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# Effects of some plant growth promoting rhizobacteria (PGPR) on rooting of grapevine rootstocks

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## Summary

In this study, effects of topical applications of two plant growth promoting rhizobacteria (PGPR) strains and their combination (*Bacillus* BA16, OSU142 and BA16+OSU142) on the rooting of 41B and *Rupestris* du Lot rootstocks were investigated. The results showed that none of the bacterial strains have significant effects on success rate at 41B and *Rupestris* du Lot alone, but BA16+OSU142 combination significantly increased the rooting rate and rooting degree of 41 B, and decreased the rooting rate and rooting degree of *Rupestris* du Lot compared with control. In addition, none of the applications had significant effects in number, length and weight of roots on cuttings of both 41B and *Rupestris* du Lot.

Our results suggest that PGPR may have a great potential to stimulate the rooting of hardwood cuttings of grapevine rootstocks, with low rooting capability.

Key words: *Rupestris* du Lot, 41 B, rooting, hardwood cutting, PGPR, *Bacillus*

## INTRODUCTION

Phylloxera resistant rootstocks play an important role in viticulture. There are several important priorities being found for grape rootstocks such as phylloxera, nematode and stress conditions resistance, easy grafting and easy rooting (Weaver, 1976; Mullins et al., 1992; Westwood, 1993; Childers et al., 1995). One of these priorities is not more important than the others. *V. berlendieri* is resistant to high calcareous soils and phylloxera, but its use is very limited due to the difficulties in rooting ability of the cuttings. Therefore, some treatments including plant growth

regulators, especially auxins, carbohydrates and other chemical substances have been applied on the cuttings for increasing the rooting percentage.

Some plant growth promoting rhizobacteria belonging to the genus *Pseudomonas* and *Bacillus* are known to have a potential (Lazarovits, Nowark, 1997) for production of phytohormones, particularly indole-3-acetic acid (IAA) (Goto, 1990; Monier et al., 1998; Benizri et al., 1998; Beyeler et al., 1999; Hedden, Phillips, 2000; Lambrecht et al., 2000; Bochow et al., 2001).

Therefore, recent studies have been demonstrated that success in rooting and basal callusing of hardwood cuttings in some plant species important in horticulture, can be achieved by use of PGPR applications (Bassill et al.,1991; Hatta et al., 1996). *Bacillus* OSU142 was reported to have a great potential with antagonistic activity against a number of plant pathogenic bacteria and fungi as well as growth promoting effect on some field and fruit production (Eşitken et al., 2002). However, there has been no attempt to study the effects of PGPR applications on rooting of hardwood cutting of grapevine rootstocks.

The aim of this study was to investigate the effects of the PGPR strains, *Bacillus* BA16 and OSU142 used alone and in combination on the rooting of grapevine rootstocks, having various rooting capability.

## MATERIALS AND METHODS

The experiment was conducted to evaluate the effects of some PGPR strains on the rooting of grapevine rootstocks at Atatürk University, Erzurum, Turkey in 2000.

Bacterial strains, culture condition, media and applications: two plant growth promoting rhizobacteria strains, *Bacillus* BA16 and OSU142 and their combination were used in this study. Bacterial strains were maintained for long term storage in nutrient broth (NB) with 15% glycerol at -80°C. For this experiment, bacterial strains were grown on nutrient agar (NA). A single colony from each strain was transferred to 250 ml flask containing NB, grown aerobically in the flask on a rotating shaker (100 rpm) for an overnight at 27°C. Bacteria grown in NB was then diluted in sterile distilled water to a final concentration of $10^9$ CFU/ml. Resulting bacterial suspensions were used for treatment of grapevine grafts.

Plant materials: Hardwood cuttings of 41B and *Rupestris* du Lot rootstocks were obtained from Vineyard of Egridir Horticulture Research Institute in 2000, and used in this experiments. From previous studies, it is known that rooting capacity of *Rupestris* du Lot cuttings is better than those of 41B rootstock which require exogenous hormone treatments for increasing rooting percentage.

Rooting experiments: The cuttings of rootstocks approximately 40 cm in length with a single bud were divided into 4 treatment groups. Three of them were separately dipped into bacterial suspension ($10^9$ CFU/ml) of BA 16, OSU142 and BA16+OSU142 combination in a 4 $dcm^3$ containers and incubated for 3 hr on a rotating shaker at 150 rpm at room temperature. The cuttings in the control group was dipped into the tap water. After treatments, they were placed in perlite in mist chamber controlled at temperature of 21±2°C and at 90±5% relative humidity for 6 weeks. The trial was laid

out according to complete randomized design with two replications and 20 cuttings per replicate (Düzgüneş et al., 1987). This experiment was repeated twice.

After incubation period, cuttings were evaluated for the rooting rate (%), number, length and weight of root (Panea et al., 1997; Kamiloğlu, Tangolar, 1997). Rooting degree was determined by using 0-4 scale; 0: No rooting, 1: 25% rooting, 2: 50% rooting, 3: 75% rooting and 4: 100% rooting at basal end of the cutting (Kısmalı, Karakır, 1990). Data were evaluated by analysis of variance and means were separated by Tukey' Least Significant Differences (LSD) test at $P< 0,05$ and 0,01 (Table 1).

## RESULTS AND DISCUSSION

Data of the experiment, summarized in Table 1, showed that all bacterial strain applications have an increasing effects on rooting rate and rooting degree at 41B having low rooting capability. However, bacterial strains applied in combination decreased the rooting rate and rooting degree at *Rupestris* du Lot having high rooting capability. All applications had no significant effects on the number, length and weight of root compared to the control even though there was a numerical increase in rooting rate of 41B and *Rupestris* du Lot and rooting degree of 41B and *Rupestris* du Lot by treatments of BA16 and OSU142, OSU142, and BA16 and OSU142, respectively.

Analysis of the data showed that there were significant differences among the treatments.

Overall evaluation of the data demonstrated that there were either numerically or statistically important increases in the rooting percentage and rooting degree of rootstock with low rooting capability by all bacterial applications (Table 1). At rootstock with high rooting capability, the bacterial combination decreased the rooting rate and rooting degree.

Table 1. Effects of the topical application of PGPR strains on rooting of rootstock cuttings

| Rootstock | Application | Rooting Rate (%)[z] | Rooting Degree (0-4) | Root Number | Root Length (cm) | Root Weight (g) |
|---|---|---|---|---|---|---|
| 41B | Control | 30.0 b | 0.6 b | 4.4 | 5.2 | 0.804 |
| | BA 16 | 50.0 ab | 1.3 a | 7.6 | 7.0 | 1.056 |
| | BA 142 | 50.0 ab | 0.7 b | 4.5 | 6.5 | 0.938 |
| | BA16+BA142 | 80.0 a | 1.4 a | 5.8 | 4.0 | 1.115 |
| | *LSD* | ** 20.281* | ** 0.480* | *ns* | *ns* | *ns* |
| *Rupestris* du Lot | Control | 90.0 a | 2.7 a | 11.0 | 7.8 | 14.3 |
| | BA 16 | 100.0 a | 3.5 a | 18.1 | 6.3 | 16.2 |
| | BA 142 | 100.0 a | 3.0 a | 15.6 | 5.8 | 17.3 |
| | BA16+BA142 | 50.0 b | 1.6 b | 16.3 | 9.3 | 14.4 |
| | *LSD* | ** 28.46* | *** 1.029* | *ns* | *ns* | *ns* |

[z] – data were transformed, ns: not significant,*: Data significantly different at 0.05, **: Data significantly different at 0.01

Positive effects of bacterial applications on rooting of rootstock having low rooting rate may be explained by auxin and/or auxin like plant growth promoting substance production of bacterial strains. This speculation has been supported by some other findings in the previous studies demonstrated that BA16 and OSU142 have capability of producing auxin and/or same antimicrobial substances (Şahin et al., 2000; Çakmakçı et al., 2001). Furthermore, Benizri et al. (1998), Beyeler et al. (1999), and Hedden and Phillips (2000) reported that some strains in the genus of *Pseudomonas* and *Bacillus* were able to produce plant growth regulators particularly indole-3-acetic acid (IAA).

It is very well known that the hormones of auxin groups can stimulate root formation of cuttings (Bonner, Galston, 1952; Leopold 1967; Hartman et al., 1990; Raven et al., 1992). Therefore, use of synthetic auxin has been recommended for rooting of grapevine rootstocks cuttings on basis of the evidence reported in the literature by a great number of researchers (Epstein et al., 1984; Watanabe et al., 1998; Jiang et al., 2000).

Application of bacterial strains in combination was found to be more effective than the treatments of each bacterial strain used alone. Similar results have been observed by Ercişli et al., (2000); they demonstrated that *Agrobacterium* strains (A 1, A16 and A18) used alone on sour cherry (*Prunus cerasus*) cuttings were less effective in root formation than strains in combinations.

In contrast to the results at 41B rootstock, mixed suspension of two bacterial strains (BA16+OSU142) decreased rooting rate and rooting degree at *Rupestris* du Lot having high rooting potential. This result was interpreted that high auxin and auxin derivatives contents inhibited adventitious root formation. Due to the bacterial strains in combination may stimulated auxin and/or auxin derivatives, endogenous auxin may reached extreme level in *Rupestris* du Lot rootstock which may having higher auxin level than 41B rootstock. So, extreme auxin level can inhibit root formation on cuttings of *Rupestris* du Lot. It is well known that endogenous auxin level in plant species of which cutting roots easy, was higher than in plants of which cutting roots very difficulty (Iştar et al., 1980). The fact that bacterial strains have different effect on root formation of cutting depending on rootstock can be also attributed to rootstock which can respond variously to auxins (Kafalı, Ergenoğlu, 1993). On the other hand, it is possible to explain this variation by capability of bacterial strains colonizing and producing metabolites at different level on basal end of the cuttings of different rootstock (Rodriques, Frago, 1999).

In conclusion, the results of the study suggest that applications of BA16 and OSU142 can increase rooting of hardwood cutting of grapevine rootstocks having low rooting capability, but not for rootstocks with high rooting capability. So it may be practical to use PGPR strains to stimulate the rooting of grapevine rootstock with low rooting capability.

A further study is necessary to examine the effect of different levels of plant growth regulators (auxins) on the rooting percentage of grapevine rootstock cuttings in addition to PGPR applications.

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## Wpływ niektórych rizobakterii stymulujących wzrost (PGPR) na ukorzenianie sadzonek podkładek winorośli

Streszczenie

Badano wpływ dowierzchołkowego zastosowania dwóch szczepów bakterii stymulujących wzrost (*Bacillus* BA16 i OSU142) oraz łącznego ich użycia na ukorzenianie zdrewniałych sadzonek podkładek winorośli 41B i *Rupestris* du Lot. Wykazano, że osobne użycie każdego ze szczepów bakterii nie miało istotnego wpływu na ukorzenianie sadzonek, ale łączne ich zastosowanie istotnie stymulowało ten proces w przypadku podkładki 41B (trudno tworzącej korzenie), jednocześnie działając ujemnie na ukorzenianie sadzonek podkładki *Rupestris* du Lot (łatwo się ukorzeniającej). Żaden z zabiegów nie miał istotnego wpływu na liczbę, długość i masę korzeni na sadzonkach obu podkładek. Sugeruje się, że bakterie stymulujące wzrost (PGPR) mogą być przydatne do stymulowania tworzenia korzeni przez zdrewniałe sadzonki podkładek winorośli trudnych do ukorzenienia.