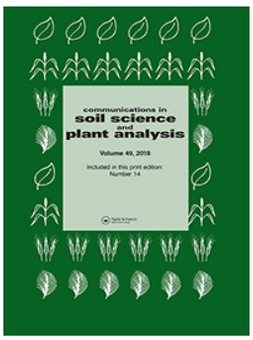

Taylor & Francis
Taylor & Francis Group

Communications in Soil Science and Plant Analysis

ISSN: 0010-3624 (Print) 1532-2416 (Online) Journal homepage: http://www.tandfonline.com/loi/lcss20

Effects of Root Plant Growth Promoting Rhizobacteria Inoculations on the Growth and Nutrient Content of Grapevine

Ummugulsum Erdogan, Metin Turan, Fadime Ates, Recep Kotan, Ramazan Çakmakçi, Yasar Erdogan, Nurgül Kitir & Sefik Tüfenkçi

To cite this article: Ummugulsum Erdogan, Metin Turan, Fadime Ates, Recep Kotan, Ramazan Çakmakçi, Yasar Erdogan, Nurgül Kitir & Sefik Tüfenkçi (2018) Effects of Root Plant Growth Promoting Rhizobacteria Inoculations on the Growth and Nutrient Content of Grapevine, Communications in Soil Science and Plant Analysis, 49:14, 1731-1738, DOI: 10.1080/00103624.2018.1474910

To link to this article: https://doi.org/10.1080/00103624.2018.1474910

Published online: 21 May 2018.

COMMUNICATIONS IN SOIL SCIENCE AND PLANT ANALYSIS
2018, VOL. 49, NO. 14, 1731–1738
https://doi.org/10.1080/00103624.2018.1474910

Effects of Root Plant Growth Promoting Rhizobacteria Inoculations on the Growth and Nutrient Content of Grapevine

Ummugulsum Erdogan[a], Metin Turan[b], Fadime Ates[c], Recep Kotan[d], Ramazan Çakmakçi[e], Yasar Erdogan[f], Nurgül Kitir[b], and Sefik Tüfenkçi[g]

[a]Department of Food Engineering, Faculty of Engineering, Bayburt University, Bayburt, Turkey; [b]Department of Genetic and Bioengineering, Faculty of Engineering, Yeditepe University, Istanbul, Turkey; [c]Viticulture Research Institute, Department of Breeding Technique, Manisa, Turkey; [d]Faculty of Agriculture, Department of Plant Protection, Atatürk University, Erzurum, Turkey; [e]Faculty of Agriculture, Department of Field Crops, Çanakkale Onsekiz Mart University, Çanakkale, Turkey; [f]Demirözü Vocational School, Department of Veterinary, Bayburt University, Bayburt, Turkey; [g]Faculty of Agriculture, Department of Bioengineering, Yüzüncü Yıl University, Van, Turkey

ABSTRACT

The objective of this study was to evaluate the effects of seven nitrogen (N_2)-fixing and/or phosphorus (P)-solubilizing and siderophore-producing microorganism based bio-fertilizers in single and triple strain combinations isolated from the acidic rhizospheric soil of native tea, grapevine, and wild red raspberries. As a result of this study, bacterial efficiency was found to be variable and depended on the bacterial strains and evaluated growth parameters. Plant growth-promoting rhizobacteria (PGPR) has improved macro- and micro-nutrient concentrations in grapevine leaves, and stimulated plant growth. Triple inoculation and single inoculation based bio fertilizers were found to stimulate overall plant growth, including shoot and leaf weight, main shoot length, leaf ground index, chlorophyll, nitrogen, zinc and iron content of grapevine cv 'Italy'. Bio-fertilizers increased the nutrients such as nitrogen, zinc and iron concentrations and consequently increased the chlorophyll content of the leaves.

ARTICLE HISTORY
Received 17 January 2018
Accepted 1 May 2018

KEYWORDS
Chlorophyll content; grapevine; nutrients; PGPR; plant growth; yield

Introduction

Grape has been planted commercially in several areas in Turkey. Commercial grapevine varieties have substantially differed in their nutrient efficiency, and there is experimental evidence that in some cases associative rhizobacteria are involved in the expression of this trait. Chemical fertilizers were used to provide nutrient at a high rate for a long time. This has resulted in pollution, decreased biodiversity in intensively farmed regions, and environmental degradation, which is increasingly widespread and sometimes irreversible. Beneficial microorganisms, including nitrogen (N_2)-fixing bacteria, provide minerals to plants and they directly correlated with efficient crop production. Bio-fertilizers could be considered as a kind of beneficial microorganisms with the ability to mobilize nutrient elements from non-usable to usable form, thereby saving considerable amount of chemical fertilizers needed by the plants. PGPR have been suggested to be important for agriculture with the aim of improving nutrients availability for plants and have been increasingly used worldwide in sustainable agriculture as biological fertilizer (Yildirim et al. 2011a). Some strains of PGPR have been reported to enhance nutrient uptake by plants. Earlier studies indicated that PGPR could improve growth, yield and nutrient uptake of vegetable crops such as tomato, lettuce and broccoli (Turan, Ataoglu, and Sahin 2007; Yildirim, Turan, and Donmez 2008; Yildirim et al. 2011b; Gunes et al. 2009).

CONTACT Ummugulsum Erdogan gulsum25@gmail.com Department of Food Engineering, Faculty of Engineering, Bayburt University, Bayburt, Turkey.

These aforementioned beneficial effects rely on the PGPR-elicited enhanced nutrient availability and nutrient use efficiency. Plant growth promoting bacteria may influence plant growth by synthesizing plant hormones or facilitating the uptake of nutrients from the soil through different direct mechanisms such as biological nitrogen fixation, solubilization of inorganic phosphate, and synthesis of siderophores for iron sequestration making the nutrients more available to plants.

The ability of PGPR to enhance host plant uptake of relatively immobile nutrients, in particular phosphorus (P), and several micronutrients, has been the most recognized beneficial effect of mycorrhiza, by expanding the absorption area. PGPR can promote the growth of phosphate solubilizing microorganisms around plant roots. Once the soluble phosphate has released, then both PGPR and plant roots could benefit from it. Though plants require adequate P in the early stages of growth for optimum crop production, excess P supply in the soil is a major environmental concern (Plenchette et al. 2005). Moreover, the reserves of P in the world are finite and are gradually being depleted. Thus, there is a need to develop agricultural systems based on the factor of meeting minimum P requirements for crops.

Parameters such as prevention of environment pollution, achievement of agricultural sustainability, and necessity to decrease the agricultural cost force the use of improved and organic farming microorganism. N2-fixing and P-solubilizing bacteria are becoming attractive in terms of plant growth and productivity increase, and becoming important in sustainable and organic farming as an alternative for inorganic fertilizers (Cakmakci and Erdogan 2012).

Dense monoculture leads to soil exhaustion and increased pollution in viticulture. Because of overdose in fertilization and high mineralization rate, crucial amount of nitrogen from vineyards is dispersed in underground waters. New and environment-friendly strategies are needed to decrease chemical fertilizer and pesticide use. Additional and alternative sources should be evaluated and nutrient intake efficiency should be increased at plant nutrition. Thus, it is necessary to identify convenient rhizobacteria species to use in viticulture, which could be isolated from local country soils involving high-throughput studies. Improved biological fertilizer formulations that are convenient for agriculture application can help to strengthen the organic grape sector by increasing the quality and productivity of organic viticulture (Cakmakci and Erdogan 2012).

While annual plants provide a lot of information about the activity of N_2-fixing and P-solubilising soil microorganisms, there is very limited information about nitrogen-fixing and phosphate-solubilizing bacteria on perennial plants such as grapevine. The inoculation of grapevine explants with PGPR isolates increase the physiologic activity and plant resistance to cold stress. *Burkholderia phytofirmans* and grapevine studies have shown that bacteria can be transferred to the young fruits after inoculation. On the other hand, it has been determined that *Burkholderia* sp. PsJN isolate excretes high amount of 1-aminocyclopropane-1-carboxylate (ACC) deaminase by decreasing ethylene hormone's preventing effect, and induces grapevine growth (Compant et al. 2005a). Some endophytic bacteria can reach the root xylem and can be colonized on flower, fruit, upper parts and seeds of the grapevine, and also they generate plant growth effects on other plants (Compant et al. 2005b). Field studies have shown that some strains isolated from plant proliferation organs can be colonized especially on grapevine rhizosphere and endosphere (Compant et al. 2010). It was reported that the widespread entophytic bacteria species on grapevine were belong to *Pseudomonas, Enterobacter* and *Bacillus* species (Lo Piccolo et al. 2010). In the studies conducted in Turkey, the bacteria that can be cultured from wild grapevine soils and that are convenient to use as biological fertilizer on viticulture according to specialities as nitrogen fixation and phosphate solubilization specialty have been detected as isolates mostly belonging to *Bacillus, Pseudomonas, Paenibacillus, Brevibacillus* and *Stenotrophomonas* species, and *Pseudomonas putida, Pseudomonas fluorescens, Bacillus megaterium* and *Stenotrophomonas maltophilia* species (Karagöz et al. 2012). Additionally, grapevine rhizospheres could be studied to determine the change in bacteria type according to population, geographic location, soil pH level and vegetation type on the research fields. It was stated that *Pseudomonas* and *Bacillus* species induced callus generation and growth (Kose et al. 2005).

Nowadays, the studies of PGPR effects on herbal growth became important and until now, hundreds of publications have been made. However, this environment-friendly technic used on grape production system is very limited, and researches are still insufficient on grape rhizosphere soils around the world. Organic viticulture gains importance by day in most of vine producer countries. Even so, organic viticulture is at the initial level in most of the countries and the amount of organic vineyard is very limited.

Turkey is one of the grape diversity centers that have a lot of desired specialty in local species growth. However, not only grapevine rhizosphere soils were captured but also detailed bacteria isolation was not performed with the aim of biological fertilizer development in Turkey. In this study, nitrogen-fixing, phosphate-solubilizing and siderophore-producing bacteria isolated from grapevine (Karagöz et al. 2012), tea (Cakmakci et al. 2010) and raspberry (Cakmakci et al. 2008) rhizosphere soils were used to test grapevine growth and their effects on chlorophyll and leaf nutrient content.

Material and methods

Study area and grapevine cultivars

This study was conducted on an experimental area at Ataturk University İspir Hamza Polat Vocational School and Faculty of Agriculture, Department of Crop Science under natural soil conditions in 2015–2016. In the study 'Italian' grape species (grafted on 5BB rootstock) seedlings grown by Manisa Viticulture Research Station were used. According to long-term data/studies (1985–2010) in the experimental area, with an average temperature of 5.7 °C and total rainfall of 402.5 mm, plant growth in the region is restricted to the period between May and October. The first year of the experiment received higher distribution of rainfall in the growing period, while average monthly air temperature was generally close in the first and the second years of the experiment. Average daily air temperature ranged between 9.4 °C and 20.0 °C in the growing season of 2015, and between 6.0 and 19.5 °C in the growing season of 2016.

Bacterial strain collection and its characteristics

The bacterial strains (*Bacillus megaterium* RC07, *Pseudomonas putida* RC06, *Bacillus subtilis* RC11, *Pseudomonas putida* FA19d, *Pseudomonas putida* FA19b, *Pseudomonas fluorescens* RC77, *Bacillus subtilis* RC63 and *Serratia marcescens* K2f) used in the study were obtained from the culture collection unit of the Department of Field Crops, Faculty of Agriculture, Ataturk University. The isolate was tested for N_2-fixing ability (Döbereiner 1988) and phosphate solubilization capacity on the National Botanical Research Institute's phosphate growth medium (NBRIP-BPB). The ability of rhizobacterial isolates to grow on Döbereiner nitrogen-free culture medium indicated their non-symbiotic N_2-fixation ability.

Phosphate solubilizing capacity of the isolates was tested qualitatively in NBRI-BPB liquid medium according to Mehta and Nautiyal (2001). Selected and purified bacterial colonies were inoculated to (50μI inoculum with approximately 1 to 2×10^9 cfu ml-1148) 5mI NBRI-BPB medium. Autoclaved and uninoculated medium served as the control (Yildirim, Turan, and Donmez 2008). It was also determined that the bacterial strain used in the study had 1875.32 ng μl-1 gibberellic acid, 810.36 ng μl-1 salicylic acid, 72.35 ng μl-1 IAA and 0.8 0.83 ng μl-1 ABA.

Seedling inoculation

The seedlings were first surface disinfected to avoid the presence of any saprophytic and/or pathogenic microorganisms on the seedling surface. Bacteria were grown in 50 ml flasks containing 20 mL of TSB medium on a rotary shaker at 27°C for 24 hours. The absorbance of the bacterial suspensions was measured spectrophotometrically at 600 nm and appropriately diluted to 1×10^8 CFU ml-1 in sd. H_2O.

Initially, before planting, inoculated seedlings were waited in liquid bio-fertilizer for 60 min, and 30 days after planting, liquid formulations were injected into the root area as 5 ml/seedling.

Field manipulation, experimental setup, and treatments

The red soils used for the studies were identified as orthic acrisols (FAO/UNESCO) or ultisols as per United States Department of Agriculture. The experiment was initiated in late April 2015 and repeated in 2016, as well. The soil of the experimental area during both years was analyzed and given in Table 1.

The experiment was arranged as a completely randomized block design including biological fertilizer formulation based on seven microorganisms and one control without bio-fertilizer application. It was repeated for three times in eight applications and in each repetition included nine plants. The study was conducted in duplicate. Seedlings were planted in unused arable land, weeding was done by hand when required, and any chemical pesticides or fertilizers not used. Plants were adequately irrigated to prevent water stress.

Chlorophyll readings

Chlorophyll contents of the top fifth and sixth leaves at mid-morning were measured using a portable chlorophyll meter (SPAD-502, Konica Minolta Sensing, Inc., Japan) to measure leaf greenness of the plants. SPAD readings were taken when average shoot length was 80 cm and 60 days after planting. Measurements were taken for each leaf from four locations and from two locations on each side of the midrib on all fully expanded leaves. The same leaves were used for chemical analyses (Alcantar et al. 2002).

Table 1. Chemical properties of the experimental field soils before the experiment (mean±standard deviation).

		2015		2016	
		Soil Depth		Soil Depth	
Soil Properties	Units	0–30 cm	30–60 cm	0–30 cm	30–60 cm
Clay	%	17.30 ± 0.87	20.50 ± 1.10	[a]ND	ND
Silt	%	31.25 ± 0.80	26.30 ± 0.90	ND	ND
Sand	%	51.65 ± 1.85	53.20 ± 1.60	ND	ND
Cation exchangeable capacity[b]	$cmol_c/kg$	23.20 ± 2.40	19.10 ± 1.35	ND	ND
Total N	g/kg	1.6 ± 0.03	1.12 ± 0.06	0.7 ± 0.02	0.3 ± 0.06
pH (1:2 soil:water)		7.30 ± 0.2	7.45 ± 1.14	7.20 ± 0.17	7.30 ± 1.10
Organic C	g/kg	16 ± 0.10	8 ± 1.70	13 ± 0.10	6 ± 1.90
$CaCO_3$	g/kg	128 ± 10	210 ± 30	110 ± 0.20	127 ± 0.20
Plant available P[c]	mg/kg	9.2 ± 1.60	8.2 ± 0.40	8.2 ± 0.70	3.9 ± 0.30
Exchangeable Ca[d]	$cmol_c/kg$	16.0 ± 2.20	19.1 ± 0.03	15.0 ± 1.10	17.4 ± 0.01
Exchangeable Mg[d]	$cmol_c/kg$	4.10 ± 0.40	3.20 ± 0.11	3.2 ± 0.60	2.5 ± 0.10
Exchangeable K[d]	$cmol_c/kg$	6.0 ± 0.80	4.2 ± 0.07	5.1 ± 0.50	3.0 ± 0.03
Exchangeable Na[d]	$cmol_c/kg$	0.80 ± 0.05	1.10 ± 0.12	0.60 ± 0.07	0.80 ± 0.10
Available Fe[e]	mg/kg	3.50 ± 0.30	3.30 ± 0.10	2.60 ± 0.20	1.25 ± 0.04
Available Mn[e]	mg/kg	3.60 ± 0.08	4.10 ± 0.07	1.25 ± 0.07	1.10 ± 0.05
Available Zn[e]	mg/kg	0.16 ± 0.15	0.11 ± 0.03	1.20 ± 0.15	1.10 ± 0.01
Available Cu[e]	mg/kg	2.10 ± 0.13	1.55 ± 0.03	1.00 ± 0.10	0.60 ± 0.02
Available B[f]	mg/kg	0.015 ± 0.005	0.014 ± 0.003	0.14 ± 0.07	0.08 ± 0.02
Electric conductivity	dS/m	1.12 ± 0.03	2.21 ± 0.01	1.15 ± 0.02	1.11 ± 0.02

[a]ND: Not done,
[b]Sodium acetate at pH 8.2 according to Sumner and Miller (1996),
[c]Sodium bicarbonate according to Olsen et al. (1954)
[d] Ammonium acetate at pH 7.0 according to Thomas (1982),
[e] DTPA extraction according to Lindsay and Norvell (1978)
[f]Azomethine-H extraction according to Wolf (1974)

Soil analysis

Soil samples were taken from 2 different depths (0–30 and 30–60 cm) to determine baseline soil properties. At each soil depth, soil cores were collected from two randomly chosen row in each of the eight treatments, composted and analyzed, and a sample consisting of two sub-samples was taken from each of three grapevine inter-rows situated in the plot center. Soil samples were air-dried, crushed and passed through a 2-mm sieve prior to chemical analysis.

Cation exchange capacity (CEC) was determined using sodium acetate (buffered at pH 8.2) and ammonium acetate (buffered at pH 7.0). The Kjeldahl method was used to determine total N, while plant-available P was determined by using the sodium bicarbonate method. Electrical conductivity (EC) was measured in saturation extracts. Ammonium acetate buffered at pH 7 was used to determine exchangeable cations. Available iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) in the soils were determined with diethylene triamine pentaacetic acid (DTPA) extraction methods. Available boron (B) was analyzed for extractable B using the azomethine-H extraction and a UV/VIS (Aqumat) spectrophotometer (Thermo Electron Spectroscopy, UK). Soil organic matter was determined using the Smith–Weldon method, soil pH was determined in 1:2 extracts, and calcium carbonate concentrations were determined according to Page, Miller, and Keeney (1982). The soil characterization data are presented in Table 1.

Plant sampling and analytical methods

Each plot had nine rooted grafted grapevine saplings. Four leaf samples were taken each plant and brought to the laboratory. The cleaning procedure was conducted by washing the leaves with pure water. Then, leaf samples were oven-dried at 65°C until their weight was constant, and were then ground and sieved through a 50-mesh screen. Kjeldahl method and a Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Germany) were used to determine total N (Bremner 1996). After extraction, tissue P, potassium (K), calcium (Ca), magnesium (Mg), Fe, Mn, Zn, and Cu were determined with an inductively Coupled Plasma spectrophotometer (Perkin-Elmer, Optima 2100 DV, ICP/OES, Perkin-Elmer, Waltham MA, USA).

Statistical analysis

All data were subjected to analysis of variance (ANOVA). Significant means were compared by Duncan's multiple range test method and analysis performed with SPSS 13.0 (SPSS Inc., Chicago, IL). Mean differences were considered significant if $P \leq 0.05$.

Results and discussions

In the study, seven selected bacteria pure were prepared in liquid porter forms from the bacterial stocks isolated different sources. Their nitrogen fixation, phosphate solving and siderophore production characteristics were analyzed in comparison to the control group. The average of the two-year data revealed that the application of PGPR strains significantly affected the morphological characteristics of grapevine (Table 2). Various growth parameters, i.e., main shoot length, shoot diameter, leaf area index, total branch+leaf weight and total leaf chlorophyll index demonstrated a significantly higher response to PGPR application compared to the control. Intriguingly, maximum response was recorded when mixed bacteria combination of *Pseudomonas fluorescens* RC77, *Bacillus megaterium* RC07 and *Bacillus subtilis* RC63 was applied.

Main shoot length values were calculated through *Pseudomonas putida* FA19d application. This is followed by analysis of *B. megaterium* RC07, triple combination (*P. fluorescens* RC77 + *B. megaterium* RC07 + *B. subtilis* RC63) and *Bacillus subtilis* RC11 based bio fertilizer applications. The differences between shoot diameter values were not found statistically important. All fertilizers increased the leaf area index except for

Table 2. Effects of different liquid microorganism based bio-fertilizers on grafted grapevine sapling growth and leaf chlorophyll content.

Application	Main shoot length (cm)*	Shoot diameter		Leaf area index*	Total branch+leaf weight (g)*	Total leaf chlorophyll index (SPAD)*	
		2. node	4. node			5. leaf	6. leaf
Control	114.8 c	6.42	5.41	2.07 c	272 c	35.1 b	34.2 b
FA19d	156.3 a	6.56	5.83	2.97 a	363 b	37.9 ab	36.6 ab
RC11	143.5 ab	6.37	5.58	2.91 a	494 a	38.1 ab	37.2 ab
RC77+ RC07+RC63	144.7 ab	6.31	5.57	2.89 a	488 a	38.4 a	36.5 ab
K2f	125.8 bc	6.09	5.60	2.42 bc	340 b	36.4 ab	36.3 ab
RC07	147.2 ab	6.11	5.74	2.99 a	470 a	39.5 a	38.9 a
RC06	139.8 a-c	6.19	5.55	2.87 a	338 b	37.7 ab	36.8 ab
FA19b	116.0 c	5.90	5.33	2.49 b	228 c	36.9 ab	36.2 ab

*Different letters within the same column indicate significant differences according to Duncan's Multiple Range Test ($p \leq 0.05$)

Serratia marcescens K2f. All applications increased the total branch+leaf weight except *Pseudomonas putida* FA19b when compared to control. Bio-fertilizer applications increased the chlorophyll content (SPAD) values that measured in 5th and 6th grapevine leafs, However, when it is compared to control group, the arising differences at 5th leaf triple combination (*P. fluorescens* RC77 + *B. megaterium* RC07 + *B. subtilis* RC63) and at *B. megaterium* RC07, if 6th leaf *B. megaterium* RC07 were found to be significant. The highest leaf ground index and total branch leaf weight increase values were obtained from *B. megaterium* RC07, *B. subtilis* RC11 and triple combination (*P. fluorescens* RC77 + *B. megaterium* RC07 + *B. subtilis* RC63) of bio-fertilizers applications prepared based on bacteria.

According to the two years data, it was evident that the application of N_2-fixing and P-solubilising PGPR strains promoted the contents of all nutrient elements of grapevine plant when compared to control. Under uninoculated control treatment, N (1.84%), P (0.37%), K (0.17%), Ca (5.22%), Mg (0.20%), Fe (189 mg kg^{-1}), Cu (132 mg kg^{-1}), Zn (40 mg kg^{-1} and Mn (98 mg kg^{-1}) in grapevine leaves were recorded, though, N (2.32%), P (0.54%), K (1.60%), Ca (5.24%), Fe (324 mg kg^{-1}), Zn (63 mg kg^{-1}) and Mn (168 mg kg^{-1}) was the highest in RC77+RC07+RC63 treatments. However, the increase in the contents of nutrient elements in grapevine leaves was higher when plants were grown under bacterial inoculations. The measured concentrations of plant nutrients were generally within the accepted critical levels (Jones, Wolf, and Mills 1991; Mills and Jones 1996).

The results presented here support the hypothesis that inoculation with PGPR alone or combined can improve plant growth and the nutrient contents of the plants. Enhancement of mineral uptake by plants should result in an increased accumulation of both dry matter and minerals in leaves of the plant. During the reproductive period, the accumulated minerals would be transferred to the reproductive parts of the plants.

Some of the previous studies with the same PGPR strains tested on sugar beet, chickpea, barley, raspberry, apricot and sweet cherry have reported similar findings supporting our data in the present work. The use of the OSU-142 and M-3 in chickpea (Elkoca, Kantar, and Sahin 2008), rocket (Dursun, Ekinci, and Donmez 2008) and strawberry (Gunes et al. 2009) stimulated macro- and micronutrient uptake such as N, P, K, Ca, Mg, Fe, Mn, Zn, and Cu.

Leaf macro and micro-element content changed with the application of multi-traits bacteria (Table 3). 6 of the 7 biological fertilizer formulations increased their N and Fe content when compared to control. All formulations increased their Mn content. Leaf P and K contents were increased with bacterial applications, however important differences were found in P content of RC77+RC07+RC63 fertilizer and K content of RC11 and RC07 fertilizer compared to control. Triple combination (*P. fluorescens* RC77 + *B. megaterium* RC07 + *B. subtilis* RC63) and single *B. subtilis* RC11 and *B. megaterium* RC07 bio-fertilizer applications prepared based on bacteria increased leaf iron, zinc and manganese content hence chlorophyll amount. The use of the RC77, RC07 and RC63 in tea (Cakmakci et al. 2013) stimulated macro- and micro-nutrient uptake, yield, and quality parameters was evaluated.

Table 3. Effects of different liquid microorganism based bio-fertilizers on grafted grapevine sapling growth and leaf macro and microelement quantity.

	Macro element quantity (%)*					Micro element mg/kg dry material*			
Application	N	P	K	Ca	Mg	Fe	Zn	Mn	Cu
Control	1.84 c	0.37 c	0.17 c	5.22 a	0.20 a	189 d	40 c-e	98 d	132 a
FA19d	2.06 b	0.50 ac	1.38 bc	5.43 a	0.10 c	264 bc	43 ce	126 c	110 ab
RC11	2.10 b	0.39 bc	2.56 a	5.43 a	0.12 bc	284 b	58 ab	161 ab	97 b
RC77+RC07+RC63	2.32 a	0.54 a	1.60 bc	5.24 a	0.11 c	324 a	63 a	168 a	97 b
K2f	2.16 b	0.46 ac	1.46 bc	5.36 a	0.11 c	228 cd	36 e	136 bc	111 ab
RC07	2.10 b	0.52 bc	1.99 b	5.52 a	0.11 c	264 bc	49 bc	141 bc	99 b
RC06	2.17 b	0.47 ac	1.46 bc	5.39 a	0.09 c	233 c	37de	138 bc	101 b
FA19b	1.81 c	0.41 ac	1.17 c	5.34 a	0.14 b	253 bc	48 b-d	126 c	104 b

* Different letters within the same column indicate significant differences according to Duncan's Multiple Range Test ($p \leq 0.05$).

It is concluded that leaf chlorophyll content increased increasing iron and related to zinc content (Shaaban, Loehnertz, and El-Fouly 2007) and leaf SPAD values show correlation with carbohydrates as needed in photosynthesis. It is stated that organic fertilizer applications increased the chlorophyll content (Belal 2006). This is related to increased generation of nitrogen, magnesium and iron intake and thus total chlorophyll amount increases (Harhash and Abdel-Nasser 2000).

Conclusions

These study results were found important especially because of these fertilizers are convenient to use on the fields with chlorosis problem. However, detailed farm trials should be performed on this subject.

(1) It is revealed that in 'Italian' grapevine species, leaf nutrient content, leaf chlorophyll amount, leaf area index and vegetative growth can be promoted by using bacteria based liquid biological fertilizer.
(2) In addition, N, Fe, Zn and Mn contents of grapevine can be increased by PGPR. The tested bio-fertilizers can be used in conventional and organic farming, and it can be said that bio-fertilizers can contribute to the prevention of chlorosis in the fields.
(3) Screening of rhizobacteria that show multiple plant growth-promoting traits suggests that they have better potential for field testing and applications in improving the growth of grapevine.

References

Alcantar, G., M. Sandoval, J. Z. Castellanos, F. Mendez, P. Sanchez, and M. N. Rodriguez. 2002. Plenary paper diagnostic methods to evaluate nutrient status of garlic, onion, and broccoli. *Communication Soil Science and Plant Analysis* 33:2585–98.

Belal, E. A. 2006. *Effect of some kinds of fertilizers on yield and quality of Thompson Seedless grapevines (Vitis vinifera L.).* PhD diss, Faculty of Agriculture, Mansoura University.

Bremner, J. M. 1996. *Nitrogen-total. Methods of soil analysis part 3: Chemical methods*, Eds. J. M. Bartels, and J. M. Bigham., 1085–121. Madison, Wisconsin: The Soil Science Society of America and the American Society of Agronomy.

Cakmakci, R., M. F. Donmez, Y. Erturk, M. Erat, A. Haznedar, and R. Sekban. 2010. Diversity and metabolic potential of culturable bacteria from the rhizosphere of Turkish tea grown in acidic soils. *Plant and Soil* 332:299–318.

Cakmakci, R., and U. Erdogan. 2012. *Organik Tarım. 3. Baskı*, 367. Erzurum, Turkey: Ataturk University. Faculty of Agriculture Lecture Publications No: 236.

Cakmakci, R., U. Erdogan, B. Oral, and M. F. Donmez. 2008. Coruh vadisinde yabani ahududu rizosfer topraklarında heterotrof azot fikseri bakteri çeşitliliği. Paper presented at the 4th National Plant Nutrition and Fertilizer Congress, Konya, Turkey, 10 October, 706–17.

Cakmakci, R., Y. Erturk, R. Sekban, A. Haznedar, and A. Varmazyari. 2013. The effect of single and mixed cultures of plant growth promoting bacteria and mineral fertilizers on tea (*Camellia sinensis*) growth, yield and nutrient uptake. *Soil Water Journal, Secial Issue for Agricasia* 2:653–62.

Compant, S., B. Duffy, J. Nowak, C. Clement, and E. Ait Barka. 2005a. Use of plant growth promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. *Applied and Environmental Microbiology* 71:4951–59.

Compant, S., B. Reiter, A. Sessitsch, J. Nowak, C. Clement, and E. Ait Barka. 2005b. Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. *Applied and Environmental Microbiology* 71:1685–93.

Compant, S., C. Clément, and A. Sessitsch. 2010. Plant growthpromoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology & Biochemistry* 42:669–678.

Döbereiner, J. 1988. Isolation and identification of root associated diazotrophs. *Plant and Soil* 110:207–12.

Dursun, A., M. Ekinci, and M. F. Donmez. 2008. Effects of inoculation bacteria on chemical content, yield and growth in Rocket (*Eruca vesicaria* subsp. *sativa*). *Asian Journal Chemistry* 20:3197–202.

Elkoca, E., F. Kantar, and F. Sahin. 2008. Influence of nitrogen and phosphorus solubilizing bacteria on the nodulation, plant growth and yield of chickpea. *Journal of Plant Nutrition* 31:157–71.

Gunes, A., N. Ataoglu, M. Turan, A. Esitken, and Q. M. Ketterings. 2009. Effects of phosphate solubilizing micro-organisms on strawberry yield and nutrient concentrations. *Journal of Plant Nutrition and Soil Science* 172:385–92.

Harhash, M. M., and G. Abdel-Nasser. 2000. Effect of organic manures in combination with elemental sulphur on soil physical and chemical characteristics, yield, fruit quality, leaf water contents and nutritional status of Flame seedless grapevines. *Journal of Agricultural Science* 25:2819–37.

Jones, Jr. J.B., B, Wolf and H.A. Mills. 1991. Plant analysis handbook: a practical sampling, preparation, analysis, and interpretation guide. Micro-Macro Publishing Inc. Athens, Georgia, USA.

Karagöz, K., F. Ateş, H. Karagöz, R. Kotan, and R. Çakmakçı. 2012. Characterization of plant growth-promoting traits of bacteria isolated from the rhizosphere of grapevine grown in alkaline and acidic soils. *European Journal of Soil Biology* 50:144–50.

Kose, C., M. Guleyuz, F. Sahin, and I. Demirtas. 2005. Effects of some plant growth promoting rhizobacteria (PGPR) on graft union of grapevine. *Journal of Sustainable Agriculture* 26:139–47.

Lindsay, W.L., and W.A. Norvell. 1978. Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Science Soceity American.J.* 42:421–428.

Lo Piccolo, S., V. Ferraro, A. Alfonzo, L. Settanni, D. Ercolini, S. Burruano, and G. Moschetti. 2010. Presence of endophytic bacteria in *Vitis vinifera* leaves as detected by fluorescence in situ hybridization. *Annals of Microbiology* 60:161–67.

Mehta, S., and C. S. Nautiyal. 2001. An efficient method for qualitative screening of phosphate-solubilizing bacteria. *Current Microbiology* 43:51–56.

Mills, H. A., and J. B. Jones. 1996. *Plant analysis handbook II. Micromacro publishing*. 183 Paradise Blvd Ste 104, Athens, Georgia.

Olsen, S. R., C. V. Cole, F. S. Watanabe, and L. A. Dean. 1954. Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate. U. S. Department of Agriculture Circular No. 939. Banderis, A. D., D. H. Barter and K.Anderson. Agricultural and Advisor

Page, A. L., R. H. Miller, and D. R. Keeney, eds.. 1982. *Methods of Soil Analysis Part 2. Chemical and microbiological properties*, 2nd ed. Agronomy. No: 9. Madison, Wisconsin, USA.

Plenchette, C., C. Clermont-Dauphin, J. M. Meynard, and J. A. Fortin. 2005. Managing arbuscular mycorrhizal fungi in cropping systems. *Canadian Journal Plant Science* 85:31–40.

Shaaban, M. M., O. Loehnertz, and M. M. El-Fouly. 2007. Grapevine genotypic tolerance to lime and possibility of chlorosis recovery through micronutrients foliar applications. *Internaional Journal of Botany* 3:179–87.

Sumner, M.E., and W.P. Miller. 1996. Cation Exchange Capacity and Exchange Coefficients. In: Sparks, D.L., Ed., Methods of Soil Analysis Part 3: Chemical Methods, SSSA Book Series 5, Soil Science Society of America, Madison, Wisconsin, 1201–1230.

Thomas, G.W. 1982. Exchangeable cations. In: Methods of Soil Analysis. (AL Page et al, eds) *Agronomy* 9:154–157, Madison.

Turan, M., N. Ataoglu, and F. Sahin. 2007. Effects of Bacillus FS-3 on growth of tomato (*Lycopersicum esculentum* L.) plants and availability of phosphorus in soil. *Plant, Soil and Environment* 53:58–64.

Wolf, B. 1974. Improvements in the azomethine-H method for determination of boron. *Communications in Soil Science and Plant Analysis* 5:39–44.

Yildirim, E., H. Karlidag, M. Turan, A. Dursun, and F. Goktepe. 2011a. Growth, nutrient uptake and yield promotion of broccoli by plant growth promoting rhizobacteria with manure. *Hort Science* 46:932–36.

Yildirim, E., M. Turan, and M. F. Donmez. 2008. Mitigation of salt stress in radish (*Raphanus sativus* L.) by plant growth promoting rhizobacteria. *Romanian Biotechnoligical Letters* 13:3933–43.

Yildirim, E., M. Turan, M. Ekinci, A. Dursun, and R. Cakmakci. 2011b. Plant growth promoting rhizobacteria ameliorate deleterious effect of salt stress on lettuce. *Scientific Resources and Essays* 6:4389–96.