



Original article

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Differentiation of Plus Trees of Scots Pine by Xylem Conditions

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Abstract. An essential stage in the establishment of seed orchards is the formation of an optimal composition, avoiding inbreeding depression in the produced seed material, which develops as a result of crossbreeding between closely related plus trees. The negative effect can be reduced by ensuring the genetic heterogeneity of the seeds. The purpose of this study is to give a relative evaluation of the plus trees of Scots pine (*Pinus sylvestris* L.) as one of the most economically significant tree species on the European continent according to their physiological conditions. The formation and lignification of the xylem of the plus trees were investigated in the Nizhny Novgorod region of Russia. A histochemical examination was performed using the reaction of phloroglucinol to lignin. Phenotypic differences in the physiological conditions of plus trees appeared on a uniform ecological background that indicates their genotypic determinism. This fact was confirmed by ANOVA. The share of the influence of differences between the proper plus trees was between 25.16±8.91 % and 53.98±5.48 %. According to the results of the factor and cluster analysis, the association of plus trees was generated by the similarity of the multidimensional estimates of the xylem conditions. The objects that were statistically most distant from the others were determined. The hereditary conditionality of the identified differences among plus trees allows us to consider their grouping into clusters as genotypically determined. This information provides a reasonable approach to the formation of an assortment of Scots pine clonal seed orchards.

Keywords: Scots pine, plus trees, clones, inbreeding depression, xylem, lignification, hereditary conditionality



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Научная статья

Дифференциация плюсовых деревьев сосны обыкновенной по состоянию ксилемы

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Аннотация. Важным моментом при создании лесосеменных плантаций является формирование их оптимального состава, позволяющего избежать проявлений инбредной депрессии получаемого на них семенного материала, которая возникает вследствие скрещивания между близкородственными плюсовыми деревьями. Этот негативный эффект можно снизить путем обеспечения наследственной гетерогенности материала для создания лесосеменных плантаций. Цель работы – дать сравнительную оценку плюсовых деревьев сосны обыкновенной (*Pinus sylvestris* L.) как одной из наиболее значимых в хозяйственном плане древесных пород на Европейском континенте по их физиологическому состоянию. Изучено образование и одревеснение ксилемы плюсовых деревьев, выделенных в Нижегородской области России. Проведено гистохимическое исследование с использованием реакции флороглюцина на лигнин. Фенотипические различия в физиологическом состоянии плюсовых деревьев проявились на выровненном экологическом фоне, что указывает на их генотипический детерминизм. Данный факт подтвержден дисперсионным анализом. Доля влияния различий между собственно плюсовыми деревьями составила от 25,16±8,91 до 53,98±5,48 %. По результатам факторного и кластерного анализа выполнено объединение плюсовых деревьев на основе сходства по всему комплексу оценок состояния ксилемы. Идентифицированы объекты, статисти-

чески наиболее удаленные от остальных в многомерном пространстве признаков. Наследственный характер различий, установленных между плюсовыми деревьями, позволяет рассматривать их группирование по кластерам как генотипически обусловленное. Результаты исследования дают возможность обоснованно подходить к формированию ассортимента клоновых лесосеменных плантаций сосны обыкновенной.

Ключевые слова: сосна обыкновенная, плюсовые деревья, клоны, инбредная депрессия, ксилема, одревеснение, наследственная обусловленность

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Introduction

The production of a balanced composition of the vegetative offspring of plus trees on clonal seed orchards (CSO) of the first order is one of the challenges in contemporary forest selection [8, 27, 31, 33, 48]. This problem is related in many ways to the risk of the occurrence of inbreeding depression in the seed progeny of plus trees in seed orchards [2, 27, 31]. According to some estimates, its effect can vary from 26 % to 65 % [18], while other studies have considered it as 28.6 % and 59.3 % [23]. Therefore, it is necessary to avoid closely related crossbreeding. They occur for 10–20 % of coniferous trees, more frequently in CSO than in natural plantings. Therefore, they are the primary source of inbreeding [26]. However, another informational source [35] indicates that the rate of self-pollination in CSO is 4 %. Thus, it is important to provide enough clones in seed-bearing plantations [22, 23]. Nevertheless, it is a fact that plus selection based on mass selection by phenotype of the best species of natural populations remains still the most effective strategy for the selective improvement of forests in many countries [8, 23, 58]. Although the issue of plus trees selection effectiveness has been discussed for a long time in literature, some tasks are still waiting for final solutions, requiring comprehensive answers to some questions arising from practice [23, 36, 37, 54–56].

It is currently widely accepted to use histochemical examination as a method to identify the biological characteristics and diversity of woody plants [4–6, 12, 29, 30]. Indicators of the plant physiological state determine the adaptability of the organism to the environment [44], the resistance to drought and xylem embolism [16], the success of xylogenesis [14, 45], the survival of the progeny [15], the biological potential for reproductive activity [29, 30], and regeneration ability [46]. The state and rate of the development of xylem in woody plants, the xylem cell formation and lignification processes, the ratio between the formation of early and late wood, as well as their hereditary conditionality are the subjects of systematic, comprehensive studies [16, 29, 30]. These aspects are of particular importance in relation to native tree species, which play an important role in the formation of forest resource potential. Among them is the Scots pine (*Pinus sylvestris* L.), which is a dominant species in forestry not only in Russia [7–9, 47], but also in many European countries, where it is constantly at the center of attention of the researchers [3, 4, 6, 10, 16, 44]. In the studies of Scots pine polymorphism, the solution to the problems associated with plus selection of this biological species is the establishment of seed orchards and

an increase in effectiveness. They are the subjects of numerous scientific studies conducted over a long period of time [26, 27, 36–38, 50, 51]. In this context, the solved scientific-theoretical and practical (economic) problem can be presented as overcoming the negative effect of inbreeding depression on the seed progeny of plus trees. Consequently, the purpose of our work is to conduct a comparative assessment of the plus trees of the Scots pine in relation to the formation and lignification of the xylem of annual shoots.

Object and methods

The object of the study was 21 plus trees of Scots pine, which were isolated from natural stands in the Nizhny Novgorod region of the Russian Federation using mass selection methods generally accepted in forestry breeding. Their vegetative progeny was cultivated in a clone archive (collection for *in situ* conservation) located in the same region. Formally, it is a part of the Semenovskiy Spetssemleskhoz State Budgetary Institution of the Nizhny Novgorod Region (a dedicated company producing forest seeds and planting material). Each plus tree (ortet) in the clonal archive was represented by 6–23 grafted seedlings (ramets). The total number was 320 pcs. They were arranged in rows on a rectangular pattern of 3×6 m. The location of the experimental site was 56°44'20" N and 44°20'59" E. This clone archive was formed in 1980 by planting 3-year-old seedlings generated through grafting of plus tree cuttings onto well-developed 2-year-old seedling rootstocks, which had been cultivated in an open ground forest nursery of the Semenovskiy Spetssemleskhoz. The trial plot was 1.6 ha. The properties of the soil, such as fertility, mechanical composition, and moisture conditions, in this plot were typical of forest areas in the Central Zone of the European part of Russia.

The studies were carried out through field stationery and laboratory methods, using such schemes as work organization and sampling construction, which allowed for the implementation of the principles of the only logical distinction, reliability, suitability, and expediency of experience. The elimination of the differentiating influence of environmental factors was achieved by comparisons of the studied objects and samples (plants and their shoots), which were carried out only within the boundaries of one experimental site, on which uniform schemes, with respect to seating and plant feeding areas, were maintained. A single regime for maintenance and cultivation was supported, and the uniformity of the initial planting material (the same type of three-year-old grafted saplings) used for the creation of the clone archive was provided. The same silvicultural and agrotechnical measures were carried out. As a result, the experience in each of the work blocks was organized under the conditions of the aligned ecological background. The rows of clones were placed in their archives in accordance with the applicable regulations regarding the creation of objects with a constant forest seed base and a single genetic breeding complex. In particular, a randomized placement of rows in the plot was provided. This eliminated the targeted provision of any of the progenies of plus trees, including benefits in terms of growth and the formation of the plants, their shoots, and in the end, their physiological characteristics. The elimination of time factor influence, causing chronographic (phenological and ontogenetic) variability of the analyzed characteristics of the studied plants, was achieved by the simultaneous harvesting of biological samples, simultaneous measurements, observation, and accounting

of the analyzed parameters, and comparison of only same-age trees and their parts. In practice, this condition was achieved by simultaneous planting and by using the same age of planting material in the creation of such objects. Thus, the minimization of the influence of background factors on the phenotypic manifestation of physiological differences between plants allowed us to explain the presence of such differences as being due to the action of the hereditary specificity of the studied objects in relation to the analyzed features. The correctness of the procurement of the initial material was provided by a preliminary audit of the assortment structure of the surveyed archives of clones, during which the identification of each registration tree (ramet) was carried out, and it was established that they each belonged to a clone of a concrete plus tree (ortet).

The primary unit of a sample in the histochemical experience was represented by temporary preparations of cross-sectional cuts from the average part of the annual growth, which were analyzed using a light microscope (Mikmed-2) after staining and fixation. The magnification was 10×20 . The lignification of cellulose in the xylem cell walls was assessed using the traditionally applied qualitative reactions of phloroglucinol to lignin: a 10 % solution of phloroglucinol in 95 % ethanol with a few drops of hydrochloric acid. As a sighting control, we used slices that were not affected by the test reagents. The color of their xylem cells corresponded to the absence of lignification [4, 32]. The number of formed cells in each accounting zone of the xylem was counted along not less than 3 radial files (transects). These slices were compared to the slices treated with appropriate reagents and placed on the following scale: 0 – no staining, no differences between the state of the painted slices and that of the control slices; 1 – weakly expressed pale pink coloration; 2 – well-fixed pale pink coloring; 3 – medium-intensity pink coloring; 4 – saturated pink coloration with an above-average intensity but not reaching the maximum; and 5 – maximum intensity bright crimson coloration [4, 32]. For each zone separately, we counted the number of rows of cells by transects in the radial direction, from the first row in the early xylem to the last visible row of the late xylem before the cambium. The degree of xylem lignification was expressed as a percentage of the total number of rows of its cells. The ratio of early and late xylem zones was estimated by the balance of the number of cell rows in each of them at the end of the vegetation period, when all cells of late xylem underwent lignification. To construct an integral assessment of the state of xylem, we considered indicators that determine the degree of its development and the level of lignification in the observed sight sector, covering all accounting zones from the core to the cambium [4, 32]:

the total number of rows of xylem cells formed at the time of accounting, which was determined by counting all rows of xylem cells in radial direct, regardless of the fact and degree of their lignification (feature 1);

the number of rows of completely lignified early xylem cells, which was determined by counting the rows of cells in the walls in which the process of lignification was fully completed, as confirmed by their uniform intense color, corresponding to an assessment of 5 points (feature 2);

the number of rows of absolutely non-lignified xylem cells, determined by counting only those cells in the walls in which the signs of lignification were not fixed and there was no staining (i.e., assessment of 0 points) (feature 3);

the number of rows of partially lignified xylem cells, determined by counting only those cells in which the process of wall lignification was fixed but not completely, as confirmed by their uniform color of different intensities from 1 to 4 points (feature 4);

the depth of the lignification of xylem cells, taking into account the coefficient of their lignification grading, determined as the sum of the products of the number of cell rows in a separate accounting of each zone of xylem, on their lignification score (from 0 to 5 points), carried to the maximum assessment (5 points). This was expressed as the number of rows of xylem cells, corrected for the degree of lignification (feature 5);

the absolute percentage of the lignification of xylem cells, which was determined as a percentage share of lignified xylem cells, in varying degrees (from 1 to 5 points), relative to their total number (feature 6);

the relative percentage of xylem cell lignification, determined as a percentage share of fully and partially lignified xylem cells, the number of which was adjusted to the degree of their lignification (feature 7);

the total sum of rows of the fully lignified (5 points) cells of early and late xylem (feature 8).

In order to obtain clear information concerning the importance of the actual analysis of the dynamics of plant physiological indicators and comparatively assess them, a static indicator fixed to the observation date was used. The sampling times were confined to the characteristic phenological plant states: the period of rest (the first period of accounting), the exit from the state of rest (the second period of accounting), the beginning of the formation of late xylem (the third period of accounting), the transition to the state of rest (the fourth period of accounting). In addition, we took into account the fundamental possibility of involving the number of analyzed parameters in multidimensional analysis in addition to the signs of direct accounting and the signs derived from them. The above signs were recorded at each seasonal accounting, at the same time, in January (the first accounting period), sign 8 did not appear since, by the indicated moment, the early xylem of the current year had not yet been formed. For this reason, only the most informative signs recorded in all phases of seasonal plant development are left in Table 1. This approach is traditional in the organization of forestry and biological research, including those conducted by methods of histochemical analysis [1–3, 7–11, 32].

The actual material obtained in the course of the studies was processed statistically using one-way ANOVA according to Snedekor's algorithm with a Tukey pairwise test. In addition, according to generally accepted algorithms, phenotypic means, standard deviation, and coefficients of variation for all studied features of each plus tree were calculated. Complex estimates of the relative genetic proximity or distance of the compared objects were obtained in the course of principal component analysis (PCA) [17] and cluster analysis [53]. These techniques are actively used in the study of tree species [52], including histochemical [3, 8, 32] and forestry studies [43, 52, 53], the estimate of primary roundwood production [28], the comparative assessment of plus trees [24, 25], including the clonal analysis of their reproductive parameters [7, 50], population genetics [59], and the determination of genetic proximity [26]. An obligatory condition of the experiment was accepted, according to which it was ensured that all plus trees had the same set of quantitative characteristics, the number of which determined the dimension of Euclidean space. Clones that did not have at least one of the characteristics or did not receive reliable estimates during the preliminary statistical processing were excluded from the scheme. The accepted order of the formation of numerical arrays

made it possible to calculate the correlation and covariance estimates of each of the characteristics with all other object features in a multidimensional comparison complex. Amendments to the unevenness of complexes were not entered since the accounting of all parameters of the multidimensional objects in the surveyed site had the same number. In the calculations, we included the standardized values of the initial parameters, obtained by the z-transformation and based on the ratio of the deviation of the characteristic value from its average value to the corresponding standard deviation. As a result, a matrix of the normalized values of the features was obtained, which served as the basis for calculating the generalized statistical distances in Euclidean space. This approach yielded uniform and dimensionless characteristics, allowing the comparison of the object coordinates in the multidimensional feature space. The cluster analysis method was an ascending hierarchical procedure. The square of the Euclidean distance was used as a metric, and the agglomeration was considered the average linkage between groups.

Results and discussion

An assessment of the physiological condition of the plus trees in the examined clone archive, considering a wide range of features describing the level of xylem development and the degree of cell lignification in the tissues of annual shoots, revealed a noticeable differentiation in the composition of the analyzed clone archive (Table 1). Since the identified phenotypic differences between the plus trees appeared in the unified environmental conditions, there is a reason to believe that the source of their occurrence is primarily genetic. Statistical confirmation was provided by analysis of variance, in which the actual value of Fisher's F-criteria for most features exceeded the minimum acceptable limit at 5 % and 1 % significant levels. Significant differences failed to be confirmed in only two cases for features 4 and 5. We validated the effect of the differences between the actual plus trees (ortets), which in such cases is considered the coefficient of heritability in its wide meaning, on signs concerning the essentiality of the differences. This effect ranged from 16.85 ± 9.90 % for feature 1 to 49.59 ± 6.00 % for feature 2, with a general background of phenotypic differences.

The statistical reliability of the obtained results and the presented evidence of the hereditary conditionality of the revealed phenotypic diversity of plus trees with respect to the physiological state of the xylem of their annual shoots created sufficient ground for conducting multivariate analyses. In the factor analysis (PCA), these characteristics were grouped according to the principal components. The results of this transformation are reflected in Fig. 1 and in Table 2.

Figure 1 displays the extraction of the principal components and indicates quite a satisfactory outcome of this variant of the transformation factor. The distribution of analyzed variables (values of investigated features) is clearly visible, systematic, and logical (Table 2). Note that features 4 and 5, as well as features 6 and 7, were always a part of the same components. In the formation of the principal components, feature 1 always participated in combination with feature 8, and feature 3 with features 6 and 7. At the same time, some specificity in the structure of the principal components was revealed on each of the observation dates. Note that the information on feature 2 in the first term of registration is not presented due to the absence of the number of rows of fully lignified cells of early xylem formed in the current year at the time of registration of the actual indicator. The general tendency in the procedure of extracting the principal components was, for all accounting periods, a convincing exclusion of insignificant values close to 0 (Fig. 1).

Table 1

Average values of indicators of the physiological status of plus trees

Plus trees, No	Feature of xylem state (M±m)						
	1	2	3	4	5	6	7
14	24.33±2.19	21.78±2.27	1.67±0.17	0.89±0.31	0.60±0.21	92.34±1.34	91.04±1.58
3	22.11±1.18	20.33±1.05	0.78±0.15	1.00±0.29	0.71±0.20	96.44±0.72	95.21±0.58
19	29.56±1.25	27.56±1.21	1.00±0.00	1.00±0.29	0.73±0.22	96.56±0.18	95.67±0.33
6	37.89±1.80	35.56±1.67	1.00±0.00	1.33±0.24	0.91±0.18	97.31±0.14	96.21±0.21
22	21.78±0.92	19.89±0.89	1.00±0.00	0.89±0.26	0.60±0.16	95.34±0.20	94.02±0.50
1	30.44±2.68	28.56±2.61	1.11±0.11	0.78±0.28	0.56±0.20	96.13±0.46	95.46±0.45
5	19.78±1.66	18.11±1.60	1.11±0.11	0.56±0.24	0.40±0.19	94.17±0.56	93.38±0.56
17	43.56±4.61	41.11±4.46	1.33±0.17	1.11±0.26	0.78±0.20	96.85±0.33	96.04±0.36
18	31.67±1.00	29.67±0.94	1.00±0.17	1.00±0.29	0.78±0.26	96.83±0.51	96.10±0.48
40	23.33±0.80	21.44±0.91	1.00±0.00	0.89±0.31	0.67±0.25	95.68±0.14	94.71±0.35
41	34.56±1.16	32.33±1.25	1.00±0.00	1.22±0.28	0.93±0.23	97.08±0.09	96.22±0.24
45	22.78±1.02	21.00±1.09	0.89±0.11	0.89±0.26	0.60±0.19	95.93±0.52	94.68±0.64
47	24.78±2.41	22.56±2.33	1.00±0.00	1.22±0.22	0.87±0.17	95.70±0.36	94.25±0.50
29	31.78±2.34	29.44±2.29	1.33±0.17	1.00±0.24	0.73±0.17	95.72±0.49	94.89±0.64
49	23.78±3.14	21.67±3.12	1.00±0.00	1.11±0.26	0.87±0.24	95.23±0.57	93.98±0.86
39	21.00±0.82	19.00±1.11	1.00±0.00	1.00±0.37	0.78±0.31	95.18±0.18	94.04±0.65
21	32.56±2.10	30.44±1.82	1.11±0.11	1.00±0.33	0.73±0.26	96.53±0.30	95.79±0.24
20	21.44±1.33	19.33±1.25	1.00±0.17	1.11±0.31	0.80±0.22	95.07±1.03	93.72±0.98
30	31.00±1.40	28.78±1.30	1.00±0.17	1.22±0.22	0.87±0.15	96.77±0.48	95.64±0.36
42	34.22±3.02	31.89±2.77	1.11±0.11	1.22±0.55	0.91±0.43	96.60±0.33	95.70±0.61
44	27.56±1.26	25.56±1.31	0.89±0.11	1.11±0.26	0.69±0.15	96.74±0.44	95.11±0.86
Total	28.09±0.62	26.00±0.60	1.06±0.03	1.03±0.06	0.74±0.05	95.91±0.14	94.85±0.16

Note: The most informative characteristics (features 1–7) are used. Abbreviations of the features are used as specified in the Methods section. M – the average of the feature; ± m – error of the representativeness of the sample mean.

Table 2

Structure of the principal components of xylem state of plus trees by the accounting periods

Signs and indicators	Principal components of the accounting period								
	the first period		the second period			the third period			
	first	second	first	second	third	first	second	third	
Feature 1	0.944	–	–	–	0.793	0.962	–	–	
Feature 2	–	–	–0.794	–	–	0.966	–	–	
Feature 3	–	–0.972	–	0.847	–	–	–	–0.931	
Feature 4	0.982	–	0.864	–	–	–	0.936	–	
Feature 5	0.989	–	0.962	–	–	–	0.939	–	
Feature 6	–	0.986	–	–0.838	–	–	–	0.763	
Feature 7	–	0.980	–	0.684	–	–	–	0.713	
Feature 8	0.980	–	–	–	0.721	0.966	–	–	
Initial eigenvalues of principal components									
Value	3.9214	2.9845	3.5994	1.6475	1.1740	5.0405	1.7811	1.0901	
The share of the variance of principal components, %									
Dispersion	56.020	42.635	44.992	20.594	14.675	63.007	22.264	13.627	
Cumulative variance of principal components, %									
Dispersion	98.655		80.261			98.897			

Note: The rotated components of factor analysis are presented herein.

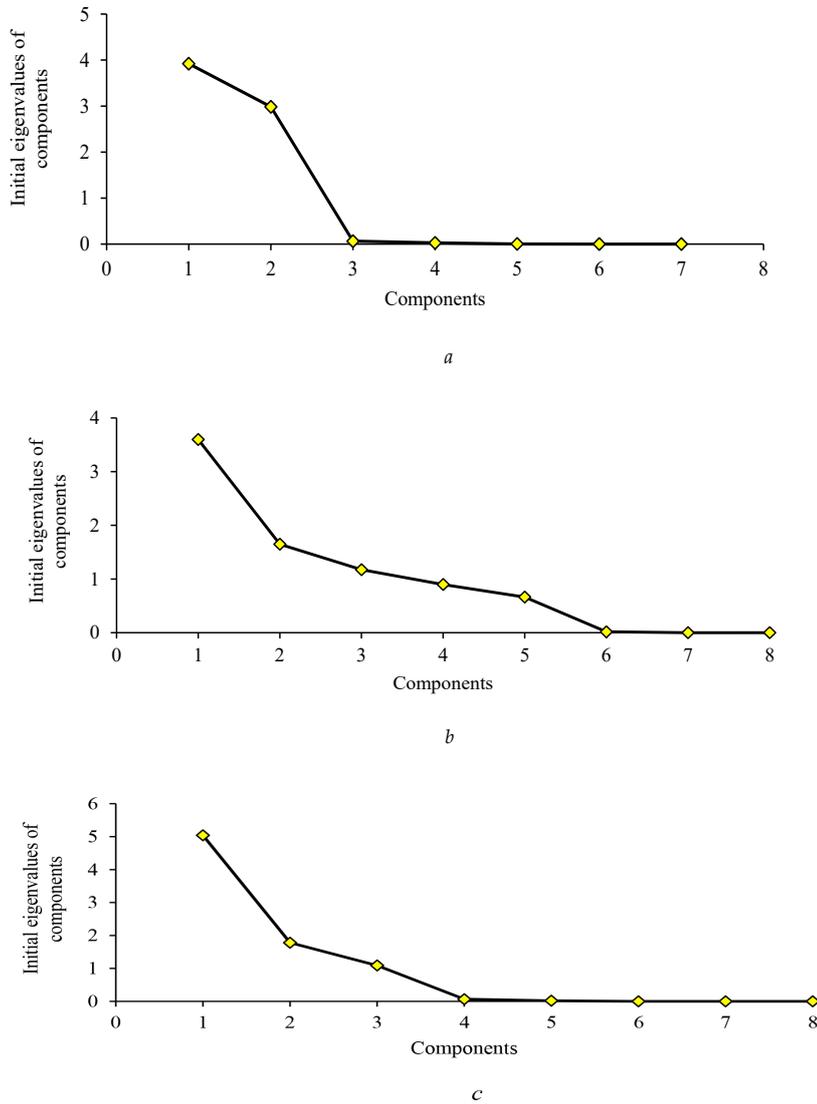


Fig. 1. Initial eigenvalues of the components with respect to the timeframes for measuring the plus tree xylem states: *a* – January; *b* – April; *c* – July

The sufficient stability and uniformity in the formation of the principal components in terms of timeframes for measuring the seasonal states of xylem during the year made it possible to perform a factor analysis (PCA) on a summary data set (Table 3, Fig. 2). It contains 23 features: 7 in the first accounting period and 8 in the second and third periods, respectively.

Figure 2 illustrates the allocation of six major components from the entire list of characteristics. The information provided in Table 3 demonstrates their structure, in which the distribution of the variables under consideration is determined, mostly seasonally, by the independent factors (principal components). The material attained at this stage of the work created an opportunity to perform a cluster analysis with the use of both normalized values of the original features (Table 4, Fig. 3) and the principal components derived on their basis (Table 5, Fig. 4).

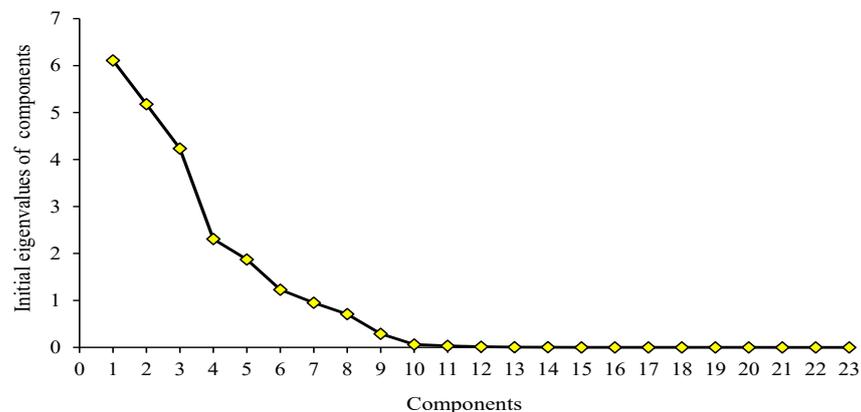


Fig. 2. Initial eigenvalues of the components for all accounting periods of the seasonal state of xylem in the shoots of plus trees

Table 3

Structure of the principal components for all accounting periods

Signs and indicators	Principal components of factor analysis on the seasonal state of xylem					
	first	second	third	fourth	fifth	sixth
Feature 1	–	0.9317	–	–	–	–
Feature 2	–	–	–	–0.9550	–	–
Feature 3	–	0.9736	–	–	–	–
Feature 4	–	0.9786	–	–	–	–
Feature 5	–	–	–	0.9641	–	–
Feature 6	–	–	–	0.9473	–	–
Feature 7	–	0.9738	–	–	–	–
Feature 8	–	–	–	–	0.6315	–
Feature 9	–	–	–0.7404	–	–	–
Feature 10	–	–	–0.7769	–	–	–
Feature 11	–	–	0.8779	–	–	–
Feature 12	–	–	0.7534	–	–	–
Feature 13	–	–	0.7449	–	–	–
Feature 14	–	–	–0.4339	–	–	–
Feature 15	0.9785	–	–	–	–	–
Feature 16	0.9733	–	–	–	–	–
Feature 17	0.9778	–	–	–	–	–
Feature 18	–	–	–	–	0.8805	–
Feature 19	–	–	–	–	–	0.7950
Feature 20	–	–	–	–	–	0.8085
Feature 21	0.7383	–	–	–	–	–
Feature 22	0.7954	–	–	–	–	–
Feature 23	0.9785	–	–	–	–	–
Initial eigenvalues of principal components						
Value	6.1104	5.1788	4.2336	2.3068	1.8718	1.2290
The share of the variance of principal components, %						
Dispersion	26.567	22.516	18.407	10.029	8.138	5.343
Cumulative variance of principal components, %						
Dispersion	91.001					

Note: The rotated components of factor analysis are presented herein.

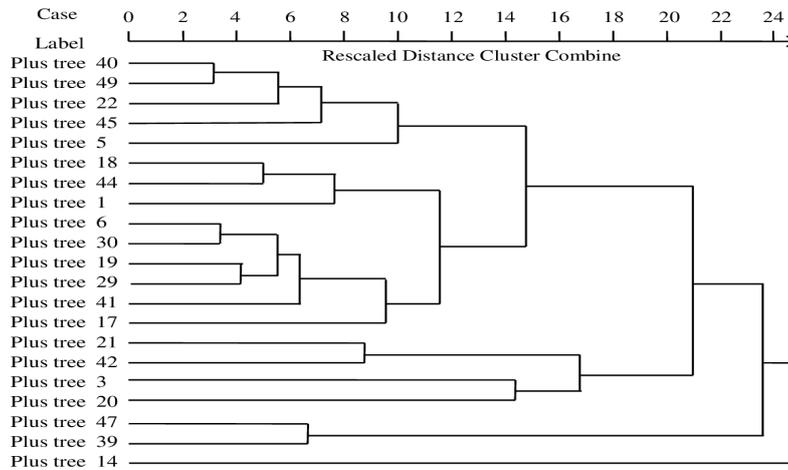


Fig. 3. Dendrogram of the similarities across plus trees based on the normalized values of the source characteristics of the physiological state of xylem

The clustering procedure was accomplished by constructing a hierarchical system, in which the plus trees were grouped according to the criterion of closest similarity for the analyzed feature combinations of the physiological state of xylem (Table 4, Fig. 3). This structure contains well-formed macroclusters with plus trees numbered 40, 49, 22, 45, 5, 18, 44, 1, 6, 30, 19, 29, 41 and 17. It has noticeable differences from the others in terms of the connection distances. Its own structure is represented by three micro-clusters of roughly the same additional density but with different numbers.

The dendrogram created with the principal components of the considered set of features (Table 5, Fig. 4) also had a discrete hierarchical composition. Meanwhile, while maintaining a certain specificity in each of the analysis options, the structures of these dendrograms were fundamentally similar. Note that the plus trees marked by the indices 40, 49, 22, 45, and 5, located on the first dendrogram (composed using the normalized values of 23 initial features) near each other, preserve the similarity of positions on the second dendrogram (created by 6 principal components). On both the first and second dendrograms, separate pairs of plus trees were detected, such as 18 and 44, 6 and 30, 47 and 39. In this case, in each of the variants of the analysis, plus tree 47 and plus tree 39 were among those that had the largest values with respect to the distance of the hierarchical cluster combination. The most specific object, the plus tree 14, remained in the same position on each of the dendrograms.

The resulting material revealed a fundamental similarity in the graphical representations of the structure with the relationship between the plus trees in the multidimensional space of features. This indicates that the observed connection related xylem state among the plus trees has a stable character.

The presence of plus trees, which have characteristics that largely distinguish them from others, indicates that these plus trees are potentially unrelated to the primary composition of the provided archive of clones.

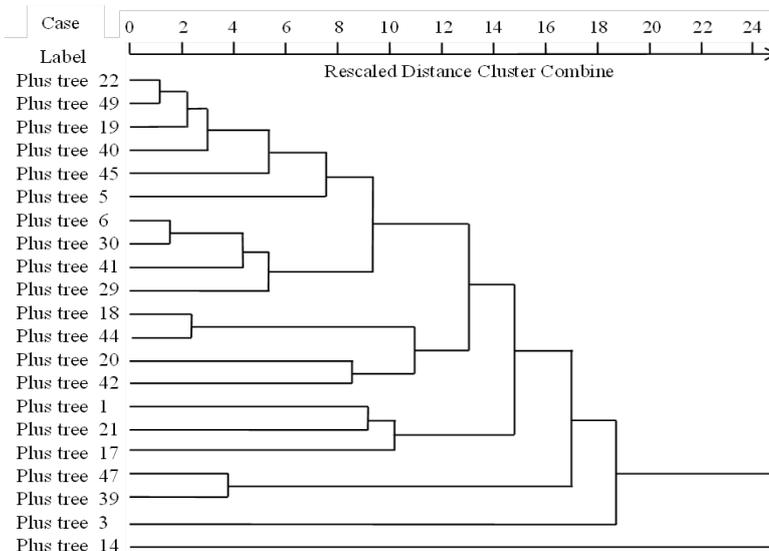


Fig. 4. Dendrogram of the similarities across plus trees based on of the principal components of physiological evaluation of the seasonal state of xylem

As the points for discussion. The results of this study provide an opportunity to test the potential genetic proximity of plus trees in practice. The tests are planned to be included in CSO. Objective information might reduce, and even eliminate completely, the risk of inbreeding depression of seed offspring. The requirement for a preliminary assessment of the reproductive compatibility of plus trees in forest seed plantations was noted by many experts and is reflected in the works of A. Lewandowski [34], D. Lindgren [36, 37], A. Sivacioglu and S. Ayan [50], T. Funda [22], O. Vanek [54], C. E. Durel [18, 19], H.P. Koelewijn [27], T. Wang [57], J.H. Russell and D.C. Ferguson [48], A.D. Bower and S.N. Aitken [13]. They emphasized the importance of generating a range of CSO at which the risk of inbreeding is minimized or eliminated entirely. Even though this problem was clear from the beginning of seed orchard development [33, 41, 42, 57], unfortunately, it is still relevant [2, 21, 23, 31]. Currently, the affords to resolve this issue are made quite widely in many countries of the world: in Poland [38], Portugal [20], Canada [51], New Zealand [1], Denmark [40], France [55], Japan [49], Finland [27], Spain [20], China [39], USA [21].

The main results of the investigation are the following:

the fact of heterogeneity was determined, and phenotypic differences for pine trees were revealed by the physiological characteristics (parameters) of the xylem state;

the hereditary dependence of the discovered physiological heterogeneity of the plus trees was proved, and the degree of genotypic conditionality of the specificity of the plus trees was determined by their ability (potential) to form a well-developed xylem and obtain a high level of its lignification;

a multiparametric assessment of the relative proximity of the studied plus trees according to the tested complex of physiological characteristics was obtained, and the most similar plus trees and those with complex differences, between which the greatest values were found;

an assessment of potential risks was given when the most similar physiological characteristics of plus trees were included in the composition of seed plantations, the

most preferred of the possible combinations of plus trees in the assortment of seed plantations were identified, ensuring minimization of the risk of inbred depression of their seed progeny.

Potential application of results – object and territorial aspects:

the results, the conclusions and the recommendations derived from them can be extended to other species of pine trees and other representatives of the pine family;

the presented methodological scheme can be applied to studies of a wide range of species of woody plants that belong to the pine family;

the tested methodical apparatus, the results that we obtained during its implementation, generalizations and conclusions based on them can be geographically extended to other regions.

Conclusions

The plus trees of Scots pine are heterogeneous with respect to the physiological state of xylem. To a large extent, their specific abilities to form xylem layers with diverse thicknesses during one vegetation period are determined by genotype. The rate of annual desiccation of shoot xylem cells (lignification) is also influenced by heredity. The carriers of these features are most favored in the composition of seed orchards. In composing clone archives, the ability to select a group of plus trees (and particular plus trees) that are the most distinct compared to others in physiological characteristics is essential. The hereditary nature of the features of such plus trees suggests that they have a minimum chance of being closely related. The use of this approach in the creation of seed orchards can reduce the risk of inbreeding depression in seed offspring and minimize its harmful consequences. The proposed method of comparative evaluation of the degree of genetic proximity of plus trees on the physiological parameters of xylem can be considered as one of the tools for testing their reproductive compatibility in the composition of clonal seed orchards.

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