# Anastomosis Grouping of *Rhizoctonia solani* and Binucleate *Rhizoctonia* spp. Isolated from Pepper in Erzincan, Turkey

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**Abstract**

Tuncer S., Eken C. (2013): **Anastomosis grouping of** *Rhizoctonia solani* **and binucleate** *Rhizoctonia* spp. **isolated from pepper in Erzincan, Turkey**. Plant Protect. Sci., **49**: 127–131.

Ninety eight isolates of *Rhizoctonia* spp. were obtained from roots of pepper (*Capsicum annuum* L.) grown in Erzincan, Turkey during the period 2007–2008. The most prevalent multinucleate anastomosis groups (AG) were AG-4 (85.2%), followed by AG-2 type 1 (7.4%), AG-6 (5.0%), and AG-3 (2.5%). The population of binucleate *Rhizoctonia* spp. comprised AG-A (82.4%), AG-K (11.8%), and AG-G (5.9%). *Rhizoctonia solani* AG-3 and AG-6, as well as binucleate *Rhizoctonia* spp. AG-G and AG-K on pepper (*C. annuum*) were firstly determined in this study. During both *in vitro* and *in vivo* pathogenicity experiments differences in virulence level between *R. solani* and binucleate *Rhizoctonia* spp. isolates were observed. Isolates of *R. solani* AG-2 type 1 and AG-4 were the most virulent, binucleate *Rhizoctonia* spp. isolates of AG-A were less virulent, whereas binucleate *Rhizoctonia* spp. isolates of AG-G and AG-K were non-pathogenic.

**Keywords**: *Thanatephorus cucumeris*; *Capsicum annuum*; hypocotyl rot; root rot; pathogenicity

Pepper (*Capsicum annuum* L.) is one of the most important vegetables in the world, including Turkey. In the 2008 growing season, an annual pepper production was estimated at 1 796 177 t in Turkey (Anonymous 2008).

*Rhizoctonia* comprises both multinucleate and binucleate species which are further divided into anastomosis groups (AGs). Currently, the multinucleate species *R. solani* Kühn (teleomorph: *Thanatephorus cucumeris* (Frank) Donk.) are divided into 14 anastomosis groups: AG-1 to AG-10, AG-BI (Sneh *et al.* 1991), AG-11 (Carling *et al.* 1994), AG-12 (Carling *et al.* 1999), and AG-13 (Carling *et al.* 2002), while binucleate *Rhizoctonia* spp. (teleomorph: *Ceratobasidium* Rogers) isolates are grouped into AG-A to AG-S (Sneh *et al.* 1991; Sharon *et al.* 2008).

*R. solani* is a widespread and ecologically diverse soilborne fungus, causing different types of diseases in many plant species (Ogoshi 1996). *R. solani* causes root rot, stem rot, fruit and seed decay, damping-off, foliar blight, stem canker, and crown rot in various crops (Baker 1970; Anderson 1982; Tu *et al*. 1996).

In pepper, *R. solani* can cause several types of damage, including hypocotyl rot and root rot (Velásquez & Victoriano 2007; Lopez *et al.* 2009). AG 4 is the major AG worldwide, causing root rot in pepper (Bolkan & Ribeiro 1985; Elias-Medina *et al.* 1997; Mikhail *et al.* 2010). Additionally, *R. solani* (AG-1) isolated from pepper has also been reported (Bolkan & Ribeiro 1985). On the other hand, *R. solani* (AG-3) has been reported to be the major causal organism of damping-off in directly seeded pepper (*Capsicum frutescens* L.) fields (Katan & Eshel 1974).

In Turkey, isolates of AG-2 type 1 and AG-4 (Tuncer & Erdiller 1990; Demirci & Döken 1995),

Supported by the Ministry of Food, Agriculture and Livestock, the General Directorate of Agricultural Research and Policies (TAGEM), Project No. TAGEM-BS-07/10-01/02-02, and the Scientific Research Fund of Atatürk University, Grant No. 2007/25.

as well as AG-8 (Tuncer & Erdiller 1990) of *R. solani,* and the binucleate *Rhizoctonia* spp. (AG-A, AG-F) have already been determined on pepper (Demirci & Döken 1995).

This research was initiated to determine the species and anastomosis groups of *Rhizoctonia* spp. present in pepper plants in the Erzincan region, and to assess the virulence characters of these isolates on pepper plants.

## MATERIAL AND METHODS

***Collection, isolation, and identification of*** *Rhizoctonia* spp. Ninety eight isolates of *Rhizoctonia* spp. were collected from 440 infected pepper plants collected during the years 2007 and 2008 from various fields in two districts (Center and Üzümlü) of Erzincan. Isolations were made from discoloured or necrotic lesions on root and hypocotyl tissues. Affected pepper tissues were washed under running tap water, surface disinfected in 0.5% sodium hypochlorite for 1 min, and placed on 1.5% water agar containing 50 mg/l streptomycin sulphate (Demirci & Döken 1993). After 48–72 h incubation at 20–25°C, hyphae from the margin of each developing colony were placed on water agar or potato dextrose agar (PDA). All isolates were maintained on PDA medium at 10°C and transferred from time to time to new medium.

Isolates of *Rhizoctonia* spp. obtained in this manner were identified on the basis of characteristics of their vegetative hyphae (Ogoshi 1975), nuclear condition (Bandoni 1979), requirement for thiamine (Rovira *et al.* 1986), and hyphal anastomosis with known tester isolates of *R. solani* (tester isolates including AG-1, AG-2 type 1, AG-2-2, AG-3, AG-4, AG-5, AG-6, AG-7, AG-8, AG-9, AG-10, AG-11, AG-12, AG-13, and AG-BI were provided by Dr. A. Ogoshi, Hokkaido University, Japan; Dr. D.E. Carling, University of Alaska Fairbanks, USA, Dr. S.M. Neate, CSIRO, Division of Soils, Australia, and Dr. D.A. Carter, University of Sydney, Australia) and binucleate *Rhizoctonia* spp. (tester isolates including AG-A, AG-Ba, AG-Bb, AG-C, AG-D, AG-E, AG-F, AG-G, AG-H, AG-I, AG-K, AG-L, AG-N, AG-O, AG-P, AG-Q, AG-J, AG-R, and AG-S provided by Dr. A. Ogoshi, Hokkaido University, Japan and Dr. M. Mazzola, Tree Fruit Research Laboratory, Wenatchee, USA) by using standardised techniques for anastomosis group determination (Parmeter *et al.* 1969).

***Pathogenicity of*** *Rhizoctonia* spp. ***on pepper***. The pathogenicity was studied using *in vitro* bioassays and *in vivo* experiments. The pathogenicity of 97 isolates representing seven anastomosis groups (Table 1) was determined on pepper (cv. Demre sivrisi) seedlings. One isolate (AG-A) was lost during storage in the laboratory.

For *in vitro* experiments, an agar plate assay was adapted from the method of Muyolo *et al*. (1993). Seeds were surface disinfested in 1.0% NaOCl for 5 min, and air-dried before use. Six seeds of pepper were placed on 10 ml of sterile 1.5% water agar in 10-cm-diameter Petri dishes. The centre of each dish was subsequently inoculated with a 6-mm-diameter mycelial disk from a 2- to 3-day-old cultures of isolates on PDA. Cultures were incubated under continuous darkness for 4 days at 25 ± 1°C, after which they were placed on a laboratory bench under 12 h light and 12 h dark. Disease severity was rated, 10 days after inoculation using a scale of 0–4, where 0 = healthy, no lesions on the hypocotyls; 1 = lesions covering < 25% of the hypocotyls; 2 = lesions covering 25–50% of the hypocotyls; 3 = lesions covering 50–100% of the hypocotyls, and 4 = seedlings were dead.

Thirteen isolates belonging to different AGs (AG-2 type 1: B418, B411; AG-3: B150, B421; AG-4: B422, B338; AG-6: B91, B93; AG-A: B199, B84; AG-G: B360, and AG-K: B82, B84) that showed the most virulence *in vitro* were chosen as inocula for greenhouse experiments. The pathogenicity of these isolates on pepper (cv. Demre sivrisi) was tested in greenhouse conditions at 25 ± 2°C. The pathogenicity tests were performed by modifying the method used by Demirci *et al*. (2002). Pepper seeds were surface disinfected in 1% NaOCl for 5 minutes. The sterilised seeds were air-dried, and washed twice with sterilised distilled water and air-dried. Ten seeds were then planted in each pot containing sterile potting mix (field soil, composted manure, sand at 1:1:1, v/v). Three-week-old seedlings were inoculated by gently removing the soil mixture from one side of the stem, placing a colonised PDA plug (1 cm diameter) in direct contact with the base of the stem and covering the inoculum with the soil mixture. Control seedlings were inoculated in a similar manner by placing a sterile PDA plug on the base of the stem. Four weeks after inoculation, plants were removed from the pot. Soil was removed from the roots by washing with running tap water and the roots and hypocotyls

of each plant were evaluated separately using the same scale as for the *in vitro* bio-assays.

***Statistical analysis***. Pathogenicity tests were carried out in a completely randomized design of four replicates. As all data showed normal distribution, they were directly subjected to analysis of variance (ANOVA) with JMP Software Version 5.0.1. (JMP, Carry, USA). Least Significant Differences (Fisher's protected *LSD*) were calculated following the significant *F* tests.

## RESULTS

### *Rhizoctonia* species and anastomosis groups

During 2007–2008, a total of 98 isolates of *Rhizoctonia* spp. were collected from pepper plants in Erzincan province, 81 were identified as *R. solani* (82.6% of the total) and 17 isolates were binucleate *Rhizoctonia* spp. (17.4%) (Table 1). Isolates of *R. solani* could be placed in four anastomosis groups: AG-2 type 1 (7.4%), AG-3 (2.5%), AG-4 (85.2%), and AG-6 (5.0%), whereas those of binucleate *Rhizoctonia* spp. were found belonging to three anastomosis groups: AG-A (82.4%), AG-G (5.9%), and AG-K (11.8%).

### Pathogenicity of *Rhizoctonia* spp. on pepper

Isolates of *R. solani* and isolates representing different anastomosis groups of binucleate *Rhizoctonia* spp. varied in virulence (Table 2). As a result of *in vitro* pathogenicity tests, it was found that the differences among the virulence of the *Rhizoctonia* spp. isolates were statistically significant. All isolates of *R. solani* AG-2 type 1 were pathogenic to pepper (cv. Demre sivrisi) seedlings. *R. solani* AG-4 isolates were found to be the most virulent, whereas some isolates of AG-4 (*R. solani*) were not pathogenic on the pepper cultivar tested. The AG-6 (B-93) isolate was found to be less virulent, whereas isolates of the other anastomosis groups of *R. solani* (AG-3 and AG-6) and binucleate *Rhizoctonia* spp. (AG-A, AG-G, and AG-K) isolates were not pathogenic on pepper cultivar tested.

Isolates B418, B411 (AG-2 type 1), B150 (AG-3), B422, B338 (AG-4), B91 (AG-6), and B199 (AG-A) were highly virulent under greenhouse conditions (Table 2). Virulence of these isolates was significantly different from that of all other isolates tested.

Isolations of *Rhizoctonia* spp. from discoloured or necrotic lesions on root and hypocotyl tissues were successful in all pathogenicity tests. The resulting cultures were paired with the original cultures and their anastomosis grouping confirmed.

Table 1. Number and proportion of anastomosis groups of *Rhizoctonia* species isolated from pepper in Erzincan, Turkey

| Anastomosis group (AG) | | Number of isolates | Percentage |
|---|---|---|---|
| *Rhizoctonia solani* | AG-2 type 1 | 6 | 7.4 |
| | AG-3 | 2 | 2.5 |
| | AG-4 | 69 | 85.2 |
| | AG-6 | 4 | 5.0 |
| Binucleate *Rhizoctonia* spp. | AG-A | 14 | 82.4 |
| | AG-G | 1 | 5.9 |
| | AG-K | 2 | 11.8 |
| Total | | 98 | |

Table 2. *In vivo* pathogenicity of *Rhizoctonia* species on pepper (cv. Demre sivrisi) seedlings

| Anastomosis group (AG) | | Isolate | Disease index |
|---|---|---|---|
| Rhizoctonia solani | AG-2 type 1 | B418 | 3.17[z] |
| | | B411 | 3.00 |
| | AG-3 | B150 | 2.67 |
| | | B421 | 1.83 |
| | AG-4 | B422 | 2.83 |
| | | B338 | 2.67 |
| | AG-6 | B91 | 2.67 |
| | | B93 | 0.83 |
| Binucleate *Rhizoctonia* spp. | AG-A | B199 | 2.50 |
| | | B84 | 0.00 |
| | AG-G | B360 | 0.33 |
| | AG-K | B82 | 0.50 |
| | | B84 | 0.33 |
| Control plants | | | 0.00 |
| *LSD* | | | 0.74 |

Disease index 0–4; 0 = healthy, no lesions on hypocotyls; 1 = lesions covering < 25% of the hypocotyls; 2 = lesions covering 25–50% of the hypocotyls; 3 = lesions covering 50–100% of the hypocotyls, 4 = seedling is dead; [z]means compared with Fisher's protected Least Significant Difference (*LSD*) ($P = 0.01$); data are averages of 4 replicates

## DISCUSSION

In the present study, 81 isolates of the multinucleate *R. solani* and 17 binucleate *Rhizoctonia* spp. isolates obtained from peppers in Erzincan, Turkey were investigated for anastomosis groupings. The most prevalent multinucleate AG was found AG-4 (85.2%), followed by AG-2-1 (7.4%), AG-6 (5.0%), and AG-3 (2.5%). The most common occurrence of *R. solani* AG-4 isolates on pepper has also been reported from other countries by several workers (Bolkan & Ribeiro 1985; Elias-Medina *et al.* 1997; Mikhail *et al.* 2010). The population of binucleate *Rhizoctonia* spp. in our study comprised AG-A (82.4%), AG-K (11.8%), and AG-G (5.9%). The anastomosis groups of *R. solani* (AG-1, AG-2 type 1, AG-4, AG-8) and of the binucleate *Rhizoctonia* spp. (AG-A and AG-F) have been reported from peppers (Bolkan & Ribeiro 1985; Tuncer & Erdiller 1990; Demirci & Döken 1995). In contrast, *R. solani* (AG-1 and AG-8) were not found among the isolates from peppers in our case. To our best knowledge, this is the first record of AG-3 and AG-6 of *R. solani* and AG-G and AG-K of binucleate *Rhizoctonia* spp. determined from pepper (*C. annuum*).

Results obtained with the *in vitro* bio-assays and with the greenhouse experiments showed a similar tendency. Among the isolates of different *Rhizoctonia* AGs, significant differences in virulence could be observed. The highest disease ratings on pepper seedlings were shown from the isolates of AG-2 type 1 and AG-4 of *R. solani*, whereas the binucleate *Rhizoctonia* spp. isolates of AG-A were less virulent and the isolates of AG-G and AG-K were non-pathogenic. Some AG isolates of binucleate *Rhizoctonia* spp. have been reported as virulent, avirulent or less virulent cultivated plants (Sanders *et al.* 1978; Burpee *et al.* 1980; Hurd & Grisham 1983; Sumner 1985; Eken & Demirci 2003, 2004). In addition, other studies have shown that binucleate *Rhizoctonia* spp. could be effective as a biocontrol agent against diseases caused by *R. solani* or *Pythium* spp. (Cubeta & Echandi 1991; Herr 1995; Villajuan-Abgona *et al.* 1996).

This study revealed the presence of various AGs among the *Rhizoctonia* isolates collected in pepper fields in Erzincan, Turkey. The predominance of AG-4 isolates demonstrated here suggests that breeding pepper for resistance to hypocotyl rot and root rot incited by *R. solani* might be primarily focused on the use of AG-4 population of this pathogen.

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Received for publication October 22, 2012
Accepted after corrections January 19, 2013

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