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Variation in cone and seed characteristics in a clonal seed orchard of Anatolian black pine [*Pinus nigra* Arnold subsp. *pallasiana* (Lamb.) Holmboe]

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Abstract: *Cone and seed characteristics of Anatolian black pine were investigated in a clonal seed orchard for two years, 2002 and 2006. The orchard, originated from Kastamonu-Karadere seed stand was established in 1993 by using 1 year-old grafts in an area of 13 ha, at Hanönü-Günlüburun, northern Turkey and includes 30 clones. The results showed that, significant variation exists among clones for 14 of cone and seed traits for 2006. The clones had cone wet weight in range of 16.92 to 38.51g, whereas this value varied in range of 11.16 to 24.06 g for cone dry weight. Cone length varied from 55.19 to 74.43 mm, while cone width varied in range of 26.66 to 36.57 mm. The range of scale number and fertile scale number varied from 80.02 to 110.64 and 38.03 to 56.20, respectively. Among the clones, the seed and filled seed number were 6.70-24.97 and 5.79-21.12, respectively. The 1000 seed weight varied in range of 20.36 to 29.73 g. The respective values of average seed length and width were 6.29 mm and 3.57 mm, while wing length and width were 19.59 mm and 7.21mm. The average seed efficiency was 13.5%. Coefficients of variation among grafts (CV_G) were mostly bigger than among clones (CV_C), indicating high variation within the population. Year to year correlation coefficients for seed and cone characteristics were varied from moderate (0.58) to strong (0.83). The respective broad sense heritability values of clone mean basis (H_2) for cone dry weight, cone width, 1000 seed weight were 0.77, 0.83 and 0.76. The seed efficiency had a H_2 value of 0.43.*

Key words: *Clonal variation, Cone, Seed, Anatolian black pine, Turkey, Heritability*
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Introduction

Anatolian black pine (*Pinus nigra* Arnold. subsp. *pallasiana* (Lamb.) Holmboe) is one of the most common and important forest tree species in Turkey due to usefulness of its wood to many commercial uses. 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 forests (Anonymous, 2006). Because of the high proportion of non-productive area, big amount of reproductive material is necessary to keep present Anatolian black pine forest stock even to continue with increasing trend by rehabilitating the degraded areas. Also, this pine species is the most important species which can be spread to the steppe regions in Anatolia. In 2000's, the semi-arid steppe regions were evaluated as potential afforestation areas. And so, the importance of Anatolian black pine is increasing with time for Turkish foresters. Actually, the seed demand for this species is mainly supplied from current 53 (428.8 ha) of seed orchards and 79 (10384.7 ha) of seed stands (Anonymous, 2008). 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 purposes.

The purpose of the present study is to investigate the variation and inheritance pattern in cone and seed characteristics of Anatolian black pine and to determine interrelationships that exist among these characteristics for forming base to improvement projection of this species.

Materials and Methods

This work reported here was carried out in the 13 ha clonal seed orchard of Hanönü, located at Tasköprü, Kastamonu. The orchard was established in 1993 and comprises 2039 grafts of 30 clones, derived from intensively selected trees in Kastamonu-Karadere seed stand. Grafts were 1 year-old at the time of establishment and were planted at a spacing of 8 x 8 m. By the time of this study (2006), the graft age is 14 years-old. So far, no pruning has been done in the orchard. First cone harvesting from this orchard started in year 2003 when the grafts were 11 years-old.

For this study, in all clones, five grafts were sampled at random and the cones were collected in November 2006, when the seeds were mature. The abundance and sources of pollen were not monitored in the orchards for 2005 spring when the time of seed-cone pollination. Each graft was divided into three levels according to crown. 12 of cones having no external damage were collected at random from the middle level of graft between one third and two third of the crown.The cones were placed in individual paper bags and kept at 4°C until analysis. The collected cones were classified into the pots and attended to flex open the cone scales into the indoor condition. Five grafts of 30 clones respectively were assessed firstly for cone traits such as; cone wet weight and

cone dry weight (CWW and CDW), cone length and cone width (CL and CW), scale number and fertile scale number (ScN, FScN). After that, the seeds were then extracted (by cone), dewinged and counted. By counting the seeds, seed number and filled seed number (SeN, FSeN) traits were determined. Also, on the seeds and wings seed length and width (SeL and SeW), wing length and wing width (WL and WW), 1000 seed weight (1000 SW), seed efficiency (SE) were determined. Seed efficiency is the ratio of the total number of filled seeds to the seed potential (Bramlett *et al.*, 1977). Seed potential is the maximum number of seeds that a cone can produce (two times the number of fertile scales) (Lyons, 1956).

Analyses of variances were performed for all 14 traits. Broad sense heritability values were estimated on both individual tree basis (H_1) and on clone mean basis (H_2) as the ratio of total genetic variance (σ^2_C) to total phenotypic variance ($\sigma^2_{C+}\sigma^2_E$) (Matziris, 1984) for the H_1 and to ($\sigma^2_{C+}\sigma^2_{E/n}$) for H_2 (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 σ^2_E =error mean square and σ^2_C = (clone mean square-error mean square) / no. of ramets per clone (Wright, 1962).

Data were subjected to one-way analysis of variance. Variables were tested for normality and homogeneity of variances and logarithmic transformation for counting, arcsine for ratio, were made. Differences were considered significant at $p<0.05$ level. Relationships between 14 of seed and cone related characters were tested using correlation analyses. Also, year to year variation between the years of 2002 and 2006 were tested with correlation analysis. Variation coefficient among clones (CV_C) and grafts (CV_G) in percent was estimated as the ratio of standard deviation of the clones/grafts to arithmetic means.

Results and Discussion

The overall means (±SE) for all characteristics assessed in 2006 along with their minimum and maximum values and their standard deviations, range and variation coefficients were determined (Table 1). The analyses of variance showed that there are significant differences among the clones at 0.001 and 0.05 probability level for all cone and seed characteristics examined (Table 2). Also, clonal variation in seed and cone traits for *Pinus halepensis* (Matziris, 1998) and *Pinus sylvestris* (Keles, 2007), in cone dimension for *Pinus contorta* (Owens *et al.*, 2005), in filled seed number/cone for *Pinus banksiana* and *Pinus taeda* (Todhunter and Polk, 1981; Schmidtling, 1983), in seed weight for *Picea sitchensis* (Chaisurisri *et al.*, 1992) were reported. Meanwhile, the clonal variation defined in this study, symbolizes the variation within the population for Anatolian black pine. The bigger genetic variation within the population than among the population was determined by Gulcu (2002) for Anatolian black pine in seedling and sampling traits. Also, it was reported by Yuksek (1997) that 92.6% of genetic variation among the 14 of Anatolian black pine population originated from variation within the population.

The CWW among the clones varied from 16.92 g to 38.51 g with an overall mean of 24.04 g (SD_C = 4.68 g) (Table 1) while, the CDW of cones varied from 11.16 g to 24.06 g with overall mean of 16.18 g (SD_C= 3.14 g). Concerning of CWW and CDW among the clones, moisture content of the cones, had a mean value of 32.7%. CL and CW, which are characteristics for determining cone size were quite variable. CL varied among clones in range of 55.19 to 74.43 mm (mean= 64.30 mm), and CW varied in range of 26.66 to 36.57 mm (mean=30.59 mm). Also, with respect to the combined mean of FScN (48.14), each Anatolian black pine cone had the potential to produce about 96 seeds.

In 2006, the overall mean of SeN and FSeN extracted were 16.32 seeds cone^{-1} and 13.53 seeds cone^{-1}, respectively. When compared the seed potential value of a cone (96 seeds cone^{-1}), these 13.53 of FSeN occupy only 14% of the full potential. In 2002, the overall mean of FSeN was reported as 8.71 seeds cone^{-1} as overall mean of 9 clone, for the studied orchard (Çilgin *et al.*, 2007) and this indicates year to year variation in the FSeN.

The mean 1000SW was 24.29 g (range = 20.36 to 29.73 g) among the clones. The mean SeL varied among clones from 5.74 mm to 7.26 mm with overall mean of 6.29 mm and standard deviation 0.30 mm. The corresponding range for SeW was 3.32 mm to 4.11 mm with overall mean of 3.57 mm (Table 1). In 2002, for the studied orchard 1000 SW varied from 16.930 g to 25.617g (Çilgin *et al.*, 2007). In spite of the non significant differences between the ranges of 1000SW for two years, the 1000 SW value increases with time. The mean 1000SW estimated in this study is higher than the values for natural populations, 22.5 g (Atay, 1959) and seed stands, 21.76 g and clonal seed orchard, 22.85 g (Deligoz and Gezer, 2005). But this mean value is smaller than reported by Ertekin (2006), 26.02 g for the seed orchard which is at the same stage (14 years-old) with the studied orchard. This result can be mostly clarified with gene pool and growing area of the seed orchard. Also, there was large variation among clones in WL (range=17.55 to 21.68 mm) and WW (range=6.42 to 8.05 mm). Part of this variation was related to variation in cone size, since these characteristics were strongly correlated with CDW (Table 3).

The average SE for all clones was 13.5%. (Table1). The 520 (21%), 525 (20%), 508 and 533 (19%) numbered clones have the biggest SE values. Therefore, the future studies and observations in this orchard, should be intensified on these clones. Meanwhile, 509 (5%), 519 and 537 (7%) number clones have the smallest SE values. For, the 13 years-old *Pinus sylvestris* seed orchard, the overall mean of SE reported as 17.9% (Sivacioglu and Ayan, 2008). Although, there are limited estimates for Turkish clonal seed orchards by using cone analysis about seed efficiency, this 13.5 and 17.9% values are quite low in comparison to literature, especially to southern pines in USA, where values of 25% for seed orchards with no protection from insects to 70% for seed orchards with maximum protection might be expected (Bramlett, 1987). The studied orchard is no protection from insects.

Table - 1: Overall means and statistical values of cone and seed traits of Anatolian black pine clonal seed orchard assessed in 2006

Traits	Unit	Among the clones					Among the grafts		
		Mean	Std. Error	Min-Max	SD_C	*CVc*	Min-Max	SD_G	CV_G
1. CWW	g	24.04	0.85	16.92-38.51	4.68	19.47	9.57-44.10	6.36	26.46
2. CDW	g	16.18	0.57	11.16-24.06	3.14	19.41	6.53-28.69	4.28	26.45
3. CL	mm	64.30	0.91	55.19-74.43	5.01	7.79	41.82-84.03	7.79	12.12
4. CW	mm	30.59	0.42	26.66-36.57	2.29	7.49	24.55-38.72	2.93	9.58
5. ScN	no	96.25	1.12	80.02-110.64	6.17	6.41	71.25-126.25	9.23	9.59
6. FScN	no	48.14	0.84	38.03-56.20	4.63	9.62	26.42-66.83	7.21	14.98
7. SeN	no	16.32	0.87	6.70-24.97	4.76	29.17	1.00-41.33	8.51	52.14
8. FSeN	no	13.53	0.74	5.79-21.12	4.04	29.86	1.50-36.54	7.00	51.74
9. 1000SW	g	24.29	0.45	20.36-29.73	2.50	10.29	16.80-35.67	3.55	14.62
10. SeL	mm	6.29	0.05	5.74-7.26	0.30	4.77	5.37-8.03	0.44	6.99
11. SeW	mm	3.57	0.03	3.32-4.11	0.17	4.76	2.99-4.64	0.25	7.00
12. WL	mm	19.59	0.21	17.55-21.68	1.16	5.92	12.86-23.68	1.91	9.75
13. WW	mm	7.21	0.08	6.42-8.05	0.44	6.10	5.23-9.35	0.70	9.71
14. SE	%	13.5	1.00	5.00-21.00	4.00	29.62	1.00-34.00	7.00	51.85

CWW = Cone wet weight, CDW = Cone dry weight, CL = Cone length, CW = Cone width, SeN = Scale number, FscN = Fentile scale number, ScN = See number, FSeN = Filled seed number, SW = Seed weight, SeL = Seed length, SeW = Seed width, WL = Wing length, WW = Wing width, SE = Seed efficiency, SDc = Standard deviation among the closes, CVc = Variation coefficient among the clones, SD_G = Standard deviation amont the grafts, CV_G = Variation coefficient among the grafts

Table - 2: Analysis of variance, variance components and heritability estimates for seed and cone traits of Anatolian black pine

Traits	Mean Squares					
	Between[1] clones	Within clones (error)	σ^2_C	σ^2_E	H_1^2	H_2
1. CWW	106.82***	23.77	16.61	23.77	0.41	0.78
2. CDW	47.80***	10.98	7.36	10.98	0.40	0.77
3. CL	115.84***	46.83	13.80	46.83	0.23	0.60
4. CW	25.78***	4.28	4.30	4.28	0.50	0.83
5. ScN	188.44***	59.22	25.84	59.22	0.30	0.69
6. FScN	108.74***	37.65	14.22	37.65	0.27	0.65
7. SeN	105.17*	63.75	8.28	63.75	0.12	0.39
8. FSeN	71.67*	43.09	5.72	43.09	0.12	0.40
9. 1000SW	31.67***	7.53	4.83	7.53	0.39	0.76
10. SeL	0.47***	0.11	0.07	0.11	0.40	0.77
11. SeW	0.15***	0.04	0.02	0.04	0.35	0.73
12. WL	7.21***	2.74	0.89	2.74	0.25	0.62
13. WW	0.98***	0.37	0.12	0.37	0.25	0.62
14. SE	0.007*	0.004	0.001	0.004	0.13	0.43

[1]***, **, * =statistically significant of the 0.001, 0.01, 0.05 probability level, respectively, $H_1^2=\sigma^2_C/\sigma^2_{C+}\sigma^2_E$, $H_{2=}\sigma^2_C/\sigma^2_{C+}\sigma^2_{E/n}$, CWW = Cone wet weight, CDW = Cone dry weight, CL = Cone length, CW = Cone width, ScN = Scale number, FscN = Fentile scale number, SeN = See number, FSeN = Filled seed number, SW = Seed weight, SeL = Seed length, SeW = Seed width, WL = Wing length, WW = Wing width, SE = Seed efficiency, σ^2_C = Total genetic variance, σ^2_E= Phenotypic variance

There are some studies about seed and cone characteristics of Anatolian black pine on natural stands (Atay, 1959; Alptekin, 1986; Ucler and Gulcu, 1999), seed stands and clonal seed orchards (Deligoz and Gezer, 2005; Ertekin, 2006; Colin *et al.*, 2007). The CDW values found 20.1 g (Atay 1959) for natural stands, 24.17 g (Deligoz and Gezer, 2005) for seed stands, 27.38 g (Deligoz and Gezer, 2005) for clonal seed orchards. With respect to 2002 data, when the studied orchard was 10 years-old, the mean CDW was 13.266 g. In this study, the mean CDW was 16.18 g (range=11.16-24.06 g). Overall mean of CDW (16.18 g) in this study was smaller than other seed orchards, natural populations and seed stands. The lower values of CDW for 2002 and 2006 for the studied orchard than the other seed stands and natural population, should be evaluated together with the high ages of them. Mean values of CL found 63.7 mm (Atay, 1959), 63.23 mm, (Alptekin, 1986), 66.35 mm, (Ucler and Gulcu, 1999), 64.00 mm (Deligoz and Gezer, 2005) for natural populations and seed stands, while these values were 29.8, 31.09 and 32.67 mm, respectively, for the CW. Also, for the clonal orchards, CL as 62.5 mm (Deligoz and Gezer, 2005) and 70.90 mm (Ertekin, 2006) were reported, while these values for the CW varied between 30.8 and 32.9 mm, respectively. In 2002, for

Table - 3: Pearson correlation coefficients of cone and seed traits of Anatolian black pine

Traits	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. CWW	1.00	0.97	0.79	0.79	0.34	0.36	0.24	0.14	0.52	0.50	0.60	0.70	0.75	0.08
2. CDW		1.00	0.82	0.93	0.32	0.38	0.31	0.24	0.54	0.51	0.61	0.73	0.80	0.17
3. CL			1.00	0.66	0.20	0.25	0.41	0.38	0.19	0.23	0.29	0.59	0.58	0.38
4. CW				1.00	0.27	0.36	0.21	0.12	0.60	0.50	0.62	0.69	0.85	0.05
5. ScN					1.00	0.85	0.24	0.15	0.02	0.08	0.05	0.15	0.03	0.04
6. FScN						1.00	0.26	0.19	0.13	0.08	0.08	0.24	0.08	0.00
7. SeN							1.00	0.95	0.08	0.00	0.22	0.14	0.12	0.88
8. FSeN								1.00	0.16	0.07	0.28	0.13	0.05	0.97
9. 1000SW									1.00	0.85	0.80	0.58	0.70	0.19
10. SeL										1.00	0.75	0.64	0.60	0.09
11. SeW											1.00	0.55	0.64	0.32
12. WL												1.00	0.65	0.12
13. WW													1.00	0.06
14. SE														1.00

CWW = Cone wet weight, CDW = Cone dry weight, CL = Cone length, CW = Cone width, ScN = Scale number, FscN = Fentile scale number, SeN = See number, FSeN = Filled seed number, SW = Seed weight, SeL = Seed length, SeW = Seed width, WL = Wing length, WW = Wing width, SE = Seed efficiency.

Table - 4: Year to year (2002, 2006) Pearson and Spearman correlation coefficients in five cone and seed characteristics of anatolian black pine

Traits	Correlation coefficient	
	[1]Pearson	Spearman
1. CDW	0.67*	0.73*
2. CL	0.58	0.60
3. CW	0.73*	0.83**
4. FSeN	0.71*	0.58
5. 1000SW	0.83**	0.59

[1]**,* Correlation is significant at the 0.01,0.05 level, probality level respectively

the studied orchard the mean CL and CW was 53.59 and 27.12 mm (Cilgin *et al.,* 2007).

There are statistically significant differences in FSeN (mean=13.53, range=5.79-21.12) among the clones. In 2002, mean values of FSeN was 8.71. The mean value of FSeN was much high for the year 2006. The differences in the FSeN between the two years, is probably to different quantities of pollen produced in the two years.

CWW is strongly correlated with CDW (r=0.97), and expected CDW and CW (r=0.93). CDW was weakly correlated with SeN (r=0.31) and FSeN (r=0.24). CDW was more moderately correlated with 1000SW (r=0.54), SeL(r=0.51), SeW(r=0.61). The heavier the cones are the larger and heavier the seeds included in them. CDW is also correlated with WL (r=0.73) and WW (r=0.80). The SE weakly correlated with most of the traits except for SeN(r=0.88) and FSeN (r=0.97) (Table 3).

Broad sense heritability of clone mean basis (H_2) estimates are shown in Table 2. CW is strongly inherited trait with H_2 value 0.83. CWW and CDW, expressing cone size are also inherited, with H_2 values 0.78, 0.77, respectively. The SeN (H_2=0.39) and FSeN (H_2=0.40) are moderately inherited traits. Seed and wing sizes that are maternally influenced are inherited quite strongly (H_2 range=0.62-0.77).

Correlation coefficients between the years 2002 and 2006 on seed and cone characteristics are shown in Table 4. There are statistically significant correlations in all characteristics between the two years examined, except for CL. The correlations varied from moderate values, *e.g.*, 0.58 for the CL to a strong correlation of 0.83 for 1000 SW. It is interesting that the year to year correlation coefficients for the FSeN was quite strong (r=0.71) indicating that there are clones which consistently produce cones with high number of full seeds (Table 4). These results are in close agreement with findings reported by Sarvas (1962), who concluded that differences between individual genotypes in the proportion of empty seed produced, remain more or less constant from year to year (Matziris, 1998).

The high variation among /within the clones and heritability values estimated in this study, indicates opportunities of high selection intensity in the breeding programs for Anatolian black 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 or establishment of new seed orchards are made. 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.

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