INSTITUTE OF FORESTRY • BELGRADE
INSTITUT ZA ŠUMARSTVO • BEOGRAD

SUSTAINABLE FORESTRY
COLLECTION 87-88, 2023

ODRŽIVO ŠUMARSTVO
ZBORNIK RADOVA 87-88, 2023

DOI: 10.5937/SustFor2388079J
UDK: 630*232.412.4(497.11)
Original scientific paper

BACTERIAL TREATMENT IMPACT ON MORPHOLOGICAL TRAITS OF ONE-YEAR-OLD SESSILE OAK SEEDLINGS OF TWO SERBIAN PROVENANCES

Sanja JOVANOVIĆ[1], Vladan POPOVIĆ[1], Aleksandar LUČIĆ[1], Ljubinko RAKONJAC[1]*

Abstract: *Plant growth promoting bacteria present a diverse group of bacteria with wide applicative potential in seedling production, especially in referring to the Green agenda concept. The effect of 2 bacterial species on one-year-old sessile oak seedling's height, root collar diameter, and their ratio was studied. Oak plants were from 2 provenances – Avala and Košutnjak. The most significant influence on measured traits in Avala had treatment with Pseudomonas koreensis, and in Košutnjak, with Viridibacillus arvi. However, a two-way analysis of variance showed no statistical significance of provenance effect and bacterial treatment on measured traits. The research needs to be repeated on a greater number of individuals in order to confirm the results since the bacteria manifested their PGP potential in vitro.*

Keywords: plant growth promoting bacteria, sessile oak, provenances, morphology, Serbia

UTICAJ BAKTERIJSKIH TRETMANA NA MORFOLOŠKE OSOBINE JEDNOGODIŠNJIH SADNICA HRASTA KITNJAKA DVE SRPSKE PROVENIJENCIJE

Sažetak: *Bakterije koje promovišu rast biljaka predstavljaju raznovrsnu grupu bakterija koje imaju širok aplikativni potencijal u proizvodnji sadnica, naročito u skladu sa načelima zelene agende. U ovom radu ispitivan je efekat 2 bakterijske vrste na prečnik korenovog vrata, visinu, i njihov odnos kod jednogodišnjih sadnica kitnjaka 2 provenijencije – Košutnjak i Avala. Na njihove vrednosti u provenijenciji Avala najveći efekat imao je tretman bakterijom Pseudomonas koreensis, a u provenijenciji Košutnjak bakterijom Viridibacillus arvi. Međutim, dvofaktorijalna analiza varijanse pokazala je da nema statističke značajnosti između provenijeničnog efekta i bakterijskog tretmana na merene osobine. Istraživanje treba ponoviti na većem broju uzoraka kako bi se potvrdili dobijeni rezultati, s obzirom da su bakterije pokazale in vitro potencijal za promociju rasta biljaka.*

Ključne reči: bakterije koje promovišu rast biljaka, hrast kitnjak, provenijencije, morfologija, Srbija

[1]Institute of Forestry, Kneza Viseslava 3, 11000, Belgrade, Serbia
*Corresponding author. E-mail: sanja.jovanovic@forest.org.rs

1. INTRODUCTION

Bacteria present a large group of very diverse organisms with enormous potential for application. Although being more known as plant pathogens, within the domain of bacteria, there is a special group of "Plant Growth-Promoting Bacteria" (PGPB), with metabolically diverse members that promote plant growth, development, coupling with its surroundings during transplantation shock, fructification, resistance to different plant pathogens, tolerance on stressful environmental conditions, etc. These bacterial species can be found all over plant organism, and there are evidence they are being inherited as well (Abdelfattah et al., 2021). PGPB are most abundant in plant rhizosphere, where their growth and reproduction are supported by root exudates, leading to even species-specific bacteria-plant relations (Backer et al., 2018; Upadhyay et al., 2022). Nowadays, these bacteria are seen as an ecologically adequate substitution for chemical fertilizers, especially in referring to the Green agenda concept.

The forestry sector in Serbia relies on traditional methods of forest seedling production. However, the rise of ecological awareness and knowledge on environmental damage caused by chemical substances that are being used leads to more restricted lists of approved chemicals that can be used in nurseries, and proper replacements need to be found and produced. Sessile oak (*Quercus petraea* (Matt.) Liebl) is one of the most common oak species cultivated in forest seedling nurseries in Serbia (Popović et al., 2019). It is also one of the most important species in Serbian forest, in general. Its forests cover more than 173,000 ha (Banković et al., 2009), and it is highly valued as a resource for many branches of the economy. Due to many problems with its natural regeneration, artificial measures take place for which healthy and top-quality seedlings are necessary. Foresters perceived the significance of different values of seedlings attributes and their association with future transplant success and field performance since the beginning of the 20th century (Grossnickle & MacDonald 2018). Subsequently, they started examining and developing different nursery production strategies to produce as good seedlings as possible, i.e., to manipulate plant traits. In order to estimate seedling quality, foresters use various morphological and physiological plant attributes that are rapid, simple, cheap, reliable, nondestructive, quantitative, and diagnostic (Zaerr, 1985). However, in the operational level, only some of them are practiced. One of the main morphological parameters of good seedling quality are greater root collar diameter and seedling height. Greater stem diameter enables a higher chance of survival and growth by limiting susceptibility to planting stress through improved water uptake and transport to foliage, while greater height provides a competitive advantage (i.e., access to light) on sites with competing vegetation (Grossnickle & MacDonald 2018). Besides plant nursery management, seedling attributes are also influenced by the provenance effect. Different origins and life histories have selected distinct genotypes that can differ in fitness and field performance when found under the same environmental conditions.

In this research, we examined the effect of two plant growth-promoting bacterial isolates on the height, root collar diameter and their ratio of one-year-old sessile oak seedlings of two provenances from Serbia, Avala and Košutnjak, with the aim of inspecting whether the bacterial treatment is positivley affecting these plant traits.

2. MATERIAL AND METHODS

The two bacterial strains were isolated from soil samples of sessile oak rhizosphere from its natural forest stands in Mountain Rudnik (unpublished data) and tested *in vitro* for plant growth-promoting traits and based on the partial 16S rDNA sequence identified as *C* (Heyrman et al., 2005) Albert et al. 2007 and *Pseudomonas koreensis* Kwon et al. 2003 (unpublished data).

Sessile oak acorns used to test bacterial treatments were collected from its natural stands on Mountain Avala and Košutnjak. After seed processing, acorns were sowed in containers for broadleaved species, and the mixture of white and black peat was used as a substrate (Baltic 70/30, The Netherlands).

Within each sessile oak provenance, three groups were formed: 1) group treated with *V. arvi*, 2) group treated with *P. koreensis*, and 3) control group treated with tap water. Each treatment was conducted on ten one-year-old seedlings of sessile oak.

Treatments contained live bacterial cells of selected bacteria (10^8cfu/ml) in saline in 10 ml volume. Seedlings were inoculated by sterile syringe at the end of May. The procedure was repeated after 7 days.

Plants were nurtured in semicontrolled conditions in the Nursery of the Institute of Forestry in Belgrade (44°46’55” N, 20°25’21” E), in half shadow conditions. They were watered every other day, without any fertilization 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 caliper with an accuracy of 0.1 mm. A two-way analysis of variance (ANOVA) followed by a Dunnett’s multiple comparisons post hoc test was performed to analyse the provenance and treatment effect. A P value less than or equal to 0.05 was considered statistically significant. Statistical analysis was done in Graph Pad Prism version 9.0.0. for Windows (Graph Pad Software, San Diego, California USA).

3. RESULTS

In table 1, descriptive statistics data of measured plant attributes is presented for Avala provenance. The minimum height value was reported in treatment with *P. koreensis*, 4.5 cm. The maximum height value was detected in the same treatment group, 21.5 cm. The root collar diameter had its minimal value in the control group, 1.96 mm, while the maximum value was recorded in treatment with *P. koreensis*, 5.36 mm. The height to root collar diameter ratio was highest in a plant from a control treatment group, 6,54, and the lowest value was detected in a plant from a treatment with *P. koreensis*, 1.73.

In table 2, descriptive statistics data is presented for the Košutnjak population. The maximum height value was detected in *V. arvi* treatment, 24 cm, while the minimum was in a control treatment, 7.5 cm. Maximum root collar diameter was reported in the control treatment, 4.61 mm, and the minimum in the *V. arvi* treatment, 2.24 mm. The height to root collar diameter ratio had the highest and lowest values in plants from *V. arvi* treatment group, 6.7 and 2.02.

Table 1. *Descriptive statistics for morphological traits of one-year-old sessile oak seedlings of Avala provenance in relation to bacterial treatment*

Treatments		Height (h)	Root collar diameter (d)	h/d
Viridibacillus arvi	Mean	*12,14*	*3,38*	*3,62*
	Min	*7.00*	*2.64*	*2.35*
	Max	*20.60*	*4.52*	*5.27*
	SD	*3,98*	*0,71*	*0,98*
	CV%	*32.76*	*20.92*	*27.03*
Pseudomonas koreensis	Mean	*13,81*	*3,66*	*3,82*
	Min	*4.50*	*2.37*	*1.73*
	Max	*21.50*	*5.36*	*5.63*
	SD	*5,50*	*0,91*	*1,42*
	CV%	*39.81*	*24.98*	*37.23*
Control	Mean	*12,89*	*3,19*	*4,02*
	Min	*6.00*	*1.96*	*2.01*
	Max	*21.30*	*3.70*	*6.54*
	SD	*6,16*	*0,54*	*1,61*
	CV%	*47.81*	*17.05*	*39.97*

Table 2. *Descriptive statistics for morphological traits of one-year-old sessile oak seedlings of Košutnjak provenance in relation to bacterial treatment*

Treatments		Height (h)	Root collar diameter (d)	h/d
Viridibacillus arvi	Mean	*12,50*	*3,28*	*3,88*
	Min	*6.80*	*2.24*	*2.02*
	Max	*24.00*	*3.81*	*6.70*
	SD	*5,50*	*0,66*	*1.60*
	CV%	*43.98*	*20.16*	*41.25*
Pseudomonas koreensis	Mean	*12,14*	*3,50*	*3,52*
	Min	*8.00*	*2.37*	*2.42*
	Max	*19.50*	*4.43*	*5.03*
	SD	*3,48*	*0,76*	*0,84*
	CV%	*28.68*	*21.70*	*23.94*
Control	Mean	*11,41*	*3,34*	*3,42*
	Min	*7.10*	*2.08*	*2.44*
	Max	*16.00*	*4.61*	*4.67*
	SD	*3,74*	*0,84*	*0,76*
	CV%	*32.76*	*25.27*	*22.29*

In the Avala provenance, coefficients of variation (CV) were the highest in the control treatment for plant height and height to root collar diameter ratio, while the highest CV for root collar diameter was observed in treatment with *P. koreensis*. The lowest CV was detected in control treatment for root collar diameter, while for height and height to root collar diameter ratio in *V. arvi* treatment. Based on CV, plant height was more variable trait than the root collar diameter.

In the Košutnjak provenance, CV values were highest in the group treated with *V. arvi* for height and height to root collar diameter ratio. In contrast, the highest value of CV for root collar diameter was observed in the control group. The lowest CV value for height trait was detected in the group treated with *P. koreensis*. For root collar diameter the lowest value was observed in the group treated with *V. arvi*, and for height to root collar diameter in the control group. On the basis of CV, plant height was the most variable trait in this provenance, and the least variable was root collar diameter.

Table 3. *Two-way analysis of variance (ANOVA) for the measured morphological characteristics of one-year-old seedlings of sessile oak from two provenances and under two bacterial treatments*

Parameter		**F (DFn, DFd)**	**p**
Root collar diameter	Treatment	*F (1.917, 27.80) = 1.398*	*0.2635*
	Provenance	*F (1, 18) = 0.01944*	*0.8907*
	Treatment x Provenance	*F (2, 29) = 0.4910*	*0.6170*
Plant height	Treatment	*F (1.803, 26.15) = 0.1601*	0.8316
	Provenance	*F (1, 18) = 0.4517*	*0.5100*
	Treatment x Provenance	*F (2, 29) = 0.2375*	*0.7901*

In order to investigate provenance and bacterial treatment influence on measured plant growth attributes, two-way ANOVA was performed. The results of a two-way ANOVA are presented in Table 3. There is no statistically significant effect of provenance and bacterial treatment and their interaction on seedling height or root collar diameter.

4. DISCUSSION

Root collar diameter and seedling height are phenotypic plant attributes affected by many factors, such as genetics, provenance effect, climate and weather, soil properties, pathogens, and other environmental elements (Vivas et al., 2019). In this paper, we investigated the influence of bacterial treatment on these two plant traits on a one-year-old sessile oak seedlings in one growing season. Although differences in the mean values exist for each of the two measured traits, they are not statistically significant.

Popović et al. (2022) measured the same growth parameters on one-year-old sessile oak seedlings that originate from healthy acorns of selected trees of the Area of Outstanding Natural Landscape "Avala". The mean height value was 22.9 cm, which is greater than in this research (13,81 cm was the highest mean value in Avala, 12,5 cm in Košutnjak). Although both of our maximum height values were obtained from the bacterial treatment group plants, the maximum height reached in the control groups was 21.3 cm and 16 cm, respectively. Regarding the seedling root collar diameter, in the mentioned study, the mean value was 4.4 mm. In comparison, in our study, these values are 5.36 mm for the Avala population (in bacterial treatment group) and 4.61 mm (the control group) for Košutnjak. Popović et al. (2022) conducted their research on a 50 seedlings sample, that gives more reliable final results and conclusion. However, since the mother trees and acorns were selected, their results could present an above-average values.

Popović et al. (2019) investigated the same traits on one-year-old sessile oak bare root seedlings. The mean root collar diameter was 3.52, and the mean height was 14.8 cm. Seedlings originated from eastern Serbia and were produced in the same nursery as seedling for this research. These results are more similar to ours, however, the experimental seedlings were bare-rooted, while seedlings in the present study were produced in containers. Significant differences in root architecture of these two types of seedlings can affect future plant characteristics.

Avala and Košutnjak are two provenances in central Serbia, with very similar climate conditions. In both provenances, the most variable trait was seedling height, while the least variable was root collar diameter.

In Avala provenance, the higher impact on observed plant traits was achieved in treatment with *P. koreensis*. However, in population Košutnjak, treatment with *V. arvi* had higher influence on seedling traits (except for root collar diameter) based on mean, maximal, and minimal values. Bacteria *V. arvi* and *P. koreensis* are soil bacteria already described as plant growth-promoting bacteria (Gu et al., 2020; Jabborova, 2022; Lyngwi et al., 2016). *In vitro* tests confirmed their PGP potential (unpublished results). Therefore, it is possible that the conditions for experiment performance were not optimal. In unpredictable and stressful field conditions, bacterial population might have decreased its number, or couldn't express its PGP potential. In addition, a low number of oak seedlings might have affected the results, and the experiment should be investigated on a larger plant sample. Another factor that should be considered is the life span of oaks. The seedlings might be in an inappropriate stage for the evaluation of bacterial treatment efficiency. Since the scarcity of similar studies in this forest species, there is a large open space for research to be conducted in the future, where all factors can and should be tested.

5. CONCLUSION

The results obtained in this study do not show a statistically significant interaction between the effects of provenance and bacterial treatment on seedling height or root collar diameter. The research should be conducted on a larger sample, and more growth parameters included. Since one of the major challenges in forestry is seedling transplantation and establishment in new field conditions, in the future, it

is recommended to investigate the bacterial influence on plant growth and survival after transplanting to the field.

Acknowledgment: *This study was carried out under the Agreement on realization and funding of scientific research activity of scientific research organizations in 2023 funded by the Ministry of Science, Technological Development and Innovation. No. 451-03-47/2023-01/ 200027.*

REFERENCES

Abdelfattah, A., Wisnieswski, M., Schena, L., & Tack, J.M.A. (2021). Experimental evidence of microbial inheritance in plants and transmission routes from seed to phyllosphere and root. *Environmental Microbiology, 23(4),* 2199–2214. doi:10.1111/1462-2920.15392

Banković, S., Medarević, M., Pantić, D., & Petrović, N. (2009). *Nacionalna inventura šuma Rešublike Srbije - Šumski fond Republike Srbije*. Ministarstvo poljoprivrede šumarstva i vodoprivrede Republike Srbje - Uprava za šume, Beograd (1-244).

Backer, R., Rokem, J.S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., & Smith, D.L. (2018). Plant Growth-Promoting Rhizobacteria: Context, Mechanisms of Action, and Roadmap to Commercialization of Biostimulants for Sustainable Agriculture. *Front. Plant Sci.*, 9:1473. doi: 10.3389/fpls.2018.01473

Gu, Y., Ma, Y., Wang, J., Xia, Z., & Wei, H. (2020). Genomic insights into a plant growth-promoting Pseudomonas koreensis strain with cyclic lipopeptide-mediated antifungal activity. *Microbiologyopen, 9(9):*e1092.

Grossnickle, S.C., & MacDonald, J.E. (2018). Seedling quality: history, application, and plant attributes. *Forests*, 9(5):283.

Jabborova, D. (2022). The effects of *Pseudomonas koreensis* IGPEB 17 and arbuscular mycorrhizal fungi on growth and physiological properties of ginger. *Turkish Journal of Agriculture and Forestry, 46(4)*:488-495. doi: 10.55730/1300-011X.3020

Lyngwi, N.A., Nongkhlaw, M., Kalita, D., & Joshi, S.R. (2016). Bioprospecting of Plant Growth Promoting Bacilli and Related Genera Prevalent in Soils of Pristine Sacred Groves: Biochemical and Molecular Approach. PloS ONE, *11(4)*:e0152951doi: 10.1371/journal.pone.0152951

Mataruga, M., Cvjetković, B., Cuyper, B.D., Aneva, I., Zhelev, P., Cudlín, P., Metslaid, M., Kankaanhuhta, V., Collet, C., Annighöfer, P., Mathes, T., Tsakaldimi, M., Paitaridou, D., Jónsdóttir, R.J., Monteverdi, M.C., de Dato, G., Mariotti, B., Kolevska, D.D., Lazarević, J., Sundheim Fløistad, I., Klisz, M., Gil, W., Paiva, V., Fonseca, T., Nicolescu, V-N., Popović, V., Devetaković, J., Repáč, I., Božič, G., Kraigher, H., Andivia, E., Diez, J.J., Böhlenius, H., Löf, M., Bilir, N., Villar-Salvador, P. (2023). Monitoring and control of forest seedling quality in Europe. *Forest Ecology and Management*, 546 doi: 10.1016/j.foreco.2023.121308

Popović, V., Lučić, A., Rakonjac, Lj., & Kerkez-Janković, I. (2019). Analysis of morphological quality parameters of one-year old bare root sessile oak (*Quercus petraea* (Matt.) Liebl) seedlings. *Sustainable forestry*, 79-80, Institute of Forestry, Belgrade, p. 59-64

Popović, V., Lučić, A., Jovanović, S., & Rakonjac, Lj. (2022). Variability of characteristics of sessile oak (Quercus petraea (Matt.) Liebl) seedlings from the area of outstanding natural landscape "Avala". *Sustainable forestry*, 85-86, Institute of Forestry, Belgrade, p. 53-63. DOI: 10.5937/SustFor2285065C

Upadhyay, S.K., Srivastava, A.K., Rajput, V.D., Chauhan, P.K., Bhojiya, A.A., Jain, D., Chaubey, G., Dwivedi, P., Sharma, B. & Minkina, T. (2022). Root Exudates: Mechanistic Insight of Plant Growth Promoting Rhizobacteria for Sustainable Crop Production. *Front. Microbiol,* 13:916488. doi: 10.3389/fmicb.2022.916488

Vivas, M., Rolo, V., Wingfield, M.J., & Slippers, B. (2019). Maternal environment regulates morphological and physiological traits in *Eucalyptus grandis*. *Forest Ecology and Management, 432*:631-636. doi: 10.1016/j.foreco.2018.10.016

Zaerr, J.B. The role of biochemical measurements in evaluating vigor. In Evaluating Seedling Quality: Principles, Procedures, and Predictive Abilities of Major Tests; Duryea, M.L., Ed.; Forest Research Laboratory, Oregon State University: Corvallis, OR, USA, 1985; pp. 137–141.

BACTERIAL TREATMENT IMPACT ON MORPHOLOGICAL TRAITS OF ONE-YEAR-OLD SESSILE OAK SEEDLINGS OF TWO SERBIAN PROVENANCES

Sanja JOVANOVIĆ, Vladan POPOVIĆ, Aleksandar LUČIĆ, Ljubinko RAKONJAC

Summary

Plant growth promoting bacteria present a diverse group of bacteria that have wide applicative potential in seedling production, especially in refer to Green agenda concept. One of the most producted forest species in Serbian forest nurseries is sessile oak. In this paper the effect of 2 bacterial species on one-year-old sessile oak seedling's height, root collar diameter, and their ratio was studied. Oak plants were from 2 provenances – Avala and Košutnjak.

The observed traits were estimated by a Vernier caliper and a ruler. The greatest influence on measured traits in Avala had treatment with *P. koreensis* bacteria, and in Košutnjak the one with *V. arvi*. Two-way analysis of variance showed there were no statistical significance of provenance effect and bacterial treatment on measured traits. The research needs to be repeated on greater number of individuals in order to confirm obtained results, since the bacteria manifested their PGP potential in laboratory. Since one of the major challenges in forestry is seedling transplantation and establishment in new field conditions, in future it is recommended to investigate bacterial influence on plant growth and survival after transplanting to the field.

UTICAJ BAKTERIJSKIH TRETMANA NA MORFOLOŠKE OSOBINE JEDNOGODIŠNJIH SADNICA HRASTA KITNJAKA DVE SRPSKE PROVENIJENCIJE

Sanja JOVANOVIĆ, Vladan POPOVIĆ, Aleksandar LUČIĆ, Ljubinko RAKONJAC

Rezime

Bakterije koje promovišu rast biljaka predstavljaju raznovrsnu grupu bakterija koje imaju širok aplikativni potencijal u proizvodnji sadnica, naročito u skladu sa načelima zelene agende. Jedna od šumskih vrsta koje se najviše proizvode u rasadnicima Srbije je hrast kitnjak. U ovom radu ispitivan je efekat 2 bakterijske vrste na prečnik korenovog vrata, visinu kao i njihov odnos kod jednogodišnjih sadnica kitnjaka 2 provenijencije – Košutnjak i Avala.

Posmatrane osobine merene su pomičnim kljunastim merilom i lenjirom. Na njihove vrednosti u provenijenciji Avala najveći efekat imao je tretman bakterijom *P. koreensis*, a u provenijenciji Košutnjak bakterijom *V. arvi*. Dvofaktorijalna analiza varijanse pokazala je da nema statističke značajnosti između provenijeničnog efekta i bakterijskog tretmana na izmerene osobine. Istraživanje treba ponoviti na većem broju uzoraka kako bi se potvrdili dobijeni rezultati, s obzirom da su bakterije laboratorijski pokazale potencijal za promociju rasta biljaka. Kako je jedan od glavnih izazova u šumarstvu presađivanje sadnica i njihovo uspostavljanje u novim terenskim uslovima, preporuka je da se u budućnosti ispita uticaj bakterija na rast i preživljavanje biljaka nakon presadnje na teren.