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Research Article/Araştırma Makalesi

The Effect of Plant Growth Regulator Bacteria on Micro Propagation of Grapevine Rootstock with Three Different Rooting Abilities

Farklı Köklenme Yeteneğine Sahip Asma Anaçlarının Mikro Çoğaltılmasına Bitki Gelişimini Düzenleyici Bakterilerin Etkisi

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ABSTRACT

In the study, 3 American rootstocks (110 R, 1103 P and 5 BB) with low, medium, and high rooting ability were used, 2 different bacterial isolates *Bacillus cereus* (ZE-7) and *Pseudomonas putida* (ZE-12) and their binary combination and the bottom of the cuttings. It was aimed to determine the effect of bacteria on rooting by treating the rooting part. During the rooting stage, hormone-free MS medium was treated with PGPRs, and in addition, hormone-free MS medium containing 1 mgl-1 IBA was used to determine the effects of PGPRs strains. At the end of the study, the root ratio of the explants was maintained; root length, number of roots, root fresh weight, root dry weight, shoot length, shoot fresh weight, and shoot dry weight data were examined. It was determined that PGPR applications generally gave higher values than control and IBA applications. Among the rootstocks, it was determined that they gave the best results in terms of root development. The highest values were obtained in the 5BB rootstock, and the lowest values were generally obtained in the 110R rootstock. Shoot development values varied according to the rootstocks and applications. In terms of the effects of the applications on root development, the highest rooting rate was 72.03%, the highest root number ratio was 1.95 on average, and the highest root fresh weight was 39.75 mg from the Bacillus Cereus application; Additionally, the highest root dry weight was obtained from *B.cereus+P.putida* application with 13.06 mg. The research highlighted this feature of 5 BB rootstock, which is considered among the easily rooting rootstocks, with its PGPR effect. 110 R, which is known as a difficult rooting rootstock and has high resistance to drought and active lime, which are the biggest threats today, showed a lower rooting rate, but when the values were examined, it was above the control and IBA applications.

Key Words: 5BB rootstock, rooting rate, *in vitro, Bacillus cereus, Pseudomanas putida*

ÖZ

Çalışmada köklenme yeteneği düşük, orta ve yüksek olan 3 Amerikan anacını (110 R, 1103 P ve 5 BB), 2 farklı bakteri izolatı (*Bacillus cereus* (ZE-7) ve *Pseudomonas putida* (ZE-12) ve bunların ikili kombinasyonu ile çeliklerin dip kısmını muamele etmek suretiyle bakterilerin köklenme üzerinde etkisini belirlemek amaçlanmıştır. Köklendirme aşamasında hormondan ari MS ortamı, BBAR'lar ile muamele edilmiş olup ayrıca, BBAR ırklarının etkilerini belirleyebilmek için hormondan ari 1 mgl^{-1} IBA içeren MS ortamları kullanılmıştır. Çalışma sonunda sürdürülen eksplantların kök oranı, kök uzunluğu, kök

sayısı, kök yaş ağırlığı, kök kuru ağırlığı, sürgün uzunluğu, sürgün yaş ağırlığı ve sürgün kuru ağırlığı verileri incelenmiştir. BBAR uygulamalarının, genel olarak kontrol ve IBA uygulamalarına oranla daha yüksek değerler verdiği saptanmıştır. Anaçlar arasında kök gelişimi bakımından en yüksek değerler 5BB anacında, en düşük değerler ise genel olarak 110R anacında elde edilmiştir. Sürgün gelişim değerleri anaçlara ve uygulamalara göre değişiklikler göstermiştir. Uygulamaların kök gelişimleri üzerine etkileri açısından en yüksek *Bacillus cereus* uygulamasından en yüksek köklenme %72.03, en yüksek kök sayısı oranı ortalama 1.95 adet ile en yüksek kök yaş ağırlığı 39.75 mg elde edilmiştir; ayrıca en yüksek kök kuru ağırlığı da 13.06 mg ile *B.cereus+P.putida* uygulamasından alınmıştır. Araştırma kolay köklenen anaçlar arasında değerlendirilen 5 BB anacının, BBAR etkisi ile bu özelliğini daha çok ön plana çıkmıştır. Zor köklenen anaç olarak bilinen, günümüzdeki en büyük tehdit olan kuraklık ve aktif kireçe dayanımı yüksek olan 110 R ise daha düşük bir köklenme göstermiştir, fakat değerler incelendiğinde kontrol ve IBA uygulamalarının üstünde olmuştur

Anahtar Kelimeler: 5BB anacı, köklenme oranı, in vitro, *Bacillus cereus, Pseudomanas putida*

Introduction

The phylloxera plague, which had only natural distribution before the 19th century, on viticulture emerged as a result of its transport to Europe before 1860. It showed devastating effects on the *Vitis vinifera* L. (*Vitaceae; Rhamnales*) species, which is known as the European grape, which was included in this distribution through the first European colonies in the early 18th century, destroying all the vineyards in the European continent after the vineyards in France. It was subsequently carried by colonization to Africa, South America, and Australia/New Zealand (Gökbayrak, 2006). Today, it has spread all over the world, except for parts of Chile, China, and Australia (Granett et al., 2001). Weakening and vine death due to vineyard phylloxera were observed in all regions where the pest spread (Gökbayrak, 2006). Regional drying out in the vineyards may spread to the entire vineyard area in the future, causing not only yield loss but also the loss of the entire vineyard. For this reason, American grapevine species with different levels of resistance to phylloxera are used as rootstocks.

Many rootstocks are used in the world that can adapt to different soil types, have different resistance to drought, lime, salinity, phylloxera, and nematodes, as well as their ability to adapt to *V. vinifera* varieties (Yağcı, 2022) Another factor affecting the selection of rootstocks is the rooting rate. Among the grapevine rootstocks, some rootstocks root easily (5 BB, 1613 C) as well as more difficult rootstocks (110 R, 140 Ru, 41 B) (Howell, 1987; Çelik, 2011).

Although the exact system of root formation is not known today, it is accepted that some phytohormones (such as auxin) prevent ethylene synthesis and accordingly provide mineralization of nutrients (Goto, 1990; Steenhoudt and Vanderleyden, 2000). In viticulture, IBA and NAA are the most preferred hormones to promote rooting in cuttings (Algül et al., 2016).

In addition to these, there have been many studies in recent years using non-pathogenic rhizobacteria (known as plant growth promoting rhizobacteria -PGPR) such as *Bacillus, Pseudomonas, Agrobacterium, Streptomyces, Alcaligenes* for rooting cuttings (Patena et al., 1988; Goto, 1990; Srinivasan et al., 1996; Tripp et al., 1997; Stomp, 1997; Ercişli et al., 2003; Eşitken et al., 2003a; Larraburu et al., 2007; Teixeira et al., 2007; Peyvandi et al., 2010).PGPR's are known as beneficial bacteria strains found in soil. They make important contributions by increasing the root development of plants (Kloepper, 1994; Glick, 1995; Cleyet-Marcel et al., 2001). Bacteria that increase rooting by producing auxin also have other beneficial effects such as inhibiting ethylene synthesis and providing mineral substance uptake (Kapulnik et al., 1985; Lifshitz et al., 1987).

Some reproduction methods: due to negative features such as allowing the production of a limited number of plants over a long period, causing the transmission of systemic diseases and undesirable mutations, needing large reproduction areas and requiring more time for the production of propagation materials, nowadays it is more rapid and massive. It is seen that tissue culture methods, which are more effective in production, gain importance in plant reproduction. Tissue culture methods; allow the

production of strong and healthy plants by creating genetically homogeneous plant populations (Murashige, 1974; Blazina et al., 1991, Figiel-Kroczyńska et al., 2022). In propagation using the tissue culture method, some problems arise in rooting woody plants (Larraburu et al., 2007). To solve these problems, some vitamins (Antonopoulou et al*.,* 2005) and tryptophan (Khalid et al., 2004; Sharma et al*.,* 2014), amino acids (Pedrotti et al., 1994), indole acetic acid (De Klerk et al., 1997) and similar methods were used.

In this study, micro cuttings of 3 American rootstocks (110 R, 1103P and 5 BB) with low, medium and high rooting ability, propagated under *in vitro* conditions, were treated with 2 different PGPR *Bacillus cereus*, *Pseudomanas putida* and their dual combination (*B. ceresus+ P. putita*) to determine the effect of PGPR on rooting, it was aimed to observe the effect of BBARs on rooting under *in vitro* conditions.

Materials and Methods

Material

Rootstocks

Plant material of 3 American vine rootstock (5BB, 1103P, 110R) with different rooting abilities (high, medium, and low) was obtained from the vineyard of Tokat Gaziosmanpasa University Application and Research Center.

5 BB; It is a strong rootstock, and its vegetation period is shorter than 420 A rootstock. 5 BB rootstock is a rootstock that can adapt to moist and clay soils. It does not like very arid soils; it is well resistant to around 20% active lime and nematodes. Although it is well rooted, some problems may arise in grafting in the vineyard. In situ inoculations in the field, too many roots are formed from the pen (Howell, 1987; Çelik, 2011; Anonymous, 2023).

110 R; It is one of the most widely used rootstocks recognized since 1945. Since it is a strong growing rootstock, it delays the ripening of the grape variety grafted on it. It is a rootstock that is very resistant to active lime and drought up to 17%. Although the 100 R rootstock, which has a very weak rooting ability, gives good results in grafting in the field, the lignification of annual sticks remains weak (Howell, 1987; Çelik, 2011; Anonymous, 2023).

1103 Paulsen (1103 P); It develops vigorously, as in 110 R, and adapts well to moist and clayey-calcareous soils and is based on active lime by 17-18%. Rooting and grafting rate of rootstock, which can withstand 0.6 g NaCl/kg salt in the soil, is high (Howell, 1987; Çelik, 2011; Anonymous, 2023).

PGPR applications

Bacillus cereus (ZE-7) and *Pseudomonas putida* (ZE-12) bacterial isolates were used in the study. The isolates used are isolated from the pepper production areas of Tokat Province and are kept as stock cultures in the Phytopathology laboratory.

Method

Establishment of in vitro Culture

The samples of rootstocks were thoroughly washed under running tap water with 1–2 drops of Tween 20 for 20 min. After defoliating micro cuttings (1–1.5 cm long) were cut and surface sterilized with 70% (v/v) ethyl alcohol. Thereafter, micro cuttings were washed with sterilized distilled water followed by a 20% (v/v) sodium hypochlorite solution (NaOCl, containing 5% active chlorine) for 20 min together with a few drops of Tween-20 under aseptic conditions of a laminar airflow cabinet. Finally, the explants were thoroughly washed three times (2, 3, and 5 min, respectively) with sterilized distilled water.

Surface sterilized explants were prepared for planting by cutting about 0.5-0.7 cm in length and with 1 active bud on it. 0.5 mgl^{-1} BA, 3% sucrose and 0.7% bacto agar were added to the MS nutrient medium used in the initial culture stage. The pH was adjusted to 5.8 without adding agar to the nutrient medium (Sivritepe, 1995). In the initial culture, 15 ml of nutrient medium was placed in 105 cc jars and autoclaved at 121ºC under 1.06 bar pressure for 20 minutes. In the establishment of in *vitro culture*, one explant was planted in each jar.

Root induction

Nutrient broth bacteria growth media (0.65

gr/50 ml) were prepared for 3 PGPR (*Bacillus cereus* (ZE-7), *Pseudomonas putida* (ZE-12) and their dual combination) in Petri dishes and sterilized in an autoclave at 121°C for 15 minutes. The development media contaminated with PGPR were covered with aluminum foils and kept in a shaker at 27 °C at 200 rpm for 24 hours in the dark. Growing bacteria were diluted with 10% distilled water in a sterile laminar cabinet, then applied to the bottom of the micro cuttings, and then the micro cuttings were planted in hormone-free MS environments. To determine the effects of PGPR strains during the rooting stage, media containing 1 mg/lt IBA and hormone free were used in MS media. The planted explants were kept in the growth chamber at a temperature of 24±2 °C and a photoperiod of 16 hours.

6 weeks after the treatments, rooting rate (%), number of roots per explant (pieces or number), root length (cm), shoot length (cm), root fresh and dry weight (mg), shoot fresh and dry weight (mg) were examined. To determine the effect of PGPR on rootstocks, the fresh weights of shoots and roots (mg) were determined with the help of precision balances (Radwag WTB200, Poland) with an accuracy of ±0.001 g. After drying the samples in an oven at 65 °C for 72 hours, root and shoot dry weights (g) were determined with the same precision balance measurement.

Statistical analyses

Experiments were conducted in randomized plots design with 3 replications with 10 saplings in each. The data were analysed with Two-way Factorial ANOVA by using SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA) software. Duncan's multiple range test was used to determine different treatment means when ANOVA showed significant differences among the means. The level of significance was set as 5%.

Results and Discussion

The results of the parameters related to the plant growth and rooting of the plantlets extracted from the rooting conditions are given in Table 1-2. In general terms; Bacteria appear to have a significant effect on rooting rootstocks. In Table 1, the effect of applications on root rate created statistical differences between rootstocks and rootstock x application interaction, and it is seen that *B. cereus* application gave the highest rooting rate (84.80%). In terms of rootstocks, the highest rooting rate was seen in 5 BB (84.80%) rootstocks (Figure 1).

Data on root development of rootstocks after applications are presented in Table 1. In the number of rootstocks, except 110R, there were statistical differences in 1103P and 5BB rootstocks (Table 1). The highest root number was seen in 5BB rootstocks with 2.61 in *B. cereus* bacteria application. When looking at the rootstocks, the highest root length was seen in 5BB rootstock, when evaluated in terms of applications, *B. cereus* application gave the highest root length value. 1103P rootstock gave the highest root length ratio with 2.14 cm in *B. cereus* application, 110R rootstock with 1.49 cm in *B. cereus* application, and 5BB rootstock with 3.13 cm in *B. cereus* application. In terms of root wet weight, when the rootstocks are examined, it is seen that the highest rootstock is 1103P. When evaluated in terms of applications, *B. cereus* application gave the highest root fresh weight. 1103P rootstock gave the highest root fresh weight value with 54.62 mg in *B. cereus* application, 110R rootstock with 31.87 mg in *B. cereus+ P. putida* application, and 5BB rootstocks with 40.57 mg in *B. cereus* application. When examined in terms of rootstocks, the lowest root fresh weight was seen in 110R rootstock with 22.51 mg. There was no statistical difference in root dry weight in rootstock, rootstock x application interaction.

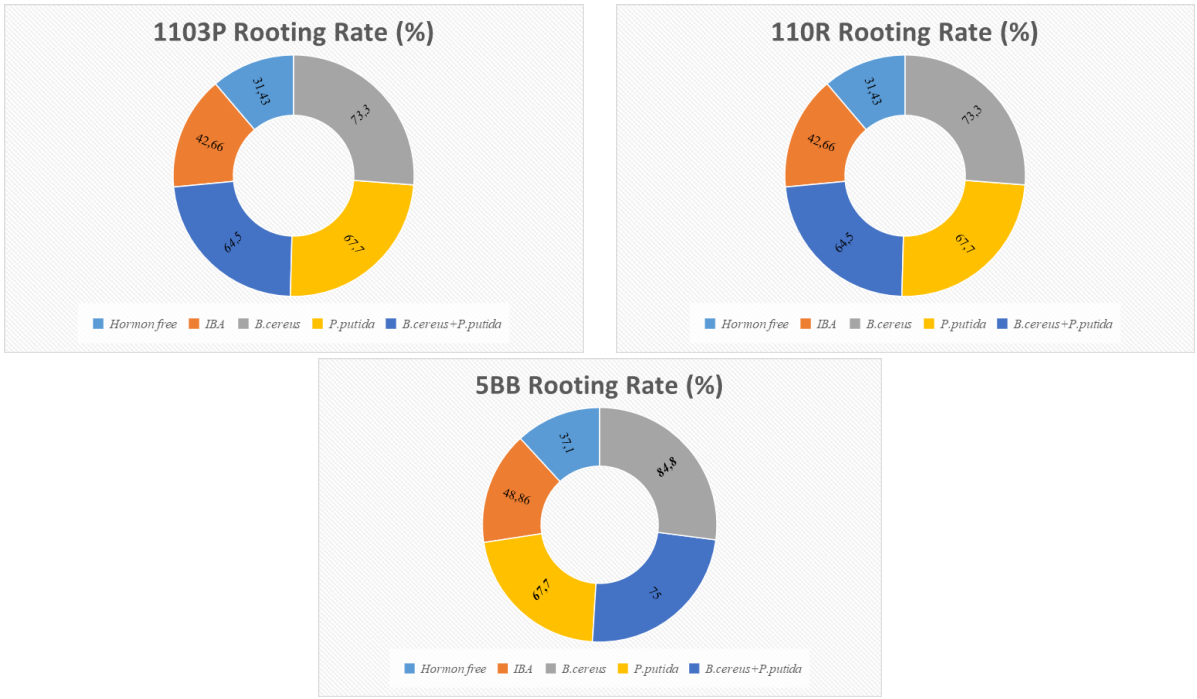


Figure 1. Rooting rates of rootstocks

Table 1. Effects of applications on roots development

	Rootstock	Hormon free	IBA	*B. cereus*	*P. putida*	*B. cereus+P. putida*	Mean
Rooting rate (%)	1103 P	CD 46.30 a	BC 48.50 a	A 58.00 c	B 51.60 b	D 44.80 c	C 49.84
	110 R	C 31.43 b	B 42.66 a	A 73.30 b	A 67.70 a	A 64.50 b	B 55.92
	5 BB	D 37.10 b	C 48.86 a	A 84.80 a	B 67.70 a	AB 75.00 a	A 62.69
	Mean	38.27 d	46.67 c	72.03 a	62.33 b	61.43 b	
Number of roots	1103 P	AB 1.76 a	A 1.97 a	AB 1.79 b	AB 1.51 b	B 1.28 a	B 1.66
	110 R	A 1.58 a	A 1.66 a	A 1.46 b	A 1.37 b	A 1.25 a	B 1.46
	5 BB	B 1.66 a	B 1.74 a	A 2.61 a	AB 2.04 a	B 1.67 a	A 1.94
	Mean.	1.67 ab	1.79 a	1.95 a	1.64 ab	1.40 b	
Root lenght (cm)	1103 P	B 1.61 b	AB 1.84 a	A 2.14 ab	AB 1.83 a	AB 1.79 ab	B 1.90
	110 R	C 0.86 c	BC 1.10 b	A 1.49 b	BC 1.05 b	AB 1.31 b	C 1.16
	5 BB	B 2.14 a	AB 2.51 a	A 3.13 a	B 2.07 a	B 2.30 a	A 2.43
	Mean.	1.53 b	1.82 b	2.35 a	1.65 b	1.80 b	
Root fresch weight (mg)	1103 P	B 15.40 a	AB 23.22 a	A 54.62 a	AB 26.98 a	AB 29.97 a	A 30.05
	110 R	B 14.76 a	B 16.27 a	AB 24.08 a	AB 25.58 a	A 31.87 a	A 22.51
	5 BB	C 18.33 a	BC 26.03 a	A 40.57 a	BC 25.29 a	B 28.95 a	A 27.83
	Mean	16.16 c	21.87 bc	39.75 a	25.95 bc	30.26 ab	
Root dry weight (mg)	1103 P	A 3.12 a	A 5.23 a	A 7.46 a	A 6.06 a	A 11.15 a	A 6.60
	110 R	A 3.63 a	A 3.77 a	A 5.29 a	A 5.66 a	A 13.34 a	A 6.32
	5 BB	A 4.28 a	A 5.97 a	A 7.88 a	A 5.14 a	A 14.70 a	A 7.65
	Mean	3.68 b	4.99 b	6.87 b	5.71 b	13.06 a	

A, B, C: →Different capital letter on the same line represent statistically significant differences among the applications ($p<0.05$). a, b, c:↓ Different lowercase letter on the same column represent statistically significant differences among the rootstocks doses ($p<0.05$).

Among the shoot length applications, *P. putida* was statistically insignificant, while IBA application gave the highest shoot length. In the rootstock x application interaction, the highest shoot length was measured as 3.72 cm in 1103P in IBA application and 3.61 cm in 5BB rootstocks (Table 2). While 5BB rootstock gave the highest shoot fresh weight to rootstocks, *B. cereus* application gave the highest shoot fresh weight ratio when evaluated in terms of applications. 1103P rootstock gave the highest rooting rate with 75.14 mg in *B. cereus* application, 110R rootstock with

129.75 mg in *B. cereus+ P. putida* application, and 5BB rootstocks with 71.53 mg in *B. cereus* application. When the lowest fresh shoot weight was examined in terms of rootstocks, it gave 43.56 mg of 1103P (Table 2). When looking at the rootstocks, 5BB rootstock gave the highest shoot dry weight ratio, while when evaluated in terms of applications, *B. cereus* application gave the highest shoot dry weight ratio. 1103P rootstock gave the highest shoot dry weight ratio with 13.66 mg in *B. cereus* application, 110R rootstock with 18.24 mg in *B. cereus* application, and 5BB rootstocks with 16.10 mg in *B. cereus* application. The lowest shoot dry weight ratio among rootstocks was 8.63 mg with 1103P (Table 2).

Table 2. Effects of applications on shoots development

	Rootstock	Hormon free	IBA	*B.cereus*	*P.putida*	*B.cereus+P.putida*	Mean
Shoots lenght (cm)	1103P	A 3.59 a	A 3.72 a	B 1.74 b	B 1.55 a	B 1.06 b	A 2.33
	110R	A 2.05 b	AB 1.83 b	BC 1.37 b	C 1.03 a	BC 1.26 b	B 1.51
	5BB	A 2.77 b	A 3.61 a	B 2.65 a	C 1.60 a	C 1.58 a	A 2.44
	Mean	2.80 a	3.06 a	1.92 b	1.39 c	1.30 c	
Shoot fresh weight (mg)	1103P	A 29.46 b	A 46.30 a	A 75.14 a	A 38.48 a	A 28.43 a	A 43.56
	110R	B 50.16 a	B 27.77 b	AB 82.08 a	B 49.46 a	A 46.67 a	A 46.66
	5BB	C 38.75 ab	BC 49.76 a	A 71.53 a	ABC 58.87 a	AB 65.74 a	A 56.93
	Mean	39.46 b	41.28 b	76.25 a	48.94 b	46.95 b	
Shoot dry weight (mg)	1103P	B 5.66 a	AB 9.96 a	A 13.66 a	AB 10.08 a	B 3.80 b	B 8.63
	110R	A 8.67 a	A 5.05 b	A 18.24 a	A 9.00 a	A 10.57 a	AB 10.30
	5BB	B 8.23 a	AB 11.68 a	A 16.10 a	AB 12.54 a	A 15.91 a	A 12.89
	Mean	7.52 b	8.90 b	16.00 a	10.54 b	10.09 b	

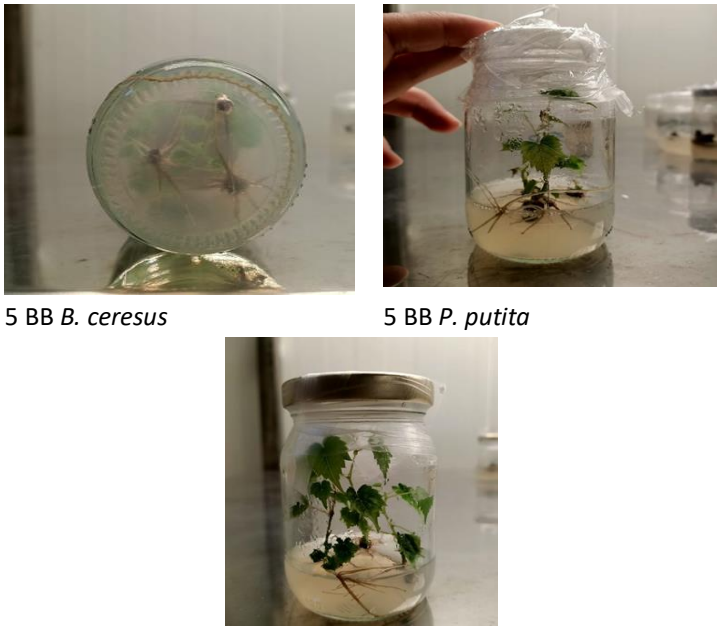
A, B, C: →Different capital letter on the same line represent statistically significant differences among the applications ($p<0.05$). a, b, c:↓ Different lowercase letter on the same column represent statistically significant differences among the rootstocks doses ($p<0.05$).

PGPR are bacteria that live in the rhizosphere and can directly and/or indirectly improve the extent or quality of plant growth. Direct promotion of PGPR involves either providing the plant with plant growth promoting agents or helping plants mobilize and uptake nutrients from the rhizosphere (Amarouchi et al., 2021). Among the main beneficial activities of PGPR are there is the conversion of minerals in the soil into the form that the plant can take, nitrogen fixation, suppression of pathogens and the production of hormones that promote plant growth (Berg et al., 2013; Mendes et al., 2013).

There is some data on the effect of beneficial microorganisms on rooting, growth promotion, and reduction of hyperhydricity in *in vitro* cultivars (Frommel et al., 1991; Burns and Schwarz, 1996; Carletti et al., 1998; Nowak 1998). In the field of viticulture, these PGPR's are used in controlling disease agent (Ferreria et al., 2022; Fu et al., 2022), in storage (Chen et al., 2022), in organic viticulture (Ostroukhova et al., 2022; Korkutal et al., 2017) and to promote plant growth in greenhouse/field conditions (Köse et al., 2005; Sabır et al., 2012).

In-vivo studies examining the effects of PGPR strains on the rooting and shoot growth of vines; showed that these bacterial groups have positive effects on root formation (Di Marco and Osti, 2008; Sabır et al., 2012; Sabır, 2013; Mahmood, 2015). Eşitken (2003b) stated that PGPR, with their various hormonal potentials, can be used for various purposes such as rooting of cutting, grafting union, fruit setting and thinning, lateral root formation, increasing tolerance against abiotic stress as well as growth, development, and biological control with root inoculation and/or spraying. However, studies on promoting rooting in *in-vitro* propagation remained limited. In a study examining the effects of bacteria on vineyard disease agents, it was stated that PsJN (*Pseudomonas* sp.) stimulated the growth of grapevine plantlets grown *in-vitro* for two

generations, and the beneficial effect of the second generation was more pronounced compared to the first generation. In addition, in the same study, it was stated that *Pseudomas* sps caused an increase in shoot and root dry weight and the number of nodes (Barka et al., 2000). When the results of the study are examined, the positive effect of PGPRs on root development is presented in parallel with the literature information given above. In the study, it is seen that bacterial applications give higher values compared to control and IBA applications. When the results are analyzed in terms of rootstocks, it is seen that 5BB rootstocks, which are considered among the easily rooted rootstocks, bring this feature to the forefront with the effect of PGPR (Figure 2).



5 BB *B. ceresus* 5 BB *P. putita*

5 BB B.*ceresus+ P. putita*

Figure 2. The effect of bacteria on rooting 5 BB rootstock (orginal photograph by Neval Topcu Altıncı, 2021)

The 110R rootstock, which is known as hard-to-root rootstock and has high resistance to drought and active lime, which is the biggest threat today, showed a lower rooting rate, but these values were above control and IBA applications. *In-vitro* studies are one of the methods frequently used in the production of plant materials that are difficult to reproduce. For this reason, it is seen as a method that can be used in seedling production or in the propagation of these grapevine rootstocks, which are difficult to root in field conditions.

Conclusions

Sustainable and environmentally friendly agricultural practices come to the force to lead a quality and healthy life in a changing, developing and increasing population. PGPRs are one of the solutions that should be used in this sector. In our study, positive effects of antagonistic bacteria on American grapevine rootstocks with different rooting abilities were observed *in vitro*. The research highlighted this feature of 5 BB rootstock, which is considered among the easily rooting rootstocks, with its BBAR effect. 110 R, which is known as a difficult rooting rootstock and has high resistance to drought and active lime, which are the biggest threats today, showed a lower rooting rate, but when the values were examined, it was above the control and IBA applications. Considering the role of *in vitro* propagation in rapid and healthy plant production, it is thought that these two factors can be used in an integrated manner to create a practical way to obtain rootstocks that root hard.

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Authors' Contribution: This article was prepared from the N.T.A. statistical analysis, writing, editing, and submitting the manuscript; F.Ç data collection and analysis and writing.

References

Algül, B. E., Tekintaş, F. E., & Dalkılıç, G. (2016). The Usage of Plant Growth Regulators and Hormone Biosynthesis Booster Applications. Journal of Adnan Menderes University Agricultural Faculty 2016; 13(2):87-95. DOI: https://doi.org/10.25308/aduziraat.294100

Amarouchi, Z., Esmaeel, Q., Sanchez, L., Jacquard, C., Hafidi, M., Vaillant-Gaveau, N., & Ait Barka, E. (2021). Beneficial microorganisms to control the gray mold of grapevine: from screening to mechanisms. Microorganisms 9:1386. DOI: . https://doi.org/10.3390/ microorganisms9071386

Anonymous (2023). https://www.plantgrape.fr/en/varieties/rootstock-varieties/21

Antonopoulou, C., Dimassi, K., Therios, I., Chatzissavvidis, C., & Tsirakoglou, V. (2005). Inhibitory effect of riboflavin (Vitamin B2) on the *in vitro* rooting and nutrient concentration of explants of peach rootstock GF 677 (*Prunus amygdalusx P. persica*), Scientia Horticulturae 106, 268-272. DOI: https://doi.org/10.1016/j.scienta.2005.02.019

Barka, E. A., Belarbi, A., Hachet, Nowak, C. J., & Audran, J. C. (2000). Enhancement of *in vitro* growth and resistance to grey mould of *Vitis vinifera* co-cultured with plant growth-promoting rhizobacteria. FEMS Microbiol. Lett. 186: 91-95. DOI: https://doi.org/10.1111/j.1574-6968.2000.tb09087.x

Berg, G., Alavi, M., Schmidt, C., Zachow, C., Egamberdieva, D., Kamilova, B., & Lugtenberg, B. J. J. (2013) Biocontrol and osmoprotection for plants under salinated conditions. Molecular Microbial Ecology of the Rhizosphere (de Bruijn FJ, ed), pp. 587–592. John Wiley & Sons, Inc., Hoboken, NJ. DOI: https://doi.org/10.1002/9781118297674.ch55

Blazina, I., Korosec-Koruza, Z., Ravinkar, M., & Gogala, N. (1991). Regeneration and micropropagation of the grapevine (*Vitis vinifera* L.' zelen ') from shoot tip meristem. Acta Horticulturae, 300: 123-126.

Burns, J. A., & Schwarz, O. J. (1996) Bacterial stimulation of adventitious rooting on *in vitro* cultured slash pine (*Pinus elliottii* Engelm.) seedling explants. Plant Cell Rep 15:405–408. DOI: 10.1007/BF00232064

Carletti, S. M., Llorente, B., Rodríguez C´aceres, E., & Tandecarz, J. (1998) Jojoba inoculation with *Azospirillum brasilense* stimulates *in vitro* root formation. Plant Tissue Cult Biotech 4:165–174

Chen, C., Zhang, X., Wei, X., Zhu, Y., Chen, W., & Han, Y. (2022). Postharvest biological control of *Botrytis cinerea* and the mechanisms underlying the induction of disease resistance in grapes by *Lactobacillus plantarum* CM-3. Biological Control 172 (Art. 104982) 15 pp., 2022. DOI: https://doi.org/10.1016/j.biocontrol.2022.104982

Cleyet-Marcel, J.C., Larcher, M., Bertrand, H., Rapior S., & Pinochet, X. (2001). Plant growth enhancement by rhizobacteria. In: MorotGaudry, J.-F. (Ed.), Nitrogen Assimilation by Plants: Physiological, Biochemical, and Molecular Aspects. Science Publishers Inc., Plymouth, UK, pp. 185–197.

Çelik, S. (2011). Bağcılık (Ampeloloji). Namık Kemal Üniversitesi, Ziraat Fakültesi, Bahçe Bitkileri Bölümü, Cilt 1, 3. Baskı, Tekirdağ.

De Klerk, G. J., Ter Brugge, J., & Marinova, S. (1997). Effectiveness of indoleaceticacid, indolebutyric acid and naphthaleneacetic acid during adventitious root formation *in vitro* in Malus 'Jork 9', Plant Cell Tissue and Organ Culture, 49,39-44.

Di Marco, S., & Osti, F. (2007). Effects of Trichoderma applications on vines grown in organic nursery IFOAM Organic World Congress, Modena, Italy, June 16-20, 2008.

Ercişli, S., Eşitken, A., Cangi, R., & Şahin, F. (2003). Adventitious root formation of kiwifruit in relation to sampling date, IBA and *Agrobacterium rubi* inoculation. Plant Growth Regulation 41: 133-137.

Eşitken, A., Ercişli, S., Şevik, İ., & Şahin, F. (2003). Effect of ındole-3- butyric acid and different strains of *Agrobacterium rubi* on adventitive root formation from softwood and semi-hardwood wild sour cherry cuttings. Turk J Agric For 27, 37-42 Tubitak.

Eşitken, A., Karlidag, H., Ercisli, S., Turan, M., & Sahin, F. (2003). The effect of spraying a growth promoting bacterium on the yield, growth and nutrient element composition of leaves of apricot (*Prunus armeniaca* L. cv. Hacihaliloglu). Aust J Agr Res 54: 377–380

Ferreira, G. M., Moreira, R. R., Jarek, T. M., Nesi, C. N., Biasi, L. A., & Mio, L. L. M. (2022). Alternative control of downy mildew and grapevine leaf spot on *Vitis labrusca*. Australasian Plant Pathology 51 (2) 193-201, 2022. DOI: https://doi.org/10.1007/s13313-021-00836-7

Frommel, M. I., Nowak, J., & Lazarovits, G. (1991). Growth enhancement and developmental modifications of *in vitro* grown potato (*Solanum tuberosum* ssp. tuberosum) as affected by a nonfluorescent *Pseudomona*s sp Plant Physiol., 96 (1991), pp. 928-936

Figiel-Kroczyńska, M., Krupa-Małkiewicz, M., & Ochmian, I. (2022). Efficient micropropagation protocol of three cultivars of highbush blueberry (*Vaccinium corymbosum* L.). Not Bot Horti Agrobo 50(4):12856. DOI:10.15835/nbha50412856

Fu, L., Cai, R., Feng, Z., Song, X., Zhu, Y., & Shi, F. (2022). Screening of biocontrol *Bacillus* spp. and its suppression efficacy on grape gray mold. Chinese Journal of Biological Control 38 (2) 440-446, DOI: 10.16409/j.cnki.2095-039x.2022.02.016

Glick, B. R. (1995). The enhancement of plant growth by free-living bacteria. Can. J. Microbiol. 41, 109–117. DOI: https://doi.org/10.1139/m95-015

Goto, M. (1990). Fundamentals of Bacterial Plant Pathology, Academic Press. Inc. San

Gökbayrak, Z. (2006). Troublesome Pest of Viticulture: Phylloxera. Alatarım 2006, 5, 37–43.

Granett, J., Walker, M., Kocsis, L., & Omer, A. D. (2001). Biology and Management of Grape Phylloxera. Annual Review of Entomology 46:387-412.

Howell, G. S., (1987). Vitis Rootstocks. In: Rom, R. C., Carlson, R. F. (Eds.), Rootstocks for Fruit Crops, A Wiley P Inter Sciece Publication, John Wiley and Sons, NewYork, Inc, p 451–472

Khalid, A., Arshad, M., &Zahir, Z. A. (2004). Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat, Journal of Applied Microbiology, 96, 473-480. DOI: https://doi.org/10.1046/j.1365-2672.2003.02161.x

Kloepper, J. W. (1994). Plant growth-promoting rhizobacteria. In: Okon, Y. (Ed.), Azospirillum/Plant Associations. CRC Press, Boca Raton, FL, pp. 137–166.

Korkutal, I., Bahar, E., & Gunes, N. (2017). Different doses effects of *Trichoderma harzianum* and *Bacillus subtilis* on cv. Syrah: I. young plants performance during growing period in organic viticulture. 2nd International Balkan Agriculture Congress, Tekirdag, Turkey, 16-18 May 2017, pp. 650-657

Köse, C., Güleryüz, M., Şahin, F., & Demirtaş, İ. (2005). Effects of some plant growth promoting rhizobacteria (PGPR) on graft union of grapevine. J Sustain Agric. 26(2): 139-147. DOI: https://doi.org/10.1300/J064v26n02_10

Larraburu, E. E., Carletti, S. M., Rodríguez Cáceres, E. A., & Llorente, B. E. (2007). Micropropagation of photinia employing rhizobacteria to promote root development, Plant Cell Reports, 26 (6), 711-717. DOI: 10.1007/s00299-006-0279-2

Mahmood, M. N. (2015). Effect of different doses of biofungicides on growth characteristics of Merlot vines grafted on to 110R rootstock. Namık Kemal University, Graduate School of Natural and Applied Sciences, Tekirdağ. 146s.

Mendes, R., Garbeva, P., & Raaijmakers, J. M. (2013). The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol. Rev. 37, 634–663. DOI: https://doi.org/10.1111/1574-6976.12028

Murashige, T., & Skoog, F. A. (1962). A Revised medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures. Physiol. Plant. 15, pp. 473-497.

Nowak, J., Asiedu, S. K., Bensalim, S., Richards, J., Stewart, A., Smith, C., Stevens, D. and Sturz, A. V. (1998) From laboratory to applications: challenges and progress with *in vitro* dual cultures of potato and beneficial bacteria. Plant Cell Tissue Organ Cult. 52, 97-103. DOI: 10.1007/978-94-015-8951-2_39

Ostroukhova ,E., Peskova, I., Levchenko, S., Vyugina, M., Belash, D., & Shadura, N. (2022). The use of a microbiological preparation based on *Bacillus subtilis* in organic viticulture Source: BIO Web of Conferences 48 (Art. 02006) 8 pp., 2022. DOI:10.1051/bioconf/20224802006

Patena, L., Sutter, E. G., & Dandekar, A. M. (1988). Root induction by *Agrobacterium rhizogenes* in a difficult to root woody species. Acta Horticulturae 227: 324-329. DOI: 10.17660/ActaHortic.1988.227.59

Pedrotti, E. L., Jay-Allemand, C., Doumas, P., & Cornu, D. (1994). Effect of autoclaving amino acids on *in vitro* rooting response of wildcherry shoot. Scientia Horticulturae 57 (1): 89-98. DOI: https://doi.org/10.1016/0304-4238(94)90037-X

Peyvandi, M., Farahani, F., Mazinani, M. H., Noormohamadi, Z., Ataii, S., & Asgharzade, A. (2010). Pseudomonas fluorescent and its ability to promote root formation of olive micro shoots. International Journal of Plant Production 4 (1)

Sabir, A. (2013). Improvement of grafting efficiency in hard grafting grape Berlandieri hybrid rootstocks by plant growth-promoting rhizobacteria (PGPR). Scientia Horticulturae (Amsterdam), 164, 24–29. DOI: https://doi.org/10.1016/j.scienta.2013.08.035.

Sabir, A., Yazici, M. A., Kara, Z., & Şahin, F. (2012). Growth and mineral acquisition response of grapevine rootstocks (*Vitis* spp.) to inoculation with different strains of plant growth-promoting rhizobacteria (PGPR). J Sci Food and Agric 92(10): 2148-2153. DOI: https://doi.org/10.1002/jsfa.5600

Sharma, V., Kamal, B., Srivastava, N., Negi, Y., Dobriyal, A. K., & Jadon, V. S., (2014). Enhancement of *in vitro* growth of *Swertia chirayita* Roxb. Ex Fleming cocultured with plant growth promoting rhizobacteria, Plant Cell Tiss Organ Cult. 121, pages215–225 (2015). DOI: 10.1007/s11240-014-0696-9

Sivritepe, N. (1995). Researches on salt resistance tests and some factors affecting salt resistance in grapevines. Uludağ University Graduate School of Natural and Applied Sciences Department of Horticulture (PhD thesis), Bursa, 176 s.

Srinivasan, M., Holl, F. B., & Petersen, D. J. (1996). Influence of indoleacetic-acid-producing Bacillus isolates on the nodulation of *Phaseolus vulgaris* by *Rhizobium etli* under gno to biotic conditions, Canadian Journal of Microbiology 42 (10): 1006-1014. DOI: https://doi.org/10.1139/m96-129

Steenhoudt, O., & Vanderleyden, J. (2000). Azospirillum, a

free-living nitrogen- fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspect, FEMS Microbiology Reviews, 24, 487-506. DOI: https://doi.org/10.1111/j.1574-6976.2000.tb00552.x

Teixeira, D. A., Alfenas, A. C., Mafia, R. G., Ferreira, E. M., De Siqueira, L., Maffia, L. A., & Mounteer, A. H. (2007). Rhizobacterial promotion of eucalypt rooting and growth. Brazilian Journal of Microbiology 38: 118-123. DOI: https://doi.org/10.1590/S1517-83822007000100025

Tripp, K. E., & Stomp, A. M. (1997). Horticultural applications of *Agrobacterium rhizogenes* (hairy-root): enhanced rooting of difficult to root woody plants. Combined Proceedings of the International Plant Propagators' Society 47: 527-535.

Yağcı, A. (2022). Bağcılık (Lecture notes). https://antalya.tarimorman.gov.tr/Belgeler/bagcilik.pdf (date of access: 12.02.2023)