










Interclonal variation in biometric parameters of the *Pinus sylvestris* L. seedlings based on multivariate analysis

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ABSTRACT

Seed origin is one of the key factors determining growth processes, morphological structure, and adaptive potential of forest tree species seedlings during early ontogenetic stages. Due to increasing climate stress, the importance of using genetically valuable and morphologically robust planting stock is becoming particularly relevant. The aim of the study was to assess interclonal variation in the main biometric parameters of the Scots pine (*Pinus sylvestris* L.) seedlings grown from seeds of different clonal origin, and to evaluate the effectiveness of integrated multivariate statistical methods for selecting promising clones. The study material consisted of the *Pinus sylvestris* L. seedlings grown from seeds of 60 plus-tree clones. Seedling height, root collar diameter, taproot and lateral root length, needle length, and seedling air-dry mass were measured. Principal component analysis (PCA) and cluster analysis were applied for the integrated assessment of interclonal differences. Significant interclonal differences were established for all biometric parameters studied. Seedling height ranged from 13.0–15.0 cm in the clones with minimum quantitative characteristics (D1, D41, D13) to 40.0–42.0 cm in the clones with high biometric parameters (D21, D22, D20, D32, D40), which consistently exceeded the control. Root collar diameter varied from 0.3–0.6 mm in the clones D1, D59, and D60 to 2.7–3.0 mm in the clones D31, D18, D10, and D51. Taproot length in clones D22, D20, and D32 was 22.0–23.3 cm, while lateral root length in the clones with high biometric parameters reached 18.0–19.1 cm, significantly exceeding the control values. Needle length in productive clones was 4.0–4.4 cm, while in low-productivity clones it did not exceed 1.2–1.5 cm. Seedling air-dry mass ranged from 16.0–18.0 g in the clones with low growth potential to 35.0–39.6 g in the clones D21, D22, D20, D32, and D40. The results of the principal component analysis showed that the first two components explained 81.1% of the total variation in biometric parameters (PC1 – 63.9%, PC2 – 17.2%) and provided clear differentiation of seedlings by clonal origin. Cluster analysis confirmed high within-clone homogeneity and distinct among-clone differences. The combined application of univariate biometric analysis, PCA, clustering, and an integrated summary diagram provided clear differentiation of Scots pine clones with high growth potential (D21, D22, D20, D32, D40) as well as identification of a low-productivity clone (D1). The results justify the application of integrated multivariate methods for identifying genetically valuable clones promising for forest seed production and tree breeding.

Keywords: *Pinus sylvestris* L., seedlings, clonal seed origin, biometric parameters, cluster analysis.

INTRODUCTION

Seed origin is one of the key factors shaping growth processes, vigour, and morphological development of Scots pine (*Pinus sylvestris* L.) seedlings. Numerous studies demonstrate that using the seeds from plus-trees and genetically

improved clones supports the production of planting stock with higher biometric parameters and enhanced adaptive capacity (Boiko et al., 2021; Mazurchuk et al., 2025). Genetically determined growth variation within species plays a key role in selecting high-quality planting stock, particularly under increasing climate

stress conditions, including prolonged droughts, temperature fluctuations, and more frequent extreme weather events (Oberhuber, Kofler, 2020). Therefore, science-based genotype selection is considered a prerequisite for establishing productive and resilient forest stands under changing environmental conditions.

The relevance of this study stems from the need for quantitative and qualitative assessment of differences among the Scots pine seedlings grown from seeds of various plus-tree clones, which will improve reforestation efficiency and enable establishment of stable, highly productive stands in afforestation regions. The subject of the study were the biometric parameters of Scots pine seedlings, including seedling height, root collar diameter, needle length, taproot and lateral root length, and air-dry mass.

The aim of the study was to assess interclonal variation in the main biometric parameters of the Scots pine (*Pinus sylvestris* L.) seedlings grown from seeds of different clonal origin, and to evaluate the effectiveness of integrated multivariate statistical methods for selecting promising clones.

BACKGROUND

Seedling biometric parameters, including height, root collar diameter, root system length, above-ground biomass, and needle length, are widely used to assess vigor and breeding value of coniferous planting stock. Numerous studies confirm that the Scots pine seedlings grown from seeds of plus-trees and genetically improved clones are characterized by higher growth rates and better-developed root systems, which positively affects their survival under field conditions (Boiko et al., 2021; Vaskiv, 2025).

Genotypic differences significantly influence the formation of the main morphometric parameters of seedlings already in the early stages of ontogenesis. According to Oberhuber and Kofler (2020), seedling growth and development are largely determined by the ecological plasticity of genotypes, as well as genetically determined growth characteristics become particularly important under abiotic stress factors, such as drought and soil degradation. In this regard, height, root collar diameter, and seedling mass parameters are considered primary selection criteria when selecting adapted planting stock (Jakoby et al., 2019).

Root system development has been established as one of the key factors in seedling competitiveness and adaptation to changing climatic conditions. The seedlings with well-developed, extensively branched root systems exhibit greater resilience to moisture deficit and superior regenerative capacity under stress conditions (Kramarets and Matsiakh, 2018; Janowski et al., 2025). At the same time, seedling biometric parameters are dynamic characteristics formed under the influence of both genetic factors and growing conditions, particularly moisture, light, and temperature regime (Oberhuber and Kofler, 2020).

Numerous studies indicate that seed origin significantly determines initial seedling growth rates and developmental stability during early ontogenetic stages. The seedlings derived from seeds of clones subjected to intensive selection exhibit greater homogeneity of biometric parameters and reduced within-population variability, enhancing the breeding value of the planting stock (Zhezhkun, 2021; Zachara, 2014). Morphometric traits of the root system and above-ground parts closely correlate with the genetic potential of parent trees, and interclonal differences are already apparent in the early stages of cultivation (Remezova et al., 2021).

Along with morphological characteristics, physiological processes play an important role in determining seedling productivity, particularly dry matter accumulation, photosynthetic pigment content, and gas exchange intensity, which also depend on the genetic origin of the seed (Pinchuk et al., 2021; Mazurchuk et al., 2025). This determines the expediency of a comprehensive approach to assessing clone prospects, combining biometric, anatomical, and physiological parameters.

Given the multifactorial nature of morphometric trait formation, multivariate statistical methods are increasingly applied in breeding research, particularly principal component analysis (PCA), which enables integrated assessment of biometric characteristics and identification of variability patterns among genotypes (Jolliffe and Cadima, 2016; Isik et al., 2017). However, despite numerous studies, comprehensive assessment of clonal differences in Scots pine seedlings based on multiple biometric parameters under both open-field and greenhouse conditions remains largely unexplored, which necessitates the present study.

MATERIALS AND METHODS

In April 2023, experimental plots were established for germinating genetically improved Scots pine seed under open-field conditions at Teteriv Forestry, a branch of the State Specialised Forest Enterprise (SFE) ‘Forests of Ukraine’. Genetically improved seeds were collected and dried separately from each clone to determine the most productive seedlings obtained from seeds of different clonal origin.

Scots pine clonal seed orchards were created through grafting of selected clones, planting of seedlings grown from plus-tree seeds, and planting of grafts produced in polyethylene greenhouses.

The orchards are located in Potashniansky Forestry (compartment No. 69, parcel No. 14, established in 2006, area 5.0 ha) and Myhalsky Forestry (compartment No. 123, parcel No. 1, area 18.6 ha). Forest site type: C2 (fresh relatively fertile site); forest type: fresh hornbeam-oak-pine mixed forest. Soils are sod-podzolic, weakly podzolized, with sandy texture. Plus-tree clones were selected from superior stands and gene conservation areas in the Polissia zone of Ukraine based on growth as well as seed production assessment of seedling and clonal progenies. A total of 60 clones were selected as the most promising for establishing seed orchards. For convenience of result presentation, each clone was assigned a designation (code) of the type D1, D2,... D60, where the letter D serves as a conditional marker for the experimental variant (clone), and the numerical index corresponds to the clone’s serial number in the sample. These designations were used in tables, graphical materials, and multivariate statistical analysis. During the first mass harvest in 2016–2017, 10,917 kg of cones were collected, yielding 168 kg of seeds with a mean extraction rate of 1.54%.

The Scots pine seedlings grown from locally sourced seed, collected following standard operational procedures from plus-trees without regard to clonal identity, were used as control.

Planting stock was grown at the Teteriv Forestry branches of the SFE ‘Forests of Ukraine’. The seeds collected from clonal seed stands were used for the study. Before sowing, the seeds were soaked for 16 hours (May 2nd, 2023). On May 3rd, 12 g of seeds were sown on each containers according to the experimental design, corresponding to 1,500 seeds per 1 m². The seed sowing rate was 120 seeds per linear meter of sowing row.

The size of one plot was 0.5 × 2.0 m (1 m²). First-class seeds were used. Uniform emergence was recorded on May 19th, 2023.

The growing medium, prepared under controlled conditions in containers, consisted of bark and peat in a 7:3 ratio. The top soil layer was loosened, levelled, and covered with a 2-cm layer of white sand. The seeds were evenly distributed over the soil surface, covered with sifted sand, rolled, and shaded. Seedlings were watered in the evening when daytime heat subsided. Watering frequency was adjusted according to environmental conditions to prevent soil drying or waterlogging. In the current year, along with pre-sowing seed treatment, foliar fertilization of seedlings (4 applications) was applied during the growing season. During the research, morphometric measurements were taken and the condition of the seedlings was analyzed.

The initial assessment of interclonal differences was based on the analysis of individual biometric parameters. For each clone, mean values of the parameters were calculated from measurements of ten seedlings.

For the integrated assessment of interclonal differences and analysis of intraclonal variability in Scots pine seedlings, multivariate statistical methods were applied – principal component analysis (PCA) and cluster analysis. Both methods were performed using individual biometric parameters of each seedling, without prior averaging of values within clones. For each clone, individual biometric parameters of 10 seedlings ($n = 10$) were used in the cluster analysis; clustering was not based on mean values within clones. The following biometric traits were used as input variables: seedling height, root collar diameter, needle length, taproot and lateral root length, and air-dry mass. To eliminate the effect of different measurement units, all parameters were standardized prior to analysis. PCA was performed on a correlation matrix to identify the main directions of variation in biometric traits and to reduce the dimensionality of the original data. Cluster analysis can be performed using a variety of methods to determine the distance between clusters: Between-groups linkage, Within-groups linkage, Nearest neighbor, Furthest neighbor, Centroid clustering and other. For the research, cluster analysis was conducted using Ward’s hierarchical method, which minimizes within-cluster variance and ensures the formation of statistically robust clusters. The clustering results are

presented as a dendrogram illustrating the degree of similarity among individual seedlings of different clonal origin.

The seedlings were lifted in September 2024 at the forest nursery of Teteriv Forestry, branch ‘Stolychnyi Forest Office’, SFE ‘Forests of Ukraine’.

RESULTS OF RESEARCH

Biometric parameters of the Scots pine seedlings were measured in September–October 2024, including seedling height, root collar diameter, needle length, length of taproot and lateral roots, and seedling air-dry mass (Figure 1).

On the basis of the mean biometric parameter values for each clone, comparative analysis and graphical interpretation of interclonal differences in the Scots pine seedlings were performed. For each parameter separately (seedling height, root collar diameter, taproot and lateral root length, above-ground air-dry mass, needle length), graphs were constructed showing clones with the highest and lowest mean values of the corresponding trait, as well as the control. This approach enabled not only revealing contrasts between clone groups with different growth and development levels, but also assessing the control relative to extreme values for each biometric parameter. Results are presented in Figure 2.

Figure 2 presents a comparative analysis of main biometric parameters of the Scots pine seedlings for clones with the highest and lowest mean trait values compared to the control, enabling visual assessment of the degree of interclonal differentiation based on morphometric parameters.

In terms of seedling height, clones with high growth potential (D21, D22, D20, D32, D40) were

characterized by mean values ranging from 40.0 to 42.0 cm, significantly exceeding the control.

In contrast, the clones with the lowest values for this parameter (D1, D41, D13) had seedling heights of only 13.0–15.0 cm, indicating significant growth lag. Clones D31, D18, D10, and D51 produced the seedlings with root collar diameter of 2.7–3.0 mm, consistently exceeding the control, while in clones D1, D59, and D60 this parameter remained at a minimum level (0.3–0.6 mm).

Analysis of root system development revealed significant interclonal differences in both taproot length and lateral root length. In clones D22, D20, and D32, taproot length was 22.0–23.3 cm, and lateral root length reached 18.0–19.1 cm, significantly exceeding control values. Meanwhile, in the clones with low growth potential (particularly D1 and D41), taproot length did not exceed 2.0–3.0 cm, indicating weak development of the below-ground plant parts. Needle length clearly differentiated clones by growth potential. In the clones with high biometric parameters (D21, D22, D20, D32, D40), mean needle length was 6.5–7.8 cm, consistently exceeding the control and indicating more intensive development of the assimilatory apparatus. In contrast, in the clones with minimal growth characteristics (particularly D1 and D41), needle length did not exceed 4.0–4.4 cm, consistent with their overall reduction in above-ground biomass and weaker seedling growth. Seedling air-dry mass values reflected distinct interclonal differences. The clones with high morphological characteristics (D21, D22, D20, D32, D40) produced seedlings with air-dry mass of 35.0–39.6 g, significantly exceeding the control and indicating normal development of above-ground and below-ground plant parts. In contrast, in the clones with low biometric parameters (D1, D41, D13), the values for this parameter remained minimal, not exceeding 16.0–18.0 g, confirming their lower productivity already in the early stages of ontogenesis.



Figure 1. Laboratory processing: measuring the biometric parameters of the Scots pine seedlings

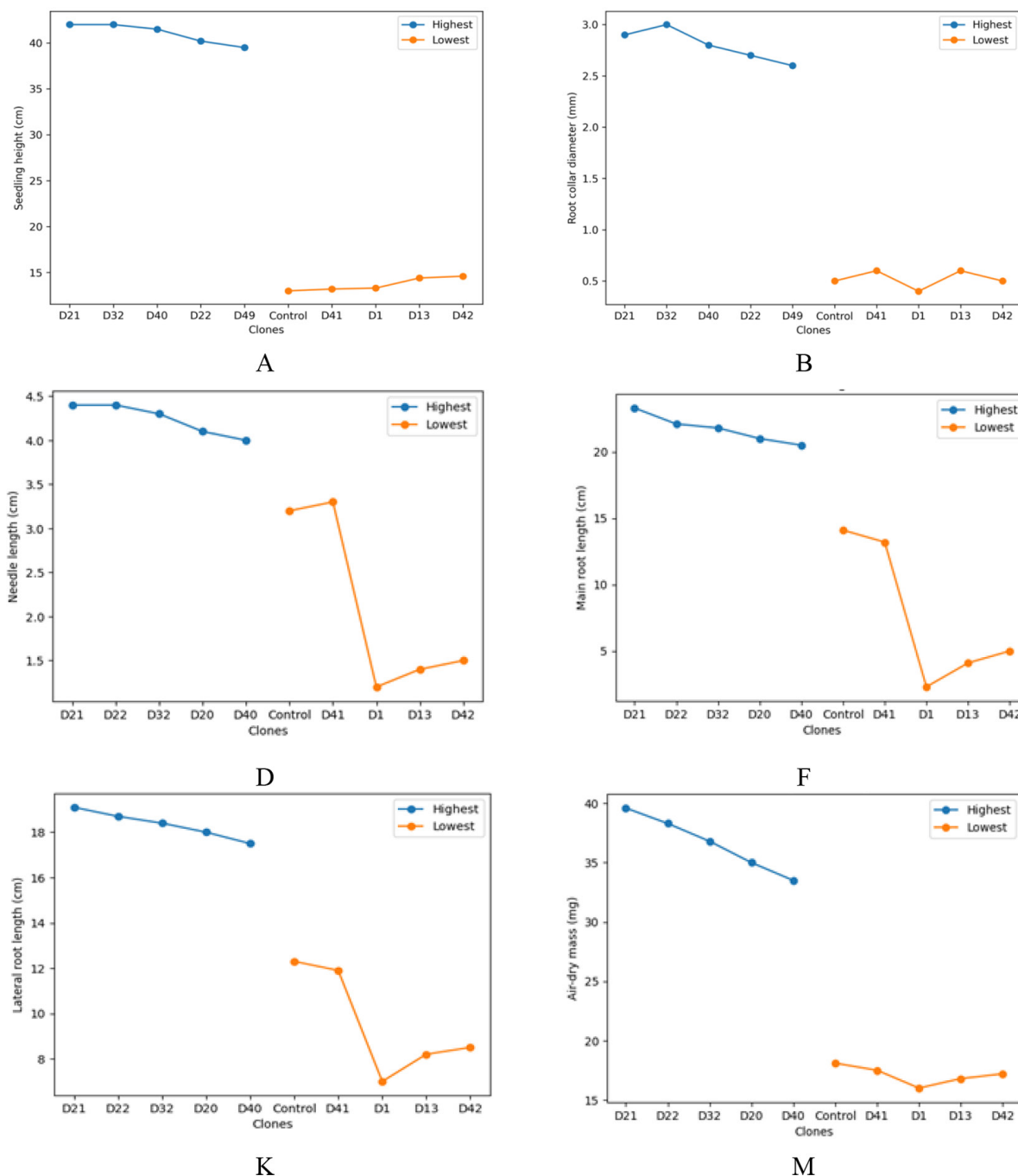


Figure 2. Comparative characteristics of biometric parameters of the Scots pine (*Pinus sylvestris* L.) seedlings grown from the seeds of clones with the highest and lowest mean biometric values: A – seedling height (cm), B – root collar diameter (mm), D – needle length (cm), F – taproot length (cm), K – lateral root length (cm), M – seedling air-dry mass (g). Designations D1–D49 correspond to clone codes

Thus, graphical comparison of mean biometric parameters of the seedlings with the control enabled clear identification of the most productive clones and confirmed the systematic nature of interclonal differences across both above-ground and below-ground traits. The presented samples illustrate the morphological heterogeneity among the seedlings of different clones based

on a complex of above-ground and below-ground traits, and serve as visual confirmation of the quantitative differences recorded in the biometric analysis results, which were used for subsequent clustering of individual seedlings.

Cluster analysis was applied to further generalize results and identify similarities among individual seedlings of different clones.

Figure 3 presents the results of clustering the Scots pine seedlings based on biometric characteristics, enabling assessment of both within-clone homogeneity level and the degree of among-clone differentiation.

Cluster analysis of the Scots pine seedlings (Figure 3) demonstrated that the majority of seedlings grouped according to clonal origin. This indicates that plants of the same clone are more similar to each other in biometric parameters than to seedlings of different clones, even under identical growing conditions.

For individual clones, particularly D21, D22, D20, D32, and D40, clusters with minimal internal variation among individual seedlings were formed, indicating stable expression of genetically determined growth traits and low within-clone variability. Groups of clones with similar complex morphometric characteristics are distinguished on the upper branches of the dendrogram. One

such group combines the clones with higher mean values of main biometric parameters (D21, D22, D20, D32, D40), while other clusters are formed by the clones with moderate or lower growth rates, particularly D1 and D54. The seedlings of clones with low growth potential do not cluster with high-performing clones but form separate dendrogram branches. This confirms that clonal seed origin is a determining factor in the formation of morphometric structure of seedlings, and the observed differences are genetically determined.

Overall, cluster analysis results clearly demonstrate the possibility of identifying groups of the Scots pine clones with different growth potential and confirm the feasibility of applying integrated multivariate methods for generalized assessment of planting stock quality.

Multivariate statistical methods were applied to summarize the obtained results and comprehensively assess interclonal differences in the

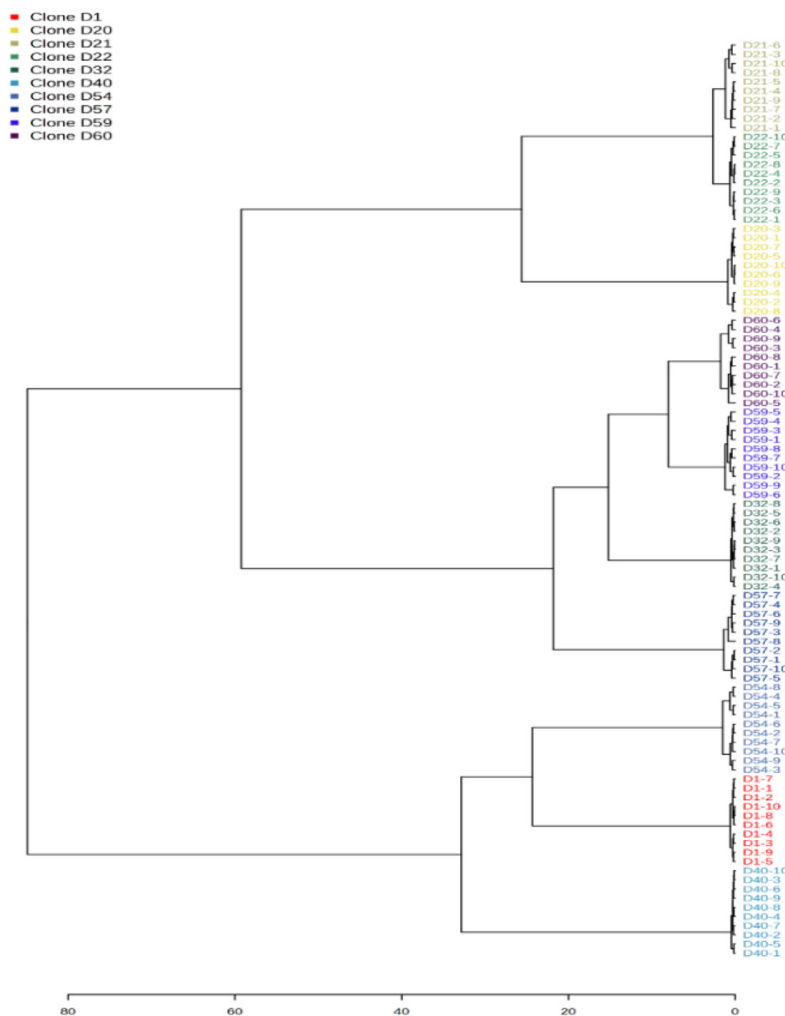


Figure 3. Results of cluster analysis of the Scots pine (*Pinus sylvestris* L.) seedlings based on biometric parameters (clustering was performed for individual seedlings, n = 10 for each clone). Designations D1–D60 correspond to conditional clone codes

Scots pine seedlings. Principal component analysis (PCA) and clustering enabled simultaneous consideration of relationships among biometric characteristics and assessment of the integrated influence of seed clonal origin on seedling morphological structure formation (Figure 4).

The first two principal components (PC1 and PC2) explain 81.1% of the total variation in biometric parameters of the Scots pine seedlings, indicating a high level of information generalization and the feasibility of using two-dimensional

projection for interpreting multivariate analysis results (Figure 4). The projection of seedlings in the space of the first two principal components, which explain the major proportion of trait variation (PC1 – 63.9%, PC2 – 17.2%), shows clear grouping of seedlings within individual clones and spatial separation between them, indicating high within-clone homogeneity and distinct among-clone differences.

The distribution of seedlings according to PC1 and PC3 components confirms these patterns

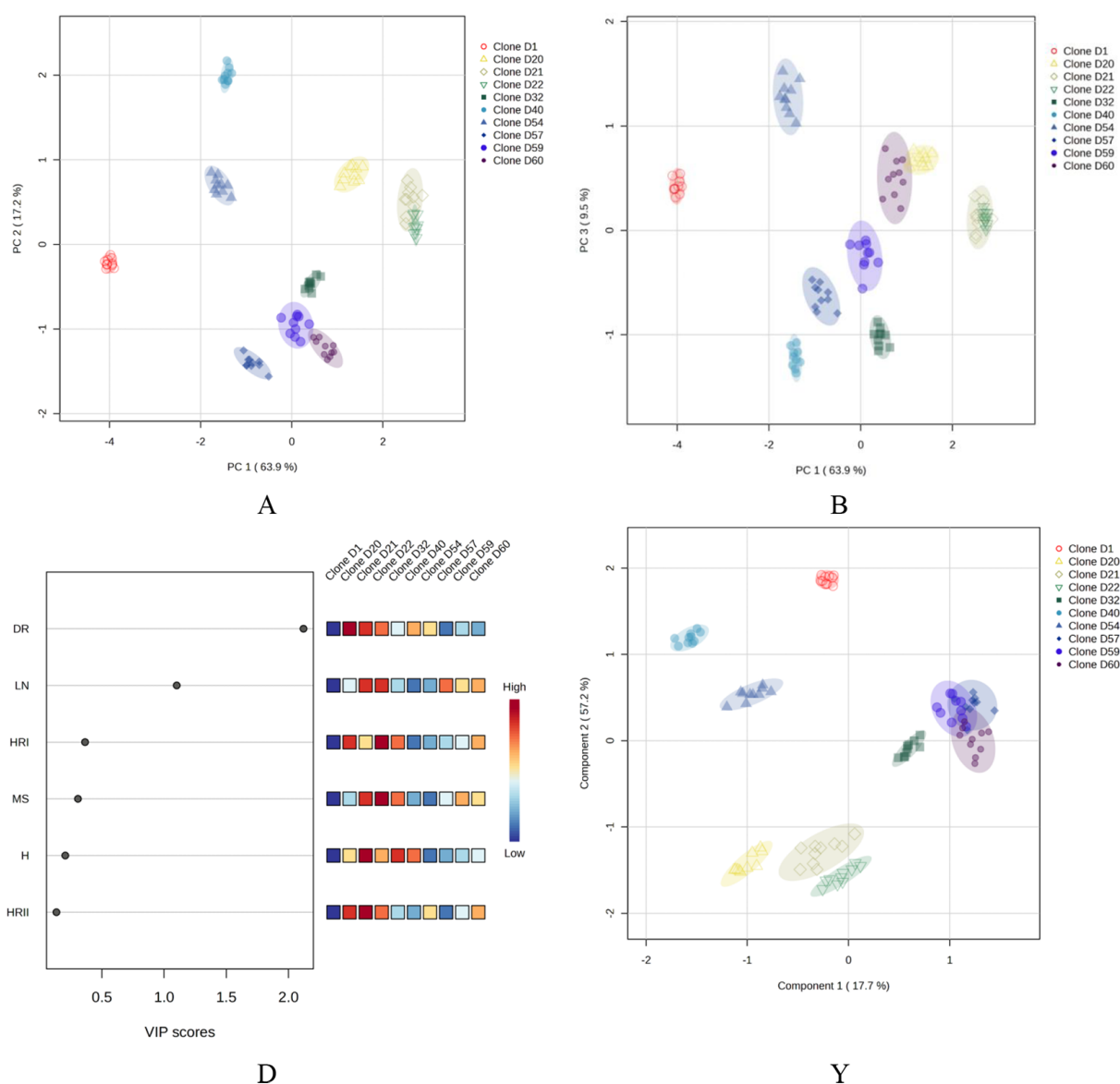


Figure 4. Projection of the Scots pine (*Pinus sylvestris* L.) seedlings in principal component space (PC1, PC2, PC3): A – projection of seedlings in the space of the first two principal components (PC1 and PC2); B – distribution of seedlings according to the first and third principal components (PC1 and PC3); D – contribution of biometric parameters to principal components (loadings); Y – integrated PCA projection illustrating the spatial grouping of seedlings based on the studied biometric parameters (each point corresponds to an individual seedling, n = 10 for each clone). Each point corresponds to an individual seedling; n = 10 for each clone; designations D1–D60 correspond to conditional clone code

and indicates that even less significant components further differentiate individual clones. Partial convergence of individual groups reflects the presence of the clones with similar growth types and close adaptive potential.

Analysis of the contribution of biometric parameters to principal component formation (based on loadings) demonstrated that the traits related to above-ground and root system development play a decisive role in clone differentiation, primarily seedling height, root collar diameter, and root parameters, while mass parameters are of secondary importance.

The generalized PCA projection clearly demonstrates the formation of clone groups with different growth potential levels: clones with higher values of main biometric parameters occupy separate regions of multidimensional space and are clearly distinguished from clones with lower values. This confirms the consistency of multivariate analysis results with single-factor assessment data for individual traits and justifies further use of integrated graphical approaches to compare the clones with the highest qualitative and quantitative parameters (Figure 5).

The integrated summary diagram, constructed based on mean values of all studied biometric parameters (Figure 5), clearly confirms the presence of stable interclonal differences in growth

and development of the *Pinus sylvestris* L. seedlings. Clones D21 and D22 are characterized by the highest integrated parameter values across all quantitative and qualitative traits, indicating balanced development of above-ground and below-ground seedling parts. Clones D20, D32, and D40 also demonstrate high integrated levels of biometric parameters but differ in specialization of certain traits.

In contrast, clone D1 is characterized by minimal normalized values for most biometric parameters, consistent with univariate analysis, PCA, and clustering results. Thus, combining analysis of individual biometric traits with multivariate statistical methods provides comprehensive assessment of interclonal differences and enables substantiated selection of the clones with high growth and adaptive potential for further use in forest seed production and tree breeding.

DISCUSSION

The obtained results indicate clearly expressed interclonal differences in the Scots pine seedlings, both for individual biometric parameters and their integrated set. The high level of clone differentiation by seedling height and root collar diameter confirms the key role of these

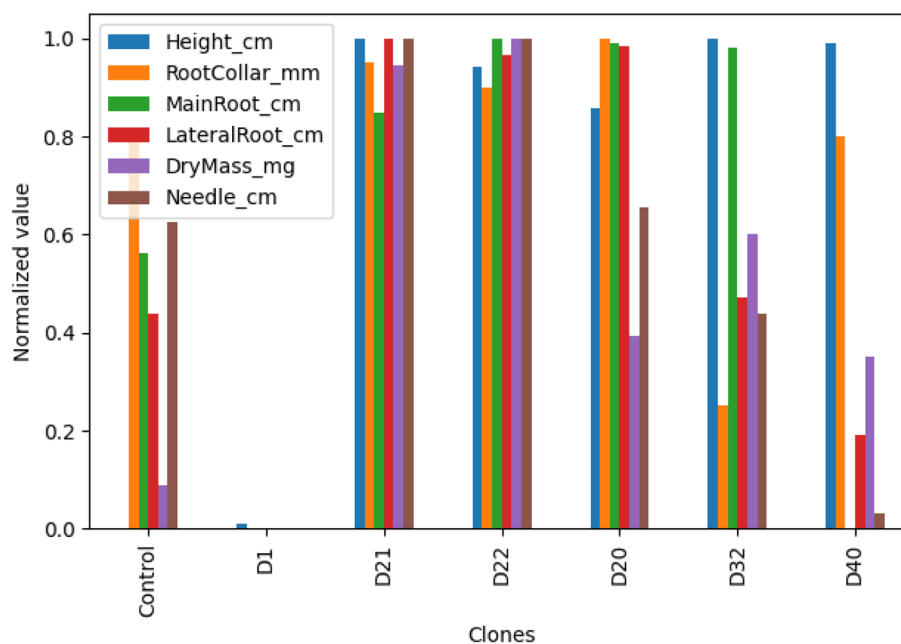


Figure 5. Integrated summary diagram of the Scots pine (*Pinus sylvestris* L.) seedlings grown from seeds of clones with the highest and lowest biometric parameters (seedling height, root collar diameter, taproot and lateral root length, air-dry mass, and needle length)

parameters as indicators of plant vigor and adaptive potential. Previous studies have repeatedly noted that root collar diameter is a more stable and prognostically significant trait than height, especially during early seedling development (Mexal and Landis, 1990; Grossnickle, 2005, 2012). The results obtained in this study are consistent with these findings, as the clones with larger root collar diameter values were simultaneously characterized by comprehensively higher morphometric parameters.

Significant interclonal differences were also found in root system development, manifested in both taproot and lateral root length. A well-developed root system is critical for ensuring water and mineral nutrition of seedlings and determines their resilience to abiotic stress factors, particularly moisture deficit. Similar patterns are described by Kramarets and Matsiakh (2018), who emphasized the close relationship between root system morphology and the adaptive capacity of woody plants. Also, a significant association was identified when examining the effects of different pre-planting preparation methods for Scots pine seedlings on their adaptive potential and survival rate in forest plantations. Changes in the anatomical features of seedling root tissues depending on the methods of preparation for planting were characterized. The morpho-anatomical features of the adaptive potential of small-sized planting material were determined (Melnychuk et al., 2013). Thus, the identified differences among clones may have long-term consequences for the formation of productive and resilient stands under field conditions.

Interclonal differences in needle length deserve special attention, as they also revealed clear differentiation among the studied clones. Needle length is an important morphological trait closely linked to photosynthetic activity, water relations, and overall plant physiological status, and thus may serve as an indirect indicator of seedling growth and adaptive potential. Previous studies have demonstrated that variation in needle parameters in coniferous species, particularly Scots pine, is genetically determined and reflects plant response to environmental conditions (Niinemets & Lukjanova, 2003; Reich et al., 1999; Grotkopp et al., 2004). The differences in needle length identified in this study, combined with high growth rates of above-ground and root systems, allow this trait to be considered a promising target

for further detailed, particularly genetic, evaluation in the most productive clones.

The application of principal component analysis enabled integration of biometric parameters and identification of the main directions of morphometric trait variation among clones. Clear spatial separation of seedlings in PCA projections indicates coordinated expression of growth traits and confirms that interclonal differences are formed not by individual parameters but by a complex of interrelated characteristics. This approach is widely used in breeding and forestry research to assess genetic variability and identify promising genotypes (Jolliffe and Cadima, 2016; Isik et al., 2017), confirming its applicability in this study.

Analysis of the contribution of individual biometric traits to the formation of principal components (based on loading coefficients) enabled assessment of the role of each parameter in clone differentiation. The traits related to above-ground and root system development had the greatest impact on data structure formation, consistent with results of other studies where morphometric characteristics are considered integral indicators of growth potential (Grossnickle, 2012; Krynytskyi and Zhezhkun, 2021). The smaller contribution of individual growth indices indicates their auxiliary, but not decisive, role in clone differentiation. Cluster analysis results complement the PCA findings and confirm the existence of functionally distinct clone groups with different growth potential levels. High within-clone homogeneity of seedlings indicates stable expression of heritable traits and reduced phenotypic variability, which is an important characteristic of genetically valuable planting stock. At the same time, clear differentiation of less productive clones emphasizes the decisive role of seed origin even under identical growing conditions, consistent with the conclusions of Ruotsalainen (2014) and Mullin et al. (2011).

Overall, the study results confirm the feasibility of a comprehensive approach to assessing planting stock quality, combining analysis of individual biometric parameters with multivariate statistical methods. This approach enables more reliable identification of the clones with high growth potential and adaptive capacity, which is of great practical importance for forest seed production, tree breeding, and establishment of highly productive and resilient Scots pine stands under increasing climate challenges.

CONCLUSIONS

As a result of this study, interclonal variation in main biometric parameters of the Scots pine (*Pinus sylvestris* L.) seedlings grown from seeds of different clonal origin was assessed, and the feasibility of using integrated multivariate statistical methods for selecting genetically valuable planting stock was substantiated. The obtained results confirm that the stated objective has been fully achieved.

Significant and systematic interclonal differences were established for all morphometric parameters examined.

The highest growth potential was found in clones D21, D22, D20, D32, and D40, which consistently exceeded the control in seedling height, root collar diameter, root system parameters, needle length, and air-dry mass. In contrast, clones D1, D41, and D13 were characterized by minimal values for most biometric traits, indicating their lower productivity in the early stages of ontogenesis.

Principal component analysis was shown to enable integrated generalization of biometric traits and identification of the main directions of interclonal differentiation. The first two principal components explain more than 80% of the total variation in parameters, with decisive contribution to data structure made by traits related to above-ground and root system development. Cluster analysis confirmed high within-clone homogeneity of seedlings and clear spatial separation of the clones with different growth potential.

The novelty of this study lies in the comprehensive combination of univariate biometric analysis with multivariate methods (PCA and clustering), enabling transition from assessment of individual traits to holistic characterization of seedling morphological structure. It has been demonstrated that the identified interclonal differences are genetically determined and manifest under identical growing conditions.

The practical outcome is the substantiated identification of a clone group (D21, D22, D20, D32, and D40) with high integrated growth and adaptive potential, recommended for further use in forest seed production and tree breeding.

Further research will focus on genetic evaluation of individual organs, particularly needles, of the most promising clones to analyze hereditary determination of growth and adaptive traits in depth, as well as their field performance, since

forest plantations have already been established using these seedlings and are subject to systematic monitoring of growth as well as stand condition.

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