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Using Arbuscular Mycorrhizal Fungi and *Rhizobium leguminosarum* Biovar *phaseoli* Against *Sclerotinia sclerotiorum* (Lib.) de Bary in the Common Bean (*Phaseolus vulgaris* L.)

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Abstract: In this study, the effects of Arbuscular Mycorrhizal Fungi (AMF) *Glomus mosseae* (Gm), *Glomus fasciculatum* (Gf) and *Rhizobium leguminosarum* biovar *phaseoli* (Rlp), which are the important members of rhizosphere and biological control agents, were examined on the patho-system of *Sclerotinia sclerotiorum* (Lib.) de Bary (Ss) and common bean. The colonization and nodulation of two biological control agents exhibited differences as a result of reciprocal interactions of these items as well as the effect of the Ss. Nodulation of Rlp particularly decreased in triple inoculation. In addition, colonization of AMF significantly decreased in treatment of Ss+AMF than control AMF. Treatments of single inoculations of AMF and Rlp isolates reduced disease severity by 10.3-24.1%. It was determined that single biological control agents inoculations were more effective than dual inoculations (AMF+Rlp). When the morphological parameters of common bean were considered, all of the morphological parameters values were decreased in treatments which present pathogen isolate. Besides this, all biological control agents increased total contents of P and N in treated plants compared to the controls.

Key words: Common bean, *Sclerotinia sclerotiorum*, arbuscular mycorrhizal fungi, *Rhizobium leguminosarum* biovar *phaseoli*, biological control

INTRODUCTION

Arbuscular mycorrhizal fungi and rhizobia are two of the most important plant symbionts. They play a key role in natural ecosystems and influence plant productivity, plant nutrition and plant resistance (Demir and Akköprü, 2007). Mycorrhizas benefit the host through mobilization of phosphorus from nonlabile sources, whereas *Rhizobium* fixes N_2 (Dar *et al*., 1997; Scheublin and Van der Heijden, 2006). The well-known activities of nitrogen-fixing bacteria improving the bioavailability of the major plant nutrients N and P, are very much enhanced in the rhizosphere of mycorrhizal plants where synergistic interactions of such microorganisms with mycorrhizal fungi have been demonstrated (Barea *et al*., 2002). A great deal of work has been carried out on the tripartite symbiosis legume-mycorrhiza-*Rhizobium* (Azcón-Aguilar and Barea, 1992; Barea *et al*., 2000, 2002). The inoculation of mycorrhizal fungi has been shown to improve nodulation and N_2 fixation. Since soil-borne pathogens, as well as symbionts, share common habitat and show differential influence on the growth of the host plant, major interest has recently focused on the relevance of arbuscular mycorrhizas and rhizobia in the control of soil borne pathogens (Dar *et al*., 1997). As it known, these pathogens are typically difficult to control through conventional fungicide applications. By the arbuscular mycorrhizal fungi consistent reduction of disease symptoms has been described for fungal pathogens such as *Phytophthora*, *Gaeumannomyces*, *Fusarium*, *Pythium*, *Rhizoctonia*, *Verticillium* and *Aphanomyces* (Azcón-Aguilar and Barea, 1996; Demir and Akköprü, 2007). However, the effect of arbuscular mycorrhizas and *Rhizobium* on plant disease, nutrient uptake and rhizosphere microbial biomass and their activities are very difficult to generalize because the interactions involving arbuscular mycorrhiza, root rot fungi and *Rhizobium* vary with the microbial species and plant cultivars (Dar *et al*., 1997).

The present study was aimed to assess the roles of AMF and *Rhizobium* in the biological control of the common bean root and basal stem rot pathogen, *Sclerotinia sclerotiorum*, in addition to their influence on plant growth and soil nutrient availability.

MATERIALS AND METHODS

This study was conducted at Yuzuncu Yil University (Van/Turkey) in 2006 and 2007. Bean seeds (*Phaseolus vulgaris* cv. Sehirali beans) were surface sterilized in

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2% sodium hypochlorite, rinsed 2-3 times in sterile distilled water and sown in plastic pots (16x18) containing a sterilized mixture of soil and sand (3:1, v/v). The following treatments, with five replications each, were included in the study: (1) *G. mosseae* (Gm); (2) *G. fasciculatum* (Gf); (3) *Rhizobium leguminosarum* biovar *phaseoli* (Rlp); (4); *Sclerotinia sclerotiorum* (Ss); (5) Gm+Rlp; (6) Gm+Ss; (7) Gm+Rlp + Ss; (8) Gf+Rlp; (9) Gf+Ss; (10) Gf+Rlp+Ss; (11) Rlp+Ss; (12) uninoculated control. All pots were placed in a growth chamber under standard conditions (14 h light (25-27°C), 65% relative humidity) in a completely randomized experimental design. Plants were watered twice a week with deionized water and 100 mL of the nutrient solution (containing 720 mg $MgSO_4.7H_2O$, 12.2 mg KH_2PO_4, 295 mg Ca $(NO_3)_2.4H_2O$, 240 mg KNO_3, 0.75 mg $MnCl_2.4H_20$, 0.75 mg KI, 0.75 mg $ZnSO_2.H_2O$, 1.5 mg H_3BO_3, 0.001 mg $CuSO_4.5H_20$, 4.3 mg FeNaEDTA and 0.00017 mg $Na_2MoO_4.2H_2O$; modified from Vosátka and Gryndler (1999) was applied three times into each pot during the experiment (10 weeks).

Inoculations: Gm and Gf inoculations were accomplished by placing 2 g soil inoculum, containing 75 spores g^{-1} soil and 50 spores g^{-1} soil respectively, per pot, where the bean seeds were to be sown. *Rhizobium*, grown in yeast extract mannitol broth, was applied to seeds, containing 3.9×10^5 cells g^{-1}. The seeds were sown immediately. Control plants did not receive any inoculum. For *S. sclerotiorum* inoculation, the wheat grains were boiled in tap water for 30 min. The boiled grains were put into 250 mL glass bottles up to half-length of the bottles and sterilized in an autoclave at 121°C for 40 min. A 5 mm disc agar covered with the mycelium of pathogen was placed in to the bottles containing the sterilized wheat grains. The bottles were incubated at 24°C for 4 weeks. After the pathogen has fully covered the grain surfaces with the mycelium the grains were then dried at room temperature. In addition, 15 sclerotia of pathogen were also added to the soil. Five grams of sterilized wheat grains were added to control pots (Irshad and Onoğur, 2002).

Plant growth and nutrient uptake: At the end of the experiment, common bean plants were harvested 10 weeks after seed sowing. Plant roots were separated dried (70°C, 48 h) and weighed. Plants were analyzed for nitrogen and phosphorus by standard methods (Kacar, 1984) and nutrient uptake recorded.

Determination of disease severity caused by *S. sclerotiorum*, root colonization by AMF fungi and nodulation of *Rhizobium*: The disease development on each bean plant grown in the growth chamber for 10 weeks was rated by using the following scale (Irshad and Onoğur, 2002): 0, no symptoms; 1, pathogen development on the basal stem, no symptoms in the plant tissue; 2, symptoms in the plant tissue, plant is still living; 3, plant died. Bean roots were dyed in order to determine the existence of Gm and Gf by a modified method of Phillips and Hayman (1970) and the colonization rates were determined by the Grid-Line Intersect Method (Giovanetti and Mosse, 1980). The number and activity of the nodules were recorded at the plant harvest (Öğüt *et al*., 2003).

Statistical analysis: The data were subject to analysis of variance and means compared using Duncan's multiple range tests.

RESULTS

Plant growth and nutrient uptake: Inoculation with arbuscular mycorrhizal fungi (Gm and Gf) and Rlp significantly increased shoot fresh weight and shoot dry weight, respectively, in comparison to the other treatments. Dry and fresh weights of root were significantly lower in control plants than those infected with Ss or inoculated with Gm, Gf, or Rlp either alone or in combination (Table 1). In the other hand, plant height was significantly increased when inoculated with Gm + Rlp.

Impacts of the biocontrol agents on the P and N contents and fresh and dry matter weights of tomato shoots and roots were also determined besides their effects on Ss. Mycorrhizal plants acquired more P, in comparison to the control, even in the presence of Ss (Table 1). The most remarkable results were obtained from the applications Gm+Rlp+Ss and Gm whose shoot and root contents were 72.5 and 80.8% higher, respectively than that of the control (Table 1). The N content of shoot in the single application of Rlp was higher than those in single or dual applications of the arbuscular mycorrhizal fungi (Table 1). The dual inoculation of Gm and Rlp had significant effect on the nitrogen content of roots (Table 1).

The number of nodules, AMF Root Colonization and Disease Severity: Colonization rates of the Gm and Gf and nodule number of Rlp are presented Table 2. As seen in Table 2, it was determined that Ss alone significantly (56.9, 53.2 and 57.1%, respectively) reduced the colonization levels of AM fungi and nodule number of Rlp compared with the non-treated control and the Ss treatments accompanied with the biological control agents (Gm+Rlp+Ss and Gf+Rlp+Ss) also reduced the colonization levels of arbuscular mycorrhizal fungi and

Table 1: Plant growth and nutrient uptake of the common bean inoculated with *G. mosseae* (Gm), *G. fasciculatum* (Gf), *R. leguminosarum* biovar *phaseoli* (Rlp) and *S. sclerotiorum* (Ss) alone or various combinations

						Plant nutrient uptake			
	Fresh weight (g plant^{-1})		Dry weight (g plant^{-1})			Phosphorus (mg kg^{-1})		Nitrogen (%)	
Treatments	Shoot	Root	Shoot	Root	Plant height (cm plant^{-1})	Shoot	Root	Shoot	Root
Control	6.95b*	0.38f	5.20c	0.23g	28.00c	488.93f	157.96e	1.36g	0.78c
Gm	9.66a	2.00cd	5.86ba	1.23cd	31.20b	1369.26cbd	824.84a	1.82edf	0.89b
Gf	9.33a	2.33cb	5.80ba	1.40b	31.13b	1296.78cbd	561.63bca	1.98edc	0.90b
R.lp	6.00cb	3.00a	6.00a	1.40b	32.03b	666.69fe	506.98bdc	2.68a	0.84b
Ss	5.46cd	2.20cbd	4.03d	1.13d	23.33ed	1196.59cd	369.57bedc	2.44ba	0.76dc
Gm+Rlp	5.73cd	3.33a	5.36bc	1.70a	34.66a	1158.03d	639.40ba	1.71egf	1.05a
Gf +Rl.p	4.90d	1.80d	3.63d	1.36cb	27.46c	1434.79cb	596.48ba	1.50gf	0.70d
Gm+Ss	3.76e	0.93e	3.03e	0.63f	18.16f	1486.07b	591.93ba	1.97edc	0.87b
G f+Ss	2.40f	1.13e	2.06f	0.76f	23.50ed	1736.56a	442.55bdc	1.40g	0.57fe
R lp+Ss	5.50cd	2.30cb	2.46f	1.46b	31.90b	1264.13cbd	298.36edc	2.27bac	0.50f
Gm+Rlp+Ss	1.90f	1.33e	1.26g	0.93e	24.50d	1779.10a	789.79a	2.23bdc	0.91b
Gf+Rlp+Ss	2.03f	2.56b	0.96g	1.46b	21.86e	775.78e	263.26ed	2.48ba	0.58e

Values are means of five replications; Means within a column for each experiment by the same letter(s) are not significantly different ($p<0.005$)

Table 2: The number of nodules, AMF root colonization and disease severity of common bean plants inoculated with *G. mosseae* (Gm), *G. fasciculatum* (Gf), *R. leguminosarum* biovar *phaseoli* (Rlp) and *S. sclerotiorum* (Ss) alone or various combinations

Treatments	The number of nodules	AMF root colonization (%)	Disease severity (%)
Control	--	--	--
Gm	--	65.0ba	--
Gf	--	77.0a	--
R.lp	7.00a*	--	--
Ss	--	--	58.0bac
Gm+Rlp	6.33ab	40.0dce	--
Gf +Rl.p	5.33b	45.0dce	--
Gm+Ss	--	28.0e	44.0d
G f+Ss	--	36.0de	48.0dc
R lp+Ss	3.00c	--	52.0bdc
Gm+R.lp+Ss	6.00ab	48.0dc	67.0a
Gf+Rlp+Ss	5.33b	56.0bc	61.0ab

Values are means of five replications; Means within a column for each experiment by the same letter(s) are not significantly different ($p<0.005$)

nodule number of Rlp between 14.2-27.2%. Percentage efficacy of the mycorrhizal fungi and Rlp combinations with both single and dual inoculations against Ss are seen in Table 2. While the single inoculations of rhizosphere members inhibited Ss at the rate of between 10.3-24%, its dual inoculations increased disease severity rates of 4.9% and 13.4%.

DISCUSSION

Pathogenic infection by Ss reduced growth and nutrient uptake by the common bean, whereas inoculation with AMF or Rlp, alone, reduced pathogenicity. The decreased severity of disease and improved plant growth and nutrient uptake, expressed that the common bean inoculated by AMF or Rlp is somewhat resistant to the root pathogen Ss. Previous reports revealed that mycorrhizal plants offer increased tolerance to fungal root pathogens (Zambolin and Schenck, 1983; Jalali *et al*., 1983; Akköprü and Demir, 2005) and bacterization of legume seeds/seedlings with *Rhizobium* significantly reduced some rot root diseases caused by soil-borne fungal pathogens (Chakraborty and Purkayastha, 1984; Chakraborty and Chakraborty, 1989; Dar *et al*., 1997). It seems that enhanced plant growth improved nutrient assimilation and possibly a physical barrier have probably imparted altered resistance to the plants (Dar *et al*., 1997), as the disease incidence was reduced in plants inoculated with AM fungi and Rlp. Since the role of altered root exudates, changes in rhizosphere microbial activities and biochemical antagonism through phytoalexin and rhizobitoxine production as mechanisms of disease tolerance induced by AMF and Rlp (Dar *et al*., 1997) cannot conduct here.

AMF and *Rhizobium*, as the most important symbionts of rhizosphere have shown stimulating (Subba Rao *et al*., 1986; Champavat, 1990; Dar *et al*., 1997; Edwards *et al*., 1998) or inhibiting (Söderberg *et al*., 2002; Scheublin and Van der Heijden, 2006) effects on each other or on the growth of plants and pathogens. This was also confirmed in our study. In dual applications of both symbionts microorganisms decreased colonization or nodulation of each other. These inhibiting effects are thought to be related to the secretion of antimicrobial substances (Walley and Germida, 1997; Mar Vázquez *et al*., 2000). The colonization of AM fungi and the nodule numbers of Rlp was also significantly reduced presence of Ss. It is not clear how AMF root colonization or *Rhizobium* nodulation is affected by soil-borne pathogens (Johansson *et al*., 2004). However, it has been hypothesized that these effects may be related to the species and varieties of microorganisms and the conditions in the rhizosphere (Siddiqui and Shaukat, 2002; Anjair *et al*., 2003).

Legume being endowed with the unique ability to utilize the vast reservoir of atmospheric nitrogen with the

help of symbiotic nitrogen fixing bacterium *Rhizobium*. Other soil microorganisms such as arbuscular mycorrhizal fungi have been credited for beneficial effects on plants. AMF absorbs phosphorus and translocates it to the roots (Champavat, 1990). Similar synergistic effect of Rlp and AM fungi has been recorded in common bean plants. It is also now well documented that inoculation of plants with AM fungi can stimulate nodulation and nitrogen fixation by legumes (Bethlenfalvay *et al*., 1982; Subba Rao *et al*., 1986; Champavat, 1990). Some workers showed that in P efficient soils, nitrogen fixation in several legumes inoculated with appropriate *Rhizobium* strain depended much on phosphorus, which could be supplied by mycorrhizal infection (Powell, 1976; Manjunath and Bagyaraj, 1984). The results reported also were confirmed in this study.

The present study concluded that suitable combinations of AM fungi and *Rhizobium* bacteria may increase the plant growth and resistance to pathogens. In future studies, therefore, more detailed investigations in various pathosystems and of the interactions between the microorganisms and the host plant are needed to develop much more efficient biocontrol of the related diseases.

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