JEB
Journal of Environmental Biology

Journal Website : www.jeb.co.in
E-mail : editor@jeb.co.in
ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

Variation and inheritance pattern in cone and seed characteristics of Scots pine (*Pinus sylvestris* L.) for evaluation of genetic diversity

Hakan Şevik[1] **and Osman Topaçoğlu**[2]
[1]Faculty of Engineering and Architecture, Kastamonu University, Kastamonu-37150, Turkey
[2]Forestry Faculty, Kastamonu University, Kastamonu-37150, Turkey
*Corresponding Author E-mail: hakansevik@gmail.com

Publication Info

Paper received:
17 April 2014

Revised received:
09 December 2014

Re-revised received:
02 February 2015

Accepted:
20 March 2015

Abstract

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. This species is classified as one of the economically important tree species for Turkish Forestry in the "National Tree Breeding and Seed Production Program". The objective of the present study was to investigate variation and inheritance pattern in cone and seed characteristics of Scots pine and to evaluate variation in cone and seed characters within and among clones and grafts. The results showed that maximum CV among the clones was found for SWe (21.95), FS (16.99) and CWe (16.88). According to the results of SAS, variation between the clones is averaged at 19.2% and variation within the clones is averaged at 24.4 %. Variation between the clones ranged from 3.6% (SW) to 34.5% (TC) and variation within the clones ranged from 12.3% (SW) to 38.1% (WL). For CW, AL, AW, WW and TC, genetic variation among clones was higher than within clones. When the results of study like compared with results obtained from natural populations, it was seen that genetic variability in seed orchard which was subjected to study was quite low. This case may have dangerous results for the future of forests.

Key words

Genetic variation, *Pinus sylvestris* L., Scots pine, Seed characters, Seed orchard

Introduction

Scots pine (*Pinus sylvestris* L.) is the most widely distributed member of family *Pinaceae* in the world. The longitudinal range of Scots pine covers over 14,000 km and extends from 8°W in Spain to 141°E in Siberia. The latitudinal range of Scots pine covers over 3,700 km and extends from 37° N in Turkey to 70°N in Norway, Sweden, and Finland (Turna, 2003). Scots pine is an important commercial forest tree species, widely planted for industrial and conservation activities such as protecting soil erosion and living snowfences. Scots pine occupies about 738.000 ha in Turkey, growing mainly in the Black Sea coastal mountains on warm southern slopes, where climate is humid. Within its distribution range, scots pine can grow under diverse ecological conditions. Such a wide range of ecological conditions favors the formation of a variety of ecotypes (Turna and Güney, 2009). Scots pine is an important component of subalpine ecosystems of Black sea region in Turkey because of its role in watershed protection, its functional natural habitat for wild life, also, because of its esthetic values. It has, therefore, great importance especially in Blacksea forestry. Scots pine is one of the important forest tree species both in Turkish Forestry and breeding program (Şevik *et al.,* 2010).

Seed orchards are the most important seed source for forestry plantation and a link between present and future forests as gene conservation areas. Also, seed orchards have more importance for conservation of unproductive forest to productive forest because of its improved seeds (Bilir and Temiraga, 2012). There are in total 110.6 ha of scots pine seed orchard at 21 places in Turkey (Anonymus, 2014) and 9.2% of the annual seed demand for plantations of the species is covered by the orchards (Dutkuner *et al.,* 2008). Determination of similarity/differences among the clones of seed orchard is an important stage in breeding program. It is known to obtain maximum variability among genotypes and minimum loss of genetic diversity in the current generation. It is also important for sustainable forestry and to transmit the current gene diversity to next generation by afforestation with suitable seed source. Besides, it can be used to determine breeding population and gene conservation areas and for establishment of seed orchards of the species. Morphological distance can play an important role for this purposes. In light of the above, the present study was carried out to investigate variation and inheritance pattern in cone and seed characteristics of Scots pine and to evaluate variation in cone and seed characters within and among clones and grafts.

Materials and Methods

The present study was carried out in Tekçam Clonal Seed Orchard, located at Taşköprü, Kastamonu. The orchard was established in 1995 and comprise of 1987 grafts of 30 clones derived from intensively selected trees in Araç-Dereyayla seed stand. Grafts were 2 year old at the time of establishment and were planted at a spacing of 6 x 6 m. Commercial cone harvesting was started in 2003 when the grafts were 10 year old. So far, no pruning has been done in the orchard.

For the present study, cones were collected from 30 clones x 7 graft x 3 cones and in total 630 cones were studied. Cone length (CL), cone width (CW), cone weight (CWe), apophisys length (AL), apophisys width (AW), number of filled seeds (FS), number of total seeds (TS), wing length (WL), wing width (WW), seed length (SL), seed width (SW), seed weight (SWe), number of open carpel (OC) number of total carpel (TC) were determined. Length and width were measured with digital microcompas (0.01 mm) from 10 samples for each sample cone (for seed, wings and carpels). Weight was measured with digital weighing machine (0.001 gr).

Data of all characters were subjected to one-way analysis of variance. Duncan test was used for determining homogeneous groups. Variables were tested for normality and homogeneity of variances and logarithmic transformation for counting values were made. Differences were considered significant at $P < 0.05$ level. Broad sense heritability values were estimated both on

Table 1 : Overall means and statistical values of cone and seed traits among the clones

Clone no	Cone length (cm)	Cone width (cm)	Cone weight (gr)	Apophisys length (cm)	Apophisys width (cm)	Filled seeds (no)	Totally seeds (no)	Wing length (cm)	Wing width (cm)	Seed length (mm)	Seed width (mm)	Seed weight (gr)	Open carpel (no)	Totally carpel (no)
11	5.39	2.54	16.76	1.09	1.00	27.50	32.90	2.14	0.65	4.80	2.96	0.33	27.95	64.20
12	5.76	2.97	21.97	1.03	1.06	28.57	38.10	2.17	0.66	5.65	3.15	0.42	30.43	77.95
13	5.57	2.70	17.38	1.16	0.97	32.70	36.75	2.32	0.69	5.42	3.14	0.45	28.80	66.55
14	5.30	2.66	17.64	1.07	1.09	27.35	33.30	2.07	0.71	5.44	3.09	0.42	27.85	66.60
15	5.40	2.91	20.04	1.09	1.11	27.89	34.53	2.07	0.72	5.49	2.83	0.40	27.63	63.68
16	5.97	2.93	23.23	1.09	0.90	30.00	37.90	2.40	0.68	5.69	3.14	0.46	36.15	79.15
17	5.05	2.77	16.63	0.88	0.85	12.65	23.71	1.96	0.64	5.37	3.17	0.21	27.24	72.88
18	5.09	2.88	18.66	0.96	0.96	27.11	29.33	2.15	0.68	5.35	3.18	0.34	25.44	79.33
19	5.80	2.81	19.89	1.14	1.06	29.38	31.71	2.24	0.67	5.59	3.02	0.40	29.24	67.14
20	6.05	2.86	23.23	1.10	1.03	29.26	40.63	2.15	0.73	5.37	3.35	0.49	30.74	68.74
21	5.67	2.83	19.72	1.08	1.14	27.94	32.65	2.11	0.73	5.33	3.34	0.48	28.47	72.53
22	5.20	3.11	22.39	0.97	1.04	24.68	27.95	2.02	0.71	5.63	3.27	0.41	24.53	74.16
23	5.48	2.99	22.01	0.95	1.07	22.68	26.79	1.99	0.65	5.40	3.37	0.35	24.16	79.05
24	5.24	2.42	13.86	0.97	0.83	25.62	28.69	2.02	0.55	5.54	2.78	0.28	29.15	70.46
25	5.23	2.49	14.27	1.00	0.97	27.80	34.85	1.94	0.58	5.60	2.81	0.37	25.30	60.25
26	5.76	2.95	21.31	1.17	1.08	25.14	30.57	2.21	0.71	5.68	3.31	0.36	28.14	71.14
27	5.22	2.81	18.79	1.01	0.99	18.42	27.11	2.06	0.63	5.39	3.15	0.27	29.47	73.53
28	5.52	3.05	22.68	1.01	1.01	29.53	34.35	2.21	0.77	5.24	3.12	0.44	29.24	78.00
29	5.61	2.86	20.72	1.10	1.04	26.59	31.18	2.21	0.68	5.78	3.41	0.45	27.18	72.65
30	5.34	2.86	19.26	0.98	0.94	23.21	29.93	2.21	0.73	5.72	3.03	0.33	26.21	73.50
31	6.07	2.89	22.50	1.08	1.00	23.45	29.40	2.22	0.72	5.42	3.32	0.40	25.20	66.10
32	4.98	2.94	18.88	0.92	1.05	25.61	33.39	1.88	0.68	5.18	3.17	0.40	29.00	71.22
33	5.16	2.79	17.54	0.96	1.02	25.38	35.31	1.98	0.64	5.42	3.26	0.38	23.31	64.00
34	5.06	2.65	15.89	0.95	0.94	21.05	28.80	1.97	0.62	5.34	3.04	0.30	25.50	64.60
35	6.00	2.92	22.54	1.17	1.00	35.12	42.35	2.39	0.69	5.98	3.24	0.62	35.47	75.12
36	6.17	2.93	22.72	1.14	0.96	33.89	39.42	2.26	0.65	5.56	3.19	0.55	33.00	71.37
37	6.02	2.89	20.60	1.04	1.03	29.40	36.00	2.20	0.61	5.59	3.06	0.43	26.73	71.20
38	5.88	3.04	22.83	1.13	1.07	24.09	36.27	2.29	0.73	5.96	3.45	0.46	25.55	72.36
39	5.47	2.67	17.37	1.02	0.96	28.62	34.90	2.08	0.65	5.47	2.98	0.48	24.71	65.19
40	6.34	3.41	31.00	1.07	1.10	33.37	38.11	2.49	0.81	5.86	3.50	0.60	30.63	78.63
Mean	5.56	2.85	20.08	1.04	1.01	26.95	33.40	2.14	0.68	5.50	3.16	0.41	28.18	70.76

individual tree basis (H_1^2) and clone mean basis (H_2) as the ratio of total genetic variance (s^2 c) to total phenotypic variance (s^2 c + s^2 E) (Matziris, 1984) for H_1 and to (s^2 c + s^2 E/n) for H_2 (n = graft number). Cloning effect variance biases heritability values, but the magnitude is negligible and can be ignored (Matziris, 1993). In the present study, heritability components were estimated as σ^2 E = error mean square and σ^2 c = (clone mean square-error mean square/ number of grafts per clone).

Data analyses were conducted using SAS program (SAS, 1987). All the traits were subjected to analysis of variance (ANOVA), first for each individual test and then with data sets combined over field sites in each population. Since error variances were consistent for each trait from site to site in each population, no adjustment was necessary to combine the data over sites. Because of the differences in statistical designs used in the seedlings, different random models were employed. Linear model for combined data over seedlings was as follows :

$$y_{ij} = \mu + \alpha_i + \beta_{j(i)} + e_{ij} \quad (i=1,\ldots, a \; j=1,\ldots,b \; k=1,\ldots,n)$$

Where, y_{ij} is observation on jth tree of ith population; μ is overall mean; α_i is the effect of ith population; $\beta_{j(i)}$ is the effect of jth tree at ith population and eij is random error.

Results and Discussion

Overall means and statistical values of cone and seed traits among the clones are shown in Table1. When the values of Table 1 was observed, clone No. 40 which had the highest value at 6 out of 40 characters and clone No. 35 which had the highest value at 5 characters, draw attraction. Likewise, clone No. 24 which had the lowest value at 5 out of 14 characters and clone No. 17 which had the lowest value at 4 out of 24 characters, draw attraction as important clones in the sense of genetic variability.

According to Table 2 maximum cone width among the clones was found for seed weight (21,95), filled seeds (16,99) and cone weight (16.88). Minimum CV values are 4.36 (seed length) and 5.7 (seed width). Greater genetic variation among the grafts than among the clones was determined for all the characters. The coefficient of variation among the grafts (CV) were higher than among the clones, indicated high variation within the population.

Broad sense heritability H^2 estimates are shown in Table 3. Wing length, apophysis length, wing width and cone weight were strongly inherited characteristics with H^2 values of 0.91, 0.90, 0.89 and 0.89 respectively. According to Table, minimum values of H^2 was cone width (0.09), seed width (0.65) and seed length (80.71). Results of SAS analysis are shown in Fig. 1.

According to the results of SAS, the average variation between the clones was 19.2% and 24.4% respectively. Variation between the clones ranged from 3.6% (seed width) to 34.5% (total carpel) and variation within the clones ranged from 12.3% (seed width) to 38.1 (wing length). Genetic variation among clones are higher than within clones for cone width, apophysis length, apophysis width, wing width and total carpel. Pearson correlation coefficient of cone and seed traits of Scots pine is shown in Table 4. Highest correlation (0.902) was calculated between cone width and cone weight. In addition, filled seeds was strongly correlated with total seeds (0.889) and seed weight (0.768). It is known that, the morphological and physiological characteristics of forest trees are inherited. These features, with growing effects of climate and environmental conditions can vary little (Şevik, 2012). Seeds in trees, cones and cone elements least affected by environmental conditions and Thus, the genetic structure of wood is considered as the most accurate representation of starting element. Therefore, particular elements of working seed, cone has special place in the study of genetic

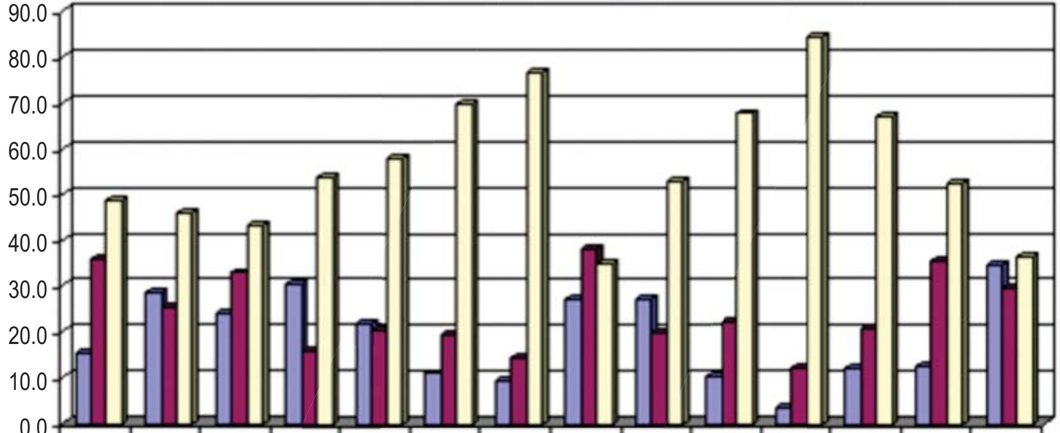
Table 2 : Overall means and statistical values of cone and seed traits among the grafts

Traits	Mean	Among the clones					Among the grafts			
		S.E.	Min	Max	SD_c	CV_c	Min	Max	SD_c	CV_c
Cone length (cm)	5.56	0.07	4.98	6.34	0.38	6.83	3.74	7.81	0.78	14.03
Cone width (cm)	2.85	0.03	2.42	3.41	0.19	6.67	1.99	3.89	0.31	10.88
Cone weight (g)	20.08	0.62	13.86	31	3.39	16.88	7.71	46.48	5.94	29.58
Apophisys length (cm)	1.04	0.01	0.88	1.17	0.08	7.69	0.65	1.66	0.14	13.46
Apophisys width (cm)	1.01	0.01	0.83	1.14	0.07	6.93	0.14	1.49	0.14	13.86
Filled seeds	26.95	0.84	12.65	35.12	4.58	16.99	5	62	10.41	38.63
Total seeds	33.4	0.82	23.71	42.35	4.47	13.38	6	83	10.65	31.89
Wing length (cm)	2.14	0.03	1.88	2.49	0.15	7.01	1.54	2.79	0.24	11.21
Wing width (cm)	0.68	0.01	0.55	0.81	0.06	8.82	0.19	0.99	0.09	13.24
Seed length (mm)	5.5	0.04	4.8	5.98	0.24	4.36	0.57	6.93	0.57	10.36
Seed width (mm)	3.16	0.03	2.78	3.5	0.18	5.7	0.33	7.66	0.47	14.87
Seed weight (g)	0.41	0.02	0.21	0.62	0.09	21.95	0.03	2.25	0.19	46.34
Open carpel	28.18	0.56	23.31	36.15	3.08	10.93	9	52	6.41	22.75
Total carpel	70.76	0.98	60.25	79.33	5.346	7.56	28	103	10.16	14.36

Table 3 : Analysis of variance, variance components and heritability estimates for studied characters

Character	Between populations	Within pop. (Error)	s^2c	s^2E	H_1^2	H^2
Cone length	2.913***	0.487	0.347	0.487	0.42	0.83
Cone width	0.733***	0.67	0.009	0.67	0.01	0.09
Cone weight	229.949***	25.362	29.227	25.362	0.54	0.89
Apophisys length	0.127***	0.013	0.016	0.013	0.55	0.90
Apophisys width	0.101***	0.014	0.012	0.014	0.46	0.86
Filled seeds	376.915***	92.557	40.623	92.557	0.31	0.75
Totally seeds	353.345***	99.377	36.281	99.377	0.27	0.72
Wing length	0.396***	0.037	0.051	0.037	0.58	0.91
Wing width	0.052***	0.006	0.007	0.006	0.54	0.89
Seed length	0.980***	0.287	0.099	0.287	0.26	0.71
Seed width	0.574***	0.200	0.053	0.200	0.21	0.65
Seed weight	0.147***	0.031	0.017	0.031	0.35	0.79
Open carpel	173.025***	33.281	19.963	33.281	0.37	0.81
Totally carpel	524.413***	78.580	63.69	78.580	0.45	0.85

***Statistically significant 0.001 probability level; $H_1^2=\sigma^2c/\sigma^2c+\sigma^2E$ and $H^2=\sigma^2c/\sigma^2c+\sigma^2E/n$



	CL	CW	CWe	AL	AW	FS	TS	WL	WW	SL	SW	SWe	OC	TC
▨ Among cones	15.5	28.5	24.0	30.5	21.9	11.0	9.4	27.0	27.2	10.3	3.6	12.3	12.4	34.5
■ Within clones	35.8	25.4	32.9	15.7	20.2	19.3	14.3	38.1	19.9	22.1	12.3	20.7	35.3	29.3
□ Error	48.7	46.1	43.1	53.8	57.9	69.7	76.3	34.9	52.9	67.6	84.1	67.0	52.3	36.2

Fig. 1 : Source of variation (y-axis %)

diversity, (Turna and Güney, 2009). Till date, many researchers have determined the genetic diversity of forest trees, only a few have used these characters for seed orchards (Sıvacıoğlu *et al.,* 2010) and seed stands (Turna and Güney, 2009; Şevik *et al.,* 2012).

High genetic diversity within-population and low among-population differentiation observed in conifers has been attributed to common lifehistory traits, such as longevity and extensive gene flow (Hamrick *et al.,* 1992; Streiff *et al.,* 1998). Salmela *et al.* (2013) reported that majority of variation was observed within populations in *Pinus sylvestris* L. In *Pinus monticola* genetic differentiation indicated that 20.1% of detected genetic variation was explained by difference among population (Kim *et al.,* 2011). High level of genetic diversity within and low genetic differentiation among population was determined in *Pinus contorta* (Parchman *et al.,* 2011).

The primary purpose of tree improvement programme is to produce genetically improved seeds. Seed orchards are one of the means used to obtain maximum genetic gains in shortest time period. The major objective of seed orchards is to produce of

Table 4 : Pearson correlation coefficients among the studied characters

	Cone width	Cone weight	Apophisys length	Apophisys width	Filled seeds	Total seeds	Wing length	Wing width	Seed length	Seed width	Seed weight	Open carpel	Total carpel
Cone length	.559**	.746**	.404**	.341**	.326**	.350**	.550**	.335**	.292**	.147**	.453**	.345**	.323**
Cone width		**.902****	.204**	.399**	.186**	.198**	.447**	.483**	.292**	.227**	.364**	.225**	.446**
Cone weight			.341**	.444**	.269**	.276**	.547**	.494**	.342**	.234**	.456**	.292**	.447**
Apophisys length				.351**	.221**	.193**	.459**	.256**	.234**	.143**	.257**	.194**	.037
Apophisys width					.126**	.112*	.154**	.321**	.130**	.143**	.195**	.011	.047
Filled seeds						**.889****	.303**	.164**	.016	-.014	**.768****	.487**	.233**
Totally seeds							.287**	.168**	.030	.011	.750**	.552**	.289**
Wing length								.496**	.471**	.253**	.443**	.339**	.358**
Wing width									.288**	.303**	.342**	.132**	.235**
Seed length										.376**	.226**	.052	.202**
Seed width											.173**	-.018	.127**
Seed weight												.444**	.303**
Open carpel													.615**

genetically improved seeds in mass quantities. The procedure by which this can be accomplished is to bring together phenotypically superior clones (or progeny from those clones) and establish them in specified design, so that the chance for cross-pollination among these selected trees high (Kaya and Işık, 2010). Seed orchards have potential to increase forest production and offer a reliable seed supply, their role and function has recently been compiled (Sıvacıoğlu *et al.,* 2010).

But, attention is required about genetic diversity of seed orchard crops and its impact on future forest. Low production of sound seeds is a common problem (Sıvacıoğlu *et al.,* 2010). Genetic diversity is richness of hereditary information in gene pole of one species. High level of inter-species genetic diversity is an assurance for adaptation to changing environmental conditions, an indication for adaptation potential of the species and an important part of ecosystem stability. Genetic diversity is also a raw material for tree improvement studies. As such, most of the research about genetic diversity is in high priority in forest trees improvement programmes (Şevik *et al.,* 2012).

The present study revealed that genetic variation among clones were higher than within clones for five (cone width, apophisys length, apophisys width, wing width and totally carpel) characters. This case can be interpreted as genetic variability is not required at seed orchard level. Maintaining forest is only possible through maintaining genetic variability. When the results of study are compared with results obtained from natural populations, it is seen that genetic variability in seed orchard which is subject to study, is quite low. This case may have dangerous impact for the future of forests.

References

Anonymus: Seed orchard of *Pinus sylvestris* L., The Research Directorate of Forest Tree Seeds and Tree Breeding, (http://www.ortohum.gov.tr/tohbah.htm), 04.12.2014

Bilir, N. and H. Temirağa: Fertility variation and status number in clonal seed orchards of Pinus sylvestris. *Pak. J. Biol. Sci.,* **15**, 1075-1079 (2012).

Dutkuner, İ., N. Bilir and M.D. Ulusan: Influence of growth on reproductive traits and its effect on fertility and gene diversity in a clonal seed orchard of scots pine, Pinus Sylvestris L. *J. Environ. Biol.,* **29**, 349-352 (2008).

Hamrick, J.L., M.J.W. Godt and S.L. Sherman-Broyles: Factors influencing levels of genetic diversity in woody plant species. New Forests, **6**, 95-124 (1992)

Kaya, N. and K. Işık: Genetic identification of clones and the genetic structure of seed crops in a *Pinus brutia* seed orchard. *Turk J. Agric. For.,* **34**, 127-134 (2010)

Kim, M.S., B.A. Richardson, G.I. McDonald and N.B. Klopfenstein: Genetic diversity and structure of western white pine (*Pinus monticola*) in North America: A baseline study for conservation, restoration, and addressing impacts of climate change, *Tree Genetics and Genomes,* **7**, 11-21 (2011)

Parchman, T.L., C.W. Benkman, B. Jenkins and C.A. Buerkle: Low levels of population genetic structure in *Pinus contorta* (Pinaceae) across a geographic mosaic of co-evolution. *Amer. J. Bot.,* **98**, 669–679 (2011).

Salmela, M.J., S. Cavers, J.E. Cottrell, G.R. Iason and R.A. Ennos: Spring phenology shows genetic variation among and within populations in seedlings of Scots pine (*Pinus sylvestris* L.) in the Scottish Highlands. *Plant Ecol. Diver.,* **6**, 523-536 (2013)

SAS Institute: SAS User's Guide. Release 6.03 Edition. Cary, North Caroline (1987).

Şevik, H.: Variation in seedling morphology of Turkish fir (*Abies nordmanniana* subsp. *bornmulleriana* Mattf). *Afri. J. Biotechnol.,* **11**, 6389-6395 (2012)

Şevik, H., S. Ayan, I. Turna and Z. Yahyaoğlu: Genetic diversity among populations in Scots pine (*Pinus silvestris* L.) seed stands of Western Black Sea Region in Turkey. *Afri. J. Biotechnol.,* **9**, 7266-7272 (2010)

Şevik, H., Z. Yahyaoglu and I. Turna: Determination of genetic variation Between populations of *Abies nordmanniana* subsp. *bornmulleriana* Mattf according to some seed characteristics, Genetic Diversity in Plants, (Eds.: M. Caliskan). INTECH Open Access Publisher, pp. 231-248 (2012).

Sıvacıoğlu, A., S. Ayan and D.A. Çelik: Clonal variation in growth, flowering and cone production in a seed orchard of Scots pine

(*Pinus sylvestris* L.) in Turkey. *Afri. J. Biotechnol.,* **8**, 4084-4093 (2010).

Streiff, R., T. Labbe, R. Bacilieri, H. Steinkellner, J. Glossl and A. Kremer. Within population genetic structure in *Quercus robur* L. and *Quercus petraea* (Matt.) Liebl. assesed with isozymes and microsatellites. *Molecular Ecology,* **7**, 317-328 (1998).

Turna, I.: Variation of some morphological and electrophoretic characters of 11 populations of Scots pine in Turkey. *Israel J. Plant Sci.,* **51**, 223-230 (2003).

Turna, I. and D. Güney: Altitudinal variation of some morphological Characters of Scots Pine (*Pinus sylvestris* L.) in Turkey. *Afr. J. Biotechnol.,* **8**, 202-208 (2009).