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ORIGINAL ARTICLE

Sustainable Mitigation of Alkaline Stress in Grapevine Rootstocks (*Vitis* spp.) by Plant Growth-Promoting Rhizobacteria

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Abstract Practical use of plant growth-promoting rhizobacteria (PGPR) on plants under stress conditions remains elusive because most of the studies focused on merely evaluating the plant growth-promoting effects on non-stressed plants. This study focused on the effect of root inoculation of different PGPRs on the growth and physiology of grapevine rootstocks 41 B, 99 R and 140 Ru grown in soilless culture with elevated pH. The rootstocks in pots under glasshouse condition were inoculated with *Agrobacterium rubi* A18 and *Bacillus subtilis* OSU 142 bacteria in early spring. To increase the pH of growth medium, the plants were watered with 250 mL plant^{-1} bicarbonate solution (840 g L^{-1} $NaHCO_3$) four times (beginning at 3–4 cm shoot growth) with one-month interval during the vegetation. Along with the bicarbonate supplementation to growth medium, root rhizosphere pH increased from an initial value 7.76 to the final values between 8.10 and 8.26. Although the bacteria population decreased progressively, they were able to alleviate the negative effects of high pH by improving vegetative growth, leaf physiology and nutrient acquisition in many cases. The bacteria strains employed in this study can be recommended to support grapevine growth and physiology under alkaline conditions for a sustainable and environment-friendly viticulture.

Keywords Grapevines · Alkaline stress · Sustainable agriculture

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Nachhaltige Reduzierung der durch alkalische Bodenverhältnisse verursachten Belastungen bei Weinreben (*Vitis* spp.) mit Hilfe von pflanzenwachstumsfördernden Rhizobakterien

Schlüsselwörter Weinreben · Alkalische Belastung · Nachhaltige Landwirtschaft

Introduction

Approximately 30% of the world's total land area is calcareous soils with their intrinsically high calcium carbonate content and alkalinity (Sánchez-Rodríguez et al. 2014). Bioavailability of certain micronutrients is low in these soils since high pH restricts the solubility of nutrients. Therefore, nutrient deficiency in plants is a worldwide problem affecting many crops cultivated in calcareous soils. Studies on grapevines revealed that the high content and reactivity of carbonate in soil adversely affected the leaf mineral content (Bavaresco and Poni 2003) and caused significant chlorosis (Sabir et al. 2010), resulting from disturbed Fe metabolism due to elevated pH in rhizosphere. To alleviate negative impacts of high pH, soil and foliar fertilizations are increasingly adopted to various crops. However, excessive use of chemical fertilizers to cope with such abiotic stress factors cause the loss or depletion of topsoil and damage the environment by toxic materials, leaching into rivers and water reservoirs, and contaminating our drinking water (Denholm et al. 2002). Fertility of soils in the last decades decreased at an alarming rate due to improper use of chemicals, causing disturbances in the ecological balance and health of beneficial microorganisms, polluting underground water, making plants more susceptible to the stress factors. Thus, a rising portion of disturbed soils in the world leads to an urgent

need of successful remediation strategies, one of which can be development of satisfactory alternatives for supplying the nutrients such as bio fertilizers and thereby protect both the environment and human health. Enhanced diversity of efficient microbial community in degraded ecosystems establish a functional equilibrium, which help maintain agricultural sustainability. Therefore, microbes play the role of chief ecological engineers in resolving the environmental problems as well as the innovative tool to reinstate the degraded ecosystems (Singh 2015). Hence, future challenges will be priory focus on enhancing ecological and sustainable methods to alleviate such stress events.

Studies revealed a wide range of beneficial influences of plant growth-promoting rhizobacteria (PGPR) such as enhanced vegetative development and mineral acquisition in apricot (Esitken et al. 2003), strawberry (Esitken et al. 2010) and grapevine (Sabir et al. 2012). PGPRs were also tested for pea under drought (Arshad et al. 2008) and for strawberries under saline (Karlidag et al. 2011) or calcareous conditions (Ipek et al. 2014). Although many studies have proven the alleviating effects of PGPRs on such stress factor, the practical application of PGPRs largely remain elusive because most of the studies focus on merely evaluating the plant growth-promoting effects under non-stressed conditions. Further, it is always a question mark to study the fate of introduced microorganisms on its survival. Therefore, this study was conducted to investigate the colonization and population changes of PGPR strains in the rhizosphere of alkaline-stressed grapevines and their expeditious mitigating effects on development, physiology and mineral acquisition of vines under stress.

Materials and Methods

Plant Materials and Study Design

This study was carried out in the research and implementation glasshouse of Selcuk University (Konya, Turkey) in 2014. The experimental layout was a two factors randomized complete block design with three rootstocks [41 B (*V. vinifera* × *V. belandieri*), 140 Ru (*V. berlandieri* × *V. rupestris*) and 99 R (*V. berlandieri* × *V. rupestris*)] and three treatments (non-treated control, *Agrobacterium rubi* A18 and *Bacillus subtilis* OSU 142). Each treatment has three replications consisted of three healthy plants. In the winter, two years old healthy vines were selected on the basis of homogeneity in growth. The vines were individually cultivated under controlled glasshouse conditions in 10 L (solid volume) pots (20 cm diameter, 28 cm height) filled with sterile peat (1.034% N, 0.94% P_2O_5, 0.64% K_2O pH 5.88, Klassman®) and perlite (0–3 mm in diameter) in equal volume. The pots were isolated from the ground with plastic sheets to prevent external infection. Before bud break, the vines were pruned to leave only the single main shoot per plant and cultivated in a controlled glasshouse under ambient light and temperature. Night and day temperatures inside the glasshouse were 18 ± 4 and 32 ± 4 °C respectively (Data logger, Ebro EBI 20 TH1). The plants were watered daily with equal amount of tap water (0.5 to 1.0 L per plant according to weather conditions) to maintain the moisture at approximately 60% water holding capacity of the cultivation medium. The shoots were tied with thread to the wires 2.5 m above the pots to let plants grow on a perpendicular position to ensure equally benefiting from the sunlight (Sabir 2013). All the vines received the same annual amount of fertilizer (20 g N, 12 g P, 20 kg K, and 1.5 g Fe chelate per vine) from April to July.

Bacterial and Bicarbonate Applications

Agrobacterium rubi A18 and *Bacillus subtilis* OSU 142 were selected as inoculants on the basis of their abilities as presented in Table 1. The bacterial strains were grown on nutrient agar (NA, containing 3 g beef extract, 5 g peptone and 15 g agar L^{-1}) for routine use. A single colony was transferred to 250 mL flasks containing nutrient broth and grown aerobically in flasks on a rotating shaker (95 rpm) for 24 h at 27 °C. Inoculation were performed by watering the plants with bacterial solutions [with the concentration of 10^9 Colony Forming Unit (CFU) mL^{-1}] seven days after bud break. The first bicarbonate ($NaHCO_3$) applications was performed when the shoots were 3–4 cm long (02.05.2014) and a total of four applications were performed with one month intervals to increase soil pH gradually. For every application, the plants were watered with 250 mL $plant^{-1}$ bicarbonate solution (840 g L^{-1} $NaHCO_3$) (Sabir et al. 2010).

Root Colonization and Soil pH

The root colonization of bacteria was tested 2 weeks after each bicarbonate application. Rhizosphere sample, consist-

Table 1 Characteristics of the bacterial strains used. (Arkhipova et al. 2005; Esitken et al. 2003; Nadeem et al. 2007, 2009; Wei et al. 2015)

Code	Species	Axuin production	Cytokinin production	ACC-deaminase activity	$Ca(HCO_3)_2$ biomineralization
A18	*Agrobacterium rubi*	+	+	+	+
OSU 142	*Bacillus subtilis*	+	+	+	+

ing of a piece of root and tightly adhering soil of each individual plant was carefully collected from the pots. In order to obtain bacterial cells from the rhizosphere soil, 1 g root samples were soaked in 9 mL of sterile saline with shaking at 200 rpm for 30 min. Serial dilutions of the cell suspensions were made and plated on lysogeny broth medium supplemented with chloramphenicol (10 mg L^{-1}) and rifampicin (50 mg L^{-1}). The plates were incubated at 28 °C for 2 days before the number of colonies was counted (Xue et al. 2009). The pH of growth medium was measured in de-ionized water two weeks after each inoculation. pH was determined electrometrically (pH meter, Seven Easy, Switzerland) on a 1:5 (w/v) dry soil:water suspension after 2 h stirring using a glass membrane electrode at 25 °C (Richards 1954).

Investigations on Plant Growth, Physiology and Nutrient Acquisition

Shoot length was (with a sensitivity of 1 mm) measured with 2–4 days' intervals (Sabir et al. 2012). Shoot diameter (measured by digital calipers at 1 cm above the second node), leaf temperature (T_{leaf}) and stomatal conductance (g_s) investigations were carried out one month after each inoculation. T_{leaf} and g_s were recorded at around 10 a. m. (Sabir and Yazar 2015) using a portable porometer (SC-1 Leaf Porometer). Measurements were performed on a total of twelve south-facing, sun-exposed mature leaves born at the top 5th to the 7th nodes per treatments (Stavrinides et al. 2010).

Investigations on other leaf characteristics were performed one month after the last bicarbonate application when the shoots elongation was approaching cessation at the end of the vegetation period (in September). Leaf greenness index (SPAD meter value mean, expressed as SPAD units) of 3rd and 4th nodes of each shoot was estimated by SPAD readings using a portable chlorophyll meter (SPAD-502; Konica Minolta Sensing, Inc., Japan) the leaves at the top. Leaf (node) number per shoot, leaf area (LA, cm^2), leaf fresh mass (FM, g), leaf dry mass (DM, g), leaf relative water content (RWC) and leaf blade element analyses were performed on fully expanded leaves of representative grapevines of each treatment (Tramontini et al. 2013). Three groups of mature leaves, consisting of fifteen leaves per treatment, were collected from the mid-shoot area of each plant (OIV 1997) in the early morning. The first group was scanned to determine single LA using WinFolia computer software program (Régent Instruments, Quebec, Canada), while the second was immediately weighed to determine FM. After weighing, they were hydrated to near maximum turgor by immersing in distilled water for four hours (Yamasaki and Dillenburg 1999). During the rehydration period, leaf samples were weighed periodically up to a constant value to ensure full rehydration. Measurements were performed after gently wiping the water from the leaf surface with tissue paper. At the end of rehydration period, leaf samples were weighed to obtain final turgid mass (TM) and placed in an oven (Turner 1981), at 70 °C for 48 h in order to obtain DM. All mass measurements were made using an analytical scale, with precision of 0.0001 g. Values of FM, TM, and DM were used to calculate RWC, using the equation suggested by Gonzalez and Gonzalez-Vilar (2003):

$$RWC\ (\%) = [(FM - DM)/(TM - DM)] \times 100.$$

The third group of leaves was dried and ground for quantitative macro and microelement analyses. This was performed by inductively coupled plasma optical emission spectrometry (Vista-Pro Axial, Varian Pty Ltd, Mulgrave, Australia) (AOAC 1970). Element analysis results were checked using certified standard reference materials obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA). At the end of the study in the following winter, quantitative pruning weight of one-year-old canes was recorded for comparison of vine baseline vigor levels.

Statistical Analysis

The collected data were subjected to statistical analysis using a factorial design. Each treatment was designed with three replicates consisting of nine pots (plants). As the rootstocks have different physiological response to environmental factors, the mean values of parameters were compared for each rootstock separately using the least significant difference (LSD) test. Statistical tests were performed at $P \leq 0.05$ using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Soil pH and Colonization Assays

Soil samplings performed two weeks after every bicarbonate application, demonstrated that the rhizosphere pH gradually increased along with the periodical bicarbonate supplementation (Table 2). Initial pH value of growth medium was 7.76 and increased to final values ranging from 8.10 (A18, 41 B) to 8.26 (A18, 140 Ru). The periodical investigations on colonization of bacteria around the grapevine root rhizosphere showed that all the bacterial strains were able to colonize the rhizosphere of genotypes (Table 3). In the first analysis, apart from control plants where no external colonization was detected, the populations of bacteria OSU 142 and A18 changed from 2.12×10^7 (140 Ru) to 5.68×10^7 (41 B) CFU g^{-1} root and from 2.86×10^7 (140

Table 2 The changes in soil pH as affected by different treatments

Genotype	Inoculant	Growth media sampling[a]				
		Initial	1	2	3	4
41 B	Control	7.76	7.92	7.85	7.99	8.12
	OSU 142	7.76	7.89	7.86	8.05	8.15
	A18	7.76	7.87	7.94	8.02	8.10
140 Ru	Control	7.76	7.95	7.85	8.08	8.24
	OSU 142	7.76	7.73	7.97	8.06	8.24
	A18	7.76	7.89	7.92	8.04	8.26
99 R	Control	7.76	7.69	7.98	8.22	8.19
	OSU 142	7.76	7.72	7.77	8.09	8.12
	A18	7.76	7.89	7.79	8.16	8.19

[a]Soil samplings were performed two weeks after every bicarbonate application ($n = 3$)

Table 3 The changes in bacteria populations ($\times 10^7$ CFU g^{-1}) as affected by different treatments

Genotype	Inoculant	Bacteria colonization (CFU g^{-1})[a]			
		1	2	3	4
41 B	Control	0.00	0.00	0.00	0.01
	OSU 142	5.68	2.44	1.83	0.72
	A18	3.84	1.87	1.02	0.62
140 Ru	Control	0.00	0.00	0.01	0.00
	OSU 142	2.12	1.72	1.57	1.12
	A18	2.86	1.64	1.04	0.93
99 R	Control	0.01	0.01	0.01	0.00
	OSU 142	5.04	2.27	1.09	0.92
	A18	2.97	2.14	1.84	1.52

[a]Soil samplings were performed two weeks after every bicarbonate application ($n = 3$)

Ru) to 3.84×10^7 (41 B) CFU g^{-1} root, respectively. The population density of bacteria gradually decreased along with the vegetation period. Two weeks after the 4th inoculation, around the end of growth season, the colony density in the root rhizosphere varied from 0.62×10^7 CFU g^{-1} to $1.52\ 10^7$ CFU g^{-1} amongst the treated groups.

Growth, Nutrient Acquisition and Physiology of Leaves

As presented in Table 4, most of the leaf growth characteristics of grapevine rootstocks grown under alkaline stress were significantly enhanced by bacteria inoculations. Inoculation with A18 strains resulted in the highest leaf number, LA and FM values in both 41 B and 140 Ru rootstocks. On the other hand, OSU 142 bacteria better performed in 99 R with the highest values in most leaf features such as leaf number, DM and LA. For example, the LA was 12.5, 12.7, and 6.7% higher in A18 to the rootstock plants 41 B, 140 Ru and 99 R respectively, as compared to control under high pH conditions without bacteria.

Bacterial inoculations significantly increased most of the leaf blade nutrients of vines compared to their controls, although a few exceptions were found (Table 5). For illustration, in 41 B, the highest Zn, Cu, and B concentrations were observed in A18 inoculated plants, while OSU142 treatment resulted in the highest K, P and Fe uptake of the plants. In 140 Ru, the highest Ca, Mg, Cu and Mn concentrations were obtained from the plants inoculated with A18 while the highest values on K, P, Fe and B were determined in OSU 142 inoculated group. On the other hand, OSU 142

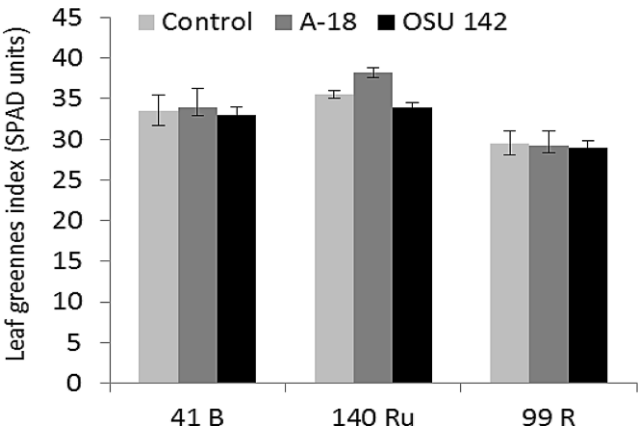

Fig. 1 The changes in leaf greenness index (SPAD readings) as affected by different treatments. Each column represents the mean of triplicate determinations with six leaves for replicate ($n = 18$). Error bar stands for the standard deviation of that mean (at $P < 0.05$ level by LSD)

Table 4 The changes in leaf characteristics as affected by different treatments

Rootstock genotypes	Inoculant	Leaf number	Leaf fresh mass (g)	Leaf dry mass (g)	Leaf RWC	Leaf area (cm^2)
41 B	Control	33.5 ± 1.80b	1.83 ± 0.03b	0.463 ± 0.01	74.2 ± 0.8c	93.9 ± 4.2b
	A18	37.7 ± 1.15a	1.97 ± 0.07a	0.457 ± 0.03	71.5 ± 1.5b	108.3 ± 2.7a
	OSU 142	35.3 ± 1.15ab	1.93 ± 0.01ab	0.492 ± 0.01	77.9 ± 0.5a	107.2 ± 3.9a
140 Ru	Control	40.8 ± 1.26b	1.59 ± 0.01b	0.397 ± 0.01	81.8 ± 0.5a	71.6 ± 3.4b
	A18	47.0 ± 2.29a	1.61 ± 0.02a	0.405 ± 0.00	76.0 ± 1.5b	80.7 ± 3.9a
	OSU 142	40.2 ± 1.58b	1.58 ± 0.02b	0.394 ± 0.02	78.1 ± 2.0b	70.7 ± 2.4b
99 R	Control	41.3 ± 1.15b	1.17 ± 0.03	0.293 ± 0.01b	80.1 ± 2.4	80.2 ± 4.0b
	A18	43.2 ± 1.04ab	1.21 ± 0.04	0.285 ± 0.00b	80.6 ± 3.5	85.7 ± 1.1ab
	OSU 142	46.2 ± 1.61a	1.19 ± 0.02	0.333 ± 0.01a	83.3 ± 1.4	88.3 ± 2.9a

All values are means ± standard error ($n = 9$). Means not connected by same letter are significantly different ($P < 0.05$) level by LSD

Table 5 The changes in leaf nutrient concentrations as affected by different treatments

Rootstock genotypes	Inoculant	K (%)	P (%)	Ca (%)	Mg (%)	Zn mg kg^{-1}	Fe mg kg^{-1}	Cu mg kg^{-1}	Mn mg kg^{-1}	B mg kg^{-1}
41 B	Control	1.06c	0.35b	2.61a	0.42	20.1b	207.6c	3.94b	39.6	41.7b
	A18	1.18b	0.33a	2.46b	0.39	23.4a	201.4b	4.76a	37.0	56.5a
	OSU 142	1.27a	0.36a	2.55ab	0.39	22.9a	219.8a	4.72a	38.2	56.1a
140 Ru	Control	1.21b	0.33b	1.92c	0.40c	18.3	208.8b	3.19b	27.1b	32.4b
	A18	1.20b	0.31b	2.36a	0.47a	18.0	206.1b	3.91a	39.4a	32.8b
	OSU 142	1.29a	0.42a	2.09b	0.44b	18.2	223.2a	3.22b	30.3b	36.5a
99 R	Control	0.91c	0.26c	1.93b	0.42b	15.1b	176.1c	2.84b	33.5	29.3c
	A18	1.08b	0.33b	1.88b	0.43ab	15.1b	188.7b	3.88a	30.7	38.3b
	OSU 142	1.22a	0.39a	2.46a	0.47a	22.1a	202.6a	4.11a	33.3	42.3a

Means not connected by same letter are significantly different ($P < 0.05$) level by LSD ($n = 19$)

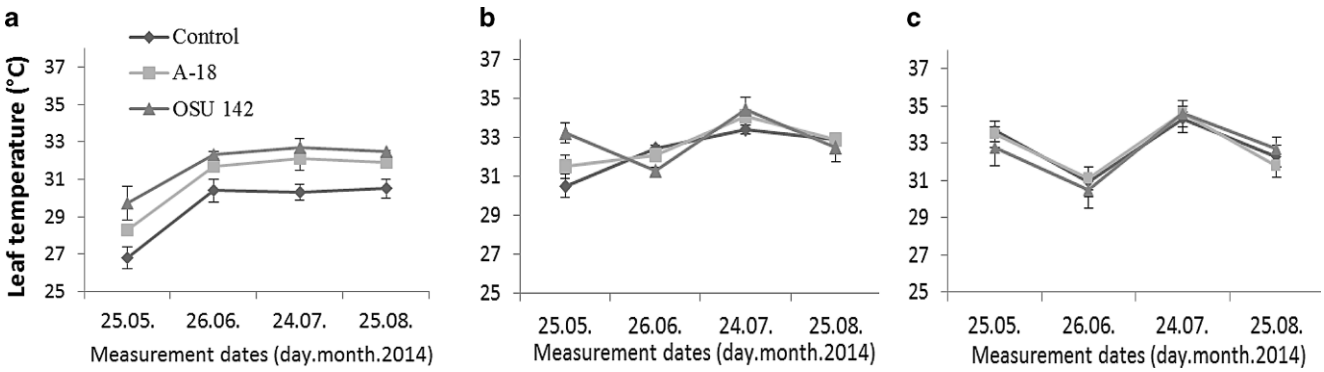

Fig. 2 Changes in leaf temperature (°C) as affected by different treatments. Each point represents the mean of triplicate determinations ($n = 18$). Error bar stands for the standard deviation of that mean (at $P < 0.05$ level by LSD). (**a** 41 B, **b** 140 Ru, **c** 99 R)

inoculation in 99 R resulted in the highest accumulation of almost all the nutrients analyzed, except for Mn.

Leaf greenness index of the rootstocks 41 B and 99 R did not show significant variation in response to the bacteria inoculation (Fig. 1). However, A18 significantly increased the chlorophyll concentration of 140 Ru (38.2 SPAD units) in comparison to control (35.4 SPAD units). T_{leaf} was significantly affected by the treatments in 41 B while it did not show significant variation in 140 Ru and 99 R (Fig. 2). In 41 B genotype, inoculation of OSU 142 resulted in the highest T_{leaf} independent from measurement time. Similar effect of A18 was also noticeable with its significantly higher values than control. With an increasing trend from the beginning of the vegetation period, g_s did not show significant variation up to the final analysis which was performed near to cessation of shoot growth (Fig. 3). However, at the final analyses (one month after the 4th bicarbonate application), there were significant changes in g_s of the rootstocks as response to the bacteria strains. All the bacteria strains gave significantly higher g_s values in comparison the bacteria-

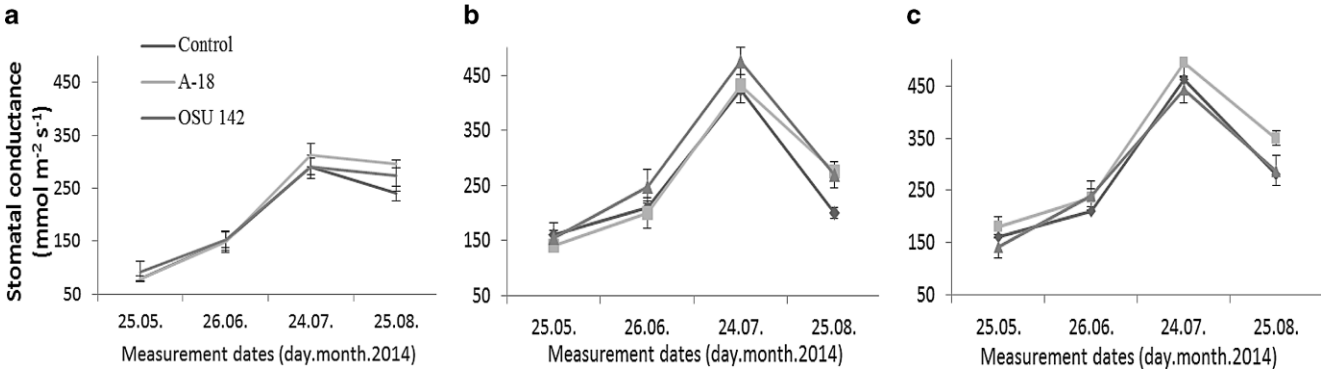

Fig. 3 Changes in stomatal conductance (mmol m^{-2} s^{-1}) as affected by different treatments. Each point represents the mean of triplicate determinations. Error bar stands for the standard deviation of that mean (at $P < 0.05$ level by LSD). (**a** 41 B, **b** 140 Ru, **c** 99 R)

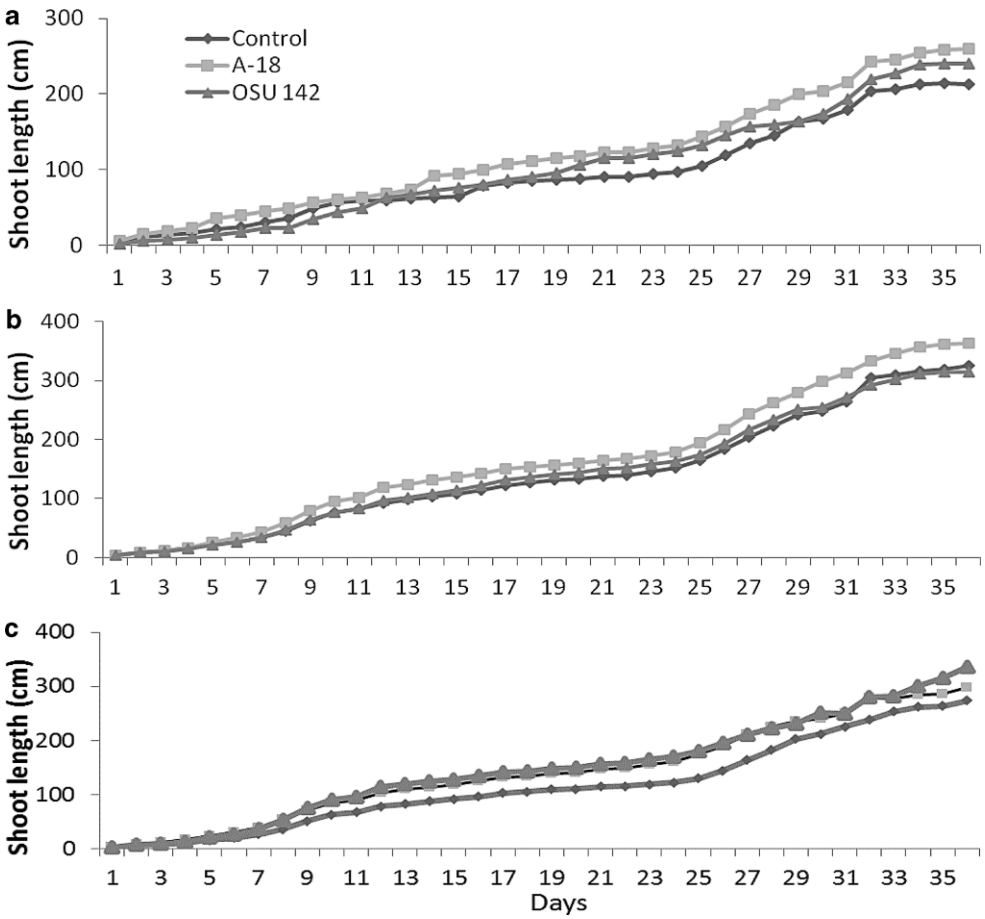

Fig. 4 Shoot development course of grapevine rootstocks as affected by different treatments ($n = 9$). (**a** 41 B, **b** 140 Ru, **c** 99 R)

free plants. Furthermore, inoculation of the A18 strains resulted in the highest values in g_s across the genotypes.

Shoot Growth

As depicted in Fig. 4a–c, there was no remarkable effect of bacteria on shoot growth up to the second inoculation (7th measurement on shoot length), however an accelerated shoot elongation was detected after the second bacteria application in most of the inoculated plants. Final measurements, when the shoot growth was near to cessation, show that A18 (*Agrobacterium rubi*) in all the genotypes and OSU 142 (*Bacillus subtilis*) in 41 B and 99 R rootstocks apparently promoted the shoot growth. Shoot diameter values for 41 B and 140 Ru genotypes demonstrated that bacteria effects were insignificant up to the final measurement (Fig. 5a–c). On the other hand, the bacterium OSU 142 had a significant promoting effect on shoot diameter as early

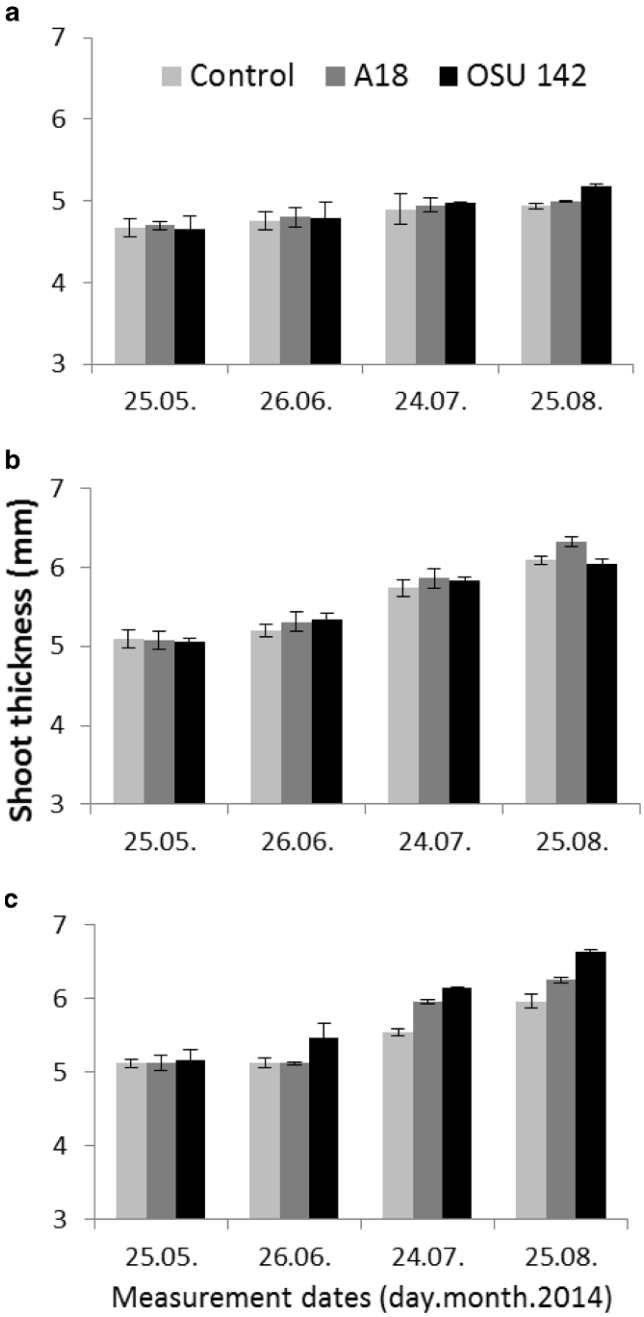

Fig. 5 The changes in shoot diameter as affected by different treatments. Each column represents the mean of nine determinations (n = 9). Error bar stands for the standard deviation of that mean (at $P < 0.05$ level by LSD). (**a** 41 B, **b** 140 Ru, **c** 99 R)

as the second measurement date with its value 5.46 mm compared to control (5.13 mm) and A18 (5.12 mm). At the end of the growth season, there were significant differences amongst the bacterial treatments for shoot diameter. OSU 142 inoculation resulted in the highest shoot diameter values for 41 B (5.18 mm) and 99 R (6.63 mm), while A18 gave the highest value for 140 Ru (6.33 mm).

As seen in Fig. 6, inoculation of A18 significantly increased the lignified shoot length by 21.0, 24.9 and 26.4% for 140 Ru, 99 R and 41 B, respectively, in comparison to

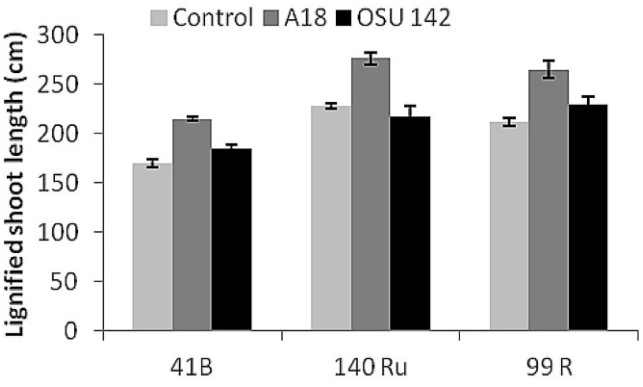

Fig. 6 The changes in lignified shoot lenght as affected by different treatments. Each column represents the mean of nine determinations (n = 9). Error bar stands for the standard deviation of that mean (at $P < 0.05$ level by LSD)

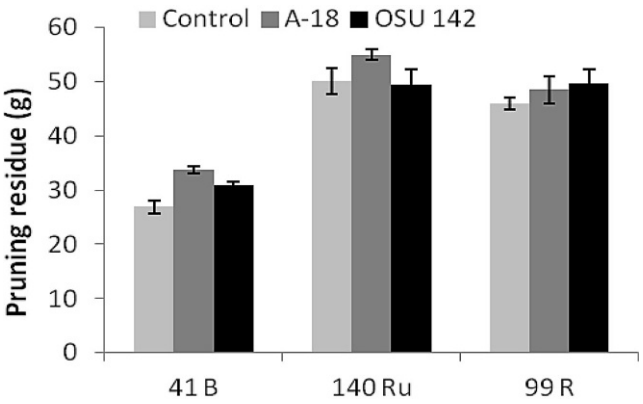

Fig. 7 The changes in pruning residue weight as affected by different treatments. Each column represents the mean of nine determinations (n = 9). Error bar stands for the standard deviation of that mean (at $P < 0.05$ level by LSD)

control. OSU 142 had also significant promoting effect on lignified shoot lengths of 41 B and 99 R although it did not significantly affect the 140 Ru genotype. Pruning residue, one of the most commonly known parameter as a good indicator for vegetative development, was significantly affected by inoculants in 41 B and 140 Ru, while the effects of the bacteria were insignificant for 99 R (Fig. 7). The highest pruning weight values were obtained from A18 for both 41 B and 140 Ru. Besides, OSU 142 had also significantly positive effect on pruning residue.

Discussion

Studies on grapevines grown on alkaline soil revealed that severe inhibition in vegetative development can occur at an early growth stage (Sabir et al. 2010; Bavaresco et al. 2003), presumably as a consequence of changes in phytohormone metabolism in connection with inhibited root

growth (Römheld 2000). It has been demonstrated that the production of root hairs was abundant with 476 hairs to a centimeter of root under slightly acid conditions (pH: 5.7) and was reduced to only 180 in slightly alkaline conditions (Winkler et al. 1974). Bates et al. (2002) also reported a decreased vegetative growth in grapevine (cv. ′Concord′) biomass above a soil pH of 7.0. Considering the overall literature knowledge, a soil pH in the range 5.5 to 6.5 is considered optimum for grapes and generally has better nutrient balance for plant growth than soils that are more acidic or alkaline. In the present study, as a first report on mitigation of lime stress in grapevines to our knowledge, grapevine rootstocks subjected to elevated pH condition exhibited better vegetative growth and mineral uptake when treated with bacterial strains A18 or OSU 142. Initially, both the two bacteria better adapted to 41 B roots than other rootstocks. This indicated a genotype-dependent association of the bacteria sources as previously stated by Pedraza (2008) and Sabir et al. (2012) in non-stress studies performed on different PGPRs. Along with the gradual increment in the soil pH, bacteria population tended to decrease. Besides, as already stated by Hrynkiewicz et al. (2010), diversity of bacteria is affected by the season, soil condition and plant age. In our study, soil condition, especially pH, was most probably the leading factor for bacterial community since the other conditions were the same. On the other hand, the presence of bacteria around the root rhizosphere did not result in remarkable variation in rhizosphere pH of this study. In contrast to our findings, Orhan et al. (2006) reported that bacterial inoculations with OSU 142 decreased pH level of experimental soil from 6.7 to 6.0. This has led us to an emphasis on selection of plant-specific beneficial bacteria that are rhizosphere competent (i. e., beneficial bacteria that effectively colonize the root system under a given stress condition). Nonetheless, the paucity of detailed studies directly comparing the effect of bacteria on soil pH make it difficult to draw any robust conclusions regarding the pH management with bacteria inoculation.

As known, 1-aminocyclopropane-1-carboxylate (ACC) deaminase facilitates plant growth by decreasing ethylene levels, inducing the tolerance to stress factors in plants (Zahir et al. 2004). A wide range of bacteria genera, including the tested strains in this study (A18 from *Agrobacterium* and OSU 142 from *Bacillus*), has known to exhibit ACC deaminase activity (Nadeem et al. 2007). According to Arshad et al. (2007), such rhizobacteria take up the ethylene precursor ACC and convert it into 2-oxobutanoate and NH_3. Consequently, the main remarkable effects of root inoculation with ACC deaminase-producing rhizobacteria are the promotion of shoot growth and enhancement in rhizobial nodulation and macro element uptake in various crops (Nadeem et al. 2007, 2009). Most of the leaf nutrient concentrations we report here were greatly similar to those found in the literature (García-Escudero et al. 2013; Sabir et al. 2014) and mostly fell within the recommended values for grapevines (Winkler et al. 1974). Normally, the availability of many micronutrients (Mn, Cu, Zn and B, for example) decreases as soil pH increases (Bavaresco et al. 2003). Considering the lower values of most nutrients in control plants, such an adverse effect of elevated pH is distinguished in the current study. The bacteria inoculation, on the other hand, obviously mitigated the restrictive effect of high pH on grapevine nutrient acquisition physiology with their several beneficial mechanisms as reported in various studies on other plants (Alikhani et al. 2006; Hrynkiewicz et al. 2010). However, Zn concentrations in control and A18 treated vines were slightly below the recommended values, while on the other hand, OSU 142 remarkably increased the Zn level reaching to the recommended level. The same critical levels and positive effect of the same bacteria strain were also detected for K concentration in 99 R. Lime-induced chlorosis is a term often used for chlorosis associated with disturbed nutrient metabolism on high Ca-containing soil (Sabir et al. 2010; Masroor et al. 2016) with high pH as one of major agricultural problem that results in reduced crop yields (Bavaresco et al. 2003) which is estimated about 1/3 of cultivated soils worldwide (Wallace and Lunt 1960). Phosphorus is an essential part of the energy transfer system in plants. Especially, the noticeably higher P concentration resulting from OSU 142 inoculation across the rootstock genotypes proves the P solubilizing capacity of *Bacillus* strains as previously explained by Esitken et al. (2010). Positive effects of various PGPR on the stimulation of uptake of several minerals have been reported by other researchers in e. g. raspberry (Orhan et al. 2006) and grapevines (Sabir et al. 2012). Actually, potential of phosphate-solubilizing microorganisms has been the subject of intensive investigations (Richardson 2001), as agricultural soils around the world are predominately alkaline and are characterized by a high pH and low amounts of plant available P (Alikhani et al. 2006). Hence, OSU 142 has a good potential for the soil with high pH to overcome P mobilization converted to sparingly soluble forms.

Physiology, morphology and growth characteristics of the plant leaf serve simple indicators of environmental stress (Sabir and Yazar 2015), since the leaf can immediately respond to ecological changes (González-Fernández et al. 2015), as was investigated in the present study. Although the effects of the strains were insignificant during the early vegetation period, the investigations around the cessation of shoot elongation revealed that the g_s was noticeably stimulated by the bacterial strains.

Conclusion

From the present investigations we conclude that (i) the population of the strains A18 and OSU 142 around the root rhizosphere gradually decreased along with the prolonged vegetation period, probably accompanied with pH increase in the soil, (ii) the strains employed in the study were able to mitigate the negative impacts of elevated pH on grapevine rootstocks by improving vegetative growth, leaf physiology and nutrient acquisition, and (iii) effects of bacteria strains were generally genotype-dependent. Therefore, the bacteria strains employed in this study can be recommended to inoculate into the soil to support grapevine growth and physiology under lime stress conditions for a sustainable and environment-friendly viticulture.

Conflict of interest U. Karaca and A. Sabir declare that they have no competing interests.

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