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THE USE OF MORPHOLOGICAL TRAITS OF EUROPEAN BEECH (*Fagus sylvatica* L.) SEEDLINGS FOR SELECTION OF REPRODUCTIVE MATERIAL FROM THE PROVENANCES LIKELY AFFECTED BY CLIMATE CHANGE

KORIŠTENJE MORFOLOŠKIH ZNAČAJKI SADNICA OBIČNE BUKVE (*Fagus sylvatica* L.) ZA SELEKCIJU REPRODUKCIJSKOG MATERIJALA IZ PROVENIJENCIJA VJEROJATNO ZAHVAĆENIH KLIMATSKIM PROMJENAMA

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SAŽETAK

Global climate change causes the reduction of tree growth and spread of European beech (*Fagus sylvatica* L.) forests, thus indicating the need to preserve tree abundance due to the changes that have occurred. The comparison of beech seedlings from various provenances in southeast and central Europe was carried out based on the dimensions of root collar diameter and height to select the proper starting material that would reduce the negative consequences of the climate. There have been statistically significant differences in root collar diameter and height between various seedlings tested in European provenances. Clear differentiation was recorded between European countries in the growth performances of one-year-old and two-year-old seedlings of *F. sylvatica* based on the nursery test. The study's results indicate the potential for producing adaptable beech reproductive material from various European provenances. This finding lays the groundwork for future precise analyses that aim to select provenances tolerant to changing climate conditions.

KEY WORDS: beech, seedlings, variability, provenance

INTRODUCTION
UVOD

The influence of climate change leads to the need for finding strategies of adaptation in order to preserve biodiversity of various species (Orlowsky and Seneviratne 2012; Felton *et al.* 2016, 2024). European beech (*Fagus sylvatica*) as one of the most important tree species in Europe will face the change in the phenology of foliation, mortality and reduced tree growth due to climate change (Gárate-Escamilla *et al.* 2019), with a particular tendency of reducing forest productivity in southern areas (Del Castillo *et al.* 2022). Also, central part of Europe to a certain extent suffers the negative effects of climate change (Zimmermann *et al.* 2015). It is estimated that by the end of the 21st century there

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will be an increase in temperature which will cause significant decrease of beech abundance in altitudes below 500 m in certain southern parts of its distribution area (Pavlović *et al.* 2019). Furthermore, as a consequence of reduced tree vitality, complex disorders caused by biotic diseases occur (Langer and Bußkamp 2023). The knowledge of key functional adaptability indicators is especially necessary in combating the impact of climate changes (Kramer *et al.* 2010). However, so far there have been no experiments that included the comparison of the selected populations from Western Balkans and Central Europe, especially as indicators of their potential for growth as a parameter of adaptation to climate changes that have occurred.

Morphological characteristics of beech seedlings are of key importance in the creation of tolerance to drought stress (García-Plazaola and Becerril 2000). Diameter and the height of seedlings of deciduous tree species are equally important for the survival and growth of seedlings in the first year from planting (Ivetić *et al.* 2016). Furthermore, the knowledge of morphological characteristics is important for the determination of tree quality, which is related to their phenological characteristics (Kembrytė *et al.* 2022). Also, previous studies have shown significant connection between the morphology of the leaves and adaptation to growth conditions (Adamidis *et al.* 2021, Petrik *et al.* 2022). However, morphological differentiation is not decisive in adapting to extremely negative effects of the drought caused by increased temperature (Mathes *et al.* 2023). Therefore, long-term research is needed, which will enable the use of the reproductive material that has been completely adapted to the new conditions. The base for these studies represents finding of initial material and the production of reproductive material which shows the potential for adaptation to new, less favourable conditions for the development of this tree species. Phenotypic plasticity, i.e. adaptability of provenances, represents stimulation of natural selection processes (Crispo 2008), which is necessary in accordance with the existing and expected negative changes in beech forests. The possibility of finding provenances that have the potential of faster growth as biological characteristic for the adaptation to more unfavourable conditions is reflected in the continuation of the study of dimen-

Table 1. Data of the studied provenances of European beech
Tablica 1. Podaci o provenijencijama obične bukve

Provenance label *Oznaka provenijencije*	Provenance name *Naziv provenijencije*	Latitude *Geografska širina*	Longitude *Geografska duljina*	Altitude (m) *Nadmorska visina*
BG1	Centralni Balkan	42.86444	24.24941	1100
BG2	Rila	42.26417	23.28333	1280
SRB1	Mali Jastrebac	43.39092	21.65006	830
SRB2	Rudnik	44.10531	20.6136	700
SRB3	Povlen	44.16111	19.69861	870
SRB4	Javor	43.44913	20.06806	1350
SRB5	Goč	43.56351	20.75001	920
SRB6	Fruška Gora	45.14194	19.62289	370
SRB7	Severni Kučaj	44.12941	21.79868	730
SRB8	V. Jastrebac	43.36242	21.56092	810
SRB9	Južni Kučaj	44.07015	21.75708	700
SRB10	Stara Planina	43.38065	22.60313	1260
SRB11	Dubašnica	44.10063	21.88801	900
SRB12	Miroč	44.57029	22.37021	450
SRB13	Kukavica	42.79124	21.97133	1200
SRB14	Boranja	44.38997	19.28981	650
SRB15	Kačer-Zeleničje	42.82314	22.21206	1180
MNE1	Borak	42.82738	19.99544	1250
MNE2	Kovač	43.40833	19.11611	960
BIH1	Javor	44.15611	18.94333	1010
BIH2	Lisina	45.02522	17.00861	400
HR1	Bukovača	45.34747	15.22334	435
HR2	Građevačka Biogora	45.79191	17.12667	185
HR3	Južni Papuk	45.50722	17.63802	685
HR4	Bublen	45.23139	15.84747	205
SI1	Hrastovec	46.35667	15.96667	300
SI2	Osankarica	46.45325	15.38333	1240
CZ1	Malužin	49.27334	16.66171	360
DE1	Harz	51.78503	10.51716	820

Country abbreviations: BG-Bulgaria; SRB-Serbia; MNE-Montenegro; BIH-Bosnia and Herzegovina; HR-Croatia; SI-Slovenia; CZ- Czech Republic; DE-Germany.

sions of seedlings from parts of Europe most affected by climate change. First of all, the use of the source of origin in accordance with the degree of threat can significantly increase the stability of stands exposed to unfavourable conditions.

In accordance with the above, the study of growth of seedlings produced from the selected parts of southeast and central Europe was carried out. The obtained results enable: i) selection of initial material for the production of reproductive material with the potential for good growth; ii) classification of different southeast and central beech provenances, which enables easier work organization when selecting certain provenances for further testing of beech adaptability to current global changes.

MATERIAL AND METHODS
MATERIJAL I METODE

Field methods and plant material

As the object of research, 29 beech provenances were selected from the territory in central and southeast Europe, whose general characteristics are presented in Table 1 and Figure 1. In the autumn of 2018 about 1 kg of visually healthy seeds was collected in each provenance. Seed collection was carried out at the provenance level, selecting seeds from multiple mother trees that were at least 50 meters apart to prevent inbreeding. The collected material was delivered and processed in the laboratory of the Institute of Forestry in Belgrade. The seeds were mechanically cleaned from impurities, and empty seeds were removed using the air current. The cleaned seeds were disinfected by 35% solution of H_2O_2 during 2 minutes.

Experimental methods

After the conducted analyses of quality and health condition in the laboratory, in the spring of 2019 a nursery test was established in the nursery of the Institute of Forestry in Belgrade. During the first growing season, regular care measures such as mechanical weeding and watering were implemented. In the autumn of 2019, 50 seedlings were chosen and labelled by provenance using the random sampling method. All of the marked seedlings survived during the second growing season and were afterwards measured. At the end of the growing seasons 2019 and 2020 measurements of height and root collar diameter of one-year-old and two-year-old seedlings were carried out. Height was measured with a ruler with a precision of 0.5 cm, and root collar diameter with a vernier calliper with a precision of 0.1 mm.

Statistical analyses

Measured morphometric characteristics of seedlings were processed in statistical program package Statistica 7.0.

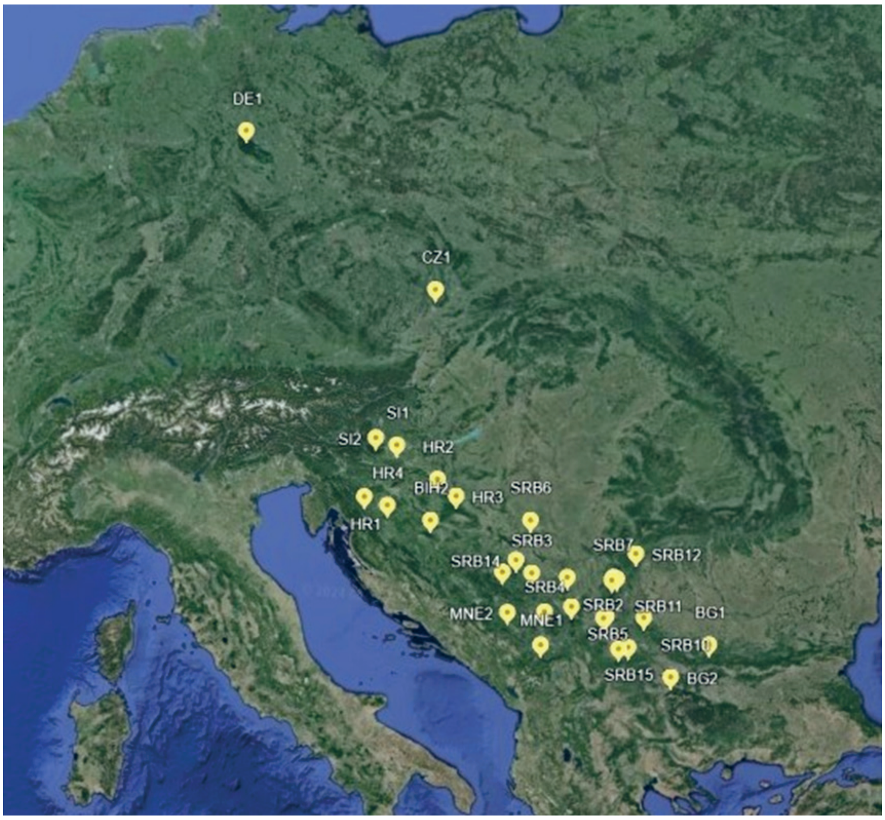


Figure 1. Map of 29 sampled provenances of European beech (*Fagus sylvatica* L.)
Slika 1. *Karta 29 uzorkovanih provenijencija obične bukve (Fagus sylvatica L.)*

Mean values of all the analysed characteristics of seedlings were obtained using the MEANS procedure. The significance of differences between mean values of the analysed parameters was verified by one factor analysis of variance (One-Way ANOVA), where the analysed factor was the provenance. Additional testing by Fisher's multiple tests (LSD) was carried out for all pairs of populations with the aim to determine exactly which populations are statistically significantly different from each other. In order to see the differences between the provenances, a multivariate statistical analysis (Agglomerative Hierarchical Clustering) was carried out, which grouped provenances according to the similarity of all the analysed characteristics of seedlings by pair-group average method.

RESULTS
REZULTATI

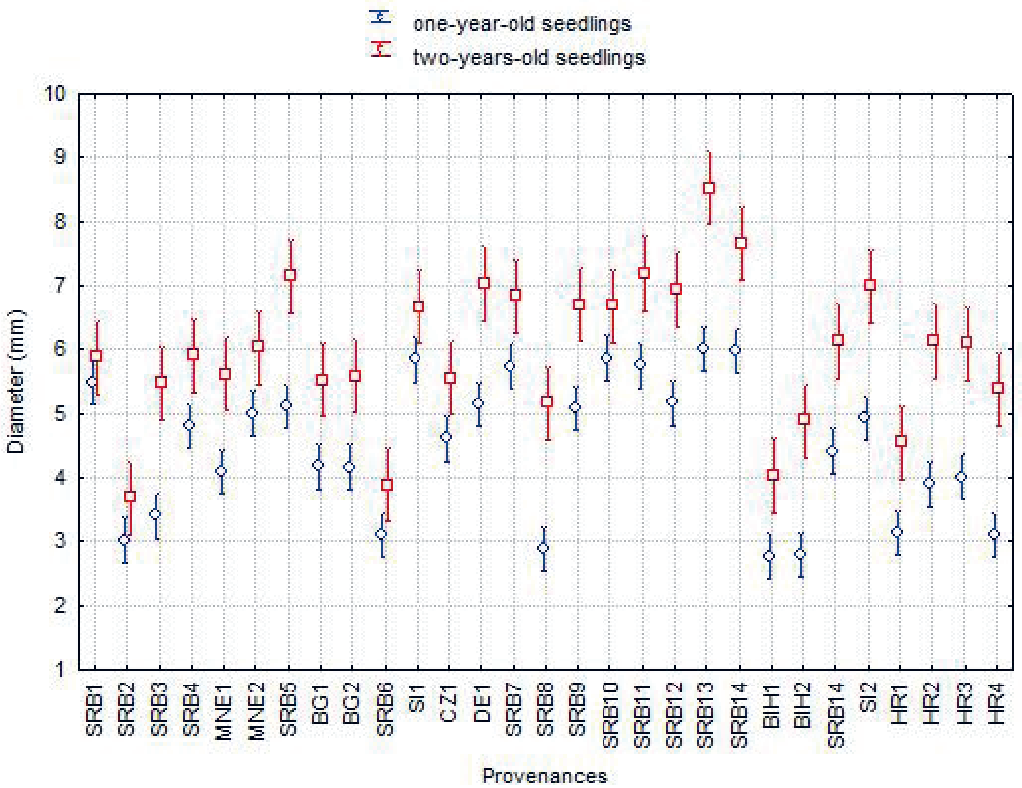
Based on the applied statistical analyses the following results were obtained and presented for each provenance (Figure 2, 3).

Mean values of root collar diameter of seedlings per provenance are presented in Figure 2. At the end of the first growing season, mean value of root collar diameter ranged from 2.8 mm (BIH1, BIH2) to 6 mm (SRB13). At the end of the second growing season the largest mean value of root collar diameter was of the provenance SRB13 (8.5 mm), while the smallest average value of this parameter was recorded in the provenance SRB2 (3.7 mm).

Figure 3 shows average values of height of one-year-old and two-years-old seedlings per provenances. The largest mean value of the height of one-year-old seedlings was measured in the provenance SI1 (51 cm), and the smallest mean value in provenances BIH2 and SRB8 (14 cm). At the end of the second growing season the largest mean value of height was measured on seedlings from provenance SI1 (64.5 cm), and the smallest mean value on seedlings from provenances BIH2 and SRB2 (24.5 cm).

Statistical significance of differences between populations was obtained for all analysed characteristics of one-year-old and two-years-old seedlings (all $p<0.0001$) based on the application of one-way ANOVA (Tables 2 and 3).

Assessment of closeness, i.e. distance of the studied provenances based on the measured morphometric characteristics of seedlings was carried out by using cluster analysis. According to the visual representation of dendrogram of similarity of provenances, four clusters were obtained. The first cluster was formed from provenances SRB1, SRB2, SRB3, SRB4, MNE1, MNE2, SRB5, BG1, BG2, SRB6 and



Vertical bars denote 0.95 confidence intervals

Figure 2. Average values of seedlings' root collar diameter (mm) per provenances

Slika 2. *Prosječne vrijednosti promjera vrata korijena sadnica (mm) po provenijencijama*

Vertical bars denote 0.95 confidence intervals

Figure 3. Average values of seedlings' height (cm) per provenances

Slika 3. *Prosječne vrijednosti visine sadnica (cm) po provenijencijama*

Table 2. Analysis of variance for root collar diameter and height of one-year-old seedlings

Tablica 2. *Analiza varijance promjera vrata korijena i visine jednogodišnjih sadnica*

Source of variation *Izvor varijabilnosti*	Degrees of freedom *Stupnjevi slobode*	Sum of square *Suma kvadrata*	Mean square *Srednja kvadratna vrijednost*	F value *F vrijednost*	Fisher test-no. of groups *Fisher test-broj grupa*
Root collar diameter/*Promjer vrata korijena* (mm)					
Between groups / *Između grupa*	28	987.93	35.28	37.45**	
Within groups / *Unutar grupa*	840	791.49	0.94		13
Total / *Ukupno*	868	1779.42			
Height/*Visina* (cm)					
Between groups / *Između grupa*	28	84699.8	3025.0	36.39**	
Within groups / *Unutar grupa*	840	69818.4	83.1		14
Total / *Ukupno*	868	154518.2			

** $P < 0.0001$.

Table 3. Analysis of variance for root collar diameter and height of two-years-old seedlings

Tablica 3. *Analiza varijance promjera vrata korijena i visine dvogodišnjih sadnica*

Source of variation *Izvor varijabilnosti*	Degrees of freedom *Stupnjevi slobode*	Sum of square *Suma kvadrata*	Mean square *Srednja kvadratna vrijednost*	F value *F vrijednost*	Fisher test-no. of groups *Fisher test-broj grupa*
Root collar diameter/*Promjer vrata korijena* (mm)					
Between groups / *Između grupa*	28	1084.12	38.72	15**	
Within groups / *Unutar grupa*	840	2171.42	2.58		13
Total / *Ukupno*	868	3255.54			
Height/*Visina* (cm)					
Between groups / *Između grupa*	28	115197	4114	34.49**	
Within groups / *Unutar grupa*	840	100307	119		15
Total / *Ukupno*	868	215504			

** $P < 0.0001$.

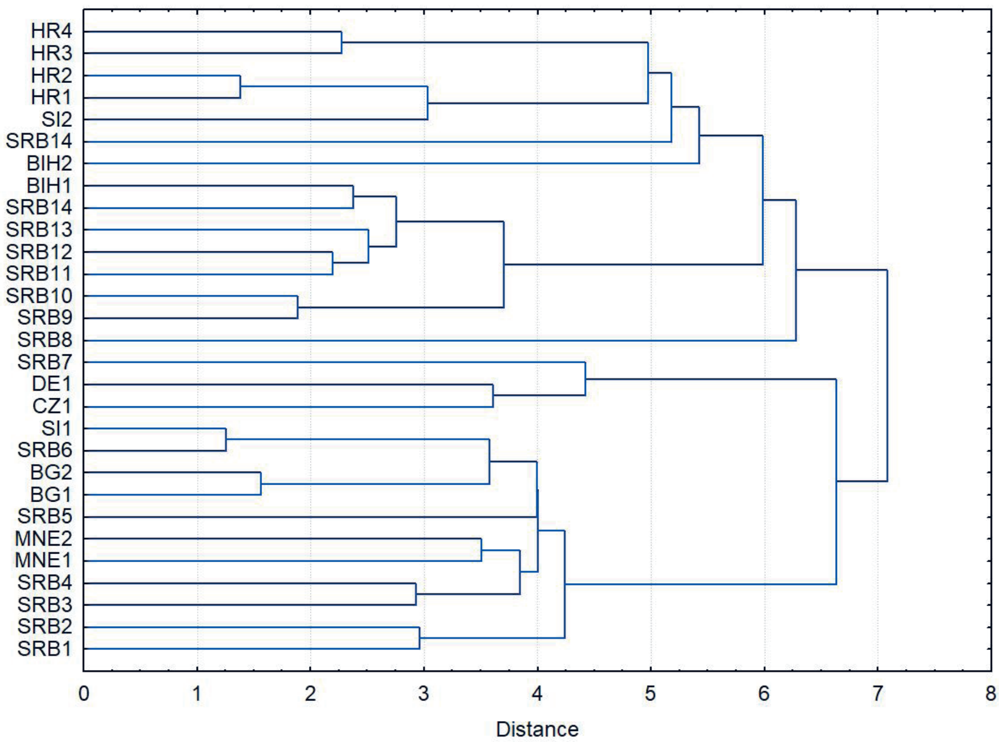


Figure 4. Dendrogram for the characteristics of height and root collar diameter
Slika 4. *Dendrogram za svojstva visine i promjera vrata korijena.*

SI1, the second cluster from provenances CZ1, DE1 and SRB7, the third cluster from provenances SRB9, SRB10, SRB11, SRB12, SRB13, SRB14 and BIH1 and the fourth cluster from provenances SI2, HR1, HR2, HR3 and HR4 (Figure 4).

DISCUSSION AND CONCLUSIONS
RASPRAVA I ZAKLJUČCI

The detailed analysis and comparison of dimensions of seedlings from the selected provenances of southeast and central Europe was carried out in this study. The obtained results confirm previous provenance tests on beech (Mekić *et al.* 2010; Ballian and Zukić 2011; Stojnić *et al.* 2015a; Bogunović *et al.* 2020; Memišević Hodžić and Ballian 2021; Popović *et al.* 2021) which showed variability of dimensions of seedlings. On the other hand, marked differentiation was observed in seedling dimensions originating from different parts of southeast and central Europe. The variability of the dimensions of the tested seedlings, following the geographical distribution, indicates a more significant genetic potential of specific provenances for the growth of seedlings with targeted characteristics. The results indicate the potential for planning simple and efficient fieldwork to select and collect materials from various parts of Europe for further analysis. This could be a starting point for the nursery production of large amounts of reproductive materials.

Provenances from the second and third cluster showed notably greater potential for growth, whereby provenances Hrastovec and Harz reached great heights. These results confirm conclusions of the study by Stojnić *et al.* (2015b), which based on the anatomy of leaves in a common garden test showed that provenances from endangered southern parts of Europe do not have to be the only source of adaptive material. Provenances Južni Kučaj, Kukavica and Boranja from Serbia have also shown pronounced height growth. The selected starting material represents the quality base for future research related to finding the individuals with long-term tolerance to adverse conditions caused by climate change. First of all, the production of this reproductive material can help in the initial stages of afforestation and reforestation of different beech forests, in order to increase the number of seedlings that have great potential for survival and further development.

Provenances from the first and fourth cluster showed notably smaller potential for growth, which is especially pronounced on Rudnik, Povlen, Javor, Central Balkan, Rila, Fruška Gora, Javor, Lisina, Bukovača and Bublen mountains. Interestingly, certain provenances from this clusters, e.g. Mali Jastrebac and Osankarica, showed marked height growth. The mentioned areas should be given priority when applying forest conservation measures, through introduction of reproductive material from other parts of Europe which showed potential for adaptability to adverse conditions. This is especially recommended in the mentioned cases of seedlings with smaller dimensions because the surrounding competition leads to additional slowdown in growth (Chakraborty *et al.* 2021), while the creation of heterogeneous conditions in stands contributes to reducing the consequences of drought caused by climate change (Schmied *et al.* 2023).

Dimensions of root collar diameter showed similar tendency as the heights of seedlings. Provenances from the second and third cluster showed significant growth of the diameter, with the exception of Javor. The above results indicate the potential of the selection of quality reproductive material that will meet the challenges of modern forestry. These conclusions are in line with the previous studies which indicated the potential of southern European beech provenances for adaptation (Nielsen and Jørgensen 2003). Also, we deem that we should adopt the recommendation based on experiences by the same authors who have stated that mixing the plant material from sensitive and resistant provenances could significantly contribute to evolutionary adaptability.

A small number of authors dealt with research related to the creation of optimal conditions for the growth of beech due to climate change. Dewan *et al.* (2019) showed that temperature in the period of reproduction had an important role in determining adaptation to climate change. On the other hand, the research related to fructification of certain populations from the endangered part of distribution area of beech showed significant variability between populations in the degree of yield, as well as a positive correlation between horizontal crown projection area and seed yield (Gavranović *et al.* 2018). Furthermore, the variability in morphological characteristics of seed of Greek provenances has been determined. (Varsamis *et al.* 2020). Our research, which dealt with dimensions of seedlings in a two-year nursery test, showed that there were provenances which showed significant potential for survival in unfavourable conditions based on the root collar diameter and height of seedlings. We deem that future research should be focused on the study of interaction of external factors, seed dimensions, and morphological characteristics of seedlings in order to separate long-term tolerant seedlings by further selection from potentially adaptive reproductive material and test the yield of trees and stands obtained from such reproductive material.

The success of afforestation as the answer to challenges brought by global changes can most efficiently be achieved by dedicated production of reproductive material, that is, of known origin and characteristics suitable for the ecological conditions in the habitats (Isajev 2022). For example, large beech trees are thought to suffer less from adverse consequences due to drought caused by climate change than small trees (Pretzsch *et al.* 2018). The spread of beech in different parts of the distribution area is characterised by large genetic diversity, which represents the potential for adaptation to adverse conditions (Dounavi *et al.* 2016). In this sense, the tested provenances which are classified in different clusters enable a precise insight into the adaptive potential of beech from various countries based on their morphological characteristics. Earlier research has determined that there is variability of ecophysiological reactions of beech seedlings between certain countries of Europe (Cocozza *et al.* 2016). The same authors recommend combined analysis of physiological and structural characteristics of seedlings in order to enable the best performance of beech in its habitats. Our research showed that dimensions of seedlings from endangered parts of Europe are clearly different and in that sense the possibility opens up for an easier analysis of their physiological characteristics. The connectedness of these characteristics will enable the best effect of planting, adapted to the degree of change in the conditions in a specific habitat.

Certain beech provenances from southern and central Europe are currently relatively healthy and with small differences they are grouped into different clusters (Ćelepirović *et al.* 2021). However, taking in consideration the previously mentioned facts on the influence of climate change on the increased occurrence of diseases caused by fungi as a consequence of decreased vitality of trees (Langer and Bußkamp 2023), it is expected that the provenances from clusters with poorer growth characteristics will show greater sensitivity to development of certain diseases. Special attention should be paid to these areas, i.e. preventive protection measures should be strengthened. Based on the obtained results, which have determined that the seedlings from these areas have slower growth, i.e. they leave the juvenile stage later, intensive protection is recommended against species from the genera *Phytophthora* and *Fusarium* which have significant tendency of spreading in beech forests (Milenković *et al.* 2012; Stępniewska *et al.* 2021; Jankowiak *et al.* 2023; Ejaz *et al.* 2023).

The growth of beech in the conditions of competition of other tree species is characterised by decreased horizontal elongation of the crown, smaller ratio of height and diameter and longer branches, as a way of adaptation (Juchheim *et al.* 2017). Also, there are differences between pure and mixed stands in the way the beech is morphologically adapted (Dieler and Pretzsch 2013). At the moment it cannot be reliably stated what habitus the tested provenances show with pronounced or slowed growth in the stands with different tree species. However, the provenances from this study that show less potential for adaptation to changed conditions, due to decreased growth, should not be excluded because they might be useful in cases of afforestation and artificial reforestation of stands where great productivity is not requested. In this way it is possible to preserve the gene pool of the individual trees that show poor adaptability to new and more adverse conditions occurred due to climate change.

The research results in the nursery test of the morphological characteristics of beech seedlings from different southeast and central European provenances showed high variability and differentiation between provenances. The seedlings produced show potential for good growth; they should be further studied to select genotypes that can survive in altered habitats caused by global changes. The use of reproductive material from provenances exhibiting good growth rates in this study will accelerate their selection and preserve endangered European beech forests. It is necessary to analyze various phenotypic properties in provenance tests to investigate the genetic diversity and structure of the selected provenances and obtain more precise results.

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SAŽETAK

Globalne klimatske promjene uzrokuju smanjenje rasta drveća i rasprostranjenosti bukovih šuma (*Fagus sylvatica* L.), odnosno ukazuju na potrebu očuvanja brojnosti drveća zbog nastalih promjena. U cilju pravilnog odabira polaznog materijala koji će umanjiti negativne posljedice klime, napravljena je usporedba sadnica bukve različitih provenijencija jugoistočne i srednje Europe prema dimenzijama promjera vrata korijena i visine. Utvrđene su statistički značajne razlike u promjeru vrata korijena i visini sadnica između ispitivanih europskih provenijencija. Uočena je jasna razlika između različitih europskih provenijencija u rezultatima rasta jednogodišnjih i dvogodišnjih sadnica obične bukve (*F. sylvatica*) na temelju rasadničkog testa. Rezultati ovog istraživanja, putem klasifikacije različitih europskih provenijencija bukve, olakšavaju proizvodnju prilagodljivog reprodukcijskog materijala i preciznu buduću analizu u cilju odabira provenijencija otpornih na promijenjene klimatske uvjete. Ograničenje ovog istraživanja u pogledu trajanja i starosti korištenog reprodukcijskog materijala polazište je za buduća istraživanja selekcije adaptivnog materijala koji će odgovoriti na izazove u šumarstvu uzrokovane klimatskim promjenama.

KLJUČNE RIJEČI: bukva, sadnice, varijabilnost, provenijencija