



Comparative analysis of one-year-old oak seedlings grown in two commercial containers

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Abstract

Container characteristics can influence seedling morphology and quality by affecting root development and seedling growth during nursery production. This study evaluated the effects of two container types, Plantagrah and Hiko, on the morphology and quality of one-year-old seedlings of *Quercus frainetto*, *Q. petraea*, and *Q. pubescens*. Twenty seedlings per species and container type were randomly selected. Morphological traits, dry mass traits, rooting intensity (ROIN), and Dickson Quality Index (DQI) were assessed. The effects of species, container type, and their interaction were analyzed using two-way ANOVA, while multivariate variation was explored using principal component analysis (PCA). Significant differences among species were observed for most measured traits. *Quercus petraea* generally exhibited the greatest diameter, height, and seedling dry mass, whereas *Q. frainetto* showed the lowest values for most growth-related traits. Container type significantly affected diameter, fine root dry mass, total root dry mass, H/D index, and DQI. Seedlings grown in Hiko containers exhibited larger diameters, greater root biomass, lower H/D values, and higher DQI values than seedlings grown in Plantagrah containers. Significant interactions between species and container type were detected only for shoot:root ratio, indicating that container effects were generally consistent across species. PCA showed clearer separation among species than among container types. The results indicate that container type influences several important indicators of seedling quality and that Hiko containers provide favorable conditions for the production of high-quality seedlings of the studied oak species during the first nursery year.

Keywords

Quercus petraea; *Quercus frainetto*; *Quercus pubescens*; Container types; Seedling quality

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1 Introduction

Oak species (*Quercus* spp.) are among the most ecologically and economically important broadleaved tree species in Europe, providing valuable timber, supporting biodiversity, and delivering numerous ecosystem services (Löf et al. 2016; Lyubenova et al. 2024). Consequently, the production of high-quality oak planting stock is an important objective of forest nurseries and forest establishment programs.

Seedling quality is strongly influenced by nursery production practices (Villar-Salvador et al. 2004; Grossnickle and MacDonald 2018; Mataruga et al. 2023) and is considered an important determinant of field establishment success (Grossnickle and Ivetić 2022; Grossnickle and MacDonald 2018). Among the factors affecting seedling development, container type plays an important role because it determines the growing space available for root development and can influence water availability, nutrient uptake, root development, and overall seedling morphology (Grossnickle and El-Kassaby 2016; Mariotti et al. 2015; Grossnickle and Ivetić 2022). Container characteristics such as cell volume, depth, shape, and growing density have been shown to affect seedling growth and quality in several forest tree species, including oaks (Tsakalimi et al. 2005; Mariotti et al. 2015; Grossnickle and El-Kassaby 2016).

Although the effects of container characteristics on seedling growth have been investigated in previous studies (Tsakalimi et al. 2005; Mariotti et al. 2015; Mijatović et al. 2022; Banach et al. 2023), comparative information for multiple oak species grown under identical nursery conditions remains limited. Oak species differ in their growth patterns, morphology, and ecological adaptations, which may result in different responses to nursery production systems (Rodríguez-Calcerrada et al. 2008; Pérez-Ramos et al. 2010; Jakanović et al. 2024). Improved understanding of these responses could contribute to more efficient nursery production and the development of high-quality planting stock.

Therefore, the aim of this study was to evaluate the effects of two container types, Plantagrah and Hiko, on the morphology and quality of one-year-old seedlings of *Quercus frainetto* Ten., *Q. petraea* (Matt.) Liebl., and *Q. pubescens* Willd. The following null hypotheses were tested:

H_{01} : container type has no effect on seedling morphology and quality, and

H_{02} : the effect of container type on seedling morphology and quality does not differ among species.

2 Material and methods

2.1 Plant material

Acorns of *Quercus frainetto*, *Q. petraea* and *Q. pubescens* were collected from healthy coppice stands in the Belgrade vicinity (*Q. frainetto* – 44.632475°N, 20.399707°E; *Q. petraea* – 44.762058°N, 20.437013°E; *Q. pubescens* – 44.763667°N, 20.431975°E), from at least 15 parent trees per population. Seeds of each species were sown in November 2023, in two commercially available hard-plastic container types: Plantagrah (Bosnaplast 18) and Hiko V-265 (Table 1), in the nursery of University of Belgrade – Faculty of Forestry (coordinates: 44.782572°N, 20.425504°E). Each container cell was filled with a mixture of Pindstrup® (Pindstrup Mosebrug, Denmark) and perlite (2:1), and one seed per cell was placed. Seedlings were irrigated 2-3 times per week up to the middle of September 2024. Foliar fertilization was applied twice per week from

the start of June to the end of July (according to producer instruction, GroGreen Starter® Lima Europe NV, Belgium).

Table 1. Characteristics of Plantagrah and Hiko containers.

Container traits	Plantagrah	Hiko
Form	hexagonal	square
Cell volume [cm ³]	220	265
Depth [mm]	180	150
Density [n/m ²]	440	368
Hole	bottom	bottom
Cell wall	slick	slick
Dimension [mm]	320 x 215 x 180	352 x 216 x 150
Number of cells per container	33	28

In November 2024, 20 seedlings per species and container type were randomly selected (N = 120) and transported to the Laboratory for seeds and seedlings at the University of Belgrade – Faculty of Forestry. Seedlings' height (± 0.1 cm) and diameter at substrate level (± 0.1 mm) were measured using measuring tape and calliper. Roots were gently washed, and fine (lateral) roots were separated from taproot. Seedlings were dried at 68 ± 2 °C for 48 hours (Ivetić 2013) and dry masses of shoot, tap and fine roots were determined. Shoot and root weights were measured on an electronic scale with an accuracy of ± 0.001 g. Total root dry mass was obtained as the sum of the tap and fine root masses, and total seedling dry mass as the sum of total root dry mass and shoot dry mass. Shoot to root (S:R) ratio was calculated as shoot dry mass divided by total root dry mass. H/D index was calculated following Roller (1977) as the ratio between seedling height and diameter at substrate level, and Dickson Quality Index (DQI) was calculated following Dickson et al. (1960). Rooting intensity (ROIN) was calculated as the ratio between total root dry mass and container cell volume (Endean and Carlson, 1975).

2.2 Statistical analysis

All statistical analyses were conducted in R version 4.5.2 (R Core Team, 2025) using the packages car (Fox and Weisberg 2019), emmeans (Lenth and Piaskowski 2025), ggplot2 (Wickham 2016), and FactoMineR (Le et al. 2008). Descriptive statistics were calculated separately for each species and container type combination and are presented as means $\pm$ standard deviations. The effects of tree species, container type, and their interaction on seedling morphological and quality traits were evaluated using two-way ANOVA. Assumptions of ANOVA were evaluated using Shapiro-Wilk tests of residual normality and Levene's tests for homogeneity of variance. For variables showing heterogeneous variances, log transformation was performed to assess whether variance homogeneity improved before the final analyses. Estimated marginal means were calculated to compare seedling quality among species and container types and to summarize overall container performance. Multivariate variation in seedling morphology and quality traits was explored using principal component analysis (PCA). Statistical significance was assessed at $\alpha = 0.05$.

3 Results

Mean values and standard deviations of all measured traits are shown in Figure 1. Regardless of container type, *Q. petraea* exhibited the largest seedlings, with the greatest diameter (4.07 ± 0.55 and 3.61 ± 0.65 mm for Hiko and Plantagrah, respectively), height (17.56 ± 3.40 and 16.75 ± 3.43 cm), shoot dry mass (0.709 ± 0.212 and 0.635 ± 0.221 g), total root dry mass (2.630 ± 0.672 and 1.961 ± 0.779 g), and total seedling dry mass (3.338 ± 0.837 and 2.596 ± 0.926 g). In contrast, *Q. frainetto* produced the smallest seedlings for most measured traits, with diameter ranging from 2.98 ± 0.35 to 3.28 ± 0.65 mm, height from 10.43 ± 2.97 to 11.92 ± 3.15 cm, and total seedling dry mass from 2.101 ± 0.726 to 2.150 ± 0.666 g. *Quercus pubescens* generally showed intermediate values for most measured traits.

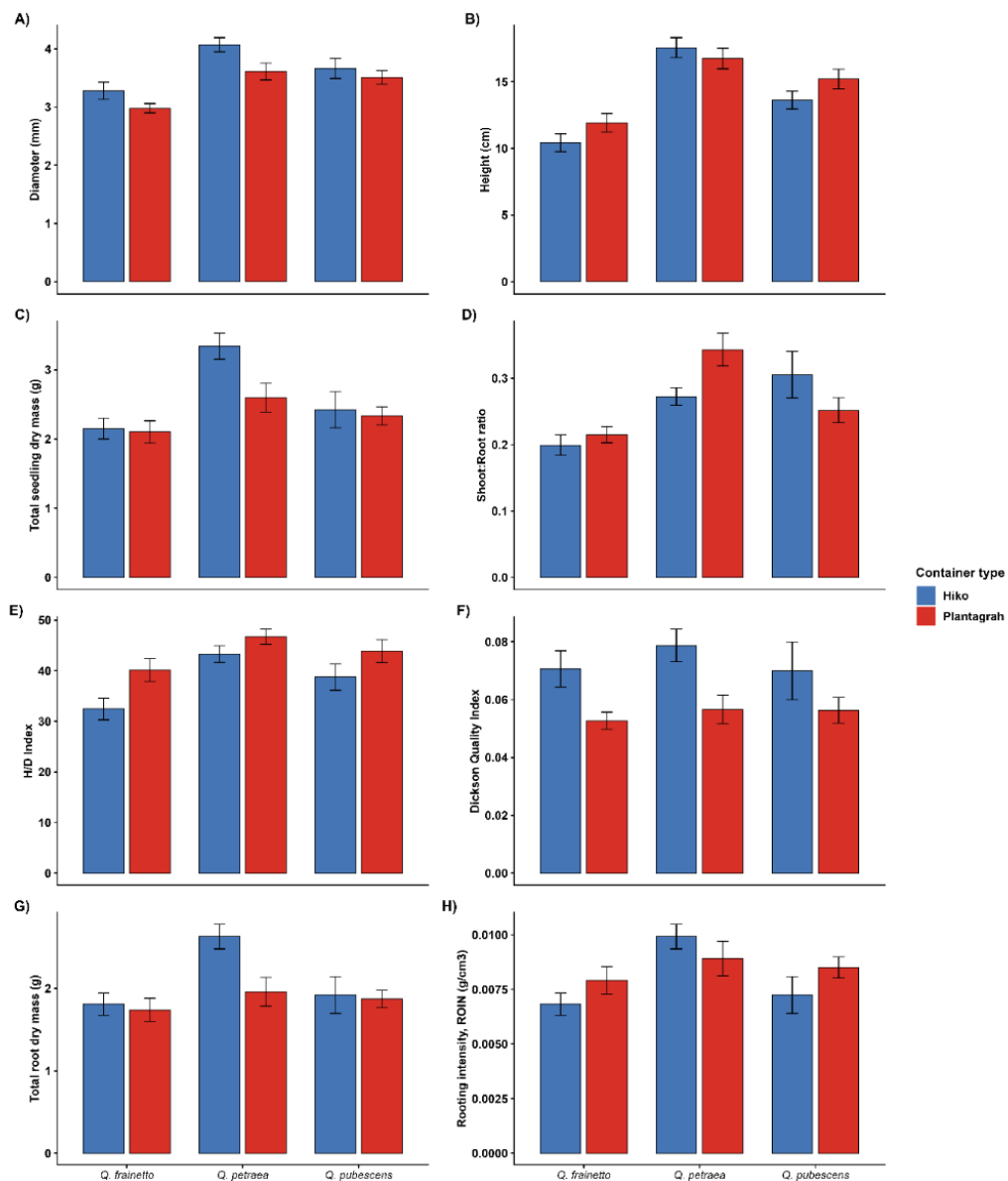


Figure 1. Bar charts of container types per species for A) diameter, B) height, C) total seedling dry mass, D) Shoot:Root ratio, E) H/D index, F) DQI, G) total root dry mass, and H) ROIN.

Mean trait values also varied between container types. Seedlings grown in Hiko containers tended to exhibit larger diameters (3.28 - 4.07 mm), greater total root dry mass (1.809 - 2.630 g), higher total seedling dry mass (2.150 - 3.338 g), and higher DQI values (0.070 - 0.079) than seedlings grown in Plantagrah containers. In contrast, seedlings grown in Plantagrah containers were generally taller and exhibited higher H/D index values than those grown in Hiko containers (Figure 1). Among all species and container combinations, *Q. petraea* grown in Hiko containers exhibited the highest diameter (4.07 mm), total seedling dry mass (3.338 g), and DQI (0.079).

The effects of tree species, container type, and their interaction on seedling morphological and quality traits are presented in Table 2. Tree species significantly affected all measured traits except DQI (Table 2). Container type significantly affected diameter, fine root dry mass, total root dry mass, H/D index, and DQI. Seedlings grown in Hiko containers exhibited greater diameters, total root dry mass, and DQI values, whereas seedlings grown in Plantagrah containers exhibited higher H/D index values (Figure 1). The interaction between tree species and container type was significant only for shoot:root ratio (Table 2), whereas all other interactions were not significant.

Table 2. Results of two-way ANOVA ($p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***).

Parameter	Term	df	F-value	p-value
Diameter	Tree species	2	14.5110	< 0.001***
	Container type	1	7.7412	0.0063**
	Tree species x Container type	2	0.6740	0.5117
Height	Tree species	2	34.8627	< 0.001***
	Container type	1	1.6721	0.1986
	Tree species x Container type	2	1.7828	0.1728
H/D index	Tree species	2	8.6177	0.0003***
	Container type	1	9.8848	0.0021**
	Tree species x Container type	2	0.5159	0.5984
Fine root dry mass	Tree species	2	4.4441	0.0139*
	Container type	1	4.2370	0.0418*
	Tree species x Container type	2	1.9925	0.1411
Total root dry mass	Tree species	2	5.8504	0.0038**
	Container type	1	4.0580	0.0463*
	Tree species x Container type	2	2.4572	0.0902
Shoot dry mass	Tree species	2	31.7529	< 0.001***
	Container type	1	0.3471	0.5569
	Tree species x Container type	2	0.6813	0.508
Total seedling dry mass	Tree species	2	10.5528	0.0001***
	Container type	1	3.6784	0.0576
	Tree species x Container type	2	2.1338	0.1231
Shoot:Root ratio	Tree species	2	15.0165	< 0.001***
	Container type	1	2.0270	0.1573
	Tree species x Container type	2	3.2162	0.0438 *
DQI	Tree species	2	0.6719	0.5128
	Container type	1	15.7279	0.0001 ***
	Tree species x Container type	2	0.2090	0.8117
ROIN	Tree species	2	5.3406	0.0061 **
	Container type	1	0.7012	0.4041
	Tree species x Container type	2	1.8789	0.1575

The first two principal components explained 82.1% of the total variation in seedling traits, with PC1 and PC2 accounting for 55.9% and 26.2% of the variation, respectively (Figure 2). Species were more clearly separated in the PCA than container types (Figure 2). *Quercus frainetto* and *Q. petraea* formed distinct groups, whereas *Q. pubescens* showed an intermediate pattern. Seedlings grown in Hiko and Plantagrah containers largely overlapped.

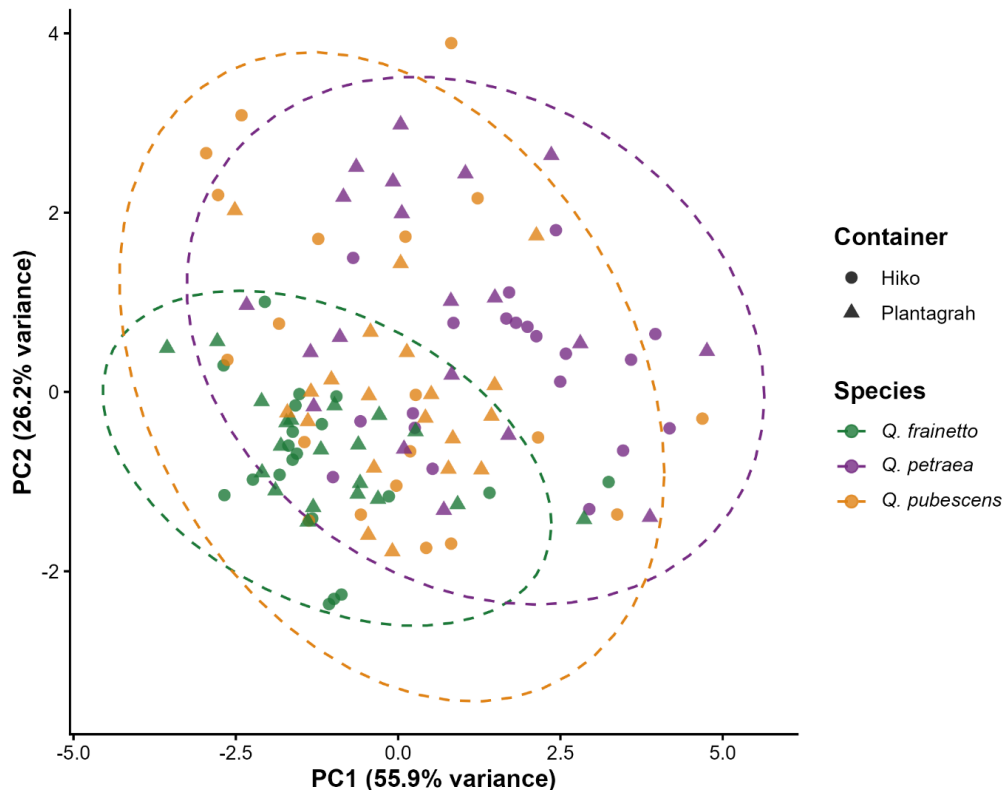


Figure 2. Principal component analysis (PCA) of oak seedlings.

4 Discussion

Significant differences in seedling morphology were observed among the oak species studied. Among the three species, *Q. petraea* generally exhibited the largest seedlings, whereas *Q. frainetto* showed the lowest values for most growth-related traits. *Quercus pubescens* displayed intermediate values for the majority of measured parameters. These differences likely reflect differences in growth among the studied oak species during early seedling development. Previous studies have shown that growth and biomass allocation can vary among oak species (Rodríguez-Calcerrada et al. 2008; Pérez-Ramos et al. 2010; Jokačević et al. 2024). The greater height, diameter, and seedling dry mass observed in *Q. petraea* suggest faster early growth under the nursery conditions of this study, whereas the lower values recorded for *Q. frainetto* suggest slower initial growth. Compared with published nursery guidelines obtained under different nursery conditions, the height of *Q. frainetto* seedlings was within the recommended range for 1+0 container seedlings, while diameter, total seedling dry mass, and DQI were lower than the recommended values (Ertas 2026). For *Q. petraea*, diameter was within the reported range for 1+0 container seedlings, whereas height

was somewhat lower than the typical range reported by Nicolescu et al. (2026). The height and diameter of *Q. pubescens* seedlings were within or above the values reported for 1+0 container-grown seedlings by Cocozza and Kerkez Janković (2026). Despite clear differences in morphology and dry mass traits, no significant species effect was detected for DQI. As DQI integrates several morphological characteristics into a single measure of seedling quality (Dickson et al. 1960), this result suggests that differences in individual traits did not translate into differences in overall seedling quality. The two container types differed in several characteristics, including cell volume, depth, shape, and growing density. Therefore, the observed differences cannot be related to any single container trait but rather reflect the combined influence of the commercial container types compared in this study (Mariotti et al. 2026).

Although species accounted for much of the observed variation among seedlings, container type also significantly affected several important indicators of seedling quality. Seedlings grown in Hiko containers exhibited greater stem diameter, higher fine root dry mass, higher total root dry mass, and higher DQI values than seedlings grown in Plantagrah containers. In addition, Hiko-grown seedlings had lower H/D values, indicating a more balanced relationship between height and diameter. In contrast, container type had no significant effect on seedling height, shoot dry mass, total seedling dry mass, or ROIN, suggesting that its effects were expressed mainly through root development and stem diameter rather than increased shoot growth. These differences may be related to the characteristics of the tested containers. Compared with Plantagrah, Hiko containers have a larger cell volume and lower growing density (Table 1), which may provide more space for root development and reduce competition among seedlings (Mariotti et al. 2015; Grossnickle and El-Kassaby 2016). Similar effects of container characteristics on root development and seedling quality have been reported in previous studies (Tsakalimi et al. 2005; Banach et al. 2023; Mijatović et al. 2022). The greater root biomass observed in Hiko-grown seedlings was accompanied by larger stem diameter and higher DQI values, suggesting that container characteristics influenced overall seedling morphology and quality. Root development and seedling morphology are widely recognized as important components of seedling quality (Grossnickle 2012; Grossnickle and Ivetić 2022).

Among the evaluated quality indicators, DQI showed the clearest response to container type. Average DQI values were consistently higher in Hiko containers across all three species, indicating that the positive effect of this container type was evident regardless of species. Significant interactions between species and container type were not detected for diameter, height, biomass-related traits, H/D index, DQI, or ROIN, indicating that container effects were generally similar across the three studied oak species. The only exception was S:R ratio, for which a significant interaction was observed, suggesting species-specific differences in biomass allocation between container types. However, because no interaction was detected for DQI or other major quality indicators, the overall effect of container type on seedling quality appeared to be largely consistent across species, which is in agreement with previous studies reporting positive effects of container characteristics on oak seedling quality (Tsakalimi et al. 2005; Banach et al. 2023). Although total root dry mass was greater in Hiko containers, ROIN did not differ significantly because total root dry mass was expressed relative to container volume. As a result, the greater total root dry mass observed in Hiko seedlings did not translate into differences in root dry mass per unit

container volume. This finding is consistent with previous studies showing that container traits influence root development (Mariotti et al. 2026).

The production of high-quality planting stock is an important objective of forest nurseries, as seedling quality can influence establishment success after planting (Grossnickle and MacDonald 2018; Grossnickle and Ivetić 2022; Villar-Salvador et al. 2004). In the present study, container type significantly affected several traits commonly used to assess seedling quality, including diameter, root biomass, and DQI. Among the tested container types, Hiko consistently produced seedlings with larger diameters, greater root biomass, and higher DQI values than Plantagrah. These results suggest that Hiko containers may provide more favorable conditions for seedling growth during the first nursery year.

5 Conclusions

The present study demonstrated that tree species and container type influenced the morphology of one-year-old oak seedlings. Significant differences among species were observed for most measured traits, with *Q. petraea* generally producing the largest seedlings and *Q. frainetto* the smallest. Despite these differences, DQI did not differ significantly among species.

Container type significantly affected several important indicators of seedling quality, including diameter, fine root dry mass, total root dry mass, H/D index, and DQI. Seedlings grown in Hiko containers generally exhibited larger diameters, greater root biomass, lower H/D values, and higher DQI values than those grown in Plantagrah containers.

Container effects were similar for most measured traits across species, indicating that the relative advantages of Hiko containers were not species-specific. These results suggest that Hiko containers may provide a suitable production system for the cultivation of *Q. frainetto*, *Q. petraea*, and *Q. pubescens* seedlings during the first nursery year. Further research is needed to determine whether the observed differences in seedling quality are reflected in survival and growth after planting.

6 Funding statement

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