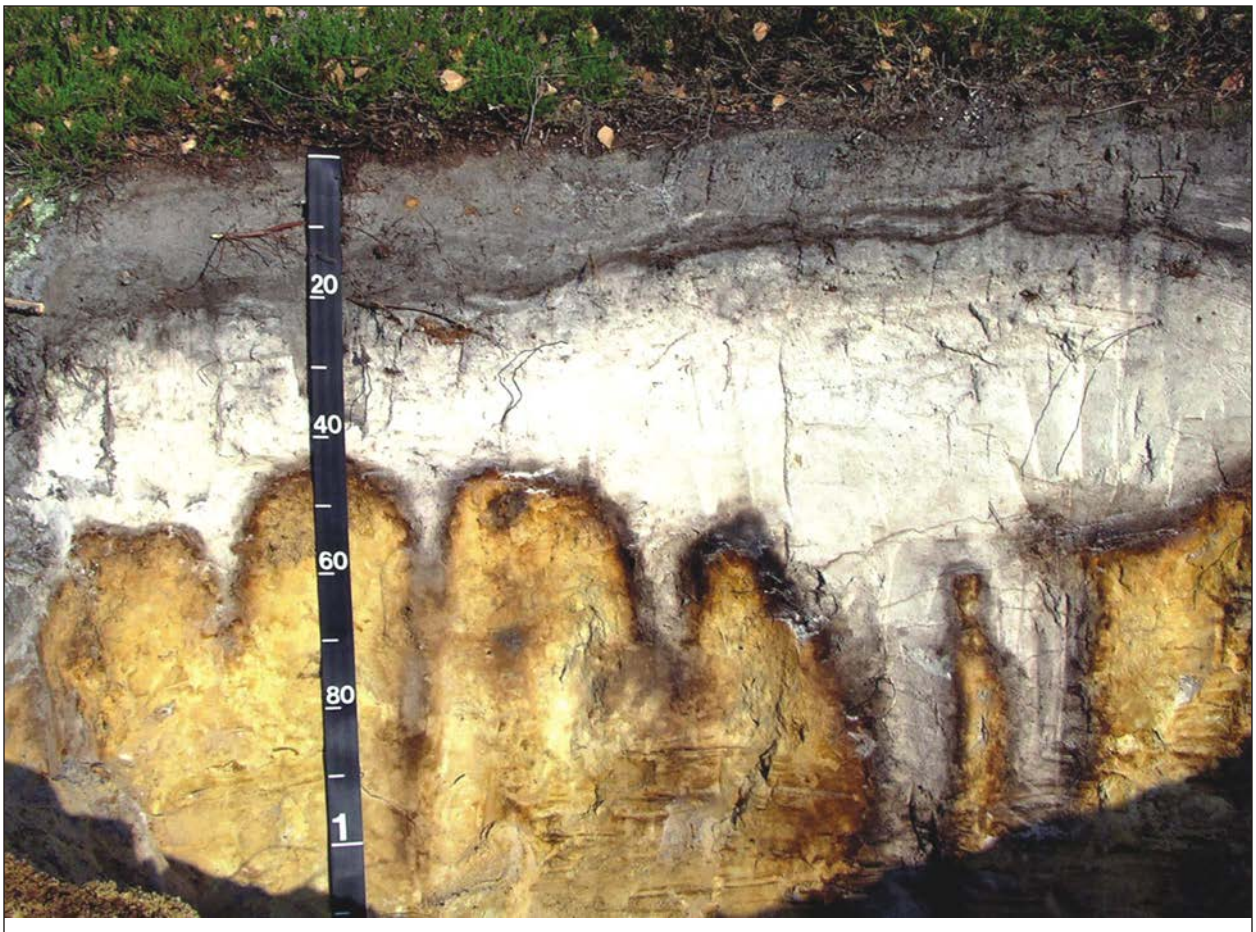

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The Effects of Plant Growth Promotion Rhizobacteria on Vegetative Growth and Leaf Nutrient Contents of Hazelnut Seedlings (Turkish hazelnut cv, Tombul and Sivri)

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ABSTRACT

The objective of the study was to explore possibilities to reduce fertilizer requirement and to identify bacterial strains that used for organically grown Turkish hazelnut cultivars. Plant growth promoting effects of *L. enzymogenes* 9/8, *B. atrophaeus* 55/1, *A. agilis* 2/3, *P. macquariensis* 59/8, *B. lentus* (Pb. validus) 29/6, *B. pyrrocinia* 13/4, *P. agglomerans* 5/8, *R. radiobacter* 42/1, *S.s maltophilia* 21/1, *Acinetobacter calcoaceticus* 47/6 and mineral fertilizer were tested on Turkish hazelnut cultivars (Tombul and Sivri) based on seedling length, total branch length, branch number trunk diameter and ionic composition of leaves. The results showed that all of bacterial treatments significantly affected the parameters tested. The highest seedling length, total branch length, branch number and trunk diameter of Tombul and Sivri of Turkish hazelnut cultivars was obtained with *A. calcoaceticus* 47/6, *R. radiobacter* 42/1, *S. maltophilia* 21/1, *P.macquariensis* 59/8, respectively and increasing ratio of seedling length, total branch length, branch number and trunk diameter of Turkish hazelnut cultivars was 24.49, 31.60, 69.28 and 18.74% for Tombul and 21.72, 68.43, 46.90 and 24.41% for Sivri, respectively compared to control. The concentrations of N, P, K, Ca, Mg, Fe, Cu, Mn, Zn, B and Al of plant tissue nutrients were significantly increased by the bacterial treatments tested. All bacterial treatments had positive effect but treatments 13/4 and 42/1 were the most effective in promoting macro and micro nutrient uptake. These results suggest that plant growth rhizobacteria (PGPR) treatments offer an economic and simple means to increase plant growth in soils with low fertility.

Key words: Hazelnut, bio fertilizer, macro-micronutrient, PGPR , vegetative growth

INTRODUCTION

Turkey is a center of origin for the hazelnut and an important producer of this crop. The black sea region which has suitable soil and ecological factors, is extremely well suited for hazelnut growing in Turkey. In fact, Turkey currently holds around 75% of the world hazelnut production. Hazelnut varieties grown at the east and west black sea regions in Turkey are *Coryllus avellana* L and *Coryyllus maxima* Mill and their hybrids. Hazelnut production in Turkey has initiated an increasing interest by farmers grow organic hazelnuts (Bostan, 2001; Ercisli, 2004).

Because of the rapid increase of world population and parallel technological development, people were directed to obtain maximum yield per unit area in agricultural production. But efforts to obtain maximum yield gives rise to economical, social and environmental problems causing deterioration of the natural balance. It has been suggested that intensive farming methods are not the solution to the hunger problem in world. Besides this, intensive agricultural practices and methods leading to that chemical residues in agricultural crops and constitutes a threat human plant and animal health, consequently the costs of crop production have increased in course of time. To minimize these problems, organic agricultural practices started. Therefore, more recently there has been a resurgence of interest in environmental friendly, sustainable and organic agricultural practices. The use of bio-fertilizers containing beneficial microorganisms instead of synthetic chemical fertilizer is known to improve plant growth through the supply of plant nutrients and may help to sustain environmental health and soil productivity (O'Connell, 1992). However, one of the important problems in organic production is the decrease in yield (Lind *et al*., 2003; Vessey, 2003). Recent studies showed that a number of bacterial species mostly associated with the plant rhizosphere, are found to be beneficial for plant growth, yield and crop quality. They are called plant growth promoting rhizobacteria (PGPR). Known as PGPR, biological fertilizers have contributed to the increase in yield significantly by increasing availability of soil macro-micro nutrient such as P, Ca, Fe, Zn using of renewable sources. PGPR either directly helps to provide nutrient to the host plant, indirectly influence root growth and morphology or aide other beneficial symbiotic relationships (Vessey, 2003; Lucy *et al*., 2004; Cakmakci *et al*., 2009).

PGPR are groups of bacteria that can actively colonize plant roots and increase plant growth. These can stimulate plant growth, increase yield, reduce pathogen infection, as well as reduce biotic or abiotic plant stress, without conferring pathogenity (Compant *et al*., 2010). The PGPR strains include *Acinotobacter, Algaligenes, Arthrobacter, Azospirillium, Azotobacter, Bacillus, Beijerinckia, Burkholdria, Enterobacter, Erwinia, Flavobacterium, Rhizobium* and *Serrotia* genera (Bashan and de-Bashan, 2005; Han and Lee, 2005). Plant Growth Promoting Rhizobacteria (PGPR) have been considered as a possible alternative to inorganic fertilizer. These PGPR strains may affect plant growth directly by synthesis of phytohormones and vitamins, through N fixation, by resistance, or by the solubilization of organic phosphate (Dobbeleare *et al*., 2002).

Many studies have been done on the mechanisms and principles of the PGPR-plant relationship, which were accepted widely as rhizosphere effect (Glick, 1995; Zhuang *et al*., 2007). Several studies found that PGPR can stimulate growth and increase yield in apple (Amarente *et al*., 2002; Aslantas *et al*., 2007; Karlidag *et al*., 2007; Pirlak *et al*., 2007), mulberry (Sudhakar *et al*., 2000), apricot (Esitken *et al*., 2003), raspberry (Orhan *et al*., 2006), sweet cherry (Esitken *et al*., 2006; Akça and Ercisli, 2010), highbush berry (Da Silva *et al*., 2000), strawberry (Esitken *et al*., 2010), tea (Erturk *et al*., 2008; Cakmakci *et al*., 2009; Cakmakci *et al*., 2010), kiwi (Ertürk *et al*., 2010) and banana (Kavino *et al*., 2010; Mia *et al*., 2010).

This study was conducted to investigate the possibility of reducing fertilizer requirement by using PGPR and to identify bacterial strains that can be used for organically grown plants. This is the first study investigating the effects of PGPR on hazelnut seedling grown organically.

MATERIALS AND METHODS

Growth conditions and plant materials: The study was conducted in the greenhouse of Hazelnut Reseach Institue in Giresun, Turkey in 2008 and 2009. Turkish hazelnut cultivars, Tombul and Sivri seedlings plants were maintained under natural light conditions, including

day/night temperature of 31/22°C and 60-75% relative humidity during the span of the experiment. Cold-stored bare rooted strawberry plants with one well-developed crown of diameter 8-10 mm were planted in the soil. Soil samples were air-dried, crushed and passed through a 2 mm sieve prior to chemical analysis. The Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Konigswinter, Germany) were used to determine total N (Bremner, 1996). Plant-available P was determined by the sodium bicarbonate method of Olsen *et al.* (1954). Soil pH was determined in 1:2 extracts according to McLean (1982). Soil organic matter was determined by Smith-Weldon method according to Nelson and Sommers (1982). Ammonium acetate buffered at pH 7 (Thomas, 1982) was used to determine exchangeable (K, Ca, Mg, Na) cations. Microelements in the soils were determined by Diethylene Triamine Pentaacetic Acid (DTPA) extraction methods (Lindsay and Norwell, 1978). The analysis results of soil are given in Table 1. Macro (P, K, Ca Mg and Na) and micro-elements (Fe, Mn, Zn , Al, B and Cu) content of plants were determined after wet digestion of dried and ground sub-samples using a HNO_3-H_2O_2 acid mixture (2:3 v/v) with three steps (first step; 145°C, 75% RF, 5 min; second step; 180°C, 90% RF, 10 min and third step; 100°C, 40% RF, 10 min) in a microwave oven (Bergof Speedwave Microwave Digestion Equipment MWS-2) (Mertens, 2005a). Tissue P, K, Ca, Mg, Na, Fe, Mn, Zn, Al, B and Cu were determined using an Inductively Couple Plasma spectrometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Shelton, CT 06484-4794, USA) (Mertens, 2005b). ICP/OES was also used to determine P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu, B and Al after extraction. The experiment had a randomized complete design with 8 N_2 fixing and P-solubilizing PGPR strain [(*Lysobacter enzymogenes* 9/8), (*Bacillus atrophaeus* 55/1), (*Arthrobacter agilis* 2/3), (*Paenibacillus macquariensis* 59/8), (*Bacillus lentus* (Pb. validus) 29/6, (*Burkholderia pyrrocinia* 13/4), (*Pantoea agglomerans* 5/8), (*Rhizobium radiobacter* 42/1), (*Stenotrophomonas maltophilia* 21/1), (*Acinetobacter calcoaceticus* 47/6)], N fertilizer applications (600 kg ha^{-1} Ammonium sulphate, 21% N) and NP fertilizer application 600 kg ha^{-1} (Amonium sulphate, 21% N + 300 kg ha^{-1} Triple Super Phosphate,

Table 1: Some chemical and physical properties of studied soil (n = 10)

Sol properties	Values
pH (1:2.5 s/w)	5.49±0.6
Organic matter (%)	5.12±0.3
Total N (%)	0.125±0.01
NH_4-N (%)	0.005±0.01
NO_3-N (%)	0.0059±0.01
CEC ($cmol_c$ kg^{-1})	16.39±2.7
K ($cmol_c$ kg^{-1})	2.62±0.4
Ca ($cmol_c$ kg^{-1})	4.75±0.6
Na ($cmol_c$ kg^{-1})	0.20±0.02
P (mg kg^{-1})	24.27±2.6
Al (mg kg^{-1})	7.80±1.1
Fe (mg kg^{-1})	3.80±0.7
Cu (mg kg^{-1})	1.68±0.3
Mn (mg kg^{-1})	22.69±0.2
B (mg kg^{-1})	0.15±0.01
Clay (%)	13.86±1.2
Silt (%)	32.99±3.4
Sand (%)	53.15±2.9

Values are Mean±SD

42% P_2O_5) and the control treatment (without mineral and PGPR application) with 3 replicates per treatment and 6 plants per replicates. Hazelnut seedlings plants were planted into the pots sterilized with 20% sodium hypochlorite solution, filled with 5 kg soil. Bacterial applications were performed using a dipping method in which plant roots were inoculated with the bacterial suspensions at a concentration of 10^9 CFU mL^{-1} in sterile water about 30 min prior to planting. The control plants were dipped into sterile water.

Bacterial strains, isolation and identification of bacteria: The bacterial strains *L. enzymogenes* 9/8, *B. atrophaeus* 55/1, *A. agilis* 2/3, *P. macquariensis* 59/8, *B. lentus* (Pb. validus) 29/6, *B. pyrrocinia* 13/4, P. *agglomerans* 5/8, R. *radiobacter* 42/1, *S.s maltophilia* 21/1, *Acinetobacter calcoaceticus* 47/6 were initially isolated from the rhizosphere of tea plants in the eastern black sea region (Rize and Trabzon province) of Turkey. The soils of the sampled sites had pH values ranging from 3.4 to 5.8. The bacteria were identified based on their whole-cell fatty acid methyl ester (FAMEs) analysis using the Microbial Identification System (MIDI).

Bacteria were grown on Nutrient Agar (NA) for routine use, and maintained in Nutrient Broth (NB) with 15% glycerol at -80°C for long-term storage. For each experiment, a single colony was transferred to 500 mL flasks containing NB and grown aerobically in flasks on a rotating shaker (150 rpm) for 48 h at 27°C (Merck KGaA, Germany). The bacterial suspension was then diluted in sterile distilled water to a final concentration of 10^9 CFU•mL^{-1} and the resulting suspensions were used to treat one year old hazelnut seedlings.

Acetylene reduction assay (ARA): Nitrogen fixation of the isolates was determined in a nitrogen-free medium by the acetylene reduction assay. Cultures for the acetylene reduction assay were prepared and incubated at 30°C for 24 and 48 h without agitation (Cakmakci *et al.*, 2001).

Quantification of Indolylacetic acid (IAA) production and phosphate-solubilizing capacity: The bacteria were also tested for auxin production (IAA-like substances) using the method of Bent *et al.* (2001) and phosphate solubilization capacity (Cakmakci *et al.*, 2001). The flasks were incubated for 18 h at 27°C with 100 rpm rotary shaking. Following this, 125 mL flasks containing 40 mL half-strength tryptic soy broth (TSB), supplemented with 0, 0.1 and 25 mg tryptophan mL^{-1} were each inoculated with 1mL of each strain. After incubation for 48, 72 and 168 h, the density of each culture was measured with a spectrophotometer (Shimadzu UV-1208) at 600 nm and then the bacterial cells were removed from the culture medium by centrifugation. The level of indoles present in the culture fluid was estimated calorimetrically. The concentration of IAA in the bacterial elutes was measured by using Salkowski's reagent (50 mL 35% $HClO_4$ +1 mL $FeCL_3$). Each reaction mixture was centrifuged. The absorbance at 530 nm was measured with a spectrophotometer. Bacterial cells were separated from the supernatant by centrifugation at 10,000 rpm for 30 min. The concentration of IAA in each culture medium was determined by comparison with a standard curve. The IAA produced by each strain was measured in triplicate. In addition, after 48, 72 and 168 h of growth, samples were taken to determine IAA content with thin-layer chromatography (TLC) and high performance liquid chromatography-mass spectrometry (HPLC-MS) analysis. Separation of indole acetic acid in ethyl-acetate fraction was carried out in chloroform-ethyl acetate-formic acid.

All isolates were tested for their phosphate-solubilizing capacities in a sucrose-tricalciumphosphate agar media by inoculating 1 mL of a 6 day-old culture (density 4×109)

in 250 mL Erlenmeyer flasks in triplicates containing 500 μg P mL^{-1} as Rock Phosphate (RP) at 30±1°C. After incubation for 6 days, water-soluble P was determined colorimetrically by the vanadomolybdophosphoric yellow color method (Pikovskaya, 1948).

Statistical analysis: All data were analyzed using Costat software (CoHortSoftware, Montery, USA). Plant nutrient content, some vegetative growth parameters and PGPR treatments were tested using a randomized complete design with tree replications. Mean comparisons were conducted using an ANOVA protected Least Significant Difference (LSD) ($p<0.05$) test.

RESULTS

Effects of bacterial application on growth parameters of Turkish hazelnut cultivars seedling. The results of this study showed that some seedling growth parameters of hazelnut plants were significantly affected by bacterial applications (Table 2). Seedling length per plant was 93.7 cm in the control treatment. However, some of bacterial applications significantly increased seedling length per plant compared with the control. The average increase of seedling length was 4.2, 12.9, 14.6, 11.0, 13.7 and 4.2% for tombul cv and 13.7, 1.0, 17.4, 1.0, 2.0 and 21.7%, respectively for sivri

Table 2: Effects of PGPR applications on some vegetative growth parameters of Turkish hazelnut cultivars (Tombul and Sivri) (two years average n = 12)

Treatment	Seedling length, cm	Total Branch length, cm	Branch number	Trunk diameter, mm
Tombul cv.				
Control	93.66bcd	78.5cd	4.33b	9.55b
N	114.33a	107.83a	4.33b	11.29a
NP	106.33ab	93abcd	4.33b	10.08ab
Lysobacter enzymogenes 9/8	97.66bcd	102ab	5.5b	10.45ab
Arthrobacter agilis 2/3	105.66ab	83.83bcd	4.66b	10.89ab
Paenibacillus macquariensis 59/8	116.6a	96.33abc	4.5b	11.34a
Burkholderia pyrrocinia 13/4	104.0abc	103.33ab	5.16b	10.28ab
Pantoea agglomerans 5/8	106.5ab	88.83abcd	4.33b	10.34ab
Rhizobium radiobacter 42/1	91.33cd	89.83abcd	5.0b	10.76ab
Stenotrophomonas maltophilia 21/1	84.0d	101.66ab	7.33a	10.80ab
Acinetobacter calcoaceticus 47/6	97.66bcd	72.83d	5.0b	9.99ab
	12.441**	19.800*	1.558*	1.360NS
Sivri cv.				
Control	81.33c	71.6d	5.33c	9.46c
N	91.0abc	113.8ab	9.5a	10.89abc
NP	0.91abc	94.5bcd	6.33bc	10.43abc
Lysobacter enzymogenes 9/8	92.5abc	101.0abc	5.33c	11.06ab
Arthrobacter agilis 2/3	80.83c	103.6abc	7.0bc	9.93bc
Paenibacillus macquariensis 59/8	95.5ab	88.8cd	5.0c	10.34abc
Burkholderia pyrrocinia 13/4	80.66c	107.2abc	7.83ab	9.92bc
Pantoea agglomerans 5/8	83.5bc	92.2bcd	5.83bc	10.82abc
Rhizobium radiobacter 42/1	92.66bc	120.6a	6.66bc	11.77a
Stenotrophomonas maltophilia 21/1	88.0abc	91.3bcd	5.16c	9.45c
Acinetobacter calcoaceticus 47/6	99.0a	84.6cd	5.16c	11.16ab
	11.630*	20.439**	2.151*	1.357*

*Values in the same column with different letters are significantly different at $p<0.05$. ** Values in the same column with different letters are significantly different at $p<0.01$. NS: Not significant, N: Nitrogen fertilizer; NP: Nitrogen±Phosphorus fertilizer

Table 3: Effects of PGPR applications on plant nutrient element content of leaves in one year old Turkish hazelnut seedling (Tombul and Sivri) (Two years average n = 12)

	N	P	K	Ca	Mg	Mn	Fe	Zn	B	Na	Al
Tombul cv.											
Control	1.34f	1.08bcd	2.65i	1.07ef	2.00c	849b	147b	88.06b	58.95b	240.5a	164.3a
N	1.42e	0.89f	2.82d	1.08def	2.09abc	585.5d	108d	48.18e	53.43bc	112.5e	137.2b
NP	1.48d	1.17bc	2.85c	1.25ab	2.18a	919a	128.5c	93.01a	75.25a	208.5bc	142.5b
9/8	1.42e	1.06bcd	2.97a	0.95g	2.07bc	520.5f	121c	45.54ef	74.43a	195.0dc	118.8c
2/3	1.79a	0.91f	2.71g	0.99fg	1.74e	642.5c	123c	43.77ef	46.84c	121e	141.1b
59/8	1.67abc	1.08bcd	2.93b	0.91g	1.62f	530.5f	84.5e	65.42d	47.05c	191.5cd	86.3d
13/4	1.66bc	1.03de	2.68h	1.17bcd	2.09abc	549.5def	122c	60.31d	53.86bc	192cd	112.6c
5/8	1.53d	0.92ef	2.80d	0.9g	1.60f	535.5ef	126c	42.17f	54.41bc	229a	112.9c
42/1	1.62c	1.18b	2.75f	1.28a	2.13ab	662c	168.5a	63.98d	53.27bc	238.5a	176.5a
21/1	1.64bc	1.05cd	2.78e	1.19abc	1.87d	582de	131c	80.61c	48.92c	222.5ab	134.4b
47/6	1.70ab	1.93a	2.65i	1.09cde	1.88d	585d	128c	63.28d	47.36c	178.5d	135.8b
	0.058**	0.110**	0.026*	0.096**	0.104*	44.601**	12.009**	4.786**	7.271*	16.755*	12.707**
Sivri cv.											
Control	1.43e	1.21e	2.61hi	1.09bc	2.12b	732b	173bc	61.72de	67.31bc	221 bcd	175b
N	1.44e	1.24de	2.60i	1.03c	2.20b	656c	154de	72.95c	61.03de	211 cd	154c
NP	1.49d	1.65a	2.73c	1.04c	2.09b	891a	189a	74.39c	90.59a	230 bc	204a
9/8	1.51cd	1.39bc	2.71d	1.13bc	2.17b	753b	143e	69.40cd	60.50e	205 d	137d
2/3	1.43e	1.17ef	2.76b	0.91d	1.91c	608cd	119f	74.83c	70.12b	207 d	119e
59/8	1.40e	1.25de	2.82a	1.16ab	2.16b	641cd	188ab	92.96a	67.57bc	228 bc	178b
13/4	1.67b	1.31cd	2.68e	1.27a	2.23ab	886a	166cd	77.11bc	63.90cde	232 b	177b
5/8	1.67b	1.10f	2.65f	1.20ab	2.38a	874a	192a	56.90e	60.21e	215 bcd	182b
42/1	1.50d	1.41b	2.74c	1.04c	2.21ab	607d	172bc	57.43e	61.54cde	212 cd	176b
21/1	1.95a	1.38bc	2.64fg	1.11bc	2.13b	648cd	160cd	92.27a	67.03bcd	251 a	155c
47/6	1.55c	1.21e	2.63gh	1.17ab	2.15b	730b	116f	84.66ab	59.81e	210 cd	147cd
	0.0454**	0.0826**	0.0175*	0.1011*	0.16*	44.137*	15.052**	8.439**	5.681*	17.61*	11.243**

*Values in the same column with different letters are significantly different at p<0.05. ** Values in the same column with different letters are significantly different at p<0.01. N: Nitrogen fertilizer; NP: Nitrogen + Phosphorus fertilizer

cv when *L. enzymogenes*, *B. atrophaeus A. agilis*, *P. macquariensis*, *B. lentus* (Pb. validus), *B. pyrrocinia*, *P. agglomerans* and *A. calcoaceticus* were applied. Similar to seedling length, total branch length, branch number and trunk diameter were also significantly increased by bacterial treatments compared with the control. But some bacterial strains such as *R.radiobacter* 42/1, *S. maltophilia* 21/1 and *A.calcoaceticus* 47/6 decreased seedling length and total branch length compared to the control in Tombul cv and *A. agilis* 2/3, *P. macquariensis* 59/8, *S. maltophilia* 21/1 and *A. calcoaceticus* 47/6 decreased seedling length and total branch number compared to the control in Sivri cv. The result of the present study suggests that maximal seedling length and trunk diameter were obtained in *P. macquariensis* 59/8 and maximum brunch number in *S. maltophilia* 21/1 in Tombul cv. The highest seedling length, total branch length, branch number and trunk diameter of Tombul and Sivri of Turkish hazelnut cultivars was obtained with *A. calcoaceticus* 47/6, *R. radiobacter* 42/1, *S. maltophilia* 21/1, *P. macquariensis* 59/8, respectively and increasing ratio of seedling length, total branch length, branch number and trunk diameter of Turkish hazelnut cultivars was 24.49, 31.60, 69.28 and18.74% for Tombul and 21.72, 68.43, 46.90 and 24.41% for Sivri, respectively, compared to control (Table 2).

Effects of bacterial application on plant nutrient element (PNE) contents of leaves: The Plant Nutrient Element (PNE) contents of leaves treated by PGPR strains may provide important

information about the effect of bacterial inoculation in PNE uptake. PGPR strains could improve production of plant growth regulators or increase plant nutrient uptake. In this study; we have found that bacterial treatments significantly increased plant nutrient element contents of hazelnut leaves compared with the control (Table 3). Root inoculation with *A. agilis* 2/3, *A. calcoaceticus* 47/6, *R. radiobacter* 42/1 and *L. enzymogenes* 9/8 strains promoted N, P and K uptake of hazelnut seedlings (Tombul varieties). The highest Fe, Na and Al contents were obtained from *R. radiobacter* 42/1 and *Lysobacter enzymogenes* 9/8 applications compared to control. Root inoculation of *S. maltophilia* 21/1, *R. radiobacter* 42/1 and *P. macquariensis* 59/8 applications promoted N, P and K uptake of hazelnut seedling (Sivri cv.). The highest Mg, Mn, Fe content of leaves were obtained from the *P. agglomerans* 5/8 application compared the control. Inoculation with PGPR strains promoted significantly seedling growth, but the growth responses were strain and variety-specific. For example, *A. calcoaceticus* 47/6 application significantly increased seedling length in Sivri varieties. In contrast, *A. calcoaceticus* 47/6 was not significant statistically for the same parameter. The positive effects of *A. agilis* 2/3, *P. agglomerans* 5/8, *P. macquariensis* 59/8, *B. pyrrocinia* 13/4, *S. maltophilia* 21/1 and *A. calcoaceticus* 47/6 on the N contents of the leaves may be related to N fixing capacity of these bacterial strains.

DISCUSSION

Two years of trials under greenhouse conditions showed that inoculations with *L. enzymogenes* 9/8, *B. atrophaeus* 55/1, *A. agilis* 2/3, *P. macquariensis* 59/8, *B. lentus* (Pb. validus) 29/6, *B. pyrrocinia* 13/4, *P. agglomerans* 5/8, *R. radiobacter* 42/1, *S.s maltophilia* 21/1, *Acinetobacter calcoaceticus* 47/6 significantly enhanced seedling length, total branch length, branch number and trunk diameter and PNE uptake of Turkish hazelnut cultivars seedling. The nutritional status of the medium was important for the plant growth period. This result may be explained by bacterial applications which may have influenced plant cytokinins and IAA hormone contents. These findings supported by PGPR strains tested on chickpea, sugar beet, barley, corn, raspberry and tomatoes in different soil conditions. In fact the positive effects of PGPR on the yield and growth of crops such as chickpea, apple strawberry, spinach, tomatoes, sugar beet, barley and wheat were explained by N_2-fixation ability, phosphate solubilizing capacity, indole acetic acid (IAA) and antimicrobial substance production (Cakmakci *et al.*, 2001, 2007a, b; Karlidag *et al.*, 2007; Esitken *et al.*, 2010).

Nutrient (N, P, K, Ca, Mg, Fe, Zn, Al, B, Cu and Mn) contents of leaves were significantly increased by bacterial treatments. Kumawat *et al.* (2000) and Hoque *et al.*, (1999) studies show that plant N content were higher in bacterial inoculation than that of N and NP applications.

In addition, exogenous IAA is proven to contribute to colonization efficiency and to the growth and survival of PGPR on host plants (Vandeputte *et al.*, 2005). The presence of high numbers of bacteria in the rhizosphere is important in order to convert insoluble forms of organic and inorganic substances into available plant nutrients, which can affect vegetative growth. Therefore, one of the possible mechanisms by which PGPR enhances hazelnut seedlings' growth is the production of plant growth regulators and available nutrients. This assertion is in agreement with the findings reported by Bent *et al.* (2001), Watanabe *et al.* (2004), Zhang *et al.* (2003), Orhan *et al.* (2006) and Erturk *et al.* (2010). In addition, bacterial inoculations increased Ca, K, Fe, Cu, Mn and Zn in leaves. This increase may also be explained by organic acids production by plants and bacteria in the rhizosphere, which decrease soil pH and stimulate the availability of Ca, Fe, K, Cu, Mn and Zn. These findings a supported by previous studies (Smith and Read, 1997; Sundara *et al.*, 2002; Shen *et al.*, 2004; Karlidag *et al.*, 2007; Cakmakci *et al.*, 2009).

CONCLUSION

The results of this study clearly indicated that the PGPR root inoculations could reduce the deleterious effects of low nutrient content on hazelnut seedling growth. PGPR root inoculation was shown to increase seedling length, branch length and number of improved hormonal metabolism. Among the various PGPR isolates tested, 59/8, 21/1, 47/6, 42/1 strains was the most effective in promoting growth and growth parameter of hazelnut, but 13/4 and 42/1 were most effective in promoting macro and micro nutrient uptake of Turkish hazelnut cultivars (Tombul and Sivri), therefore reducing the need for chemical fertilizers for hazelnut seedlings. Further studies are needed to determine the effect of PGPR tested at these levels and the efficiency of PGPR under natural field condition and other plant species.

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