG K(-)	(-)	AG F_a	99.84
Rs7	AG 1(-), AG 3(-), AG 4(+), AG 5(-), AG 2-1(-), AG E(-), AG K(-)	AG 4(+)	AG 4	99.83
Rs9	AG 1(-), AG 3(-), AG 4(-), AG 5(-),AG 2-1(-), AG E(-), AG K(-)	(-)	AG F	99.84
Rs10	AG 1(-), AG 3(-), AG 4(+), AG 5(-), AG 2-1(-), AG E(-), AG K(-)	AG 4(+)	AG 4-HGI	96.73
Rs14	AG 1(-), AG 3(-), AG 4(-), AG 5(-),AG 2-1(-), AG E(-), AG K(-)	(-)	AG F	99.83

*: No fusion between testes isolates and hyphae, **: Fusion between testes isolates and hyphae

isolates (Figure 3). The phylogenetic tree of isolates clearly showed that the isolates were grouped into two distinct main clusters (Figure 3). The first cluster consisted of binucleate *Rhizoctonia* AG- F and the second cluster consisted of multinucleate *Rhizoctonia solani* AG-4 isolates.

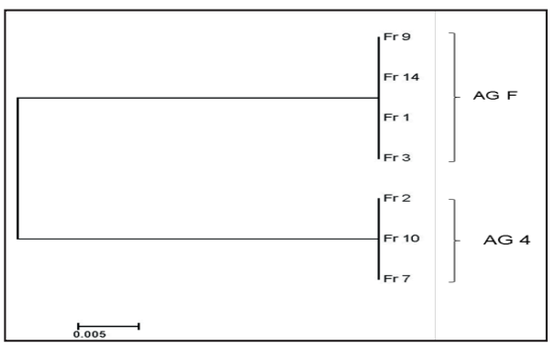

Figure 3. Phylogenetic tree for the seven isolates of *Rhizoctonia* based on their ITS sequences

4. Discussion and Conclusion

Soil-borne pathogens are the main causes of root rot and dryings in pistachio. The most important soil-borne pathogens were reported as *Phytophthora capsici*, *Phytophthora citricola*, *Phytophthora citrophthora*, *Phytophthora cryptogea*, *Phytophthora nicotianae*, *Phymatotrichopsis omnivora*, *Eutypa lata*, *Cytospora terebinthi*, *Scelerotinia sclerotiorum*, *Verticillium dahliae*, *Fusarium* spp. and *Rhizoctonia* solani (Chitzanidis, 1995; Michailides et al., 1995; Eskalen et al., 2001; Teviotdale et al., 2002; Alaei et al., 2017; Aydın, 2019a, 2019b). Soil pathogens or non-pathogenic saprophyte fungi can survive together in the plant root zone and in the soil (Reeleder, 2003; Agrios, 2005). In this study, saplings with disease symptoms in pistachio nurseries were selected. Isolations were collected from the root-crown root and different fungi were isolated. Since the study was focused on *Rhizoctonia* and anastomosis groups, only *Rhizoctonia*-like isolates were selected. The pathogenicity study showed that some *Rhizoctonia* isolates were not virulent, thus we concluded that these isolates live together with some other pathogenic fungi. Non-pathogenic *Rhizoctonia* (Np-R) isolates intensively colonize on the plant surface, subsurface plant organs, stems and leaves. Therefore, Np-R isolates are in physical competition with virulent isolates to compete for nutrients or to colonize on the infection site (Sneh, 1996). In another study conducted in nurseries of the same region reported that *Fusarium* spp., *Rhizoctonia* spp., *Macrophomina* spp., and *Verticillium* spp. were isolated from the infected plant roots (Aydın, 2019a).

Rhizoctonia solani Kühn. [Sexual circuit: *Thanatephorus cucumeris* (FR) Donk] is a soil-borne pathogen and has a large number of hosts, including pistachio. The fungus can survive for many years in the form of sclerotia on the plant or in the soil. When saplings are planted in contaminated soils, the pathogen attacks the plant and causes root and crown rot. The plants can die in a short time when environmental conditions are optimal for the pathogen. In this study, isolates were obtained from saplings. The pistachio seedlings are especially highly sensitive to soil pathogens in their young period. Some of the previous studies reported that *R. solani* is more effective on young trees planted in nurseries and orchards (Holtz et al., 1996; Holtz and Teviotdale, 2016; Aydın, 2019b).

Rhizoctonia has many species or biotypes that cause different levels of disease in plants. These are also called anastomosis groups (Sneh, 1996). The hyphae of close relatives tended to fuse (anastomosis) (Burpee et al., 1980; Ogoshi et al., 1983). The anastomosis groups cause disease depending on the host (Carling et al., 2002). For example, studies reported that AG-3 causes disease in potatoes (Demirci and Döken, 1993, 1995; Callerus et al., 2000; Truter and Wehner, 2004; Tsror, 2010). In this study, the anastomosis groups of Rs2, Rs7, and Rs10 isolates were determined as AG-4. The Rs2 was identified as the most pathogenic isolate. Studies conducted in Iran, which is one of the most important pistachio producers in the world, reported that damping-off, root, and stem rot diseases caused by *R. solani* AG-4 in many pistachio saplings cause significant and severe economic losses (Ashkan and Abusaidi, 1995; Ilkhan et al., 2011). *Rhizoctonia solani* AG-4 causes disease in many host plants other than pistachio. For example, although the pathogen does not form sclerotia in the tubers of potatoes, it causes deep wounds in the crown root and stem (Sneh, 1996; Virgen-Callerus et al., 2000; Aydın and Turhan, 2013). In addition, Ünal et al. (2015) reported that the pathogen causes root rot in wheat. Another study conducted in the Van region showed that the pathogenic *R. solani* isolates obtained from the tomato plant were AG-4 (Durak and Ok, 2019).

The majority of *R. solani* AG's are considered pathogens in various plants, while the binucleate *Rhizoctonia* species are generally regarded as non-pathogenic fungi in plants. Therefore, the BNR species have been used as a biological control agent against some diseases (Sneh et al., 1986;

Sneh et al., 1989; Sneh and Auster, 1998; Trillas, 2006). The fungus is mostly found in soil organic matter or plant debris (Andersen and Rasmussen, 1996; Sneh, 1996). Non-pathogenic *Rhizoctonia* spp. (Np-R) can be found among all AG population groups (Sneh, 1996). The results of this study showed that Rs9 and Rs14 isolates were not pathogenic in pistachio saplings and they were identified as *Rhizoctonia* AG-F based on molecular detection. However, many studies reported that some BNR species are pathogenic and cause damping-off, root rot, stem rot, leaf burn, and fruit rot in some plants (Parmeter and Whitney, 1970; Tanaka et al., 1994; Ünal et al., 2014). The disease severity of *Rhizoctonia* Rs3 was 40.63%. Molecular identification showed that *Rhizoctonia* Rs3 is AG-Fa. The isolate does not have very high virulence. But the isolate caused moderate disease in pistachio saplings. To our knowledge, this is the first record in pistachio in Turkey. Similarly, Alaei et al. (2017) reported that binucleate *Rhizoctania* AG-F causes root and stem rot in pistachio seedlings in Iran.

In this study, two methods were used to determine the anastomosis groups of *Rhizoctonia* isolates. In the first method, the test isolates were compared with the isolates collected from pistachio saplings and the anastomosis reactions of the hyphae were examined under the microscope. In this method, the Rs2, Rs7, and Rs10 isolates were determined as AG-4, while the Rs1, Rs3, Rs9, and Rs14 isolates could not be identified because they did not fuse with any test isolates used in the study. The DNA-based molecular method was used in the second method. The unidentified isolates in the first method were determined as *Rhizoctonia* AG-F. The AG-F isolate was not included in the first method that used the test isolates, thus, the anastomosis groups of some isolates were only identified by the molecular method. There are many *Rhizoctonia* groups including binucleate and multinucleate (Sneh et al., 1998; Sharon et al., 2008). Therefore, obtaining and using all the test isolates are very difficult in terms of time and labor conditions. In addition, advanced subgroups cannot be identified by the classical method. Therefore, the use of DNA-based molecular methods would be appropriate for future studies on *Rhizoctonia* and anastomosis groups.

Rhizoctonia solani is a soil-born pathogen that creates stable structures like sclerots, which are difficult to control and can cause yield losses. *Rhizoctonia solani* infects many fruit trees and vegetables, including pistachio. In pistachio, *Rhizoctonia solani* causes dry rot and drying in the subsoil parts of the plant, especially during the sapling period.

Some *Rhizoctonia*, such as the binucleate AG-F known as NpR, causes a slight disease in pistachio seedlings. The observations during the study showed that soil and soil-like materials used in pistachio nurseries were not sterilized. This practice may cause drying of trees in the early stages of development due to transferring the material and saplings contaminated with some other soil pathogens including *Rhizoctonia* to the newly established orchards. In addition, the results revealed that the use of the molecular method in the identification of *Rhizoctonia* species and anastomosis groups provided quick and reliable results.

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