

EFFECTS OF PLANT GROWTH-PROMOTING RHIZOBACTERIA ON SOME MORPHOLOGIC CHARACTERISTICS, YIELD AND QUALITY CONTENTS OF HUNGARIAN VETCH

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ABSTRACT

This research study was designed to determine the effects of plant growth-promoting rhizobacteria (PGPR) on some morphological characteristics, yield and quality contents of Hungarian vetch (*Vicia pannonica* Crantz.) in the Kelkit Aydın Dogan Vocational Training School Research Area during 2008-2009 and 2009-2010 plant growing season. The research consisted of a control (without plant growth-promoting rhizobacteria) and 12 different plant growth-promoting rhizobacteria (*Pseudomonas putida* PPB310, *Bacillus cereus* BCB51, *Pantoea agglomerans* PAB58, *Pseudomonas fluorescens* PFC82, *Pseudomonas fluorescens* PF84, *Arthrobacter mysorens* AM235, *Paenibacillus polymyxa* PP315, *Pantoea agglomerans* PAA362, *Bacillus atrophaeus* BA361, *Bacillus megaterium* BMA424, *Bacillus megaterium* BMA479 and *Bacillus subtilis* BS521) seed inoculations and three replicates. Some morphological characteristics (stem diameter, plant height and leaf number), dry matter yield, crude protein, crude protein yield, ADF, NDF, macro and micro-elements (B, Ca, Na, K, Mg, P, S, Cu, Fe, Mn and Zn) were tested in the research study. According to the results of the research study, some of the PGPR treatments had positive effect on morphological characteristics (especially PFC82, BA361 and PAB58), dry matter yield (very little PAA362), crude protein (very little PFC82), ADF and NDF (especially BA361) and macro and micro-elements (especially PAA362) of Hungarian vetch.

Keywords: Hungarian vetch, plant growth-promoting rhizobacteria, dry matter yield, crude protein, macro and micro-elements

INTRODUCTION

Organic animal production activities have begun to spread throughout the world in recent years. As a result of these phenomena, the requirement of organic forage crops production increases for organic livestock. Organic forage crops production in the fertilization especially shows important differences compared to conventional production. Crop rotation systems, intercropping mixtures, green manures, solid and liquid farmyard manures, poultry manures, compost, zeolite and biological fertilizers are used by farmers in the organic agriculture instead of chemical fertilizers (Yolcu 2010).

Uses of plant growth-promoting rhizobacteria isolated from various plants have started to spread in organic agriculture areas by the aim of plant nutrition. Micro-organisms promote the circulation of plant nutrients and reduce the need for chemical fertilizers (Cakmakci et al., 2007a). N₂-fixing and P-solubilizing bacteria may be important for plant nutrition by increasing N and P uptake of the plants (Cakmakci et al., 2006). Improvement of plant growth can be achieved by the direct application of plant growth-promoting rhizobacteria to seeds (Cakmakci et al., 2007b). Plant growth-promoting rhizobacteria

(PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth using a wide variety of mechanisms (Ashrafuzzaman et al., 2009).

Vetches are commonly used in organic animal feeding throughout the world. Hungarian vetch seeded in the autumn in cold climates doesn't generally suffer from the effects of harsh winters. Hungarian vetch growing faster by benefiting from snow water and early spring rains competes better against weeds. As a result of this, Hungarian vetch provides plenty quality hay production for organic animal feeding.

Numerous studies have been conducted on different crops on the topic of the effects of PGPR (Javaid 2009; Yolcu et al. 2011; Khanna and Sharma 2011; Krey et al. 2011). However, there are currently no adequate number of studies on the topic of the effects of PGPR on the morphological characteristics, yield and quality contents of Hungarian vetch. In this regard, the aim of the present study was to investigate the effectiveness of twelve PGPR on some morphological characteristics, yield and quality contents of Hungarian vetch.

MATERIALS AND METHODS

Characterisation and isolation of bacterial strains

Twelve plant growth-promoting rhizobacteria, 2 fix N₂ (*Arthrobacter mysorens* AM235 and *Bacillus subtilis* BS521) and 10 solubilise P and fix N₂ (*Pseudomonas putida* PPB310, *Bacillus cereus* BCB51, *Pantoea agglomerans* PAB58, *Pseudomonas fluorescens* PFC82, *Pseudomonas fluorescens* PF84, *Paenibacillus polymyxa* PP315, *Pantoea agglomerans* PAA362, *Bacillus atrophaeus* BA361, *Bacillus megaterium* BMA424 and *Bacillus megaterium* BMA479) were used in this study. All strains used in the present study were previously isolated from the acidic rhizosphere of tea growing areas (Cakmakci et al., 2010).

Field experiment and growth conditions

A research study was carried out at the Kelkit Aydin

Dogan Vocational Training School Research Area of Gumushane University in the Northeast of Turkey (1412 m elevation, 40° 08' N, 39° 25' E). Hungarian vetch was sown on October 14, 2008 and October 17, 2009 at a seeding rate of 80 kg ha⁻¹ (Acikgoz 2001). The study design was a randomised complete block with thirteen treatments replicated three times. Each plot size was 3.0 m x 1.68 m, with a 24 cm row spacing and plots were separated by a 2.5 m buffer zone. The treatments were: control (without plant growth-promoting rhizobacteria), 2 fix N₂ (AM235 and BS521) and 10 solubilise P and fix N₂ (PPB310, BCB51, PAB58, PFC82, PF84, PP315, PAA362, BA361, BMA424 and BMA479). The plots were irrigated twice with 15 day intervals after the rains stopped (Serin and Tan, 2001) and were harvested at the embedment period of bottom fruits (Acikgoz 2001) in 2009 and 2010. Climatic data during the years of study and long term means were shown in Table 1.

Table 1. Climatic data of the location in 2008, 2009, 2010 and the long-term average (1986-2006) at Kelkit, Turkey.

	J	F	M	A	M	J	J	A	S	O	N	D	
Years	Total Precipitation (mm) (Monthly)												Total
2008	40.8	23.3	38.4	51.4	28.4	35.8	2.6	20.0	30.3	35.2	21.1	34.4	361.7
2009	21.3	45.6	57.9	96.3	63.6	25.3	37.4	0.0	71.0	35.1	127.2	33.0	613.7
2010	73.2	24.1	58.2	49.2	57.5	93.5	12.8	0.0	8.2	87.1	1.2	14.2	479.2
1986-2006	33.1	35.5	38.3	57.7	68.3	45.1	14.8	13.8	26.3	50.6	45.1	37.6	466.2
	Mean air temperature (°C) (Monthly)												Mean
2008	-6.1	-4.4	8.1	11.6	11.7	16.6	20.1	21.5	17.1	11.9	6.6	-0.7	9.5
2009	-0.2	3.2	3.8	7.8	12.9	18.1	19.6	18.0	14.8	13.0	4.7	4.2	10.0
2010	2.0	4.3	6.3	9.0	14.4	19.4	22.3	23.5	19.6	11.9	7.9	5.3	12.2
1986-2006	-1.8	-1.0	3.1	9.4	13.3	16.8	20.2	20.1	16.3	11.3	4.4	0.5	9.4
	Mean relative humidity (%) (Monthly)												Mean
2008	70.7	71.4	63.0	65.0	68.3	69.6	68.5	69.4	68.3	73.0	72.9	73.2	69.4
2009	71.2	68.7	67.9	63.5	65.5	66.4	67.6	65.8	72.8	67.7	77.2	73.7	69.4
2010	73.0	68.1	65.7	69.1	67.2	66.9	65.5	59.5	65.2	74.6	65.2	64.5	67.0

Plant analysis

Stem diameter, plant height and leaf number were determined as the mean of six plants. Plant samples were oven-dried at 68°C for 48 hours and weighted to find the dry matter yield for each year. Dried Hungarian vetch samples were ground with a Wiley mill to pass a 1-mm screen and then analyzed for chemical characteristics. The total nitrogen was found by using the Kjeldahl method and the crude protein was calculated by multiplying the N content by 6.25 (Bremner 1996). The ADF and NDF concentrations of samples were determined according to Van Soest (1963). B, Ca, Na, K, Mg, P, S, Cu, Fe, Mn and Zn concentrations of Hungarian vetches were found as a result of wet digestion of dried and ground sub-samples using a HNO₃-H₂O₂ acid mixture (2:3 v/v) in three steps (first step: 145°C, 75%RF, 5 minutes; second step: 180°C, 90%RF, 10 min and the third step: 100°C, 40 %RF, 10 minutes) in a microwave

(Bergof Speedwave Microwave Digestion Equipment MWS-2) (Mertens 2005a), and inductively Couple

plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA) (Mertens 2005b). Chemical characteristics of research soils (0-45 cm) in 2008 and 2009 were shown in Table 2.

Table 2. Chemical characteristics of research soils (0-45 cm) in 2008 and 2009.

Soil Properties	Units	2008	2009
Cation exchangeable capacity	cmol _c kg ⁻¹	23.2	20.1
Total N	mg kg ⁻¹	5.3	13.7
pH	(1:2 soil:water)	7.9	7.4
Organic matter	g kg ⁻¹	1.1	2.7
CaCO ₃	g kg ⁻¹	25.3	28.7
Available P	mg kg ⁻¹	14.2	12.8
Exchangeable Ca	cmol _c kg ⁻¹	15.0	17.0
Exchangeable Mg	cmol _c kg ⁻¹	1.51	1.71
Exchangeable K	cmol _c kg ⁻¹	2.2	2.5
Exchangeable Na	cmol _c kg ⁻¹	0.7	0.5
Available Fe	mg kg ⁻¹	5.4	5.0
Available Mn	mg kg ⁻¹	6.1	7.7
Available Zn	mg kg ⁻¹	2.2	2.2
Available Cu	mg kg ⁻¹	2.3	2.6

Statistical analysis

The results were subjected to analysis of variance (ANOVA) and significant means were compared with Duncan's multiple range test method using the SPSS 13.0 statistical package program (SPSS Inc., 2004).

RESULTS AND DISCUSSION

Morphological characteristics

Most of PGPR inoculants provided higher stem diameter than that of the control as the mean of 2009 and 2010 (Table 3). The greatest stem diameter of Hungarian vetch was found in BA361 (1.89 mm) PGPR inoculant. This inoculation was followed by BMA479 (1.86 mm) and PFC82 (1.86 mm) plant growth-promoting rhizobacteria inoculants. Many of the PGPR inoculants had a higher plant height than that of the control in the mean of 2009 and 2010 (Table 3). The

highest plant height was obtained in PFC82 (51.0 cm) followed by PP315 (49.9 cm), PF84 (49.4 cm) and PAB58 (48.4 cm) rhizobacteria inoculants. Similarly, Cakmakci et al. (2007a) in barley and Javaid (2009) in blackgram [*Vigna mungo* (L.) Hepper] reported differences in terms of plant height with bacteria inoculations. PAB58 (19.4), PFC82 (18.7) and BA361 (18.5) rhizobacteria inoculants had higher leaf numbers than the control (17.0) in the mean of 2009 and 2010. The other rhizobacteria inoculants caused similar or lower leaf numbers compared to the control (Table 3). In addition, other researchers reported that plant growth-promoting rhizobacteria affected the leaf numbers of soybean (Dashti et al., 1997) and fodder maize (Hamidi 2006). Effects ($p < 0.01$) of plant growth-promoting rhizobacteria on all morphological characteristics in 2009 and 2010 were shown in Figure 1.

Table 3. Effects of plant growth-promoting rhizobacteria (PGPR) on some morphological characteristics, dry matter yield and forage quality in Hungarian vetch (throughout two years)

Treatments	Stem Diameter (mm)	Plant Height (cm)	Leaf Number (leaf plant ⁻¹)	Dry Matter Yield (kg ha ⁻¹)	Crude Protein (%)	C. Protein Yield (kg ha ⁻¹)	ADF (%)	NDF (%)
Control	1.71ef	43.9e	17.0cd	4593ab	16.8ab	771a	31.6c-e	51.3ab
PPB310	1.80bc	45.0de	17.6b-d	4170c	16.5a-c	688b	30.1e	47.7de
BCB51	1.80bc	45.6c-e	16.4de	4191bc	16.0b-d	671b	32.9bc	44.9fg
PAB58	1.74c-e	48.4a-c	19.4a	2524f	15.9b-e	401e	30.8e	50.6a-c
PFC82	1.86ab	51.0a	18.7ab	2932e	17.4a	510c	31.7c-e	48.7cd
PF84	1.79cd	49.4ab	16.5de	4303a-c	16.1b-d	693b	34.5a	49.1b-d
AM235	1.66f	45.3de	18.3a-c	3682d	14.8e-g	545c	33.9ab	42.9g
PP315	1.66f	49.9ab	15.6e	4538a-c	14.4fg	653b	32.6b-d	43.7g
PAA362	1.71ef	43.9e	16.4de	4653a	13.9g	647b	30.6e	46.6d-f
BA361	1.89a	45.0de	18.5ab	4450a-c	15.4d-f	685b	28.7f	44.6fg
BMA424	1.73de	42.6e	17.7b-d	4354a-c	14.9e-g	649b	31.4de	52.5a
BMA479	1.86ab	47.4b-d	17.5b-d	3116e	15.6c-e	486cd	31.5c-e	46.1ef
BS521	1.78cd	47.2b-d	16.9de	2897e	15.2d-f	440de	30.7e	48.3c-e
Mean	1.77	46.5	17.43	3877	15.6	603	31.6	47.5
2009	1.72b	41.3b	16.04b	2961b	15.9a	470b	30.8b	47.0b
2010	1.81a	51.7a	18.82a	4794a	15.3b	736a	32.4a	48.0a
T	**	**	**	**	**	**	**	**
Y	**	**	**	**	**	**	**	*
T*Y	**	**	**	**	**	**	**	**

T : Treatments, Y: Year, *Significant at %5 level. **significant at %1 level

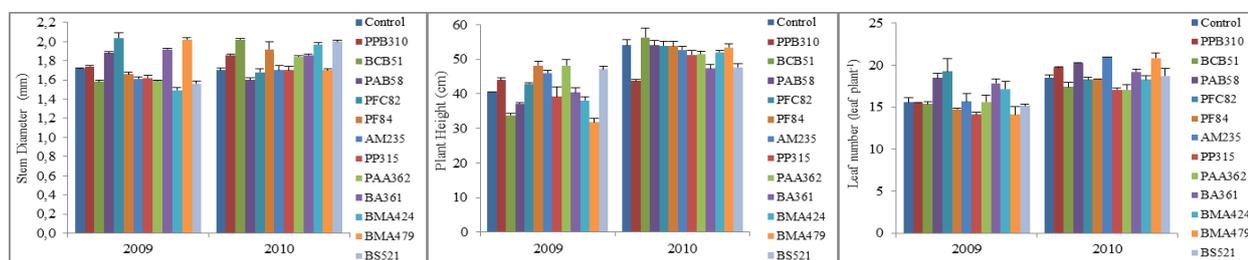


Figure 1. Effects of plant growth-promoting rhizobacteria (PGPR) on stem diameter, plant height and leaf number in 2009 and 2010.

Yield and Quality

Inoculation of Hungarian vetch seeds with plant growth-promoting rhizobacteria caused significant differences in the dry matter yield of Hungarian vetch

in the mean of 2009 and 2010 (Table 3). The highest dry matter yield was determined in PAA362 (4653 kg ha⁻¹) rhizobacteria inoculation. However, PAA362 (4653 kg ha⁻¹), the control (4593 kg ha⁻¹), PP315 (4538 kg ha⁻¹), BA361 (4450 kg ha⁻¹), BMA424 (4354 kg ha⁻¹),

¹) and PF84 (4303 kg ha⁻¹) rhizobacteria inoculations had statistically similar yields in terms of dry matter. The other inoculation applications yielded lower dry matter than that of the control (Table 3). Also in other studies, Dashti et al. (1997) in soybean, Cakmakci et al. (2001) and Canbolat et al. (2006) in barley, Rugheim and Abdelgani (2009) in faba bean reported an increase of yield with bacteria inoculations. However, Yolcu et al. (2011) determined that one of five PGPR decreased the dry matter yield of Italian ryegrass. Furthermore, Berggren et al. (2005) reported that the *P. putida* strain A313 reduced pea dry matter production. Conversely, other researchers stated that microorganism strains had no effect on the growth or yield of pea (Chanway et al., 1989) and soybean (Javaid and Mahmood, 2010).

The highest crude protein concentration in Hungarian vetch was found in PFC82 (17.4 %) rhizobacteria inoculant in the mean of 2009 and 2010 (Table 3). However, the PFC82 rhizobacteria inoculant was similar to the control and PPB310 rhizobacteria inoculants. The other rhizobacteria inoculants had similar or lower crude protein concentration than that of the control (16.8 %). Similarly, Estevez et al. (2009) stated that the symbiotic N₂ fixation of legume plants does not always produce advantageous results with PGPR

and rhizobia co-inoculation. Furthermore, Zaidi et al. (2003) reported that the soil nitrogen content did not show appreciable differences as a result of the inoculation in chickpea. In addition, Berggren et al. (2005) determined that the *Pseudomonas putida* strain A313 reduced the pea nitrogen content. The highest crude protein yield was determined in the control (771 kg ha⁻¹) in the mean of 2009 and 2010. All treatments had a significantly lower crude protein yield than that of the control (Table 3). Also in another study, Yolcu et al. (2011) reported that one of five PGPR inoculants decreased the crude protein yield in Italian ryegrass. ADF and NDF concentrations were affected by different PGPR inoculations in the mean of 2009 and 2010. BA361 (28.7%) rhizobacteria inoculants gave lower ADF concentration than that of the control (31.6 %). Most of the PGPR inoculations had lower NDF concentrations than that of the control (Table 3). Similarly, Mishra et al. (2008) stated that Dual inoculation, i.e., *Azospirillum* with indigenous AM consortia decreased ADF and NDF concentrations of *Panicum maximum*. Effects of plant growth-promoting rhizobacteria (PGPR) on dry matter yields, crude protein concentrations, crude protein yield, ADF and NDF in 2009 and 2010 were shown in Figure 2.

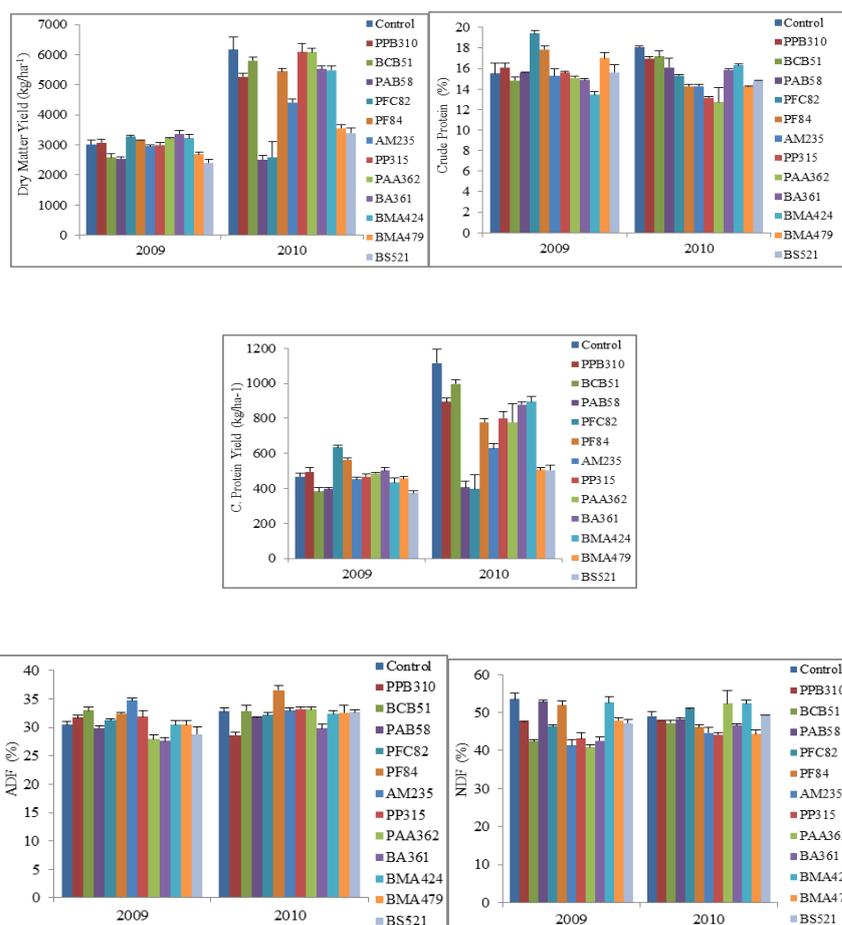


Figure 2. Effects of plant growth-promoting rhizobacteria (PGPR) on dry matter yield, crude protein concentration, crude protein yield, ADF and NDF in 2009 and 2010.

Table 4. Effect of plant growth-promoting rhizobacteria (PGPR) on macro- and micro-nutrient concentrations (mg kg⁻¹) in Hungarian vetch (throughout two years)

Treatments	B	Ca	Na	K	Mg	P	S	Cu	Fe	Mn	Zn
Control	43cd	24781c	2665a	8671f	2814c	1733d	2730e	18.5bc	999d	63b	113f
PPB310	46b	25225c	2639a	9368e	2439e	1710d	3015c	17.7bc	941d	57c	125de
BCB51	42de	23904d	2597a	9763d	2301fg	1625e	2662e	17.6 c	679g	56cd	124de
PAB58	45bc	23026ef	2682a	8374g	2339ef	1720d	2569f	20.9a	723fg	57c	147b
PFC82	40e	23368e	2678a	7953h	2606d	1511f	2875d	19.1 b	1452a	66ab	130cd
PF84	46b	24222d	2611a	9755d	2743c	1717d	2883d	17.5 c	764ef	53de	128cde
AM235	43cd	26672b	2639a	11943b	2762c	2288b	2463g	18.9bc	583h	51e	125de
PP315	42de	25108c	2739a	10095c	3085b	1736d	2738e	17.8bc	1158b	67a	134c
PAA362	50a	27854a	2590a	12979a	3279a	2190c	3754a	20.9a	818e	67a	124de
BA361	38f	20822g	2684a	8700f	2227g	2339b	2697e	18.1bc	931d	65ab	124de
BMA424	42de	22537f	2447b	4985i	1366h	2954a	2229h	18.3bc	1073c	53de	115f
BMA479	43cd	18570h	2638a	13019a	2827c	1745d	3241b	18.9 b	771ef	56c	156a
BS521	45bc	24942c	2646a	9892cd	2717c	1676de	3181b	17.5 c	757ef	57c	120ef
Mean	43	23925	2635	9654	2577	1919	2849	18.6	896	59	128
2009	43	26833a	2615	10489a	3010a	1647b	2825b	18.8	1208a	69a	142a
2010	43	21018b	2655	8819b	2145b	2190a	2873a	18.4	585b	49b	114b
T	**	**	*	**	**	**	**	**	**	**	**
Y	ns	**	ns	**	**	**	**	ns	**	**	**
T*Y	**	**	**	**	**	**	**	**	**	**	**

T : Treatments, Y: Year, *Significant at %5 level. **significant at %1 level

Macro- and micro-nutrient concentrations

All PGPR inoculants caused variation on macro and micro – nutrient concentrations of Hungarian vetch in the mean of 2009 and 2010. Inoculants of PAA362, PPB310 and PF84 in B, PAA362 and AM235 in Ca, BMA479, PAA362, AM235, PP315, BS521, BCB51, PF84 and PPB310 in K, PAA362 and PP315 in Mg and BMA424, BA361, AM235 and PAA362 in P had higher concentrations than that of the control in the mean of 2009 and 2010 (Table 4). The other PGPR inoculants had similar or lower B, Ca, K, Mg and P concentrations than that of the control. Similarly, Yolcu et al. (2011) reported that some PGPR treatments increased concentrations of K, Ca, Mg and B, and also the other PGPR treatments caused similar or lower concentrations than that of the control in Italian ryegrass. Elkoca et al. (2010) determined an increase of P, K, Ca, B and Mg concentration in common bean with PGPR treatments. Furthermore, Cakmakci et al. (2009) found that PGPR treatments caused a similar or higher concentration of P, K, Ca and Mg than that of the control in spinach leaves. Conversely, it was reported that PGPR treatments had no effect on the K and Mg concentration of barley (Cakmakci et al., 2007a).

Inoculants of PAA362, BMA479, BS521, PPB310, PF84 and PFC82 in S, PAA362 and PAB58 in Cu,

PFC82, PP315 and BMA424 in Fe, PAA362 and PP315 in Mn and BMA479, PAB58, PP315, PFC82, PF84, PPB310, AM235, BCB51, PAA362 and BA361 PGPR treatments in Zn had higher concentrations than that of the control in the mean of 2009 and 2010 (Table 4). The other PGPR inoculants had similar or lower S, Cu, Fe, Mn and Zn concentrations than those of the control. Similarly, Yolcu et al. (2011) reported that some PGPR treatments increased the concentrations of S, Cu and Zn, and also other PGPR treatments had a similar or lower concentration than that of the control in Italian ryegrass. Furthermore, Cakmakci et al. (2007a) stated that treatments of PGPR had a similar or higher Fe, Mn, Zn and Cu concentration than that of the control in barley. Elkoca et al. (2010) found significant increases of Fe, Mn, Cu and Zn concentration in the common bean with PGPR treatments. In addition, Shirmardi et al. (2010) reported that rhizobacteria inoculants produce higher Zn and Cu, similar Fe and similar or lower Mn concentration than that of the control. Conversely, it was determined that PGPR treatments had no effect on the Fe, Mn and Zn concentration of spinach leaves (Cakmakci et al., 2009). Effects of plant growth-promoting rhizobacteria (PGPR) on B, Ca, Na, K, Mg, P, S, Cu, Fe, Mn and Zn concentration in 2009 and 2010 were shown in Figure 3, 4, 5 and 6.

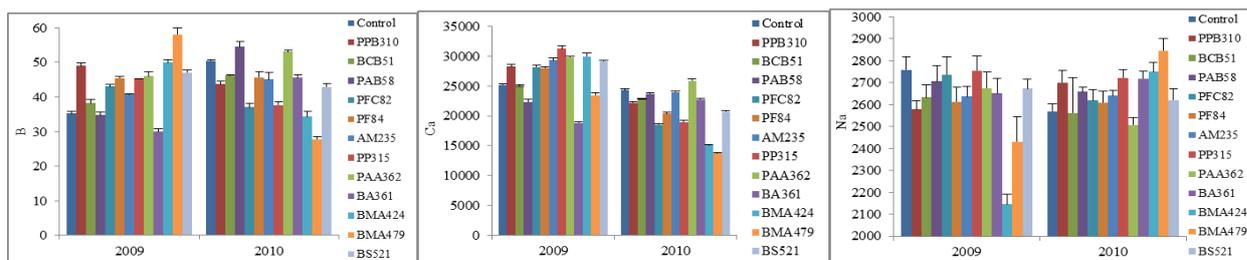


Figure 3. Effects of plant growth-promoting rhizobacteria (PGPR) on B, Ca and Na concentrations (mg kg^{-1}) in 2009 and 2010.

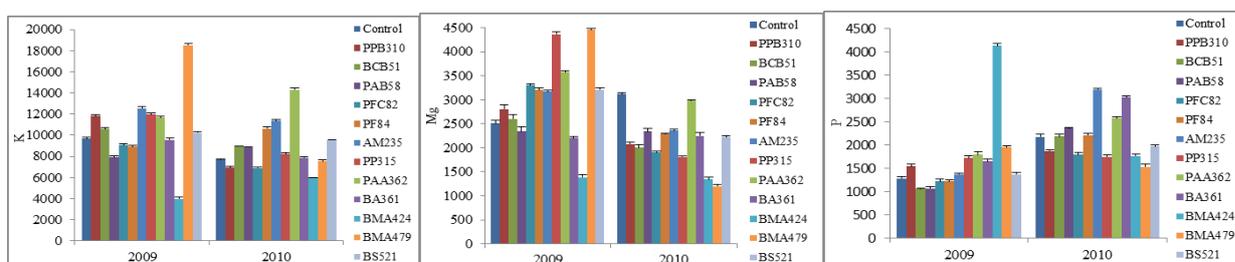


Figure 4. Effects of plant growth-promoting rhizobacteria (PGPR) on K, Mg and P concentrations (mg kg^{-1}) in 2009 and 2010.

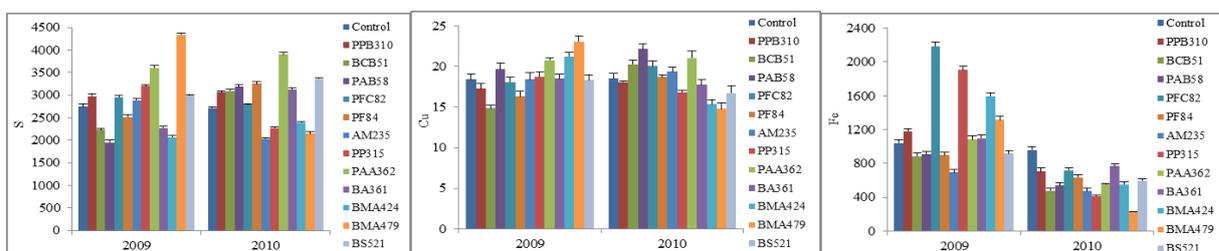


Figure 5. Effects of plant growth-promoting rhizobacteria (PGPR) on S, Cu and Fe concentrations (mg kg^{-1}) in 2009 and 2010.

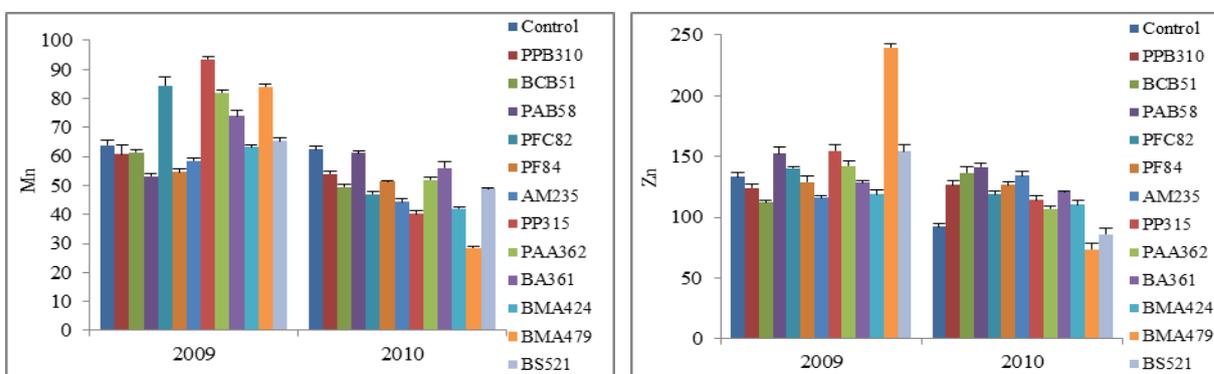


Figure 6. Effects of plant growth-promoting rhizobacteria (PGPR) on Mn and Zn concentrations (mg kg^{-1}) in 2009 and 2010.

CONCLUSIONS

Overall, some of the PGPR treatments (especially PFC82, BA361 and PAB58) had a positive effect on the morphological characteristics of Hungarian vetch. PAA362 in dry matter yield and PFC82 in crude protein concentration produced a very slight increase. *Rhizobium* spp are naturally present in the root zone of legumes. However, the addition of PGPR may cause competition or incompatibility between native *Rhizobium* spp and PGPR. Therefore, this competition and incompatibility may lead to the reduced efficiency of both bacteria in terms of dry matter yield, crude protein concentration and crude

protein yield. Some of the rhizobacteria (especially BA361) was effective in terms of ADF and NDF. Some rhizobacteria (especially PAA362) increased the macro and micro-element concentrations (B, Ca, K, Mg, P, S, Cu, Mn and Zn) of Hungarian vetch.

The results of our research study show that inoculation of a legume plant seed with the plant growth-promoting rhizobacteria does not cause a significant increase in dry matter yield and hay quality except macro and micro-element concentrations. However, our results should be tested by studies to be conducted with various PGPR in different legume forage crops.

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