

**BACTERIAL TREATMENT IMPACT ON ONE-YEAR-OLD SESSILE OAK
(*QUERCUS PETRAEA* (MATT.) LIEBL) SEEDLINGS OF THREE SERBIAN
PROVENANCES**

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Abstract

Plant growth-promoting bacteria support plant health, development and functioning throughout their life without negative environmental side effects. Their application in the forestry sector is not examined enough. Sessile oak is one of the most valuable forest species in the Serbian forest fond. This research studied the effect of *Viridibacillus arvi* and *Pseudomonas koreensis* on the height and root collar diameter of one-year-old sessile oak seedlings. The results indicate no significant improvements in plant height and root collar diameter as a consequence of bacterial treatment. However, there were notable differences between plants of different provenances. Further research needs to be conducted in order to verify obtained results and get more detailed information on plant-bacteria relations.

Keywords: *bacteria, sessile oak, seedlings, provenances, Serbia.*

Introduction

Demands for good quality seedlings on the global market are increasing. The rise of awareness among the population on environmental pollution and toxicity of different chemical fertilizers and pesticides, used in seedling production, opened space for green solutions, among which biofertilizers and biostimulants take place. In the basis of this concept are different microorganisms and their products that help plant growth. Plant growth-promoting bacteria (PGPB) are a diverse group of bacteria that live on plants or inside their body, making an important part of the plant holobiont (Sanchez-Cañizares et al., 2017). They stimulate plant growth, fructification, resistance to different pathogens and extreme environmental conditions, influence root architecture, and support their development and wellbeing. While helping them to overcome harsh periods of time, they also enhance their adaptivity potential, which is very important in fighting climate change issues (Abdelaal et al., 2021; Fiodor et al., 2021). PGPBs are producers of hormones (auxin, cytokinin, gibberellin), enzymes, acids that dissolve limiting nutrients, siderophores, blocators of ethylene synthesis, and many of them also fix nitrogen (Glick, 2012). Also, through processes of interspecies competence, they indirectly help their plant host (Fira et al., 2018).

PGPB potential for usage in forestry as a "green alternative" for fertilizers and pesticides is not well studied. Serbia has 29.1% forest coverage, according to the latest official data (Banković et al., 2009) and plans to achieve 41 % by 2035, as stated in The Spatial Plan of the Republic of Serbia from 2021 to 2035. Sessile oak (*Quercus petrea* (Matt.) Liebl) is one of the most valuable forest tree species in the Serbian forest fond that has a wide ecological range, and occupies an elevation between 300 and 1300 m. For its wood's excellent technical properties, sessile oak is used in many branches of economy. However, it is equally important in an ecological context, since it is an edicator of numerous forest types that present a significant habitat for the main game species. Compared with other Central European oak species, sessile oak is a more drought-tolerant and different studies predict it will expand its range due to climate change forecasts (Mette et al., 2013; Stimm et al., 2021). Consequently, it

is one of the most commonly grown species in Serbian nursery production (Popović et al., 2019).

Nowadays, sessile oak forests struggle with intense dieback, due to multiple physiological problems associated with forest age. An additional problem is the low success of natural regeneration, which together emphasizes the need for artificial regeneration measures, which further indicates a greater need for top-quality seedlings. Production of forest seedlings is fundamental for future successful establishment of healthy and high yielding economically profitable forest stands (Binotto et al., 2010). In seedling quality evaluations, stem height and root collar diameter are the main morphological parameters for both bare-root and container-grown seedlings' status (Grossnickle and MacDonald, 2018; Ivetić et al., 2017). Grossnickle and MacDonald (2018) reported these morphological attributes are important markers of future survival because, after replanting, seedlings retain these characteristics for an extended time interval as a result of transplanting shock.

The aim of this study was to investigate the effect of bacterial treatments on the stem height and root collar diameter of one-year-old sessile oak seedlings of three Serbian provenances as a principal seedling quality parameters.

Material and Methods

The one-year-old seedlings used in this paper were produced from acorns collected in the Autumn of 2020 from three Serbian provenances – mountain Rudnik in Central Serbia, mountain Rogozna in the south-west part of Serbia, and Grabova reka in eastern Serbia, from their natural forest stands. Container seedlings filled with peat (Freepeat, Holland) were nurtured in the nursery of the Institute of Forestry, in half-shadow conditions and watered every second day (unless it rained).

Two bacterial species – *Viridibacillus arvi* and *Pseudomonas koreensis*, used in seedling treatment, were isolated from rhizosphere soil samples from sessile oak stands on Mount Rudnik and underwent them laboratory testing for *in vitro* plant growth-promoting traits (unpublished data).

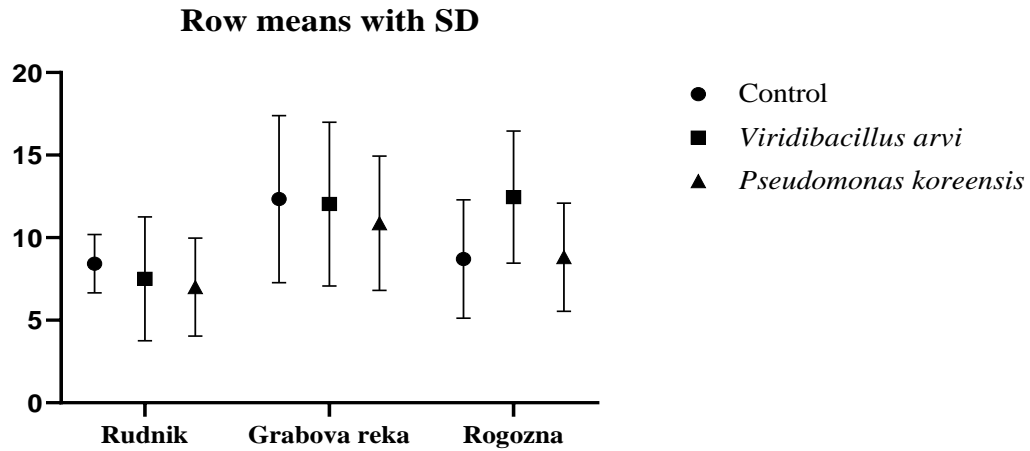
Ten seedlings per treatment were inoculated with 10 ml of bacterial preparations at the end of May, by sterile syringe. The procedure was repeated after 7 days. No additional fertilizers or pesticides were used. Each of the two bacterial treatments contained live bacterial cells of selected bacteria (10^8 cfu/ml) in saline. Tap water was used as a control treatment.

At the end of the growing season, seedling height was measured by a ruler with an accuracy of 0.5 cm. The seedling root collar diameter was estimated by Vernier calliper with an accuracy of 0.1 mm. The morphological characteristics of seedlings were described by descriptive statistics features. A two-way analysis of variance (ANOVA) followed by a Tukey's test were performed to analyse provenance and treatment effect. All statistics were done in GraphPad Prism version 9.0.0. for Windows.

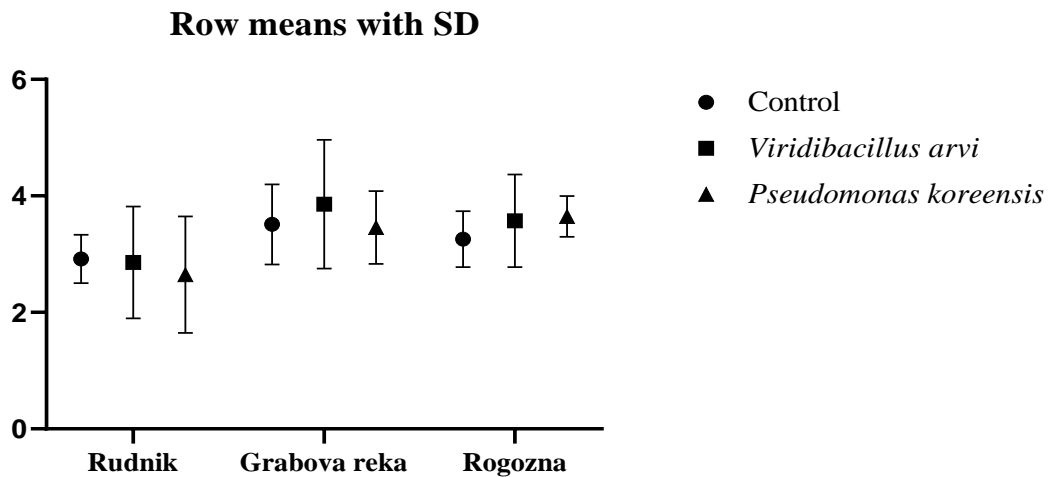
Results and Discussion

Graphs 1 and 2 presents the mean values of seedlings height and root collar diameter regarding provenance and bacterial treatment factors. The maximal mean height seedling value was measured for the group treated with *V. arvi* in the Rogozna population – 12.46 cm, while the lowest was measured for the Rudnik population group treated with *V. arvi* – 6.76 cm. The biggest seedling root collar mean value was detected in a group treated with *V. arvi* in the Rogozna population – 3.57 mm, and the lowest in Rudnik population plants treated with the same bacteria – 2.57 mm. The coefficient of variability value for seedling height ranges from 32.05 % (*V. arvi* treatment in Rogozna population) to 62.94 % (*V. arvi* treatment

in Rudnik population). The coefficient of variability value for seedling root collar diameter ranges from 14.74 % (control treatment in Rogozna population) to 49.7 % (*V. arvi* treatment in Rudnik population). Sessile oak seedlings mean sturdiness quotients with standard deviations and coefficient of variations are presented in Table 1.



Graph 1. Mean height (cm) and standard deviation of sessile oak seedlings



Graph 2. Mean root collar diameter (mm) and standard deviation of sessile oak seedlings

Table 1. The sturdiness quotient (SQ) of sessile oak seedlings

Provenances	SQ								
	<i>V. arvi</i>			<i>P. koreensis</i>			Control		
	M	SD	CV	M	SD	CV	M	SD	CV
Rudnik	24.72	7.71	31.20	27.49	8.18	29.76	29.42	7.39	25.13
Grabova reka	30.73	7.65	24.89	31.08	7.59	24.42	34.41	8.74	25.42
Rogozna	36.12	13.54	37.48	24.25	8.62	35.55	26.38	8.37	31.74

M – mean value, SD – standard deviation, CV – coefficient of variation

In order to study provenance and bacterial treatment influence on measured plant growth attributes, two-way ANOVA was performed (Tables 2 and 3).

Table 2. Analysis of variance for seedling height

Parameter	SS Effect	DF Effect	MS Effect	F	P value
Interaction	63.88	4	15.97	F (4, 76) = 1.092	P=0.3664
Provenance	236.2	2	118.1	F (2, 76) = 8.080	P=0.0007
Treatment	44.44	2	22.22	F (2, 76) = 1.520	P=0.2253

Table 3. Analysis of variance for seedling root collar diameter

Parameter	SS Effect	DF Effect	MS Effect	F	P value
Interaction	1.368	4	0.3420	F (4, 76) = 0.5963	P=0.6664
Provenance	10.51	2	5.256	F (2, 76) = 9.163	P=0.0003
Treatment	0.6838	2	0.3419	F (2, 76) = 0.5961	P=0.5535

For *Pseudomonas koreensis* was shown to produce ammonia, siderophore, protease, amylase and cellulase (Gu et al., 2020). It increased *Arabidopsis* plant fresh weight and lateral root development and antagonized plant pathogenic fungi. Jabborova (2022) reported *P. koreensis* IGPEB 17 to significantly increase plant height, leaf number, length, and width compared to control treatment in *Zingiber officinale*. *Viridibacillus arvi* was first described by Heyrman et al. (2005) as a novel species isolated from soil, and further reclassified by Albert et al. (2007). Both isolate species in our research demonstrated PGP traits (unpublished results). However, in the pot test with sessile oak seedlings, they didn't express a significant impact. A low number of plants might be one reason for this result. Also, concerning the life span of oaks, first year is maybe too early for measuring the effect of bacterial treatment. Some studies reported that PGPB beneficial effect can often be more visible in unfavorable environmental conditions (Aguilera-Torres et al., 2023), which wasn't our case. However, the provenance effect on seedling height and root collar diameter was expressed. Origin impact on seedling attributes is well known and recorded in many studies (Caliskan, 2014). The three presented populations derive from central, eastern and western part of Serbia, regions that differ in site conditions. Different evolution histories affect greatly on seedling's expressed attributes.

Conclusions

The results obtained in this study showed that bacterial treatment of sessile oak seedlings of different provenances didn't significantly improve plant height and root collar diameter. However, there were notable differences between provenances. The obtained results need to be verified, and further tests need to be conducted with a greater plant sample and more plant growth parameters included. Also, plant growth and survival should be examined after transplanting to the field.

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