Provenances vs. microhabitat on field performance of *Quercus robur* seedlings

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Abstract

Provenance trials, as the subject of continuous analyses, provide empirical information about the plasticity of tree species. Changing climate and environmental conditions likely favor genotypes with high levels of plasticity. Finding the suitable provenance for the reintroduction of pedunculate oak to habitats that are threatened and where this species no longer exists provides important information for the targeted use of the available gene pool. The dominant ecological factors in the development of pedunculate oak forests are groundwater level and changes in the hydrological regime of habitats. In this study, we established nursery and field provenance trials to test two pedunculate oak seed provenances from different hydrological conditions to investigate the influence of seedlings' provenance and field microhabitat on growth parameters and survival. In the nursery trial, the height and ground level diameter were measured. After three years in the pilot object, the height and diameter were analyzed again, as well as the survival. To determine the microhabitat influence planting area was divided in two ways: three repetitions and two planting blocks. In this study, significant differences in analyzed growth parameters between the chosen provenances were obtained at the end of the first vegetation period. In later ontogenetic phases influence of the provenance is missing. As the difference between provenances disappears, the influence of microhabitat occurs (significant differences between repetition or planting blocks). After the second year of development, no significant difference was observed between the two provenances of different hydrological regimes, but there are significant differences between the microhabitats in the afforested area (established pilot object).

Keywords

Pedunculate oak; Provenances; Nursery trial; Growth characteristics; Survival

Contents

1 Introduction

Natural regeneration and silviculture that is close to nature have been often promoted and considered a priority in most European countries. High forest, coppice-with standards and coppice are the three main silvicultural regimes for pedunculate oak forests, as one of the economically and ecologically most important species in Europe (Ducousso and Bordacs 2004). However, besides many insects and pathogens (Eaton et al. 2016), human pressure and the transformation of land into agricultural use (Bozzano and Turok 2003), changes in rainfall distribution and incidence of stress-induced pathogens (Vannini et al. 1996), Acute Oak Decline (AOD) (Denman et al. 2014), forest dynamic and the evolution of forestry practices (forsaking of coppicing, aging of the populations) and introduction of exotic genotypes through plantations, the considerable risk for European oak forests is oak forest management, which seems very conservative regarding genetic resources (Ducousso and Bordacs 2004). Due to difficulties in the natural regeneration of oak forest (Johnson 1979; Povak et al. 2008; Mölder et al. 2019), artificial regeneration and sometimes reintroduction is required. In terms of reintroduction genetic quality and suitability of the forest reproductive material is crucial. However, sometimes, it is impossible to use local reproductive material and choosing the right provenance could be an issue.

Provenance trials, as the subject of continuous analyses (Šijačić-Nikolić and Milovanović 2010), provide, among others, empirical information about the plasticity of tree species. Phenotypic plasticity is not unlimited buffering capacity (Mátyás 2007) and it differs among populations and species (Aitken et al. 2008). However, changing climate and environmental conditions likely favor genotypes with high levels of plasticity, whereas low plasticity may lead to extinction (Rehfeldt et al. 2001). In changing environments and climates pedunculate oak forests are threatened mostly by an anthropogenic factor, lowering the level of groundwater or the absence of flooding, and air and water pollution (Eaton et al. 2016).

In Serbia, pedunculate oak forests mostly belong to a complex of alluvial-hygrophilous forests in large river basins of Sava, Danube, Morava and their tributaries. A dominant ecological factor in the development of these forests is a ground water level and changes in the hydrological regime of habitats. Finding the suitable provenance for the reintroduction of pedunculate oak to habitats that are threatened and where this species no longer exists provides important information for the targeted use of the available gene pool.

Due to many challenges in the natural regeneration of pedunculate oak (e.g. Annighöfer et al. 2015; Grossnickle and Ivetić 2017; Gerzabek et al. 2020) many studies have been carried out both at the European and regional level from the aspects of field performance (e.g. Mariotti et al. 2015a; Mariotti et al. 2015b; Popović et al. 2015; Sevillano et al. 2016; Zadworny et al. 2021) to morphological characteristics of acorns and growth parameters (Gradečki et al. 1993; Roth 1999; Nikolić and Orlović 2002; Roth et al. 2009; Roth et al. 2011; Ivanković et al. 2011; Bauer-Živković et al. 2015; Memišević Hodžić and Ballian 2016; Popović et al. 2016; Ivetić et al. 2017; Devetaković et al. 2019; Gavranović Markić et al. 2022).

In this study, we established nursery and field provenance trials (pilot object) to test two pedunculate oak seed provenances both representing registered seed sources, but from different hydrological conditions to investigate the influence of seedlings' provenance and field microhabitat on growth parameters and survival as parameters of gen-ecological potential.

2 Material and methods

2.1 Seed source

Two seed provenances were selected: Mitrovica and Sombor (Figure 1). Mitrovica (44.99738, 19.22392) is located in FMU “Morović“, FE “Sremska Mitrovica“, ecologically belongs to the wetter variety of pedunculate oak and common hornbeam forests (*Carpino - Quercetum roboris*) in non-flooded areas and represents the largest complex of pedunculate oak forests in Serbia. Since 1938, 90% of these forests have been separated from the Sava River by an embankment, while the existing network of drainage channels further reduces the groundwater level in the forest. This former forest floodplain has been left without a large amount of water, which has a bad effect on the quality, health and spread of the pedunculate oak. Sombor provenances (45.79157, 18.90216) is located in SNR “Upper Danube Region“, FE “Sombor“. This region stretches along the left bank of the Danube river as a part of a large ridge complex that extends through two neighboring countries (Hungary and Croatia) and, viewed as a whole, represents one of the last large floodplains in Europe.

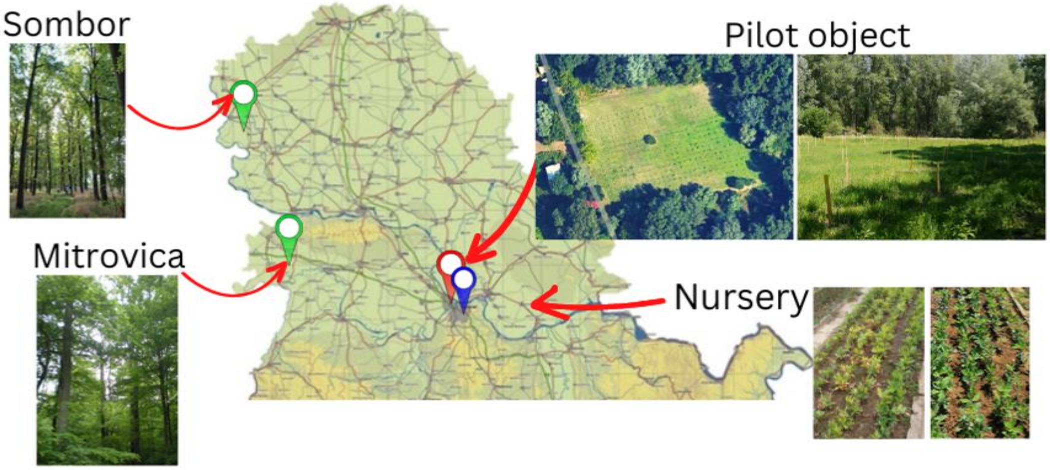

Figure 1. Locations of the provenances (Sombor and Mitrovica), nursery and pilot object.

2.2 Nursery cultivation

Acorns from both provenances were collected in the autumn of 2017 and separately sown in modified Duneman seebeds in the nursery of the Faculty of

Forestry, University of Belgrade, in natural forest soil. If necessary, the seedlings were weeded, watered and hoeed. During the entire vegetation period, foliar liquid fertilizer (Fitogal S®- Galenika) was applied according to producer prospects. Seedlings were cultivated for two years (2018-2019).

2.3 Establishment of pilot object

In November 2019, a two-year-old bareroot seedlings were lifted from the nursery to the area prepared for planting on the Great War Island, Belgrade, Serbia (44.831597, 20.427377) (Figure 1). The protected area "Great War Island", created as a sedimentary and alluvial accumulative formation due to the slowing down and stopping of sand drifts at the confluence of the Sava and Danube rivers, makes up 59.6% (123.62 ha) of the forested area, of which the majority are high natural stands of soft hardwoods (92.6 ha) and a smaller part of artificially raised stands (7.4 ha). It is characterized by an insufficient degree of forest cover, the wider area is largely unvegetated by forest and its structure is characteristic of island-type floodplains (2011-2020).

The pilot object was established according to the principle of three repetitions for each provenance (Šijačić-Nikolić et al. 2019), according to a pre-prepared planting scheme (Figure 2). After planting, in December 2019, a significant number of seedlings died due to the occurrence of soil frost damage, which destroyed almost half of the planted seedlings (Figure 3). After the frost damage in early spring 2020 all the damaged seedlings were replaced, but in order to keep a homogeneous sample in terms of its characteristics, primarily the age of the planting material, the time of planting, care measures, the way the surface is maintained and the same weather conditions, replaced seedlings were not taken into account for further measurements.

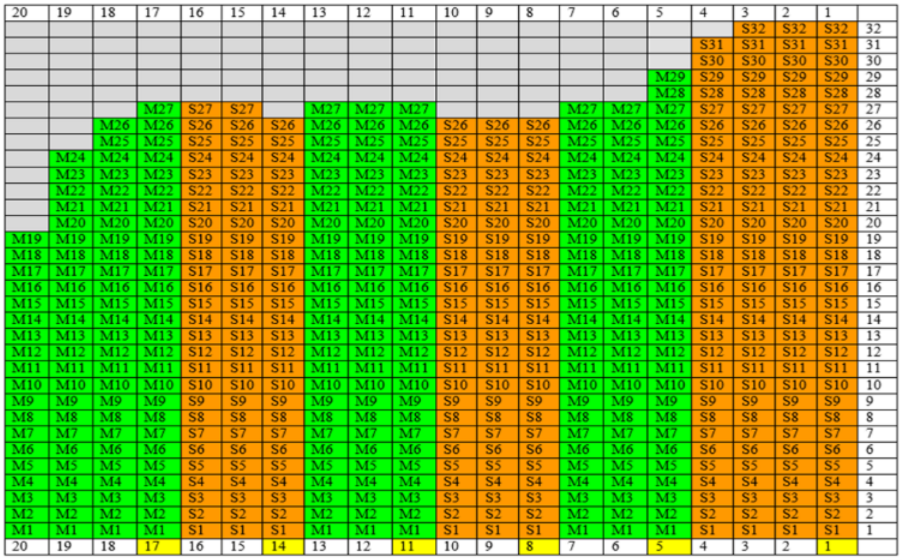

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Figure 2. Planting scheme plots arrangement in pilot object in the (November 2019).

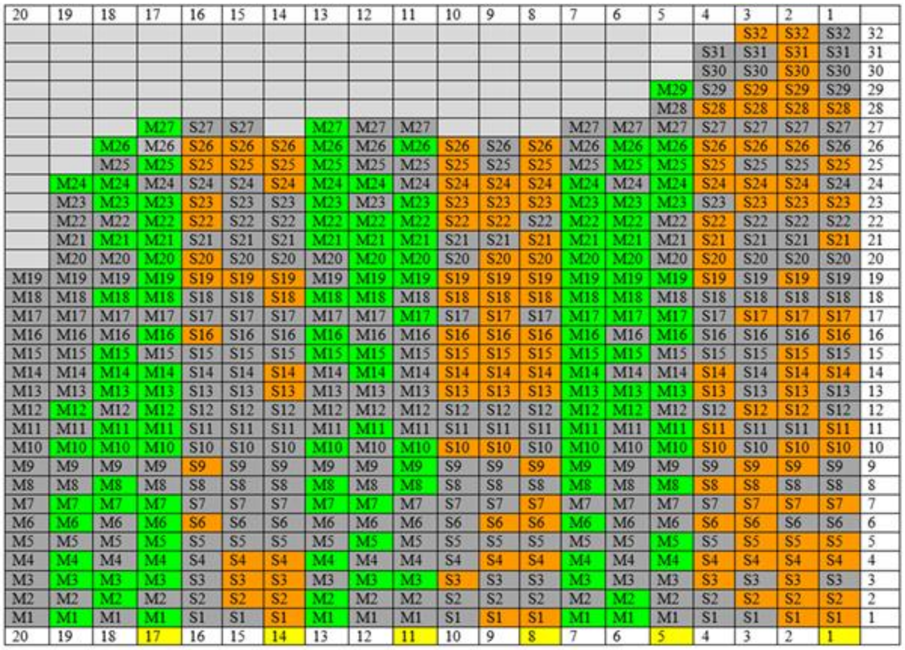

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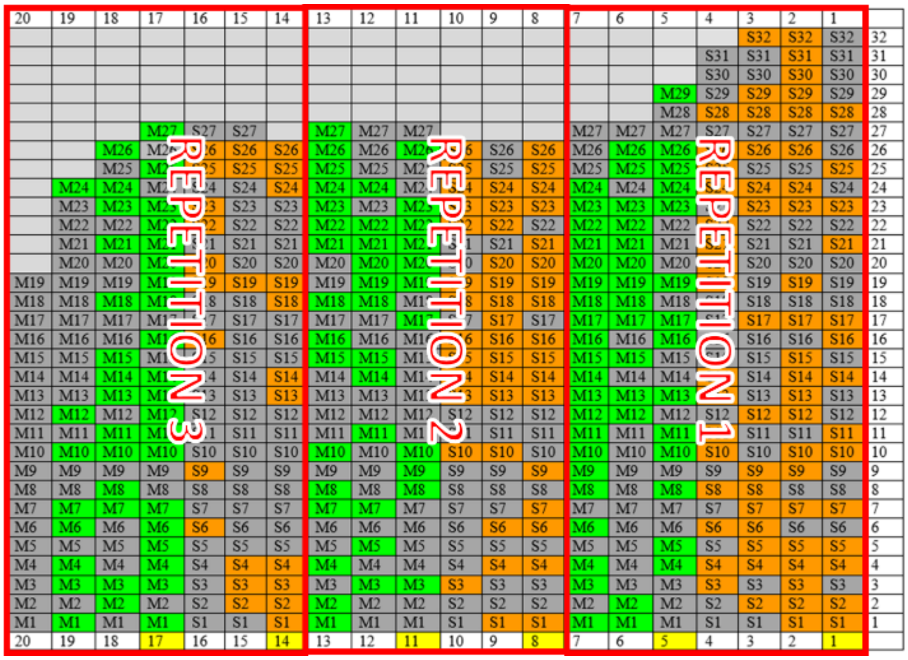
Figure 3. Planting scheme plots arrangement after the frost damage (December 2019).

2.4 Assessment of gen-ecological potential and microhabitat influence

In the nursery trial, after the first (autumn 2018) and the second (autumn 2019) vegetation period, the height (H) and ground based diameter (D) of the 120 seedlings were measured (60 seedlings of Mitrovica and 60 of Sombor provenance). After three years in the pilot object, in the autumn of 2022, when the seedlings were five years old for all survived seedlings the height and diameter were analyzed again, as well as the survival. Sampling included 265 seedlings in the pilot object, 129 seedlings originating from the Mitrovica provenance and 136 seedlings originating from the Sombor provenance – all the seedlings that survived in the period from planting (2019) to the end of 2022, with the absence of any mechanical damage (Figure 4). The height of the seedlings was measured from ground level to the terminal bud, using a ruler with an accuracy of 1 mm and the ground based diameter (D) was measured using a digital caliper with an accuracy of 0.01 mm. In addition to these two measured parameters, the index of seedling diameter and height was also calculated. Based on these measurements, the parameters of the genetic potential of the seedlings of two provenances (Mitrovica and Sombor) in the area of the Great War Island were determined. To determine the gen-ecological potential, descriptive statistics parameters were analyzed for the features of observation: - mean value, SD - standard deviation, CV (%) - coefficient of variation, min-max - minimum and maximum value and one-way analysis of variance (ANOVA).

To determine the microhabitat influence, planting area was divided in two ways: the first is into three repetitions (Figure 5), and the second into two planting blocks (Figure 6), excluding the influence of the origin of the seedlings. Repetitions included the division of the planting area into three parts, each of which included one replicate from each provenance (S or M). The first repetition included 114 seedlings in total, the second 83, and the third 68. Planting blocks included the division of the planting area into two blocks, the first block included 13 seedlings in each planting row (total of 114 analyzed seedlings), and the second rest of the planting area (total of 151 analyzed seedlings). This way of dividing the area does not include an even number of seedlings, but an even spatial distribution of repetitions/blocks. One-way ANOVA was used to test differences between mean values of measured characteristics between the repetition or the planting blocks. Mean values were separated using an LSD post-hoc test, with a significance level of p<0.05 (α=0.05). All analyzes were performed in STATGRAPHICS Centurion XVI.I.

Figure 4. Pedunculate oak seedlings in pilot object: A – two-year-old (spring 2020) and B – five-year-old (autumn 2022).



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M9	M9	M9	M9	S9	S9	S9	M9	M9	M9	S9	S9	S9	M9	M9	M9	S9	S9	S9	S9	9
M8	M8	M8	M8	S8	S8	S8	M8	M8	M8	S8	S8	S8	M8	M8	M8	S8	S8	S8	S8	8
M7	M7	M7	M7	S7	S7	S7	M7	M7	M7	S7	S7	S7	M7	M7	M7	S7	S7	S7	S7	7
M6	M6	M6	M6	S6	S6	S6	M6	M6	M6	S6	S6	S6	M6	M6	M6	S6	S6	S6	S6	6
M5	M5	M5	M5	S5	S5	S5	M5	M5	M5	S5	S5	S5	M5	M5	M5	S5	S5	S5	S5	5
M4	M4	M4	M4	S4	S4	S4	M4	M4	M4	S4	S4	S4	M4	M4	M4	S4	S4	S4	S4	4
M3	M3	M3	M3	S3	S3	S3	M3	M3	M3	S3	S3	S3	M3	M3	M3	S3	S3	S3	S3	3
M2	M2	M2	M2	S2	S2	S2	M2	M2	M2	S2	S2	S2	M2	M2	M2	S2	S2	S2	S2	2
M1	M1	M1	M1	S1	S1	S1	M1	M1	M1	S1	S1	S1	M1	M1	M1	S1	S1	S1	S1	1
20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	

Figure 5. Repetition scheme plots arrangement.

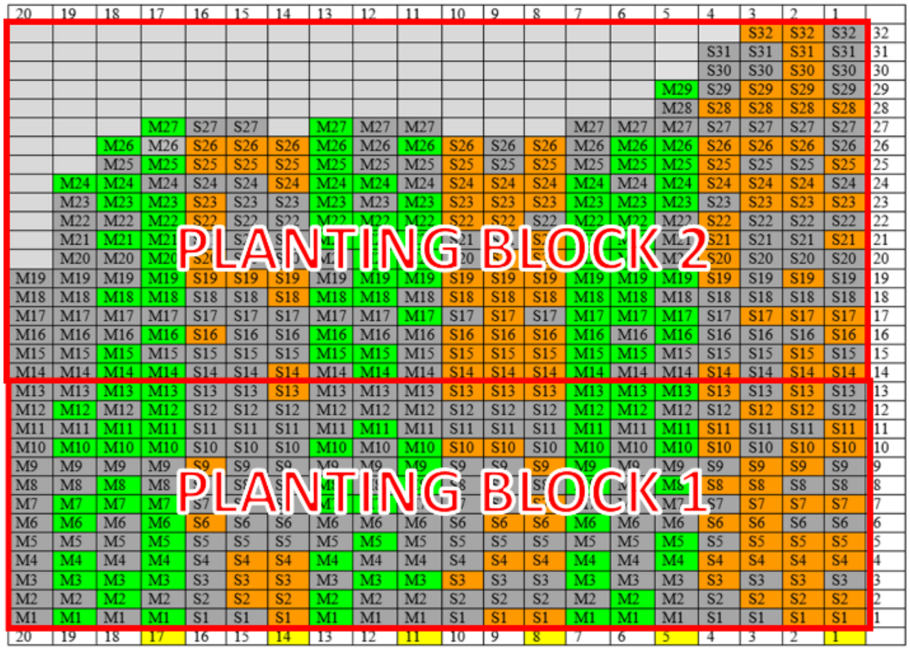

Figure 6. Planting blocks scheme plots arrangement.

3 Results

3.1 Assessment of growth parameters in nursery trial

At the end of the first vegetation period (2018) the results indicated statistically significant differences ($p<0.05$) for all analyzed growth parameters, and lower coefficient variation for seedlings originating from Sombor provenance compared to seedlings originating from Mitrovica provenance (Table 1). At the end of the second vegetation period (2019) the results indicate a uniform degree of variation between all measured parameters (CV% values) for both provenances with no statistically significant differences ($p>0.05$), (Table 2).

Table 1. Descriptive statistics and ANOVA for measured growth parameters for seedlings at the end of the first vegetation period in nursery trial.

Provenance	N	**Ground based diameter 2018 (cm)**			**Height 2018 (cm)**			**Diameter/ height ratio 2018**		
		X±SD	CV (%)	min-max	X±SD	CV (%)	min-max	X±SD	CV (%)	min-max
Mitrovica	60	0.72±0.15	20.39	0.52-1.06	37.89±9.02	23.81	18.0-63.1	0.020±0.004	21.39	0.013-0.035
Sombor	60	0.79±0.12	14.71	0.53-1.09	52.46±9.81	18.70	28.5-74.0	0.015±0.002	16.04	0.011-0.023
Total	120	0.76±0.14	18.10	0.52-1.09	45.18±11.90	26.34	18.0-74.0	0.018±0.004	22.89	0.011-0.035
ANOVA		**SS**	**F-Ratio**	**P-Value**	**SS**	**F-Ratio**	**P-Value**	**SS**	**F-Ratio**	**P-Value**
(between provenances)		0.15588	8.83	0.0036	6371.46	71.73	0.0000	0.000514855	43.54	0.0000

Table 2. Descriptive statistics and ANOVA for measured growth parameters for seedlings at the end of the second vegetation period in nursery trial.

Provenance	**N**	**Ground based diameter 2019 (cm)**			**Height 2019 (cm)**			**Diameter/ height ratio 2019**		
		X±SD	CV (%)	min-max	X±SD	CV (%)	min-max	X±SD	CV (%)	min-max
Mitrovica	60	0.90±0.23	25.76	0.51-1.54	74.12±20.35	27.46	32-136	0.012±0.002	19.31	0.009-0.021
Sombor	60	0.87±0.23	26.64	0.32-1.38	74.33±20.64	27.77	35-160	0.012±0.003	21.52	0.006-0.019
Total	120	0.89±0.23	26.16	0.32-1.54	74.23±20.41	27.50	32-160	0.012±0.002	20.45	0.006-0.021
ANOVA		**SS**	**F-Ratio**	**P-Value**	**SS**	**F-Ratio**	**P-Value**	**SS**	**F-Ratio**	**P-Value**
(between provenances)		0.035604	0.66	0.4177	1.40833	0	0.9539	9.97139E-06	1.61	0.2069

In the first vegetation period, seedlings originating from Sombor had an advantage in height and growth diameters compared to the seedlings originating from Mitrovica. However, in the second vegetation period, the values have reversed for diameter growth and had been similar for height growth. By comparing the average values of height and diameter increment of seedlings from 2018 to 2019, the growth of seedlings originating from Sombor provenance is lower than the growth of seedlings originating from Mitrovica provenance. The average annual increase in diameter for seedlings originating from Sombor provenance is 0.07 cm, while for seedlings originating from Mitrovica provenance it is 0.18 cm (Figure 7 – left). The average annual increase in height for seedlings originating from Sombor provenance is 21.87 cm, while for seedlings originating from Mitrovica provenance is 36.23 cm (Figure 7 – right).

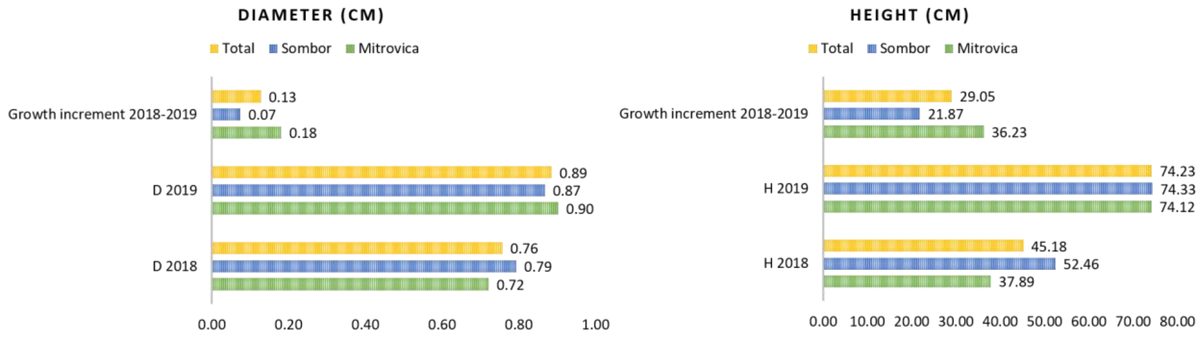

Figure 7. Average annual growth increase in the average diameter (left) and average height (right) of two-year old seedlings in nursery trial (2019-2022).

3.2 Assessment of growth parameters in the pilot object

3.2.1 Survival

During the establishment of the pilot object in November 2019, a total of 544 seedlings were planted, of which 259 were from the Mitrovica provenance and 285 from the Sombor provenance. After three years, 138 seedlings from the Mitrovica provenance and 130 from the Sombor provenance remained in the pilot object, which is a total of 268 seedlings (Figure 8). Observed as a percentage, survival from the establishment of the pilot object to the end of the third growing season in the field, total survival was 49% - 53% of seedlings originating from the Mitrovica provenance and 46% of seedlings originating from the Sombor provenance.

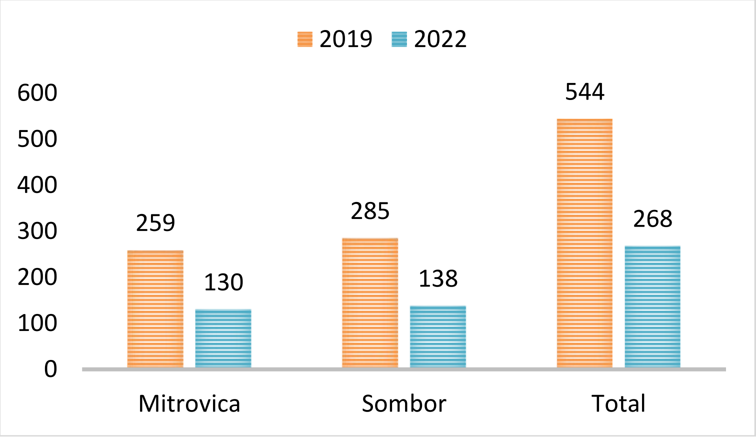

Figure 8. Number of planted (2019) and survived (2022) seedlings in the pilot object.

Based on the results shown above, it can be concluded that the seedlings originating from the Mitrovica provenance had a higher percentage of survival compared to the seedlings originating from the Sombor provenance.

3.2.2. Growth parameters

The results indicated a relatively uniform degree of variation between the measured parameters (CV% values) for both provenances with no statistically significant differences (p>0.05) for diameters and heights, and statistically significant differences for the diameter/height ratio values (Table 3).

Table 3. Descriptive statistics and ANOVA for measured growth parameters for two different provenances.

Provenance	N	**Ground based diameter (cm)**			**Height (cm)**			**Diameter/ height ratio**		
		X±SD	CV (%)	min-max	X±SD	CV (%)	min-max	X±SD	CV (%)	min-max
Mitrovica	129	3.62±1.249	34.47	1.73-6.87	231.82±94.73	40.86	85-505	0.016±0.006	38.63	0.01-0.04
Sombor	136	3.66±1.261	34.44	1.65-7.95	251.29±82.12	32.68	80-480	0.015±0.005	36.31	0.01-0.02
Total	265	3.64±1.253	34.39	1.65-7.95	241.81±88.85	36.74	80-505	0.015±0.006	38.08	0.01-0.04
ANOVA		**SS**	**F-Ratio**	**P-Value**	**SS**	**F-Ratio**	**P-Value**	**SS**	**F-Ratio**	**P-Value**
(between provenances)		0.0959278	0.06	0.8053	25083.9	3.2	0.0746	0.00023281	6.85	0.0094

By comparing the average values of height and diameter of seedlings from 2019 and 2022, the growth of seedlings is more or less uniform. The average three-year-increase in diameter for seedlings originating from Sombor provenance is 2.79 cm, while for seedlings originating from Mitrovica provenance it is 2.72 cm (Figure 9 – left). The average three-year increase in height for seedlings originating from Sombor provenance is 177.0 cm, while for seedlings originating from Mitrovica provenance is 157.7 cm (Figure 9 – right). Based on the above results, it is seen that there are no significant differences in the average growth values of seedlings of different provenances, which is in accordance with the above results (Table 1).

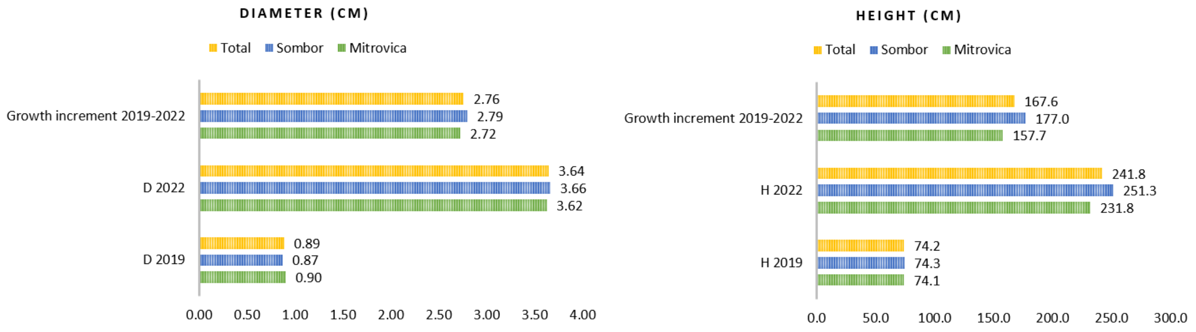

Figure 9. Average growth increase in the average diameter (left) and average height (right) of five-year-old seedlings for the period from 2019-2022.

3.3 Assessment of microhabitat influence in pilot object

Given that no greater significant differences between seedlings originating from different provenances were confirmed, we tried to determine whether there are differences between seedlings concerning the position of the seedlings within the pilot object itself. For this purpose, the seedlings growth differences were found between three repetitions (R1, R2, R3), and two blocks (PB1, PB2). In addition to descriptive statistics and one-way analysis of variance (ANOVA), an LSD test was performed, as well as an analysis of variance between pairs of repetitions/planting blocks.

Based on the results of descriptive statistics and one-way analysis of variance, it was determined that there are statistically significant differences for all the measured growth parameters of five-year-old seedlings from three different repetitions (R1, R2, R3), however, these results were further tested with the LSD post-hoc test (Table 4) as well as the variance check (Table 5).

Table 4. Descriptive statistics, ANOVA and LSD test for measured growth parameters for three different repetitions.

Repetition	N	**Ground based diameter (cm)**			**Height (cm)**			**Diameter/ height ratio**		
		X±SD (LSD)	CV (%)	min-max	X±SD (LSD)	CV (%)	min-max	X±SD (LSD)	CV (%)	min-max
R1	114	3,74±1,15(b)	30,76	1,65-6,51	271,92±74,13 (b)	27,26	130-470	0,013±0,005(a)	35,50	0,01-0,02
R2	83	3,94±1,53(b)	38,88	1,73-7,95	254,16±98,95(b)	38,93	80-505	0,016±0,006(a)	37,94	0,01-0,03
R3	68	3,11±0,81(a)	25,95	1,90-5,51	176,27±61,35(a)	34,81	100-345	0,019±0,006(a)	32,49	0,01-0,04
Total	265	3,64±1,25	34,39	1,65-7,95	241,81±88,85	36,74	80-505	0,015±0,006	38,08	0,01-0,04
ANOVA		**SS**	**F-Ratio**	**P-Value**	**SS**	**F-Ratio**	**P-Value**	**SS**	**F-Ratio**	**P-Value**
(between repetitions)		28,0998	9,52	0,0001	408154	31,9	0,00000	0,000622	20,57	0,00000

Table 5. Variance check between pairs of repetitions of measured growth parameters of five-yearold seedlings in the pilot object.

	Ground based diameter (cm)		**Height (cm)**		**Diameter/ height ratio**	
Repetitions pairs	**F-Ratio**	**P-Value**	**F-Ratio**	**P-Value**	**F-Ratio**	**P-Value**
R1/R2	0,562476	**0,0046**	0,561159	**0,0045**	0,65316	**0,0360**
R1/R3	2,03372	**0,0019**	1,45991	0,0930	0,607095	**0,0194**
R2/R3	3,61566	**0,0000**	2,60159	**0,0001**	0,929473	0,7481

The results of descriptive statistics and ANOVA observed by planting blocks confirmed the influence of microhabitat, as well as in the case of different repetitions. Based on the obtained results, significant differences were determined for the measured diameters and heights of five-year-old seedlings from two different blocks, while no statistically significant differences were found for the value of the diameter/height ratio (Table 6), however, these results were further analyzed with the LSD test (Table 6) and variance check (Table 7). Variance checks between planting blocks show significant differences for ground-based diameter.

Table 6. Descriptive statistics, ANOVA and LSD test for measured growth parameters for two planting blocks.

Planting block	N	**Ground based diameter (cm)**			**Height (cm)**			**Diameter/ height ratio**		
		X±SD (LSD)	CV (%)	Min-max	X±SD (LSD)	CV (%)	Min-max	X±SD (LSD)	CV (%)	Min-max
PB1	114	3,10±0,98(a)	31,53	1,65-6,5	201,39±74,38(a)	36,93	80-460	0,016±0,006(a)	39,43	0,01-0,04
PB2	151	4,05±1,28(b)	31,69	1,73-7,95	272,33±86,87(b)	31,90	85-505	0,015±0,006(b)	37,01	0,01-0,03
Total	265	3,64±1,25	34,39	1,65-7,95	241,81±88,85	36,74	80-505	0,015±0,006	38,08	0,01-0,04
ANOVA		**SS**	**F-Ratio**	**P-Value**	**SS**	**F-Ratio**	**P-Value**	**SS**	**F-Ratio**	**P-Value**
(between blocks)		58,9972	43,63	0,00000	326950	48,93	0,00000	2,02E-05	0,58	0,4466

Table 7. Variance check between pair of planting blocks of measured growth parameters of five-year-old seedlings in the pilot object.

	Ground based diameter (cm)		**Height (cm)**		**Diameter/ height ratio**	
Planting block pair	**F-Ratio**	**P-Value**	**F-Ratio**	**P-Value**	**F-Ratio**	**P-Value**
PB1/PB2	0,579196	**0,0025**	0,733037	0,0827	1,2194	0,2558

4 Discussion

For long-lived species such as forest trees, it is often assumed that local populations are superior to non-local populations. However, there have been several studies demonstrating contradictory results (Bischoff et al. 2006; Crespi 2000). Non-local genotypes may suffer from maladaptation to the local environment resulting in lower fitness (Vander Mijnsbrugge et al. 2010). Translocation of plant material from the site to the site always involves at least one of the risks: maladaptation, changing environmental conditions, disturbance of the ecosystem, etc. However, if there is no locally available material, translocation and reintroduction is the only solution. When choosing the right provenance of forest reproductive material environmental criteria such as geographical distance, climate, geomorphology and hydrology of both provenance and planting site should be considered. Populations that are further away but from similar habitats may be better adapted than neighboring populations from a contrasting habitat (Vander Mijnsbrugge et al. 2010). When choosing the right provenance, the size of the selected seed source should be considered also. If using limited seed sources (few individuals or small isolated population), inbreeding depression, genetic drift, and low genetic diversity/low heterozygosity may result in low establishment rates and a low fitness of the restored population (Williams 2001; Hufford and Mazer 2003; Vergeer et al. 2003). In this sense, the degree of heterozygosity might be more important than plant provenance (Procaccini and Piazzi 2001), but still, it is unclear how much diversity is needed (Vander Mijnsbrugge et al. 2010). A lower coefficient of variation and no significant difference for the most observed parameters in this study might be the result of lower genetic diversity, or result of well adaptability of selected provenances. To be sure which of the two assumptions is correct seedlings in the provenance trial (pilot object) should be further tested on a molecular basis to assess the level of genetic diversity. Since it is cost-demanding, assessing the genetic diversity of seed sources or seedling stock is a present and future issue. However, for ecological restoration purposes, maintenance of the genetic diversity is a priority and it is better to avoid any type of selection during seed collection, seed processing and in nursery practices (Vander Mijnsbrugge et al. 2010). A high level of genetic diversity in a population increases adaptability, so forest reproductive material with high levels of genetic diversity should be chosen to maintain genetic adaptability at a particular site (Konnert et al. 2015).

In this study, significant differences for all analyzed parameters between the chosen provenances were obtained at the end of the first vegetation period which is consistent with the view that in the first stages of ontogeny the variability of plants is the greatest and that it decreases with age (Šijačić-Nikolić and Milovanović 2010). High level of diversity is expected and in accordance with previous research on oak seedlings (Roth et al. 2009; Roth et al. 2011; Ivanković et al. 2011; Memišević Hodžić and Ballian 2016; Devetaković et al. 2019; Ballian and Memišević Hodžić 2022) and as the variability of acorns (Gradečki et al. 1993; Nikolić and Orlović 2002; Bauer-Živković et al. 2015; Popović et al. 2016; Gavranović Markić et al. 2022). In this study in later ontogenetic phases influence of the provenance is missing (no significant differences between Sombor and Mitrovica were founded). As the difference between provenances disappears, the influence of microhabitat occurs (significant differences between repetition or planting blocks). It is already recorded that seedling establishment is highly dependent on microhabitats in Mediterranean environments (Mariotti et al. 2015a; Villar-Salvador et al. 2012). Since evident climate change across the Western Balkans region, the impact of global warming is most visible through the change of two meteorological parameters: temperature and precipitation (Knez et al. 2022). In the Republic of Serbia, the climate varies from moderately continental to continental in the mountains to Mediterranean subtropical and continental in the southwest (Bogdanović et al. 2012). The occurrence of temperature extremes and dry periods due to uneven distribution of precipitation during the growing season will increasingly have an impact on seedling field performance in this region also as in the Mediterranean. Plant mortality during summer is the main factor limiting the regeneration of many woody species (Villar-Salvador et al. 2012). To meet European Green Deal recommendations one of the efforts that the Republic of Serbia should make to reduce the harmful impact of climate change is, among others, afforestation as a desirable strategy in the sector of land-use change and forestry (Knez et al. 2022). To meet this recommendation forestry nursery production should be improved starting from the seed sources as a base for the production of genetically quality forest reproductive material, through the improvement of nursery practice, to the improvement of forest plantation care measures. According to Knez et al. (2022), the implementation of specific activities to meet European Green Deal recommendations is at a low level for most of the Western Balkans region countries including the Republic of Serbia. To optimize afforestation, according to Konnert et al. (2015) research, particularly provenance research and molecular genetics and ecology, will be foremost in helping design proper strategies and legal requirements and regulations for implementing them.

5 Conclusions

Based on the obtained results of research on the influence of provenances of different hydrological regimes (Sombor and Mitrovica) and microhabitat on survival and growth parameters of *Quercus robur* seedlings, we can conclude:

- After the first year of development (in nursery trial) significant differences for all analyzed parameters between the chosen provenances were obtained.
- High-level diversity of 1+0 seedlings is confirmed and in accordance with previous research.
- In the first vegetation period seedlings originating from Sombor provenance had an advantage over the height and diameter growth compared to the seedlings originating from Mitrovica provenance.
- After the second year of development (in nursery trial), a uniform degree of variation between all measured parameters for both provenances with no statistically significant differences ($p > 0.05$) were observed.
- The annual growth (2018-2019) of seedlings originating from Sombor provenance was lower than the growth of seedlings originating from Mitrovica provenance according to the average values of height and diameter increment of seedlings.
- There were no significant differences in the average growth values of seedlings of different provenances comparing the average values of height and diameter of seedlings from 2019 and 2022 – the growth of seedlings was more or less uniform.
- The influence of microhabitat (both for repetitions and planting blocks) is confirmed.
- There were significant differences in the measured diameters and heights of five-year-old seedlings from two different blocks (PB1 and PB2), while no statistically significant differences were found for the value of the diameter/height ratio.
- There were statistically significant differences for all the measured growth parameters of five-year-old seedlings from three different repetitions (R1, R2, R3).

- It is needed to continue further in provenance research, molecular genetics, and ecology of the seed sources and planting material and greater efforts should be made in terms of optimizing the field performance of seedlings.

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