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Research Article

Determination of Disease Severity and Anastomosis Groups of *Rhizoctonia solani* Isolates from Chickpea Plant in Konya Province

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ABSTRACT

This study was carried out to determine the anastomosis groups and disease severity of *Rhizoctonia solani*, which causes root and root collar rot in chickpea production areas in Konya. A total of eleven isolates of *Rhizoctonia* spp. were obtained in the isolations made from the root and root collar of the plants in the surveys. *Rhizoctonia* isolates examined microscopically were grouped as multinucleate, binucleate according to the number of nuclei. Ten isolates were multinucleate and identified *R. solani*, while one isolate was binucleate *Rhizoctonia* sp. In pathogenicity tests, five multinucleate isolates were found to be pathogenic. Disease severity was determined as 100% for 1.2 A and 1.2 B coded isolates, 86% for 1.1 A coded isolate, 75% for 1.1 B coded isolate, and 44% for 1.2 C coded isolate. It was observed that the other multinucleate five isolates caused only a shortening in plant heights. No disease symptoms were observed in the plants inoculated by the binucleate isolate. All isolates were characterized using the internal transcribed spacer (ITS) region of ribosomal DNA, and two different anastomosis groups were defined accordingly. It was determined that 5 virulence multinucleate *R. solani* isolates belonged to AG-4 HGII and 5 other multinucleate isolates to AG BI anastomosis group.

1. Introduction

In terms of cultivation area in Turkiye, legumes take the most important place after cereals. Considering the total legume production, Turkey is among the largest producers in the world (FAOSTAT, 2021). Among nine legume species grown in Turkey, chickpea (*Cicer arietinum* L.) is the most common. While chickpea cultivation area constitutes 57.6% of the legume cultivation and 51.2% of the production amount area in the country (TURKSTAT, 2021).

Chickpea, which is an important source of vegetable protein, has been used in human and animal nutrition and green fertilization both in Turkiye and in the world, especially in the Near East, Far East, Mediterranean, South America, and Central America countries, which has been cultivated since ancient times (Eser, 1976; Reddy and Singh., 1984; Reddy and Kabbabeh, 1985). It can be grown in almost every region in Turkey, and the region where it grows most is the Central Anatolia region. Almost 65.7% (414 thousand tons) of the total chickpea production takes place in the Central Anatolia region. According to the provinces, Ankara ranks first with 93 thousand tons in chickpea production, Yozgat ranks second with 86 thousand tons, Kırşehir ranks third with 78 thousand tons, and Konya ranks fourth with 50 thousand tons (TURKSTAT 2021).

Although it has a large cultivation area, it is thought that the desired yield cannot be obtained for many reasons in chickpea. Abiotic and biotic stress factors are the most important factors limiting production, including diseases and pests. It is seen that the soil-borne agents also cause yield losses in recent years, together with the *Ascochyta rabiei* factor. *Fusarium oxysporum* f. sp. *ciceris F. solani F. acuminatum F.moniliforme F.sambucinum F. equiseti Rhizoctonia solani* Kühn., *Macrophomina phaseolina* (Tassi) Goid, and *Cylindrocarpon tonkinense* Bugnic. were determined as soil-borne agents that causing wilt and root rot in chickpea (Aydın and İnal, 2019; Dolar 1996; Dolar and Nirenberg, 1998; Soran, 1977; Yücel and Güncü, 1991).

In particular, fungi of the genus *Rhizoctonia* are common in many parts of the world and have a wide host range. It causes a variety of diseases in important cultivars worldwide, including species in Solanaceae, Fabaceae, Asteraceae, Poaceae, and Braccicaceae, as well as ornamental and forest trees (Ogoshi, 1975; Ogoshi et al., 1996).

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Rhizoctonia fungi are a large and complex group that varies within itself (Carling and Summer, 1992). They are examined in three groups in terms of the number of nuclei in hyphae cells. These; They are called multinucleic (MN, multinucleated), binucleic (BN, binucleated) and uninucleic (UN, mononuclear) (Sneh et al.,1991). Within the multinucleic and binucleic *Rhizoctonia* genera, there are subgroups whose hyphae are compatible with each other and can fuse where they come into contact, and these fused subgroups are called the anastomosis group (AG) (Vilgays and Cubeta, 1994; Sneh et al., 1996). Anastomosis groupings are classically based on the characterization of hyphal anastomosis reactions of *Rhizoctonia* hyphae, and deoxyribonucleic acid (DNA)-based methods are increasingly used to identify anastomosis groups (AGs) (Vilgalys and Gonzalez, 1990; Cubeta et al., 1991; Carling et al.,2002).

R.solani, one of the important species in the *Rhizoctonia* genus, was named by Kühn in 1858 and the most important species of the *Rhizoctonia* genus was introduced to the scientific world with the naming of the telemorph *Thanatephorus cucumeris* (Frank) Donk and became the most studied and known species in the genus (Carling and Sumner, 1992). Matsumoto reported the presence of hyphal anastomosis for the first time in *R.solani* in 1921. Schulz, on the other hand, classified isolates according to their anastomosis abilities for the first time in 1936.There are 14 anastomosis groups of *R.solani*, AG 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 and AGB1 (Carling et al. 1999; 2002; Yang and Li, 2012) groups detected in chickpea are AG-1, AG-2-2, AG-2-2LP, AG-2-3, AG-3, AG-4 and AG-5 (Dubey et al. 2011).AG-4 and AG-5 groups are also available in chickpea cultivation areas in our country (Tuncer and Erdiller,1990) Demirci et al.,1999; Başbağcı et al., 2019).

In this study, it was aimed to determine the anastomosis groups of *Rhizoctonia* isolates obtained from chickpea cultivation areas in and around Konya and to reveal their virulence.

2. Materials and Methods

2.1.Materials

2.1.1. Plant material

The main material of the study is the diseased plant samples collected from chickpea cultivation areas in Konya province of Turkey and the disease-sensitive ILC-482 chickpea variety used in the pathogenicity tests of *Rhizoctonia* isolates.

2.1.2. Fungal material

In the study,eleven isolates of *Rhizoctonia* isolates were obtained from diseased chickpea plants and grouped according to nucleus and anastomosis properties.

2.1.3.Plant growing media and chemicals

In pathogenicity experiments, peat, garden soil and perlite were used in the ratio of 2:1:1 as the growing medium. Potato dextrose agar (PDA) and water agar (WA) with the addition of streptomycin sulfate were used in fungal isolations and purifications. Safranin O solution was used to determine the number of nuclei of *Rhizoctonia* isolates. In the isolations, 1% NaOCl (Sodium hypochlorite) was used for surface disinfection, and barley seeds were used for long-term storage of the isolates.

2.2.Methods

2.2.1. Collection of diseased plant samples

Diseased plant samples were collected from April to July of 2019 according to guided sampling. Samples were taken considering typical disease symptoms with dark brown lesions on the root and root collar. The samples were labeled and brought to the laboratory in paper bags and stored at +4 °C until isolation.

2.2.2. Fungal ısolation from diseased plant samples

Collected plant samples were washed under tap water. The washed plant samples were allowed to dry on blotting papers.Then, with the help of a sterile scalpel, sections including the healthy part along with the area showing disease symptoms were taken on the root and root collar parts. The sections taken were disinfected superficially in 1% NaOCI for 1-2 minutes, passed through sterile distilled water three times and left to dry for 5-10 minutes in a laminar flow cabinet on sterile blotting papers.

Dried plant sections were placed in Potato Dextrose Agar (PDA, Merck + Streptomycin sulfate) medium, 5-6 pieces per petri dish. Petri dishes were incubated at 25 °C for 3-5 days. At the end of this period, mycelial discs with a diameter of 5 mm from the developing colonies were transferred back to fresh PDA medium and grown for 7-10 days at 25 °C to obtain pure cultures.

2.2.3. Storage of Rhizoctonia isolates

Barley seeds were taken into capped glass tubes and sterilized in an autoclave for one hour at 121 °C for two consecutive days. Then, mycelial discs with a diameter of five mm, taken from *Rhizoctonia* cultures that were previously grown in PDA medium for 5-6 days, were placed in these tubes and incubated at 24±1 °C for 20-30 days and the hyphae were expected to cover the seeds (Başbağcı., 2020). Then, *Rhizoctonia* isolates, which were wrapped in sterile barley seeds, were placed in a refrigerator operating at +4 °C for long-term storage (Olaya and Abawi, 1994).

2.2.4. Identification and determination of groups

Identification of fungal isolates obtained from diseased plants was made according to Carling and Summer (1993), taking into account the morphological characteristics of *Rhizoctonia* spp. In order to determine the number of nuclei of the isolates identified as *R. solani*, firstly nuclei were stained to determine multinucleate (multi-nucleated), binucleate (two-nucleated) isolates. To prepare 100 ml of safranin O solution; 6 ml of 0.5% saffron prepared with distilled water was poured into a

flask, and 10 ml of 3% KOH solution prepared with distilled water, 5 ml of glycerine and 79 ml of distilled water were added and mixed (Akın, 2001). A drop of Safranin O solution was dropped on a slide for examination. Hyphae tips taken from *Rhizoctonia* isolates developed by transferring to PDA and WA medium and incubating in the dark at 25 °C with the help of a coverslip were placed on the solution in the slide. In the preparations prepared in this way, the number of nuclei in the hyphae was determined by taking into account the number of nuclei in at least 20 cells (Ogoshi et al.,1990, Carling et al., 1994, Karaca et al., 2002).

Molecular characterization studies to determine the anastomosis groups of *Rhizoctonia solani* isolates were carried out in Bolu Abant İzzet Baysal University, Faculty of Agriculture, Department of Plant Protection Laboratory. In the relevant laboratory, anastomosis groups were determined by amplification of *R.solani* isolates using ITS gene region 1F (CTTGGTCATTTAGAGGAGTAA) and ITS 4B (CAGAGACTTGTACACGGTCCAG)primers (Gardens and Bruns 1993).

2.2.5. Pathogenicity Test

ILC-482 chickpea cultivar known to be sensitive to *R.solani* was used in the studies. For this, sterile growing media containing a 2:1:1 mixture of peat, garden soil, and perlite were filled into 2-liter sterile pots.After the sterile growing medium was filled into the pots, 3 pieces of chickpea seeds, which were pre-germinated under sterile conditions in a medium containing 1% WA, were planted in each pot.

The inoculation process was carried out by placing one each of the barley seeds on which *R.solani* hyphae had been wrapped before, next to each germinated chickpea seed sown. After the inoculation process, the seeds were covered with sterile growing medium. As a control, healthy chickpea seeds germinated in a medium containing 1% WA disinfected with 2% NaOCl for 5 minutes were planted in pots containing the same amount of sterile growing medium.3 pots were used for each isolate and one pot was considered a replicate. All pots were left to develop at 20-25 °C under controlled climate room conditions. Approximately 45 days after planting, the plants were removed and disease severity values were calculated according to the Townsend-Heuberger formula given below, taking into account the 0-4 scale given in Table 1 (Townsend-Heuberger, 1943; Kim et al., 1997; Demirci, 1998; Paulitz et al., 2000).

Table 1
0-4 scale used in the evaluation of disease severity of *Rhizoctonia* isolates

Scale degree	Symptom
0	No symptom (root and root collar)
1	Slight discoloration or roots emerging from seed less than 3 cm
2	One or more small lesions (< 0.5 cm) or roots emerging from the seed less than 2 cm
3	One or more small lesions (> 0.5 cm) or roots emerging from the seed less than 1 cm
4	Severe lesion, completely dead or rootless seedling

Disease Severity (%) = Σ(nxV/ZxN)x100

n: number of plants with different disease degrees on the scale

V: scale degree

Z: highest scale degree

N: total number of plants observed

2.2.6. Statistical analysis

The SPSS statistical program (SPSS Inc., version 17.0) was applied to disease severity values obtained from pathogenicity experiments. The difference between the applications was determined by the Tukey multiple comparison test (P≤0.01).

3. Results and Discussion

Eleven *Rhizoctonia* isolates were obtained as a result of isolations and identifications made from 204 diseased plant samples collected in 2019 by guided sampling method in chickpea cultivation areas of Konya Province.

R.solani isolates, which were examined microscopically using the prepared 0.5% Safranin O solution, were grouped as multinucleate (multinucleated), binucleate (binucleated) according to the number of nuclei, and 10 isolates were multinucleated (1.1A, 1.1B, 1.2A, 1.2B, 1.2C, KK-11E, KK-11A, KK-11B, KK-11C, KK-11D (Figure 1) and 1 isolate was binucleate (KAR-5 (Figure 2).

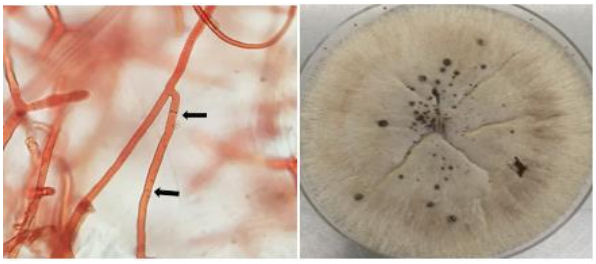

Figure 1
Microscopic view (left) and petri dish (right) of the multinucleate *Rhizoctonia solani* isolate

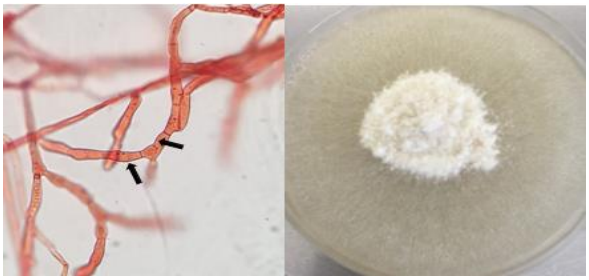

Figure 2
Microscopic view of binucleate *Rhizoctonia* sp. isolate (left) and petri dish (right)

As a result of the analyzes performed considering the molecular characterization of a total of eleven *Rhizcotonia* isolates, it was determined that the existing multinucleate isolates were included in the AG-4 HGII and AG-BI anastomosis groups. The anastomosis group of the binucleate isolate (KAR-5) could not be determined (Table 2).

Table 2
Anastomosis groups of *Rhizoctonia* isolates determined according to ITS primers

Isolate number	Isolate number	Anastomosis groups according to ITS primers
1	1.1 A	AG-4 HGII
2	1.1 B	AG-4 HGII
3	1.2 A	AG-4 HGII
4	1.2 B	AG-4 HGII
5	1.2 C	AG-4 HGII
6	KK-11 E	AG-BI
7	KK-11 A	AG-BI
8	KK-11 B	AG-BI
9	KK-11 C	AG-BI
10	KK-11 D	AG-BI
11	KAR-5	Not determined

The disease severity values of 11 *R.solani* isolates in chickpea plants as a result of the pathogenicity tests carried out with three replications are given in Table 3.

Table 3
Disease severity rates of *Rhizoctonia* isolates on chickpea plants (%)

Isolate number	Isolate number/anastomosis group	Disease severity (%)
1	1.1 A/AG-4 HGII	86.00 b
2	1.1 B/AG-4 HGII	75.00 c
3	1.2 A/AG-4 HGII	100.00 a
4	1.2 B/AG-4 HGII	100.00 a
5	1.2 C/AG-4 HGII	44.00 d
6	KK-11 E/AG-BI	0.00 e
7	KK-11 A/AG-BI	0.00 e
8	KK-11 B/AG-BI	0.00 e
9	KK-11 C/AG-BI	0.00 e
10	KK-11 D/AG-BI	0.00 e
11	KAR-5-/not determined	0.00 e
12	Control	0.00e

P≤0.01 (There is no statistical difference between the means expressed with the same letter in the same column)

While different rates of disease severity were observed in five isolates as a result of pathogenicity tests, no disease severity value could be calculated in six isolates. All of the isolates that were determined with different rates of disease belonged to the AG-4 HGII anastomosis group, with the highest disease severity in 1.2A and 1.2B isolates detected. These two isolates were followed by isolate 1.1A with a disease severity value of 86%. The lowest disease severity was obtained from isolate 1.2 C with 44% (Table 3). Symptoms such as seed infection, pre-emergence and post-emergence damping-off and visible lesions on the root and collar were observed in plants infected with the disease at different levels by inoculation of isolates in the AG-4 HGII anastomosis group (Figure 3, 4).

Figure 3
Infection of *R.solani* isolate no. 1.2 A on ILC-482 chickpea seeds

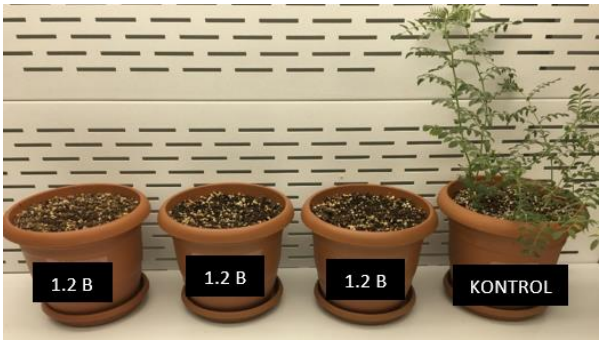

Figure 4
The image that emerged as a result of pre-emergence and post-emergence damping-off infection on chickpea plants(ILC-482 variety) in the pathogenicity test of isolate 1.2 B of *R.solani*

In the pathogenicity experiment, none of the symptom types described in the 0-4 scale given in Table 1 of the multinucleate five isolates with the AG BI anastomosis group were observed in plants, so numerical disease severity values could not be determined in them. Therefore, the disease severity of all of these isolates was evaluated as 0%.Although these isolates did not have the disease symptoms specified in the 0-4 scale, it was observed that they caused a partial shortening of the plant heights compared to the control. KAR-5 isolate, which was detected as a binucleate isolate among *R. solani* isolates, did not cause any disease symptoms on plants (Figure 5).

Figure 5
Image of inoculated chickpea plants(ILC-482 variety) with binucleate KAR-5 isolate of *Rhizoctonia* sp.

Although the morphological characters of the obtained isolates are variable, eleven *R. solani* isolates are divided into three categories according to colony color and most of them have light brown colony color (Ganeshamoorthi and Dubey, 2015).

Rhizoctonia spp. it is a complex group that includes pathogen species that cause economic loss in many agriculturally important plants, as well as non-pathogenic species (Sneh et al., 1996). There are disease records of different *Rhizoctonia* spp. in various legumes grown in various ecological conditions of the world. Generally, binucleated *Rhizoctonia solani* isolates are not considered pathogenic in plants, although some exceptions have been reported (Ogoshi, 1987; Hua et al., 2014; Türkkan et al., 2018). In our study, it was determined that the binucleate *Rhizoctonia solani* isolate that we obtained did not show any disease symptoms in plants, that is, it was not pathogenic. On the other hand, multinucleated *Rhizoctonia solani* isolates cause root and crown diseases in many plants, including legumes (Verma, 1996; Sneh et al., 1996; Erper et al., 2016; Yıldırım and Erper, 2017; Türkkan et al., 2020). In our study, among eleven *R. solani* isolates whose virulence was tested, five isolates in the multinucleate AG-4 HGII anastomosis group were found to cause pre-emergence damping-off at different percentages. It was observed that the other five isolates in the multinucleate, AG-BI anastomosis group caused only stature shortening in plants. *R.solani* AG-4 isolates isolated from chickpea were found to be moderately and highly virulent, and some of them caused pre-emergence damping-off (Hwang et al., 2003).

R.solani, which is in the AG-4 anastomosis group, has been reported many times worldwide to be pathogenic for chickpeas (Hwang et al.,2003; Dubey et al., 2011, 2014; Ganeshamoorthi and Dubey, 2013, 2015). Dubey et al. (2011) reported that the severity of disease caused by AG-4 isolates ranged from 11% to 100% in chickpea, and the highly virulent isolate level was 75.6% in chickpea. However, Ganeshamoorthi and Dubey (2015) found that 4 of 50 *R.solani* isolates of AG-4 were moderately virulent in chickpea. This information supports our findings, and it was found that our isolates in the AG-4 anastomosis group caused between 44% and 100% disease severity on chickpea plants.

Five AG groups (AG-1, AG-2, AG-3, AG-4 and AG-5) of *R.solani* have been determined on chickpea in the world (Hwang et al., 2003; Mikhail et al., 2010; Youssef et al., 2010; Dubey et al., 2011, 2014; Ganeshamoorthi and Dubey, 2013, 2015).

In Turkey, AG-4 of *Rhizoctonia solani* was found in barley (Demirci, 1998; Ünal and Kara, 2017), beans (Eken and Demirci, 2004; Kılıçoğlu and Özkoç, 2013), pepper (Tuncer and Eken, 2013), tomato (Yıldız and Döken, 2002), soybean (Erper et al., 2011), cotton (Kural et al., 1994), Johnson grass (Demirci et al., 2002), and wheat (Demirci, 1998; Ünal et al., 2015) has been reported. AG-4 and AG-5 groups with high virulence are present in chickpea cultivation areas in our country (Tuncer and Erdiller 1990; Demirci et al., 1999;Başbağcı et al., 2019). This study showed that the AG-BI anastomosis group of *R.solani* is also present in our country.

Since the isolates of *R.solani* fungus do not have the potential to cause disease at the same severity in chickpea plants, and some isolates do not have any effect on disease formation, not every *R.solani* isolate obtained is considered as a pathogen and considering the compatibility of such isolates with pathogenic isolates, they can be used as biological control agents. With the anastomosis that may occur between pathogen and apathogen *R.solani* isolates, it may be possible to determine effective biological control agents by activating the hypovirulence mechanism.

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