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Variation in Cone and Seed Characteristics in Three Populations of Anatolian Black Pine (*Pinus nigra* subsp. *pallasiana*) in Turkey

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ABSTRACT

Anatolian black pine is a native coniferous tree species of Turkey. It is economically important species and especially suited to use in water deficit areas for afforestation studies, therefore it has great importance. Currently, only scattered populations Anatolian black pine have remained in Central Anatolian region of Turkey. Three populations from Nevşehir, Turkey were evaluated for cone and seed related characters. Hodul population was the remnant population whose seeds were used for afforestation of two different sites in 1980s (Işbank and Avanos populations). Cones from the randomly selected representative trees were evaluated for carpel number per cone, cone width, length and weight and seed number in three populations. Seeds obtained from those cones were evaluated for seed width, length and weight in 2011. No statistically significant differences were detected for carpel number per cone, cone width, length and weight as well as seed length. Seed weight, seed width and weight was important. The highest (57.8) and the lowest (27.9) numbers of seeds were obtained from Avanos and Hodul populations; respectively. The lowest seed width was found in Avanos population (3.21 mm). Avanos and Işbank populations had higher average seed weight than the remnant population. Correlation coefficients were calculated between the characters and 8 significant correlations were detected. Among these 6 were positively correlated while seed width and seed weight were negatively correlated. Results indicate that newly established orchards could be used as alternative seed sources for afforestation studies in Central Anatolian semi dry regions.

Key words: Afforestation, cone, *Pinus nigra*, remnant population, rehabilitation, seed

INTRODUCTION

Black pine is an economically important conifer species and it was first recognized as a separate species in 1768 (Vidakovic, 1991). It is found in Mediterranean countries, Balkans, Crimean peninsula and Anatolia (Arbez and Millier, 1971). It is scattered through large geographical area and therefore it contains many subspecies and varieties. Anatolian black pine (*Pinus nigra* Arnold subspecies *pallasina* (Lamb.) Holmboe) is one of the botanical forms of black pine and is usually found in Western Anatolia as pure or mixed stands. Destruction and degradation of natural habitats also created remnant populations which are common throughout Central Anatolia.

Black pine has the third largest distribution (4.2 million ha) among other forest species after *P. brutia* and oaks in Turkey (Anonymous, 2006). Black pine is among the primarily used tree

species for reforestation and rehabilitation studies in Turkey. Hence Anatolian black pine is suitable for afforestation of high altitude lands with dry climatic conditions, it is the primarily used conifer species for Anatolian steppes (Koski and Antola, 1993; Kaya and Temerit, 1994). In order to produce necessary seedlings for rehabilitation studies many clonal seed orchards have been established recently (Anonymous, 2001; Kaya *et al.*, 2003). Utilization of seed orchards depends on a high and uniform seed production, which is convenient to collect. Differences in growth and seed related characters in seed orchards will have effects on the collected seeds and produced seedlings (Urgenc, 1998).

Black pine has large genetic diversity among coniferous plants. Studies have shown that diversity within and between populations are important sources for genetic diversity in this species (Bonnet-Masimbert and Bikay-Bikay, 1978; Nikolic and Tucic, 1983; Alptekin 1986; Velioglu *et al.*, 1998; Gulcu and Ucler, 2008). Among the remnant populations within Turkey variation largely depends on diversity within populations (Kaya and Temerit, 1994). For rehabilitation studies clonal seed banks are being established but they are not enough for required seed production at present (Sivacioglu *et al.*, 2009). Anthropologic conditions damaged natural distribution and stands of this species throughout Central Anatolia (Uslu, 1958). Therefore, it was necessary to use remnant populations as seed banks due to difficulties in obtaining large amounts of seeds necessary for seedling production from clonal seed banks and also adaptive abilities of these populations to the region. There is limited information about seed related characters of remnant populations and orchards established using seeds from the remnant populations.

The aim of this preliminary study was to evaluate suitability of two reforested areas for seed production by comparing cone and seed characters of those populations to the original remnant population.

MATERIALS AND METHODS

Study area: The study was carried out in Hodul Mountain, Nevşehir (1586 m). Hodul population was the remnant source of seedlings for Işbank and Avanos populations and comprised over 10 ha and contained 79 relic Anatolian black pine trees over 250 years old. Işbank (38°43' N, 34°52' S) and Avanos (38°44' N, 34°53' S) populations were planted in 1980 and 1985, respectively. Average altitudes of populations were 1015 and 990 m for Işbank and Avanos, respectively. The closest meteorological observation station is located in Urgup (1060 m) and mean temperature (°C), relative humidity (%) and average precipitation (mm) for 42 years (1970-2012) were given for the region in Table 1.

Sampling: Each selected tree was located from each other at least 70 m apart. Cone harvest was done in November 2011. A total of 30 trees from Hodul population and 5 trees from Işbank and Avanos populations were selected at random to test differences for cone and seed related characters. Cones were harvested from each tree (60-70) and bagged separately. Number of carpels per cone, cone width (mm), cone length (mm) and cone weight (g) were determined from 10 cones for each tree. In order to collect seeds, cones were incubated in an oven at 50°C for 24 h. After seed

Table 1: Long year average of meteorological conditions of the study area

Parameter	Jan.	Feb.	Mar.	Apr.	May.	Jun.	July.	Aug.	Sep.	Oct.	Nov.	Dec.
Mean temperature (°C)	-1.2	0.3	4.8	10.1	14.2	18.5	21.7	21.0	16.2	10.9	4.9	0.8
Relative humidity (%)	75.0	72.0	66.1	61.5	60.1	54.2	49.6	50.4	54.8	64.0	70.4	74.3
Average precipitation (mm)	36.0	30.8	34.3	53.6	52.7	35.0	9.9	3.2	11.5	31.7	31.7	37.6

harvest, number of seeds per cone, seed width (mm), seed length (mm), seed weight (g) were recorded for each cone. Seed weight was calculated as average seed weight produced by per cone for the study.

Data analysis: Data was subjected to one-way analysis of variance (ANOVA) with Minitab statistical program. Means were separated using Tukey's procedure. Correlation coefficients between the examined characters were calculated using Minitab software.

RESULTS

Long year average of selected meteorological conditions of the study area was given in Table 1. The coldest months were January and February. Average yearly precipitation of the study area was 368 mm and the highest amount of precipitation occurred in April (53.6 mm) and May (52.7 mm) whereas August (3.2 mm) was the driest month based on 42 years of meteorological data.

Magnitude of variation for the examined characters and variations observed for the characters between the populations were given in Table 2. No statistically significant difference

Table 2: ANOVA results of cone and seed characters and their magnitude of variation from 3 populations of *Pinus nigra*

Variable	Mean±SE	Minimum	Maximum
Carpel number			
Avanos	79.80±6.70	64.30	104.50
Işbank	68.28±5.37	54.50	86.50
Hodul	72.89±1.82	51.60	101.00
Cone width			
Avanos	31.13±1.61	26.80	36.68
Işbank	30.75±0.63	28.59	32.56
Hodul	31.79±0.38	28.44	38.61
Cone length			
Avanos	63.41±3.29	56.99	75.29
Işbank	60.80±0.54	59.26	62.61
Hodul	62.83±1.27	46.05	77.84
Cone weight			
Avanos	13.56±1.90	9.66	20.76
Işbank	13.68±0.73	10.98	15.34
Hodul	14.67±0.53	9.40	22.54
Seed number			
Avanos	57.82±4.79	46.80	74.00
Işbank	35.64±7.18	17.60	58.20
Hodul	27.94±2.59	8.00	55.30
Seed width			
Avanos	3.21±0.05	3.08	3.35
Işbank	3.80±0.16	3.31	4.22
Hodul	3.56±0.04	3.01	3.94
Seed length			
Avanos	6.04±0.26	5.37	6.73
Işbank	6.19±0.21	5.61	6.73
Hodul	6.51±0.11	5.45	7.73
Seed weight			
Avanos	0.76±0.10	0.49	0.99
Işbank	0.57±0.06	0.42	0.75
Hodul	0.30±0.03	0.07	0.79

was detected for carpel number per cone, cone width, length and weight and seed length. It was also observed that populations did not differ from each other for those characters.

Statistically significant differences were detected for number of seeds per cone, seed width and seed weight (Table 2). The highest number of seeds per cone was obtained from Avanos population with a mean of 57.8 seeds and the lowest value was observed from the remnant population. As a result, Avanos population was ranked differently than Işbank and Hodul populations. Mean seed number was 40.72 and ranged from 8-74 between the populations. For seed width Avanos population ranked lower than the Işbank and Hodul populations. Mean seed width was 3.52 mm for the populations examined. Seed weights for per cone for the populations ranged between 0.36-0.76 g and the mean seed weight of three populations was 0.54 g. The lowest seed weight was observed in the remnant population and the highest seed weight was found in Avanos population.

Average cone weight of three populations was found to be 13.97 g. Average numbers of carpels for the three populations were 73.66 in the study. Average number of carpels for Avanos and Işbank populations was 74.04 higher than the remnant population (72.89) (Table 2). Average cone width and cone length was 31.22 mm and 62.35 mm, among the three populations, respectively. The highest number of seeds was obtained from Avanos population (57.82) followed by Işbank (35.64) and the remnant population (27.94) (Table 2). Seed width was lower in Avanos population than the other two populations. Seed length did not exhibit large variation between the populations.

Black pine produces light and dark seeds. One thousand seed weight changes between light and dark colored seeds and 1000-seed weight for dark and light colored seeds are 24.5 and 18.5 g, respectively. Therefore, 1000-seed weight for populations was not given instead average seed weight produced per cone was reported in this study. Average seed weight per cone was 0.76 and 0.57 g for Avanos and Işbank populations whereas seed weight of the remnant population was 0.30 g.

In order to find associations between examined characters correlation coefficients were calculated. Correlations between seven characters were found to be statistically significant at 5 and 1% level of significance (Table 3). Six characters were found to be negatively correlated. Among the negatively correlated characters, correlation coefficient between seed width and seed number (-0.484) was important.

Correlation coefficients for cone width with carpel number (0.559), cone weight with cone width (0.820) and cone length (0.671) and seed weight with seed number (0.881) was statistically significant at 1% level. Cone length with cone width (0.352) and cone weight with carpel number (0.391) was important at 5% significance level.

Table 3: Correlation coefficients and importance levels of cone and seed characters in *Pinus nigra*

	Carpel no.	Cone width	Cone length	Cone weight	Seed No.	Seed width	Seed length	Seed weight
Carpel No.	-							
Cone width	0.559**	-						
Cone length	0.177	0.352*	-					
Cone weight	0.391*	0.820**	0.671**	-				
Seed number	0.309	0.191	0.261	0.254	-			
Seed width	-0.275	0.091	-0.037	0.160	-0.484**			
Seed length	0.002	0.234	0.170	0.283	-0.246	0.143	-	
Seed weight	0.201	0.098	0.204	0.201	0.881**	-0.268	-0.160	-

***Significant at 5 and 1% level of significance, respectively

DISCUSSION

Black pine exhibits large variation for flower related characters, cone and seed related characters (Matziris 1993; Kaya and Temerit, 1994; Ertekin, 2010; Sivacioglu and Ayan, 2010) Aim of the present study was to detect variations between populations to evaluate their suitability for alternative seed production sources for afforestation studies. In the present study, no statistically important variation for carpel number, cone width, length, weight and seed length were observed between the populations. Important differences were detected for seed number, seed width and seed weight. Most of the variation found in seed banks of black pine results from variations within a population (Gulcu, 2002; Sivacioglu and Ayan, 2010). Cone production is under strong genetic control in black pine (Matziris, 1993). Therefore, high cone producing trees could potentially continue to produce large amounts of cones and seeds. Large and old trees usually produce more cones than the young trees (Ordonez *et al.*, 2005; Haymes and Gordon, 2012). Therefore, it is expected that cone and seed production of Avanos and Işbank populations will increase with time.

Black pine has the potential to produce up to 96 seeds (Sivacioglu and Ayan, 2010). Seed production depends on the number of carpels produced by cones. There was no statistically significant difference for carpel number between the populations (Table 2). Seeds from the remnant population were used for Işbank and Avanos populations suggesting that seed production of these two populations could exceed seed production of the remnant population in coming years as seed production increases with increased age of trees (Ordonez *et al.*, 2005; Haymes and Gordon, 2012).

Average cone width of three populations found in the present study was 31.22 mm and it was within the range of previously reported values for black pine (Ucler and Gulcu, 1999; Deligoz and Gezer, 2005). Cone length ranged from 63.23 to 66.35 mm for natural black pine forests (Alptekin, 1986; Ucler and Gulcu, 1999) and it could reach up to 70.9 mm for clonal seed orchards (Ertekin, 2006). Cone length of the three populations was 62.35 mm, a value lesser than the reported average cone lengths for black pine.

Cone weight of natural and clone orchards of black pine have been reported previously. Deligoz and Gezer (2005) found average cone weight 24.17 g for natural populations. In this study, average cone weight of the populations was found to be 13.97 g. On the other hand, cone weight was found to be 27.38 g (Deligoz and Gezer, 2005) and 16.18 g (Sivacioglu and Ayan, 2010) for clonal seed orchards. Sivacioglu and Ayan (2010) reported that average cone width increased from 27.12 to 30.59 mm, cone length from 53.59 to 64.30 mm and cone weight from 13.26 to 16.18 g between 2002 to 2006 for a clonal black pine seed orchard. These results suggest that Avanos and Işbank populations' cone characters could increase over time as trees grow more.

Seed number for a clonal seed orchard was reported to be 16.32 for black pine (Sivacioglu and Ayan, 2010). Results presented here are higher than the selected clones and it is possible to produce more seeds from these populations, especially from Işbank and Avanos populations. Seed width and length of 11 natural black pine populations ranged from 2.99 to 3.38, 5.57 to 6.23 mm, respectively (Deligoz and Gezer, 2005) and 3.57 and 6.29 mm for a clone population (Sivacioglu and Ayan, 2010). Results of this study are close to previously reported results for seed width and length of black pine. Average seed weight per cone was 0.76 g for Avanos and was 0.57 g Işbank population whereas seed weight of the remnant population was 0.30 g. New established populations have more and heavier seeds which are desired to obtain more seeds per cone.

CONCLUSION

For successful and sustainable reforestation and afforestation activities it is important to establish seed production regions. Without considering the ecological conditions of seed sources,

forestation studies may be unsuccessful. Although, Anatolian black pine forests are divided into regions and sub regions according to the ecological conditions in Turkey, remnant population in this study could serve as new seed sources for future forestation studies. As a consequence, results of this study demonstrated that newly established orchards are very similar in terms of cone and seed related characters to the remnant population and could be used as alternative seed sources for afforestation and reforestation studies in Central Anatolian semi dry regions. In order to select parents for seed production, other important characters; such as growth and wood production should also be studied within Avanos and Işbank populations.

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