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## Determination of the Anastomosis Grouping and Virulence of *Rhizoctonia solani* Kühn Isolates Associated with Bean Plants Grown in Samsun/Turkey

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**Abstract:** Anastomosis groupings and virulence of 229 *Rhizoctonia solani* isolates, obtained from bean plants and soils in Samsun province, were determined. About 59 % of the isolates belonged to anastomosis group AG 4, 31 % to AG 2-2 and the remaining 10 % to AG 5. All the isolates selected for the pathogenicity test were found to be virulent at varying degrees to eight plants from different families. Isolate HAF 1-3 belonging to AG 4 were found to be the most virulent isolate. Sugarbeet was the most susceptible plant species while corn and leek seemed to be rather resistant. The virulence of the isolates on different bean cultivars varied. AG 4 and AG 2-2 group isolates caused severe symptoms of root rot on all cultivars. AG 5 isolate was highly to moderately virulent regarding the susceptibility of the bean cultivars. Horoz was found to be the most susceptible bean cultivar.

**Key words:** AG 2-2, AG 4, AG 5, pathogenicity, root-rot

### Introduction

*Rhizoctonia* group fungi are among soil-borne pathogens causing economic yield losses on their host plants. They exist in nature as strains differing from each other in characteristics such as cultural conditions and virulence. Vegetative incompatibility exists among the hyphae of different strains and this group of fungi are considered in 12 anastomosis groups. Morphology and virulence of the isolates in the same anastomosis group may also be different from each other. Some anastomosis groups are widespread all over the world. Distribution of pathogenic groups vary with respect to the distribution of their host plants, while that of saprobic ones vary according to the vegetation and climatic factors (Muyolo *et al.*, 1993 a; Ogoshi, 1996). Although root and hypocotyl rot of bean plants was commonly caused by AG 4, other groups such as AG 1, AG 1-IB, AG 2-1, AG 2-2 and binucleate CAG-5 were also isolated from diseased bean plants (Warren *et al.*, 1972; Galindo *et al.*, 1982; Sumner, 1985; Phillips, 1991; Tu *et al.*, 1996). It was reported that *Rhizoctonia solani* frequently infected plants younger than two weeks, since hypocotyls with completely developed cuticle were resistant against infection, but older plants could also be infected if the cuticle had any kind of injury (Stockwell & Hanchey, 1985). Pathogen delayed emergence, decreased development rate and increased shoot:root ratio on bean plants especially when the temperature was higher than 18° C and soil moisture was low (van Bruggen *et al.*, 1986 a). Disease severity increased with respect to the increase of the inoculum density and reached maximum with the dose of 250-350 sclerotia/kg soil (van Bruggen *et al.*, 1986 b). It was determined that the fungus decreased the population by affecting the plants during the first 30 days after emergence (Sanchez-Anguinano & Cardenas-Alonso, 1988). In addition, susceptibility of bean cultivars and lines against different AG groups were also investigated and resistant varieties and lines were determined (Sumner, 1985; Pastor-Corrales & Abawi, 1986; Guevara-Torres *et al.*, 1986; Vallejos, 1988; Camara *et al.*, 1988, Muyolo *et al.*, 1993 b). Investigations on the isolation of *Rhizoctonia* group fungi causing diseases on various plants and on the determination of the anastomosis groupings were carried out, in Turkey. In a study performed in the middle Anatolian region, isolates belonging to AG 2-1, AG 3, AG 4, AG 5, AG 6 and AG 8 were obtained as a result of isolations from different plants such as barley, wheat, potato, pepper, tomato, chickpea, bean, soybean, tobacco, sugarbeet, alfalfa, carrot and carnation and from soil. It was determined that the isolates obtained from bean plants belonged to AG 5 (Tuncer & Erdiller, 1990). It was reported that most of the *Rhizoctonia solani* isolates obtained from potato plants in Erzurum province

were AG 3, and that AG 2-1, AG 2-2, AG 4 and AG 5 anastomosis groups were also isolated in lower rates (Demirci & Döken, 1993). Multinucleate AG 1, AG 2-1, AG 2-2, AG 3, AG 4, AG 5, AG 9, AG 10 and binucleate AG A, AG E, AG F, AG G, AG I and AG K isolates were obtained from various cultivated plants sampled from different provinces. Among them, AG 4, AG 5, AG A, AG E, AG I and AG K isolates were reported to be obtained from bean hypocotyl tissues as well as other plants (Demirci & Döken, 1995). As a result of a study performed in Samsun province in 1995, it was determined that *Rhizoctonia* group fungi were among the most common pathogens causing root-rot on bean plants and isolation frequency of the fungi were found to be 17.3 % (Hatat & Özkoç, 1997). The aim of the present study was to determine the anastomosis groupings of *Rhizoctonia solani* isolates previously obtained from the roots and rhizosphere soil of bean plants and their virulence on cultivated plants from different families. In addition, *in-vitro* reactions of some bean cultivars, commonly cultivated in the region, against some isolates from different anastomosis groups of *Rhizoctonia solani* were also investigated.

### Materials and Methods

**Source of isolates:** *Rhizoctonia solani* isolates were obtained from the roots and rhizosphere soils of bean plants showing root rot disease symptoms in Samsun province, Turkey, during 1995 vegetation period. Cultures were maintained and stored as test tube slants of PDA at room temperature. In order to determine the nuclear condition of the isolates, they were transferred to PDA and incubated at 25°C in the dark. Developing mycelia of the isolates were stained with safranin O (Bandoni, 1979) and nuclei numbers were observed for at least 15 different cells. Isolates with multinucleate vegetative cells were characterized as *R. solani* and included in this study.

**Anastomosis grouping:** A practical method modified after Bandoni (1979) and Kronland & Stanghellini (1988) was used for the determination of the anastomosis groupings of the isolates. *R. solani* isolates and the representative 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 *R. solani* isolates and the tester isolates were cut from the margin of a growing colony and transferred to water agar plates, being on the opposite sides of the coverslip. After incubation at 25°C in the dark for 24-48 hours, when overlapping mycelia of the two isolates were observed, the coverslip was removed from the plate and placed on the mixture of one drop of Safranin O and one

drop of 3 % KOH on a slide. Stained hyphae were then examined microscopically and anastomosing hyphae were traced back to their source in order to confirm the anastomosis between our isolate and the tester isolate. For the anastomosis testing, all the pairs were examined twice.

**Pathogenicity tests:** In order to determine the virulence of *Rhizoctonia solani* isolates, one or two isolates were randomly selected from each group. The following plants from different families were evaluated as hosts for the selected isolates:

Tomato	<i>Lycopersicon esculentum</i> Mill.	Solanaceae
Bean	<i>Phaseolus vulgaris</i> L.	Leguminosae
Cucumber	<i>Cucumis sativum</i> L.	Cucurbitaceae
Cabbage	<i>Brassica oleracea</i> L.	Cruciferae
Corn	<i>Zea mays</i> L.	Gramineae
Leek	<i>Allium porrum</i> L.	Liliaceae
Sugarbeet	<i>Beta vulgaris</i> L.	Chenopodiaceae
Lettuce	<i>Lactuca sativa</i> L.	Compositae

Bean varieties Öz Ayse-16, Gibbs, Yalova-5, Horoz, Balkiz and Beta Alman Ayse-sirik, commonly cultivated in the region were also used in the pathogenicity test, in order to determine the virulence of *R. solani* isolates on different bean cultivars.

Agar-plate assay was used in the pathogenicity tests (Ichielevich-Auster *et al.*, 1985). Isolates were transferred to YEDA (1% yeast extract - 0.5 % pepton - 0.5 % dextrose - 1.7 % agar) and incubated at 26°C for two days. Agar plugs with mycelia from the growing edge of the cultures were then transferred to 2 % water agar plates and incubated under same conditions for two more days. Seeds of the plants and bean cultivars selected for the pathogenicity test were surface sterilized in 70 % ethyl alcohol for 2 minutes and then in 1 % NaOCl for 10 minutes, rinsed with sterilized distilled water and blotted dry. Five seeds were plated on each petri dish adjacent to the margin of the growing mycelia, under aseptic conditions. After 7-8 days incubation at 26 ° C, roots and hypocotyls of the seedlings were examined and disease severity ratings were made (Ichielevich-Auster *et al.*, 1985) by using 1-5 scale modified after Muyolo *et al.* (1993 a), where 1= healthy seedling, 2= very little superficial lesions on roots and hypocotyls, 3= deep and large lesions on the roots or on the hypocotyl, 4= severe root-rot, lesions surrounding hypocotyl, partially restricted root length, and 5= complete root-rot, collapsed hypocotyl with wilted leaves or dead seedlings. Five replicate plates were used for each plant or cultivar-isolate combination. Disease severity data were subjected to analysis of variance and means were compared by using Fisher's Protected LSD test (P= 0.05).

## Results

**Anastomosis groups of the isolates:** It was determined in the study that 59 % of the 229 *R. solani* isolates obtained from the roots and from the rhizosphere soil of the bean plants grown in Samsun province belonged to AG 4, 31 % to AG 2-2 and 10 % to AG 5.

**Virulence of the isolates:** As a result of the pathogenicity tests, it was found that the differences among the virulence of *R. solani* isolates were statistically significant. Virulence differences existed also among the isolates from the same anastomosis group (Fig. 1). AG 4 isolate Haf 1-3 was found to be the most virulent isolate and AG 2-2 isolate Çaf 2-1 followed this. Disease severity means caused by all isolates were significantly different from that of control plates. This indicated that all isolates caused different levels of disease on the plants. Similarly, susceptibility levels of the plants differed from each other (Fig. 2). When the effects of all isolates were examined together, sugarbeet was found to be the most susceptible plant species, while corn and leek were rather resistant.

*R. solani* isolates caused similar symptoms on the plants used in the pathogenicity test. Lesions as reddish brown superficial lines

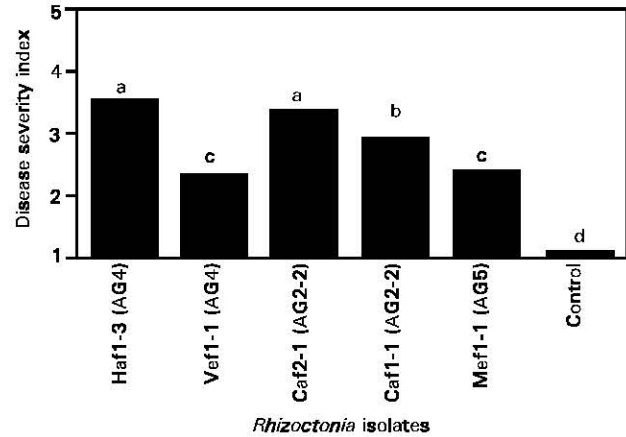


Fig. 1: Virulence of *Rhizoctonia solani* isolates on different plants (Bars followed by the same letter(s) do not significantly different from each other according to Fisher's Protected LSD test, P= 0.05)

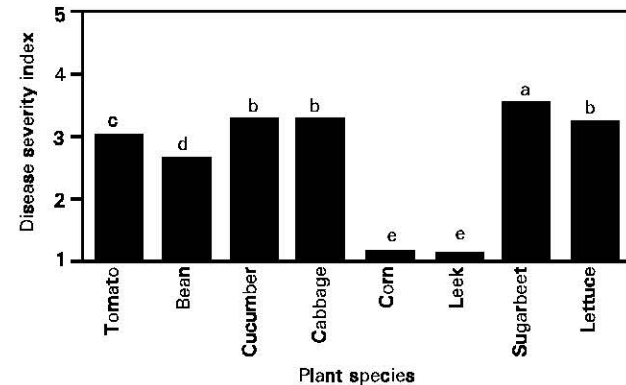


Fig. 2: Disease severity on different plant species infested with *Rhizoctonia solani* isolates (Bars followed by the same letter(s) are not significantly different from each other according to Fisher's Protected LSD test, P= 0.05)

on the roots and hypocotyls of the plants existed at the beginning. These lesions developed causing browning and rotting of the lateral roots and deep necrosis surrounding the main root or the hypocotyl in the susceptible plant-virulent isolate combinations, and finally young plants thoroughly rotted and died.

When the effects of the isolates were examined on each plant separately, it could be seen that the virulence of the isolates changed within plant species (Table 1). AG-4 isolate HAF 1-3, which was the most virulent isolate on tomato plants, was arranged in the same group with isolates causing the highest disease severity on other plants. Although it was also in the same anastomosis group, isolate VEF 1-1 usually caused less severe symptoms on the plants. ÇAF 2-1 and ÇAF 1-1 isolates, in the same anastomosis group (AG 2-2) caused same level of disease on tomato, bean and corn plants, while the virulence of the first isolate was higher on other plants. AG 5 isolate MEF 1-1 usually caused moderate disease symptoms, but its virulence was high on sugarbeet plants while it was found in the same group with control on corn and leek plants. When the results of the pathogenicity test were examined in terms of the susceptibility levels of the plants; the most resistant plants were corn and leek against all isolates tested. Plants were more susceptible against AG 2-2 and AG 4 group isolates.

Table 1: Virulence of *R. solani* isolates obtained from beans grown in Samsun on different plants

Disease severity means*								
<i>R. solani</i> isolates	Tomato	Bean	Cucumber	Cabbage	Corn	Leek	Sugarbeet	Lettuce
HAF 1-3 (AG 4)	4.56 A b**	3.20 A c	4.96 A ab	5.00 A a	1.00 B d	1.24 AB d	4.16 AB b	4.40 A b
VEF 1-1 (AG 4)	3.24 B a	2.32 B b	2.84 C ab	2.84 B ab	1.12 AB c	1.00 B c	2.76 C ab	2.64 C ab
ÇAF 2-1 (AG 2)	2.84 B c	3.52 A b	5.00 A a	4.48 A a	1.20 AB d	1.00 B d	4.76 A a	4.52 A a
ÇAF 1-1 (AG 2)	3.16 B b	3.12 A b	3.64 B ab	3.24 B b	1.36 A c	1.36 A c	3.92 B a	3.52 B ab
MEF 1-1 (AG 5)	3.08 B b	2.16 B c	2.04 D c	2.80 B b	1.08 B d	1.04 B d	4.12 AB a	3.12 BC b
Control	1.00 C b	1.00 C b	1.00 E b	1.00 C b	1.00 B b	1.00 B b	1.56 D a	1.00 D b

\* Disease severity was assigned to each plant on a scale of 1-5, in which 1= healthy, 5= wilted or dead plant

\*\* Means in a column followed by the same capital letter, and means in a row followed by the same lowercase letter are not significantly different from each other according to Fisher's Protected LSD test (P= 0.05)

Table 2: Virulence of *R. solani* isolates obtained from beans in Samsun on different bean cultivars

Disease severity means*						
<i>R. solani</i> isolates	Öz Ayşe-16	Gibsy	Yalova-5	Horoz	Balkiz	Beta Alman Ayşe Sirik
HAF 1-3 AG 4	2.52 B d**	3.84 A bc	3.32 AB cd	5.00 A a	3.72 A bc	4.24 A ab
VEF 1-1 AG 4	2.44 B bc	2.96 BC b	2.88 B bc	4.88 A a	2.60 B bc	2.28 B c
ÇAF 2-1 AG 2	3.52 A bc	3.44 AB bc	3.60 A bc	5.00 A a	3.20 AB c	3.88 A b
ÇAF 1-1 AG 2	3.28 A c	3.08 BC c	3.20 AB c	5.00 A a	3.30 AB c	3.80 A b
MEF 1-1 AG 5	2.44 B d	2.36 CD d	3.60 A c	5.00 A a	3.48 A c	4.28 A b
Control	1.00 C b	2.00 D a	1.00 C b	1.00 B b	1.14 C b	1.18 C b

\* Disease severity was assigned to each plant on a scale of 1-5, in which 1= healthy, 5= wilted or dead plant

\*\* Means in a column followed by the same capital letter, and means in a row followed by the same lowercase letter are not significantly different from each other according to Fisher's Protected LSD test (P= 0.05)

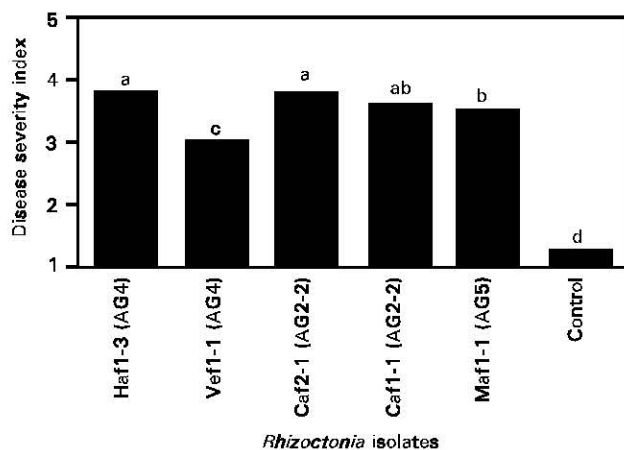


Fig. 3: Virulence of *Rhizoctonia solani* isolates on different bean cultivars (Bars followed by the same letter(s) do not significantly different from each other according to Fisher's Protected LSD test, P= 0.05)

Virulence of the isolates on different bean varieties were also different from each other. As in the plant species, virulence of the isolates belonging to the same anastomosis group were different from each other. However, AG 4 isolate Haf 1-3 and AG 2-2 isolates caused severe disease on bean varieties (Fig. 3, Table 2). According to the susceptibility of the cultivars, AG 5 isolate caused severe symptoms on some of them and less severe symptoms on the others. Susceptibility of the bean cultivars against *R. solani* isolates were also significantly different from each other (Fig. 4). Regarding the effects of all isolates, Horoz found to be the most susceptible cultivar and Öz Ayşe-16 the most resistant one. When the virulence of each isolate was examined separately, it could be seen that highest disease severity means were obtained with Horoz cultivar in all isolates, while other cultivars were severely or moderately affected from the isolates (Table 2).

According to these results, it can be suggested that none of the bean cultivars used in this study were resistant to *R. solani*. But Öz Ayşe-16 which was less affected from the isolates can be recommended for the region.

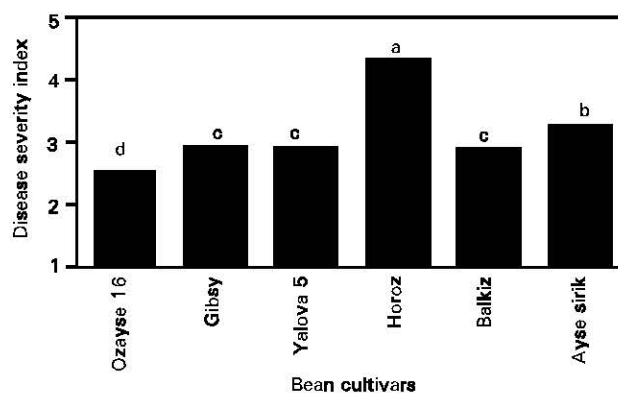


Fig. 4: Disease severity on different bean cultivars infested with *Rhizoctonia solani* isolates (Bars followed by the same letter(s) do not significantly different from each other according to Fisher's Protected LSD test, P= 0.05)

## Discussion

In present study more than half of the 229 *R. solani* isolates obtained from the roots and from the rhizosphere soil of the bean plants grown in Samsun province belonged to AG 4, and the others to AG 2-2 and to AG 5. It was reported in the previous publications that *R. solani* isolates causing disease on bean plants were mostly from AG 4, but isolates from AG 1 and AG 2 were also found on bean plants (Tu *et al.*, 1996). However, in our country isolates from AG 1, AG 4 and AG 5 were obtained from beans (Tuncer & Erdiller, 1990; Demirci & Döken, 1995). This may be because of the ecological differences among the regions.

As a result of the pathogenicity tests, it was found that the differences among virulence of the *R. solani* isolates and the susceptibility levels of the plants were statistically significant. AG 4 isolate Haf 1-3 was the most virulent isolate and AG 2-2 isolate Çaf 2-1 followed this. It was also found in the study that sugarbeet was the most susceptible plant species, while corn and leek were rather resistant. Likely, Phillips (1991) found that AG 4 isolates caused pre-emergence death and hypocotyl lesions on bean and soybean plants while the virulence of all the isolates were

lower on corn plants. However, Sumner & Bell (1982) reported that AG 2 and AG 4 isolates caused root-rot of corn, and that disease symptoms were more severe especially on the irrigated fields of monoculture cultivation or where corn was cultivated in turn with peanut and soybean. It was reported that AG 4 isolates had a wide host range and they could infect many plants such as cotton and onion (Ogoshi, 1996). However, isolates from this group caused very weak symptoms on leek plants belonging to the same family with onions, in the present study. Virulence of AG 5 isolate MEF 1-1 was usually lower, but it caused severe root-rot symptoms on sugarbeet plants. It was reported that AG 5 group isolates were weakly pathogenic or not pathogenic on plants (Ogoshi, 1996). But there are some other reports suggesting that they caused severe root-rot on sugarbeet plants like AG 2 and AG 4 isolates (Herr, 1991; Olaya & Abawi, 1994). This group of isolates were previously isolated from bean plants in our country, but their pathogenicity was not mentioned (Tuncer & Erdiller, 1990; Demirci & Döken, 1995). It was also reported in another study performed in Turkey that, AG 5 group isolates caused reddish-brown hypocotyl lesions on cotton plants (Kural *et al.*, 1994).

In present study the *R. solani* isolates, obtained from beans were found to belong to AG 2-2, AG 4 and AG 5 anastomosis groups that caused varying degrees of root-rot on plants from different families and on different bean varieties. It was suggested by various researchers that the host range and the diseases caused by the isolates from different anastomosis groups were different, and so determination of the anastomosis groupings of the isolates was very important (Parmeter *et al.*, 1969; Kronland & Stanghellini, 1988). In addition, it was reported that the virulence of *R. solani* isolates on different bean cultivars and lines also differed (Sumner, 1985; Pastor-Corrales & Abawi, 1986; Guevara-Torres *et al.*, 1986; Vallejos, 1988; Camara *et al.*, 1988; Muyolo *et al.*, 1993 b).

There is no doubt that this group of fungi can cause economic yield losses on plants grown in Samsun province where vegetable growing is common. For that reason, cultural practices such as the use of resistant cultivars and the improvement of soil conditions will be useful. Cultivation of resistant plants such as corn or of rather resistant cultivars such as Öz Ayse-16 can be recommended for the areas where fungus population is high. It is known that chemical control is not so efficient against soil borne pathogens like *Rhizoctonia* group fungi and that pesticides have negative side effects on the environment and on human health. Therefore, it will be useful to determine biocontrol agents that could be effective against *R. solani* and to evaluate their efficiency by *in vivo* and *in vitro* studies.

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