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GENETIC DIVERSITY AMONG POPULATIONS IN BLACK PINE (*PINUS NIGRA* ARNOLD. SUBSP. *PALLASIANA* (LAMB.) HOLMBOE) SEED STANDS IN TURKEY

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Abstract

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Anatolian Black Pine (*Pinus nigra* Arnold. subsp. *pallasiana* (Lamb.) Holmboe) is one of the widespread and important forest tree species in Turkey. The stands of this species occupy roughly 4.2 million ha, which about 1.8 million ha of that are considered to be non productive forests. Anatolian Black Pine can be spread to the steppe regions in Anatolia. The semi-arid steppe regions are evaluated as potential afforestation areas. Actually, the seed demand for this species is mainly supplied from current 53 seed orchards and 79 seed stands.

The objective of this study was to investigate the genetic variation in Anatolian Black Pine seed stands. The number of 12 morphological characters was measured and observed on juvenile seedling (root-collar diameter, percentage of germination and living, ratio of live to germination, hypocothyl length, cotyledon number, cotyledon length and width) and seedling (root-collar diameter, epycotyl length, needle length and width) from 14 seed stands in Turkey. The obtained data was analyzed by ANOVA, Cluster and Duncan test. Cluster and ANOVA tests showed that there were significant differences within the *Pinus nigra* seed stands for the characters.

Key words: Anatolian Black Pine; seed stands; genetic variation; morphological characters

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). It grows from an elevation of 100 m up to 1800 m but mainly from 500-800 up to 1200-1600 meters (Bassiotis, 1967). It is considered one of the most important coniferous species and is extensively used in reforestation programs throughout the country (Matziris, 1978; Palmerg, 1983).

Anatolian black pine 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 (Yaltirik 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.

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 55 of seed orchards and 79 of seed stands.

In this study, we investigated genetic variation in morphological characters of *Pinus nigra* Arnold. subsp. *pallasiana* (Lamb.) Holmboe from 14 seed stands in Turkey. Our main objective was to examine the distribution of genetic variation among populations in terms of some seedling characters.

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Material and Methods

Seed collection and sowing

Open pollinated seed materials from 14 different populations of *Pinus nigra* Arnold. subsp. *pallasiana* (Lamb.) Forest Tree Seed and Tree Breeding Research Directorate in Turkey provided Holmboe from 14 seed stand in Turkey. Bulked seeds for each population were collected from already established stands (Table 1).

In this study, Black pine seeds were sown by using conventional methods at Nursery in Kastamonu (Altitude: 850 m) with 4 replications to river sand, forest soil and peat in 1:1:2 proportion, respectively, and covered with perlite.

Seed and seedling morphological variables studied and data collection

While cotyledon number (CN), length (CL) and width (CW), percentage of germination (GP) and survival (AP), root collar diameter (RCD), hypocotyls length (HL) were measured from all juvenile seedlings in June and root collar diameter (RCD1) epycotyl length (EL), needles length (NL) and width (NW) were measured from all seedlings in October. In addition, ratio of survival to germination was calculated (AGR=AP/GP).

Statistical Analyses

Data were subjected to multi-way analysis of variance, Duncan test and Hierarchical Cluster analysis with SPSS statistical package program. Relationships between 13 related characters were tested using correlation analyses.

Moreover, collected data was determined with Penrose formula. Data were standardized before the calculations and the morphological distance among populations were estimated as; Where $Z_{i,k}$ is standardized values of the k^{th} characteristics of the i^{th} population, $X_{i,k}$ is original average of the k^{th} characteristics of the i^{th} populations for the k^{th} characteristics and S_k is the standard deviation of the studied populations for the k^{th} characteristics (Þevik et al., 2010).

$$D_{i,j} = \sum_{k=1}^{p} \frac{(\mu_{ki} - \mu_{kj})^2}{p.V_k} \quad ,$$

where, D_{tj} is the morphological distance between the i^{th}, population and the j^{th} populations, n is the number of studied characteristics, μ_{kj} is the standardized values of the k^{th} of the I^{th} population, μ_{kj} is the standardized values of the k^{th} characteristics of the j^{th} population, V_k is the variance of standardized averages of the k^{th} characteristics (Yahyaoglu et al,, 2001) was applied by standardized values in SPSS statistical package program (Þevik et al., 2010).

$$Z_{i,k} = \frac{(X_{i,k} - \bar{x}_k)^2}{S_k}$$

Results

The analysis of variance showed that there were significant differences among populations at 0.5 for epycotyl length and seedling root collar diameter, 0.01 for juvenile seedling root collar diameter, cotyledon number and needle width and 0.001 for percentage of germination and alive, cotyledon length. The results of variance show that there are no significance differences between the populations according to ratio of germination to alive, hypocotyls length, cotyledon width

Table 1
The locations of seed stands

Pop. No	Region	District	Unit	Altitude, m
1	Çanakkale	Yenice	Asar	270
2	Çanakkale	Kalkım	Kalkım	550
3	Bursa	Bursa	Bursa	950
4	Kütahya	Tavşanlı	Alabarda	1050
5	Balıkesir	Alaçam	Gölcük	1050
6	Kütahya	Simav	Kiçir	1100
7	Bursa	İnegöl	Boğazova	1200
8	Ankara	Nallıhan	Uluhan	1250
9	Bursa	Keleş	Sorgun	1350
10	Balıkesir	Bigadiç	Aktuzla	1378
11	Kütahya	Domaniç	Dereçarşamba	1400
12	Kütahya	Tavşanlı	Balıköy	1500
13	Denizli	Çal	İnceler	1560
14	Isparta	Sütçüler	Tota	1600

and needle length. Mean values and multiple comparisons of studied morphological characters shown in Table 2. According to the table minimum GP (15%), AP (15%) and maximum AGP (100%) values are determined to population 7. Maximum GP (68.75%) and AP (67.50%) values are determined to population 11. Population 12 shows maximum values of RCD (1.15mm) and CN (8.38). According to results of Duncan test, population 5 is in the last homogeny groups for all characters.

Table 2
Mean values and multiple comparisons of studied morphological characters

POP	GP		AP		AGR	RCD		HL	CN	
1	22.50	ab	21.25	ab	93.75	1.07	ab	17.63	**7.38**	a
2	40.00	bcd	40.00	bcdef	100.00	1.02	a	17.00	7.77	abc
3	30.00	abc	28.75	abc	95.83	1.06	ab	**15.80**	7.50	a
4	56.25	def	53.75	defg	92.00	0.98	a	18.04	8.00	abc
5	61.25	ef	**56.25**	efg	90.13	1.04	ab	**20.70**	8.00	abc
6	61.25	ef	56.25	efg	92.78	0.97	a	20.32	7.80	abc
7	**15.00**	a	**15.00**	a	**100.00**	0.97	a	17.29	7.70	ab
8	41.25	bcde	40.00	bcdef	97.78	**0.96**	a	16.47	7.53	a
9	56.25	def	51.25	defg	86.98	0.99	a	18.29	8.00	abc
10	35.00	bc	33.75	abcd	93.33	1.05	ab	19.45	8.36	c
11	**68.75**	f	**67.50**	g	98.44	0.99	a	17.71	8.00	abc
12	47.50	cde	37.50	bcde	**80.21**	**1.15**	b	18.73	**8.38**	c
13	56.25	def	48.75	cdefg	85.42	0.98	a	17.26	7.88	abc
14	68.75	f	60.00	fg	86.46	0.99	a	18.60	8.19	bc
Average	47.14		43.57		92.37	1.02		18.09	7.89	
F Values	6.737***		5.249***		1.451ns	2.263**		1.285ns	2.342**	
Max.	68.75		67.50		100.00	1.15		20.70	8.38	
Min.	15.00		15.00		80.21	0.96		15.80	7.38	
Diff. (%)	358.00		350.00		25.00	20.00		31.00	14.00	

Table 2 (Continue)

POP	CL		CW	RCD1		EL		NL	NW	
1	**18.33**	a	0.77	1.42	abc	11.76	ab	**19.55**	**0.78**	a
2	19.43	ab	0.78	1.40	abc	13.66	abc	**25.86**	0.90	bcd
3	22.81	abcd	0.80	1.34	abc	14.90	bc	21.82	0.94	bcd
4	23.01	abcd	**0.82**	1.40	abc	11.55	ab	23.74	0.94	bcd
5	**30.31**	e	**0.82**	1.52	c	14.52	abc	25.06	**1.01**	d
6	25.59	cde	0.81	1.42	abc	**15.60**	c	24.60	1.00	d
7	24.38	bcd	0.79	1.47	bc	13.95	abc	22.83	0.86	abc
8	20.68	abc	0.76	1.42	abc	14.06	abc	23.92	0.90	bcd
9	24.66	bcd	0.74	**1.52**	c	15.02	bc	24.58	0.94	cd
10	22.82	abcd	0.78	1.47	bc	15.31	c	23.71	0.88	abc
11	26.43	de	0.77	1.49	bc	15.60	c	23.47	0.92	bcd
12	21.31	abcd	0.74	1.38	abc	13.69	abc	23.33	0.86	abc
13	22.92	abcd	**0.73**	**1.25**	a	**11.38**	a	20.85	0.82	ab
14	22.93	abcd	0.79	1.29	ab	12.72	abc	22.38	0.84	abc
Average	23.26		0.78	1.41		13.84		23.26	0.90	
F Values	3.244***		.864ns	1.939*		2.055*		1.357ns	2.966**	
Max.	30.31		0.82	1.52		15.60		25.86	1.01	
Min.	18.33		0.73	1.25		11.38		19.55	0.78	
Diff. (%)	65.00		12.00	22.00		37.00		32.00	29.00	

Similarly, population 1 is in the first homogeny group for all characters. At same time, minimum values of CN (7.38), CL (18.33mm), NL (19.55mm) and NW (0.78mm) are calculated to population 1. Morphological distance and grouping according to Penrose formula are shown in Table 3.

Maximum values are calculated between the population 7 and population 13 (4.42) and population 1 (3.84). The third highest value is calculated between population 5 and population 12 (3.84). Minimum values are 0.5 (population10 and population14), 0.58 (population8 and population3) and 0.61 (population10 and population 3). According to totally values, maximum values are shown by population 5 (36.63) and population 1 (35.17). Minimum totally values are shown by population 14 (18.27) and population 3 (18.73).

On the cluster dendrogram constructed based on Euclidean distances with the use of the nearest neighbor method for 12 quantitative morphological traits, two distinct groups can be noticed: the first is population 5 and the others. The second group cans distinct two groups, population1 and the others. According to these results, it can be said that there are three main groups (Figure 1).

Results of correlation analyses are shown in Table 4. Positive significant correlation was found between many characters (Table 4). The maximum values are 0,961 (GP and AP), 0.587 (NL and EL) and 0.536 (NW and NL). According to these results, altitude is relation with only for GP and AP.

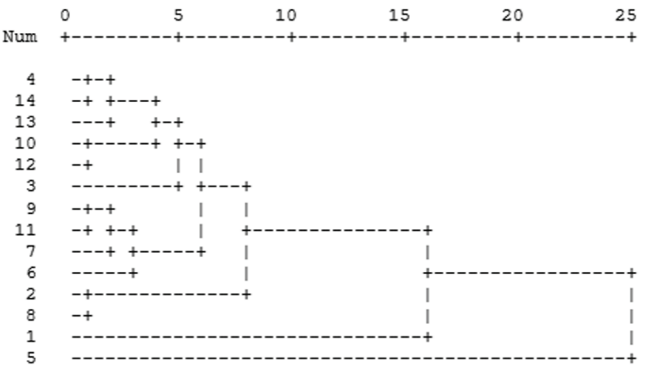

Fig. 1. Dendrogram of 14 populations of Black Pine based on morphological characters

Discussion

Genetic variation can be determined with morphological characters (Sevik, 2012; Sevik et al., 2012), isoenzymes analysis (Muona et al., 1987; Bilgen and Kaya, 2007; Turna, 2003) and DNA markers (Clark et al., 2000; Goldstein, 1995). Many researchers use morphological characters for determination to genetic variation on *Pinus ponderosa* (Linhart et al., 1981), *Pinus sylvestris* (Glowacki and Stephan, 1994; Quencez and Bastien, 2001; Þevik et al., 2010) and *Pinus oocarpa* (Romero et al., 2006). In this study, according to results of Duncan test, penrose Formula and cluster dendogram, it can be said that population-5 and population-1 are genetically the most varried population from the others. According to Table 1, the altitude of population-5 is 1250 m and altitude of population-1 is 1100 m. Generally, these altitudes are optimum for black pine but maximum and minimum values are determined in these altitude. This results show that local conditions more effective on morphological features than altitude. Similar results were shown to other studies; Aguinagalde et

Table 3
Results of penrose analysis

	2	3	4	5	6	7	8	9	10	11	12	13	14	TOTAL
1	2.4	2.18	2.7	3.13	1.92	**3.8**	2.53	2.75	2.41	2.75	3.26	3.11	2.26	35.17
2		1.43	2	3.14	2.01	2.94	1.06	1.47	1.92	1.67	3.57	2.97	1.67	28.23
3			1.4	2.41	0.95	2.17	**0.6**	1.19	**0.61**	1.49	1.71	1.8	0.78	18.73
4				3.61	1.15	2.57	1.51	0.71	1.38	0.97	2.23	1.52	1.16	22.93
5					1.37	2.51	2.68	2.88	2.94	2.37	**3.84**	3.17	2.58	36.63
6						2.34	1.39	1.49	1.57	1.57	2.42	1.73	1.54	21.45
7							1.59	3.04	1.07	1.1	2.49	**4.42**	1.27	31.35
8								1.62	0.7	1.26	1.14	3.26	1.19	20.51
9									1.86	1.32	2.44	0.66	0.86	22.29
10										0.76	1.93	2.93	**0.5**	20.58
11											2.6	2.38	0.65	20.89
12												3.82	2.2	33.65
13													1.61	33.38
14														18.27

Table 4
Results of correlation analysis

	GP	AP	AGR	RCD	HL	CN	CL	CW	RCD1	EL	NL	NW
ALT	.365**	.331**	-0.090	-0.092	0.008	0.062	-0.004	-0.092	-0.017	-0.017	-0.048	0.066
GP		**.961****	0.071	-0.133	.272**	.201**	.359**	0.107	0.020	-0.023	0.056	.196**
AP			.369**	-0.130	.278**	.172*	.383**	0.124	0.071	-0.011	0.069	.226**
AGR				-0.056	0.053	-0.070	0.103	0.061	0.132	0.038	0.039	0.125
RCD					0.014	0.117	0.033	0.032	.187*	0.069	0.033	0.036
HL						.212**	0.104	.294**	0.067	0.003	0.138	0.139
CN							0.066	0.081	0.084	.154*	.277**	0.064
CL								0.128	.224**	.182*	.239**	.330**
CW									0.070	0.062	0.026	0.128
RCD1										.430**	.308**	.333**
EL											**.587****	.382**
NL												**.536****

**. Correlation is significant at the 0.01 level; *. Correlation is significant at the 0.05 level.

al. (1997) determined to relationships among five black pine populations of European using morphometric and isozyme markers. They found that Corsican population is the most distant, with relative lack of genetic variation, probably due to its geographic isolation. Nicolic and Tucic (1983) reported that a correlation with geography seems to exist. Population differentiation exists in Black Pine, but also that differences in allelic frequencies do not follow geographic pattern. *Pinus nigra* have many geographical variants are often not clearly separable, because much of its variation shows a clinical pattern (Aguinagalde et al., 1997). Aguinagalde and Bueno (1994) examined *P. nigra* subsp. *salzmanii* from the same forest area of the Iberian Range, looking for morphometric and/or isoenzymatic markers capable of differentiating between these very close populations.

The populations are not homogeneous with regard to the morphological characteristics. The difficulties in Black Pine taxonomy explain the several studies on morphology, terpenoids composition and more recently on isozyme variation that have been conducted with this species (Nikolic and Tucic, 1983; Scaltsoyiannes et al., 1994).

These results could be used in preparation of gene map, seed transfer zones, determination of breeding populations, gene conservation areas, geographic variation and resulting of provenance trials of the species in short period.

Conclusion

The main implication of this paper is that tree populations had more or less different characteristics in 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 forming to *Pinus nigra*.

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