Clonal Variation Based on Some Morphological and Micromorphological Characteristics in the Boyabat (Sinop/Turkey) Black Pine (*Pinus nigra* subsp. *pallasiana* (Lamb.) Holmboe) Seed Orchard

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Seed orchards with high hereditary qualities and the improvement studies used are of great importance. This study was carried out on individuals in a Boyabat grafted black pine seed orchard, Sinop. The morphological and micromorphological measurements of the characteristics were performed on needle samples taken from individuals, and the genetic diversity was determined on a clonal basis. According to the analysis of variance applied to the data obtained from the measurements and the morphological and micromorphological characters of the clones, it was determined that there was a significant difference among the clones at the P<0.001 confidence level. In this context, according to Duncan's Range test, the creation of a large number of groups is an indicator of it. The highest heritability rates were obtained in needle diameter, stipule diameter, number of the dorsal stoma, and needle length characteristics.

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INTRODUCTION

Climate types, quite different from each other, prevail across Turkey, and accordingly, there is a wide variety of species in forested areas (Atalay and Efe 2015). However, they enable us to study domestic and foreign species together in afforestation studies. Thus, the opportunities to establish healthy forest stands are increasing in terms of both quality and quantity. In afforestation studies, it is necessary to select appropriate species of the appropriate origin, to follow the principles of improvement studies, and to pay attention to afforestation techniques. For this purpose, the selection of seed sources with high hereditary qualities and the improvement studies used are of great importance (Wu *et al.* 2015; Kaviriri *et al.* 2020; Weng *et al.* 2020).

Improved seeds can increase wood production by up to 40% (Üçler and Turna 2005; Yahyaoğlu and Ölmez 2005). Seeds of a certain origin ensure afforestation and provide economic and ecological benefits (Wu *et al.* 2015). The most important purpose of tree breeding is to promote ecological and economic benefits (Dyjakon 2019).

Black pine (*Pinus nigra* Arnold.) is the most important species which can be spread to the steppe regions in Türkiye. Away from its native areas Black pine is planted for its timber production purposes (Topacoglu *et al.* 2016). Black pine, which is one of the dominant species in the forest assets of Türkiye, is a primary forest tree species that has a very wide distribution area starting from South Europe up to Türkiye. It can be argued that black pine is a typical south European forest tree species that is ecologically and economically important in the abovementioned distribution area (Gülsoy and Cinar 2019).

Depending on changing environmental conditions, it is almost impossible to predict what kinds of threats forests will face in the future. Ensuring the continuity of the genetic diversity of species will make the presence of individuals carrying genes that will be needed in the upcoming years possible (Sevik *et al.* 2013).

It is preferable for improvement studies that the genetic diversity of the selected populations is as high as possible. It is easier to find proper improvement materials, and the chance of success is higher with populations having a broad genetic base (Velioğlu *et al.* 2002; Lindgren *et al.* 2008). High intraspecific genetic diversity is a guarantee of adaptation to changing environmental conditions. Genetic diversity determines the adaptation potential of a species and is an important part of ecosystem stability. Therefore, the conservation of genetic diversity is essential to maintain adaptability. Genetic diversity is a raw material that will be shaped in improvement studies and through which results can be obtained accordingly. High genetic diversity evenly increases the chance of genetic staff to choose genotypes and populations appropriate for their study objectives (Şevik 2012). Seed orchards are one of the most important seed sources in terms of creating a connection between present and future, such as gene conservation areas, and future forest plantations (Bilir and Temiraga 2012).

Seed orchards are areas where seeds are produced in large quantities to obtain the highest genetic gain as cheaply and fast as possible (Wu *et al.* 2015). According to another definition, they are plantations that are operated for the frequent, abundant, and easy harvesting of forest tree seeds, with use of selected clones or fertilizers, and where pollen flow from isolated or external sources can be blocked or reduced (Zobel and Talbert 1984). They are also shown as the most appropriate way to put the genetic gain obtained from tree breeding studies into practice (Tulukçu *et al.* 2002).

Genetic diversity can be determined by physiological and morphological characteristics or molecular markers (Suangtho *et al.* 1999). To date, genetic variation studies have been generally initiated based on morphological characteristics, and after obtaining sufficient data, detailed information has been reached through isoenzyme and DNA studies. However, morphological characteristics were mainly examined in the studies carried out, and the number of studies carried out based on anatomical characteristics has remained quite limited (Donnelly *et al.* 2016). Studies on needle sizes and anatomical characteristics of conifers showed significant differences between and within populations (Bobowicz and Korczyk 1994; Urbaniak *et al*. 2003; Androsiuk and Urbaniak 2006). Many studies have been carried out on clonal seed orchards in terms of the morphological characteristics of seeds and cones (Deligöz and Gezer 2005; Çılgın *et al.* 2007; Hauke-Kowalska *et al.* 2019; Kaviriri *et al.* 2020; Weng *et al.* 2020).

Variation among clones and within clones is an important factor in terms of the seed production (Prescher *et al.* 2007). Many studies have been carried out on the variations of the productivity of forest tree species (Kang and Lindgren 1998; Benowicz and El-Kassaby 1999; Kang 2001; Bilir *et al.* 2002; Sengün and Semerci 2002; Bilir *et al.* 2004;

Lindgren *et al.* 2009).

The study was carried out on grafted black pine individuals in the Boyabat seed orchard, Sinop. The Boyabat *Pinus nigra* seed orchard, Sinop, was established by taking seed stands of Kastamonu origin. Clones were planted by grafting to seedlings at a distance of 8 × 8 m. The seed orchard is 10.9 Ha in size and located at an altitude of 450 m. This study determined the structuring of adaptive genetic diversity on a clonal basis by analyzing the morphological and micromorphological characteristics of needle samples taken from individuals. For this purpose, the characters measured were analyzed using the SPSS package program, and it was attempted to determine the structuring of adaptive genetic diversity on a clonal basis.

EXPERIMENTAL

The study was carried out in the Boyabat black pine seed orchard, Sinop. The black pine seed orchard affiliated to Sinop Provincial Directorate was planted by the Forest Trees and Seed Improvement Institute in 1995 with 30 clones. From the administrative aspect, the seed orchard is within the boundaries of Kastamonu Regional Directorate of Forestry, Boyabat Forest Management Directorate, Bürnük Forest Sub-district Directorate, and has a size of 10.1 ha (Fig. 1).

Within the scope of the study, genetic variation attributes in the seed orchards were determined with the help of some morphological and anatomical characteristics. Morphological characteristics have been used to determine genetic variations in many studies carried out to date (Sengün and Semerci 2002; Sevik *et al.* 2013). However, the number of studies in which anatomical characteristics have been used to determine genetic variations is very limited. However, it is known that all phenotypic characters, including anatomical characters, are formed under the mutual interaction of genetic structure and environmental conditions (Yayla *et al.* 2022; Kuzmina *et al.* 2023; Cobanoglu *et al.* 2023). The most dominant factors affecting phenotypic characters are climatic (Koç 2022; Dogan *et al.* 2023) and edaphic (Cetin *et al.* 2022; Key *et al.* 2022) factors. It can be accepted that in seed orchards where climatic and edaphic factors are relatively homogeneous, anatomical characters, like other phenotypic characters, are shaped largely depending on the genetic structure, since they are established in a limited and nearly flat area. In fact, it is accepted that anatomical characters are less affected by environmental conditions and therefore reflect genetic structure more clearly (Yigit 2016; Yigit *et al.* 2021). Therefore, anatomical characters are important instruments that can be used especially in genetic variation studies, but the number of studies on this subject is negligible.

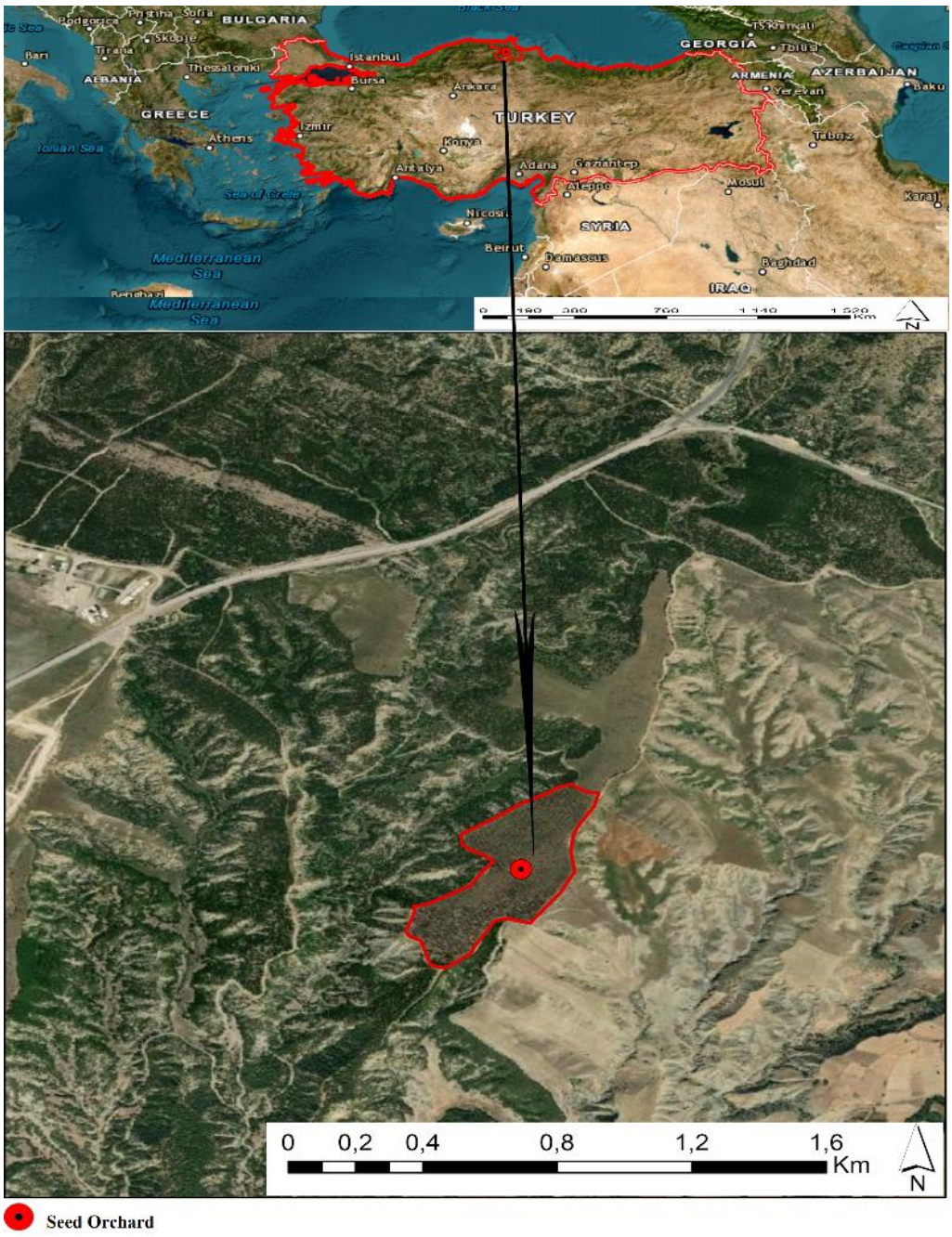

Fig. 1. Location of the Boyabat black pine seed orchard on the map

Within the scope of the study, needle samples were collected in December, except for the vegetation season. Needle samples were collected from a total of 90 trees, three ramets from each of 30 clones, and last year's needles from the same direction, labeled, airtight packed, and brought to the laboratory.

Morphological characteristics, such as needle length (NL) (cm), needle width (NW) (mm), needle diameter (ND) (mm), stipule diameter (SD) (mm), number of the dorsal stoma (NDS), number of dorsal stoma channels (NDSC), number of the ventral stoma (NVS), and number of ventral stoma channels (NVSC), were determined on the needle

samples collected. Furthermore, ash determination (A) was performed as follows: an empty crucible and cover were dried for 15 min on a heater or in an indirectly heated furnace at approximately 600 °C. The porcelain crucibles were allowed to wait in the desiccator for 45 min and weighed with a precision of 0.1 mg. The sample was put in the crucible. The cover removed and the crucible and its contents were put in an indirectly heated furnace and burned until all carbon was removed. Initially, temperature was gradually increased to avoid volatilization. The crucible was allowed to wait for 3 h at 575 ± 25 °C in the furnace. At the end of this period, the sample in the crucible should be completely bleached, or the particles should have been lost. Ash was calculated as follows,

$$\text{Ash\%} = (A*100)/B$$

where A is the ash weight, and B is the complete dry sample weight (g).

Anatomical characteristics, such as needle wet weight (WW), the weight values of needles taken when they were fresh (g), needle dry weight (DW), and oven-dried weights of samples (at a precision of 0.001 g), were determined.

The data obtained were analyzed using the SPSS 20.0 statistical package program. The analysis of variance was applied to clones in terms of the characters measured, and Duncan's test was applied to the data in case of statistically significant ($P \leq 0.05$) differences as a result of the analysis. As a result of Duncan's test, new homogeneous groups were determined (Kalıpsız 1994; Ercan 1995).

Broad sense heritability values were estimated both on individual tree basis (H_1) and clone mean basis (H_2) as the ratio of total genetic variance (s^2c) to total phenotypic variance ($s^2c + s^2E$) for H_1 and to ($s^2c + s^2E/n$) for H_2 (n = graft number). Cloning effect variance biases heritability values, but the magnitude is negligible and can be ignored. In the present study, heritability components were estimated as σ^2E= error mean square and σ^2c = (clone mean square-error mean square/ number of grafts per clone). This formula has been used in different articles (Sevik and Topacoglu 2015).

RESULTS AND DISCUSSION

As a result of the measurements and calculations performed on the samples taken in the Boyabat black pine clone orchard, A, WW, DW, NL, NW, ND, SD, NDS, NDSC, NVS, and NVSC values were determined. The study was carried out on 141 trees of 30 different clones, and the analysis of variance was applied to the values obtained as a result of the calculations. The results are presented in Table 1. Significant differences were found in terms of all the morphological characteristics studied at a confidence level of 99.9% both among the clones and within the clones. Duncan's test was applied for each anatomical characteristics to determine how the clones were grouped within themselves, and the test results are presented in Table 2.

According to Duncan's test results of the A character, clone 81 (1.839) formed a class alone, and clone 87 had the highest value (1.361) and was included only in the last homogeneous group according to the ash content of the needle. According to the wet weight values of the needle, clone 95 with the lowest value (1.833) was included only in the first homogeneous group, and clone 82 with the highest value (2.11) was included only in the last homogeneous group. According to the results of Duncan's test applied to the dry weight values of the needle, Clone 95 was included only in the first homogeneous group

(0.817) according to the dry weight values of the needle and that clones 1 and 82 (2.003 and 2.087) were included only in the fifth and last homogeneous group (Table 2).

In terms of the NL morphological character, the clones formed 16 different classes among themselves. Clone 85 was included in the first homogeneous group with a needle length value of 10.5 cm and clone 93 was included in the last homogeneous group with a needle length value of 12.6 cm. In terms of the ND morphological character, it is observed that three different groups were formed. Clone 88 was included in the first homogeneous group, clone 93 was included in the second homogeneous group, and all other clones were in the 1st class. In terms of the NW character, the test revealed that 15 different groups were formed. When the groups were examined, clone 85 was included in the 1st homogenous group, clones 81 and 85 were included in the 2nd homogenous group, and clone 8 was included in the last homogeneous group alone. In terms of the SD morphological character, clone 85 formed a homogeneous group alone, and clone 81 formed the 2nd homogenous group. Clone 93 was included in the last homogenous group (Table 2).

The clones were grouped in 11 different groups in terms of the NDS character according to Duncan's test results of micromorphological characteristics. Clone 98 formed the first and single group and clones 95 and 97 formed the second group together. Clone 89 formed the last class alone. When Duncan's test results were examined in terms of the NDSC character, 7 groups were formed. Clone 82 formed the last class alone and clones 85 and 89 formed a class together. When Duncan's test results were examined in terms of the NVS micromorphological character, clones 86, 93, 88, 96, and 10 formed a class by themselves. Clone 8 formed the last class alone. When the results of Duncan's test applied to micromorphological characteristics were examined in terms of the NVSC character, clones 7, 93, and 99 formed a class by themselves and clone 8 formed the last class.

Correlation analysis was performed to determine the relationship between the elements, and the results are presented in Table 3. The studied relationship that was not measured in the correlation analysis was related to the linear part of the relationship between the variables.

Table 1. Analysis of Variance Applied to All Characteristics

Characters		Sum of Squares	Degree of Freedom	Means Square	F-Value	Significant
A	Between groups	59.454	29	2.05	6.462	.000*
	Within groups	47.591	150	0.317		
	Total	107.045	179			
WW	Between groups	41.932	29	1.446	3.113	.000*
	Within groups	69.682	150	0.465		
	Total	111.614	179			
DW	Between groups	17.533	29	0.605	2.577	.000*
	Within groups	35.188	150	0.235		
	Total	52.721	179			
NL	Between groups	573.725	29	19.784	8.463	.000*
	Within groups	4488.475	1920	2.338		
	Total	5062.2	1949			
NW	Between groups	68.01	29	2.345	5.486	.000*
	Within groups	820.713	1920	0.427		
	Total	888.723	1949			
ND	Between groups	15.938	29	0.55	20.027	.000*
	Within groups	52.688	1920	0.027		
	Total	68.626	1949			
SD	Between groups	27.168	29	0.937	10.036	.000*
	Within groups	179.229	1920	0.093		
	Total	206.397	1949			
NDS	Between groups	35876.015	29	1237.104	9.192	.000*
	Within groups	19650.167	146	134.59		
	Total	55526.182	175			
NDSC	Between groups	184.917	29	6.376	3.604	.000*
	Within groups	258.333	146	1.769		
	Total	443.25	175			
NVS	Between groups	44654.432	29	1539.808	4.395	.000*
	Within groups	51149	146	350.336		
	Total	95803.432	175			
NVSC	Between groups	216.045	29	7.45	4.467	.000*
	Within groups	243.5	146	1.668		
	Total	459.545	175			

P<0.05 (95% confidence level); P<0.01 (99% confidence level); P<0.001 (99.9% confidence level)
ns: Not significant

Table 2. Duncan's Test Results of Ash Content in All Characteristics

Clone No	A	WW	DW	NL	NW	ND	SD	NDS	NDSC	NVS	NVSC
1	2.929gh	2.926defghı	2.087e	10.872 abcdef	1.4020 a	1.7849 cdef	2.09 bcdefgh	119.33ıjk	10defg	137ghı	11.67efgh
2	2.602efgh	2.693cdefg	1.694cde	11.375 efghıjkl	1.3847 a	1.7899 cdef	2.03 bcde	81.67abc	8abc	112.67abcdefg	9.67abc
3	1.966abcdef	3.068efghı	1.257abcd	11.733 hıjklmn	1.4325 a	1.7769 cde	2.08 bcdefg	90.67abcde	10defg	115abcdefgh	11.67efgh
4	2.361cdefgh	2.215bcd	1.549bcde	11.944 klmno	1.5104 a	1.9331 lmn	2.27 jkl	110ghıj	9.33bcdefg	140hı	11.67efgh
5	1.794abcdef	3.546hıj	1.326abcd	10.635 ab	1.4245 a	1.8715 hıjklm	2.13 efghı	102.67defgh	10defg	125bcdefghı	12.33gh
6	1.819abcdef	3.130efghı	1.235abcd	10.831 abcde	1.4869 a	1.8836 ıjklm	2.11 efghı	102.33defgh	9.67cdefg	116.67abcdefgh	10.67abcdefg
7	1.520abc	3.361fghıj	1.145abc	11.263 cdefghıj	1.4657 a	1.8589 ghıjk	2.16 efghıj	99.33defgh	9bcdef	100.33ab	9a
8	2.654fgh	3.573hıj	1.906de	12.205 nop	1.5717 ab	1.9843 no	2.27 jkl	125jk	10.33efg	148.33ı	13.33gh
9	1.657abcde	2.460bcde	1.224abcd	10.720 abcd	1.3606 a	1.7526 bc	1.98 bcd	86.5abcd	9.5bcdefg	100.5ab	10.5abcdef
10	2.069abcdefg	3.685ıj	1.547bcde	11.548 ghıjklm	1.5542 ab	1.9377 mn	2.23 ıjk	97.25cdefgh	9bcdef	97a	9.75abcd
81	1.839abcdef	1.126a	1.328abcd	10.975 abcdefg	1.4128 a	1.7081 b	1.95 b	107.33fghı	10defg	132.33defghı	11.67efgh
82	3.175h	2.116bc	2.003e	11.295 cdefghıj	1.4140 a	1.8035 cdefgh	2.11 efghı	112.5hıj	11g	134defghı	11.5defg
83	1.315ab	2.626cdef	0.961ab	11.771 ıjklmn	1.5367 ab	1.9224 klmn	2.21 hıjk	90.67abcde	9.67cdefg	108abcd	10.67abcdefg
84	1.880abcdef	2.956defghı	1.443abcde	12.089l mnop	1.4433 a	1.896jklm	2.14 efghı	94abcdefg	10defg	102ab	12fgh
85	1.678abcde	3.186efghı	1.294abcd	10.463 a	1.3713 a	1.5581a	1.81 a	119ıjk	10.67fg	136.67fghı	11.67efgh
86	2.353cdefgh	2.786cdefgh	1.681cde	11.636 hıjklmn	1.4487 a	1.7656bcd	2.06 bcdef	91.67abcdef	8.33abcd	92.67a	10.33abcdef
87	1.361ab	4.015j	1.058abc	11.334 defghıjk	1.4892 a	1.8502ghıj	2.14 efghı	97bcdefgh	10defg	116.33abcdegh	11.33cdefg
88	1.640abcde	3.086efghı	1.129abc	11.093 bcdefgh	2.562 c	1.8302defghıj	2.31 kl	81.33abc	8abc	94.67a	9.33ab
89	2.187bcdefg	2.866cdefgh	1.719cde	11.597 ghıjklmn	1.4148 a	1.8333defghıj	2.16 efghıj	132k	10.67fg	135.33efghı	11.67efgh
90	1.730abcdef	2.665cdef	1.161abc	12.458 op	1.4709 a	1.8976jklm	2.16 efghıj	101.33defgh	8.67abcde	130cdefghı	11.67efgh
91	1.827abcdef	2.673cdef	1.193abc	10.683 abc	1.4229 a	1.8639hıjk	2.07 bcdefg	81.33abc	8.33abcd	101.33ab	10.33abcdef
92	2.188bcdefg	3.095efghı	1.670cde	11.643 hıjklmn	1.4307 a	1.7953cdefg	2.10 defgh	105.33efghı	9.33bcdefg	118.33abcdefgh	10.33abcdef
93	2.061abcdefg	3.193efghı	1.550bcde	12.611 p	1.7853 b	2.0127o	2.36l kl	88abcd	9bcdef	93a	9a
94	2.057abcdefg	3.056efghı	1.529bcde	11.151 bcdefghı	1.4747 a	1.8349efghıj	2.29 kl	105.33efghı	9bcdef	128.33cdefghı	11bcdefg
95	1.134a	1.833b	0.817a	11.480 fghıjkl	1.3482 a	1.7092b	1.98 bcd	80.67cbefghı	9.33bcdefg	104abc	10abcde
96	2.516defgh	2.790cdefgh	1.593bcde	11.879 jklmno	1.5681 ab	1.9225klmn	2.20 ghıjk	81.33abc	7.67ab	96a	9.67abc
97	1.304ab	3.466ghıj	0.936ab	12.152 mnop	1.4705 a	1.8672hıjkl	2.19 fghıjk	80.67ab	7a	110.33abcdef	9.67abc
98	1.672abcde	2.835cdefgh	1.239abcd	12.047l mnop	1.368 a	1.7868cdef	1.97 bc	78.67a	8abc	101ab	9.33ab
99	2.4312cdefgh	2.235bcd	1.727cde	11.573 ghıjklmn	1.4475 a	1.8673hıjkl	2.22 hıjk	82.33abc	7a	110abcde	9a
100	1.600abcd	2.620cdef	1.215abc	11.852 jklmno	1.3783 a	1.8257defghı	2.07 bcdefg	94.67abcdefg	8.33abcd	110.67abcdefg	10.33abcdef

The correlation coefficient calculated as a result of the correlation analysis was indicated with r and took values between –1 and +1. The fact that the coefficient was close to +1 indicates that there was a good correlation between the two variables, and the fact that it was close to -1 indicates that there was a good but inverse correlation, in other words, one of the variables increased while the other one decreased. Upon evaluating the results in this respect, it is observed that the level of relationship between some elements was high.

Table 3. Correlation Analysis Results

	NW	ND	SD	A	WW	DW	H	A%	NDS	NDSC	NVS	NVSC
NL	-.105	.544**	.447**	-.007	.001	.043	.061	-.113	.063	-.057	.014	.038
NW	1	-.144	-.062	.079	.066	.101	.160	.044	.128	.130	.157	.108
ND		1	.682**	.350**	.006	.013	.049	-.005	-.016	.047	-.090	.092
SD			1	.334**	-.045	-.013	.045	.030	.029	-.070	.016	-.010
A				1	-.198	-.127	-.134	.002	.050	-.007	-.134	-.056
WW					1	.950**	.895**	-.163	.212	.046	.239*	.189
DW						1	.915**	-.196	.311**	.104	.289*	.242*
H							1	-.221	.361**	.152	.334**	.273*
A%								1	-.212	-.113	-.061	-.075
NDS									1	.743**	.716**	.634**
NDSC										1	.433**	.634**
NVS											1	.800**

There was a positive correlation in general with respect to the measured characteristics. When the results were examined in this context, the relationship between some elements was observed to be quite high. For example, the correlation coefficients between WW and DW (0.950), NVS and NVSC (0.800) were quite high. Similarly, the correlation coefficient calculated between A and WW (-0.198) was negative but very strong. Very strong relationships were observed among many characteristics. Analysis of variance, variance components and heritability estimates for studied characteristics are shown in Table 4.

Table 4. Analysis of Variance, Variance Components and Heritability Estimates for Studied Characteristics

Characteristics	Between Populations	Within Populations	s^2c	s^2E	H_1^2	H^2
A	2.05	0.317	0.347	0.317	0.52	0.87
WW	1.446	0.465	0.196	0.465	0.30	0.72
DW	0.605	0.235	0.074	0.235	0.24	0.65
NL	19.784	2.338	3.489	2.338	0.60	0.90
NW	2.345	0.427	0.384	0.427	0.47	0.84
ND	0.55	0.027	0.105	0.027	0.80	0.96
SD	0.937	0.093	0.169	0.093	0.65	0.92
NDS	1237.104	134.59	220.503	134.59	0.62	0.91
NDSC	6.376	1.769	0.921	1.769	0.34	0.76
NVS	1539.808	350.336	237.894	350.336	0.40	0.80
NVSC	7.45	1.668	1.156	1.668	0.41	0.81

When the table values were examined, it was seen that the characters with the highest heritability were ND, SD, NDS, and NL. According to the table values, the H_2 value was 0.90 and above in terms of these characters.

The images of dorsal and ventral stoma channels and stomas taken on the needles during the measurements performed to determine micromorphological characteristics are presented in Fig. 2.

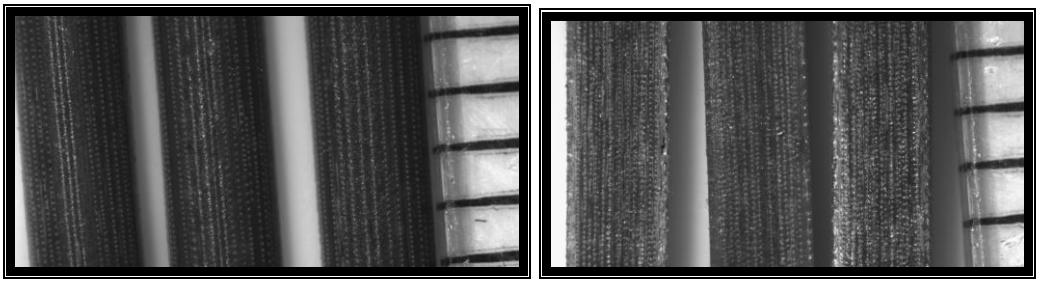

Fig. 2. The images of dorsal and ventral stoma channels

The results of the analysis of variance show that there were statistical differences at a confidence level of at least 95%, in terms of all the micromorphometric characteristics examined in the Boyabat clonal seed orchard. There were statistically significant differences among the clones in terms of all the characteristics studied. According to the results of Duncan's test, it can be said that the creation of a large number of groups is an indicator of it.

It is possible to interpret the results in a way that the genetic diversity in the seed orchard is sufficient. Most studies of clonal variations of seed orchards are morphological, physiological, and phenological, and the determination of anatomical characteristics brings a different dimension to such studies. Afforestation studies conducted on thousands of hectares of land are performed with the seeds obtained from seed orchards. The degree of success of the outcomes of afforestation studies emerges after many years. Genetic differences among clones in seed orchards should be clearly revealed by determining anatomical characteristics to observe successful outcomes in a shorter time.

Genetic variation studies were initiated based on morphological characteristics. As well as any phenotypic characteristic, plant metabolism is shaped by the mutual interaction between genetic structure (Özel *et al.* 2022; Tandogan *et al.* 2023; Kurz *et al.* 2023) and environmental conditions (Varol *et al.* 2022; Ghoma *et al.* 2022; Cetin *et al*. 2023). Therefore, genetic variation studies have been carried out on many species based on morphological characteristics. Topaçoğlu (2013), Güney *et al*. (2014), and Sevik (2012) determined genetic variations based on morphological characteristics in *Pinus nigra, Pinus brutia*, and *Abies*, respectively. In addition to these, there are many studies aimed at determining genetic variations among clones in seed orchards (Cilgin *et al.* 2007; Buğday 2008).

Morphological and anatomical characteristics are a result of the interaction between genetics and the environment (Yigit *et al.* 2018a) and are shaped by the effects of genetic factors (Sevik *et al.* 2017; Yigit *et al.* 2018b) and environmental factors (Yigit *et al.* 2016; Turkyilmaz *et al.* 2020). This can be explained by the effect of microenvironment conditions on the clones. Microenvironment conditions affect morphological characteristics significantly (Cetin *et al.* 2018; Yucedag *et al.* 2019). This is a seed orchard. Therefore, the genetic structure is thought to be the same on a clone basis. However, it is thought that the differences arise from environmental conditions. Therefore, micro environmental conditions are quite effective on clones.

In this study, the clones differed significantly at a confidence level of 99.9% in terms of all characteristics and formed a large number of homogeneous groups, according to Duncan's test results. This can be interpreted based on a hypothesis that the genetic diversity in the seed orchard is high. Genetic diversity is desired to be high, especially in the population. In the studies carried out, it was determined that intrapopulation genetic diversity in many species was higher than interpopulation genetic diversity. In the studies carried out, it was determined that the ratio of interpopulation variation was 9% in *Pinus contorta* (Wheeler and Guries 1982), 6% in *Pinus nigra* (Velioğlu *et al.* 2002), 6.1% in *Pinus strobus* (Rajora *et al.* 1998), 1.5% in *Abies sachalinensis* (El-Kassaby *et al.* 1992), 2.6% in *Abies mariesii* (Suyama *et al.* 1992), 4.8% in *Abies cephalonica* (Fady and Conkle 1993), and 13.3% in *Abies alba* (Vendramin *et al.* 1999). Considering that the interclone variation was quite high in this study, it can be said that the results obtained in the literature are generally compatible with the results of the present study.

There are many genetic variation studies that have been carried out using morphological characteristics to date. However, both the number of studies, in which genetic variation was determined using anatomical characteristics, and the number of the characteristics used are quite limited. Matziris (1993) and Lamhamedi *et al*. (2000) determined the variation using needle resin channels and electron microscope images, respectively. The number of studies in which wood density was used to determine genetic variation is quite high (Hernandez and Adams 1991; Zhang and Morgenstern 1995; Hylen 1997; Chave *et al.* 2006). The anatomical characteristics of wood have been generally analyzed to investigate whether the fiber source is suitable for papermaking in the paper industry (Ay and Şahin 1996; İstek *et al.* 2009).

Seed orchards continue to make significant contributions to improved seed production until the genetic values of clones in Turkey are completed. The protection and maintenance of these orchards should be sustained meticulously. Along with the establishment of seed orchards, it is aimed to meet all seed needs that will arise in the future from seed orchards. Furthermore, it has been aimed to provide resources for seed orchard genetic studies and to protect populations that are of good quality and under the danger of extinction.

CONCLUSIONS

1. This study determined the genetic diversity by needle morphological and micromorphological characteristics in the Boyabat black pine clonal seed orchard. There were statistically significant differences at a confidence level of 99.9% among the clones in terms of all the studied characteristics.
2. There were differences among the clones in terms of revealing the hereditary value of the clonal seed orchard in this respect and most of the characteristics studied. One of the largest deficiencies of seed orchards is the narrowing of the gene pool.
3. There were statistical differences at a confidence level of at least 95% according to the results of the analysis of variance in terms of most of the characteristics examined in the seed orchard. This result can be interpreted as the genetic diversity in the seed orchard is sufficient.

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Data Availability

All data included in this study are available upon request by contact with the corresponding author.

Declariation

The authors declare no conflict of interest.

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