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# The influence of origin on the quality of pedunculate oak (Quercus robur L.) seedlings

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#### Abstract

Oak forests are among the most ecologically and economically significant in Europe. Due to the consequences of climate change on the environment, successful restoration of these ecosystems has been reduced in recent times. In order to increase the regeneration of oak forests, the analysis of quality parameters of one hundred and twenty pedunculate oak (Quercus robur L.) seedlings of ten different half-sib families have been carried out in the nursery of the Institute of Forestry in Belgrade (Serbia). This study showed the influence of origin (mother trees) on the morphological characteristics of pedunculate oak seedlings. A high degree of variability has been determined for root collar diameter, height, weight of aboveground part, root weight, Roller's sturdiness coefficient and seedlings quality index. The largest variability has been determined for the height of seedlings where a large number of transitional halfsib families is noticed between the largest and the smallest values. A small number of half-sib families (33.33%) showed high quality of seedlings, based on the cluster analysis of quality parameters. There was a high degree of correlation between root weight and aboveground part weight, giving the potential for the selection of quality reproductive material with a properly formed habitus. There was a high degree of correlation between root weight and aboveground part weight, which suggests the potential for selecting quality reproductive material with a properly formed habitus. This study facilitates the selection of suitable reproductive material sources for regenerating pedunculate oak forests within this particular region of distribution.

#### Keywords

Forestry; Pedunculate oak; Quality parameters; Variability; Selection

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## **1** Introduction

The genus *Quercus* contains about 430 deciduous and coniferous tree and shrub species (Cvjeticanin et al. 2016). Pedunculate oak (*Quercus robur* L.) represents one of the most significant species of this genus in Europe. In addition to ecological significance, it has played a significant role in human culture since ancient times (Cvjeticanin et al. 2016; Eaton et al. 2016). Furthermore, pedunculate oak stands out as the most productive tree species in habitats with alluvial-diluvial soils found in river terraces across specific regions worldwide (Kostin and Manaenkov 2019). Due to valuable trees, pedunculate oak forests are especially significant for maintaining various functions of production, biodiversity and social functions, which are achieved by choosing different management strategies (Lof et al. 2016). Recently, there is a need for more intensive planning of management methods in order to harmonize ecological values of these forests with sustainable forestry (Molder et al. 2019a). All of the above indicates the need for selection of high-quality seedlings of pedunculate oak which will facilitate the development of this tree species.

More intensive development of pedunculate oak seedlings is significant also in competition with other vegetation which has a negative effect on transpiration processes (Jensen et al. 2011). In the forests of pedunculate oak, a large number of parasitic and saprophytic species of fungi were found (Karadzic et al. 2017, Marciulynas and Menkis 2024). In this sense, a large number of species of fungi was recorded on healthy seedlings, indicating their importance due to the loss of seedling vitality (Jankowiak et al. 2022). Furthermore, it was found that large diversity of the fungi species on the root of pedunculate oak has more of an inhibitory than stimulating effect on its growth (Kwasna and Szewczyk 2016).

Increased growth of pedunculate oak leads to shortening the time of fruiting, which has great benefits for regeneration of oak forests (Olave et al. 2021). Larger acorns of pedunculate oak give larger germination percentage than smaller acorns (Devetakovic et al. 2019). In the continuation of these research, it is necessary to examine the quality of pedunculate oak seedlings and thus obtain the knowledge on morphological characteristics of reproductive material of pedunculate oak from this part of range of distribution. In this way, detailed data regarding the potential of starting material selection is obtained.

# 2 Material and methods

## 2.1 Field methods

In October 2017, seed material was collected in the seed stand RS-2-2-qro-11-828 (X 7431343, Y 4956045), on the territory of the city of Belgrade (Serbia). The selection of mother trees was carried out based on phenotypic characteristics and abundance of yield. On the whole area of the seed stand, ten mother trees were selected, under which crowns 5 kg of visually healthy and undamaged acorns was collected, regardless of dimensions. After the collection, acorns were dried to 35% of humidity and stored at the temperature of 3-5 °C. After processing the seeds were sown

in containers LIECO type L15 of volume of 390 cm<sup>3</sup> in the nursery of the Institute of Forestry in Belgrade. During the first and the second growing season regular care measures were carried out, including mechanical weeding, watering and chemical treatment with fungicides against powdery mildew caused by fungus *Microsphaera alphitoides*.

At the end of the second growing season, in November 2019, 12 seedlings per mother tree were randomly selected for morphometric analyses. The seedlings were carefully removed in order to reduce root damage. The following parameters were measured: height, root collar diameter, weight of aboveground part of the seedling in absolutely dry state and root weight in absolutely dry state. The height of seedlings was measured by ruler with the precision of 0.1 cm, and root collar diameter by vernier calliper with the precision of 0.01 mm. Thereafter, aboveground part was separated from the root, and they were dried separately in the dryer of the type Binder, at the temperature of 105 °C for 48 hours. The weight of the aboveground part and the weight of root were measured on an electronic scale with an accuracy of 0.01 g. Sturdiness coefficient (SQ) was calculated according to Roller 1977, and quality index (QI) according to Dickson et al. 1960.

#### 2.2 Statistical methods

Preliminary screening of the data included the analysis of fulfilment of the conditions for application of the selected tests. Linearity and homoscedasticity of general linear model are verified based on the dot chart. The normality of residuals for each quality indicator was carried out by Kolmogorov-Smirnov test with Lilliefors correction.

General linear model (GLM) with Tukey's HSD post hoc test was used for comparison of values of root collar diameter, height, Roller's sturdiness coefficient and quality index between different genotypes. For values of root weight and aboveground part weight the conditions for application of GLM were not fulfilled, which is why nonparametric tests were used. Kruskal-Wallis test with Dunn's post hoc test was used for comparing weight of the aboveground part and root weight between different half-sib families.

Statistical analyses were carried out using software packages Microsoft Office 2021 (Microsoft Corp.) and SPSS 27 (IBM Corp.).

# 3 Results

There was statistically significant difference in size of root collar diameter, height, Roller's sturdiness coefficient and quality index between different half-sib families based on GLM (Table 1). Kruskal-Wallis test showed statistically significant difference in weight of the aboveground part (H = 40.831; p < 0.001) and root weight (H = 29.623; p < 0.001).

Tukey's HSD test showed statistically significant difference between different combinations of half-sib families in dimensions of root collar diameter, height, ratio of height and root collar diameter, and quality index (Table 2).

According to table 2, the seedlings from half-sib family 5 had the largest root collar diameter. The seedlings from half-sib families 1, 3 and 6 had the smallest root collar diameter. Based on root collar diameter measurements, the highest number of genotypes was observed at the transition point between these seedlings.

Table 1. The influence of half-sib family to on various quality parameters of pedunculate oak seedlings.

Tested parameter	Type III sum of squares	Degrees of freedom	Mean square	F-value	Significance
Root collar diameter	95.545	9	10.616	3.010	0.003
Shoot height	11082.242	9	1231.360	11.502	< 0.001
Sturdiness coefficient	88.125	0	9.792	10.657	< 0.001
Quality index	2.617	9	0.291	8.907	< 0.001

Table 2. Tissue nutrient contents of *G.arborea* seedlings inoculated with AM fungi and PGPR (mean of 5 replicates).

Half-sib family	Root collar diameter (mm)	Shoot height (mm)	Sturdiness coefficient	Quality Index
1	7.89 ± 1.18a	42.33 ± 6.17ad	5.45 ± 1.03ac	1.50 ± 0.10a
2	8.81 ± 2.52ab	43.42 ± 11.67ad	5.05 ± 1.17a	1.50 ± 0.10a
3	7.91 ± 1.63a	46.67 ± 10.39ach	5.96 ± 1.06ad	1.46 ± 0.13a
4	10.15 ± 1.71ab	55.42 ± 8.03dfgh	5.53 ± 0.79ac	1.56 ± 0.14ac
5	10.53 ± 1.36b	67.33 ± 8.68fg	6.46 ± 0.92bc	1.82 ± 0.19b
6	7.91 ± 2.01a	57.17 ± 14.26bcfg	7.29 ± 0.89b	1.75 ± 0.21bc
7	9.21 ± 1.92ab	64.33 ± 11.73g	7.10 ± 1.08bd	1.86 ± 0.31b
8	8.54 ± 2.03ab	49.50 ± 9.81acdh	5.90 ± 0.82ad	1.56 ± 0.14ac
9	8.73 ± 1.91ab	40.83 ± 12.29ae	4.67 ± 0.90a	1.48 ± 0.22a
10	8.11 ± 2.15ab	38.08 ± 7.76ae	4.84 ± 0.85a	1.45 ± 0.18a

Notes: a-h: homogeneous groups determined using Tukey's test, p < 0.05.

The height of seedlings of different half-sib families showed great variability. Seedlings of half-sib families 5 and 7 had the largest height, while seedlings from halfsib family 6 had similar height and, they were on the transition toward the seedlings of smaller dimensions. A large number of seedlings had smallest heights or were at the transition between the largest and the smallest heights (Table 2).

According to the same table, the seedlings from the half-sib family 6, which in terms of height were close to the tallest seedlings but had the lowest value of the root collar diameter, also had the largest ratio of height and diameter. In addition, the seedlings from the half-sib families 5 and 7 had the largest quality index.

Dunn's post hoc test showed statistically significant difference between different half-sib families in the weight of aboveground part and root weight (Table 3).

As expected, the seedlings from half-sib families 5 and 7 had the largest weight of aboveground part. Nevertheless, root weight was the largest in half-sib family 4 followed by half-sib families 5 and 7. When measuring weight of aboveground part, less variability was observed compared to the root weight (Table 3).

According to Table 4, there was statistically significant correlation between morphological traits of seedlings and the weight of seedlings, as well as between the weight of aboveground part and the root weight of seedlings. The seedling diameter showed the largest correlation with the aboveground part weight and root weight.

Cluster analysis showed clear differentiation of seedlings of different half-sib families compared to their quality parameters (Figure 1). The seedlings from half-sib families 4, 5 and 7 showed joint characteristics of the highest quality. For the half-sib families 8, 3, and 6, the values of quality indicators were in the middle. The remaining seedlings formed separate clusters, showing the lowest values of quality parameters (Figure 1).

Table 3. Mean value	Table 3. Mean values (± standard deviation) of oak seedling traits.				
Half-sib family	Shoot dry weight (g) Root dry weight (g				
1	5.11 ± 2.37a	10.18 ± 4.40ac			
2	7.24 ± 6.21a	13.96 ± 9.63a			
3	5.19 ± 2.35a	11.24 ± 4.00a			
4	12.04 ± 5.66b	21.16 ± 5.75e			
5	14.04 ± 5.68b	17.18 ± 5.03bde			
6	7.71 ± 6.18ad	9.49 ± 5.59a			
7	12.46 ± 6.77b	14.52 ± 6.91abc			
8	7.41 ± 5.28a	12.92 ± 7.62ad			
9	5.92 ± 3.86a	12.11 ± 5.29a			
10	5.39 ± 3.79a	11.60 ± 6.55a			

Table 4. Correlation between quality parameters of pedunculate oak seedlings.

Parameter	Statistics	Root collar diameter	Shoot height	Shoot dry weight	Root dry weight
	Pearson	1	.631	0.834	0.860
Root collar diameter	Р	-	< 0.001	< 0.001	< 0.001
	Ν	120	120	120	120
	Pearson	.631	1	.783	.560
Shoot height	Р	< 0.001	-	< 0.001	< 0.001
	Ν	120	120	120	120
	Pearson	0.834	0.783	1	0.816
Shoot dry weight	Р	< 0.001	< 0.001	-	< 0.001
	Ν	120	120	120	120
	Pearson	.860	.560	.816	1
Root dry weight	Р	< 0.001	< 0.001	< 0.001	-
	Ν	120	120	120	120

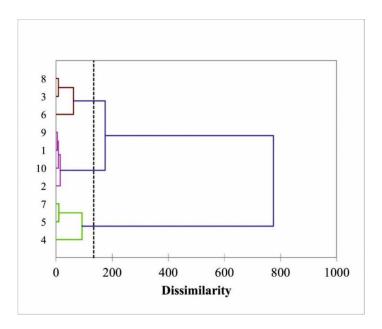


Figure 1. Dendrogram of cluster analysis of quality of pedunculate oak seedlings of different half-sib families.

#### 4 Discussion

This study showed significant possibility of selection of starting material of pedunculate oak based on the morphometric traits in progeny test. The determined morphological variability of root collar diameter and the height of seedlings in this study is in line with the previous study on the variability of leaf dimensions of pedunculate oak trees (Batos et al. 2017). The obtained results enable more precise insight into potential of pedunculate oak trees in this part of the range of distribution for production of high-quality seedlings which will meet the needs in forestry for regeneration of pedunculate oak forests and production of wood of the highest quality.

The successful regeneration of pedunculate oak relates to maintaining proximity of mother trees in order to maintain ecological continuity in oak forests (Frouz et al. 2015; Molder et al. 2019b). The use of seedlings obtained from these mother trees can significantly facilitate the work on the process of regeneration of these forests, reducing the necessity of maintaining proximity of mother trees by silvicultural measures. In addition, quality reproductive material of pedunculate oak is also significant in order to study the processes which will enable different ways of its more successful planting and resistance to stress caused by low temperatures (Saha et al. 2013; Szuba et al. 2022).

Conservation of pedunculate oak relates to management of dominant and vital trees, which will enable high productivity of these stands (Stimm et al. 2022). High quality seedlings which have the potential for reaching these dimensions represent the starting point for establishment of stable and productive stands. The studies related to the effect of the type of containers to morphological performances of pedunculate oak seedlings showed various results (Mariotti et al. 2014; Popovic et al. 2014; Mijatovic et al. 2022). We deem that in order to speed up the production it is necessary to favor the production of the said genotypes in different types of containers. The density of seedlings in containers can be reduced to increase the growth and production of quality seedlings (Banach et al. 2023). Therefore, it is important to further investigate the potential for improving the growth parameters of the lower-quality genotypes in this study.

In case of drought stress, the adaptive mechanism of pedunculate oak activates reduced growth (Deligoz and Esra 2018). Therefore, we deem that on the habitats exposed to short-term drought stress preference should be given to the reproductive material produced from genotypes with higher quality, which will be due to the reduction in dimensions still preserve the productivity of these habitats. On the other hand, in case of habitats with longer duration of drought stress, special detailed studies are necessary that will determine whether there is a possibility of use of seedlings with smaller dimensions. The vitality of pedunculate oak trees is one of the most important factors that have effect on diversity of fungi that appear on this tree species (Agostinelli et al. 2018). Accordingly, it is recommended to use half-sib families of high quality in order to reduce future pathogen damage that may occur in these forests.

Planting of pedunculate oak in groups instead of traditional planting in lines can contribute a lot to survival, growth and quality of the trees (Saha et al. 2012). We believe that the use of seedlings of the pronounced quality can significantly contribute to positive aspects of this planting technique. In addition, it is necessary to test the use of lower-quality seedlings in these conditions because they may grow better.

The seedlings of pedunculate oak produced from the larger acorns had larger dimensions than seedlings produced from smaller acorns (Roth et al. 2011). In this research the examination of the effect of seed morphology to the seedling morphology was not carried out. Different morphological indicators clearly differentiated highquality seedling genotypes, which can be used to investigate the influence of acorn morphology on their characteristics in the future.

# **5** Conclusion

In this study, the analysis of various quality parameters of pedunculate oak seedlings produced from the seeds of different mother trees was carried out. The obtained results and conclusions can be presented as follows:

- Significant differences were found in root collar diameter, height, aboveground part weight, root weight, Roller's sturdiness coefficient, and quality index among seedlings from different half-sib families.
- The height of seedlings exhibited the greatest variability, with numerous categories reflecting various degrees of height development.
- A statistically significant and strong correlation was observed between the weight of the aboveground part and root weight. This suggests a realistic potential for cultivating seedlings with optimal characteristics from this stock.
- Grouping seedlings based on the tested parameters revealed distinct clusters representing seedlings of highest, medium, and lowest quality among all tested half-sib families.
- Seedlings from half-sib families demonstrating the highest quality should be prioritized for use whenever feasible to minimize damage caused by competing vegetation and the diverse fungal species commonly found in pedunculate oak habitats.

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