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Full Length Research Paper

Evaluation of seed production of scots pine (*Pinus sylvestris* L.) clonal seed orchard with cone analysis method

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This research was carried out to investigate seed production in a 13 years-old scots pine (*Pinus sylvestris* L.) clonal seed orchard, including 30 clones. Eight of cone and seed traits as number of fertile and infertile scales, cone volume, cone number, filled and empty seed number, seed efficiency and 1000 seed weight were studied. Significant differences existed between the clones in all traits. The overall mean of fertile scale number was 30.1 with a range of 19.8 to 42.2 among the clones while infertile scale number was 41.1 with a range of 34.1 to 51.5. Overall mean of filled seed number, empty seed number and filled seed percentage was 11.6, 15.0 and 43.6%, respectively. The cone number and cone volume varied 33.4 to 287.88 and 6.6 to 18.2 cm^3 among the clones, respectively. The average seed efficiency for all clones was rather low and 17.9%. The overall mean of 1000 seed weight was found 10.9 g with a range of 8.6 to 13.2 g. As to 1000 seed weight and filled seed number values, 0.68-19.33 kg of filled seeds per hectare varying among the clones can be produced in this orchard. The cluster dendrogram produced two groups; 18, 27, 26, 30 numbered clones in the first group and the others.

Key words: *Pinus sylvestris*, seed, orchard, clone, cone.

INTRODUCTION

Scots pine (*Pinus sylvestris* L.) is one of the most common and important forest tree species in Turkey due to usefulness of its wood to many commercial uses. Pure stands of this species occupy roughly 750 000 ha (Genç, 2004). This species is classified as one of the economically important tree species for Turkish Forestry in the "National Tree Breeding and Seed Production Program" (Koski and Antola, 1993). For importance of this species, in Turkish Forestry, planning, economical and biological success of plantation are thus important issues. Of course big amount of reproductive material is necessary to keep present scots pine forest stock even to continue with increasing trend by rehabilitating the degraded areas. Presently, the need of scots pine seeds is approximately 715 kg per year (Bilir et al., 2007).

Demand for scots pine seeds and seedlings in Turkey is obtained mainly by domestic production, but currently only 9.2% of the Turkish seed demand in scots pine is supplied from current 111 ha of seed orchards (Cengiz, 2003; Bilir et al., 2007). Most of these seed orchards are still rather young. Nevertheless, new orchards have been established to cover the demand of improved seed for afforestation according to the national seed and tree breeding program (Koski and Antola, 1993). High and genetically improved seed yield from the seed orchard is an integral part in the success of a tree breeding program. If high seed production is to be maintained, yields must be monitored, causes of seed losses must be identified in the seed orchards. So, as all over the world, in Turkey, orchard managers need a procedure to identify and quantify seed production losses in scots pine. In order to manage seed orchards effectively and efficiently, managers need simple, reliable and practical methods to

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monitor the quantity and quality of seed crops, from the time of seed-cone pollination to cone harvest.

There are some techniques for determining seed production/efficiency in seed orchards. The cone analysis method which is one these methods first used for red pine (*Pinus resinosa* Ait.) by Lyons (1956). The method was modified and further developed as a procedure for evaluating seed production and seed efficiency, seed losses in seed orchards (Bramlett, 1972). This method provides information which is needed to evaluate and modify orchard management procedures so that seed yield can be maximized.

In this study, the seed production in a scots pine clonal seed orchard was assessed by using cone analysis method. The information obtained is 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 clonal seed orchards in Turkey.

MATERIALS AND METHODS

This study was carried out in Tekçam clonal seed orchard, located at Taşköprü, Kastamonu ($41^0$36' N, $35^0$05' E, 1160 m). The orchard was established in 1995 and comprises 1987 grafts of 30 clones derived from intensively selected trees in Araç-Dereyayla seed stand. Grafts were 2 years old at the time of establishment and were planted at a spacing 6 x 6 m. Commercial cone harvesting started in the 2003 when the grafts were 10 years old. By the time of this study (2006), the graft age is 13 years. So far, no pruning has been done in the orchard.

In all clones, ten replications (grafts) were sampled at random and the cones, were harvested, and counted separately by graft (ramet). Cones were collected in autumn 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 height. 12 of cones having no external damage were collected at random from the middle level of graft between 1/3 and 2/3 of the height. 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. Ten grafts of 30 clones respectively were assessed for number of fertile/infertile scales of graft (FScN and IFScN), number of cones/graft (CoN), cone volume/graft (CoV), filled/empty seed number of graft (FSeN and ESeN), seed efficiency (SE) and 1000 seed weight (g)/graft (1000SW).

After opening the scales, they were systematically removed, one by one, with forceps to determine the number of fertile and infertile scales and remove the seeds. Fertile scales were located 4 in the central portion of the cone and had two well developed ovules in the form of a mature seed for pine species (Lyons, 1956; Bramlett et al., 1977). After removing the seed wing, extracted seeds were floated on water during 12-16 h. After that, number of empty and filled seeds was counted. Cone number for per graft was counted and cone volume was determined by thinking the cones as a geometric cone. 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).

Data were subjected to multi-way analysis of variance. Variables were tested for normality and homogeneity of variances and logarithmic transformation for FScN, IFScN, FSeN, ESeN, CoN, arcsine for SE, were made. All pair wise comparisons of individual means were done by the least significant differences (LSD). Differences were considered significant at $P < 0.05$ level. Coefficient variation 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. Relationships between eight of seed related characters were tested using correlation analyses. Moreover, dendrograms were constructed on the basis of Euclidean distances by the nearest neighbourhood method to analyze the similarity between clones as to the studied characters (Zar, 1999).

RESULTS AND DISCUSSION

The analysis of variance showed that there was significant differences among clones at 0.001 and 0.01 probability level for all cone and seed characteristics examined (Tables 1 and 2). All clones combined, scots pine cones had a mean of 30.1 FScN with range among the clones from 19.8 (clone 24) to 42.2 (clone 26). Thus, as to the combined mean of FScN (30.1), each scots pine cone had the potential to produce about 60 seeds. The IFScN varied among the clones from 34.1 (clone 3) to 51.5 (clone 8) with overall mean 41.1 (Table 1). The bigger CV_G values than CV_C show the bigger variation within the clones than among the clones in the orchard.

As overall mean of the clones, there were 166.1 of cones/graft in the orchard. The biggest value (287.88) of CoN was estimated for clone 23, while the lowest value (33.4) for clone 1 (Table 1). The big value of standard error for CoN shows the variation within the clones.

It was found that cone volume was under strong genetic control (Verheggen and Farmer, 1983; Stoehr and Farmer, 1986). In this study, CoV varied among the clones from 6.6 (clone 15) to 18.2 cm^3 (clone 30) with overall mean of 10.7 cm^3 (Table 1).

The overall mean of FSeN and ESeN per clone was 11.6 and 15.0, respectively. It was found that filled seed number per cone 9.9 for the same orchard in 2005 (Bilir et al., 2007). This indicates the effect of age increasing to seed production. FSeN, varied among the clones from 3.3 (clone 7) to 22.8 (clone 18) while ESeN varied from 6.5 (clone 9) to 25.8 (clone 2) (Table 2). The FSeN variation in this study (3.3-22.8) is in the lower range with Yazdani et al. (1995), who reported that total seeds per cone varied between 9.3 and 21.8 in a scots pine clonal seed orchard. Also, overall mean of filled seeds percentage in this study was 43.6%. This value is varied at 21.9-66.1% interval among the clones and at 8.22-81.33% interval within the clones. The overall mean value (43.6 %) of filled seed percentage is in the lower range of what (Yazdani et al., 1995) reported. They showed that the percentage of empty seeds per cone in clonal seed orchard of scots pine ranged from 12% to 39%. Furthermore, the result is lower than 12% in average proportion of empty seeds (88% of filled seeds) in open pollinated

Table 1. Mean values and multiple comparisons of studied cone related characters.

Clone	Cone related characters							
	FScN		IFScN		CoV		CoN	
1	31.1±0.7[1]	ef[2]	39.1±1.3	fghi	7.4±0.2	mn	33.4±7.9	n
2	36.4±0.9	bc	42.6±1.4	bcdef	10.6±0.3	efghi	80.2±25.3	kl
3	24.9±0.8	jkl	34.1±1.0	j	10.5±0.5	efghij	152.4±31.8	cdefghij
4	26.1±0.9	hijk	39.5±1.8	fghi	10.7±0.3	efghi	161.2±34.8	cdefghij
5	27.6±1.0	fghij	37.6±1.6	ghij	10.0±0.7	ghijk	74.8±29.6	lm
6	35.9±1.0	bcd	41.9±1.5	bcdefg	9.5±0.3	ghijk	219.6±50.4	abcde
7	25.5±0.8	hijk	46.7±1.4	abcd	8.2±0.3	klmn	119.6±27.2	ghijk
8	25.0±0.9	jkl	51.5±2.5	a	9.2±0.4	ghijkl	255.6±38.8	ab
9	25.6±0.7	ijkl	35.4±1.1	ij	10.8±0.3	efgh	135.2±34.9	efghij
10	33.7±1.0	cde	39.0±1.3	fghi	12.1±0.4	cde	250.8±49.4	abc
11	29.9±1.5	fghi	41.0±1.6	cdefg	11.0±0.8	efg	192.8±56.5	bcdefghij
12	31.4±0.9	def	43.3±1.5	bcdef	11.9±0.5	cdef	145.6±39.6	efghij
13	29.0±0.6	fgh	41.3±1.6	cdefg	10.4±0.6	efghij	94.6±16.5	ijkl
14	29.2±0.7	fgh	44.7±1.4	bcde	7.7±0.2	lmn	190.0±17.4	abcdef
15	27.0±0.7	ghij	34.3±1.0	j	6.6±0.2	n	186.6±28.9	abcdefg
16	24.0±1.0	kl	41.0±1.6	cdefgh	9.1±0.3	hijklm	121.4±43.8	jkl
17	30.6±1.0	efg	40.5±2.0	efghi	10.4±0.3	efghij	106.8±24.3	hijk
18	38.6±1.2	ab	47.0±1.3	ab	13.7±0.4	bcd	112.6±15.5	fghij
19	31.0±1.0	ef	37.2±1.6	ghij	9.9±0.5	ghijk	153.6±44.3	defghij
20	27.8±0.9	fghij	41.9±1.5	bcdefg	8.9±0.2	ijklm	200.6±48.7	abcdefg
21	30.8±0.6	ef	39.2±1.1	fghi	13.8±0.4	bc	190.4±27.7	abcdefg
22	30.0±0.8	eg	41.3±1.3	cdefg	10.1±0.4	fghij	158.0±27.0	bcdefghi
23	23.0±1.2	l	40.2±1.4	efgh	8.7±0.5	jklm	287.8±64.8	a
24	19.8±0.7	m	36.6±1.7	hij	7.4±0.2	mn	250.4±60.2	abcd
25	34.3±1.4	cde	41.0±1.7	defgh	11.9±0.6	def	197.0±63.0	abcdefgh
26	42.2±0.8	a	44.3±1.6	bcdef	14.3±0.3	b	255.2±35.6	ab
27	38.2±1.1	ab	41.1±1.8	cdefg	15.0±0.4	b	105.2±16.1	hijk
28	29.2±0.8	fgh	41.7±1.2	bcdefg	12.2±0.7	cde	251.8±34.1	ab
29	29.7±0.9	efg	40.7±1.6	efgh	9.3±0.5	ghijkl	241.2±35.6	abc
30	36.1±1.1	bc	46.0±1.6	abc	18.2±0.7	a	57.8±23.3	mn
Overall Mean	30.1±0.2		41.1±0.3		10.7±0.1		166.1±5.8	
F value /Clone[3]	14.022***		4.262***		1.880**		8.276***	
LSD[4]	0.055		0.054		5.491		0.241	
F value /Graft	0.003ns		0.679ns		1.521ns		0.552ns	
CV_G (%)	21.49		15.97		30.53		60.98	
CV_C (%)	16.86		9.21		24.12		40.58	

[1]Values are the means of 10 grafts and show as mean ± standard error.
[2]For each character, mean values with the same letter are not significantly different at $P < 0.05$ level;
[3] ***: significant at $P < 0.001$; **: significant at $P < 0.01$; ns: none significant; [4]The values of FScN, IFScN and CoN are transformed.

Pinus sylvestris, reported by Koski (1971). However, Bilir et al. (2007) reported that the percentage of filled seeds increases with increasing orchard age. So, the low filled seeds percentage in this seed orchard can be explained with the orchard age (13 years old as of 2006). The percentage of filled seeds per cone is of vital interest because it reflects the effectiveness of fertilization and thus the amount of self-fertilization. Selfing causes empty seeds in scots pine, and selfing results more often in empty seeds than in vital selfed seeds (Lindgren, 1975).

The average SE for all clones was 17.9%. Although, there is no estimates for Turkish clonal seed orchards by using cone analysis about seed efficiency, this17.9% value is quite low in comparison to literature, especially to

Table 2. Mean values and multiple comparisons of studied seed related characters.

Clone	Seed related characters							
	FSeN		ESeN		SE		1000SW	
1	8.5±0.6	def	20.0±0.7	bc	13.4±0.9	jk	8.6±0.1	m
2	11.1±0.7	cde	25.8±1.2	a	14.7±0.9	hij	10.8±0.4	ghi
3	11.5±0.9	cde	10.5±0.7	j	22.6±1.4	bcd	12.5±0.3	abc
4	10.3±0.8	cde	9.8±0.9	j	18.9±1.2	defghi	10.9±0.2	fghi
5	12.7±1.1	cde	11.3±0.7	hij	21.2±1.6	cdef	10.0±0.2	jkl
6	12.6±0.8	cde	10.4±0.5	j	17.1±1.0	fghij	11.1±0.2	efghi
7	3.3±0.3	k	12.5±0.8	ij	5.3±0.6	m	10.5±0.2	ijk
8	6.1±0.7	fgh	11.1±1.0	j	10.4±1.1	kl	8.9±0.2	lm
9	12.7±0.8	bc	6.5±0.5	k	23.9±1.3	bcd	11.8±0.2	cdef
10	18.2±0.9	ab	21.4±0.9	ab	26.4±0.9	ab	11.8±0.2	cdefg
11	13.1±1.2	cde	10.0±0.7	j	20.3±1.3	defghi	12.0±0.3	bcd
12	12.2±0.9	bcd	18.1±0.7	bcde	19.1±1.3	defghi	11.4±0.2	defgh
13	12.2±0.6	bcd	19.1±0.9	bcd	20.7±0.9	cdef	10.8±0.3	fghi
14	9.9±0.5	cde	19.0±0.9	bcd	16.9±1.0	fghij	9.2±0.4	lm
15	7.9±0.6	efg	20.5±0.9	ab	14.5±1.0	ij	8.8±0.1	m
16	5.4±0.7	hij	13.3±0.9	fghi	9.8±1.0	kl	9.1±0.3	lm
17	10.3±0.8	cde	16.4±1.1	cdef	15.5±1.2	ghij	10.3±0.2	ijk
18	22.8±1.0	a	15.6±0.7	def	29.7±1.3	a	10.3±0.2	ijk
19	8.9±0.9	efg	15.4±1.1	defg	13.5±1.2	jk	11.9±0.3	bcd
20	5.7±0.6	ghi	11.4±0.8	hij	9.4±0.9	kl	10.8±0.2	hij
21	12.8±0.6	bc	16.1±0.7	cdef	20.7±0.9	cdef	13.1±0.2	a
22	10.5±0.6	cde	21.2±1.0	ab	17.2±0.8	efghij	11.4±0.2	defgh
23	4.9±0.7	ij	13.6±1.1	fgh	7.1±0.9	lm	9.8±0.3	kl
24	3.7±0.4	j	11.0±1.1	hij	7.2±0.8	lm	9.1±0.2	lm
25	14.2±1.1	bc	16.0±1.0	def	19.9±1.0	defg	11.9±0.3	bcde
26	21.3±0.9	a	17.6±0.7	bcde	25.2±1.0	abc	12.8±0.2	ab
27	21.4±1.2	a	21.5±1.1	ab	27.5±1.3	ab	11.5±0.2	cdefgh
28	13.4±0.8	bc	7.7±0.9	k	22.5±1.2	bcd	11.7±0.2	cdefgh
29	13.5±1.0	bc	14.9±0.8	efg	22.4±1.5	bcde	10.3±0.2	ijk
30	15.5±1.3	bc	12.2±0.9	ghij	19.9±1.5	defgh	13.2±0.4	a
Overall Mean	11.6±0.2		15.0±0.9		17.9±0.3		10.9±0.1	
F value /Clone	14.174***		16.915***		15.349***		14.668***	
LSD[5]	0.170		0.160		3.660		0.968	
F value /Graft	0.002ns		0.685ns		0.138ns		0.003ns	
CV_G (%)	53.72		38.30		44.63		15.33	
CV_C (%)	42.72		31.75		35.59		12.02	

[5] The values of FSeN and ESeN are transformed.

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. The lowest (5.3) SE was observed in clone 7 while the highest (29.7) for clone 18 (Table 2).

Overall mean of 1000SW was found 10.9 g with range among the clones from 8.6 g (clone 1) to 13.2 g (clone 30) (Table 2). One of the advantages with seed orchard seed is that the seed weight normally is higher than for seed stand (Wennstrom, 2001). This is also shown in the study where the average seed weight per seed was 10.9 mg. In accordance with this result, Bilir et al. (2007) found mean weight of a seed 11.1 mg for three clonal seed orchard of scots pine in Turkey. Boydak (1977) reported a seed weight of 9.2 mg in natural populations of *P.*

Table 3. Pearson correlation coefficients among eight cone and variables.

Variable	1	2	3	4	5	6	7	8
1.FScN	1.000	0.198**	0.769**	0.473**	0.544**	0.639**	0.363**	-0.195
2.IFScN		1.000	0.128*	0.052ns	-0.005ns	0.195**	-0.061ns	0.026ns
3.FSeN			1.000	0.274**	0.925**	0.659**	0.355**	-0.096ns
4.ESeN				1.000	0.139*	0.177**	0.004ns	-0.266ns
5.SE					1.000	0.542**	0.325**	-0.098ns
6.CoV						1.000	0.659**	-0.188ns
7.1000SW							1.000	0.019ns
8. CoN								1.000

ns: Non significant, **: significant at the 0.01 level, *: significant at the 0.05 level.

sylvestris in the middle part of Turkey. Also, Turna (2003) and Şevik (2005) reported 9.9 and 10.1 mg from eleven and nine Turkish scots pine populations, respectively.

The FSeN and 1000SW per cone or clone are important genetic characteristics for estimation of seed harvest from seed orchards or per hectare. In this study, the mean FSeN is varied between 3.3-22.8 among the clones and 0-33.1 within the clones. Also, the 1000SW values are varied in 8.6-13.2 g interval among the clones and in 7.1-15.9 g within the clones. As to these values in this orchard 0.68-19.33 kg of filled seeds per hectare varied among the clones can be produced. The seed yield value of only one clone is above 15 kg/ha. For this reason, in the second generation seed orchards, the clones capable to produce copious seed should be widely used in point of seed production. Also, in the seed orchard the higher seed production is contributed by the fact that the density is higher, stimulating early seed production in this orchard. Seed crop per hectare in scots pine may surpass 30 kg under climatically optimal conditions of Central Europe and 15 kg in northern Sweden (Matyas, 1991). In spite of the favorable climatic conditions when compared to those locations, the seed production is rather low in the studied orchard. The low seed yield in this orchard may be explained with young age of the orchard and disharmony in synchronization among the clones.

When examine the relationships among the cone and seed traits; the FScN had a stronger positive correlation with CoV (r = 0.639) than IFScN (r = 0.195). Similarly, the association between CoV and FSeN (r = 0 .659) was greater than the association between CoV and the ESeN (r = 0.177). There was a strong correlation between the FScN and FSeN (r = 0.769) but correlation between FScN and IFScN was weak (r = 0.198). SE was weakly correlated with some of the cone measures (r = -0.005 to 0.139) except the FSeN (r = 0.925) as would be expected and FScN (r=0.544). There was no correlation between SE and IFScN at p≤0.01 level. The number of ESeN was not strongly correlated with any of the other cone traits. The 1000SW is strongly correlated with CoV. There was no significant correlation between CoN per clone and all the other traits (Table 3).

The results in this study of correlations between characters are mainly in accordance with previous studies in *P. sylvestris* and other species. Positive significant correlation (r=0.95) was found between yield of filled seed per cone and total number of seeds per cone in a clonal seed orchard of *Pinus halepensis* (Matziris, 1998). Also, positive significant correlations were reported among seed and cone characters in *Pinus contorta* (Ying et al., 1985) and *Pseudotsuga menziesii* (Reynolds and El-Kassaby, 1990). Positive and significant correlation was also found between seed-cone crop size and filled seed yield (r=0.93) in *P. menziesii* (El-Kassaby and Cook, 1994). Greene and Johnson (1994) found that seed production is highly, inversely, correlated with the mean seed mass.

On the cluster dendrogram constructed on the basis of Euclidean distances with the use of the nearest neighbourhood method for 8 quantitative seed and cone traits, two distinct groups can be noticed: the first composed of clones 18, 27, 26, 30 and the others (Figure 1).

Variation in cone production within the clones (5-465) is greater than among the clones (33.4-287.8) as it is shown by El-Kassaby et al. (1989) for *P. menziesii*. Also, El-Kassaby and Cook (1994) reported that both seed-cone and filled-seed production were under genetic control in that species. Clonal variation in both seed and cone production capability will not affect genetic composition of seed orchard seeds, but also the balance between maximizing genetic gain and maintaining seed production capacity at the time of seed orchard rogueing (Ying and Illingworth, 1985). 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 (Prescher et al., 2005). 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 of Prescher et al. (2005) seem to be about half as heavy as in studied orchard of

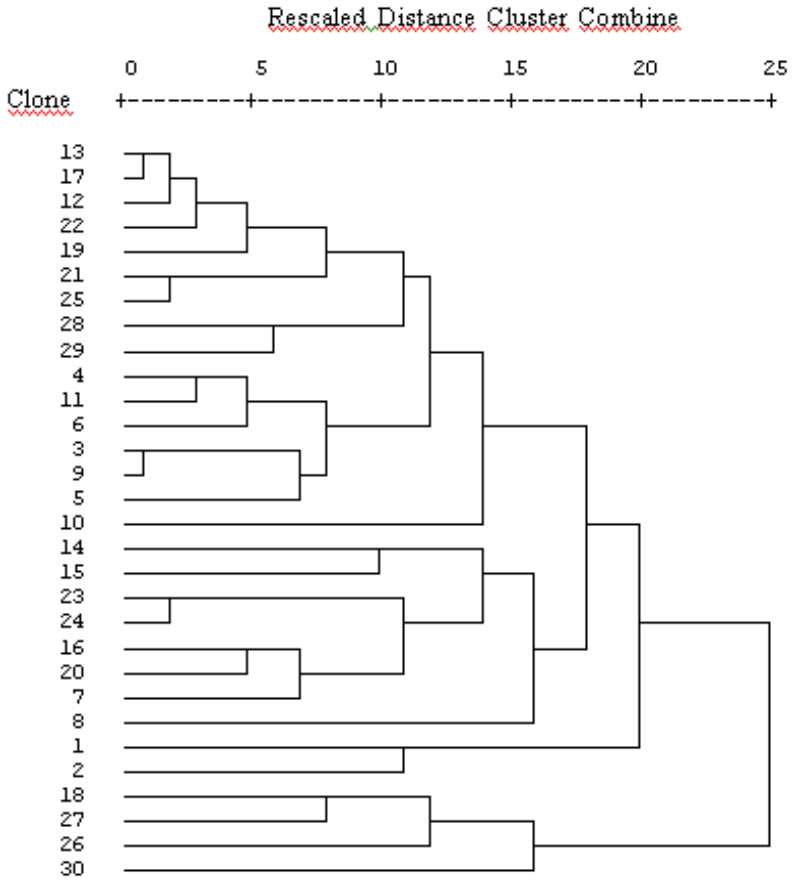

Figure 1. Dendrogram of 30 clones of *Pinus sylvestris* based on 8 seed and cone traits.

Turkey.

The variation between clones indicates that production and quality of seed of scots pine can be improved by selection. The size of genetic base must not be reduced. Also, attention must be paid to these characteristics when management of existed or establishment of new and second generation seed orchards are made.

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