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ORIGINAL PAPER

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VINE GROWTH AND YIELD RESPONSE OF ALPHONSE LAVALLÉE (*V. vinifera* L.) GRAPEVINES TO PLANT GROWTH PROMOTING RHIZOBACTERIA UNDER ALKALINE CONDITION IN SOILLESS CULTURE

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ABSTRACT

High carbonate content in soil negatively affect plant growth, because the availability of nutrients is restricted due to high pH. The present investigations were carried out to reveal possible alleviating effects of the exogenous root inoculation PGPRs on development and physiology of soilless-grown grapevines cultivated under alkaline stress in controlled glass house. pH of growth medium was increased from 7.5 to the values ranging from 7.9 (control) to 8.1 (A18) according to the bacterial inoculations by $NaHCO_3$ supplementations. Bacteria inoculations did not result in statistically significant differences in pH values of growth media. The bacterial population density found in the rhizosphere of grapevines ranged from 6×10^8 CFU mL^{-1} (M-3) to 9×10^8 CFU mL^{-1} (Ca-637). The highest value of shoot thickness was obtained from Ca-637 (5.3 mm), followed by A18 (5.2 mm), while M3 did not significantly affected the shoot thickness. The greatest pruning residue per vine was obtained from A18 treatment (81.5 g), followed by Ca-637 (80.8 g) while the lowest value was determined in control. Vine yield was the greatest with A18 (1128 g) treatment and was followed by Ca 637 (1059 g). Considering the general observations, root inoculation of PGPRs A18 and Ca-637 may be recommended in enhancing bioremediation of alkali growth media.

Key words: grapevines, PGPRs, abiotic stress, bioremediation

INTRODUCTION

Studies revealed that high carbonate in soil negatively affect mineral acquisition of grapevines [Bavaresco and Poni 2003], because the availability of nutrients is restricted due to high pH due to elevated carbonate in soil [Sabir et al. 2010]. About 30% of the world's area is calcareous soils with high calcium carbonate content [Sánchez-Rodríguez et al. 2014], adversely affecting the leaf mineral content [Bavaresco and Poni 2003] and disturbing Fe metabolism [Sabir et al. 2010]. Around the world, grapevine is generally cultivated in regions where alkaline soils with low iron availability prevail. High bicarbonate solution in alkaline soils is the main cause of lime-induced chlorosis with its well-investigated negative effects on both yield and quality of grapes [Bates et al. 2002]. Several agents have been examined for

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their biostimulant or biofertilizer potential to promote plant growth under stress conditions [Nadeem et al. 2007, Karlidag et al. 2011, Dilek and Sabir 2016]. Generally, fertilization management is relevant to the application of suitable amount of non-chemical and chemical fertilizers, which result in an increase of soil quality, plant nutrition, cultural advantages, and finally, obtaining of suitable yield [Shakeri et al. 2016]. For non-chemical sustainable fertilization strategies, manipulation of symbiotic and free-living plant-growth-promoting rhizobacteria (PGPR) has become a significant component in many intensive cropping systems [Bashan et al. 2004, Rolli et al. 2017].

Dense populations of microorganisms colonize the root zone (rhizosphere) of plants because rhizosphere is an attractive habitat with a rich organic carbon provided by plant roots [Dimkpa et al. 2009]. Plants supply organic carbon to their surroundings in the form of root exudates. Rhizobacteria respond to root exudates by means of chemotaxis towards the exudate source; and in such scenario, competent bacteria tend to modulate their metabolism towards optimizing nutrient acquisition [Hardoim et al. 2008]. Many root-associated bacteria have been shown to produce IAA [Ali 2015], and inoculation of various plant species with such bacteria has resulted in increased plant growth [Sabir et al. 2012]. Another widespread characteristic among rhizosphere bacteria is ACC deaminase activity, and regulation of ACC is a principal mechanism by which bacteria exert beneficial effects on stressed plants [Saleem et al. 2007]. ACC-possessing bacteria can use the ethylene precursor ACC as a source of nitrogen. Bacterial hydrolysis of ACC leads to a decrease in plant ethylene level, which, in turn, results in increased plant growth [Belimov et al. 2009]. Certain bacteria employed for their beneficial effects under abiotic stress conditions, such as *Bacillus* sp., have been shown to induce plant systemic resistance [Chakraborty et al. 2006], and the primed physiological state of an inoculated plant could, thus, be one explanation for increased resistance against abiotic stresses. Creus et al. [2004] reported on reduced grain yield losses and higher Mg, K and Ca contents in grains of *Azospirillum*-inoculated wheat exposed to water shortage. Similarly, inoculation of salt-stressed maize with ACC deaminase containing *Pseudomonas syringae*, *P. fluorescens* and *Enterobacter aerogenes* resulted in higher K/Na ratio in combination with high chlorophyll proline contents [Nadeem et al. 2007]. Further, inoculation of grapevines with *Burkholderia phytofirmans* PsJN, lowered the rate of biomass reduction during cold treatment (4°C), and promoted post-chilling recovery [Barka et al. 2006].

PGPRs were examined for pea under drought [Arshad et al. 2008] and for strawberries under saline [Karlidag et al. 2011] or alkaline conditions [Ipek et al. 2014]. However, there is insufficient literature knowledge on PGPRs as elicitors for grapevines under alkaline stress. Their practical application largely remain elusive since the studies mostly focused on merely assessing the plant growth inducing impacts under unstressed conditions. Further, it is always a question mark to study the fate of introduced microorganisms on its survival. Therefore, the main objectives of this study was (a) to analyze the effects of bicarbonate induced high pH condition on vegetative development and physiology of grapevine genotype, (b) to assess the colonization capacity of different PGPRs on grapevine roots under elevated pH, and (c) to reveal possible alleviating effects of PGPRs on alkaline stress in grapes.

MATERIALS AND METHODS

Plant material and cultivation. Two years old grapevine (*Vitis vinifera* L. cv. Alphonse Lavallée) plants grafted on 41 (*V. vinifera* cv. Chasselas × *V. berlandieri*) rootstock were cultivated in soilless growth system under controlled glasshouse condition Selcuk University (Konya, Turkey) in 2015. At the beginning of the experiment, healthy vines were selected on the basis of homogeneity in vegetative growth. Soilless growth was established in 50 L pots containing 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 the ratio 1 : 1 (v/v). Bacterial strains of *Bacillus megaterium* M3, *Agrobacterium rubi* A18 and *Alcaligenes eutrophus* Ca-637 were used to assess their potential effects on mitigating the alkaline stress in comparison with untreated control vines. Treatments were replicated three times with three vines

per replicate. The pots were isolated from the ground with plastic sheets to prevent possible external infection. Prior to bud break, the vines were spur pruned to leave 4 winter buds per plant (two spur canes with two buds on each). Night and day temperatures inside the glasshouse were 17 ±4 and 33 ±4°C respectively (Data logger, Ebro EBI 20 TH1). The plants were watered daily with equal amount of fresh tap water (0.7 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 summer shoots were tied with thread to the wires 2.2 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 (approx. 45 g N, 18 g P, 40 kg K per vine) from April to August.

Bacterial inoculation and bicarbonate supplementation. Strains of the PGPR *Bacillus megaterium* M3 (auxin and cytokinin producing, N-fixing, Ca $(HCO_3)_2$ solubilizing), *Agrobacterium rubi* A18 (auxin and cytokinin producing, Ca $(HCO_3)_2$ solibilizing) and *Alcaligenes eutrophus* Ca-637 (auxin producing, Ca $(HCO_3)_2$ solubilizing) 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 units/mL) one week after bud break. Bicarbonate ($NaHCO_3$) applications were performed when the shoots were 3–4 cm long (one week after bacteria inoculation) and was replicated one month later to increase soil pH gradually. For applications, the plants were watered with 500 mL/plant bicarbonate solution (840 g L^{-1} $NaHCO_3$) [Sabir et al. 2010].

Root colonization of bacteria and soil pH. The root colonization capacity of bacteria was tested 2 months after the second root inoculation. Rhizosphere sample, consisting 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]. To check pH alcalinization, about 100 g growth medium (peat ant perlite mixture) samples adhering to the grapevine roots at 10–20 cm depth were collected from each plant. The pH was measured in de-ionized water (1/5 w/v) [Richards 1954] two weeks after each inoculation.

Vine growth, yield and quality. Shoot length (using a tapeline with a sensitivity of 1 mm) and shoot thickness (measured by digital calipers at 1 cm above the second node) were measured at the end of growth period around the ceasation of shoot elongation.

For berry sampling, approximately twelve clusters per treatment were used at maturity. At harvest time (when all the experimental vines attain at least 15 °Brix juice total soluble solid), productive and qualitative parameters of grapes were recorded, as follows; grape yield (g $plant^{-1}$), total cluster number per vine, cluster weight (g), cluster length (cm) and cluster width (cm). As for chemical analyses of grape juice, total soluble solid content (TSS) content (°Brix, digital refractometer), titratable acidity (%) and pH were determined.

Statistical analysis. The row data were subjected to statistical analysis using factorial design. The mean values of parameters were compared using the least significant difference (LSD) test. Statistical tests were performed at $P < 0.05$ using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Changes of pH and bacteria populations in growth medium. pH level of growth media was regularly monitored to reveal alkalinity level. Changes in pH values of growth medium in response to $NaHCO_3$ applications and bacteria inoculations has been presented in Table 1. Initially, pH value of growth medium was 7.5. $NaHCO_3$ supplementations led to gradual increase in pH of growth media to the values ranging from 7.9 (control) to 8.1 (A18) after the second application. Bacteria inoculations did not result in statisti-

Table 1. Changes in pH values of growth medium in response to $NaHCO_3$ applications and bacteria inoculations

Inoculant	Measurement times		
	Initial	1st application	2nd application
Control	7.5	7.7	7.9
M3	7.5	7.9	7.9
A18	7.5	7.9	8.1
Ca-637	7.5	7.8	7.9
Significance	–	n.s.	n.s

n.s. – not significant at 5% level by LSD

Table 2. Bacterial populations determined 2 months after the bacteria inoculation

Inoculant	Bacterial population (CFU mL^{-1})
Bacillus megaterium M3	6×10^8 CFU
Agrobacterium rubi A18	5×10^8 CFU
Alcaligenes eutrophus Ca-637	9×10^8 CFU
Control	n.d.

CFU – colony forming unit, n.d. – not detected

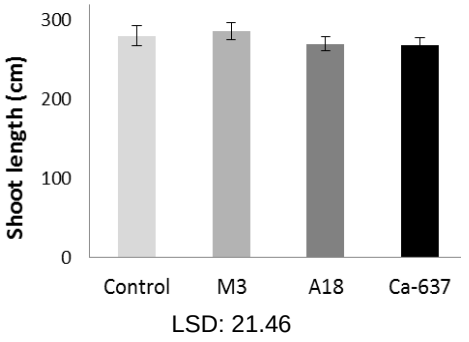

Fig. 1. Changes in shoot length of grapevines in response to bacteria inoculations. Each column represents the mean of nine determinations. Error bar stands for the standard deviation of that mean

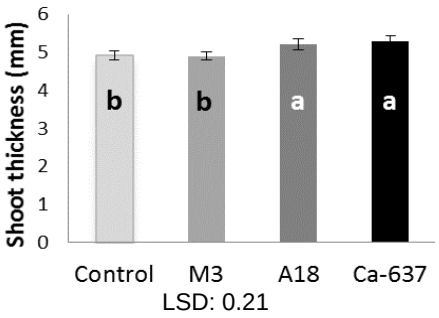

Fig. 2. Shoot thickness response of grapevines to bacterial inoculations. Each column represents the mean of nine determinations. Error bar stands for the standard deviation of that mean

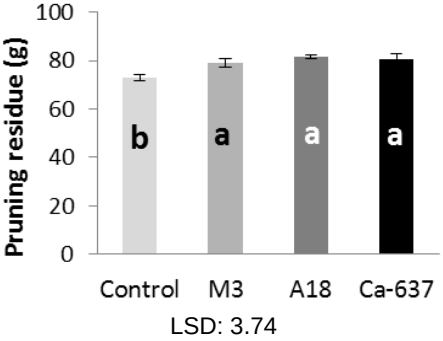

Fig. 3. Pruning residue response of grapevines in response to bacteria inoculations. Each column represents the mean of nine determinations. Error bar stands for the standard deviation of that mean

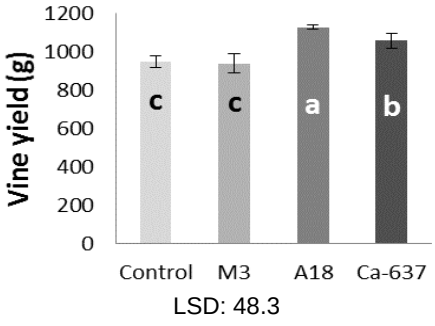

Fig. 4. Vine yield response of grapevines to bacterial inoculations. Each column represents the mean of nine determinations. Error bar stands for the standard deviation of that mean

Table 3. Changes in berry number per cluster, cluster width and cluster length in response to bacteria inoculations

Inoculant	Berry number per cluster	Cluster width (cm)	Cluster length (cm)
Control	26.1 ±2.0 b	8.6	14.0 ±0.5 c
M3	26.4 ±2.1 b	8.8	17.1 ±0.1 a
A18	32.3 ±3.0 a	9.3	18.2 ±0.8 a
Ca-637	31.3 ±2.0 a	8.9	15.0 ±0.5 b
LSD	4.35	n.s.	0.90

All values are means ± standard error (n = 9). Means not connected by same letter are significantly different at 5% level by LSD

Table 4. Changes in pH, TSS and titratable acidity in response to bacteria inoculations

Inoculant	pH	TSS (Brix)	Titratable acidity (%)
Control	4.15 ±0.02	16.3 ±0.20 a	0.45 ±0.00 a
M3	4.11 ±0.00	16.6 ±0.03 a	0.40 ±0.05 b
A18	4.16 ±0.02	15.5 ±0.34 b	0.43 ±0.00 a
Ca-637	4.06 ±0.03	15.1 ±0.36 b	0.44 ±0.02 a
LSD	n.s.	0.43	0.03

All values are means ± standard error. Means not connected by same letter are significantly different at 5% level by LSD

cally significant differences in pH values of growth media. The colonization capacity of bacteria was determined to investigate their activity around the root rhizosphere (tab. 2). The investigations showed that all the strains used were able to colonize the rhizosphere of grapevine. The bacterial population found in the rhizosphere of grapevines ranged from 5×10^8 CFU mL^{-1} (A18) to 9×10^8 CFU mL^{-1} (Ca-637).

Vine growth response to bacteria. The effects of inoculation with PGPR on the shoot length of the vines were statistically insignificant (fig. 1). However, there were significant differences in shoot thickness in response to bacteria inoculations (fig. 2). The highest value of shoot thickness was obtained from Ca-637 (5.3 mm), followed by A18 (5.2 mm), while M3 did not significantly affected the shoot thickness. Inoculations of grapevine roots with Ca-637 and A18 bacteria strains resulted in 9.4% and 7.8% thicker shoots, respectively in comparison to those of non-inoculated vines. Pruning residue was also determined in order to investigate the effects of bacteria treatments on grapevine vegetative development. As illustrated in Figure 3, all the bacteria inoculations significantly increased the pruning residue values. The greatest pruning residue per vine was obtained from A18 treatment (81.5 g), followed by Ca-637 (80.8 g) while the lowest value was determined in control. Pruning residue values indicated that A18 and Ca-637 inoculations led 9.1% and 8.3% higher plant biomasses, respectively compared to those of control vines.

Yield and quality changes. Vine yield exhibited statistically significant variation among the treatments (fig. 4). The greatest yield was achieved with A18 (1128 g) treatment and was followed by Ca-637 (1059 g) while the effect of M3 was insignificant.

The vine yield in response to A18 bacteria inoculation was 17.3% higher than that of control. There were significant differences in response to the treatments with respect to berry number per cluster and cluster length, although cluster width did not show significant variation (tab. 3). The highest values for berry number per cluster (32.3) and cluster length (18.2 cm) were obtained from A18 treatment. Ca-637 treatment also resulted in significant increases in berry number (31.3) and cluster length (15.0) charactersitics in comparison with the control. TSS and titratable acidity levels of the Alphonse Lavallée grape must was significantly affected by the treatments, while the changes in pH were statistically insignificant (tab. 4). TSS values of A18 (15.5 °Brix) or Ca-637 (15.1 °Brix) treated grapes were apparently lower than those M3 (16.6 °Brix) or control (16.3 °Brix) groups probably in relation with yield per vines. Inoculation of M3 resulted in decrease in titratable acidity of grape must.

DISCUSSION

Elevated concentrations of bicarbonate (HCO_3) as a consequence of impeded gas exchange of wet compacted calcareous soils is involved in the observed element deficiency chlorosis in grapevines [Sabir et

al. 2010] and other deciduous fruit trees [Kassa 2015]. Studies revealed that root and shoot developments of grapevines are negatively affected above a soil pH of 7.0 [Winkler et al. 1974, Bates et al. 2002]. Vineyard soils with a pH greater than 7.5 typically cause nutrient imbalances in grapevines [Bates and Wolf 2008]. Many studies have been conducted to ameliorate the negative effect of alkaline stress conditions on plant growth and yield. Recent experiments have demonstrated that certain PGPR can be an innovative ecological approach for mitigation of alkaline stress [Xun et al. 2015]. But the survival and population density and root colonization capacity can vary according to the ecological condition [Karakurt and Aslantas 2010] and genotypes [Sabir et al. 2012]. It is well established that microbial communities showing different activities or producing altered signals may, in the long term, either result in the establishment of altered communities and/or in the elicitation of different plant responses [Compant et al. 2010]. Therefore, effectiveness of rhizosphere bacteria is related with their adaptive strategies to unflavored conditions such as elevated pH as established in the current research. Present investigations displayed that the bacteria strains formed colonies around the grapevine roots under alkaline condition, although their colonization capacity differed among the species. Rousk et al. [2010] also investigated soil bacterial and fungal communities across long-term liming experiment (pH 4.0–8.3) in an arable soil to analyze the direct influence of pH on the abundance and composition of the fungi and bacteria. They hypothesized that bacterial communities would be more strongly influenced by pH than fungal communities. They further emphasized that both the relative abundance and diversity of bacteria were positively related to pH, the latter nearly doubling between pH 4 and 8. To our knowledge, no attempts have been made to study the effects of PGPR on growth, physiology and productivity in grapevines grown under alkaline stress. Studies revealed that the shoot growth of grapevine is significantly decreased when the soil pH exceeds 7.0 [Bates et al. 2002]. The results of the present soilless experiment, focused on the effect of the exogenous root inoculation of three different PGPRs on the plant growth and yield of grapevines under alkaline conditions, demonstrated obvious mitigating effects of bacteria strains *Agrobacterium rubi* A18 and *Alcaligenes eutrophus* Ca-637 on grapevines as the shoot thickness, pruning residue and yield parameters were markedly higher when these strains were used. Similar promoting effects of various PGPRs on shoot thickness were also reported by Karakurt and Aslantas [2010] in five apple cultivars, and Sabir et al. [2012] in two grapevine rootstocks. Similar to the results related to vegetative growth, berry number per cluster, cluster length and vine yield parameters revealed that the reproductive development of the grapevines under high pH conditions were promoted by A18 and Ca-637. The increase in grape yield by bacterial inoculation may have particularly resulted from the increased berry number per cluster, because the berry numbers in response to A18 and Ca-637 treatments were 23.8% and 20.0% greater than those of control. Similarly, fruit number increase response to PGPRs in strawberries grown under alkaline stress were also reported by Ipek et al. [2014].

CONCLUSIONS

The present investigations revealed that the exogenous root inoculation PGPRs, particularly A18 and Ca-637, markedly enhanced shoot thickness and pruning residue weight of grapevines grown in soilless culture under alkaline condition (pH 7.92–8.10). All the strains used were able to colonize the rhizosphere of grapevines. Bacteria inoculations did not greatly influenced the pH values of growth media. Considering the general observations, root inoculation of PGPRs A18 and Ca-637 may be recommended in enhancing bioremediation of alkali growth media. Inoculation with PGPR can benefit the growth of vines, relieves the negative impact caused by stress on the plant, and leads to more rapid rates of remediation in alkaline growth media. This study showed encouraging results suggesting that the use of bacteria such as A18 and Ca-637 may reduce the use of expensive chemical fertilizers and help to introduce sustainable agriculture production even under degraded high pH soil conditions existing

in most viticultural regions worldwide including the dense grape growing areas of Turkey.

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