

Genetic variation in seed and cone characteristics in a clonal seed orchard of Scots pine (*Pinus sylvestris* L.) grown in Kastamonu-Turkey

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Abstract

*Cone and seed characteristics of scots pine (*Pinus sylvestris* L.) were investigated in a clonal seed orchard for 2006. The orchard was established in 1995 in an area of 7.2 ha, at Taşköprü/Kastamonu, northern Turkey and includes 30 clones. The results showed that significant genetic variation exists among clones for cone wet weight and dry weight; cone width and length; total, fertile and infertile scale number; total, sound and empty seed number; 1000 seed weight; apophysis length, width and thickness; seed length and width; wing length and width; seed potential, seed efficiency and cone volume. Coefficients of variation within clones were mostly bigger than among clones, indicating high variation within the population. The respective broad sense heritability values of clone mean basis for cone dry weight, cone width, 1000 seed weight were 0.59, 0.58 and 0.57. The broad sense heritability and variation data obtained in this study are very valuable in the management practices of the existing Scots pine clonal seed orchards and can be also used for better planning of advanced generation of clonal seed orchards in Turkey.*

Keyword: Scots pine, orchard, seed, cone, Kastamonu, Turkey.

Introduction

Scots pine (*Pinus sylvestris* L.) is one of the most important forest tree species in Turkey for valuable characters of wood in varied commercial sectors, and also for biomass production. Stands of this species occupy roughly 1239578 ha and also 523935 ha of them are classified as unproductive [1,2]. Thus, roughly 42% of scots pine forest area is unproductive. These unproductive areas have been evaluated as potential areas for rehabilitation practices to diminish the wood supply deficit in Turkey. Of course, big amount and high quality of reproductive material is necessary to keep present scots pine forest stock even to continue with increasing trend by rehabilitating the degraded areas [3]. Presently, Turkey has 21 Scots pine seed orchards on 109 ha [4], only 9.2% of annual seed demand for plantations is covered from the orchards at the beginning of the millenium [5]. As to some projections, the need of scots pine seed is 715 kg per year [6]. Only definite amount of this seed demand was supplied from of seed orchards in Turkey. But, most of these seed orchards are still rather young [7]. New orchards have been established to cover the demand of improved seed for afforestation according to the national seed and tree-breeding program of Turkey [8,9]. Seed orchards have the potential to increase forest production and offer a reliable seed supply, their role and function has recently been compiled [10]. They are by far the most important outlets to forestry of breeding programs and they can be useful in gene conservation. Investment in seed orchards is often by the most cost-efficient way of increasing future forest production [9]. For that reason, it is desirable to improve the function of seed orchards. There are worries about the genetic diversity of the seed orchard crops and its impact on the future forest. Low production of sound seeds is a common problem [11]. Seed production and collection is often

expensive. Basic data on seeds and cones in seed orchards and their occurrence and variation and the possible causes of variations are desirable to get a better understanding of possible improvements of seed orchard function, economy and impact to the forest [11]. Thus, the evaluation of the clonal variation for more effective orchards is a very urgent issue for Turkish foresters.

The aim of this study was to evaluate the variation in cone and seed characters within and among clones and grafts and to discuss on the establishment and management of seed orchards.

Material and Method

The studied clonal seed orchard of scots pine, located in Taşköprü (Kastamonu) (41°36'N, 35°05' E, 1160 m), comprises 1987 ramets of 30 clones and was established on a 7.2 ha of area by Forest Tree Seeds and Tree breeding Research Directorate in 1995, by using 2 years-old grafts at a spacing of 6 m x 6 m. The orchard was established with phenotypically selected plus trees from Araç-Derayayla seed stand. For seedling propagation, cone harvesting started in the 2003, when the ramets were 10 years old. By the time of this study (2006), the ramet age was 13 years-old. From each of these clones ten grafts were selected at random. A random sample of 12 mature cones was obtained from the middle part of each ramet crown, during November 2006. On these cones, for each graft the following registrations were assessed. The cones were weighted (*CWW*) and lengths (*CL*) and diameters (*CW*) (across the broadest portion) were measured with digital caliper. The air-dry cones were weighted (*CDW*). The seeds were then extracted (by cone), dewinged and counted. The mean values of graft were calculated as the average of the values in 12 cones. Also, the clone means was estimated as the average of 10 grafts (120 cones per clone). In this way, overall clonal means of total seed number (*TSeN*), number of sound seeds (*SSeN*) and empty seeds (*ESeN*) were determined. The same treatments were implemented for scale number (*TScN*), fertile (open after heating) scale number (*FScN*) and infertile (closed after heating) scale number (*IFScN*). These traits were firstly evaluated on cone base, and after converted to ramet and clonal values. A sample of 1000 seeds (*1000 SW*) was weighted. Prior to dewinging, a random sample of 6 seeds from each cone (72 seeds per ramet) was taken and lengths (*SeL*) and widths (*SeW*) of both seeds and wings (*WL* and *WW*) were measured. Cone volume (*CoV*) for each cone was determined by thinking the cones as a geometric cone. Also, apophysis length (*ApL*), width (*ApW*) and thickness (*ApT*); seed potential (*SeP*), seed efficiency (*SeE*) and cone volume (*CoV*) were determined.

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 21 of seed and cone related characters were tested using correlation analyses. 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. Individual tree basis broad sense heritability values (H^2) were estimated as the ratio of total genetic variance (σ^2_C) to total phenotypic variance ($\sigma^2_C + \sigma^2_E$) [12]. Cloning effect variance biases the heritability values, but the magnitude is negligible and can be ignored [13]. 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 [14].

Results and Discussion

The descriptives of all studied characters were presented on Table 1. Meanwhile, the analyses of variance (Table 2) showed that there are significant differences among the clones at 0.001 probability level for all cone and seed characteristics examined. Similarly, for the other pine species clonal variation in seed and cone traits for *Pinus halepensis* [15] and *Pinus sylvestris* [16,17] in cone dimension for *Pinus contorta* [18] in filled seed number cone⁻¹ for *Pinus banksiana* and *Pinus taeda* [19,20] are reported. Meanwhile the clonal variation defined in this study symbolizes the variation within the population for scots pine. Differences among the scots pine populations in some seedling and morphological-electrophoretic characters were found [21,22]. Genetic variation among the clones of *Pinus contorta* for cone length, width and dry weight is reported [23]. Also, total seed number per cone varied between 9.3 and 21.8 in a Scots pine clonal seed orchard in northern Sweden [24]. In this study, the seed number per cone varied in a range of 3.1 to 22.8.

Table1. Descriptives of cone and seed traits of studied Scots pine clonal seed orchard.

Character	unit	Among the clones						Among the grafts			
		mean	SE	Min	Max	SD	CV	Min	Max	SD	CV
1.CWW	g	1419	0.55	9.09	23.29	3.03	21.35	6.59	29.09	3.88	27.31
2.CW	mm	27.34	0.37	23.33	33.18	2.03	7.43	21.74	36.55	2.56	9.36
3.CL	mm	52.47	0.99	44.10	65.30	5.46	10.41	37.63	70.89	6.76	12.89
4.CDW	g	10.90	0.43	6.99	17.64	2.35	21.56	2.45	21.79	2.94	26.94
5.TScN	no	71.19	1.34	56.31	86.51	7.33	10.30	39.53	107.06	10.11	14.20
6.FScN	no	30.11	0.93	19.75	42.17	5.11	16.97	15.00	47.08	6.50	21.58
7.IFScN	no	41.08	0.69	34.14	51.48	3.81	9.27	24.53	61.46	6.54	15.91
8.TSeN	no	26.31	1.44	13.78	42.93	7.87	29.91	7.92	56.17	9.70	36.86
9.SSeN	no	11.52	0.91	3.10	22.83	4.97	43.14	1.00	33.08	6.23	54.07
10.ESeN	no	15.05	0.85	6.77	25.82	4.68	31.10	3.75	32.92	5.67	37.65
11.1000 SW	g	10.91	0.24	8.68	13.25	1.31	12.01	7.17	16.00	1.66	15.26
12.ApL	mm	6.62	0.09	5.60	7.65	0.52	7.85	4.90	8.48	0.63	9.49
13.ApW	mm	8.72	0.09	7.76	10.31	0.54	6.19	7.09	11.02	0.66	7.52
14.ApT	mm	3.05	0.06	2.46	3.87	0.35	11.48	2.26	7.63	0.46	14.99
15.SeL	mm	4.98	0.04	4.46	5.34	0.22	4.42	4.19	5.83	0.29	5.84
16.SeW	mm	2.71	0.03	2.35	3.33	0.16	5.90	2.11	6.69	0.29	10.59
17.WL	mm	16.59	0.30	14.04	20.28	1.62	9.76	12.78	22.56	1.97	11.86
18.WW	mm	4.96	0.08	4.19	6.08	0.42	8.47	3.68	10.56	0.60	12.14
19.SeP	no	58.26	2.29	29.09	84.33	12.57	21.58	15.17	94.17	15.68	26.92
20.SeE	%	0.18	0.01	0.05	0.29	0.06	33.33	0.02	0.42	0.08	43.92
21.CoV	cm ³	10.68	0.48	6.57	18.83	2.62	24.53	4.77	22.92	3.25	30.38

Considerable variation among the clones both *CWW*, *CW*, *CL*, *CDW* (*CV* ranged 7.43-21.56 and *TScN*, *FScN*, *IFScN* (*CV* ranged 9.27-16.97) was found. Also, the bigger genetic variation (*CV* ranged 9.36-27.31 in cone traits and 14.20-21.58 in scale traits) among the grafts than among the clones was determined. Moreover, apophysis traits as *ApL*, *ApW*, *ApT* showed bigger variation among the grafts than among the clones. *CoV* varied among clones from 6.57 to 18.83 cm³ (*CV*=24.53) while it varied among grafts from 4.77 to 22.92 cm³ (*CV*=30.38) (Table 1). *SeL* and *SeW* characters have smaller *CV* values both among the clones

and among the grafts. Among the clones, the mean *TSeN*, *SSeN* and *ESeN* were 26.31, 11.52, 15.05 respectively. The overall mean of *1000 SW* was 10.91 g with a range from 8.68 to 13.25 g. Also, among the clones, the average *WL* and *WW* were 16.59, 4.96 mm, respectively (Table 1). The coefficients of variation among grafts (*CV*) were mostly bigger than among clones, indicates high variation within the population (Table 1).

Table 2. Analysis of variance, variance components and heritability estimates for seed and cone traits of Scots pine.

Characteristics	MEAN SQUARES		σ^2_C	σ^2_E	*H ²
	Among ¹ clones	Within clones (Error)			
1.CWW	91.94***	6.76	8.52	6.76	0.56
2.CW	41.23***	2.82	3.84	2.82	0.58
3.CL	298.61***	18.57	28.00	18.57	0.60
4.CDW	55.14***	3.63	5.15	3.63	0.59
5.TScN	536.81***	55.47	48.13	55.47	0.46
6.FScN	261.42***	18.67	24.28	18.67	0.57
7.IFScN	145.35***	31.69	11.37	31.69	0.26
8.TSeN	619.48***	37.59	58.19	37.59	0.61
9.SSeN	247.35***	16.40	23.10	16.40	0.58
10.ESeN	219.17***	12.02	20.72	12.02	0.63
11.1000 SW	17.18***	1.22	1.60	1.22	0.57
12.ApL	2.67***	0.15	0.25	0.15	0.63
13.ApW	2.88***	0.17	0.27	0.17	0.61
14.ApT	1.19***	0.10	0.11	0.10	0.52
15.SeL	0.48***	0.04	0.04	0.04	0.52
16.SeW	0.27***	0.06	0.02	0.06	0.26
17.WL	26.11***	1.48	2.46	1.48	0.62
18.WW	1.75***	0.21	0.15	0.21	0.42
19.SeP	1579.22***	102.69	147.65	102.69	0.59
20.SeE	0.04***	0.003	0.00	0.003	0.55
21.CoV	68.56***	4.30	6.43	4.30	0.60

¹*** =statistically significant of the 0.001 probability level. *H²= $\sigma^2_C / (\sigma^2_C + \sigma^2_E)$ (σ^2_C =Total genetic variance; σ^2_E =Phenotypic variance)

Broad sense heritability of clone mean basis (H²) was estimated as shown in Table 2. The H² values varied in a range of 0.56-0.60 for cone related characters. Also, *CoV*, expressing cone size is one of the strongly inherited characters (H²=0.60).

Seed potential (*SeP*) is the maximum number of seeds that a cone can produce (two times the number of fertile scales [25], and the seed efficiency is he ratio of the total number of filled seeds to the seed potential [26]. *TScN*, *FScN* and *IFScN* were quite variable among the clones. *TScN* varied among clones from 56.31 to 86.51 (mean= 71.19) while *FScN* varied from 19.75 to 42.17 (mean= 30.11) (Table 1). Also, *IFScN* varied among clones from 34.14 to 51.48 (mean = 41.08). Thus, as to the combined mean of *FScN* (30.11±0.93) and *SSeN* (11.52±0.91) each scots pine cone had the potential to produce about 60 seeds and the average *SE* is 18% in the studied orchard. Although there is no estimates for Turkish clonal seed orchards by using cone analysis about seed efficiency, this 18 % value is 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 [27].

Table 3. Pearson correlation coefficients of cone and seed traits of Scots pine.

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1.CWW	1																				
2.CW	0.93	1																			
3.CL	0.90	0.73	1																		
4.CDW	0.98	0.91	0.87	1																	
5.TSeN	0.63	0.58	0.55	0.64	1																
6.FSeN	0.71	0.57	0.73	0.73	0.87	1															
7.IFSeN	0.26	0.34	0.09	0.24	0.75	0.33	1														
8.TSeN	0.54	0.42	0.61	0.57	0.63	0.83	0.09	1													
9.SSeN	0.76	0.67	0.81	0.79	0.64	0.82	0.12	0.81	1												
10.ESeN	0.06	-0.03	0.11	0.07	0.34	0.46	0.04	0.76	0.24	1											
11.1000SW	0.77	0.71	0.73	0.79	0.31	0.48	-0.05	0.31	0.54	-0.10	1										
12.ApL	0.57	0.40	0.78	0.54	0.02	0.31	-0.38	0.27	0.54	-0.15	0.69	1									
13.ApW	0.77	0.82	0.61	0.71	0.26	0.35	0.04	0.36	0.52	0.04	0.56	0.39	1								
14.ApT	0.54	0.67	0.47	0.52	0.25	0.21	0.21	0.13	0.32	-0.13	0.54	0.44	0.43	1							
15.SeL	0.35	0.28	0.50	0.34	0.08	0.26	-0.18	0.14	0.35	-0.17	0.61	0.60	0.28	0.37	1						
16.SeW	0.72	0.77	0.52	0.72	0.31	0.27	0.24	0.02	0.31	-0.31	0.72	0.39	0.63	0.55	0.22	1					
17.WL	0.80	0.61	0.90	0.80	0.45	0.65	-0.00	0.45	0.71	-0.05	0.78	0.81	0.43	0.39	0.52	0.50	1				
18.WW	0.70	0.75	0.5	0.72	0.25	0.36	0.00	0.20	0.46	-0.20	0.74	0.44	0.69	0.41	0.33	0.77	0.58	1			
19.SeP	0.67	0.53	0.67	0.73	0.79	0.96	0.22	0.82	0.80	0.46	0.54	0.31	0.29	0.19	0.27	0.28	0.64	0.40	1		
20.SeE	0.64	0.59	0.71	0.68	0.40	0.62	-0.07	0.70	0.94	0.12	0.53	0.60	0.50	0.33	0.40	0.28	0.65	0.50	0.65	1	
21.CoV	0.98	0.95	0.89	0.96	0.60	0.67	0.25	0.53	0.77	0.03	0.76	0.58	0.79	0.64	0.37	0.74	0.77	0.70	0.63	0.67	1

As to the Pearson correlation coefficients between all combinations of the characteristics (Table 3), *CWW* was strongly correlated with *CDW* ($r=0.98$), *CoV* ($r=0.98$) and as expected *CW* ($r=0.93$), *CL* ($r = 0.90$). There was more significant positive correlation between *CDW-SSeN* ($r=0.79$) than *CDW-ESeN* ($r=0.07$). Similarly, significant positive correlations are reported between cone weight-number of filled seeds per cone and between cone weight-seed weight in *Pinus sylvestris* while correlation between cone weight-number of empty seed per cone ($r = 0.61$ and 0.24 for two grafts) changed for grafts [28]. In accordance with the findings [15] in *Pinus halepensis*; *CWW* was significantly correlated with *CDW* ($r=0.98$), *TSeN* ($r=0.54$) and *1000 SW* ($r=0.77$). Positive significant correlation ($r = 0.95$) was also found between yield of filled seed per cone and total number of seeds per cone in a clonal seed orchard of *Pinus halepensis* [15]. This value was estimated as 0.81 (between *TSeN* and *SSeN*) in this study. Cone fresh weight was significantly correlated with cone dry weight total number of seeds per cone and seed weight in *Pinus halepensis* [15]. Positive significant correlations among cone and seed characters (cone length, cone width, cone dry weight, seed weight and number of filled seed per cone) are reported in *Pinus contorta* [23]. In accordance with this result, correlations between *SSeN* and other cone traits varied in a range of 0.67 - 0.81 , while they varied in a range of 0.71 - 0.79 for *1000 SW* (Table 3). Also, positive and significant correlation among cone and seed crops [29] and between seed-cone crop size, filled seed yield [30] were found in *Pseudotsuga menziesii*.

In Swedish seed orchards the percentage of filled seeds is 88 and 73% at the top and bottom of grafts, respectively. Also, the weight of filled seeds varied between 6.4 to 6.6 mg [31]. In this present study, the filled seed percentage among the clones varied between 21.9 to

66.1%. And, also the weight of filled seeds varied from 8.6 to 13.2 mg. In that case, orchard seeds seem to be about half as heavy as in studied orchard of Turkey [31].

In this study, for the studied seed orchard high variation both among the clones and among the ramets (grafts) indicates the high selection capacity in the breeding programs for Scots pine. Also, the high heritability values for some of the studied characters show high selection intensity. The bigger variation within the clones than among the clones confirms that the genotypes have responses against the heterogeneity of growing area in seed orchard. Therefore, attention must be paid to these traits in the management of existed orchards or for the establishment of new seed orchards. In the same time, the data of the periodical and comparative experiments in the same orchard, will support the breeding perspectives of this species, and these results will serve as a sample for similar evaluations in other species.

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