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Genetic Variation in Cone and Seed Characteristics in a Clonal Seed Orchard of Aleppo Pine Grown in Greece

By D. MATZIRIS[1]

Summary

Cone and seed characteristics of Aleppo pine (*Pinus halepensis* MILL.) were investigated in a clonal seed orchard for two successive years, 1994 and 1995. The orchard was established in January 1987 in an area of 10 ha, at Amphilochia, west Greece and includes 76 clones. The results showed that significant genetic variation exists among clones for cone wet and dry weight, number and volume of seeds extracted, weight and volume of 1000 seeds, percentage of full seeds and cone, seed and wing lengths and widths. Only cone moisture content was predominantly influenced by the environment. Cone weight at the time of harvesting (June) varied among clones from 30 g to 77.2 g ($\bar{x}$ = 48.99 g), while the dry weight varied from 27.2 g to 70.2 g ($\bar{x}$ = 44.1 g). Cone length varied from 6.6 cm to 11.6 cm with overall mean 9.3 cm. Year to year correlation coefficients for seed characteristics were varied from moderate (0.46) to strong (0.81). These correlations indicated that the clones are quite stable from year to year, in production and in seed quality and size. Broad sense heritability (H^2) estimates were variable. Cone length and width were strongly inherited with H^2 values 0.74 and 0.73 respectively. The respective H^2 values for wet and dry cone weight were 0.79 and 0.78. The number of seeds per cone and the number of full seeds were moderately inherited characteristics with H^2 values 0.47 and 0.36 respectively. Seed volume and weight based on a 1000 seed sampling were strongly inherited (H^2 = 0.75 and 0.73, respectively). The percentage of full seed had a H^2 value 0.41.

Key words: *Pinus halepensis*, Aleppo pine, seed orchard, heritability, correlation, maternal effect.

FDC: 164.7/8; 165.3; 165.51; 232.311.3; 232.312.31; 174.7 (*Pinus halepensis*; (495).

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Introduction

Aleppo pine (*Pinus halepensis* MILL.) is a circum-Mediterranean species with discontinous geographic distribution and is very well adapted to dry sensitive to fire ecosystems. The species flowers at an early age and produces a large number of serotinous cones, that persist on branches for many years. Cone and seed characteristics vary among species, provenances and individual genotypes in *Pinus* L.. Cones vary in size from 2 cm to 3 cm in length (*P. montana* MILL.) to 50 cm to 60 cm (*P. lamberteana* DOUGL.) (MIROV, 1967). In weight the variation is even greater (2 g in *P. montana* MILL., 1100 g in *P. coultery* D. DON.). Cone and seed sizes vary also widely among seed orchard clones (BERGMAN, 1968). It is well known that double fertilization in gymnosperms does not take place (cf. CHAMBERLAIN, 1966) as in the angiosperms and the endosperm (female gametophyte) has a haploid number of chromosomes (n = 12) instead of being diploid (2n = 24). The endosperm is enclosed in a seed coat which is diploid, developed also from maternal tissue. Therefore the characteristics of the seed coat including seed wings are inherited from the female parent alone. CLAIR and ADAMS (1991) stated that the variation in seed weight of different female parents of coniferous species are the result of three factors: (i) the diploid gametophyte of embryo, (ii) the haploid genotype of the megasporophyte (endosperm), and (iii) the enviromental effect on the mother tree during seed development. As the endosperm in coniferous seeds, that is the nutrient source of embryo, is exclusively maternal in origin, the female parent determines the characteristics "e. g., endosperm weight" of the nutrient source. The maternally inherited diploid seed coat also determines phenomena related to seed dormancy and germination (EL-KASSABY et al., 1992).

The purpose of the present study is to investigate the variation and inheritance pattern in cone and seed characteristics of

Aleppo pine and to determine interelationships that exist among these characteristics.

Material and Methods

Plant Material

The study was conducted in a 10 ha clonal seed orchard of Aleppo pine, located in Amphilochia, west Greece, during the years 1994 and 1995. The seed orchard was established in January 1978 and includes 76 clones, derived from intensively selected plus trees in the natural forests of Euboia island. Clones (one ramet per clone) were randomly assigned within replications with one only restriction. No grafts of the same clone were planted closer than 30 m. A random sample of 10 mature cones were obtained from the middle part of each tree's crown in three replications during June, 1994 and 1995.

Measurements

A total of 16 characteristics were evaluated. In a laboratory, the cones were weighted and lengths and diameters (across the broadest portion) were measured. The cones were then dried at 50° for 24 h and weighted. The moisture content of each cone was calculated by subtracting the dry weight from wet weight. The seeds were then extracted (by tree), dewinged, counted and the weight and volume calculated. The number of full seeds was determined by dissection, and a percentage of full seeds were calculated. A sample of 1000 seeds were weighted and the volume calculated. Prior to dewinging, a random sample of 10 seeds from each tree was taken and lengths and widths of both seeds and wings were measured.

All characteristics were evaluated in 1994. In 1995, only the total number of seeds extracted from 10 cones, seed weight, number of full seeds, and weight of 1000 seed sampled were calculated.

Statistical Analyses

Analyses of variance were performed for all 16 characteristics. Variance components were estimated by equating mean squares to their expectations and solving the resultant equations. The variance between clones (σ^2_c) is interpreted as the total genetic variance, while the variance between ramets within clones (σ^2_w) was ascribed to enviromental variance. The standard errors of the components of variances were computed using the appropriate equation (ANDERSON and BANCROFT, 1952; NAMKOONG, 1979; BECKER, 1985). Broad sense heritability values (H^2) were estimated as the ratio of total genetic variance (σ^2_c) to total phenotypic variance (σ^2_c) + (σ^2_w) (MATZIRIS, 1984). Cloning effect variance biases the H^2 values, but the magnitude is negligible (MATZIRIS, 1993) and can be ignored.

PEARSON and SPEARMAN (SNEDECOR and COHRAN, 1968, page 194) correlation coefficients were estimated among the characteristics. It is worth mentioning here that SPEARMAN's coefficient of rank correlation applies to data in the form of ranks and does not require the assumption of bivariate normal distribution (STEEL and TORRIE, 1960).

Results and Discussion

The overall mean values for all characteristics assessed in 1994 and 1995 along with their minimum and maximum values and their standard deviations are presented in *table 1*. The analyses of variance (*Table 2*) showed that there are significant differences among clones at 0.01 probability level for all cone and seed characteristics examined with the exception of moisture content. An examination of the standard errors of between clone variances (*Table 2*) reveals that the components of variances were estimated with adequate accuracy.

Cone Characteristics

The wet weight of 10 cones per clone (at the time of collection) varied from 300 g to 772 g with an overall mean of 489.9 g (S. D. = 111.203) (*Table 1*). The dry weight of cones varied from 272 g to 702 g with overall mean 441.4 g (S. D. = 102.77 g). The corresponding moisture content of the cones, had a mean value of 9.1% and ranged from 6.1% to 12.6% among clones. Although the moisture content of the mature cones was quite low in June, the cones still remained closed. This significant adaptive feature is related to the fire regeneration of the species.

Table 1. – Overall means, ranges and standard deviations of cone and seed characteristics of Aleppo pine grown at the seed orchard of Amphilochia Greece.

	Characteristic	unit	mean	min	max	S.D.
	A. Data 1994					
1.	Cone wet weight	g	489.9	300.0	772.0	111.203
2.	Cone dry weight	g	441.4	272.0	702.0	102.779
3.	Moisture content	%	9.1	6.1	12.6	1.204
4.	Total num. of seeds/10 cones	no	246.5	29.0	616.0	92.044
5.	Volume of seeds	ml	16.7	0.7	40.5	6.497
6.	Weight of seeds	g	7.1	0.5	17.4	2.751
7.	Number of full seeds	no	171.8	8.0	435.0	70.906
8.	Volume of 1000 seeds	ml	69.3	47.5	97.4	11.545
9.	Weight of 1000 seeds	g	28.7	18.4	40.8	5.163
10.	Percentage of full seed	%	67.4	25.5	81.3	9.616
11.	Cone length	cm	9.2	6.6	11.6	0.911
12.	Cone width	cm	3.9	3.2	4.6	0.312
13.	Seed length	mm	7.9	6.8	8.9	0.497
14.	Seed width	mm	4.2	3.5	4.8	0.266
15.	Wing length	mm	24.6	19.3	30.8	2.554
16.	Wing width	mm	9.8	5.1	12.8	0.950
	B. Data 1995					
	Total number of seeds	no	872.5	2.0	1261.0	229.204
	Weight of seeds	g	22.7	3.5	35.5	6.382
	Percentage of full seeeds	%	91.4	55.3	99.3	7.532
	Weight of 1000 seeds	g	25.7	17.6	33.1	3.788

Table 2. – Analyses of variance, variance components, standard error of variances and broad sense heritability (H^2) estimates of cone and seed characteristics of Aleppo pine grown in the seed orchard of Amphilochia Greece.

Characteristics	MEAN SQUARES[1] Between clones	Within clones	σ^2_C	$S.E.(\sigma^2_C)$	H^2
A. Data 1994					
1. Cone wet weight	36219.391**	2986.387	110771.671	2189.672	0.79
2. Cone dry weight	30411.764**	2566.122	9281.881	1838.767	0.78
3. Moisture content	5.121**	5.681	0.000	0.393	0.00
4. Total num. of seeds/10 cones	27536.088**	7415.728	6706.787	1691.542	0.47
5. Volume of seeds	125.018**	27.583	32.478	7.637	0.54
6. Weight of seeds	23.607**	6.349	5.753	1.451	0.47
7. Number of full seeds	16106.213**	5946.211	3386.667	1004.919	0.36
8. Volume of 1000 seeds	409.038**	40.003	123.012	24.747	0.75
9. Weight of 1000 seeds	81.045**	8.971	24.025	4.906	0.73
10. Percentage of full seed	296.507**	96.902	66.535	18.371	0.41
11. Cone length	2.243**	0.232	0.670	0.136	0.74
12. Cone width	0.319**	0.035	0.095	0.019	0.73
13. Seed length	0.738**	0.123	0.205	0.045	0.62
14. Seed width	0.210**	0.040	0.057	0.013	0.59
15. Wing length	19.384**	2.220	5.721	1.174	0.72
16. Wing width	1.923**	0.225	0.566	0.116	0.72
B. Data 1995					
Seeds per 10 cones	117607.675**	11694.400	35304.2	7115.492	0.75
Seeds weight	108.980**	11.935	32.347	6.597	0.73
Filled seeds %	111.894**	21.050	30.281	6.812	0.58
Weight 1000 seeds	37.680**	5.837	10.614	2.288	0.65
Expected M. squares	$\sigma^2_w + 3\sigma^2_c$	σ^2_w			
Degrees of freedom	59	121			

[1]** Statistically significant of the 0.01 probability level
σ^2_C = variance due to differences between clones = total genetic variance
σ^2_W = within plot variance : S.E. (σ^2_C) = standard error of variance

Cone length and cone width, which are characteristics determining cone size were quite variable. Cone length varied among clones from 6.6 cm to 11.6 cm ($\bar{x}$ = 9.2 cm), and cone width varied from 3.2 cm to 4.6 cm ($\bar{x}$ = 3.9). The mean values for cone size found here are higher from those reported by PANETSOS (1975) from wild stands. He found mean values of cone length and width among Greek provenances of Aleppo pine ranging from 7.3 cm to 8.11 cm and 3.99 cm and 4.01 cm, respectively. The higher means in the present study probably reflect the fertile soil and intensive management environment of a seed orchard.

Seed Characteristics

The differences among clones for all seed characteristics were found statistically significant (*Table 2*). In 1994, the overall mean number of seeds extracted from 10 cones per tree was 246.5, i.e., 24.6 seeds/cone, with mean weight 7.1 g, volume 16.7 ml and number of full seeds 171.8 (67.4%) (*Table 1*). The mean weight and volume of 1000 seeds were 28.7 g (range = 18.4 g to 40.8 g) and 69.3 ml (range = 47.5 ml to 97.4 ml respectively.

The mean seed length varied among clones from 6.8 mm to 8.9 mm with overall mean 7.9 mm and standard deviation 0.497 mm. The corresponding range for seed width was 3.8 mm to 4.8 mm with overall mean 4.2 mm (*Table 1*).

The mean weight of 1000 seeds is higher than reported in previous studies. In a range wide provenance study of Aleppo pine, TOCCI (1979) found mean weight of 1000 seeds 19.77 g with range among provenance from 12 g to 31 g. PANETSOS (1981), studied natural populations of Aleppo pine grown in Greece and found weight of 1000 seeds ranging from 13.55 g to 21.92 g. SCHILLER and WAISEL (1989) have found mean seed weight of 1000 seeds 17.7 g with a range among provenance from 14.7 g to 21.8 g, while DEBAZAK and TAMASSONE (1965) found mean provenance values from 15.8 g up to 29.6 g. However, these studies were of trees growing in natural stands or plantations without intensive management of Aleppo pine and therefore, are not directly comparable with results found in the present study. The heavier seeds produced by the seed orchard trees are attributed to the better environmental conditions found in the seed orchards.

There was large variation among clones in wing length and width. Part of this variation was related to variation in cone size, since these characteristics were moderately to strongly correlated with cone length and width (*Table 3*).

Overall seed quality is related to a number of factors, the most important among which appears to be the proportion of empty seeds. It is evident from the analyses of variance that there are statistically significant differences among clones in

Table 3. – PEARSON (above diagonal line) and SPEARMAN (below diagonal line) correlation coefficients of cone and seed characteristics of Aleppo pine (1994 data only).

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Cone wet weight		0.96	-0.13	0.41	0.60	0.58	0.41	0.39	0.37	0.11	0.82	0.82	0.26	0.34	0.66	0.52
2. Cone dry weight	0.95		-0.19	0.37	0.56	0.54	0.37	0.40	0.38	0.09	0.79	0.81	0.25	0.34	0.61	0.53
3. Moisture content	-0.21	-0.25		0.05	-0.02	-0.01	0.08	-0.20	-0.15	0.30	-0.27	-0.05	-0.23	-0.08	-0.22	-0.06
4. Total num. of seeds/10 cones	0.37	0.30	0.07		0.88	0.89	0.95	-0.15	-0.01	0.30	0.40	0.27	0.07	-0.11	0.16	0.11
5. Volume of seeds	0.56	0.50	-0.06	0.83		0.97	0.85	0.29	0.37	0.32	0.53	0.48	0.35	0.23	0.30	0.25
6. Weight of seeds	0.54	0.47	-0.02	0.83	0.96		0.90	0.26	0.42	0.44	0.50	0.47	0.33	0.22	0.27	0.25
7. Number of full seeds	0.42	0.35	0.06	0.92	0.82	0.89		-0.11	0.10	0.52	0.38	0.31	0.08	-0.08	0.16	0.14
8. Volume of 1000 seeds	0.35	0.36	-0.22	-0.20	0.29	0.25	-0.12		0.91	0.16	0.26	0.48	0.62	0.81	0.23	0.33
9. Weight of 1000 seeds	0.33	0.33	-0.17	-0.07	0.37	0.42	0.10	0.91		0.49	0.24	0.47	0.60	0.77	0.20	0.33
10. Percentage of full seed	0.20	0.15	0.07	-0.30	0.32	0.48	0.59	0.12	0.40		0.07	0.19	0.18	0.15	0.02	0.21
11. Cone length	0.80	0.75	-0.29	0.42	0.54	0.49	0.38	0.27	0.21	0.08		0.51	0.23	0.13	0.64	0.31
12. Cone width	0.84	0.82	-0.10	0.22	0.47	0.46	0.30	0.47	0.47	0.25	0.52		0.29	0.43	0.53	0.56
13. Seed length	0.26	0.24	-0.24	0.04	0.35	0.32	0.05	0.61	0.58	0.02	0.25	0.30		0.42	0.27	0.22
14. Seed width	0.28	0.31	-0.10	-0.19	0.26	0.25	-0.09	0.79	0.79	0.13	0.10	0.40	0.40		0.07	0.31
15. Wing length	0.63	0.58	-0.26	0.88	0.33	0.30	0.26	0.21	0.18	0.07	0.62	0.52	0.27	0.03		0.35
16. Wing width	0.49	0.51	-0.01	0.00	0.22	0.25	0.12	0.43	0.43	0.27	0.17	0.54	0.25	0.51	0.22	

the proportion of full seeds (*Table 2*). In 1994, mean values were varied from 25.5% to 81.3% with overall mean of all clones 67.34% (S. D. = 9.62%). In contrast, the proportion of full seed was much higher for the year 1995; the mean clone values were ranked from 55% to 99% with overall mean 91.4% (S. D. = 7.15%). The larger differences in the proportion of full seeds found between the two years is probably due to different quantities of pollen produced in the two years.

It is interesting that the year to year correlation coefficient for the percentage of full seed was quite strong (r = 0.69), indicating that there are clones which consistently produce cones with high percentage of full seeds. All seeds examined were fully developed and no insects or other damages were observed. These results are in close agreement with findings reported by SAVRAS (1962), who concluded that the differences between individual genotypes in the proportion of empty seed produced, remain more or less constant from year to year.

It is well known that the empty seeds in pine are primarely caused by the abortion of embryo (SAVRAS, 1962, 1968; CHAMPERLAIN, 1966). Pollination has no direct effect on the formation of empty seeds, since unpollinated ovules do not develop to full size seed. However the abundance of pollination, the capacity of pollen chamber, the number of archegonia in the different ovules and most importantly, the genetic load expressed by the number of embryonic lethal genes are important factors influencing not only the proportion of full seed but also the infiltration rate of the lethal genes. It has been shown by many investigators that the abortion due to homozygosity of embryonic lethal genes is the main factor responsible for the formation of empty seed (SAVRAS, 1962; SORENSEN, 1967; FRANKLING, 1969; ORR-EWING, 1967; KOSKI, 1971; RUDOLPH, 1980; SNIEZKO, 1984; MATZIRIS, 1996).

Interelationship Between Characteristics

PEARSON and SPEARMAN rank correlation coefficients between all combinations of the characteristics studied are shown in *table 3*. As can be seen, there is close agreement between PEARSON and SPEARMAN correlation coefficients, indicating that there are no abnormalities in the distribution of the variables examined. The most meaningful relationships are briefly discussed below.

Cone weight at the time of harvesting is strongly correlated with cone dry weight (r = 0.96) and as expected cone length and width (r = 0.82). Cone weight was more moderately correlated with the number of seeds per cone, seed weight, and volume of seed extracted (r = 0.41, 0.50 and 0.60 respectively), number of full seeds (r = 0.41), weight and volume of 1000 seeds (r = 0.37 and 0.39, respectively). The larger and heavier the cones are, the larger and the heavier and the seeds included in them. Cone weight is also correlated with wing length (r = 0.82) and wing width (r = 0.86) but weakly correlated with seed length and width (r = 0.26 and 0.34 respectively).

The yield of full seeds per cone is strongly correlated with the total number of seeds per cone (r = 0.95). This is in close agreement with results reported by BROWN (1971) in Scots pine (*P. sylvestris* L.). He observed that as the amount of pollen reaching to a strobilus increases, the number of ovules pollinated and the number of pollen chamber filled to capacity also increases. Correspondingly, the possibility that all archegonia in the ovule are fertilized, which consequently raises the probability for a resulting embryo to develop into full seed.

Year to year (1994, 1995) correlation coeffients for seed characteristics are presented in *table 4*. There were statistically significant correlations in all seed characteristics between the two years examined. The correlations were varied from moderate values, e.g., 0.46 for the number of seeds per cone, to a strong correlation of 0.81 for the weight of 1000 seeds. The percentage of full seeds had a correlation of 0.69 between the two years. SPEARMAN rank correlations had similar values, indicating that the observed change in ranking of clones is highly significant or expressing it in other way, that there are no significant changes between clones for the two years examined. All values indicated that the clones are generally quite stable from year to year in production and seed size and quality.

Table 4. – Year to year (1994, 1995) Pearson and Spearman correlation coefficients in four seed characteristics of Aleppo pine.

Characteristics	Correlation Coefficient	
	Pearson	Spearman
Number of seeds per cone	0.46	0.48
Weight of seeds per cone	0.68	0.60
Percentage of full seeds	0.69	0.47
Weight of 1000 seeds	0.81	0.82

Heritability Estimates

Broad sense heritability H^2 estimates are shown in *table 2*. Cone length and cone width are strongly inherited characteristics with H^2 values 0.74 and 0.73 respectively. Cone wet weight and cone dry weight, expressing cone size, are also highly inherited, with H^2 values 0.79 for the first and 0.78 for the second.

The number of seeds per cone and number of full seeds are moderately inherited characteristics with H^2 values 0.47 and 0.36 respectively. Seed and wing sizes that are maternally influenced characterised are inherited quite strongly. Volume and weight of 1000 seed had H^2 value 0.75 and 0.73 respectively, while the percentange of full seeds 0.41. Moisture content of cones was the only one characteristic that was found to be primarily influenced by the environment.

The variation between clones and the heritability values estimated indicate that production and quality of seed of Aleppo pine can be improved by selection. Therefore, attention must be paid to these characteristics when management of existed or establishment of new seed orchards are made.

Conclusions

From the analyses of 16 cone and seed characteristics in a clonal seed orchard of Aleppo pine grown in Greece, the following conclusions can be drown:

1. The variation among clones is statistically significant for all characteristics (cone wet weight and dry weight, number and volume of 1000 seeds, percentage of full seeds and cone, seed and wing length and widths). Only moisture content of the cones did not vary among clones.

2. Seed characteristics of clones are quite stable from year to year with respect to production and seed quality and size.

3. Cone length and width are strongly inherited characteristics with broad sense H^2 values of 0.74 and 0.73 respectively. The number of seeds per cone and the number and percentage of full seeds are moderately inherited, while the seed size, expressed by the weight and volume of 1000 seeds is strongly inherited. Moisture content of the cones is predominately under environmental control.

Literature

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