Genetic Variation in Taşköprü-Tekçam Scots Pine (*Pinus sylvestris* L.) Clonal Seed Orchard According to Some Needles Characters

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Abstract

Scots pine (*Pinus sylvestris* L.) is one of the most common and important forest tree species in Turkey due to usefulness of its wood to many commercial uses. This species is classified as one of the economically important tree species for Turkish Forestry in the "National Tree Breeding and Seed Production Program". Presently, the need of scots pine seeds is approximately 715 kg per year. Demand for scots pine seeds and seedlings in Turkey is obtained mainly by domestic production, but currently only 9.2% of the Turkish seed demand in scots pine is supplied from current 111 ha of seed orchards. High and genetically improved seed yield from the seed orchard is an integral part for the success of a tree breeding program. Genetic variation between the clones must be identified in the seed orchards to maintain high seed production.

The objective of this study was to investigate the genetic variation in Kastamonu Taşköprü-Tekçam Scots pine (*Pinus sylvestris* L.) 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: *Pinus sylvestris* L.; Scotch Pine; Seed Orchard; Genetic Variation; Needles Characters

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INTRODUCTION

Genetic variation is the fundamental component, which ensures survival and thus the stability of forest ecosystems as its quantity and quality determines the potential of population to adapt the changing in environmental condition. This is particularly important with changing population and climatic condition and when the long-term stability of forest ecosystems is increasingly threatened by environmental stress. Thus, a genetic characterization of natural forest resources is the first step necessary for a better understanding of genetic resources for implementation of insitu and exsitu conservation activities (Turna et al., 2006).

Scots pine (*Pinus sylvestris* L.) is the most widely distributed member of the family *Pinaceae* in the world. The longitudinal range of Scots pine covers over 14,000 km and extends from 8°W in Spain to 141°E in Siberia. The latitudinal range of Scots pine covers over 3,700 km and extends from 37° N in Turkey to 70°N in Norway, Sweden, and Finland (Turna, 2003; Critchfield, 1966). The species covers a diverse array of physiographic regions. Throughout much of its native range in Turkey, Scots pine is an important commercial forest tree species, widely planted for industrial and conservation activities such as protecting soil erosion and living snowfences. Scots pine occupies about 738,000 ha in Turkey, growing mainly in the Black Sea coastal mountains on warm southern slopes, where the climate is humid. In the inner mountains further south, which are also parallel on the Black Sea coast, the tree species grows primarily on humid northern slopes. The southern boundary of its geographical distribution in Turkey lies at around 38° 34' N latitude. On the eastern coast of the Black Sea, it grows at altitudes of 2000 - 2700 m. on the eastern region, it ranges from 1200 to 1600 m. In exceptional cases, it is distributed vertically from sea level (Trabzon-Çamburnu) up to the Alpine Zone (Zigana Mountain) as high as 2400 m in Northeastern Black Sea Region. Within its distribution range, scots pine can grow under diverse ecological conditions. Such a wide range of ecological conditions could favor the formation of a variety of ecotypes (Turna, 2003; Turna and Guney, 2009).

Scotch pine is an important component of subalpine ecosystems of Blacksea region in Turkey because of its role in watershed protection, its functional natural habitat for wild life, also, because of its esthetic values. It has, therefore, great importance especially in Blacksea forestry. Scotch pine is one of the important forest tree species both Turkish Forestry and breeding program. Although it has 21 seed orchard in 109,4 ha and 36 seed stands 4878,3 ha, 91% of seed demand is covered from seed stands for afforestation in the species (Ayan et al,

2005).

Seed orchards are the most important seed source in the species at this stage. Determination of similarity/differences among the clones of seed orchard is an important stage of breeding program. It should known to obtain maximum variability among genotypes and minimum loss of genetic diversity in current generation. It is also important for sustainable forestry and to transmit the current gene diversity to next generation by afforestation with suitable seed source. Besides it can be used to determination of breeding population and gene conservation areas and for establishment of seed orchards in the species. Morphological distance can play important role for the purposes.

In this study, we investigated genetic variation in needles traits of *Pinus silvestris* L. clonal seed orchard from Taşköprü-Kastamonu. Our objectives were to examine the distribution of genetic variation among and within populations in terms of some needle anatomical and morphological characters

MATERIALS AND METHODS

This study was carried out in Tekçam clonal seed orchard, located at Taşköprü, Kastamonu (41°36' N, 35°05' E, 1160 m). The orchard was established in 1995 and comprises 1987 grafts of 30 clones derived from intensively selected trees in Araç-Dereyayla seed stand. Grafts were 2 years old at the time of establishment and were planted at a spacing 6 x 6 m. commercial cone harvesting started in the 2003 when the grafts were 10 years old. By the time of this study (2010), the graft age is 17 years. So far, no pruning has been done in the orchard. In all clones, tree replications (grafts) were sampled at random and the needle, were harvested, and counted separately by graft (ramet). 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 AND DISCUSSION

Clonal means of 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)	
11	65	b c d e f g h	1,59	a b c d e f g	1,62	c d e f g h	1,72	c d e
12	63	b c d e f g	1,44	a b c d e	1,48	a b c d e f	1,53	a b c d e
13	68	c d e f g h i j k	1,40	a b c d	1,44	a b c d	1,55	a b c d e
14	66	b c d e f g h i j	1,52	a b c d e f g	1,56	a b c d e f g h	1,48	a b c d
15	67	c d e f g h i j k	1,46	a b c d e	1,47	a b c d e f	1,49	a b c d
16	67	c d e f g h i j k	1,58	a b c d e f g	1,67	d e f g h	1,71	c d e
17	58	a b c d	1,64	c d e f g	1,57	a b c d e f g h	1,58	a b c d e
18	75	h i j k	1,64	c d e f g	1,68	e f g h	1,74	d e
19	58	a b c d	1,48	a b c d e f	1,51	a b c d e f g	1,50	a b c d e
20	60	a b c d e f	1,43	a b c d e	1,41	a b c	1,54	a b c d e
21	75	g h i j k	1,63	c d e f g	1,62	c d e f g h	1,54	a b c d e
22	72	f g h i j k	1,62	c d e f g	1,70	f g h	1,49	a b c d
23	74	g h i j k	1,64	c d e f g	1,72	g h	1,61	a b c d e
24	78	j k	1,64	c d e f g	1,65	c d e f g h	1,70	c d e
25	59	a b c d e	1,31	a b	1,35	a	1,36	a b
26	69	d e f g h i j k	1,57	a b c d e f g	1,55	a b c d e f g h	1,51	a b c d e
27	57	a b c	1,40	a b c d	1,47	a b c d e f	1,36	a b
28	77	h i j k	1,75	f g	1,68	e f g h	1,54	a b c d e
29	73	g h i j k	1,54	a b c d e f g	1,59	a b c d e f g h	1,49	a b c d
30	75	h i j k	1,64	c d e f g	1,54	a b c d e f g h	1,64	b c d e
31	72	f g h i j k	1,30	a	1,38	a b	1,48	a b c d
32	77	i j k	1,51	a b c d e f g	1,56	a b c d e f g h	1,59	a b c d e
33	78	k	1,54	a b c d e f g	1,62	b c d e f g h	1,64	b c d e
34	69	c d e f g h i j k	1,36	a b c	1,49	a b c d e f g	1,43	a b c d
35	76	h i j k	1,69	e f g	1,70	f g h	1,76	d e
36	77	i j k	1,78	g	1,78	h	1,83	e
37	65	b c d e f g h i	1,46	a b c d e	1,45	a b c d e	1,38	a b c
38	49	a	1,59	b c d e f g	1,49	a b c d e f g	1,65	b c d e
39	55	a b	1,41	a b c d e	1,38	a b	1,28	a
40	70	e f g h i j k	1,65	d e f g	1,62	b c d e f g h	1,76	d e
Average	**68,13**		**1,54**		**1,56**		**1,56**	
Maximum	**78**		**1,78**		**1,78**		**1,83**	
Minimum	**49**		**1,30**		**1,35**		**1,28**	
Diff. (%)	**59,18**		**36,92**		**31,85**		**42,97**	

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 Pper cm of Ventral Face		Number of Rows of Stomata in The Dorsal Face		Number of Rows of Stomata in The Dorsal Face	
11	14,03	b c d e f g	1767,77	e f g h	13,67	c d e f	1810,00	e
12	12,87	a b c d e	1521,10	a b c d e f g	12,47	a b c d e f	1314,43	a b c
13	13,00	a b c d e	1550,00	a b c d e f g	11,43	a b c d	1385,57	a b c d
14	12,33	a b c d	1397,80	a b c d	13,37	b c d e f	1396,67	a b c d e
15	10,57	a	1191,10	a	11,53	a b c d	1295,53	a b c
16	13,30	a b c d e f	1415,53	a b c d e f	10,43	a	1256,67	a b
17	17,00	h i	1984,43	h	14,00	d e f	1683,37	c d e
18	15,33	e f g h i	1697,80	c d e f g h	14,57	e f g	1774,43	d e
19	13,20	a b c d e f	1572,20	b c d e f g	12,20	a b c d e	1588,87	b c d e
20	12,20	a b c d	1566,67	b c d e f g	11,90	a b c d e	1498,90	a b c d e
21	14,70	c d e f g h	1616,67	b c d e f g	12,23	a b c d e	1358,90	a b c d
22	14,77	c d e f g h	1745,53	d e f g h	13,30	b c d e f	1488,90	a b c d e
23	15,67	e f g h i	1691,10	c d e f g h	14,57	e f g	1598,87	b c d e
24	15,00	d e f g h i	1578,90	b c d e f g	13,43	b c d e f	1633,33	b c d e
25	11,53	a b	1296,70	a b	10,20	a	1118,90	a
26	12,90	a b c d e	1404,47	a b c d e	12,43	a b c d e f	1481,10	a b c d e
27	12,00	a b c	1495,53	a b c d e f	11,90	a b c d e	1416,67	a b c d e
28	15,90	f g h i	1780,03	f g h	13,43	b c d e f	1631,10	b c d e
29	12,23	a b c d	1520,00	a b c d e f g	12,43	a b c d e f	1576,67	b c d e
30	14,00	b c d e f g	1593,33	b c d e f g	12,43	a b c d e f	1410,03	a b c d e
31	12,47	a b c d	1431,10	a b c d e f	12,67	a b c d e f	1548,90	b c d e
32	13,10	a b c d e f	1498,20	a b c d e f	10,90	a b	1330,00	a b c
33	13,10	a b c d e f	1573,33	b c d e f g	12,33	a b c d e f	1564,43	b c d e
34	12,33	a b c d	1461,13	a b c d e f	12,77	a b c d e f	1563,30	b c d e
35	16,33	g h i	1868,90	g h	13,57	b c d e f	1566,67	b c d e
36	17,67	i	1983,23	h	16,77	g	1751,77	d e
37	12,10	a b c	1375,57	a b c	12,33	a b c d e f	1526,70	a b c d e
38	15,30	e f g h i	1583,30	b c d e f g	15,00	f g	1500,00	a b c d e
39	11,43	a b	1332,23	a b c	11,20	a b c	1261,10	a b
40	13,77	b c d e f g	1632,23	b c d e f g	13,77	c d e f	1639,97	b c d e
Average	**13,67**		**1570,86**		**12,77**		**1499,06**	
Maximum	**17,67**		**1984,43**		**16,77**		**1810,00**	
Minimum	**10,57**		**1191,10**		**10,20**		**1118,90**	
Diff. (%)	**67,17**		**66,6**		**64,41**		**61,77**	

The average values of studied characters are shown in Table 1. According to Table 1 average needle length is 68,13 mm, needle width is 1,54 mm, needle thickness is 1,56 mm, sheath thickness is 1,56 mm. Average there is 13,67 row of stoma in the ventral face and 12,77 in the dorsal face. There is 1570,86 stomata in the ventral face and 1499,06 stomata in the dorsal face per cm.

The analysis of variance showed that there were significant differences among clones at 0.001, 0.01 and 0.05 probability level in all studied characters. 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 characters

Character	Between populations	Within populations (Error)	σ^2c	σ^2E	H_1^2	H^2
NL	0,019***	0,004	0,00075	0,004	0,157895	0,789474
NWi	0,047**	0,02	0,00135	0,02	0,063232	0,574468
NTh	0,038**	0,014	0,0012	0,014	0,078947	0,631579
ShTh	0,054***	0,028	0,0013	0,028	0,044369	0,481481
NRSV	9,229***	2,053	0,3588	2,053	0,148769	0,777549
NSV	105972***	33621,5	3617,525	33621,5	0,097143	0,682732
NRSD	5,913***	1,853	0,203	1,853	0,098735	0,686623
NSD	81525*	42765,5	1937,975	42765,5	0,043352	0,475431

***, **,* Statistically significant of the 0.001, 0.01, 0.05 probability level,

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

There were large differences among the characters for broad-sense heritability (H_1^2 and H^2) (Table 2). As to the average values, the H_1 values never exceeded 0.5 and this reflects that the variation among grafts caused by environmental variation was always higher than that caused by the genetic variation in the studied orchard. In sight, there is uniform ecological condition in the studied orchard. But, because of micro-ecological differences, the environment was more important than genetics in the orchard.

In this study, large within-clone variation is attracted attention. Meanwhile, there are large variations as to the morphological and anatomical characters among the grafts. Similarly, considerable difference was reported among the grafts by Sıvacıoğlu et al. (2009) in a clonal seed orchard of *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).

Pearson correlation coefficients among the grafts and clones as to the overall mean of the studied characters are shown in Table 3.

Table 3. Pearson correlation coefficients among the studied characters

	NWi	NTh	ShTh	NRSV	NSV	NRSD	NSD
NL	0,357**	0,523**	0,335**	0,295**	0,244*	0,171	0,170
NWi		0,897**	0,783**	0,732**	0,619**	0,564**	0,497**
NTh			0,763**	0,671**	0,583**	0,561**	0,503**
ShTh				0,578**	0,526**	0,431**	0,414**
NRSV					0,888**	0,633**	0,500**
NSV						0,556**	0,520**
NRSD							0,815**

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

According to Table 3, there are significantly relationships between all characters except between needle length and stomata (number and row number). The highest correlation (0,897) is calculated between needle width and needle thickness. In addition, in Scots pine seed orchard positive correlation among the number of rows of stomata in the ventral face and number of rows of stomata in the ventral face (0,888), the number of rows of stomata in the ventral face and number of rows of stomata in the ventral face (0,815).

The high variation within the clones and heritability values estimated in this study indicates opportunities of high selection intensity in the breeding programs for Scots pine. Also, the bigger 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 results 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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