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Rhizoctonia species, anastomosis groups, and pathogenicity isolated from common bean in Lake Van Basin, Turkiye

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Abstract
Common bean (*Phaseolus vulgaris* L.) is an important vegetable crop grown in Lake Van Basin. Local genotypes are widely grown in the region. *Rhizoctonia* root rot induced by *Rhizoctonia solani* Kühn is an important soilborne plant disease that leads to global economic losses as well as in Turkey. The present study was conducted to determine anastomosis groups and pathogenicity of *Rhizoctonia* spp. obtained from bean plants in Lake Van Basin in 2013 and 2014. A total of 236 *Rhizoctonia* isolates in 5 anastomosis groups were obtained from bean plant roots. It was observed that AG- 4 (112) was the most isolated group in beans, followed by AG- 2 (41), AG- 3 (28), AG- 5 (33), and binucleate AG- K (22) isolates. Pathogenicity test conducted in thirty isolates in 5 anastomosis groups was analyzed for A64 (Bitlis/ Adilcevaz), TR68557 genotypes, and Gina (cv.) under growth chamber conditions. The study findings demonstrated that all tested isolates could infect the bean plant with different degrees of severity; however, the most virulent group was AG- 4. It was determined that the most virulent isolate was Isolate No. 19 in the A64 genotype, Isolate No. 2 in TR68557, and Isolate No. 18 in Gina cv. in *in vivo* tests. The identification and pathogenicity determination of *Rhizoctonia* isolates are the first steps towards an efficient control strategy for bean diseases caused by *Rhizoctonia* species. In order to obtain quality and productive products in the Van Lake Basin, where intensive bean production is carried out, precautions should be taken by considering the damage caused by *Rhizoctonia* spp. on plants.
Keywords: Anastomosis group, Bean, Pathogenicity, *Rhizoctonia* spp.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.), which is an edible leguminous plant, is significant in local and international agriculture and is commonly consumed in Turkey, as well as in the world. Beans are rich in minerals, vitamins, and protein (18- 31.6%) and highly important for human nutrition (Suárez-Martínez et al., 2016; Ntatsi et al., 2018; Ekincialp and Şensoy, 2018; Gavilanes et. al., 2020). China ranks first in world bean production and Turkey ranks fourth with 630.347 tons, which accounts for approximately 2.6% of the world production (Faostat, 2017). The yield and quality of this legume plant, which is significant for the nation's economy, are adversely affected by various biotic (fungal, bacterial, and viral disease agents) and abiotic factors (Costa-Coelho et al., 2014). *Rhizoctonia* spp., which is one of the most important pathogens in cultivated plants, causes substantial losses in bean production (Yıldırım and Erper, 2017). Disease agent causes yield loss of up to 90% in bean (Palacıoglu et al., 2019). *Rhizoctonia* spp. causes diseases such as wilt and root rot, could emerge in the seedling period as well as in the latter stages of growth and causes early infections that result in plant death (Jaiswal et. al., 2014). It was determined that this pathogen caused

damping-off disease in the seedling phase of plants and root, hypocotyl, capsule, grain rot, and web blight in the latter phases of the plant development (Muyolo et al., 1993; Valentín Torres et. al., 2016).

The number of nuclei in *Rhizoctonia* cells helps with their morphological classification. Those, commonly with two nuclei in their hyphae cells, were called binucleate *Rhizoctonia* and those with multinucleate were called *R. solani*. The other polynuclear species *Waitea circinata* is subdivided into two groups named *Rhizoctonia zeae* and *Rhizoctonia oryzae* (Sneh et al., 1991). Multinucleate *Rhizoctonia solani* (MNR) and binucleate *Rhizoctonia* (BNR) were divided into anastomosis groups (AG) based on the anastomosis reactions between the hyphae of isolates (Oladzad et al., 2019; Nandeesha et. al., 2021). While *R. solani* is divided into 14 anastomosis groups (AG- 1, AG- 13, including AG-BI as a subset of AG-2), several anastomosis groups were also divided into groups in themselves (Sharon et al., 2008; Yıldırım and Erper, 2017). *Rhizoctonia* anastomosis groups with binucleate were divided into 18 groups (AG- A, AG-W) (Sharon et al., 2008; Yang et al., 2015; Dong et al., 2017; Marcou et al., 2021).

Studies reported that both *R. solani* and binucleate *Rhizoctonia* AG were isolated from bean plants. Such isolates exhibited different degrees of virulent properties. *R. solani* AG- 4 was found to be the most common anastomosis group in the world that caused root rot in bean plants (Papavizas et al., 1975; Bolkan and Ribeiro, 1985; Karaca et al., 2002; Kılıçoğlu and Özkoç, 2013). Furthermore, other AGs (AG- 1, AG- 1- IB, AG- 2- 1, AG- 2- 2 and AG- 5) were reported to be prevalent in bean plants and to cause damage in several regions (Muyolo et al., 1993; Valentín Torres et. al., 2016). Binucleate *Rhizoctonia,* which were commonly isolated, were reported to be AG- A, AG- F, and AG- K (Bolkan and Ribeiro, 1985; Muyolo et al., 1993; Nerey et al., 2010).

In Turkey, various AGs were determined in studies conducted on *R. solani* and the anastomosis groups (AG) of the binucleate *Rhizoctonia.* In the Central Anatolia Region, *R. solani* AG- 5 was isolated prevalently from bean plants (Tuncer and Erdiller, 1990). It was reported that BN *Rhizoctonia* AG- K was isolated from hypocotyl in Erzurum province and was followed by BNR AG- A, AG- I, AG- E and MNR AG- 4 and AG- 5, respectively (Demirci and Döken, 1995). A study conducted in Samsun province indicated that *R. solani* AG- 4, AG- 5, and AG- 2- 2 were isolated (Karaca et al., 2002) and another study conducted in the same province concluded that *R. solani* AG- 1IB, AG- 4, AG- 5, AG- 6 and binucleate *Rhizoctonia* as AG- A, AG- Bc and AG- K were isolated from the bean plant and the rhizosphere soil (Erper, 2003). In another study 38 Rhizoctonia isolates were obtained from bean pods (AG-1 IB (1 isolate), AG-2-1 (4 isolates), AG-4 (24 isolates), AG-5 (5 isolates), AG-E (2 isolates) and AG-K (2 isolates). When conducting a pathogenicity test on bean leaves, it was found that the AG-1 IB isolate was the most virulent, followed by the AG-4 and AG-5 isolates, respectively (Akarca and Demirci, 2022). The surveys conducted in the bean plantation sites in Van province by Temizel and Ertunç (1992) indicated that *A. alternata, F. solani, M. phaseolina, Drechslera sorokiniana, R. solani, Stemphylium sp.,* and *Botrytis cinerea* were determined as important disease agents encountered.

Bean cultivation is widely practiced within the Van Lake Basin, encompassing both indigenous and commercial varieties. The utilization of local strains holds particular significance for resource preservation efforts, aiming to minimize harm to plant populations. Particularly in areas afflicted by prevalent diseases, the adoption of specific varieties remains limited. Soil-borne diseases pose a significant threat to bean crops, with root and root collar rot emerging as a primary concern. It can be defined as the most common disease in beans. It was determined as the most common problem in the survey areas visited in the Van Lake Basin. In the study of Temizel and Ertunç (1992), it was stated that *R. solani* was among the disease agents encountered as an important problem in bean plants in Van, but anastomosis groups were not mentioned. It can be taken into account that with the knowledge of anastomosis groups of this pathogen, new sustainable strategies can be developed to determine and control the host status.

The present study was intended to isolate *Rhizoctonia* species from common bean plants cultivated in Lake Van Basin, to identify anastomosis groups, and to determine the plant response of local A64 and TR68557 genotypes and commercial Gina (cv.) cultivars against disease agents. Therefore, in this study, *Rhizoctonia* density and anastomosis groups, their distribution and virulence were determined for the first time in beans in Van basin.

MATERIALS AND METHODS

The A64 bean genotype used in the present study was obtained from the Adilcevaz district in Bitlis province which is located within the boundaries of Lake Van Basin. TR68557 bean genotype was obtained from the gene bank of the Aegean Agricultural Research Institute.

Survey areas and sampling

Field surveys were conducted during the bean cultivating season of 2013 and 2014. Districts of Van (Erciş, Gevaş, Edremit) and Bitlis (Ahlat, Adilcevaz, Tatvan), where bean cultivation was carried out intensively in the basin, were selected as the survey areas. Based on the size of the growing area, 10 to 15 plant samples, which showed symptoms of root rot, were retrieved. The samples were collected randomly through diagonal movements between the corners

of the growing area. The plant samples were placed in polyethylene bags, brought to the laboratory in an icebox, and stored at 5 °C in the refrigerator until the isolation procedure.

Isolation of Rhizoctonia isolates

The roots of the plant samples were washed with tap water and were cleared from soil residues. Pieces were cut from the stem and hypocotyls in sizes of 1 to 2 millimeters, both from the tissues with necrosis and the healthy tissues. The cut pieces were kept in 0.5% sodium hypochlorite (NaOCl) solution for 1 minute for surface sterilization. The pieces were rinsed twice in distilled sterile water and then were retrieved to sterile blotting papers. Subsequently, these pieces were placed in Petri dishes containing 1.5% water agar (WA), to which 50 mg/L of streptomycin sulfate was added, to prevent bacterial contamination (Demirci and Döken, 1993). The Petri dishes were incubated at 23- 25 °C for 48 to 72 hours. Hyphae, which had the general characteristics of *Rhizoctonia* genus, were left to Potato Dextrose Agar (PDA) with hyphae tip isolation to obtain pure culture and were incubated in the dark for 3 to 5 days at 25 °C. The isolates, which were obtained as pure cultures, were stored in test tubes containing PDA at 5 °C.

Determination of anastomosis groups of Rhizoctonia isolates

The isolates obtained in the present study were *R. solani* and binuclear *Rhizoctonia* and were identified based on Ogoshi's (1975) method, depending on the growth, morphological characteristics, sclerotia presence in the isolates incubated in PDA in the dark, at 25 °C, for 7 days and the microscopic characteristics of the isolates incubated in SA, in the dark, at 25 °C for 7 days. The *Rhizoctonia* isolates were identified by utilizing standardized techniques for anastomosis group determination, based on the characteristics of their vegetative hyphae (Ogoshi, 1975), requirement for thiamine (Rovira et al., 1986), and hyphal anastomosis with known tester strains of *Rhizoctonia* AGs. Test isolates used to determine anastomosis groups were obtained from the culture collection of Atatürk University and the Mycology Laboratory of the Plant Protection Department in the Faculty of Agriculture at Yüzüncü Yıl University.

The isolates obtained in this study and test isolates were kept in PDA for 7 days at 25 °C and were paired in 1.5% SA. Therefore, mycelium discs, retrieved from the test isolates and the isolates obtained from the plants with a 5 mm diameter sterile cork borer, were correspondingly placed at a distance of 4 cm, were incubated at 25 °C for 48 to 72 hours, and later were examined directly under light microscopy to determine whether there were the conditions of a cell wall and cytoplasmic interconnection between the hyphae in the aligned colonies (Parmeter et al., 1969). If anastomosis was observed between the hyphae of two paired isolates, these isolates were defined as the same AG (Demirci and Döken, 1992).

Pathogenicity test

Two bean genotypes (A64, TR68557) and a commercial Gina cultivar were used in the pathogenicity tests of *Rhizoctonia* isolate retrieved from different regions. Pathogenicity test was designed with 30 *Rhizoctonia* isolates (3 from AG-K, 5 from AG-2, 3 from AG-3, 15 from AG-4, and 4 from AG-5), to determine the virulence of anastomosis groups. Bean seeds were subjected to surface sterilization for 5 minutes in 1% NaOCl and then were dried.

Pathogen isolates were developed on PDA at 25 °C for one week, to prepare the inoculum. Wheat grains used as the inoculum medium were moistened with pure water and boiled for a while, placed in closed Petri dishes, and autoclaved for 2 days in a row for one hour at 121 °C. Mycelial pieces retrieved from the isolates developed in PDA were inoculated with sterile wheat grains and the Petri dishes were incubated in the dark at 25 °C for two weeks.

Sterilized bean seeds were planted in pots with a mixture of peat and perlite with a 2:1 ratio. The pathogenicity study was carried out by placing 5 wheat grains colonized with isolate around the root collar and 5 sterile wheat grains in the control pots during the first true leaf formation period of the seedlings. Three plants were used for each isolate, and the experiment was repeated twice. For control, 10 plants were used in each experiment (Erper et al., 2011). After keeping the plants at 25 ± 2 °C for 16 hours in the light and 8 in the dark for 6 to 8 weeks, the plants were uprooted, their root lengths were measured and disease severity was rated based on a 0- 4 scale adopted from Muyolo et al. (1993). Each strain was isolated from the plant again and was confirmed by test isolates.

Statistical analysis

The data obtained in the study were evaluated by one-way analysis of variance using the SPSS statistical program with a significance level of $p \leq 0.05$. In the analysis of the data, the differences between the statistically significant means were grouped according to the Duncan Multiple Comparison Test.

RESULTS AND DISCUSSION

Species and anastomosis groups of Rhizoctonia isolates

Distribution of *Rhizoctonia* isolates obtained from the diseased plant samples taken from Erciş, Gevaş, Edremit, Tatvan,

Ahlat, and Adilcevaz districts in Lake Van Basin were given in Table 1. Field studies were carried out during September-October of 2013- 2014. A total of 236 *Rhizoctonia* isolates were obtained from bean plants in Lake Van Basin. According to these results, 48 isolates were obtained from the Erciş district in 2013 and 20 isolates were obtained in 2014; 21 isolates were obtained from Gevaş in 2013 and 11 isolates were obtained in 2014; 16 isolates were obtained from Edremit in 2013 and 15 isolates were obtained in 2014; 22 isolates were obtained from Ahlat in 2013 and 17 isolates were obtained in 2014; 32 isolates were obtained from Adilcevaz in 2013; 34 isolates were obtained from Tatvan in 2013 and 2014.

A total of 236 *Rhizoctonia* spp. obtained common beans were paired with test isolates, it was defined that 112 isolates belonged to *R. solani* AG-4, 41 to *R. solani* AG-2, 33 to *R. solani* AG-5, 28 to *R. solani* AG-3, and 22 binucleate *Rhizoctonia* AG-K.

Table 1. Number of isolates of *Rhizoctonia* species and anastomosis groups isolated from common bean plants according to years and locations

Rhizoctonia spp.	AGs	Districts and Sampling Year												Total
		Erciş		Gevaş		Edremit		Ahlat		Adilcevaz		Tatvan		
		2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	
Binucleate *Rhizoctonia*	K	8	2	2	1	-	2	-	-	5	-	2	-	22
R. solani	2	12	5	6	3	2	-	3	1	4	-	3	2	41
R. solani	3	3	-	2	-	2	3	5	4	3	-	4	2	28
R. solani	4	24	10	11	3	5	10	14	8	15	-	5	7	112
R. solani	5	1	3	-	4	7	-	-	4	5	-	3	6	33
Total		48	20	21	11	16	15	22	17	32	-	17	17	
General Total		68		32		31		39		32		34		236

Pathogenicity test

As a result of the surveys carried out in bean cultivation areas within the Lake Van Basin, pathogenicity tests were performed for 30 isolates obtained from the roots of the diseased plants, the plants were uprooted, and root lengths were measured and evaluated according to 0-4 scale.

The results of the pathogenicity tests, performed for 15 isolates that were selected from the 112 obtained AG-4 isolates, were presented in Table 2. It was determined that there was no statistically significant difference between isolates, based on the effect on plant root length ($p \leq 0.05$). The same case applied to genotypes in terms of root length. AG-4 isolates had significant differences from the control group ($p \leq 0.05$). The highest scale was determined for isolates 19 and 18 and the lowest scale was determined for isolate 20 for the A64 genotype. It was determined that isolate 2 was the most virulent among tested isolates for the TR68557 genotype and isolate 18 was more virulent for the Gina cultivar and they were not much affected by the others.

Of 41 AG-2 isolates obtained, pathogenicity tests were performed with randomly selected 5 isolates considering each district, and the results were presented in Table 3. There was no significant difference between isolates and genotypes in terms of root length values (Duncan Multiple Comparison Tests $p \leq 0.05$). However, the difference in the disease severity scale was significant when compared with the control group ($p < 0.05$). In the A64 genotype, isolate 14 had higher scale values, while isolate 9 was more effective for TR68557 9, and isolates 9 and 27 were more effective for Gina. It was also observed that the highly effective isolate for TR68557 and Gina was found to be the least effective isolate for A64.

Pathogenicity tests were conducted on 33 AG-5 isolates, taking into account each district, four isolates were randomly selected for each test and the results were shown in Table 4. There were no significant differences between the isolates in terms of root length ($P \leq 0.05$). Although there were statistically significant differences between the isolates in terms of scale values, the values were close to each other and the control group. Isolate 10 on A64 and TR68557 and isolate 5 on Gina caused the highest scale values.

Table 2. Root length and scale values of AG-4 isolates according to pathogenicity results in genotypes

Isolates AG-4	Genotypes					
	A64		TR68557		Gina	
	Root length (cm)	Scale*	Root length (cm)	Scale	Root length (cm)	Scale
1	27.5_{NS}±2.18	1.55_{ab}±0.96	36.89_{ab}±8.07	0.78_{ab}±0.39	37.11_{NS}±2.22	0.89_{bc}±0.51
2	38.2±9.06	1.83_{ab}±0.76	40.30_{ab}±10.89	2.11_{a}±1.84	27.05±5.20	0.67_{bc}±0.29
3	41.61±15.51	1.28_{a-c}±1.11	35.05_{ab}±0.67	1.89_{a}±0.77	33.67±5.20	1.44_{b}±1.0
4	31.26±7.89	1.22_{a-c}±0.51	35.55_{ab}±4.14	1.11_{ab}±0.69	33.33±7.51	0.78_{bc}±0.69
7	36.70 ±22.60	1.05_{a-c}±0.82	31.11_{b}±7.35	1.55_{a}±1.26	32.69±12.34	1.39_{b}±1.09
8	33.64±4.30	1.66_{ab}±0.58	37.44_{ab}±6.50	0.88_{ab}±0.69	28.89±0.84	1.16_{bc}±0.60
11	35.6 ± 8.41	1.28_{a-c}±0.75	35.27_{ab}±1.46	1.22_{ab}±0.51	30.61±2.34	1.05_{bc}±0.63
12	34.55±6.05	1.11_{a-c}±0.39	39.38_{ab}±5.09	1.16_{ab}±0.44	37.93±7.87	1.61_{ab}±0.79
13	27.97±13.14	2.11_{ab}±0.84	35.15_{ab}±3.03	1.11_{ab}±0.39	35.16±1.92	0.83_{bc}±0.44
18	37.17±8.98	2.33_{a}±0.67	36.55_{ab}±10.22	1.67_{a}±0.58	39.17±1.89	2.67_{a}±0.58
19	33.61±9.75	2.44_{a}±0.96	36.77_{ab}±4.11	1.44_{a}±0.51	52.39±39.58	0.44_{bc}±0.51
20	37.66±3.18	0.77_{bc} ±0.20	32.48_{b}±6.67	0.99_{ab}±0.34	48.61±28.07	0.89_{bc}±0.20
21	28.66±6.51	1.22_{a-c} ±0.84	39.92_{ab}±6.43	1.39_{ab}±0.98	27.66±6.08	0.10_{bc}±0.34
22	33.39±4.58	1.22_{a-c} ±0.70	38.54_{ab}±3.04	1.22_{ab}±0.19	40.33±25.79	1.22_{bc}±1.17
23	40.88±12.32	1.11_{a-c} ±0.84	36.44_{ab}±1.39	1.89_{ab}±0.19	32.78±8.59	0.94_{bc}±0.42
Control	37.89±6.68	0_{c}	46.27_{a} ±10.74	0_{b}	37.67±8.40	0_{c}

*: Plants were evaluated using the 0-4 scale; There were significant differences among the different letter(s) at P ≤ 0.05 level (according to Duncan's multiple comparison test.); NS: Not Significant (P ≤ 0.05)

Table 3. Root length and scale values of AG-2 isolates according to pathogenicity results

Isolates AG-2	Genotype					
	A64		TR68557		Gina	
	Root length (cm)	Scale*	Root length (cm)	scale	Root length (cm)	scale
6	25.94_{NS}±14.64	1.22_{a}±0.19	36.33_{NS}±2.65	1.66_{b}± 0.88	44.22_{NS}±10.19	0.89_{a}±0.54
9	25.75 ±13.07	0.61_{ab} ±0.67	33.83±0.71	3.0_{a} ± 1.0	35.67±10.07	1.28_{a}±0.25
14	28.83±3.76	1.44_{a} ±0.19	33.05±4.99	1.55_{b} ± 0.51	36.22±7.18	0.83_{a}±0.29
16	34.77±6.77	1.22_{a} ±0.63	34.69±2.47	1.72_{b} ± 0.54	29.78±2.55	1.22_{a}±0.38
27	29.54 ±1.06	1.11_{a} ±0.84	37.66±8.20	1.55_{b} ± 0.51	45.55±8.44	1.28_{a}±0.75
Control	37.89 ±6.68	0_{b}	46.27±10.71	0_{c}	37.67±8.40	0_{b}

*: Plants were evaluated using the 0-4 scale; There were significant differences among the different letter(s) at P ≤ 0.05 level (according to Duncan's multiple comparison test.); NS: Not Significant (P ≤ 0.05)

Table 4. Root length and scale values of AG-5 isolates according to pathogenicity results

Isolates AG-5	Genotype					
	A64		TR68557		Gina	
	Root length (cm)	scale *	Root length (cm)	scale	Root length (cm)	scale
5	35.89_{NS}±14.42	0.67_{ab}±0.29	34.22_{ab}±1.07	0.77_{ab}±0.51	42.67_{NS}±13.05	1.67_{a} ±0.76
10	33.82±7.93	0.99_{a}±0.58	35.11_{ab}±1.26	1.11_{a}±0.51	36.22±5.39	0.94_{ab}±0.63
15	32.95±11.86	0.89_{a}±0.51	33.22_{b}±3.34	0.99_{a}±0.34	27.61±3.46	1.33_{a} ±0.58
17	29.67±7.51	0.89_{a}±0.52	37.55_{ab}±8.18	0.77_{ab}±0.77	41.89±17.48	0.66_{ab}±0.88
Control	37.89±6.68	0_{b}	46.27_{a}±10.74	0_{b}	37.67±8.40	0_{b}

*: Plants were evaluated using the 0-4 scale; There were significant differences among the different letter(s) at P ≤ 0.05 level (according to Duncan's multiple comparison test.); NS: Not Significant (P ≤ 0.05)

Table 5 presents the pathogenicity results of the three AG-3 isolates from 28 AG-3 isolates obtained. The comparison of the A64 genotype root isolates with the control group indicated a significant difference (P≤ 0.05). Although there existed statistically significant differences between the isolates and the control group in terms of scale values, there were no significant differences among the isolates (P≤ 0.05). Isolate 24 was the highest-ranking isolate in terms of both root length and the scale. No significant differences were observed between the isolates and the control group in terms of the root length and scale for TR68557 (P≤ 0.05). A similar condition was observed for the root length for Gina and isolate 26 had the highest scale value.

Table 5. Root length and scale values of AG-3 isolates according to pathogenicity results in genotypes

Isolates	Genotype					
	A64		TR68557		Gina	
AG-3	Root length (cm)	scale *	Root length (cm)	scale	Root length (cm)	scale
24	36.55_{a}±4.69	1.28_{a}±0.25	34.94_{NS}±0.26	1.33_{NS}±1.15	33.93_{NS}±0.81	1.28_{a}±0.75
25	25.10_{b}±4.98	1.33_{a}±0.58	32.99±8.08	1.11±0.39	36.39±18.57	0.78_{b} ±0.48
26	31.11_{ab}±0.39	1.11_{a}±0.69	41.22±3.34	0.99±0.58	31.22±4.44	1.77_{a}±0.51
Control	37.89 a ±6.68	0_{b}	46.27±10.74	0	37.67±8.40	0_{b}

*: Plants were evaluated using the 0-4 scale; There were significant differences among the different letter(s) at P ≤ 0.05 level (according to Duncan's multiple comparison test.); NS: Not Significant (P ≤ 0.05)

A total of 22 AG-K isolates were obtained, and three were used for pathogenicity. The examination of the root length and scale values in Table 6 indicated that there were no statistically significant differences between the isolates, however, the comparison of TR68557 and Gina with the control group indicated a significant difference and they were placed in different groups (P≤ 0.05).

Table 6. Root length and scale values of AG-K isolates according to pathogenicity results in genotypes

Isolates	Genotype					
	A64		TR68557		Gina	
AG-K	Root length (cm)	scale *	Root length (cm)	scale	Root length (cm)	scale
28	37.20_{NS}±1.97	0.89_{NS}±0.84	37.72_{NS}±2.94	1.22_{a}±0.19	30.37_{NS}±2.28	1.22_{a}±0.19
29	36.55±6.15	0.89±0.51	34.11±3.47	1.44_{a}±0.51	36.60±10.91	1.22_{a}±0.48
30	31.27±6.34	1.05±0.63	37.11±5.34	1.11_{a}±0.19	31.54±7.73	1.33_{a}±0.67
Control	37.89±6.68	0	46.27±10.74	0_{b}	37.67±8.40	0_{b}

*: Plants were evaluated using the 0-4 scale; There were significant differences among the different letter(s) at P ≤ 0.05 level (according to Duncan's multiple comparison test.); NS: Not Significant (P ≤ 0.05)

The pathogenicity test for detecting virulence more or less indicated the signs of disease in most applications, except for the control plants. Once the all results were examined, it was determined that the group that caused the greatest damage was AG-4. In the majority of the applications with isolates, there were no significant differences in root lengths with the control group.

The mean root lengths and scale values of all isolates from different anastomosis groups are presented in Table 7. Accordingly, it was determined that the root length was statistically significant between groups and genotypes, except for Gina. In A64 and TR68557, the AG-2 isolate decreased the root length at the highest value when compared to the control group. In scale values, AG-4 caused the highest disease severity on the A64 genotype, AG-2 on TR68557. Gina's genotype was affected by all anastomosis groups.

Table 7. Root length and scale results of all isolates of anastomosis groups according to genotypes

Anastomosis groups	Genotype					
	A64		TR68557		Gina	
	Root length (cm)	scale *	Root length (cm)	scale	Root length (cm)	scale
AG-4	34.56_{ab}±9.54	1.48_{a}±0.79	36.37_{b}±5.38	1.29_{b}±0.71	35.83_{NS}±14.39	1.13_{a}±0.76
AG-2	28.97_{b}±8.67	1.12_{ab}±0.56	35.21_{b}±4.39	1.89_{a}±0.84	38.29±9.19	1.09_{a}±0.45
AG-5	33.08_{ab}±9.51	0.86_{b}±0.43	35.03_{b}±4.18	0.91_{b}±0.49	37.09±11.55	1.15_{a}±0.73
AG-3	31.22_{ab}±5.71	1.24_{ab}±0.48	36.39_{b}±5.74	1.14_{b}±0.69	33.85±9.81	1.28_{a}±0.67
AG-K	35.01_{ab}±5.33	0.94_{ab}±0.59	36.31_{b}±3.89	1.26_{b}±0.32	32.84±7.36	1.26_{a}±0.43
Control	37.89_{a}±5.64	0_{b}	46.27_{a}±9.07	0_{b}	37.67±7.10	0_{b}

*: Plants were evaluated using the 0-4 scale; There were significant differences among the different letter(s) at P ≤ 0.05 level (according to Duncan's multiple comparison test.); NS: Not Significant (P ≤ 0.05)

Bean cultivation is extensive in the Lake Van Basin. Both local and commercial varieties are used for cultivation in the region. The use of local varieties is particularly important in terms of conserving resources. Conservation is also possible by minimizing damage to plants. Specifically, the use of varieties is quite limited in areas where diseases are prevalent. Among all diseases, soil-borne diseases in beans are highly significant. It is possible to state that root and root collar rot is the most prevalent disease in bean plants. It was specified as the frequently encountered problem

in the surveyed areas in Lake Van Basin. The general symptoms in plants can be summarized as the slowing down of development, shortening of the upper parts of the plant and root section, and drying in later phases. The main fungus species causing this disease are *Rhizoctonia*. *Rhizoctonia* spp. is a fungal pathogen that is common and polyphasic throughout the world (Naik et al., 2016; Aydın and Ünal, 2021). A total of 236 *Rhizoctonia* isolates were obtained as a result of survey studies conducted in the basin areas. The identification of the species and anastomosis groups of the isolates indicated that the majority of the isolates were *R. solani* AG-4 (112). Several studies reported that the most common anastomosis group in beans was AG-4. This was followed by *R. solani* AG-2 (41), *R. solani* AG-5 (33), *R. solani* AG-3 (28), and Binucleate *Rhizoctonia* AG-K *(22)*, respectively. Other studies also mentioned that *Rhizoctonia* groups were isolated from beans. Tuncer and Erdiller (1990) reported that they isolated *R. solani* AG-5 in Central Anatolia Region, Demirci and Döken (1995) reported the isolation of AG-4, AG-5 and AG-K from the hypocotyl of bean plants, and Eken and Demirci (2004) stated that they isolated AG-2-1, AG-3, AG-4, AG-5 and AG-K from bean cultivation areas in Erzurum. The isolation from the beans in Samsun indicated that AG-4 was the most widespread group and was followed by AG-5 (Erper et al., 2011). Spedaletti et. al. (2016), reported AG 2-2 from bean in Argentina. Mora-Umaña et. al. (2013) detected different anastomosis groups (AG 1-IA, AG 1-IB, AG 1-IC, AG 1-ID, AG 2-2, AG 2- 2IIIB, AG 2-2IV and AG 4) in Costa Rica. For instance, in Japan, eight of the forty-five Rhizoctonia strains that were isolated from thirty different types of crops, including beans, were BN, while 37 were MN (Misawa and Kurose 2019). From the necrotic roots of 425 symptomatic bean plants that were collected from nine provinces in Turkey, 65 isolates of *Rhizoctonia* spp. were identified. *Rhizoctonia solani* was identified in fifty isolates with multinucleate hyphae. These isolates belonged to seven anastomosis groups: AG-1 (one isolate), AG-1-IA (one isolate), AG-2-1 (six isolates), AG-4-HGI (18 isolates), AG-4-HGII (17 isolates), AG-4-HGIII (five isolates), and AG-5 (one isolate). In contrast, fifteen isolates, AG-F (7) and AG-K (8), were *Rhizoctonia* sp. with binucleate (BN). In pathogenicity tests, three of these anastomosis groups (AG-1, AG-1-IA, and AG-2-1) were found to be non-pathogenic, whereas the remaining groups caused disease severities ranging from 71 to 100% on bean plants (Canpolat et al., 2023). Salman and Boyraz (2023), collected *Rhizoctonia* spp. isolates from plant samples in Konya, Turkey. Ten *R. solani* isolates obtained from beans. Nine of the bean isolates were determined as multinucleic. One isolate from the bean was determined as binucleic. Accordingly, the anastomosis groups of Fa 3.2 (97%), Fa 2.2 (89%) and Fa 1 (86%) in beans were characterized as AG 4HGI.

The anastomosis groups of the isolates identified in the present study are consistent with similar studies. Temizel and Ertunç (1992) indicated that *R. solani* is one of the important disease factors encountered in bean plants in Van however anastomosis groups were not specified. Therefore, the present study determined the *Rhizoctonia* concentration and anastomosis groups and their virulence for the first time in bean plants cultivated in the Lake Van Basin.

As a result of the surveys carried out in bean cultivation areas within the Lake Van Basin, pathogenicity tests were performed for 30 isolates obtained from the roots of the diseased plants. Total of 15 from 112 AG-4 isolates, 5 of 41 AG-2 isolates, 4 of 33 AG-5 isolates, 3 of 28 AG-3 isolates, and 3 of 22 AG-K isolates were used for pathogenicity testing. In selecting the isolates to be used in pathogenicity testing, the distribution of each group in districts was taken into consideration. The results indicated no statistically significant difference between the isolates and genotypes in terms of root lengths; however, there existed decreased root lengths in plants with the disease. It was also determined that the scale values of the isolates were statistically significantly different ($p \leq 0.05$). An overall wilt, growth deficiency, yellowing, and drying in the upper parts of the plants were observed in the plants affected by the disease. The uprooted plants indicated symptoms such as shortness of the roots, black roots, and darkened color of the root collar. The pathogenicity test for detecting virulence more or less indicated the signs of disease in most applications, except for the control plants. The examination of the average of the results indicated that the group causing the greatest damage to plants was AG-4. Correspondingly, Eken and Demirci (2004) stated that the most virulent AG group in beans were the isolates that belonged to AG-5 and AG-4 groups. In Samsun, AG-4 was determined as the most virulent isolate in pathogenicity tests (Erper et al., 2011). In another study, AG-2, AG-4 and AG-5 isolated from faba beans had high virulence, while AG-K was found to be weak (Genç Kesimci et al., 2022). In addition, Omar et al. (2021), obtained from the bean cultivation areas were *Fusarium* (62.5%) followed by *Rhizoctonia* (27.5%) and *R. solani* AG-4 were ranging from 26.7%–50% in the pathogenicity test. It was stated that *R. solani* AG-4 was the most common group that caused bean root rot in the world (Papavizas et al., 1975; Bolkan and Ribeiro, 1985; Karaca et al., 2002; Matloob and Juber, 2013).

The examination of the average root lengths and scale values of all isolates belonging to anastomosis groups, of which the pathogenicity tests were performed, indicated that the root lengths were statistically significant between groups and genotypes except Gina. Scale values suggested that the A64 genotype was mostly affected by AG-4, TR68557 genotype was mostly affected by AG-2, and Gina was affected by all isolates. Based on these outcomes, it was possible to suggest that Gina cultivar is sensitive to *Rhizoctonia* spp. Cankara (2019) study was to determine the reactions of some bean varieties commonly grown in Turkey to *R. solani*. There were significant differences between the pathogen isolates in terms of theirs symptoms and virulence. The reaction of bean varieties against the disease

varied significantly depending on the pathogen isolate. In another study examining the reactions of some bean cultivars against *R. solani* AG-4, 13 cultivars were evaluated as moderately resistant and 17 as susceptible (Palacıoğlu et al., 2019). In a study in which anastomosis groups of *R. solani* isolates obtained from different provinces of Turkey were identified according to sequence analysis of the rDNA-ITS region, it was determined that the most common group was AG-4. In the pathogenicity tests performed with different bean varieties, it was determined that there were significant differences between the variety reactions depending on the pathogen isolates. When all the results were analyzed, no cultivar showed a resistant reaction against all isolates (Palacıoğlu et al., 2024).

CONCLUSION

The study aimed to isolate *Rhizoctonia* fungi from bean plants in the Lake Van Basin, identify anastomosis groups (AGs), and assess their virulence through pathogenicity tests. Five AGs were isolated, with AG-4 being the most virulent and AG-5 the weakest, indicating severe disease factors in the basin. Due to its soil origin, *Rhizoctonia* disease in beans is difficult to control, with cultural methods showing limited effectiveness (Conner et al., 2014; Gossen et al., 2016). Therefore, integrated management strategies, including antagonistic microorganisms or chemical control, are necessary. However, chemical control poses environmental and health risks and can lead to resistance in microorganisms. Breeding studies are a crucial aspect of disease management in Turkey, but they are influenced by the presence of various *Rhizoctonia* AGs on beans. Effective resistance breeding and rotation strategies depend on identifying *Rhizoctonia* AGs and subgroups, as their host suitability varies. Screening AGs among isolates and selecting resistant cultivars are essential for managing *Rhizoctonia* disease in common bean production, emphasizing the importance of understanding *Rhizoctonia* diversity and pathogenicity.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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