Genetic Variation in Hanönü (Kastamonu)-Günlüburun Anatolian Black Pine (*Pinus nigra* Arnold. subsp. caramanica (Lamb.) Holmboe) Clonal Seed Orchard According to Some Needles Characters*

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Abstract

Anatolian Black Pine (*Pinus nigra* Arnold. subsp. *pallasiana* (Lamb.) Holmboe) is one of the most common and important forest tree species in Turkey. Meanwhile, the stands of this species occupy roughly 4,2 million ha, of which about 1,8 million ha are considered to be non productive forest. Also, this pine species is most important species which can be spread to the steppe regions in Anatolia. The semi-arid steppe regions evaluate as potential afforestation areas. Actually, the seed demand for this species is mainly supplied from current 53 of seed orchards and 79 of seed stands. Most of these seed orchards are still rather young and mainly established after 1990's. The main objective of Anatolian black pine seed orchards is the production of genetically improved seed for reforestation purpose.

The objective of this study was to investigate the genetic variation in Hanönü-Günlüburun Anatolian Black Pine (*Pinus nigra* Arnold. subsp. *pallasiana* (Lamb.) Holmboe) clonal seed orchard using eight morphological and anatomical needles characters. For this purpose; 3 sample trees from 30 clones and 10 needles from each samples tree were selected. Totally 900 sample needle were used for this study. Nine morphological and anatomical traits (needle length, needle width, needle thickness, sheath thickness, number of rows of stomata in the dorsal and ventral faces, number of stomata per cm of needle length in the dorsal and ventral faces) were determinate in 900 needle. The results showed that; there were significant differences between clones according to eight morphological and anatomical needles characters.

Keywords: Anatolian Black Pine; Seed Orchard; Genetic Variation; Needles Characters
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Introduction

Black pine (*Pinus nigra* Arnold.) is one of the most widely distributed species of the Mediterranean basin, particularly in southern Europe and Anatolia. It occurs naturally as small clusters in Algeria and Morocco (Davis, 1965; Kaya and Temerit, 1993). Altitudinally it grows from an elevation of 100 m up to 1800 m. but mainly from 500-800 up to 1200-1600 meters (Bassiotis, 1967). Silviculturally it is considered one of the most important coniferous species and is extensively used in reforestation programs throughout the country (Matziris, 1978; Palmerg, 1982).

Anatolian black pine [*Pinus nigra* Arnold. subsp. *pallasiana* (Lamb.) Holmboe] is one of the four recognized subspecies of black pine. It is native to a vast area extending from the Balkans to southern Carpathian Mountains, Crimea, Cyprus, Syria, Thrace and Anatolia (Alptekin, 1986). It occurs throughout Turkey except the northeast Black Sea region and covers the second largest area of all native commercial forest tree species of Turkey (Yaltırık and Efe, 2000). Meanwhile, the stands of this species occupy roughly 4,2 million ha, of which about 1,8 million ha are considered to be non productive forest. Thus, Anatolian black pine is a high priority species in the National Forest Tree Breeding Programme in the country.

Subspecies *pallasiana* has been the most preferred black pine for reforestation in dry areas. Therefore, there is a need to study its genetic differentiation to facilitate proper allocation of seed sources to planting sites and obtain genetic parameters for tree breeding purposes (Gülcü and Üçler, 2008). The seed demand for this species is mainly supplied from current 53 of seed orchards and 79 of seed stands. Most of these seed orchards are still rather young and mainly established after 1990's. The main objective of Anatolian black pine seed orchards is the production of genetically improved seed for reforestation purpose.

In this study, we investigated genetic variation in needles traits of *Pinus nigra* Arnold. subsp. *pallasiana* (Lamb.) Holmboe clonal seed orchard from Hanönü-Kastamonu. Our main objective was to examine the distribution of genetic variation among and within populations in terms of some needle anatomical and morphological characters.

Material and Methods

This study was carried out in Hanönü clonal seed orchard, located at Hanönü, Kastamonu (400 m). The orchard was established in 1993 and comprised 2039 grafts of 30 clones derived from intensively selected trees in Karadere seed stand. Grafts were 2 years old at the time of establishment and were planted at a spacing 8 x 8 m (Erkan, 2008) So far, no pruning has been done in the orchard.

Needles were collected in winter 2010 when the trees were dormant. The needles were placed in individual paper bags and kept at 4°C until analysis. Three grafts of 30 clones and 10 needles of each graft respectively were assessed for needle length (NL), needle width (NWi), needle thickness (NTh), sheath thickness (ShTh), number of rows of stomata in the dorsal (NRSD) and ventral faces (NRSV), number of stomata per cm of needle length in the dorsal (NSD) and ventral faces (NSV).

Data of all characters were subjected to one-way analysis of variance. Duncan test was used for determining homogenous groups. Variables were tested for normality and homogeneity of variances and logarithmic transformation for counting values were made. Differences were considered significant at $P < 0.05$ level. Broad sense heritability values were estimated both individual tree basis (H_1^2) and clone mean basis (H^2) as the ratio of total genetic variance ($\sigma 2$ c) to total phenotypic variance ($\sigma 2$ c + $\sigma 2$ E) (Matziris, 1984) for the H1 and to ($\sigma 2$ c + $\sigma 2$ E/n) for H2 (n =graft number). Cloning effect variance biases the heritability values, but the magnitude is negligible and can be ignored (Matziris, 1993). In this study, heritability components were estimated as $\sigma 2$ E = error mean square and $\sigma 2$ c = (clone mean square-error mean square/ number of grafts per clone) (Wright, 1962).

Results

Clonal means of the studied morphologic characters for average, results of variance analysis, groups of Duncan test, maximum and minimum values of determined characters and % difference between the minimum and maximum values are shown in Table 1.

Table 1. Clonal means of the studied morphologic characters and Duncan test results

Klon	Needle Length (mm)		Needle Width (mm)		Needle Thickness (mm)		Sheath Thickness (mm)	
1	123	a	1,59	c d e f g h i	2,14	c d e f g h i	2,4	c d e f g h
2	155	c d e f g h i	1,73	h i j k	2,29	h i	2,44	c d e f g h e
3	140	a b c d e f	1,62	d e f g h i j	2,22	g h i	2,47	d e f g h e
4	159	e f g h i	1,67	d e f g h i j	2,17	e f g h i	2,2	b c d
5	165	g h i	1,76	j k	2,3	h i	2,51	e f g h
6	162	f g h i	1,71	f g h i j k	2,28	h i	2,67	
7	135	a b c d	1,58	c d e f g h	2,01	b c d e f	2,34	b c d e f g e
8	143	a b c d e f g	1,69	e f g h i j k	2,03	b c d e f g	2,35	b c d e f g h
9	151	b c d e f g h	1,51	b c d	1,97	b c d e f	2,34	b c d e f g e
10	129	a b	1,59	c d e f g h i	2,15	d e f g h i	2,29	b c d e f e
11	163	f g h i	1,7	e f g h i j k	2,14	c d e f g h i	2,52	e f g h e
12	145	a b c d e f g h	1,83	k	2,32	i	2,59	g h
13	145	a b c d e f g h	1,46	b c	1,94	b c d	2,1	a b e
14	174	i	1,66	d e f g h i j	1,96	b c d e	2,51	e f g h
15	154	c d e f g h i	1,63	d e f g h i j	2,1	b c d e f g h	2,39	c d e f g h
16	125	a	1,3	a	1,71	a	1,87	a e
17	142	a b c d e f g	1,5	b c d	1,97	b c d e f	2,2	b c d
18	151	b c d e f g h	1,78	j k	2,18	f g h i	2,55	f g h e
19	131	a b	1,53	b c d e	2,06	b c d e f g	2,22	b c d
20	160	e f g h i	1,67	d e f g h i j	2,1	b c d e f g h	2,35	b c d e f g h
21	157	d e f g h i	1,56	b c d e f	2,04	b c d e f g	2,5	e f g h
22	150	b c d e f g h	1,57	b c d e f g	1,94	b c d	2,22	b c d e
23	158	e f g h i	1,66	d e f g h i j	2,24	g h i	2,57	g h
24	156	d e f g h i	1,54	b c d e	2,06	b c d e f g	2,22	b c d
25	137	a b c d e	1,53	b c d e	1,99	b c d e f	2,25	b c d e
26	141	a b c d e f	1,51	b c d	1,93	b c	2,21	b c d
27	159	e f g h i	1,72	g h i j k	2,16	e f g h i	2,62	h
28	166	h i	1,74	i j k	2,13	b c d e f g h i	2,63	
29	151	b c d e f g h	1,41	a b	1,91	b	2,16	b c
30	134	a b c	1,64	d e f g h i j	2,09	b c d e f g h	2,33	b c d e f g
Average	**149**		1,61		2,08		2,37	
Maximum	174		1,83		2,32		2,67	
Minimum	123		1,3		1,71		1,87	
Diff. (%)	41,5		40,77		35,67		42,78	

Table 1. Clonal means of the studied morphologic characters and Duncan test results

Klon	Number of Rows of Stomata in The Ventral Face	Number of Stomata per cm of Ventral Face	Number of Rows of Stomata in The Dorsal Face	Number of Rows of Stomata in The Dorsal Face
1	10,44 a b c d e	1127,78 a b c d e f g h	11,67 a b c	1347,78 a b c d e
2	10,11 a b c d	1092,22 a b c d e f	13,44 c d e f g	1502,22 d e f
3	10,78 a b c d e f	1262,22 c d e f g h i j	13,44 c d e f g	1586,67 e f g
4	10,89 a b c d e f	1222,22 b c d e f g h i	14,22 g	1594,44 e f g
5	12,78 h i j	1273,33 d e f g h i j	13,67 d e f g	1410 a b c d e
6	12,89 i j	1582,22 k	14,44 g	1864,44 h
7	10 a b c d	1104,44 a b c d e f	11,78 a b c d	1348,89 a b c d e
8	10,89 a b c d e f	1365,56 h i j k	14,22 g	1696,67 f g h
9	10 a b c d	1102,22 a b c d e f	10,67 a	1148,89 a
10	10,78 a b c d e f	1108,89 a b c d e f g	11,44 a b c	1172,22 a
11	13,44 j	1476,67 j k	13,89 e f g	1511,11 d e f
12	11,22 b c d e f g h	1283,33 e f g h i j	12,22 a b c d e f	1411,11 a b c d e
13	9,56 a b	981,11 a b	11,56 a b c	1230 a b c
14	12,56 g h i j	1367,78 h i j k	13,89 e f g	1478,89 c d e f
15	11,44 c d e f g h i	1213,33 b c d e f g h i	13,44 c d e f g	1453,33 c d e f
16	9,33 a	1014,44 a b c	10,78 a	1154,44 a
17	9,78 a b c	960 a	11,56 a b c	1153,33 a
18	11,89 e f g h i j	1228,89 b c d e f g h i	13,89 e f g	1555,56 e f
19	9,33 a	1067,78 a b c d e	12 a b c d e	1386,67 a b c d e
20	11,67 d e f g h i	1272,22 d e f g h i j	13,33 c d e f g	1442,22 b c d e f
21	11,89 e f g h i j	1360 h i j k	13 b c d e f g	1456,67 c d e f
22	10,11 a b c d	1027,78 a b c d	11,89 a b c d	1248,89 a b c d
23	12,11 e f g h i j	1330 f g h i j	13,67 d e f g	1501,11 d e f
24	9,56 a b	1065,56 a b c d e	11,22 a b	1276,67 a b c d
25	11 a b c d e f g	1165,56 a b c d e f g h i	11,89 a b c d	1350 a b c d e
26	9,78 a b c	1093,33 a b c d e f	11,56 a b c	1346,67 a b c d e
27	10,89 a b c d e f	1247,78 c d e f g h i j	13,22 c d e f g	1501,11 d e f
28	11,67 d e f g h i	1353,33 g h i j k	14 f g	1607,78 e f g
29	9,44 a	994,44 a b	11 a	1188,89 a b
30	12,33 f g h i j	1386,67 i j k	14,67 g	1805,56 g h
Average	10,95	1204,37	12,72	1424,41
Maximum	13,44	1582,22	14,67	1864,44
Minimum	9,33	960	10,67	1148,89
Diff. (%)	44,05	64,81	37,49	62,28

The average values of the studied characters are shown in Table 1. According to Table 1 average needle length is 149 mm, needle width is 1,61 mm, needle thickness is 2,08 mm, sheath thickness is 2,37 mm. There is 10,95 row of stoma in the ventral face and 12,72 in the dorsal face. There is 1582,22 stomata in the ventral face and 1424,41 stomata in the dorsal face per cm.

The analysis of variance showed that there were significant differences in all studied characters among clones ($p< 0.001$). The results of statistical analysis and calculated H_1^2 and H^2 values are shown in Table 2.

Table 2. Analysis of variance, variance components and heritability estimates for studied haracters

Character	Between populations	Within populations (Error)	σ^2c	σ^2E	H_1^2	H^2
NL	1533,3***	402,31	125,6656	402,31	0,238014	0,737618
NWi	12,284***	2,126	1,128667	2,126	0,346784	0,826929
NTh	1739,583***	353,72	153,9848	353,72	0,303296	0,796664
ShTh	3043,524***	620,121	269,267	620,121	0,302755	0,796249
NRSV	12,396***	2,195	1,133444	2,195	0,340533	0,822927
NSV	219563,2***	48019,63	19060,4	48019,63	0,284144	0,781295
NRSD	13,692***	3,105	1,176333	3,105	0,274759	0,773225
NSD	312309,2***	55166,204	28571,44	55166,204	0,341202	0,823360

***Statistically significant of the 0.001 probability level,

$H_1 = \sigma^2_G / \sigma^2_G + \sigma^2_E$, $H_2 = \sigma^2_G / \sigma^2_G + \sigma^2_{E/n}$

There were large differences among the characters for broad-sense heritability (H_1^2) and H^2) (Tables 1 and 2). As to the average values, the H_1 values never exceeded 0.5 (Table 2). This indicates that the variation among grafts caused by environmental variation could be always higher than that caused by the genetic variation in the studied orchard. It is expected that the studied orchard should have uniform ecological condition. But, the results of the present study indicate that the environmental conditions can be more important than genetics due to the micro-ecological differences in the orchard.

In this study, higher variation within-clone is attracted more attention. Meanwhile, there are large variations as to the morphological and anatomical characters among the grafts. Similarly, the considerable difference was reported among the grafts by Sıvacıoğlu et al. (2009) in a clonal seed orchard for *Pinus silvestris*. For this reason, to create desirable characters in seed orchard trees, there ought to be a large potential of management, which affects ‘‘the environment’’ (Bilir et al., 2006).

Table 3. Pearson correlation coefficients among the studied characters

	NL	NWi	NTh	ShTh	NRSV	NRSD	NSV
NWi	.529**						
NTh	.483**	.803**					
ShTh	.537**	.739**	.700**				
NRSV	.423**	.589**	.446**	.513**			
NRSD	.396**	.675**	.505**	.526**	.613**		
NSV	.358**	.535**	.402**	.493**	.852**	.553**	
NSD	.302**	.585**	.473**	.455**	.477**	.812**	.480**

** Correlation is significant at the 0.01 level.

There are significantly relationships between all characters (Table 3). The highest correlation (r=0,852) is calculated between number of stomata and number of rows of stomata in the ventral face. In addition, in Scots pine seed orchard positive correlation among the number of rows of stomata in the dorsal face and number of rows of stomata in the dorsal face (r=0,812), needle width and sheath thickness (r= 0,803).

The high variation within the clones and heritability values estimated in this study, indicates opportunities of high selection intensity in the breeding programs for Black pine. Also, the higher variation within the clones than among the clones confirms that the genotypes have responses against to heterogeneity of growing area in seed orchard. Therefore, attention must be paid to these traits when management of existed orchards or establishment of new seed orchards. Meanwhile, the periodical and comparative experiments in the same orchard will support to the breeding perspectives of this species and this result will evaluate as a sample for the other species. Thus, the establishment of the seed orchards by using various origins should be discussed in the tree breeding strategies of Turkey.

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