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Altitudinal variation of some morphological characters of Scots pine (*Pinus sylvestris* L.) in Turkey

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The objective of this study was to investigate the altitudinal variation within and between Scots pine (*Pinus sylvestris* L.) populations in Turkey using 23 morphological characters. Seeds were collected from 149 open-pollinated parents (trees) from five populations sampled from different altitudes in the natural distribution range of the species in northern Turkey and raised in a nursery along the coast. The populations were compared based on phenotypic variation of cones, seeds, seed-wings, juvenile seedlings and seedlings morphology, to study the pattern of natural variation of the species. ANOVA and Duncan test showed that the morphological characters differed by populations. Also, the cluster modeling performed on the morphological characters suggested that altitude was a major factor to differentiate the groups. This study showed that there were significant differences within and between the populations in *P. sylvestris* for the morphologic characters studied.

Key words: Genetic variation, morphological characters, Turkey, *Pinus sylvestris*, seed, seedling.

INTRODUCTION

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 snow-fences. 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 western re-

gion, 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). Within its altitudinal and horizontal distribution range, *P. sylvestris* exhibits considerable variation in various form and growth characteristics (Turna, 2003; Eliçin, 1971).

Genetic variation is the fundamental component of adaptation and thus, of stability of the forest ecosystems. This is particularly important when the long-term stability of forest ecosystems is increasingly threatened by environmental stress and mismanagement. Thus, a genetic characterization of natural forest resources is an essential step for a better understanding of genetic resources for the implementation of *in situ* and *ex situ* conservation activities.

The objectives of this study were to investigate the altitudinal variation among Scots pine populations in Turkey, and determine the extent of within population variation, using 23 different morphological characters. This study reports the genetic variability found in nursery (potted seedling) growth of *P. sylvestris* seedlings from

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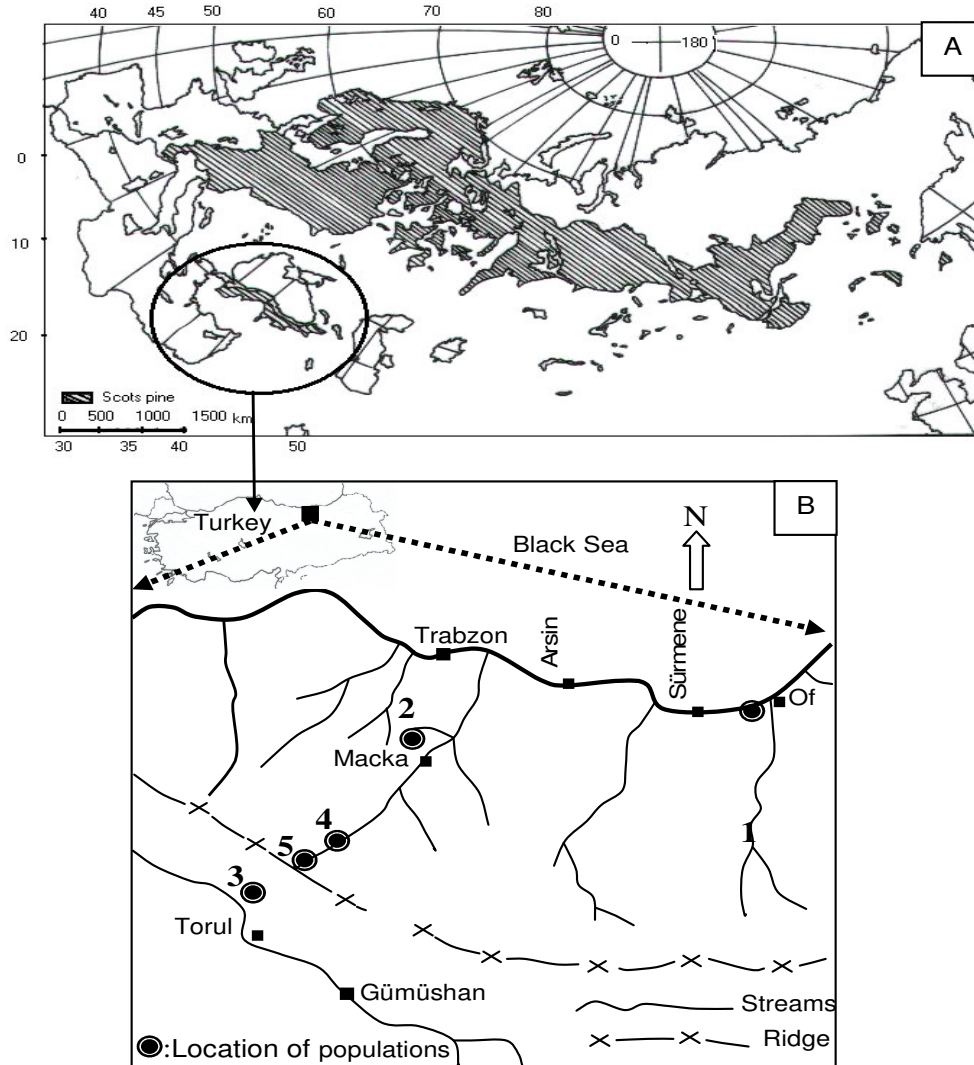


Figure 1. Geographic distribution range (A) and locations of sampled populations (B) of *Pinus sylvestris*. (Figure 1-A from Critchfield and Little, 1966).

149 open-pollinated parents trees sampled from five different populations located at different altitudes. Specific objectives of the study were to: i.) determine patterns of genetic variation within and among families and populations, ii.) describe patterns of variation in association with altitude in the region, and iii.) obtain seedling material to be used in common garden experiments to be established on various elevations for future selection and breeding studies (Işık, 1986; Işık and Kara, 1997).

MATERIALS AND METHODS

Sampling and seed collection

The natural populations of Scots pine (*Pinus sylvestris* L.) were sampled from five different altitudinal zones near Trabzon in Northeastern Turkey, starting from sea level to 450 m (pop No. 1), from 450 to 900 m (pop No. 2), from 900 m to 1350 m (Pop No. 3)

from 1350 to 1800 m (pop No. 4), and from 1800 to 2250 m (pop No. 5). Populations are numbered sequentially according to altitude above sea level (Figure 1). Each population was selected in each 450 m altitudinal range

The seed collection was carried out to serve a relatively large scale research program initiated on genetic diversity and eco-physiological studies Scots pine. The methods for sampling of individual trees and measurements on morphological characters were based on literature on some previous studies, such as Maley and Parker (1993), Koral et al. (1995), Lee et al. (1998), Matziris (1998), Turna (2004) and Boulli et al. (2001). The principal geographic characteristic of all populations were summarized in Table 1.

Sampled trees were chosen from the related populations using randomized sampling design at each elevational zone. Average distance between any two sampled trees was about 150 m. On the average, 30 trees were sampled from each population (Table 1), and about 15 - 20 healthy mature cones were collected from the last year's crops at the upper part of the crown of each tree. The cones were sun dried and extracted manually, cleaned and stored until measurements.

Table 1. Description of the five *P. sylvestris* populations sampled in northern Turkey.

Population number	Population names	Number of trees sampled	Average Altitude (m)	Altitudinal range (m)	x*	y*
1	Çamburnu	28	225	0 - 450	4531000-4530000	603000-602000
2	Maçka	30	675	450 - 900	4527500-4527000	557250-556750
3	Zigana-3	31	1125	900 - 1350	4498500-4496000	529500-527000
4	Zigana-2	30	1575	1350 - 1800	4502750-4501000	535500-534000
5	Zigana-1	30	2025	1800 - 2250	4501000-4499000	535000-533000

*UTM coordinate system.

Experimental design and nursery treatment

The seeds were sown by family in a randomized complete block design with three replications in April, 2001. Seeds were not pretreated prior to sowing. Additional seeds from each parent tree (family) were also sown in trays containers (5 x 5 x 15 cm dimension), each with 28 cells. The seeds were sown with 70% peat molds + 30% tea waste in the containers at "State Forestry Nursery" in Trabzon (altitude 5 m). For the seedlings grown in the nursery, stream sands were used as seed covering material. Aside from the labeling required by the experiment, general nursery production methods were applied on the growing stock.

Characters and their measurements

A total of 23 morphological characters, belonging to either cone, seed or seedling traits were observed or measured. Cone characters were measured on each 10 cones per tree:

- i.) Cone length (CL).
- ii.) Cone width (CW).
- iii.) Cone length to width ratio (CL/CW).

For the measurement of seed characters, 10 winged seeds were measured for each sampled tree. The seeds were chosen randomly from a composite sample derived from several cones of the same parent tree. The wings were removed and both the seed and wing traits were quantified by using:

- i.) Seed length (SdL).
- ii.) Seed width (SdW).
- iii.) Seed length to width ratio (SdL/SdW).
- iv.) Wing length (WL).
- v.) Wing width (WW).
- vi.) Wing length to wing width ratio (WL/WW).

According to experimental design, the number of germination seedlings was counted twice a week, starting on the 15th day and continuing through the 60th day after sowing. The number of days was recorded to reach 50% germination rate.

The juvenile seedling traits are those that we measured just after the completion of 50% germination. To measure the juvenile seedling traits, 10 juvenile seedlings per container (trays) were uprooted and measured for:

- i.) Dry root weight of juvenile seedling (DRWJS).
- ii.) Dry stem weight of juvenile seedling (DSWJS).
- iii.) Total dry weight of juvenile seedling (TDWJS).
- iv.) The number of the cotyledon (CN).
- v.) Hypocotyls length (HL).

The mature seedling traits are those that we measured at the end

of the growing season. We manually uprooted 10 seedlings per container and prepared them for study and measurement. These were:

- i.) The seedling fresh root weight (FRWS).
- ii.) The seedling fresh stem weight (FSWS).
- iii.) The seedling total fresh weight (TFWS).
- iv.) The seedling dry root weight (DRWS).
- v.) The seedling dry stem weight (DSWS).
- vi.) The seedling total dry weight (TDWS).
- vii.) Seedling length (SL).
- viii.) Root collar diameter (RCD).
- ix.) Lateral branch number (BN).

Data analysis

Analysis of morphological variations was based on the measurements of 23 characters related to cones, seeds, seed wings, juvenile seedlings and mature seedlings. Variations among and within populations were analyzed using ANOVA-one way. Correlation between pairs of morphological characters and altitude was evaluated using Pearson's correlation coefficient (Snedecor and Cochran, 1968; Turna, 2004). We also examined overall variations of morphological characters among populations using cluster analysis using SPSS 11.5.

RESULTS

The mean values and standard deviations of the cone, seed, seed- wing, juvenile seedlings and mature seedling characters by populations are shown in Table 2. The overall means, and standard deviations, (between populations and within populations), F, and significant levels are also presented in Table 2. The results showed that there were high levels of variation in most of the morphological characters, both among and within populations. The analysis of variance revealed that there were significant differences between and within populations at 0.01 probability level on all morphological characteristics with the exception of fresh root weight of seedling and the ratio of seed length to weight. In addition, in terms of 7 of the 9 seedling characters, population number 1 exhibited the highest values, and according to Duncan tests was significantly different from the others (Table 2). This may be due to marginal and unique nature of this population, which is the only population that is located to the sea level and on the most

Table 2. Results of analyses of variance and Duncan tests for characters in *P. sylvestris*.

Characters (unit) ***		Population No , Means and Standard Deviation*					Overall mean	F	P
		1	2	3	4	5			
Cone	CL(cm)	3.546 ± 0.70 c	4.176 ± 0.55 b	4.572 ± 0.67 a	4.670 ± 0.67 a	4.525 ± 0.47a	4.31 0 ± 0.73	15.818	0.000
	CW(cm)	1.815 ± 0.26 d	2.050 ± 0.24 c	2.233 ± 0.28 b	2.421 ± 0.28 a	2.429 ± 0.19 a	2.195 ± 0.34	30.606	0.000
	CL/ CW	1.94 ± 0.19b	2.04 ± 0.17c	2.046 ± 0.15c	1.926 ± 0.11ab	1.863 ± 0.13a	1.965 ± 0.17	7.782	0.000
Seed	SdL (mm)	4.561 ± 0.54c	4.945 ± 0.44 b	5.390 ± 0.55a	5.388 ± 0.54 a	5.448 ± 0.35 a	5.156 ± 0.59	18.018	0.000
	SdW (mm)	2.548 ± 1.19 c	2.728 ± 0.16 b	3.029 ± 0.23a	2.997 ± 0.19 a	3.003 ± 0.17a	2.867 ± 0.27	36.876	0.000
	SdL/SdW	1.789 ± 0.16a	1.81 ± 0.121a	1.780 ± 0.12 a	1.795 ± 0.101 a	1.816 ± 0.11a	1.799 ± 0.12	0.474	0.755
Wing	WL (mm)	13.75 ± 2.47c	16.07 ± 1.64 b	18.023 ± 2.26 a	17.715 ± 2.62 a	17.397 ± 1.77 a	16.638 ± 2.65	18.623	0.000
	WW (mm)	4.786 ± 0.45 d	5.052 ± 0.38 c	5.358 ± 0.47 b	5.473 ± 0.48 ab	5.662 ± 0.39a	5.723 ± 0.53	18.514	0.000
	WL / WW	2.864 ± 0.36 a	3.182 ± 0.25 b	3.362 ± 0.29 c	3.233 ± 0.36 bc	3.081 ± 0.32 b	3.150 ± 0.36	10.021	0.000
J.Seedling	DRWJS (g)	0.014 ± 0.005b	0.015 ± 0.004 b	0.012 ± 0.005 b	0.018 ± 0.007 a	0.019 ± 0.007a	0.0156 ± 0.01	8.117	0.000
	DSWJS (g)	0.120 ± 0.043a	0.09 ± 0.019b	0.0568 ± 0.02c	0.1110 ± 0.04a	0.103 ± 0.03ab	0.096 ± 0.04	18.151	0.000
	TDWJS (g)	0.1339 ± 0.05a	0.1084 ± 0.02 b	0.0690 ± 0.02c	0.1291 ± 0.05 a	0.1225 ± 0.04 ab	0.112 ± 0.04	16.095	0.000
	CN (number)	6.278 ± 0.47b	6.316 ± 0.38b	6.616 ± 0.53a	6.575 ± 0.50a	6.487 ± 0.43ab	6.458 ± 0.48	3.163	0.016
	HL (mm)	20.969 ± 3.49 a	16.924 ± 1.35cd	16.377 ± 1.61 d	17.968 ± 2.08bc	18.429 ± 1.65b	18.151 ± 2.73	16.511	0.000
Mature Seedling	FRWS (g)	2.224 ± 0.53 a	2.144 ± 0.42 a	1.885 ± 0.47 a	2.143 ± 0.47 a	2.087 ± 0.41 a	2.094 ± 0.47	2.324	0.059
	FSWS (g)	3.093 ± 0.82 a	2.543 ± 0.54 b	1.413 ± 0.33 d	1.918 ± 0.45 c	1.841 ± 0.32 c	2.145 ± 0.78	47.059	0.000
	TFWS (g)	5.317 ± 1.25 a	4.686 ± 0.87 b	3.298 ± 0.74 d	4.061 ± 0.88c	3.928 ± 0.67 c	4.238 ± 1.12	21.722	0.000
	DRWS (g)	0.868 ± 0.22 a	0.692 ± 0.12 bc	0.640 ± 0.14 c	0.702 ± 0.13 bc	0.728 ± 0.15 b	0.723 ± 0.17	9.00	0.000
	DSWS (g)	1.127 ± 0.31 a	0.795 ± 0.14 b	0.530 ± 0.12 d	0.705 ± 0.15 bc	0.671 ± 0.12 c	0.759 ± 0.27	43.526	0.000
	TDWS (g)	1.995 ± 0.51 a	1.488 ± 0.24 b	1.170 ± 0.25 c	1.407 ± 0.27 b	1.399 ± 0.25 b	1.483 ± 0.41	26.974	0.000
	SL (cm)	8.13 ± 0.82a	7.319 ± 0.87b	5.228 ± 0.40 d	5.919 ± 0.70c	5.759 ± 0.46c	6.445 ± 4.38	94.034	0.000
	RCD (mm)	3.135 ± 0.43a	2.755 ± 0.21 b	2.475 ± 0.30 c	2.696 ± 0.27b	2.723 ± 0.23 b	2.750 ± 0.36	18.793	0.000
	BN (number)	2.858 ± 0.79a	2.766 ± 0.69 a	2.033 ± 0.59 c	2.397 ± 0.68 b	2.509 ± 0.50ab	2.505 ± 0.71	7.455	0.000

***For abbreviations of the characters, see the text, Materials and Methods.

*Means with the same letter on a given row are not significantly different (at the 0.05 level) from each other.

northeastern flank within the distribution in Turkey (Figure 1).

Once the variation between the populations was revealed, measured characters were grouped using Duncan test (Table 2). Thus, 4th (Zigana 2) and 5th (Zigana 1) populations were grouped in different groups regarding the morphological characters

investigated.

Morphological traits in *P. sylvestris* were found to be quite variable. Cone length varied from 2.51 cm (pop No. 1) to 6.13 cm (pop No. 4) with a mean value of 4.310 cm. The cone width varied from 1.33 cm (pop No. 1) to 2.98 cm (pop No. 4) with a mean value of 2.195 cm. Seed length

varied from 3.40 mm (pop No. 1) to 6.90 mm (pop No. 3) with a mean value of 5.156 mm. The seed width varied from 2.25 mm (pop No. 1) to 3.70 mm (pop No. 3) with a mean value of 2.867 mm. Wing length varied from 9.35 mm (pop No. 1) to 23.15 mm (pop No. 4) with a mean value of 16.638 mm. The wing width varied from 3.90 mm

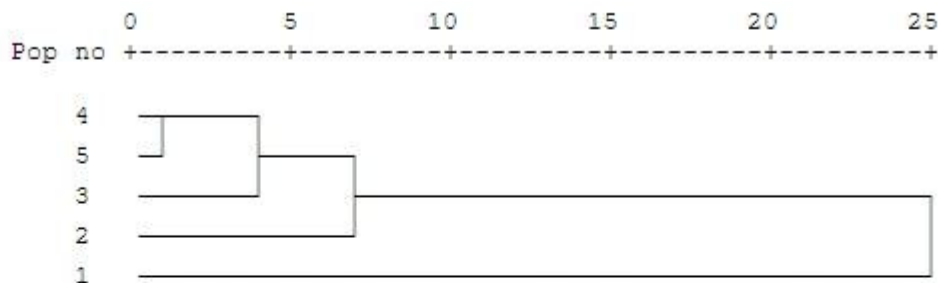


Figure 2. Hierarchical cluster of five different populations of Scots pine, based on morphological characters.

(pop No. 1) to 6.80 mm (pop No. 3) with a mean value of 5.723 mm.

Dry root weight of juvenile seedling varied from 0.0063 g (pop No. 3) to 0.035 g (pop No. 5) with a mean value of 0.0156 g. Dry stem weight of the juvenile seedling varied from 0.013 g (pop No. 1) to 0.23 g (pop No. 1) with a mean value of 0.096 g. Total dry weight of juvenile seedling varied from 0.032 g (pop No. 1) to 0.25 g (pop No. 1) with a mean value of 0.112 g. Number of the cotyledon varied from 5.2 (pop No. 5) to 7.16 (pop No. 3) with a mean value of 6.458. Hypocotyl length varied from 13.80 mm (pop No. 2) to 33.0 mm (pop No. 1) with a mean value of 18.151 mm.

Fresh root weight of seedling varied from 0.99 g (pop No. 3) to 3.63 g (pop No. 1) with a mean value of 2.094 g. Fresh stem weight of seedling varied from 0.80 g (pop No. 3) to 5.32 g (pop No. 1) with a mean value of 2.145 g. Total fresh weight of seedling varied from 2.03 g (pop No. 3) to 8.10 cm (pop No. 1) with a mean value of 4.238 g. Dry root weight of seedling varied from 0.37 g (pop No. 4) to 1.43 cm (pop No. 1) with a mean value of 0.723 g. Dry stem weight of the seedling varied from 0.31 g (pop No. 3) to 1.98 g (pop No. 1) with a mean value of 0.759 g. Total dry weight of seedling varied from 0.74 g (pop No. 3) to 3.26 cm (pop No. 1) with a mean value of 1.483 g.

Seedling length varied from 4.38 cm (pop No. 3) to 10.49 cm (pop No. 1) with a mean value of 6.445 cm. Root collar diameter varied from 1.83 mm (pop No. 3) to 4.17 mm (pop No. 1) with a mean value of 2.750 cm. Branch number varied from 1.0 (pop No. 4) to 6 (pop No. 1) with a mean value of 2.505 (Figure 2).

In order to determine degree of similarity or dissimilarity among populations based on morphological characters, the cluster analysis was also conducted (Figure 2). At the 5.0 distance unit, hierarchical clustering process leads to 3 different groups. The first group included population No. 3 (900 - 1350 m), population No. 4 (1350 - 1800 m) and population No. 5 (1800 - 2250 m). Population No. 1 (0 - 450 m) and population No. 2 (450 - 900 m) were in different groups.

Relationships between all morphological traits were expressed in a correlation matrix in Table 3. According to this table, 19 different morphological features are signi-

ficantly correlated at 0.05 or 0.01 significant level. This can be regarded on indicator of high variation in *P. sylvestris*. Therefore each population should be conserved their own places (*in situ*).

DISCUSSION

Since the morphometric variation in several tree species in Turkey has been relatively less studied, it should therefore be given a high priority in forest breeding and selection system (Işık and Kara, 1997). The aim of the present study was to extend the knowledge of the geographic (altitudinal) patterns of morphological variation of Scots pine in Turkey.

The results of this study reflecting some geographical parameter (altitude) clearly showed that ecological differentiation along gradients of various watersheds is the major driving force behind cone, seed, wing, juvenile seedling and seedling variation. By comparing characteristics of cone seed wing juvenile seedling and seedling in different environmental conditions, some general understanding about the effects of environmental factors on altitude can be obtained.

Considering the clustering groups of Scots pine, the grouping was statistically significant. Namely, a clear geographic pattern typified by altitude grouping was revealed in this study. Adjacent populations (for example pop No. 3, pop No. 4 and pop No. 5) that presented close relationships among ecological features tended to cluster into the same group. In addition, Pearson's coefficient correlations analyses were in agreement with the general tendency of relationships between the most of characters and altitude.

This results of study showed that the populations are not homogeneous with regard to the characteristic of cone, seed, wing, juvenile seedling and seedling. Populations consist of the trees having more or less different characteristics in cone, seed, wing, juvenile seedling, and seedling. The reason of the fact that the grouping and differences existed among the studied population in terms of the morphological characters may explain that there were different origins or varieties form-

Table 3. Pearson correlation coefficients between pairs of morphological characters of the *P. sylvestris* populations studied.

***	DSWS	DRWS	TDWS	FSWS	FRWS	TFWS	SL	RCD	BN	CL	CW	WL	WW	SdL	SdW	HL	CN	DRWJS	DSWJS	
DSWS	1	0.776*	0.966*	0.967*	0.565*	0.909*	0.750*	0.805*	0.611*	-0.432*	-0.407*	-0.447*	-0.359*	-0.461*	-0.544*	0.396*	-0.118	0.024	0.488*	
DRWS		1	0.913*	0.720*	0.801*	0.836*	0.449*	0.733*	0.459*	-0.292*	-0.235*	-0.304*	-0.216*	-0.319*	-0.358*	0.338*	-0.068	0.091	0.381*	
TDWS			1	0.922*	0.694*	0.932*	0.670*	0.822*	0.584*	-0.400*	-0.360*	-0.414*	-0.322*	-0.429*	-0.499*	0.395*	-0.105	0.053	0.472*	
FSWS				1	0.585*	0.940*	0.803*	0.767*	0.642*	-0.434*	-0.431*	-0.438*	-0.372*	-0.471*	-0.568*	0.345*	-0.121	0.015	0.467*	
FRWS					1	0.826*	0.332*	0.579*	0.326*	-0.201**	-0.146	-0.181**	-0.163**	-0.240*	-0.277*	0.233*	-0.032	0.134	0.343*	
TFWS						1	0.698*	0.776*	0.583*	-0.386*	-0.361*	-0.380*	-0.327*	-0.428*	-0.511*	0.338*	-0.097	0.066	0.468*	
SL							1	0.583*	0.439*	-0.394*	-0.475*	-0.410*	-0.370*	-0.414*	-0.554*	0.299*	-0.160	-0.040	0.392*	
RCD								1	0.529*	-0.278*	-0.195**	-0.310*	-0.184**	-0.342*	-0.386*	0.423*	-0.100	0.132	0.542*	
BN									1	-0.223*	-0.178**	-0.196**	-0.116	-0.206**	-0.233*	0.164**	-0.043	0.181	0.428*	
CL										1	0.867*	0.880*	0.629*	0.763*	0.645*	-0.197**	0.349*	0.140	-0.105	
CW											1	0.797*	0.719*	0.734*	0.676*	-0.146	0.339*	0.281*	0.000	
WL												1	0.716*	0.823*	0.724*	-0.187**	0.342*	0.096	-0.149	
WW													1	0.732*	0.757*	-0.099	0.318*	0.244*	-0.024	
SdL														1	0.818*	-0.121	0.380*	0.243*	-0.085	
SdW															1	-0.194**	0.453*	0.238*	-0.150	
HL																1	0.056	0.162**	0.456*	
CN																	1	0.164**	0.011	
DRWJS																		1	0.673*	
DSWJS																				1

***For abbreviations of the characters, see the text, Materials and Methods.

**Significant at the 0.01 and 0.05 levels, respectively.

ing the Scot pine stands. Variation in most of these characteristics appeared to be related mostly to altitude. The result is similar to the findings of Chmura (2006) for *Picea abies* and Kara et al. (1997), Işık and Kara (1997) and Işık (1986) for *Pinus brutia*. Also these variations and differences were caused by divergent gene and genotype frequencies between and within populations. The result reported here for *P. sylvestris* are in accordance with the findings of Alia et al. (2001), Harju et al. (1996), Nilsson and Walfridsson (1995), Prus-Glowacki and Stephan (1994), Hertel and Kohlstock (1994) and Ayan et al. (2005) and Bilgen and Kaya (2007).

This study exhibited higher adaptational plasticity and higher stability of the species under varying environmental conditions. It could be possible that adaptive strategy of coniferous species in marginal populations is connected with a high genetic variability as already reported by Szmidi and Muona (1985) for northern Swedish populations of *P. sylvestris*, by Kinloch et al. (1986) for Scottish populations of *P. sylvestris*, by Dangasuk and Panetsos (2004) for populations of *P. brutia*, and by Tigerstedt (1973) and Bergmann (1978) for populations of *P. abies*. The seed transfer region for this taxon in which the longest vertical distance should be realized in a total of

450 m is proposed. This study supported both the previous findings, and provided the flexibility among the altitudes.

P. sylvestris grows from 1000 to 2500 m. In this respect, the variation in the population 1 (0 - 450 m) and population 2 (450 - 900 m) is important. Within its distribution, Scots pine can grow in completely different ecological conditions. A successful tree breeding program may be prepared by considering these different characteristics.

Scots pine can be adapted various ecological conditions in Turkey. For instance, it can be observed by the sea level in Trabzon-Çamburnu (population 1) as an extra ordinary example of its

range. Moreover fire threats include in Trabzon-Çamburnu. Thus, high variations of *P. sylvestris* in this research are, particularly in pop No. 1, the need for in-situ conservation.

This study provided and suggested the information that forestry practices in the Scots pine stands, such as selection of seed sources and determination of seed transfer zones, should strongly consider elevation gradients in this region. In this species for which genetics tests have not been established, important differences have been found among the populations from different altitudes.

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