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# Isolation and Identification of Binucleat *Rhizoctonia* spp. from Wheat Field Soils in the Central Anatolia Region, Turkey

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**Abstract**

Current study was conducted to identify the anastomosis groups and pathogenicity of binucleate *Rhizoctonia* species from soil samples in wheat production areas of Konya, Ankara, Yozgat, Eskişehir, Kırıkkale, Kayseri, Kırşehir, Nevşehir and Aksaray provinces in the Central Anatolia Regions during 2009-2012 growing season. One thousand and two hundred fifty six (1256) soil samples were collected from wheat fields and isolations were made from the soil that using colonization of bait tissue. Species and anastomosis groups identification was done according to the basis of hyphal and colony morphology, anastomosis reaction with known tester isolates. Pathogenicity test was conducted with agar-plate assay with all isolates. Fifty one isolates were identified as binucleate *Rhizoctonia* and those isolates were found to be belonged to AG A, AG C, AG D (*R. cerealis*), AG E, AG G, AG H, AG I and AG K. In consequence of the pathogenicity tests performed, the groups other than AG D were not found to be pathogenic on susceptible wheat cultivar. AG C identified in this study is first record for Turkey, and also AG A, AG E, AG G and AG H groups were determined to be first in the wheat field soils in Turkey.

**Key words**: Binucleat *Rhizoctonia* spp., anastomosis group, wheat, soil

**Özet**

Bu araştırma 2009-2012 üretim sezonunda, İç Anadolu Bölgesi'nde Konya, Ankara, Yozgat, Eskişehir, Kırıkkale, Kayseri, Kırşehir, Nevşehir ve Aksaray İlleri buğday üretim alanlarından toplanan toprak örneklerindeki binükleat *Rhizoctonia* türlerinin anastomosis gruplarını ve patojenisitelerini belirlemek amacıyla yürütülmüştür. Buğday tarlalarından 1256 toprak örneği toplanmış ve toprak örneklerinden izolasyonlar tuzak bitki kullanılarak yapılmıştır. Tür ve anastomosis grupları, izolatların hif yapısı, koloni morfolojileri ve test izolatları ile anastomosis reaksiyonlarına göre yapılmıştır. Tüm izolatların patojenisite testleri petri denemeleri ile yapılmıştır. AG A, AG C, AG D (*R. cerealis*), AG E, AG G, AG H, AG I ve AG K anastomosis gruplarına ait 51 binükleat *Rhizoctonia* izolatı tanımlanmıştır. Hassas buğday çeşidi kullanılarak yapılan patojenisite çalışmaları sonucunda AG D dışındaki tüm izolatlar buğdayda patojen bulunmamıştır. AG C grubu Türkiye için ilk kayıttır. Ayrıca AG A, AG E, AG G ve AG H grupları ise Türkiye'de buğday tarla toprağında ilk kez tespit edilmiştir.

**Anahtar Kelimeler**: Binükleat *Rhizoctonia* spp., anastomosis grup, buğday, toprak

## Introductıon

*Rhizoctonia* genus is a soilborne pathogen with a wide host range. It is a species complex composed of several pathogen and non-pathogen anastomosis groups (AGs). The classic taxonomy of the anamorph "form-genus" *Rhizoctonia*, which includes three major groups: multinucleate *Rhizoctonia* (MNR) (teleomorphs *Thanatephorus* and *Waitea*), binucleate *Rhizoctonia* (BNR) (teleomorphs *Ceratobasidium* and *Tulasnella*), and uninucleate *Rhizoctonia* (UNR) (teleomorph *Ceratobasidium*) has been essentially based on hyphal fusion that divided *Rhizoctonia* spp. into the well-established anastomosis groups and further into subgroups (Sharon et al., 2006). Binucleate

*Rhizoctonia* isolates are grouped into 16 AGs (AG A, AG B, AG C, AG D, AG E, AG F, AG G, AG H, AG I, AG K, AG L, AG O, AG P, AG Q, AG R and AG S) (Sharon et al., 2008).

Binucleate *Rhizoctonia* spp. represent a diverse group of organisms that have been isolated from soils and plants throughout the world. These fungi can exist saprophytically in soil and plant debris or may establish parasitic relationships with plants (Sneh et al., 1996; Herr, 1995).

In studies conducted so far in the world, among BNR isolates; the groups other than AG D (*R. cerealis*) were not found to be pathogenic on wheat. Sharp eyespot caused by *R. cerealis* before commonly reported cause of damaged root and crown tissue in wheat-producing areas of Turkey (Aktaş et al. 1995, 1996, 1999; Muratçavuşoğlu, 1995; Tunalı et al. 2008). It is a potentially economically important crown pathogen of wheat in Central Anatolia Region. Binucleate AG I and AG K were also isolated from crown and subcrown of wheat and barley plant by Demirci (1998) in Erzurum, Turkey.

The aim of the present study was to determine the anastomosis groups of BNR isolates obtained from wheat field soils and their virulence on wheat.

## Materials And Methods

### Soil collection and isolation

In order to determine the anastomosis groups and pathogenicity of binucleate *Rhizoctonia* species in wheat field soils, 1256 soil samples were collected from Konya, Ankara, Yozgat, Eskişehir, Kırıkkale, Kayseri, Kırşehir, Nevşehir and Aksaray provinces in Central Anatolia Region, Turkey during 2009-2012 growing seasons. Sterile wheat straws were used for binucleate *Rhizoctonia* spp. isolation from soil samples. Soil samples from the respective fields were transferred to pots on a greenhouse bench (20±2$^0$C). Pots were then watered to field capacity. About 4 cm long internodal segments of mature, four dried wheat straws were inserted vertically in per pot, and left for 3 or 4 days after that straws were removed, washed, blotted and placed on acidified water agar (WA). Isolates of *Rhizoctonia* were transferred to potato dextrose agar (PDA).

### Identification

In order to determine hyphal diameter and the number of nuclei per cell of the isolates, *Rhizoctonia* isolates were maintained on PDA at 25°C and in the dark. Developing mycelia were stained with safranin O (Sigma, USA) and 3% KOH and observed under phase contrast microscopy at x 400 magnification.Hyphal diameter was determined by measuring 10 cells. Nuclei were counted in 15 cells. Anastomosis was tested by pairing isolates with representative testers of binucleate. *Rhizoctonia* isolates and binucleate tester isolates were activated on PDA at 25 °C in the dark.

Coverslips, sterilized by dipping in 95 % ethyl alcohol and flaming, were coated with a thin layer of 0.5 % PDA and placed on water agar plates. Agar plugs with mycelia of *Rhizoctonia* isolates and the tester isolates were cut the magrin of a growing colony and transfered to water agar plates on the opposite sides of the coverslip. After incubation at 25 °C for 24-48 h in the dark, when overlapping mycelia of two isolates were observed, the coverslip was removed from the plate and placed on a slide in the mixture of one drop of safranin O and one drop of 3 % KOH. Stained hyphae were observed microscopically. Anastomosing hyphae were traced back to their source in order to confirm the anastomosis between our isolates and the tester isolates (Kronland and Stanghellini, 1988). For the anastomosis testing, all pairs were examined twice.

### Pathogenicity tests

Agar- plate assay was used in the pathogenicity tests. Isolates were incubated on PDA at 25°C for 2 days, mycelial discs (4mm) from an actively growing edge of the fungal culture were transferred to 2% WA and incubated at the same conditions for 2 days. Seeds of the susceptible wheat cultivar (cv. Kate A-1). were disinfected by dipping in 1% NaOCl for 5 min, blotted dry with sterile paper towels, then six seeds were placed adjacent to the growing edge of the isolates in each Petri dish in sterile conditions. PDA discs were used as controls. Five replicate plates were used for each isolate after incubation for 7–8 days at 26°C, roots and hypocotyls of the plants were examined and evaluated for disease severity (Ichielevich-Auster et al., 1985). Disease severity was assessed using a scale of 1 to 5, where 1 = healthy roots or <2% discoloration on the roots, 2 = 2 to 10%, 3 = 11 to 50%, 4 = >50% discoloration and necrosis on the roots, and 5 = plants dead. These scale values were converted to disease severity values (Xi et al., 1990) using the following formula:

**Disease Severity**= [∑ (no. of plant in category x category value)] x 100 / Total no. of plants x max. category value)

## Results

The nucleus number that was found in each hypha cell was 2, and width of the main runner hyphae was less than 7µm (Figure 1a). According to the cellular nucleus number, width of the main runner hyphae, colony morphology and the

anastomosis test, 51 isolates were identified as binucleate *Rhizoctonia* and those isolates were found to be belonged to AG A, AG C, AG D, AG E, AG G, AG H, AG I and AG K (Table 1). As a result of anastomosis test, these isolates anastomosed with high fusion frequency (C 3 and C2 reactions) with binucleate tester isolates (Figure 1b). AG C identified in this study is first record for Turkey, and also AG A, AG E, AG G and AG H groups were determined to be first in the wheat field soils in Turkey.


**Figure 1**. (a) Binucleate *Rhizoctonia* cell, (b) anastomosis between hyphae (C2)

Table 1. Number of isolates of binucleate *Rhizoctonia* and anastomosis groups obtained from wheat field soils in nine provinces, Turkey

| **Name of Provinces** | **Anastomosis groups of binucleate *Rhizoctonia* isolates** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **AG A** | **AG C** | **AG D** | **AG E** | **AG G** | **AG H** | **AG I** | **AG K** |
| **Konya** | 1 | | 4 | | | | 1 | |
| **Ankara** | 2 | 2 | 4 | 3 | 3 | 3 | 8 | 3 |
| **Yozgat** | | | 2 | | | | | |
| **Eskişehir** | | 2 | | | | | 2 | |
| **Kırıkkale** | | | | | | | 3 | |
| **Kayseri** | | | | | | | 4 | 1 |
| **Kırşehir** | | | | | | | | |
| **Nevşehir** | | | | | | | | |
| **Aksaray** | **2** | | | | | | | **1** |

Morphological features of isolates on PDA were similar with descriptions of Sneh et al., (1994) and isolates of BNR growing on PDA were generally white initially and turned whitish beige to dark brown (AG D) or tan (AG G) in 3 weeks. They constituted rarely sclerotia out of AG D and AG G and they were 0.5–1.3 mm in diameter, almost globose, produced singly or in clumps, and yellowish (AG G) or light brown to dark brown (AG D) (Figure 2).

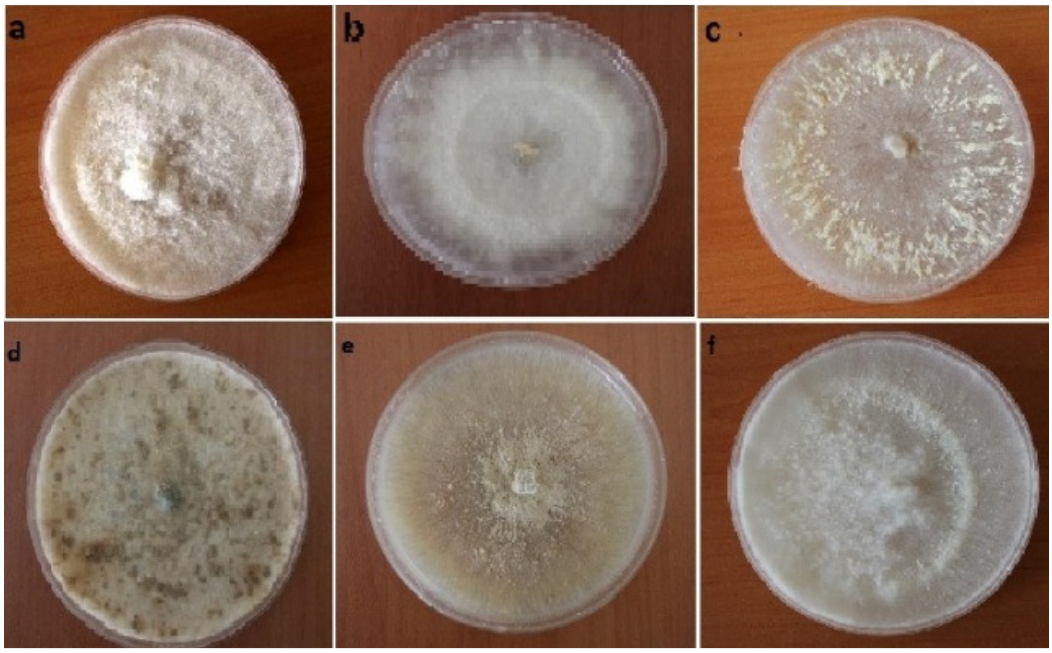


**Figure 2.** Colony appearance of some binucleate *Rhizoctonia* isolates on potato dextrose agar (a) AG A, (b) AG C, (c) AG D, (d) AG E, (e) AG G, (f) AG I.

Regarding the pathogenicity test on agar plates, all binucleate *Rhizoctonia* isolates were found to be non-pathogenic on wheat apart from AG D on susceptible wheat cultivar (cv. Kate A-1). All AG D isolates included in the group of binucleate and known as sharp eyespot disease agent in wheat were with 41-83% disease severity values assessed as pathogens. Non-inoculated plants remained healthy. BNR isolates were reisolated from plants.

**Discussion**

The present study is the first study in our country in terms of soil isolation for wheat. Also in the studies performed in different regions of the world, different groups of anastomosis leading and not leading to diseases due to wheat soil were determined, and the groups detected in our study showed similarity to them. In a study performed in Northwestern Pacific Region of America, the BNR isolates taken from the soils of 45 wheat and barley fields were defined as AG C, AG D, AG E, AG H ve AG K (Ogoshi et al., 1990). Similarly, in a study performed by Juan-Abgona et al. (1996) in Japan, 248 *Rhizoctonia* isolates were obtained from the soil, and 143 of these isolates were composed of BNR (AG A, AG Ba, AG Bb, AG G ve AG O) isolates. In a study performed in Taiwan, BNR AG A, AG Bo, AG F, AG G, AG L, AG Q, AG R ve AG S anastomosis groups were isolated as a result of insulations from 429 samples of soil (Chen and Chuang, 1997).

The detected AG A, AG E, AG G, AG H groups were determined to be first in the wheat field soils in Turkey. These groups were isolated previously in our country not from wheat but from different plants (Demirci and Döken, 1995; Demirci et al., 2002; Eken and Demirci, 2004; Durak Demirer, 2011). AG C isolates are the first records in Turkey and they were isolated in Ankara and Eskişehir provinces. In the present study, the number of the most isolated binucleat AG I group was 18 and they were obtained in Konya, Ankara, Eskişehir, Kırıkkale and Kayseri. In our country, AG I was isolated before from wheat plant and different hosts (Demirci and Döken, 1995; Demirci, 1998; Eken and Demirci, 2003).

In our study, all binucleat isolates except for AG D were not found to be pathogen. When the studies carried out in the world were examined, no pathogen BN species was found in the wheat except for AG D. Although there are few studies reporting that binucleat *Rhizoctonia* spp. show pathogenicity in some other hosts, these species generally show saprophytic character in the soil. Among them, low-virulent or non-virulent species show hypo-virulent property (Sneh et al., 1996; Tewoldemedhin et al., 2006). These groups which can also be used for the studies of biological control are generally composed of binucleat species (Cardoso and Echandi, 1987 a,b; Roberts and Sivasithamparam 1986; Gutierrez and Torres, 1990; Sneh et al., 1994; Herr, 1995).

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