

## The growth rate and genetic variability of Scots pine (*Pinus sylvestris* L.) half-sibs in test crops of Northern Kazakhstan

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### Abstract

Scots pine (*Pinus sylvestris* L.) is a valuable tree species, playing an important role in maintaining the ecological and economic balance not only in Kazakhstan, but also in many countries around the world. The purpose of this study was to assess the growth rate and to identify the genotypes of the half-sib progeny of Scotch pine plus trees in order to further obtain high-quality seeds with valuable genetic traits. A molecular genetic analysis was carried out using six microsatellite and 20 isoenzyme loci on 15 Scots pine half-sib families, represented by two samples of seeds collected in the subsequent harvesting periods (planted in 1986 and 1987), that evaluated the taxonomic characteristics such as tree height and trunk diameter (DBH, measured at 1.3 meters). Families with the highest growth rate in both samples were selected as candidates for the elite group. During the SSR analysis, a decrease in the level of expected heterozygosity (0.2377-0.5362 with an average value of 0.4593) was observed in *P. sylvestris* L. half-sibs compared to the sample of maternal plus trees (0.5444), with similar results to that of the “founder effect”. At the same time, the level of observed heterozygosity remained sufficiently high (0.3333-0.7037 with an average value of 0.4980 and 0.4556, respectively). During the isoenzyme analysis, the level of genetic variability either decreased or was similar to that of the maternal trees. Rare and unique alleles were found in seed samples of the analysed tree families. In general, this research showed that a comprehensive evaluation combining both traditional breeding methods (genetic assessment of progeny in the course of growth) and molecular genetic analysis is required for the selection of elite trees.

**Keywords:** genetic diversity; growth rate; isoenzymes; microsatellites; *Pinus sylvestris* L.

### Introduction

Scots pine (*Pinus sylvestris* L.) covers the largest area of its species in Eurasia and occupies a vast territory, extending from the European to the Asian part of the continent (Przybylski *et al.*, 2015). By playing an important role in maintaining ecological and economic balance, Scots pine has been the focus of forestry science for a long time. Interest in this species is due to its high genetic potential, genetic variability and ecological

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plasticity. Although Scots pine (*Pinus silvestris* L.) is one of the main forest-forming tree genera in Kazakhstan, it occupies an insignificant area that covers only 6.5% of the forested land (Pojasnitelnaja zapiska ..., 2021). This tree species grows mainly in the Northern and Eastern parts of the country. According to previous studies, in Kazakhstan, isolated areas of Scots pine have been identified in elongated, ribbon-like pine forests of Turgai region, isolated island forests of the Kazakh Upland, ribbon forests of Irtysh and pine granite hills of Karkaraly lowlands (Shulga, 1974). According to Gorchakovskiy (1987), the pine forests of Kazakhstan formed during the Pleistocene Epoch. Once they constituted a single forest massif that stretched from the Ural to the Altai.

The plus trees are genetic and selected objects of conservation and have been added to the Forest Legislation Code of the Republic of Kazakhstan. Such trees are entered in the state registry and are subject to protection. Plus trees are selected based on the following criteria: in comparison to the trees in close proximity, plus trees exceed the average values in height by 10% and 30% in diameter, have a trunk that has a high zone without branches and have been resistant to insects and diseases. In order to evaluate the hereditary properties of plus trees test cultures are created by producing seedlings through germinating seeds and by the process of vegetative propagation - a form of asexual reproduction. In the created test cultures, phenotypic and genotypic traits are being observed and based on the findings, valuable genotypes are selected (Egorov, 2002; Rosvall *et al.*, 2003; Kowalczyk, 2005; Bessega *et al.*, 2015; Calleja-Rodriguez *et al.*, 2019; Ishibashi *et al.*, 2021; Raevskiy *et al.*, 2022). One of the main hurdles of research is to determine the optimal age of test crops, upon reaching which it becomes possible to accurately assess the properties of fertile offspring and to predict its further growth. Experts express their opinions differently on this topic. For short-term assessment the age ranges from 5 to 15 years (Hanaoka and Kato, 2022), and for long-term between 15 to 30-40 years (Haapanen *et al.*, 2015).

The biological basis for the improvement of forest plants by breeding methods is the intraspecific diversity of forest species (White *et al.*, 2007). Morphological characteristics for Scots pine include the color and size of seeds, cones, male strobilus, bark fracturing, branching type and crown habitus (Krakau *et al.*, 2013). In general, phenotypic characteristics are considered to be the main elements for identifying differences / similarities in anatomical form or structure within the same species or genus (Rédei *et al.*, 2018). For example, the differences in tree height and the quality of timber can depend on the local environmental conditions (Leeuwen *et al.*, 2011). Since phenotypic markers are influenced by the environment, they are less useful for characterizing neutral genetic diversity (Lande and Thompson, 1990; Volk *et al.*, 2004; Cholestova and Knotova, 2012; Ganopoulos *et al.*, 2015; Sun *et al.*, 2015). A lengthy reproduction phase and an overall lengthy period of ontogenesis of tree species, complicates the process of selecting the most valuable genotypes (Isik, 2014). One of the ways to study the genome of trees is sequencing. Research in this direction can increase knowledge in the field of plasticity and adaptation of trees to the natural conditions, their phenotypic variability and the genetic structure of populations (Prunier *et al.*, 2016). However, sequencing is a laborious and expensive research method for trees. Therefore, available methods such as SSR molecular marker technologies have become widely used to study the genetic structure. The use of genetic markers allows the selection of valuable populations because they are not affected by the environment and can be detected at any stage during ontogenesis of the species (Stinchcombe *et al.*, 2002).

Nuclear markers, primarily microsatellite markers, should be used to study the genetic resources of coniferous species. The reason why is, firstly, it is because they have bisexual inheritance; secondly, in the genomes of conifers, such markers are numerous, codominant, variable and located in all parts of the genome; and thirdly, microsatellites allow detecting the highest level of heterozygosity, and are the most informative tool for studying the variability of living organisms today. Thus, nuclear microsatellite markers are widely used to assess biodiversity and differentiate populations of different pine species, certification of seed plantations and test crops. Currently, SSR (simple sequence repeats) microsatellites are considered highly polymorphic DNA markers for studying genetic variability of forest trees. Microsatellite loci belong to the most variable regions of the genome, where polymorphism is mainly determined by the difference in the number of repeats

of the main nucleotide motif (Rakoczy-Trojanowska and Bolibok, 2004; Kalia *et al.*, 2011; Senan *et al.*, 2014; Taheri *et al.*, 2018; Dar *et al.*, 2019).

The purpose of this study is to analyse the growth rates and to identify genotypes of the half-sib progeny of Scots pine plus trees with the use of molecular markers to be able to obtain high-quality viable seeds with the most valuable genetic traits.

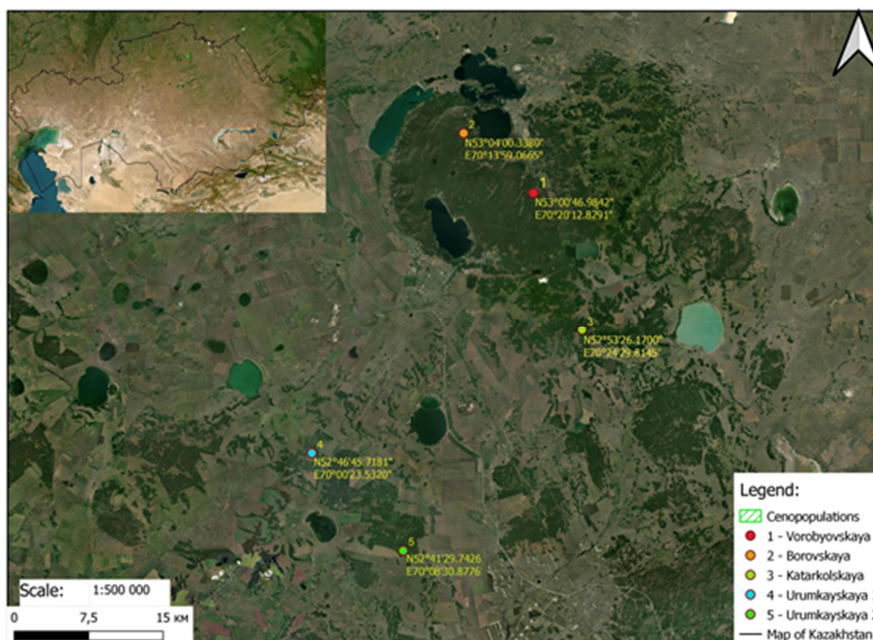
## Materials and Methods

### *Object of study*

This research was conducted in the Akmola region of Northern Kazakhstan on two test sites that contained two different tree samples – 37 and 38-year-old half-sibs Scots pine plus trees. The half-sib seeds had originated from the same mother but from different father trees due to cross-pollination. Two-year-old seedlings harvested in 1984 and 1985 were planted on test sites in 1986 and 1987 respectively. Seedlings of half-sibs were planted with an open root system by hand under the sword of Kolesov. Plant placement was 2×2 m and the experimental plot size was 16×16 m (256 m<sup>2</sup>). For both sites each of the 15 families had 3 plots with 64 seedlings planted in each plot. During the research, no less than 20 trees were used out of each plot, triplicating the number of test samples for each of the 15 families taken from both sites. The placement of experimental plots on test sites was random. The offspring of regular trees harvested through cross-pollination served as the control group. Control plots were placed after every 10 test plots. The mother trees of these families were also included during the molecular genetic analysis.

Pine half-sibs were planted in test areas with the same conditions as the mother trees. The microrelief is a slight elevation with micro dips. The soils are ordinary chernozems. The depth of groundwater is 5-7 m. The depth of effervescence is observed at the level of 160 cm in the alluvial horizon. The content of humus in the soil in the arable horizon ranges from 3.03-3.69%, in chernozems – 10.69%. Soils are not saline. The absorption capacity is 36-45 meq/100 g of soil. The levels of mobile forms of nitrogen in soil is high (content up to 60 mg/1000 g), phosphorus is low (10 mg/100 g), and for potassium it is moderate to high (15-18 mg/100 g).

To study the growth rates and genotypes of Scots pine half-sibs, the progeny of 15 plus trees with stable growth throughout the entire period of ontogenesis were selected. Mother trees originated from five coenopopulations: Borovskaya – plus trees No. 6, 17, 45; Vorobyovskaya – plus trees No. 25, 27, 28, 30, Katarkolskaya – plus trees No. 22, 44, Urumkayskaya I – plus trees No. 34, 36, 38, 71 and Urumkayskaya II – plus trees No. 48, 53 (Figure 1). Table S1 shows the silvicultural and taxonomic characteristics of parent plus trees.



**Figure 1.** Location of Scots pine coenopopulations, where maternal plus trees of half-sibs families were selected (Copyright © 2007 Free Software Foundation, <http://sasgis.org>)

The age of Scots pine half-sibs meets the criteria established by the regulatory documents. According to them, the preliminary genetic assessment of plus plantations or Scots pine plus trees on the territory of the state forest fund of Kazakhstan is carried out upon reaching the age of 21-40 years by their seed offspring (half-sibs), and the final one, upon reaching 60-80 years old (Prikaz ..., 2011; Prikaz ..., 2013)

#### *Growth measurement*

For analysing the growth rate and determining the genetic differences between the families of half-sibs, and for further selection of promising samples of offspring of plus trees on the sample plots, measurements of the main taxonomic indicators were carried out. The total height was measured using the Haglöf electronic altimeter (Haglöf Sweden®, Sweden) with an accuracy of  $\pm 0.2$ . The trunk diameter (DBH - diameter at breast height) was measured at the height of 1.3 m using the Haglöf measuring fork (Haglöf Sweden®, Sweden) with an accuracy of 1 mm.

#### *DNA isolation and microsatellite analysis (SSR)*

Plant material was taken from trees in the form of needles, which were placed into Eppendorf tubes, sealed and then transported to a laboratory at 4-8 °C, where it was stored at -20 °C until DNA was isolated. The collected plant material weighed 20 mg. Total genomic DNA was isolated by the STAB method (Doyle and Doyle, 1990). Spectrofotometer TM NanoDrop 2000 (Thermo scientific, USA) and electrophoresis in 2% agarose gel were used to determine DNA concentration and quality. All samples were diluted to 20-30 ng/ml with deionized water and stored at -20 °C.

A molecular genetic analysis was performed using six polymorphic microsatellite markers (Auckland *et al.*, 2002; Sebastiani *et al.*, 2012). The sequences of the primers used are shown in Table 1. Direct (Forward) primers at the 5'-end were labelled with a set of fluorochrome dyes: HEX, FAM, TAMRA (Table 1). Each of the six microsatellite loci was amplified in an independent multiplex reaction. Then the amplification products for each individual tree were mixed in one sample.

**Table 1.** Characteristics of amplified microsatellite loci of nuclear DNA *P. sylvestris* L.

Locus	Recurring motif	Primer sequence 5' → 3'	Range allele size, b.p.
Psyl2 (HEX)	(GCT) <sub>5</sub>	F: TTGCTTTTGCAGAACATTTCG R: GTCCTGCAGGCAATCAAAAT	199–211
Psyl17 (HEX)	(TA) <sub>7</sub>	F: TGGTCTGCAAATCAATCGAA R: GGGTAGGAATGCAAGTTAGGC	219–251
Psyl36 (HEX)	(GTC) <sub>7</sub>	F: TATCATCGAGAGCCCCAAAA R: GAAAGGCGAAAAGCAAAAGTG	245–257
Psyl42 (FAM)	(TC) <sub>9</sub>	F: CAACTTCAGCCTTGCAACAA R: CGACTTCATTTGGAACACCA	171–179
PtTx4001 (TAMRA)	(CA) <sub>15</sub>	F: CTATTTGAGTTAAGAAGGGAGTC R: CTGTGGGTAGCATCATC	197–231
PtTx4011 (FAM)	(CA) <sub>20</sub>	F: GGTAACATTGGGAAAACACTCA R: TTAACCATCTATGCCAATCACTT	230–284

The SSR (Simple Sequence Repeats) method was used for genetic certification and evaluation of the genetic diversity of half-sibs families, as well as the maternal plus trees. PCR reactions were carried out in 25 microns of the reaction mixture. Each reaction mixture contained 20 ng matrix DNA, 10×PCR buffer with 2.5 mM MgCl<sub>2</sub>, 10 mM dNTP, 10 mM each primer, and 2 units μl of DNA polymerase Taq («Primtech», Minsk, Belarus). The amount of reaction mixture was brought to 25 μl PCR-water. DNA amplification was performed in the thermal cycler Genemax-Tc-S/96 (Bioer Technology, China) according to the standard SSR-method program: pre-denaturation at 94 °C for 4 min.; 35 cycles at 94 °C for 30 sec.; 55 °C for 30 sec.; 72 °C for 40 sec. The last cycle of elongation lasted 5 minutes at 72 °C. Genotyping by capillary electrophoresis of all DNA loci was performed using the ABI Prism 3500 Genetic Analyzer (Applied Biosystems, USA). Using the standard GeneScan500 LIZ molecular mass marker (Applied Biosystems, USA), allele lengths were estimated using Gene Mapper (Applied Biosystems version 4.0, USA).

#### *Isoenzyme analysis*

After removing the integumentary scales, diploid tissues of vegetative buds were used as an experimental material. Data was collected on enzyme mobility variants at 20 loci encoding 11 gene-enzyme systems, using horizontal starch gel electrophoresis. Homogenization and isolation of enzymes were carried out in an extracting buffer with the following composition: sucrose, 0.81 g; EDTA – 1.5 mg; dithiothreitol – 1.6 mg; ascorbic acid – 1.76 mg; bovine serum albumin – 11.0 mg; NAD - 2.7 mg; NADP – 2.2 mg; pyridoxal – 0.5 mg; β-mercaptoethanol – 0.066 ml; triton X-100 – 0.25 ml; PVP – 0.8 g per 10 ml of distilled water, pH is adjusted to 6.7 with 1 M tris-(hydroxymethyl)-aminomethane solution. Electrophoretic fractionation of isoenzymes was carried out in a 12% starch gel using three buffer systems (tris-EDTA-borate, pH 8.6; tris-citrate, pH 6.2; tris-citrate/tris-HCl, pH 6.2/8.0) (Goncharenko *et al.*, 1989) with minor modifications. Histochemical staining of enzymes was performed according to standard methods described in a number of manuals (Conkle *et al.*, 1982; Cheliak and Pitel, 1984; Goncharenko *et al.*, 1989). The following enzymes were studied: aspartateaminotransferase (AAT; E.C. 2.6.1.1, three loci), malatedehydrogenase (MDH; E.C. No. 1.1.1.37, three loci), alcoholdehydrogenase (ADH; E.C. No. 1.1.1.1, two loci), diaphorase (DIA; E.C. No. 1.6.4.3, two loci), leucineaminopeptidase (LAP; E.C. No. 3.4.11.1, two loci), 6-phosphogluconatedehydrogenase (6-PGD; E.C. No. 1.1.1.44, two loci), phosphoglucoisomerase (PGM; E.C. No. 5.3.1.9, 2 loci), fluorescent esterase (FLE; E.C. No. 3.1.1.2, one locus), glutamate dehydrogenase (GDH; E.C. No. 1.4.1.2, one locus), phosphoglucoisomerase (GPI; E.C. No. 5.3.1.9, one locus), isocitrate dehydrogenase (IDH; E.C. No. 1.1.1.42, one locus). Gels were scanned and evaluated using ImageMaster®VDS software (Pharmacia Biotech, USA).

*Statistical processing*

When analysing the growth of half-sib families, the main statistical indicators were calculated, and the analysis of variance (ANOVA) was applied. To assess the significance of differences in height and diameter between the progeny of plus trees and the control group, a comparative analysis of Student's t-test was carried out. Values of  $P < 0.05$  were considered statistically significant. The entire volume of the collected material was subjected to statistical processing using computer programs MS Excel 2021, Statistica 10.

The level of genetic variability was evaluated using commonly accepted population indicators: the proportion of polymorphic loci (P), the average number of alleles per locus (A), and the average number of non-rare (with a frequency of over 1%) alleles per locus ( $A_{1\%}$ ), observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity. Mathematical processing of the obtained data and construction of a dendrogram were carried out using computer programs BIOSYS-1 (Swofford and Selander, 1981), POPGENE Version 1.32 (Yeh *et al.*, 1999) и GenAlEx 6.501 (Peakall and Smouse, 2012).

**Results**

It is assumed that the height and diameter of the stem at 1.3 m is influenced by the genotype. After conducting the analysis of variance (ANOVA), the results revealed significant differences in tree height and trunk diameter between the 15 families on the first test site. The same differences were identified for the 15 families on the second site (Table 2).

**Table 2.** Analysis of variance (ANOVA) of the dependence of indicators on the individual characteristics of half-sib plus trees

Indicator	Source of variation	Sum of squares SS	Number of degrees of freedom df	Mean square MS	F-Test	P-Value
Test Site 1 (Planted in 1986) (F critical: 1.6796)						
Height	Between groups	486.499	15	32.433	18.421	4.33E-42
	Within groups	1,338.143	760	1.761		
	Total	1,824.642	775			
Diameter	Between groups	1,291.769	15	86.118	6.644	1.4639E-13
	Within groups	9,850.937	760	12.962		
	Total	11,142.71	775			
Test Site 2 (Planted in 1987) (F critical: 1.6802)						
Height	Between groups	801.329	15	53.422	32.392	1.07E-70
	Within groups	1,199.01	727	1.649		
	Total	2,000.339	742			
Diameter	Between groups	774.956	15	51.664	4.155	2.22E-07
	Within groups	9,040.648	727	12.436		
	Total	9,815.604	742			

According to the ANOVA data, the effect of the half-sib genotype on the studied parameters was significant. The analysis found that the height of half-sibs is determined by genotype by 26.66% and 40.06%, and the stem diameter depends on the origin by 11.59% for the first test site and 7.90% for the second respectively.

Further analysis of the indicators found that the average value of all heights of the 15 families on both test sites in the study was by 2.02-2.49% higher than in the control group, and the diameter indicators were by

0.65% and 3.44% lower than the control values. What follows from this is that in the progeny of plus trees, height indicators have an advantage over control compared to stem diameter.

At this stage, it is important to identify certain families that have the best performance and outperform the control group. The results of the comparative analysis are presented in Table 3.

**Table 3.** The differences in height and in diameter in test groups and in the control group presented in %

Family number	Test site 1		Test site 2	
	$\bar{X} \pm m_x$	%	$\bar{X} \pm m_x$	%
Height (m)				
Control	16.48 ± 0.27	100	16.37 ± 0.24	100
22	19.72 ± 0.30*	119.66	21.11 ± 0.26*	128.96
38	18.17 ± 0.24*	110.25	17.03 ± 0.18*	104.03
36	17.57 ± 0.29*	106.61	17.12 ± 0.17*	104.58
45	17.33 ± 0.15*	105.16	17.01 ± 0.17*	103.91
44	17.40 ± 0.24*	105.58	16.29 ± 0.17	99.52
34	17.15 ± 0.12*	104.07	16.88 ± 0.23	103.16
48	16.96 ± 0.18	102.89	16.08 ± 0.20	98.24
53	16.88 ± 0.13	102.46	17.15 ± 0.13*	104.76
17	16.70 ± 0.22	101.34	16.16 ± 0.18	98.76
30	16.21 ± 0.19	98.35	16.47 ± 0.14	100.65
6	16.20 ± 0.16	98.29	16.81 ± 0.14	102.72
25	16.16 ± 0.15	98.08	16.42 ± 0.18	100.34
27	16.13 ± 0.24	97.88	15.68 ± 0.19*	95.78
71	16.03 ± 0.18	97.27	16.81 ± 0.18	102.71
28	15.49 ± 0.25*	93.99	15.50 ± 0.24*	94.69
Stem diameter-DBH (1.3 m), (cm)				
Control	20.62 ± 0.70	100	20.15 ± 0.64	100
45	22.34 ± 0.46*	108.34	21.13 ± 0.53	104.86
25	21.78 ± 0.53	105.62	19.88 ± 0.47	98.64
30	21.64 ± 0.54	104.96	21.86 ± 0.44*	108.49
17	21.43 ± 0.89	103.93	19.06 ± 0.43	94.58
28	21.30 ± 0.66	103.29	19.59 ± 0.59	97.24
38	19.83 ± 0.56	96.19	19.07 ± 0.40	94.63
36	19.81 ± 0.56	96.10	20.16 ± 0.66	100.05
34	19.77 ± 0.31	95.87	21.05 ± 0.58	104.46
53	19.63 ± 0.37	95.22	19.50 ± 0.41	96.79
27	19.34 ± 0.76	93.78	19.30 ± 0.46	95.80
48	18.80 ± 0.52*	91.17	17.80 ± 0.57*	88.34
71	18.71 ± 0.50*	90.74	19.51 ± 0.48	96.81
6	18.39 ± 0.49*	89.19	21.28 ± 0.47	105.62
44	18.34 ± 0.43*	88.94	20.46 ± 0.54	101.56
22	17.90 ± 0.65*	86.81	18.72 ± 0.50	92.88

\* – significant differences with control, at  $P < 0.05$

According to the results of the analysis, the only family No. 45 showed a noticeable difference over the control group values. The differences in height were observed on both test sites and diameter on one of the test sites only. Of all families, No. 22 had the tallest trees that exceeded the control values significantly. The values of the trunk diameter of this family, however, did not exceed the control group values, on the contrary, they were below the group averages.

*Microsatellite analysis*

Analysis of the allelic diversity of half-sibs of the 1st and 2nd test sites revealed 35 and 38 alleles, respectively. The trees in the 1st test site had 15 rare and unique alleles in their microsatellite loci (Pysl42<sup>168</sup>, Pysl42<sup>174</sup>, Pysl42<sup>177</sup>, Pysl2<sup>196</sup>, PtTx4001<sup>207</sup>, PtTx4001<sup>217</sup>, PtTx4001<sup>223</sup>, Pysl17<sup>211</sup>, Pysl17<sup>215</sup>, Pysl17<sup>221</sup>, Pysl36<sup>244</sup>, Pysl36<sup>245</sup>, Pysl36<sup>249</sup>, PtTx4011<sup>266</sup>, PtTx4011<sup>281</sup>). Fourteen rare and unique alleles were identified in the microsatellite loci 2nd test sites (Pysl42<sup>168</sup>, Pysl42<sup>174</sup>, Pysl42<sup>177</sup>, PtTx4001<sup>203</sup>, PtTx4001<sup>207</sup>, PtTx4001<sup>216</sup>, PtTx4001<sup>217</sup>, Pysl17<sup>211</sup>, Pysl17<sup>223</sup>, Pysl36<sup>244</sup>, Pysl36<sup>257</sup>, PtTx4011<sup>264</sup>, PtTx4011<sup>266</sup>, PtTx4011<sup>280</sup>). Rare and unique alleles were found in families No. 48, 22, 71, 45, 25, 44, 36, 30, 6, 17. The level of microsatellite loci variability was 100%.

An assessment of the level of genetic variability (Table 4) of Scots pine half-sibs of the 1st test site showed the following: the value of the average number of alleles per locus ( $n_a$ ) varies from 1.6667 (family No. 27) to 3.500 (family No. 45). The smallest value of the effective number of alleles ( $n_e$ ) was found in the family No. 17, the largest No. 45. The average value of the Shannon index ( $I$ ), which reflects the complexity of the sample structure according to the quantitative representation of objects in the population (varies from 0 to 5), and calculated for all 6 SSR loci, is  $0.8275 \pm 0.1651$  for half-sibs of the 1st test site.

**Table 4.** Values of genetic variability parameters in half-sib families of the 1st and 2nd test sites of Scots pine (microsatellite analysis)

Family	Test site									
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
	$n_a$		$n_e$		$I$		$H_e$		$H_o$	
38	2.0000	2.5000	1.5971	1.6495	0.4695	0.5390	0.3268	0.3179	0.5556	0.4359
36	2.1667	1.6667	1.5713	1.4369	0.4671	0.3398	0.3053	0.2377	0.4833	0.3833
53	1.8333	1.5000	1.4274	1.4118	0.3946	0.3144	0.2857	0.2444	0.3333	0.4000
48	2.6667	2.6667	1.4947	1.8454	0.4610	0.6764	0.2769	0.4153	0.4386	0.5222
44	2.1667	3.3333	1.6098	1.8734	0.5231	0.7930	0.3508	0.4760	0.5583	0.5000
30	2.1667	2.0000	1.8063	1.7429	0.6189	0.5793	0.4470	0.4248	0.6944	0.7037
28	2.5000	1.8333	1.9603	1.5168	0.7081	0.3920	0.4607	0.2694	0.5556	0.3958
71	3.0000	2.6667	2.0952	1.8565	0.8360	0.6960	0.5362	0.4444	0.6250	0.5741
27	1.6667	2.5000	1.5366	1.5088	0.4008	0.4808	0.3111	0.2974	0.5333	0.4270
45	3.5000	3.8333	2.2175	1.8255	0.8731	0.7223	0.5227	0.4041	0.5298	0.5216
22	1.8333	2.5000	1.5226	1.5095	0.4167	0.4526	0.2908	0.2731	0.4630	0.4206
25	2.5000	2.5000	1.8533	1.7509	0.6149	0.6275	0.3939	0.4031	0.5667	0.4899
6	2.1667	2.5000	1.7711	1.7688	0.6142	0.5981	0.4368	0.3815	0.6333	0.5000
17	1.8333	2.1667	1.4089	1.4909	0.3516	0.4455	0.2397	0.2956	0.4028	0.4500
34	2.0000	2.5000	1.4683	1.6531	0.4097	0.5833	0.2753	0.3762	0.3889	0.4167
General	5.8333	6.3333	1.9413	1.8905	0.8275	0.8309	0.4659	0.4526	0.5223	0.4737
$\bar{Q}(+)trees$	4.1667		2.4326		1.0149		0.5444		0.4556	

$n_a$  – average number of non-rare alleles per locus;  $n_e$  – effective number of alleles;  $I$  – Shannon index;  $H_e$  – expected heterozygosity;  $H_o$  – observed heterozygosity.

The lowest level of heterozygosity  $H_e$  was established for family No. 17 (23.97%), heterozygosity  $H_o$ , as in the case of isoenzyme analysis, for family No. 53 (33.33%). Family No. 71 is characterized by one of the highest average values of both heterozygotes ( $H_e = 53.62\%$ ;  $H_o = 62.50\%$ ). Also, a high level of expected heterozygosity was revealed for family No. 45 (52.27%), observed - for families No. 30 (69.44%) and No. 6 (63.33%).

In half-sib families of the 2nd test site, the average number of alleles per locus ( $n_a$ ) varies from 1.500 (family No. 53) to 3.8333 and 3.3333 (families No. 45 and 44, respectively). The lowest value of the effective number of alleles ( $n_e$ ) was found in the family No. 53 (1.4118), the highest was found in the family No. 44 (1.8734). High values of  $n_e$  were also identified for families No. 71, 48 and 45 (1.8565; 1.8454 and 1.8255,

respectively). The average value of the Shannon index (I), which reflects the complexity of the sample structure according to the quantitative representation of objects in the population (varies from 0 to 5), and calculated for all 6 SSR loci, is  $0.8309 \pm 0.1606$  for half-sibs of the 2nd test site.

The lowest levels of  $H_e$  и  $H_o$  heterozygosities were established for family No. 36 (23.77 and 38.33%, respectively). It should be noted that in the case of isozyme analysis, family No. 36 was also characterized by low values of these indicators. Family No. 71 is characterized by one of the highest average values of both heterozygotes ( $H_e = 53.62\%$ ;  $H_o = 62.50\%$ ). The highest level of expected heterozygosity was found for family No. 44 (47.60%), as it was similarly observed in the case of microsatellite analysis of half-sibs of the 1st test site, for family 30 (70.37%). Families No. 45, 48, 71 were also characterized by high values of  $H_e$  and  $H_o$  (40.41 and 52.16%; 41.53 and 52.22%; 44.44 and 57.41%, respectively). For family No. 25, a rather high level of  $H_e$  (40.31%) was revealed, for family No. 6 -  $H_o$  (50.00%).

### Isoenzyme analysis

Allelic diversity analysis of the 1st and 2nd test site half-sibs revealed 55 and 52 alleles, respectively. Out of the 20 analyzed isozyme genes, 18 were polymorphic in the case of the 1st test site, 19 - in the 2nd test site. 10 loci (Fe, Dia-1, Gdh, Adh-1, Adh-2, 6-Pgd-1, 6-Pgd-2, Mdh-3, Aat-2, and Aat-3) turned out to be the most variable, their mean  $H_e$  in the generalized sample of half-sibs Scots pine exceeded 25%. For six genes (Gpi, Pgm-1, Mdh-1, Mdh-2, Lap-1, and Lap-2), the  $H_e$  value varied from 5 to 25%, which allows them to be classified as loci with an average level of polymorphism. The least variable were Idh and Dia-2, since their average heterozygosity did not exceed 5%. No polymorphism was found for the Pgm-2 and Aat-1 loci. The alleles of isozyme loci of trees of the 1st test site were found to contain two rare (Gpi<sup>0.85</sup>, 6-Pgd-2<sup>0.80</sup>) and four unique (Dia-2<sup>0.80</sup>, Dia-2<sup>1.15</sup>, Adh-2<sup>0.70</sup>, 6-Pgd-2<sup>0.95</sup>), 2nd test site- one rare (Aat-3<sup>1.90</sup>) and three unique (Dia-2<sup>0.80</sup>, Idh<sup>1.20</sup>, Aat-1<sup>1.20</sup>).

The results of the assessment of the level of genetic variability of Scots pine half-siblings are presented in Table 5.

**Table 5.** Values of genetic variability parameters in half-sib families of the 1st and 2nd test sites of Scots pine (isozyme analysis)

Family	Test site															
	1st		2nd		1st		2nd		1st		2nd		1st		2nd	
	$P_{95}$		$P_{99}$		A		$A_{1\%}$		$H_e$		$H_o$		$n_e$			
38	0.650	0.600	0.850	0.700	2.050	1.900	2.050	1.900	0.243	0.169	0.284	0.195	1.409	1.261		
36	0.700	0.550	0.700	0.750	2.050	2.100	2.050	2.100	0.242	0.219	0.297	0.247	1.429	1.393		
53	0.550	0.550	0.750	0.700	1.950	1.950	1.950	1.950	0.179	0.185	0.197	0.205	1.273	1.283		
48	0.650	0.600	0.650	0.650	1.950	1.950	1.950	1.950	0.240	0.234	0.284	0.277	1.438	1.436		
44	0.650	0.650	0.700	0.700	2.050	2.100	2.050	2.100	0.237	0.256	0.260	0.277	1.422	1.451		
30	0.650	0.550	0.650	0.700	1.850	2.000	1.850	2.000	0.226	0.203	0.270	0.240	1.395	1.347		
28	0.600	0.600	0.750	0.750	1.850	1.950	1.850	1.950	0.186	0.214	0.211	0.221	1.302	1.359		
71	0.550	0.650	0.550	0.650	1.850	1.900	1.850	1.900	0.221	0.245	0.242	0.252	1.411	1.430		
27	0.650	0.650	0.700	0.800	2.000	2.000	2.000	2.000	0.235	0.262	0.274	0.305	1.420	1.480		
45	0.600	0.750	0.800	0.800	2.350	2.350	2.350	2.350	0.254	0.255	0.303	0.297	1.497	1.446		
22	0.650	0.700	0.700	0.700	1.850	2.000	1.850	2.000	0.246	0.235	0.272	0.257	1.470	1.442		
25	0.600	0.700	0.650	0.800	1.850	2.100	1.850	2.100	0.218	0.220	0.237	0.247	1.371	1.363		
6	0.650	0.600	0.650	0.750	2.050	1.950	2.050	1.950	0.264	0.254	0.310	0.302	1.470	1.473		
17	0.750	0.650	0.750	0.750	2.050	2.050	2.050	2.050	0.256	0.257	0.305	0.275	1.425	1.439		
34	0.600	0.600	0.700	0.600	2.000	1.750	2.000	1.750	0.230	0.204	0.272	0.245	1.414	1.361		
General	0.600	0.700	0.900	0.800	2.750	2.600	2.450	2.400	0.254	0.250	0.270	0.258	1.432	1.427		
♀(+)-trees	0.750		0.900		2.450		2.250		0.280		0.313		1.490			

$P_{95}$  – the proportion of polymorphic loci (with a frequency of the common locus allele not exceeding 95%);  $P_{99}$  – the proportion of polymorphic loci (with a frequency of the common locus allele not exceeding 99%);  $A$  – average number of alleles per locus;  $A_{1\%}$  – average number of non-rare alleles per locus;  $H_e$  – expected heterozygosity;  $H_o$  – observed heterozygosity;  $n_e$  – effective number of alleles.

The proportion of polymorphic loci in half-sibs of the 1st test site ranges from 0.550 (family No. 71) to 0.800 and 0.850 (families No. 45 and 38, respectively) according to the 99% criterion ( $P_{99}$ ) and from 0.550 (families No. 53 and 71) to 0.750 (family No. 17) according to the 95% criterion ( $P_{95}$ ). It should be noted that in families 45 and 38, the largest number of rare and unique alleles was identified (three and four, respectively). The values of the average number of alleles per locus ( $A$ ) and the number of non-rare alleles per locus ( $A_{1\%}$ ) equally vary from 1.850 (families No. 22, 25, 28, 30, 71) to 2.350 (family No. 45). The lowest value of the effective number of alleles ( $n_e$ ) was found in the family No. 53, the highest was found in the family No. 45.

The values of  $H_e$  and  $H_o$  in families of the 1st test site range from 17.90 to 26.40% and from 19.70 to 31.00%, respectively. Family No. 53 is characterized by the lowest level of  $H_e$  and  $H_o$  (17.90 and 19.70%, respectively). Also, low values of indicators were established for family No. 28 (18.60 and 21.10%, respectively). The highest values of heterozygosity were found in family No. 6 ( $H_e = 26.40\%$ ;  $H_o = 31.00\%$ ). The level of observed heterozygosity above 30.00% was established for families No. 17 and 45.

The proportion of polymorphic loci in the 2nd test site half-sibs ranges from 0.550 (families 30, 36, 53) to 0.750 (family No. 45) according to the 99.00% criterion ( $P_{99}$ ) and from 0.600 (family No. 34) to 0.800 (families No. 25, 27, 45) according to the 95.00% criterion ( $P_{95}$ ). The values of the average number of alleles per locus ( $A$ ) and the number of non-rare alleles per locus ( $A_{1\%}$ ) equally vary from 1.750 (family No. 34) to 2.350 (family No. 45). The lowest value of the effective number of alleles ( $n_e$ ) was found in the family No. 38 (1.261), the highest - 27 (1.480).

The values of  $H_e$  and  $H_o$  in families of the 2nd test site range from 16.90 to 26.20% and from 19.50 to 30.50%, respectively. The lowest level of  $H_e$  and  $H_o$ , in contrast to the half-sibs of the 1st test site, is characterized by family No. 38 (16.90 and 19.50%, respectively). Also, low values of indicators were established for family No. 36 (18.50 and 20.50%, respectively). The highest values of heterozygosity were found in family 2 No. 7 ( $H_e = 26.20\%$ ;  $H_o = 30.50\%$ ). A high level of expected heterozygosity was also established for families No. 17, 44, 45 and 6 (25.70; 25.60; 25.50 and 25.40%, respectively), observed - for families No. 6 and 46 (30.20 and 29.70 % respectively).

## Discussion

For selection purposes, families that showed higher growth rates than the control group on both test sites were of particular interest. If there was a significant increase or decrease in growth rates compared to the control group in several seed progeny of plus Scots pine trees, then it follows that the offspring will inherit the traits from the parent tree and will exhibit similar phenotypic characteristics.

At the same time, practically no attention is paid to such an issue raised in our study as the reproducibility of the results of the genetic evaluation of the half-sibs progeny of plus trees of different test sites obtained from seed harvests in different years. Our research showed that different test sites of the same families mostly differed in height from each other. Such differences were found within the family either in height or in diameter.

Although the trees on the 2nd test site were planted later, in families No. 22 and 53 the height of the trees on the 2nd test site had a higher growth rate as compared to the 1st test site - by 1.4 m and 0.3 m respectively. In families No. 36, 45, 48, 17, 30, 6, 25, 71, and 28, the height did not differ significantly across test sites. Family No. 22 had the highest values on both test sites by height - 19.72 and 21.11 m, but the smallest diameter - 17.90 and 18.72 cm respectively. The trunk diameter for the 1st test site ranged between 17.90-22.34, and between 17.8 to 21.86 cm for the 2nd test site. Family No. 45 had the largest diameter values on the 1st test site and was among the highest on the 2nd test site. Higher diameter values were observed on the 2nd test site in comparison with the 1st test site across 7 families (families No. 30, 36, 34, 71, 6, 22, 44) by 0.22-2.89 cm. Based on the above observations, the growth rates of half-sib offspring depended on the genetic factors (received during pollination) and overall micro ecological conditions.

The results obtained during this study were backed up by the research of Efimov M.N. (1981). From his study, it follows that seeds planted at different test sites often have different growth rates. Out of 12 pairs compared in his research, in 8 pairs (1.1-2.0 m) by the age of 25 he found significant differences in average height (at  $P < 0.05$ ). At the same time, with an increase in the age of the progeny of two reproductions, a certain tendency was manifested to equalize the rank position of families in the compared populations. In the compared pairs of progenies of 9 clones, two-factor analysis showed that the year of harvest had a more noticeable effect on the growth of half-sib families in height than the clonal affiliation of mother trees (Efimov, 1994). A similar phenomenon was also confirmed in the experiments of other researchers (Giertych, 1994; Mullin *et al.*, 1995).

Taking into account the unequal nature of the growth of half-sib progenies of the same maternal genotypes grown from seeds of different test sites, it can be assumed that their growth rate is partly due to the non-additive effect of genes, i.e., here, to some extent, a specific combinational ability is manifested. Studies of the nature of pollen dispersion showed that the pollination of individual ramets mainly involves the pollen of neighbouring trees. The progeny of each clone obtained by free pollination represents the sum of full sibs from pollination with its pollen of a very limited number of paternal clones. At the same time, individual successful combinations can have a decisive influence on the overall positive shift, taken as "general combinative ability" (Efimov, 2010).

It is hypothesized that the effectiveness of positive selection of woody plants on productivity can be evaluated by the results of studying the growth of seed progeny (families) of plus trees in experimental (test) cultures. If the selected feature, for example, the average height of the tested population of families statistically differs from the control, then mass selection in this population is effective (Vidyakin, 2010). It was found that on the 1st test site, six families out of 15 of the analysed families had higher values than the control group, and on the 2nd test site, this was observed in five families. The excess over control varied from 3.91% to 28.96%, depending on the family and test site. According to the parameters of the stem diameter, in most cases no significant differences were found or the indices of the families were lower than the control.

In our study, the height over the control on two test sites was found in four families No. 38, 36, 45 and 22. Poor growth in height that resulted in not exceeding the control variants, was detected in 28. Families No. 6, 17, 25, 30, 48, 71 had no significant differences from the control variants on either test site.

According to the diameter of the stem in family 48, this parameter was less on average by 10.25% for both test sites. It should be noted that in the 1st test site the proportion of families with a diameter less than the control was 33.33%, while in the 2nd test site it was 6.70%. Only 45 and 30 families had a larger diameter than the control variants in different variants.

Based on the results obtained in terms of height of half-sibs plus trees No. 22, 38, 36, 45 were classified as candidates for the elite group. Their progeny consistently exceeded the control on both test sites by an average of 10.40%. It should be noted that, in general, the progeny of plus trees height measures exceeds the control values to a greater extent compared to the measures of the trunk diameter. This is due to the initial arrangement of planting spots for half-sibs ( $2 \times 2$  m), which to a greater extent contributed to the growth of trees precisely in height, especially after crown closer.

The emerging competitive conditions between plants for illumination forced individual trees to accelerate their growth and allowed certain semi-sibs, in our case, the above-mentioned offspring to occupy a dominant position in the plantation. Despite the fact that the studied families showed relative uniformity in terms of the increase in stem diameters, this indicator must also be taken into account in the final genetic assessment of the progeny. Thus, a study of half-sibs' progeny in test forest plantations of Scots pine planted in Bulgaria revealed significant differences in stem diameters (Zhelev and Evtimov, 2017). According to the results of measurements carried out in 1996 and 2012, it was found that the best families 30 and 46 years after sowing were the same, but statistically significant changes were recorded in the rank of families in general. This suggests that the half-sib families selected by us on the basis of a mid-term genetic assessment will continue to be characterized by intensive growth and increased productivity. Currently, it is relevant to carry out not only

a selection genetic assessment, but also to establish the level of genetic diversity of plantations, progeny, individual trees using molecular markers. In genetics, “diversity” is “the set of differences between species, breeds within species, and individuals within breeds, expressed as a consequence of differences in their DNA” (Hedrick, 2003). The presence of genetic diversity and its assessment are of great importance for the selection of woody plants and their adaptation to changing environmental conditions. It is known from the literature that the level of tree heterozygosity is one of the leading factors in the stability of its development, and that there is a negative relationship between these two traits (Tikhonova, 2015).

Preservation of allelic diversity in populations of forest species is a fundamental strategy that aims to obtain valuable seeds for selective breeding. (Gargiulo *et al.*, 2019). The need to maintain a balance of genetic diversity in forests is supported by some research work. Thus, in studying structural plantations occupied by *Pinus densiflora* done based on microsatellite markers, it is possible to identify populations with the lowest genetic diversity. Due to shrinking of areas occupied by *Pinus densiflora*, it has become necessary to develop new strategies for conservation, management and renewal of forest resources that take into account genetic diversity (Ahn *et al.*, 2021). The selection of trees with high genetic diversity in populations with limited distribution is especially important. For example, the analysis of nuclear molecular markers in plantations occupied by *Abies hidalgensis* revealed a decrease in genetic diversity due to human impact, which may lead to the death of this population in the future (Rosales-Islas and Octavio-Aguilar, 2023).

The relationship between genetic diversity and the mass production of forest reproductive material is a matter of debate among scientists. When planting slow-growing forest species with a long period of ontogenesis and reproduction phase such as Scots pine, the reduction in genetic diversity increases the risk of producing seedlings that are not resistant to adverse factors. Only a small part within species can contribute to the pool of gametes and pass on strong genes to the next generation, which is important to take into account during selective breeding (Ivetić *et al.*, 2016). It is known that the offspring of plus trees is grown in clonal seed orchards in order to preserve genotypes and to carry out selective breeding. SSR molecular marker technologies are used to analyse genetic diversity and genetic structure. This method allows for the assessment of germplasm, trace the transmission of genetic diversity from parent to offspring, and to perform selective breeding of forested species. (Nielsen and Hansen, 2012; Wojacki *et al.*, 2019; Pakull *et al.*, 2021; Liesebach *et al.*, 2021; Yan *et al.*, 2023).

Since genetic diversity provides the adaptive potential of species, and, as a result, its biological stability and productivity, it is important to identify families of half-sibs with the highest level of genetic variability for their further use in breeding and reforestation work.

Based on microsatellite analysis, it was we found that for most parameters of genetic variability in the 1st test site, family No. 45 is characterized by one of the highest values, as well as by the level of heterozygosity – family No. 6; in the 2nd test site – families No. 44, 45, 48 and 71. A relatively low level of genetic diversity was found in families No. 34, 48 (1st test site), 27, 28, 36 (2nd test site), 17, 22, 53 (both test sites). For all half-sib families of the 1st and 2nd test sites, the estimates of expected heterozygosity ( $H_e$ ) were 0.7-24.7% and 2.4-27.89% lower, respectively, than the observed heterozygosity ( $H_o$ ). This indicates an established excess of heterozygotes in the progeny. The reason for the deviation of the genotypic structure from the equilibrium according to Hardy-Weinberg and the decrease in the level of expected heterozygosity may, apparently, be a process similar in its consequences to the “founder effect”, since within the family all individuals descend from the same maternal tree. In the natural populations of Scots pine, as well as in the sample of maternal plus trees of the analysed half-sib families, the opposite situation is generally observed – the level of  $H_e$  is slightly higher compared to  $H_o$ .

Based on isoenzyme analysis in both test sites, family No. 45 is characterized by the highest values for most parameters of genetic variability; and family 6 is also characterized by the heterozygosity level. A high level of expected heterozygosity in the 2nd test site was also found in families No. 17, 27 and 44, observed heterozygosity -in families No. 27 and 46. A relatively low level of genetic diversity in both test sites was found

in families 28, 53, in the 2nd test site in family 38. As in microsatellite analysis, in all families of the 1st and 2nd test sites,  $H_e$  values are lower than those of  $H_o$  (1.80-4.90% and 0.70-4.80%, respectively).

An important aspect is to identify the relationship between the productivity of woody plants and their heterozygosity (Fischer *et al.*, 2017). In similar studies of three populations of *P. sylvestris*, no significant relationship was found between heterozygosity, determined by 12 allozyme loci, and six quantitative traits (Savolainen and Hedrick, 1995). If such associations arise, then only in individual plants. A positive correlation between the annual radial growth and the level of heterozygosity for 14 polymorphic allozyme loci was found in 156 clones of aspen poplar (*Populus tremuloides* Michx.) only in the case of division of clones into heterozygosity classes (Jelinski, 1993). Some studies have established a positive relationship between heterozygosity and growth rate in selected populations of woody plants (Mitton and Grant, 1984). However, this relationship is not universal. So, for example, neither the height nor the stem cross-sectional area in F2 hybrids of *Populus trichocarpa* Torr. & A. Gray ex Hook. × *Populus deltoides* W. Bartram ex Marshall did not correlate with heterozygosity as determined by RELP markers, and none of the correlation coefficients exceeded 0.16 (Bradshaw and Stettler, 1994). A study by Blumenröther *et al.* (2001) showed an association of heterozygosity at certain loci with a smaller tree size.

The microsatellite analysis showed that the expected heterozygosity values of the analysed half-sib families in both test sites are in most cases lower than those established for natural populations of Scots pine in Europe and Asia (Floran *et al.*, 2010; Belletti *et al.*, 2012; Tóth *et al.*, 2017; Şofletea *et al.*, 2020; Konecka *et al.*, 2021; Przybylski *et al.*, 2021; Kavaliauskas *et al.*, 2022; Sheller *et al.*, 2023). We have identified a wide range of parameters of genetic diversity. Thus, the values of the expected and observed heterozygosity of  $H_e$  and  $H_o$  are within the limits: 1st test site - from 23.97 to 53.62% and from 33.33 to 69.44%, respectively; 2nd generation - from 23.77 to 47.60% and from 38.33 to 70.37%, respectively. The reason for this, apparently, is the origin of the progeny of half-sib families from one maternal tree. This, as noted above, led to a result similar in its consequences to the “founder effect”. In addition, two loci (Pysl2 and Pysl36) were included in the analysis, characterized by an average level of variability (22-23%).

According to isoenzyme analysis, in general, the revealed level of genetic variability of half-sibs was within the limits established in other papers (Dzialuk and Burczyk, 2002; Bilgen and Kaya, 2007; Kosinska *et al.*, 2007; Ivanek *et al.*, 2013; Przybylski *et al.*, 2015; Mańka *et al.*, 2015; Yanbaev *et al.*, 2020; Przybylski *et al.*, 2020; Tikhonova *et al.*, 2021). According to our isoenzyme analysis data the values of the expected and observed heterozygosity of  $H_e$  and  $H_o$  are within the following limits: 1st test site - from 17.90 to 26.40% and from 19.70 to 31.00%, respectively; 2nd test site - from 16.90 to 26.20% and from 19.50 to 30.50%, respectively.

Of particular interest are families in which rare and unique alleles have been identified. According to some studies, rare alleles may contain genetic variation that will allow a population to adapt to a changing environment in the future, although their current impact on the population may be negligible (Müller-Starck, 1995; Schaberg *et al.*, 2008; Konecka *et al.*, 2021). What was identified was that a set of alleles of microsatellite loci of half-sibs of the first and second test sites differed between each other. In the sample of genotypes of families of the 2nd test site, alleles PtTx4001<sup>223</sup>, Pysl36<sup>245</sup>, PtTx4011<sup>281</sup> identified in half-sibs of the 1st generation were not found. At the same time, the alleles Pysl42<sup>176</sup>, PtTx4001<sup>203</sup>, PtTx4001<sup>216</sup>, Pysl17<sup>223</sup>, Pysl36<sup>257</sup>, PtTx4011<sup>261</sup> were identified in the families of the 2nd test site, which were not found in the 1st test site Scots pine trees. The emergence of rare alleles in families is not surprising. Pollen contamination of background populations is one of the main factors explaining the emergence of new alleles. The dynamics of rare alleles in plant populations requires further study using more extensive material.

## Conclusions

This study shows that the reproducibility of the results of the selection and genetic evaluation of half-sib progeny of plus trees obtained from seed crops collected in two subsequent years differs. It was evident that the growth rates on both testing sites for seeds planted in 1984 and in 1985 varied among the same tree families. The variance analysis showed that the height of half-sibs was determined by the genotype by 26.66 and 40.06%, and the trunk diameter by 11.59 and 7.90% for test sites 1 and 2 respectively. Thus, the growth of half-sibs was affected by both genetic factors (such as a chronological order of genes or the changes in pollen composition each year) and by the influence of external factors.

Based on the results obtained, the following families of half-sibs No. 22, 38, 36, 45 were selected for further breeding. Their seeds proved to have valuable selective properties as they exceeded the height values of the control group on both test sites by 10.40%.

The conducted molecular genetic analysis indicates that in order to identify elite trees, a comprehensive assessment is required that combines both the traditional breeding method (a genetic evaluation of offspring) and the molecular genetic analysis. Out of the most promising offspring of plus trees that contained families No. 22, 38, 36, 45, the best genetic characteristics were identified in the half-sib family 45.

The results of the study revealed that mother trees of half-sib families No. 22, 38, 36, 45 produce high-quality seeds and can be used to obtain offspring with valuable genetic traits.

15 rare and unique alleles were identified in the alleles of microsatellite loci. Families with rare and unique alleles: 48, 22, 71, 45, 25, 44, 36, 30, 6, 17 drew special attention. Such families are not only objects of conservation of the gene pool of the species, but are also the carriers of adaptive genetic variability.

The established high level of genetic variability within individual families indicates that, in general, *P. sylvestris* is an economically suitable species for breeding programs in Kazakhstan. It means that a high level of genetic variability will bring higher genetic benefit and serve as an indicator to best adaptability to likely environmental changes in the future. The molecular genetic analysis makes it possible to identify and select genotypes and their samples characterized by a high level of individual and group genetic variability.

## Authors' Contributions

Y.K., N.C. and S.I.: methodology; N.C. and D.K: validation; Y.K., D.K., A.K. and S.I.: data curation; Y.K. and A.K.: supervision; Y.K., N.C., Y.V., A.K. and D.K.: participation in the experiment of this study, data analysis. All authors read and approved the final manuscript.

## Ethical approval (for researches involving animals or humans)

Not applicable.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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