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The Role of Arbuscular Mycorrhizal Fungi (AMF) in the Control of *Rhizoctonia* Root Rot in Local Bean Genotypes of the Van Lake Basin

Emre DEMİRER DURAK [1], Aytekin EKİNCİALP[2], Hasret GÜNEŞ[3] ✍, Çeknas ERDİNÇ [4]
[1] Van Yuzuncu Yil University, Faculty of Agriculture, Department of Plant Protection, Van, Turkiye, [2] Baskale Vocational School, University of Van Yuzuncu Yil, Van, Turkiye, [3] Department of Plant Protection, Faculty of Agriculture, Adıyaman University, Turkiye, Department of Agricultural Biotechnology, Faculty of Agriculture, Van Yuzuncu Yil University, Turkiye
[1] https://orcid.org/0000-0001-5757-6332, [2]https://orcid.org/0000-0003-1500-3215, [3]https://orcid.org/0000-0003-3155-2695
[4]https://orcid.org/0000-0003-1208-032X
✉: hasretgunes@adiyaman.edu.tr

ABSTRACT
Beans (*Phaseolus vulgaris* L.), one of the main vegetables common in the Van Lake Basin, are frequently grown in the region with local genotypes. *Rhizoctonia solani* root rot, an important soil-borne disease that negatively affects beans, especially in this region, causes economic losses in our country and worldwide. Therefore, a viable and eco-friendly alternative to chemical control in the treatment of such significant soil-borne plant diseases is the application of Arbuscular Mycorrhizal Fungi (AMF). This study aimed to investigate the effects of commercial AMF (ERS) and *Funneliformis mosseae* (Fm) against *R.solani* (Rs) root rot disease in V29 and TR 50763 (T71) bean genotypes obtained from Van-Gevaş. For this purpose, some plant growth parameters, total phenol, total antioxidant capacity, root colonization, and disease severity parameters were investigated. Fm and ERS significantly increased plant morphological parameters and reduced disease severity in both bean genotypes (V29 and T71) despite the *R.solani* inoculated pathogen. Furthermore, Fm was shown to be the most effective in plant growth among AMF treatments. ERS was the most effective treatment in total phenol concentration, antioxidant activity, and AMF root colonization. In general, it was observed that genotype T71 had a different effect on plant growth parameters and total antioxidant activity than V29. The study's results show that AMF, a sustainable agricultural technique, enhanced plant growth and reduced the damage caused by the important *Rhizoctonia* root rot disease in beans, regardless of genotype diversity. This study can be positively evaluated in the context of investigating sustainable agricultural measures against this disease in the field.

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Van Gölü Havzası Yerel Fasulye Genotiplerinde *Rhizoctonia* Kök Çürüklüğünün Kontrolünde Arbusküler Mikorizal Fungus (AMF) un Rolü

ÖZET
Van Gölü Havzası'nda yaygın olan ana sebzelerden biri olan fasulye (*Phaseolus vulgaris* L.), bölgede yerel genotiplerle sıklıkla yetiştirilmektedir. Özellikle bu bölgede fasulyeyi olumsuz etkileyen toprak kökenli önemli bir hastalık olan *Rhizoctonia solani* kök çürüklüğü, ülkemizde ve dünya genelinde ekonomik kayıplara neden olmaktadır. Bu nedenle, bu tür önemli toprak kaynaklı bitki hastalıklarının tedavisinde kimyasal kontrole uygun ve çevre dostu bir alternatif, Arbusküler Mikorizal Fungusların (AMF) uygulanmasıdır. Bu çalışmada, Van-Gevaş'tan temin edilen V29 ve TR 50763 (T71) fasulye genotiplerinde *R.solani* (Rs) kök çürüklüğü hastalığına karşı ticari AMF (ERS) ve *Funneliformis mosseae* (Fm)'nin etkilerinin araştırılması amaçlanmıştır. Bu amaçla, bazı bitki büyüme parametreleri, toplam fenol, toplam antioksidan kapasite, kök kolonizasyonu ve hastalık şiddeti parametreleri araştırılmıştır. Fm ve ERS, *R.solani* ile inokule patojene rağmen her iki fasulye genotipinde (V29 ve T71) bitki morfolojik parametrelerini önemli ölçüde artırmış ve hastalık şiddetini azaltmıştır.

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Ayrıca, Fm'nin AMF uygulamaları arasında bitki büyümesinde en etkili olduğu gösterilmiştir. ERS, toplam fenol konsantrasyonu, antioksidan aktivite ve AMF kök kolonizasyonunda en etkili uygulama olmuştur. Genel olarak, T71 genotipinin bitki büyüme parametreleri ve toplam antioksidan aktivitesi üzerinde V29'dan farklı bir etkiye sahip olduğu gözlemlenmiştir. Çalışmanın bulguları, sürdürülebilir bir tarım tekniği olan AMF'nin bitki büyümesini artırdığını ve genotip çeşitliliğinden bağımsız olarak fasulyede önemli *Rhizoctonia* kök çürüklüğü hastalığının neden olduğu zararı azalttığını göstermektedir. Bu çalışma, sahada bu hastalığa karşı sürdürülebilir tarımsal önlemlerin araştırılması bağlamında olumlu olarak değerlendirilebilir.

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INTRODUCTION

Bean (*Phaseolus vulgaris* L.) is a leguminous plant widely consumed in Turkey and worldwide. It plays an important role in both local and international agriculture. Beans are essential for human nutrition as they are rich in protein, minerals, and vitamins (Ekincialp & Şensoy, 2018; Gavilanes et al., 2020; Durak et al., 2024). Turkey ranks fourth with 519.713 tons of beans produced worldwide, or about 2.2% of the total. China is the world leader in bean production (Food and Agriculture Organization of the United Nations, 2022). Many biotic (fungal and bacterial, etc.) and abiotic variables negatively affect the productivity and quality of this significant legume crop for the national economy (Costa-Coelho et al., 2014).

Common bean production, which is significant worldwide, is susceptible to various diseases at different stages of the plant life cycle (Meziadi et al., 2016; Martins et al., 2018). With a wide range of hosts, *Rhizoctonia solani* Kühn is a soil-borne plant pathogen that causes the serious disease known as root rot in common bean production areas worldwide. Due to its facultative parasitic capacity, it can survive as a saprotroph in soil (Otten et al., 2001; Zhao et al., 2005). Symptoms of *R. solani* include plant wilt and necrotic lesions on leaves (Ghini & Zaroni, 2001). This disease agent can reduce bean production by up to 90% (Palacıoglu et al., 2019). They can also cause early infections that kill plants and occur during the seedling stage or later in growth. This pathogen has been found to cause root and grain rot and net blight in the final stages of plant development (Valentín Torres et al., 2016).

Bean cultivation in the Van Lake Basin is a common practice involving both local and commercial species. The use of native species is particularly important for resource conservation initiatives that seek to reduce damage to plant populations. Adoption of some varieties is still limited, especially in areas affected by widespread diseases (Durak et al., 2024).

In recent years, many microbial biocontrol agents have been shown to reduce soil-borne diseases (Soylu et al., 2005; Sharifi & Ryu, 2017; Soylu et al., 2020; Soylu et al., 2021). Particularly *R. solani* and *Fusarium* spp., the Arbuscular Mycorrhizal Fungi (AMF) have a significant potential for suppressing soil-borne fungi (Akköprü & Demir, 2005; Sohrabi et al., 2015; Güneş et al., 2025). The AMF species *Rhizophagus intraradices*, in addition to improving growth and yield indices, substantially reduces the adverse effects of *R. solani* when colonizing bean roots (Abdel-Fattah et al., 2011; Nasir Hussein et al., 2018). Many studies have examined the basis of these interactions, including the induction of Mycorrhizal Induced Resistance (MIR), which is characterized by the induction of systemic resistance through the stimulation of defense molecules such as morphological changes in host root tissues, infection sites, and competition for nutrients (Dalpé, 2005; Saldajeno et al., 2008). It was also emphasized that when applied in communities with the additional beneficial effect of each AMF species, the observed good yields could be a result of positive interrelationships between AMF, increasing plant diversity, and production (Singh, 2015; Eke et al., 2016). A substantial body of research, conducted with a variety of plant species, has demonstrated that different genotypes may exhibit disparate responses to mycorrhizal growth (Sensoy et al., 2007; Erdinc et al., 2017; Berger &Gutjahr, 2021; Felföldi et al., 2022).

Considering the above-mentioned statements, finding a suitable combination of AMF can be beneficial in applying this technology to increase crop yields. The present study aimed to evaluate the effects of commercial AMF and *F. mosseae* on plant growth parameters in two different bean genotypes of the pathogen *R. solani*. Furthermore, to analyze the effects of AMF on both pathogen severity and root rot and plant wilt disease. The hypothesis that

different AMF inoculations increase the severity of *R. solani* disease and positively influence colonization and the effect between genotypes was tested.

MATERIAL and METHOD

Materials

The study employed two distinct genotypes of green beans (The reason for using these genotypes in the study should be stated.). TR 50763 (T71) is one of the collections of the Aegean Agricultural Research Institute genebank, and genotype V29 was obtained from the Van-Gevaş region. Two AMF inoculums suitable for plant growth and resistance were used in this study (Gunes et al., 2023). The first was Endo Roots Soluble (ERS)-a Bioglobal company commercial AMF inoculum containing 25 spores per gram. The second was *F. mosseae* (Fm) (T.H. Nicolson and Gerd.) Schüßler & Walker, 2010). *F. mosseae* inoculum was obtained from the stocks of the Mycology Laboratory of the Department of Plant Protection, Faculty of Agriculture, Van Yuzuncu Yil University. *R. solani* AG-4 (Rs, code 18) pathogen (70% virulent), which was isolated from beans in previous studies (Durak et al., 2024), was obtained from the culture stocks of Van Yuzuncu Yil University, Faculty of Agriculture, Mycology Laboratory.

Sampling Method

Design of experiment

Bean seedlings were grown in a fluorescently illuminated growth chamber with a temperature of 23±2 °C, 60-70% relative humidity, and 16:8 light: dark period conditions according to a randomized plot experimental design with four replicates. Treatments are controlled, V29 genotype only, T71 genotype only, ERS only, Fm only, Rs only, V29 + ERS, V29 + Fm, V29 + Rs, T71 + ERS, T71 + Fm, T71 + Rs. Twelve different treatments, four repetitions of each treatment, and one plant in each repetition were used in a randomized experimental design.

The containers were sterilized by soaking them in a 5% sodium hypochlorite (NaClO) solution for 2 minutes and then washing them in tap water. Sterile 3 kg pots filled with sterile peat-perlite (2:1 ratio) were used to grow the bean seeds. Before planting, was infected with 10 g of *F. mosseae* and ERS AMF isolates (150 spores per g soil). Sterile sand was used instead of AMF isolates in the seedling beds of control treatments. *R. solani* was applied to bean seedlings three weeks after (when the first true leaves appeared) sowing. Therefore, wheat grains were used as an inoculum medium for *Rs* isolates. Using the wheat wrapping method, grains soaked in distilled water were placed in petri dishes (9 cm) and autoclaved at 121°C for one hour for two days. Then, sterile wheat grains were infected with three mycelial fragments obtained from a one-week-old immature *Rs* isolate. Petri dishes were then incubated at 25°C for four weeks. Fifteen wheat grains infected with mycelia were added to each pot (Ichielevich-Auster et al., 1985; Botha et al., 2003; Sharon et al., 2007). The pots of the control group contained 5 sterile wheat grains (Erper et al., 2011). The experiment was terminated after eight weeks when the plants showed symptoms of *R. solani*.

Laboratory Analysis

Evaluation of the characteristics related to plant growth

Assessment of the plant growth parameters after harvest, various morphological and biochemical parameters of the plant were measured, including shoot length (SL-cm), root length (RL-cm), stem diameter (SD-mm), TP (total phenol), TAC (total antioxidant capacity), shoot fresh weight (SFW-g), root fresh weight (RFW-g), shoot dry weight (SDW-g), root dry weight (RDW-g), AMF root colonization (%), relative mycorrhizal dependency (RMD) and *Rhizoctonia* disease 0-4 scale. The lengths of plants were determined with a ruler; total fresh weights were determined by weighing; total dry weights were measured by weighing dried at 70 °C for 48 h (Kacar, 1984). The shoot diameter was measured with a caliper (digital caliper, Insize, Chinese).

Total antioxidant capacity (TAC) and total phenol (TP)

The total phenol content was determined by the methodology established by Swain & Hillis (1959), while the total antioxidant content was quantified through the application of the ferric reducing antioxidant power (FRAP) method, as originally proposed by Benzie & Strain (1996).

A total of 150 µl of the extract was taken, 2400 µl of distilled water was added, and the solution was vortexed for 3-4 seconds. Subsequently, 300 µl of 20% sodium carbonate was added, and the mixture was kept at room temperature in the dark for 60 minutes. Finally, the solution was read at a wavelength of 760 nm in a spectrophotometer (Thermo Scientific Genesys 10S Model UV-VIS spectrophotometer Waltham, MA, USA.). In the calculation, the equivalent of gallic acid was employed, and the results were expressed as GA 100 mg FW^{-1}.

To ascertain the antioxidant activity of the extract, 150 µl of the extract was combined with 2850 µl of FRAP solution, comprising acetate buffer, TPTZ (TPTZ should be written clearly), and ferric chloride. The solution was transferred to a 10 ml glass tube and incubated in the dark for 30 minutes at room temperature. The absorbance was then measured at a wavelength of 593 nm using a spectrophotometer. The results were calculated according to the Trolox equivalent and are expressed as µmol TE (TE should be written clearly) 100 mg FW^{-1}.

Evaluation of the severity of the disease

R. solani infected plants were evaluated on a 0-4 scale (0: healthy seedling, 1: very small brown superficial lesions on roots or stem, 2: deep and extensive lesions on roots or stem, retarded root growth, 3: severe root rot, deep lesions surrounding the main root or stem, significantly reduced root length, 4: dead plant) (Muyolo et al., 1993). Following the scale-based assessment, the Townsend & Heuberger (1943) formula (Equation 1) was used to determine the severity of disease (DS).

$$DS\ (\%) = [\sum(S \times L) / (M \times Smax)] \times 100 \quad (1)$$

where M is the total number of plants (plant leaves), L is the number of plants (plant leaves) evaluated on the scale, Smax is the highest scale value, and S is the scale value.

AMF colonization rate (% AC) in roots was determined according to Equation 2 (Giovannetti & Mosse, 1980).

$$AC\ \% = ACR/R \times 100 \quad (2)$$

where R is the total number of roots and ACR is the number of roots colonized by AMF.

$$RMD\ (\%) = \left[\frac{A-B}{A}\right] x100 \quad (3)$$

where A: Dry weight of the mycorrhizal plant, B: Dry weight of the nonmycorrhizal plant.

Statistical Analysis

The data obtained in the study were subjected to variance analysis using the SPSS statistical program, in accordance with the randomized experimental design. In the analysis of the data, statistically significant averages were grouped according to the Duncan Multiple Comparison Test. A principal component analysis (PCA) was performed using the XLSTAT statistical program to define the dependent variable parameters corresponding to the independent variables (genotype and AMF) under *Rhizoctonia* and non-*Rhizoctonia* circumstances (Vidal et al., 2020). Additionally, a heat map cluster (ClustVis) and correlation analysis were accomplished through the utilization of the PAST 4 package program (Metsalu & Vilo, 2015).

RESULTS

Table 1 describes the effect of two different AMF treatments on SL, RL, SD, TP, and TAC in two different bean genotypes treated with *R. solani*. In general, the difference between the two bean genotypes and AMF species was statistically significant ($p \leq 0.05$). Especially in SL, the V29 control group was higher than T71 in both *Rhizoctonia* (+) and *Rhizoctonia* (-), and the difference between them was statistically significant ($p \leq 0.05$). In addition, AMF treatments increased SL despite the pathogen, especially in V29, where the Fm+Rs value increased by about 110% compared to the T71 Fm+Rs treatment (Table 1). At the same time, when the difference in shoot length between AMF types was statistically analyzed ($p \leq 0.05$), it was determined that Fm had a greater effect on the increase than ERS.

In root length, ERS in V29 was higher than in the control group despite the Rs, but about 58% lower than in Fm (Table 1). In the genotype T71, root length was higher in the control group than in the no-treatment group despite Rs. However, regarding AMF, the effect of ERS on root length in this genotype under Rs conditions was 40% higher than Fm (Table 1). It was also determined that there was no statistically significant difference between Rs (-) and Rs (+) ($p \leq 0.05$).

In shoot diameter, one of the plant growth parameters, the difference between the genotypes, AMF treatments, and pathogens was found to be statistically insignificant ($p \leq 0.05$) (Table 1). However, the difference between the AMF x genotype and AMF x Rs was found statistically significant ($p \leq 0.05$) (Table 1).

In total phenolic concentration, AMF treatments were higher than the control groups according to both genotypes (V29 and T71), and the total phenolic value of ERS was higher (42%) among AMF treatments than Fm. The difference between them was statistically significant ($p \leq 0.05$). In terms of the pathogen, the highest value was observed in the ERS+Rs (171.57 mg GAE 100 g dry weight) treatment in the T71 genotype, while the lowest total phenolic value was observed in the control + Rs (89.73 mg GAE 100 g dry weight) treatment in the V29 genotype and the difference between them was 91%. The difference between AMF treatments was statistically significant ($p \leq 0.05$), but there was no statistically significant difference between genotypes in this parameter ($p \leq 0.05$). At the same time, there was a statistically significant ($p \leq 0.05$) difference between treatments with and without Rs (Table 1).

In total antioxidant capacity, there was no statistical difference between genotypes ($p > 0.05$), while the difference between AMF treatments was statistically significant ($p < 0.05$). The difference between Rs treatments was also significant ($p \leq 0.05$). The highest total antioxidant capacity value was in V29 genotype ERS + Rhz (+) (77.60 Trolox µmol TE 100 g dry weight) combination, while the lowest value was in V29 Fm + Rs (-) (26.49 Trolox µmol TE 100 g dry weight) treatment (Table 1). The highest total antioxidant capacity value among AMF treatments was higher in ERS than Fm in both genotypes (V29 and T71). Similarly, the total antioxidant capacity of the pathogen-treated groups in both genotypes in Rs was higher than that of the untreated groups (Table 1).

Table 1. Effects of two different AMF treatments on shoot length (SL), root length (RL), stem diameter (SD), total phenolic concentration (TP), and total antioxidant activity (TAC) in two different bean genotypes treated with *Rhizoctonia solani* (mean ± standard deviation)

Çizelge 1. *Rhizoctonia solani ile muamele edilen iki farklı fasulye genotipinde iki farklı AMF uygulamasının sürgün uzunluğu (SL), kök uzunluğu (RL), gövde çapı (SD), toplam fenolik konsantrasyon (TP) ve toplam antioksidan aktivite (TAC) üzerine etkileri (ortalama ± standart sapma)*

Genotype	AMF	*Rs*	SL (cm)	RL (cm)	SD (mm)	TP (mg GAE / 100 g FW)	TAC (Trolox µmol TE 100 g FW)
V29	Control	(-)	52.28±6.94	27.13±3.23	3.80±0.33	100.29±7.70	31.20±3.20
		(+)	55.45±13.99	24.15±3.89	4.28±0.15	89.73±4.66	32.04±7.98
	ERS	(-)	32.70±2.00	46.50±2.00	5.60±1.00	180.07±2.00	39.55±2.00
		(+)	57.40±7.10	30.75±7.75	4.20±0.20	151.73±23.33	77.60±10.25
	Fm	(-)	57.17±16.24	27.13±5.16	3.90±0.49	94.07±14.10	26.49±9.63
		(+)	88.37±5.75	48.43±11.11	4.30±0.39	130.73±0.66	34.39±9.47
T71	Control	(-)	42.38±7.02	24.57±2.90	4.53±0.69	94.29±23.51	31.76±11.79
		(+)	43.88±5.16	27.90±6.05	4.50±0.46	99.51±8.05	34.79±4.30
	ERS	(-)	53.30±9.90	35.10±2.10	4.45±0.35	123.32±11.75	37.58±3.80
		(+)	47.30±12.21	45.80±13.50	4.07±0.20	171.57±12.83	58.55±11.81
	Fm	(-)	51.53±5.38	29.57±7.78	4.40±0.45	97.40±0.67	47.96±6.44
		(+)	42.00±7.17	32.63±5.48	4.68±0.45	120.07±24.00	47.05±7.40
Genotype (G)							
V29			56.89±18.14 A	33.50±11.27	4.29±0.70	124.44±35.50	38.74±18.02
T71			46.40±8.11 B	32.59±9.31	4.45±0.46	117.69±30.37	42.03±10.99
AMF (A)							
Control			48.49±9.81 B	25.80±3.98 B	4.27±0.50	95.96±12.12 C	32.33±6.57 B
ERS			47.68±12.26 B	39.54±9.79 A	4.59±0.81	156.67±25.97 A	52.84±18.76 A
Fm			57.91±19.20 A	34.44±10.91 A	4.31±0.49	110.57±19.95 B	38.01±12.11 B
***Rhizoctonia* (Rs)**							
(-)			48.29±10.94 B	31.67±8.44	4.39±0.77	114.91±33.42 B	35.06±9.37 B
(+)			54.50±17.30 A	34.38±11.75	4.35±0.36	127.22±31.76 A	45.93±18.38 A
p values (≤0.05)							
Genotype			0.001	0.529	0.554	0.159	0.296
AMF			0.002	<0.001	0.263	<0.001	<0.001
Rs			0.011	0.154	0.472	0.014	<0.001
GxA			0.001	0.265	0.012	0.202	0.001
GxRs			<0.001	0.287	0.668	0.010	0.136
AxRs			0.394	0.030	0.007	0.029	<0.001
GxAxRs			0.015	0.002	0.147	0.002	0.323

There is no statistically significant difference between the same letters ($p \leq 0.05$) according to Duncan's test findings.
Mean (M): Mean is the sum of all data points in our dataset divided by the total number of data points, Standard deviation (SD): It is a method that measures closeness and agreement of observations in a dataset. The p-value is a function used to measure how extreme the observed sample results are, conditional on a statistical model.
ERS: Commercial AMF, Fm: *F. mosseae,* Rs: *Rhizoctonia solani, TP: Total phenolic concentration, TAC:* Total Antioxidant Capacity

The percentage distribution of AMF treatments and *Rs* combination treatments in V29 and T71 genotypes is given in Figure 1. Accordingly, ERS and Fm % values were close to each other in both V29 and T71 genotypes, and the highest mycorrhizal dependence value was observed in Fm. In the V29 + Rs combination, it was determined that the Fm value was around 40% while ERS was around 0% (Figure 1). In the T71 + Rs combination, while the Fm value was around 25%, ERS was determined to be in the opposite direction with negative (-25%) values (Figure 1).

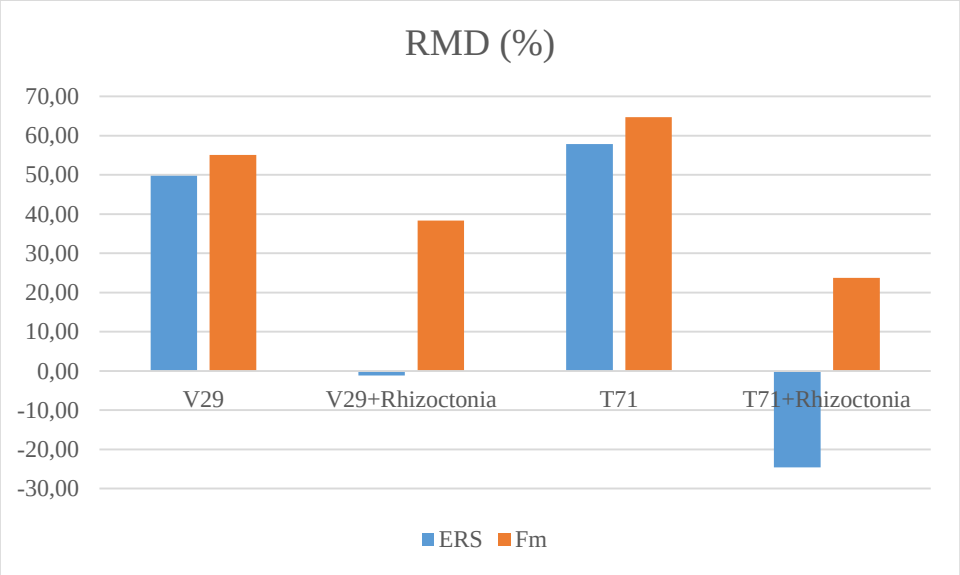

Figure 1. Percentage relative mycorrhizal dependency distribution of the combination of commercial AMF (ERS) and *F. mosseae* treatments with Rs in two different bean plant genotypes (V29 and T71)
Şekil 1. Ticari AMF (ERS) ve F. mosseae uygulamalarının Rs ile kombinasyonunun iki farklı fasulye bitkisi genotipinde (V29 ve T71) yüzde nispi mikorizal bağımlılık dağılımı

Table 2 describes the effect of two different AMF treatments on shoot fresh weight (g), root fresh weight (g), shoot dry weight (g), root dry weight (g), AMF root colonization (%), and *Rhizoctonia* disease scale in two different bean genotypes treated with *R. solani*. Accordingly, in general, the difference between genotypes and pathogen treatments in the colonization parameter or between genotypes in the scale parameter was found statistically insignificant ($p \leq 0.05$). In all other parameters, the difference between treatments was statistically significant ($p < 0.05$). In addition, it was determined that the T71 genotype had a greater effect than the V29 genotype in all plant growth parameters except root colonization and pathogen scale. Similarly, it was determined that Fm AMF treatment had a greater effect than ERS treatment on all plant growth parameters except root colonization and pathogen scale. ERS was more effective than Fm in root colonization and scale parameters (Table 2).

In the shoot fresh weight parameter, the highest value (55.05 g) was observed in the Fm+ Rs (+) combination in the T71 genotype, while the lowest value (22.59 g) was determined in control+ Rs (-) treatment in the V29 genotype. There was a significant difference of about 144% (Table 2). It is noteworthy that both AMF treatments had higher shoot fresh weight values than the control group, despite the Rs pathogen, especially in the V29 genotypes ($p \leq 0.05$) (Table 2). However, it was determined that the shoot fresh weight value of control+ Rs (+) was higher than control+ Rs (-) in the T71 genotype ($p \leq 0.05$) (Table 2).

The difference between AMF treatments in the root fresh weight parameter was found statistically insignificant ($p \leq 0.05$). The highest root fresh weight value (13.58 g) was observed in the ERS + Rs (-) treatment in genotype V29, while the lowest value (2.43 g) was observed in the control + Rs (-) treatment in the same genotype. A difference of approximately 459% was observed between them (Table 2). It was determined that the root fresh weight value of the non-Rs (-) group was higher than the Rs (+) group, and the difference between them was statistically significant ($p \leq 0.05$) (Table 2).

In the shoot dry weight parameter, AMF treatments were higher than the control groups, despite the Rs pathogen in both bean genotypes. When AMF treatments were compared, it was determined that the compatibility with the T71 genotype was better, and Fm was higher than ERS (Table 2). It was determined that the highest shoot dry weight value (9.16 g) was in the Fm + Rs (+) combination in the T71 genotype, and the lowest value (3.56 g) was in the control + Rs (-) treatment in V29 genotype, with a difference of approximately 157% (Table 2).

In the root dry weight parameter, the highest value (1.27 g) was in the Fm + Rs (+) combination in the T71 genotype, the lowest value (0.21 g) was in the ERS + Rs (+) treatment in the V29 genotype and a difference of 505% was determined between them. The highest value in this parameter was in the T71 genotype, Fm, and Rs (+) treatments (Table 2). In genotype V29, there was a 92% difference between ERS + Rs (-) treatment (0.48 g) and Fm + Rs (-) (0.25), while there was a 48% difference between ERS + Rs (+) (0.21 g) and Fm + Rs (+) (0.31 g). In the T71 genotype, there was a 176% difference between ERS + Rs (+) treatment (0.46 g) and Fm + Rs (+) (1.27 g), and the difference between them was found to be statistically significant ($p \leq 0.05$) (Table 2).

In the root colonization parameter, ERS (63.31%) was about 15% higher than Fm (54.94%) for both genotypes, and the difference between them was statistically significant ($p \leq 0.05$) (Table 2). The highest root colonization % value was observed in both the V29 genotype ERS+ Rs (-) (65.25%) and T71 ERS+ Rs (+) combination. The lowest value was observed in the T71 Fm+ Rs (-) (52.75%) treatment, and the difference between them was approximately 24% and statistically significant ($p \leq 0.05$). In V29, when AMF treatments (ERS and Fm) were compared with Rs treatments (+ and -), the colonization value decreased in Rs (+), and the opposite was the case in the T71 genotype, and the root colonization value of AMF treatments increased in combination with Rs (+).

The highest pathogen scale value (69.00) was observed in the control+Rs (+) combination of genotype V29, while the lowest value (17.67) was observed in the ERS+Rs (+) treatment of the same genotype Thus, the ERS reduced the value of the disease scale by almost 74% (Table 2). However, AMF had a significant effect on the pathogen scale value, but the difference between them was statistically insignificant ($p \leq 0.05$). In addition, while the difference in scale value between V29 and T71 genotypes was insignificant ($p \leq 0.05$), the T71 control+Rs (+) group was lower than the V29+Rs (+) combination, and a difference of approximately 21% was determined between them. However, the *R. solani* scale value decreased by approximately 57% with the *F. mosseae* T71 genotype (Table 2).

PCA, Correlation, and Heatmap Analysis

To determine the impact of AMF species (commercial AMF and *F. mosseae*) on *R. solani* in two different bean genotypes (V29 and T71), a Principal Component Analysis (PCA) was used to identify the traits contributing to the significant variation in the observed factors. The eigenvalues, variances, and contributions (PCA loadings) of the traits that differed between treatments were determined using the previously mentioned methods.

In *R. solani*, four principal components (PC) loadings were found when principal component analysis (PCA) was performed on twelve additional disease-related traits, as shown in Table 3. As indicated in Table 3, the initial component (PC1) is responsible for 35.89% of the variation in *Rhizoctonia* disease. SD, SFW, RFW, SDW, and RDW are the most effective explanatory traits for variation in *Rhizoctonia*. The second component (PC2) was found to account for 23.50% of the variation in *Rhizoctonia* disease. The most important contributors to this component are RL, TP, and Colonization. PC3 was found to explain 15.11% of the variation in *Rhizoctonia* with TAC, Scale, and RMD. The fourth component (PC4) accounted for 11.48% of the variation observed in *Rhizoctonia*, with only SL as the most significant contributing trait.

The effects of PCA1 and PCA2 components Fm and ERS on *R. solani* disease stress in two different bean genotypes were generated on a scorecard shown in Figure 2. Unstressed V29+ERS, T71+ERS, T71+Fm, and T71+ERS+*Rs* treatments are close to each other and in PCA1 and PCA2 positive regions. T71+Fm+*Rs* and T71+*Rs* treatments are in PCA1-positive and PCA2-negative regions. However, V29, T71, and V29+*Rs* treatments are in both PCA1 and PCA2 negative regions. V29+ERS+*Rs*, V29+Fm+*Rs*, and V29+Fm treatments were in the PCA1 negative PCA2 positive region. This was found to be associated with AMF interaction with bean genotypes. Therefore, the T71 bean genotype was more effective in reducing *Rs* biotic stress damage with Fm AMF treatment (Figure 2).

The correlation between the variables examined to determine the efficacy of ERS and Fm AMF treatments against *Rs* disease in V29 and T71 bean genotypes is shown in Figure 3. The data show a positive correlation between TP-Colonization, TAC-TP, SDW-RDW, SFW-RDW, SFW-SDW, SD-RFW, RL-TP, and RL-Colonization against *Rs* disease. Conversely, the correlation between SD-SL, RFW-SL, RDW-SL, and RMD scale was found to be negative (Figure 3).

Heat map clustering of traits of bean genotypes V29 and T71 with commercial AMF and *F. mosseae* is given in Figure 4. Red indicates an increase in the attribute in question, while blue indicates a decrease. In the heat maps showing the relationship between the T71 genotype and V29, especially concerning Fm, the predominance of the increase in most traits is striking in red color. The genotype-AMF heat map revealed two distinct clusters.

Table 2. Effects of two different AMF treatments on shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), AMF root colonization (%), and *Rhizoctonia* disease scale in two different bean genotypes treated with *Rhizoctonia solani* (mean ± standard deviation)

Çizelge 2. İki farklı AMF uygulamasının Rhizoctonia solani ile muamele edilen iki farklı fasulye genotipinde iki farklı AMF uygulamasının sürgün taze ağırlığı (SFW), kök taze ağırlığı (RFW), sürgün kuru ağırlığı (SDW), kök kuru ağırlığı (RDW), AMF kök kolonizasyonu (%) ve Rhizoctonia hastalık skalası üzerine etkileri (ortalama ± standart sapma)

Genotype	AMF	*Rs*	SFW (g)	RFW (g)	SDW (g)	RDW (g)	Colonization (%)	Scale
V29	Control	(-)	22.59±2.95	2.43±1.07	3.56±0.91	0.23±0.13	-	-
		(+)	32.28±3.09	4.41±1.29	4.06±1.03	0.30±0.04	-	69.00±6.68 A
	ERS	(-)	36.47±2.00	13.58±2.00	5.08±1.00	0.48±0.10	65.25±7.13	-
		(+)	24.38±2.20	4.24±1.14	3.81±0.50	0.21±0.02	61.75±6.55	17.67±6.42 D
	Fm	(-)	29.96±0.62	5.74±0.78	5.25±0.10	0.25±0.16	56.00±7.39	-
		(+)	33.07±5.20	6.19±0.15	5.36±0.07	0.31±0.10	55.00±8.52	27.75±6.39 CD
T71	Control	(-)	28.14±6.10	7.02±0.56	3.87±1.09	0.60±0.19	-	-
		(+)	43.27±5.55	10.39±0.81	7.39±0.12	0.94±0.18	-	57.25±3.77 B
	ERS	(-)	35.20±3.50	7.92±0.90	5.69±1.18	0.60±0.12	61.00±16.12	-
		(+)	37.61±8.60	4.65±1.68	5.59±1.22	0.46±0.19	65.25±10.24	32.00±14.93 C
	Fm	(-)	35.70±3.18	7.56±1.21	5.99±0.43	0.58±0.11	52.75±10.59	-
		(+)	55.05±7.71	11.09±3.57	9.16±1.98	1.27±0.07	56.00±6.16	24.75±5.25 CD
Genotype (G)								
V29			29.41±5.74 B	6.09±3.79 B	4.52±0.96 B	0.29±0.13 B	59.50±7.94	40.00±24.08
T71			39.38±9.88 A	8.10±2.66 A	6.28±1.95 A	0.75±0.31 A	58.75±11.28	38.55±16.91
AMF (A)								
Control			31.76±9.27 B	6.06±3.22 B	4.72±1.78 B	0.55±0.33 A	-	63.13±8.04 A
ERS			33.41±6.90 B	7.59±4.10 A	5.04±1.16 B	0.44±0.18 B	63.31±9.78 A	24.83±12.93 B
Fm			38.45±11.06 A	7.64±2.74 A	6.44±1.87 A	0.55±0.42 A	54.94±7.57 B	26.25±5.65 B
Rs								
(-)			30.88±6.11 B	7.37±3.55 A	4.91±1.18 B	0.45±0.20 B	58.75±10.94	-
(+)			37.91±10.93 A	6.83±3.29 B	5.89±2.12 A	0.58±0.40 A	59.50±8.39	-
p values (≤0.05)								
Genotype			<0.001	0.001	<0.001	<0.001	0.827	0.966
AMF			0.004	0.027	0.001	0.019	0.021	<0.001
Rs			<0.001	0.292	0.006	0.009	0.827	-
GxA			0.136	<0.001	0.412	0.001	0.913	0.006
GxRs			0.001	0.002	0.001	0.001	0.385	-
AxRs			<0.001	<0.001	0.005	<0.001	0.913	-
GxAxRs			0.319	0.183	0.410	0.052	0.799	-

Capital letters indicate the comparisons of the means of the applications and small letters indicate the interaction GxA for the scale. There is no statistically significant difference between the same letters (p≤0.05) according to Duncan's test findings.

Mean (M): Mean is the sum of all data points in our dataset divided by the total number of data points, Standard deviation (SD): It is a method that measures the closeness and agreement of observations in a dataset. The p-value is a function used to measure how extreme the observed sample results are, conditional on a statistical model.

ERS: Commercial AMF, Fm: *F. mosseae,* Rs: *Rhizoctonia solani*

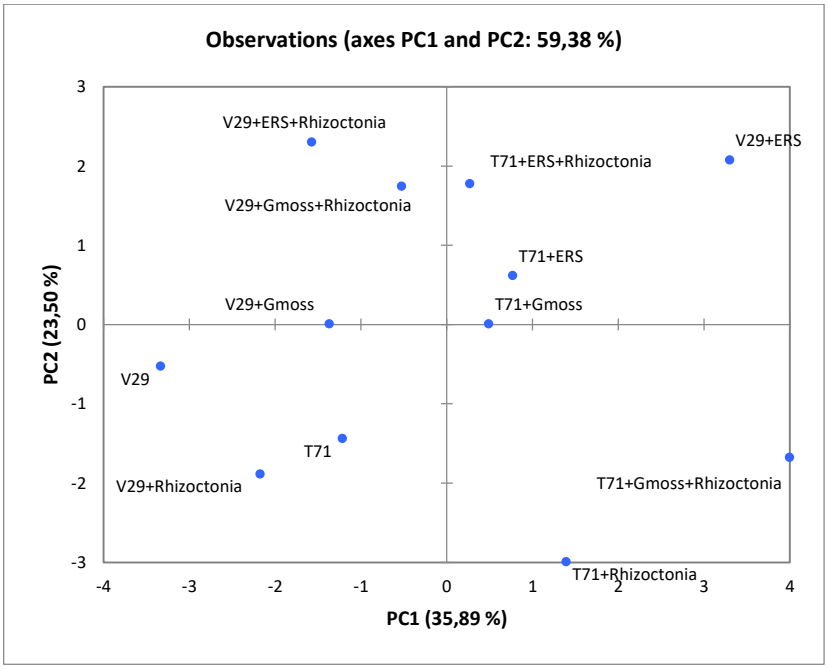

Figure 2. Plot of Principal Component Analysis (PCA) scores of the first two main components.
Şekil 2. İlk iki ana bileşenin Temel Bileşen Analizi (PCA) puanlarının grafiği.

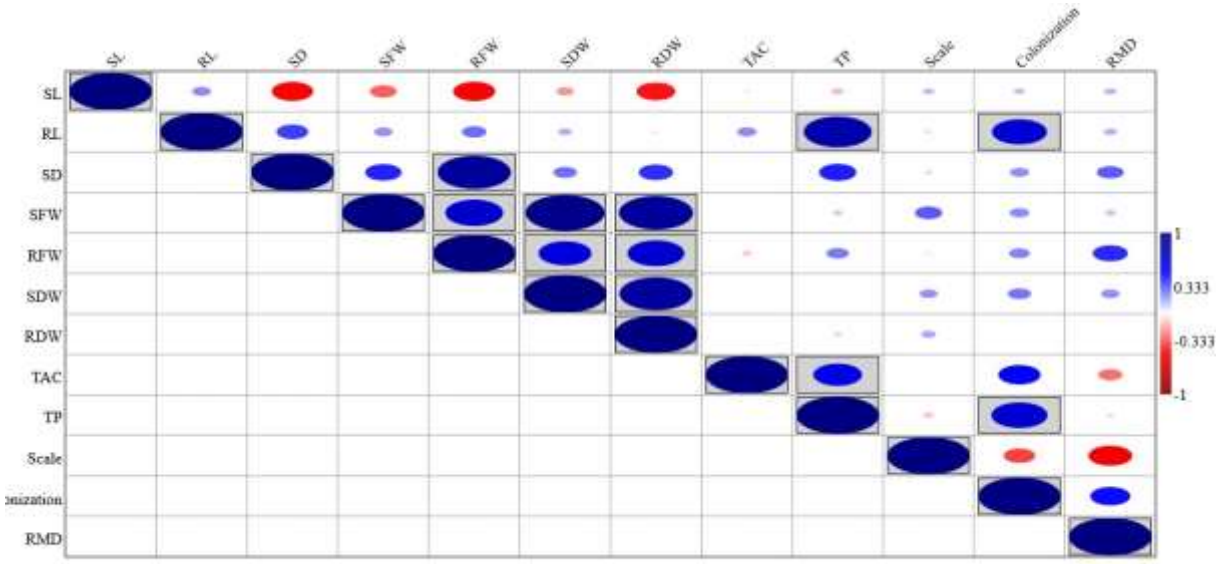

Figure 3. *Rhizoctonia solani* pathogen correlation between the traits used in the study; values in the bold frame are different from 0 and the significance level is alpha: 0,05.
Şekil 3. Çalışmada kullanılan özellikler arasındaki Rhizoctonia solani patojen korelasyonu; kalın çerçevedeki değerler 0'dan farklıdır ve anlamlılık düzeyi alfa'dır: 0,05.

Heat map clustering of traits of *R. solani* inoculation of bean genotypes V29 and T71 with commercial AMF and *F. mosseae* is given in Figure 5. Notably, despite disease stress, heat maps showing the relationship between traits in both genotypes and AMF treatments show a dominant decrease in most of the variables (blue color). The highest increase (red color) is between the V29+*Rs* application and the scale.

However, when *F. mosseae* was combined with the same treatment (V29+*Rs*+Fm), the heat map value decreased, and the color became blue. It was concluded that Fm was significantly affected, especially the scale parameter in the V29 genotype. In other words, AMF treatments increase plant growth characteristics by reducing the pathogenic effect.

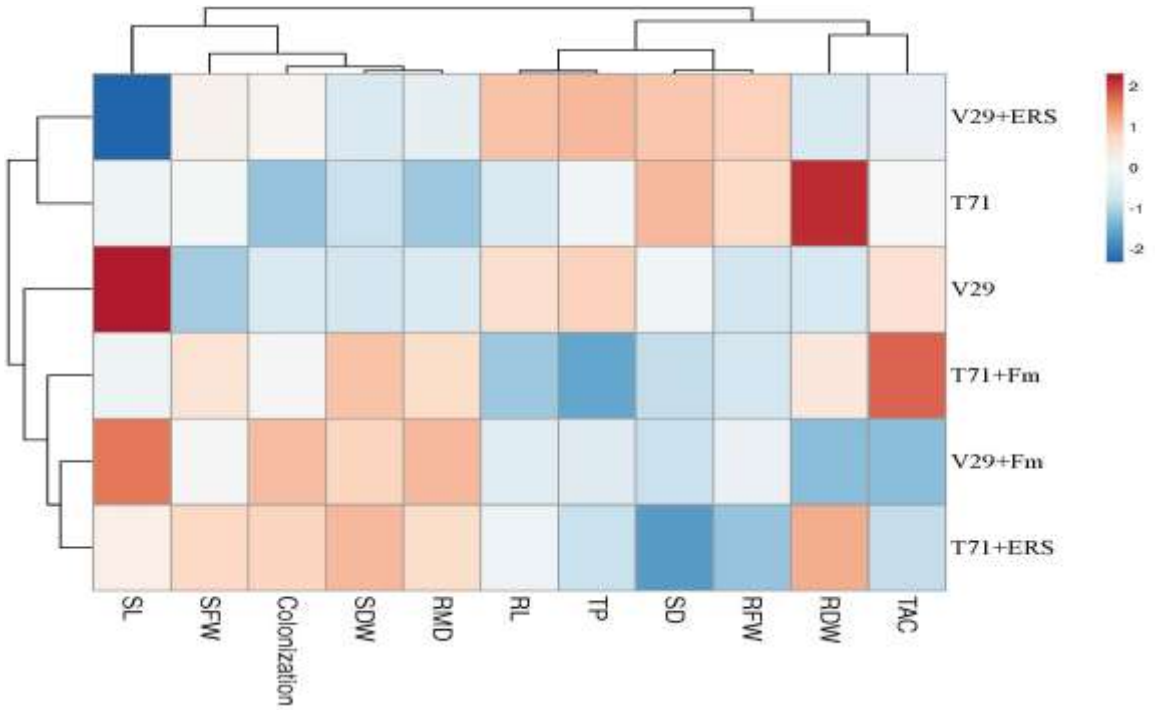

Figure 4. Heat map clustering of traits of bean genotypes V29 and T71 with commercial AMF and *Funneliformis mosseae*

Şekil 4. Fasulye genotiplerinin Ticari AMF ve Funneliformis mosseae ile V29 ve T71 fasulye genotiplerinin özelliklerinin ısı haritası kümelenmesi

Another noteworthy point in Figure 5 is that T71+*Rs* and T71+Fm+*Rs* treatments turned slightly pink in plant growth parameters (except SL and RL). In V29+Fm+*Rs* treatment, SL, RMD, and RL parameters, and V29+ERS+*Rs* treatment, TAC, TP, and colonization map temperatures were close to pink and increased. The heat map of *R. solani* disease revealed that genotypes V29 and T71 formed two distinct clusters, grouped with ERS and Fm. Heat maps propagating along the studied features and grouping ERS and Fm without exceedance showed the improvements made with AMF systems. This result was also correlated with genotype differences.

Table 3. Principal component analysis results for the characters of the study.
Çizelge 3. Çalışmanın karakterleri için temel bileşen analizi sonuçları.

	PC1	PC2	PC3	PC4
Eigenvalue	4.306	2.820	1.814	1.377
Variability (%)	35.885	23.496	15.114	11.476
Cumulative %	35.885	59.381	74.495	85.971
SL	-0.218	0.173	0.018	-0.649
RL	0.211	0.427	-0.105	-0.169
SD	0.365	0.061	0.143	0.360
SFW	0.415	-0.202	-0.154	-0.239
RFW	0.440	-0.071	0.174	0.149
SDW	0.387	-0.199	-0.087	-0.355
RDW	0.372	-0.321	-0.090	-0.056
TAC	0.064	0.304	-0.444	0.102
TP	0.204	0.465	-0.259	0.196
Scale	-0.005	-0.252	-0.506	-0.208
Colonization	0.224	0.456	0.043	-0.233
RMD	0.158	0.124	0.614	-0.254

*Values marked in yellow are different from 0, and the significance level is alpha: 0.05

** SL: Shoot Length, RL: Root Length, SD: Shoot Diameter, SFW: Shoot Fresh Weight, RFW: Root Fresh Weight, SDW: Shoot Dry Weight, RDW: Root Dry Weight, TAC: Total Antioxidant Concentration, TP: Total Phenolic, Scale: *R. solani* 0–4 scale, Colonization: AMF Root Colonization, RMD: Percentage Distribution

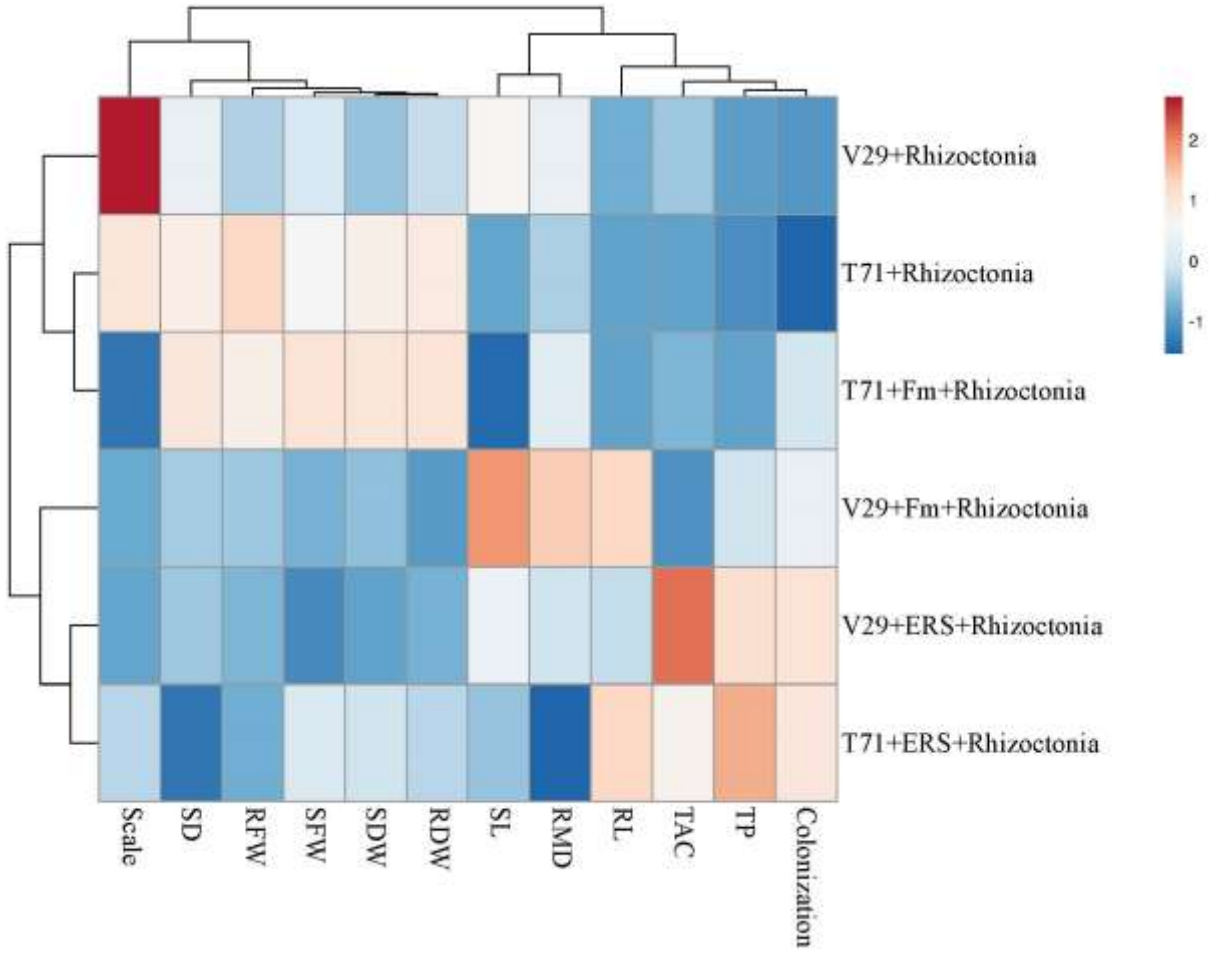

Figure 5. Heat map clustering of traits with commercial AMF and *F. mosseae* in *R. solani* inoculation of bean genotypes V29 and T71

Şekil 5. V29 ve T71 fasulye genotiplerinin R. solani inokulasyonunda ticari AMF ve F. mosseae ile özelliklerin ısı haritası kümelenmesi

DISCUSSION

In this work, we investigated the effects of commercial AMF and *F. mosseae* applications against the *R. solani* pathogen in two different bean genotypes (V29 and T71) (Table 1). Several studies have shown that *R. solani* root rot infects bean genotypes with different severity. For example, when Durak et al. (2024) investigated the anastomosis groups and pathogenicity of *Rhizoctonia* spp. Isolated from various bean genotypes grown in the Van Lake Basin, the most virulent group was AG-4. Isolate number 19 in genotype A64, isolate number 2 in TR68557, and isolate number 18 in cv. Gina was shown to be the most pathogenic isolate in *in vivo* studies.

Regardless of genotype, we found that AMF applications elevated plant growth metrics despite the *R. solani* pathogen (except RDW in ERS). According to a study by Moarrefzadeh et al. (2023), the first four of the six AMF species (*F. mosseae, Glomus claroideum, G. etunicatum, G. margarita, G. caledonium*, and *G. versiform*) significantly improved root length, shoot length, fresh-dry weight, and reduced disease incidence compared to the infected control. The highest root colonization was found in *Glomus fasciculatum*. ERS commercial AMF (mixture) showed the highest root colonization in our study (Table 2).

Furthermore, according to Moarrefzadeh et al. (2023), *F. mosseae* and *G. claroideum* had the lowest disease severity and incidence, respectively. In contrast, in our study, there was no statistically significant difference between AMF species in this respect, while ERS had the lowest value (Table 2). Beneficial microbes, such as arbuscular mycorrhizal fungi, offer a way to replace or reduce the use of fungicides in agriculture (Alabouvette et al., 2006).

AMF is useful in the control of plant diseases and improves ecosystem sustainability and productivity (Gianinazzi et al., 2010). The damage caused by *R. solani* to both bean genotypes was reduced by the use of mycorrhizal plants in this study. ERS and *F. mosseae* were found to reduce the disease scale value, and this effect was explained by

the disease severity in mycorrhizal plants. Previous reports on common beans or other plants have documented the anti-*R. solani* effect of some AMF species.

According to Aljawasim et al. (2020), disease severity induced by *R. solani* in cucumber plants decreased from 65% to 21% when *F. mosseae* mycorrhized. Furthermore, to improve growth parameters and reduce the severity and incidence of *R. solani* in common beans, Hafez et al. (2013) investigated the effect of a mixture containing five AMF species, *F. mosseae, Rhizophagus intraradices, G. clarum, Gigaspora margarita*, and *Gigaspora gigantea*, and found it to be significantly effective. According to Bagy et al. (2019), AMF primarily reduces the infection of plant roots by various soil pathogens, which in turn reduces disease incidence and severity index.

AMF has been proposed to work in several ways to strengthen plant defenses against soil-borne plant diseases (Kareem & Hassan, 2014). AMF is involved in the absorption and transport of nutrients to the roots, which reduces root damage (Liu et al., 2019). It also enhances plant growth by expanding their outer mycelium and activates plant defense mechanisms against soil pathogens through biochemical, physiological, and structural changes in the plant. These mechanisms include competition with pathogens in plant roots in the rhizosphere for host photosynthetic products and increased resistance/tolerance of AMF-inoculated plants to invasion by some plant pathogens (Amer & Abou El, 2008).

In the present study, *R. solani* did not show variable results between both bean genotypes (V29 and T71) or between both AMF treatments (ERS and *F. mosseae*), but ERS reduced the disease scale value by 74% in the V29 genotype and about 57% in the Fm T71 genotype. Moarrefzadeh et al. (2023) reported that AMF species *F. mosseae* and *G. claroideum* caused more than a 40% reduction in disease severity in beans compared to *R. solani*-infected control, which is in agreement with our findings. The fact that the biocontrol effect of AMF depends on several variables, including substrate, host plant, and even variety, may help explain these variations (Yao et al., 2002; Moarrefzadeh et al., 2022).

CONCLUSION

The results of the study suggest that AMF has a strong chance of biologically controlling *R. solani* in bean genotypes V29 and T71. As a result, AMF species had different beneficial effects. For example, plants inoculated with ERS had lower disease severity than plants inoculated with Fm, while *F. mosseae* exhibited the greatest positive effect on plant growth. Thus, regardless of the bean genotype, inoculation with appropriate AMF species can help plants compensate for the damage caused by *R. solani* and reduce the harmful effects of the pathogen. In conclusion, the mycorrhizal treatments used in this research can be considered viable, economical, and ecologically sound biological control strategies to deal with this soil-borne disease and as long-term biostimulants to promote bean plant growth. Further studies are needed to determine how well these AMF applications also work in the field to suppress this pathogen in different bean genotypes and how effective they are in combination with additional biostimulants.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors declare that there is no conflict of interest between them.

Research involving human participants and/or animals is not applicable

Neither humans nor animals were used in the study.

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