GENETIC STRUCTURE OF FOUR KAZDAĞI FIR (*ABIES EQUITROJANI* ASCHERSON ET SINTEN) POPULATIONS IN KAZDAĞI, TURKEY AS ASSESSED BY ADAPTIVE SEEDLING TRAITS

F. Filiz Çiçek¹, Zeki Kaya^{1*}, Burcu N. Çengel^{1,2} & E. Velioğlu²

¹⁾ Department of Biological Sciences, Faculty of Art and Sciences, Middle East Technical University, 06531 Ankara,

Turkey.

²⁾ Forest Tree Seeds and Tree Breeding Research Directorate, Ministry of Environment and Forestry, Ankara, Turkey * Corresponding author: Zeki Kaya, Department of Biological Sciences, Faculty of Art and Sciences, Middle East Technical University, 06531 Ankara, Turkey. Fax: 90+ (312) 210 1298, email: kayaz@metu.edu.tr

Received December 3, 2003; accepted May 13, 2005

ABSTRACT

To investigate the magnitude and pattern of genetic variation in adaptive seedling traits, seedlings of 126 families from four natural populations of Kazdağı-fir (*Abies equitrojani*) from Kazdağı in Turkey were raised in nursery environments and evaluated for 12 adaptive traits (including growth and phenological traits).

Significant population and family within population differences were observed for the most studied traits. Although the components of total variation attributable to the families within populations were greater than that of populations, variance components due to populations (range: 0-5.7 %) and families within populations (range: 0-20.7 %) were low. Estimated family heritabilities were generally low and did not exceed 0.51 (for SURV), but magnitude of family heritabilities for growth traits suggests (range: 0.17-0.20) that considerable genetic improvement could be achieved if early selection is practiced.

Genetic and phenotypic correlations between adaptive traits were in the same direction in sign, but genetic correlations were higher than phenotypic correlations. The families with higher cotyledon number had more growth, in turn, larger seedling size after the first two growing seasons indicating maternal effects. The families with later budset and bud burst dates had less growth in the second growing season.

Two major *Gene Management Zones* (GMZ) for Kazdağı fir were suggested for *in situ* conservation program; Gürgendağ population as a large-core reserve and Çan population as the second one being isolated, genetically less similar to others and having high genetic variation in adaptive seedling traits.

Keywords: Kazdağı, *Abies equitrojani*, genetic variation, heritability, genetic correlation, phenotypic correlation, Gene Management Zone (GMZ)

INTRODUCTION

Fir species are widely distributed tree species in Turkey (Figure 1). There are four native species belonging to this genus growing in pure and mixed stands in the country. These are *Abies nordmanniana* Stev. (Caucasian fir), *A. bornmulleriana* Mattf. (Bornmüller's fir), *A. cilicica* Carr. (Cilician fir) and *A. equitrojani* Aschers. et. Sinten. (Kazdağı fir) (AYTUĞ 1959, KAYACIK 1965, SAATÇIOĞLU 1969). Kazdağı fir, a narrow endemic, is found only in the Kazdağı in western Turkey (SAATÇIOĞ-LU 1969, VIDAKOVIĆ 1991, AKMAN 1995) (Figure 2).

Kazdağı fir is distributed in small disjointedareas in Kazdağı and forms mixed forests with *Quercus* species and *Pinus nigra* Arnold subsp. *pallasiana* (Lamb.) Holmboe and *Fagus orientalis* Lipsky (ATA 1975). GÜLBABA *et al.* (1998) reported that the total distribution of Kazdağı fir is only about 3,600 ha. Optimum distribution of Kazdağı fir is between 1,000–1,400 m on the northern slopes in the Kazdağı (ATA 1975, GEMICI *et al.* 1998).

Kazdağı fir is included in the priority species list to be conserved in the National Plan for in situ Conservation of Plant Genetic Diversity in Turkey (KAYA et al. 1997). The plan was presented to the Ministries of Environment and Forestry, and Agricultural and Rural Affairs as a part of the in situ Conservation of Plant Genetic Resources Project (KAYA et al. 1997). This project was funded by the ÇIÇEK, F.F. ET AL.: GENETIC STRUCTURE OF FOUR KAZDAĞI FIR (ABIES EQUITROJANI) POPULATIONS



Figure1. Distribution of for species in Turkey.



Figure 2. Locations of sampled Kazdağı fir populations.

World Bank as a special Global Environmental Facility (GEF) and it aimed to conserve the genetic diversity of forest tree species with global and national importance as well as wild relatives of cultivated plants in the selected pilot sites. Considering the Project, the Kazdağı and Bolkar Mountains, and the Ceylanpınar State Farm are the pilot sites to be conserved. Most of the natural populations of tree species possess a great amount of genetic variation. This variation can be estimated either by neutral marker studies (allozyme markers, and DNA markers), or by common garden studies (morphological, adaptive traits). Although common garden experiments are time consuming and expensive, they are the most effective way to measure adaptive genetic diversity in trees (NEALE 1998); because neutral markers may fail to identify important adaptive variants (LEDIG 1998). Thus, neutral marker studies should be supported by common nursery studies for making right decision in conservation studies. Researchers agree on two ways of conservation efforts; ex situ and in situ (FRANKEL & SOULÉ 1981, ZOBEL & TALBERT 1984, LEDIG 1988, BROWN 1992, KAYA et al. 1997, LEDIG 1998, IŞIK 1999). In situ conservation aims at preserving species in their natural habitats so that entire biological communities and co-adapted gene complexes can be preserved, thus allowing the dynamic process of evolution to continue (LEDIG 1988). It is proposed that wild plant species should be conserved in situ whereas domesticated species should be in ex situ collections (FRANKEL & SOULÉ 1981, BROWN 1992). Thus, in situ conservation is the desired option for forest trees (LEDIG 1998).

There are various approaches for *in situ* conservation. It is suggested that the Gene Management Zone (GMZ) approach could be more suitable for wild relatives of crop species and commercially important tree species. The primary function of GMZs is protection of genetic resources of either a single target species or entire community. These areas could be also managed for other economic benefits, such as grazing or timber harvest, unless other uses do not threaten its primary function (LEDIG 1988, KAYA *et al.* 1997). With the GMZ approach, genetic diversity in target plant species can be maintained in natural and seminatural areas where populations are free to evolve in their natural environment (KAYA *et al.* 1997).

The purpose of this study is to determine the magnitude and pattern of genetic variation among four Kazdağı fir populations sampled from Kazdağı by studying adaptive seedling traits (namely growth and phenology, related to adaptation of the species to its environment) in common nursery environment and to evaluate populations for their potentials as GMZ sites.

MATERIAL AND METHODS

Sampling procedure

Open pollinated seeds were collected from 126 parent trees (families) in four Kazdağı fir populations (Gürgendağ, Kapıdağ, Eybekli, Çan) in the summer of 1994. The seeds were assumed to be half sibs. Parent trees were selected at random without considering any phenologic characteristic. It was also taken into consideration that parent trees must be at least 100 m apart form each other to reduce the probability of including related trees in samples.

Gürgendağ can be considered as the core population since it has a central location and the largest distribution of the species. Geographic and topographic information on sampled populations are given in Table 1.

Seeds were sown in three nursery beds in both Kızılcahamam Forest Nursery on November 1995 (Experiment 1) and Ankara Forest Nursery on October 1996 (Experiment 2). Each nursery bed was 1.30 m. in width and 15 m. in length. The seeds from each family were randomly allocated to row plots. In each row plot, there were five experimental seedlings. Each whole plot was surrounded by a single row of buffer seedlings. Buffer seedlings were planted in two rows at each end of the nursery beds. Seeds of Abies nordmanniana were used as buffer seedlings. Five seedling row plots of 126 families were randomly allocated to plot location in a randomized complete block design with three replications. Experimental design of the nursery beds is shown in Figure 3.

Data was collected from two separate nurseries and combined to yield four replications by dropping one replication from each nursery. The dropped replications had the lowest survival in both Experiment 1 (18 % survival) and Experiment 2 (31 % survival). Since these two experiments were carried out in different locations (K1z1lcahamam and Ankara) and times, the combined data was

Population	Latitude	Longitude	Altitude	Area (ha)	Population	n size – trees
	(N)	(E)	(m)		Total	Sampled
Eybekli	39° 42' 35''	27° 07' 30''	1000	600	240000	25
Čan	39° 56' 00''	27° 07' 00''	750	123	43100	22
Gürgendağ	39° 46' 00''	26° 57' 00"	1300	2400	732000	56
Kapidağ	39° 43' 20''	26° 52' 00''	1450	250	87500	23

Table 1. Geographic and topographic information on sampled Kazda ğı fir populations.



Figure 3. Experimental design in the nursery.

standardized. Standardization of the data was carried out by subtracting experimental means of each trait for each location (nursery) from plot means and dividing them by standard deviation. The following formula (SNEATH & SOKAL 1973) was used in standardization:

$$z_i = \frac{\bar{x}_i - \bar{x}}{s_{\bar{x}_i}}$$
[1]

where, z_i =standardized value of trait x, x_i = value of trait x for a seedling in a nursery, \bar{x} = experimental mean of trait x in a given nursery and $s_{\bar{x}_i}$ = standard deviation of \bar{x} .

Eleven traits expressing survival, number of cotyledons, timing of bud set in the first year (number of days from January 1st, 1996), timing of bud set in the second year (number of days from January 1st, 1997), timing of bud burst in the second year (number of days from January 1st 1997), degree of environmental stress (damage indicated by a scale of 1 "the least" to 5 "the most"), number of buds, number of branches, total height growth in the first and second years and total diameter in the second year were recorded. The descriptions and units of these traits are listed in Table 2.

Data Analysis

Analysis of variance (ANOVA) was performed in order to determine differences between populations within location and families within populations. ANOVA was computed by using generalized least square procedure of GLM-SAS (SAS, Statistical User's Guide 1988) since this program gives unbiased estimates of all mean squares when a data set has missing plots, and it was based on plot means (family means in each replication). The following statistical model was used in the data analysis:

$$Z_{ijk} = \mu + B_k + P_{j(k)} + F_{i(j)} + e_{ijk}$$
[2]

Where μ is the experimental mean, Z_{jkl} is the mean performance of the *l*th family in the *j*th population in *k*th replication. B_k = the fixed effect of *k*th replication $[E(B_k) = 0, \operatorname{Var}(B_k) = \kappa_k^2]$; P_j = fixed effect of *j*th population $[E(P_j) = 0, \operatorname{Var}(P_j) = \kappa_j^2]$; $F_{(l)j}$ = random effect of *l*th family in *j*th population $[E(F_{(l)j}) = 0; \operatorname{Var}(F_{(l)j}) = \sigma_{(l)j}^2]$; e_{jkl} = experimental error $[E(e_{jkl}) = 0, \operatorname{Var}(e_{jkl}) = \sigma_e^2]$.

The phenotypic correlations between traits x and y were estimated according to the statistical model above (*Eq. 2*) by using the following formula:

$$r_{p(xy)} = \frac{MCP_{f(xy)}}{[MS_{f(x)} \ MS_{f(y)}]}$$
[3]

where, $r_{p(xy)}$, phenotypic correlation between traits x and y, $MCP_{f(xy)}$, mean cross products between families within populations for traits x and y, $MS_{f(x)}$, mean square between family within populations for trait x, $MS_{f(y)}$, mean square between family within populations for trait y (KAYA *et al.* 1989).

Code of traits in combined data	Definition of traits	Units
Growth traits		
СОТ	Number of cotyledons	counts
BUDNUM	Number of buds in the seedling	counts
BRANUM	Number of branches in the seedling	counts
HTI	Total height in the first year	mm
HT2	Total height in the second year	mm
D2	Total diameter in the second year	mm
Survival traits		
SURV	Survival	1 (alive): 0 (dead)
ESTR	Degree of frost damage (Kızılcahamam) and summer drought damage (Ankara)	1 to 5 (1 – excellent; 5 – worst)
Phenological traits		
BS1	Date of bud set in the first year	davs
BS2	Date of bud burst in the second year	davs
BS3	Date of bud set in the second year	days

Date of bud set in the second year

Table 2. Desriptions of the studied seedling traits.

Genetic correlations between two characters were calculated by using the following formula (FALCONER 1989):

$$r_{g(xy)} = \frac{COV_{f(xy)}}{\sqrt{\sigma_{f(x)}^2} \sqrt{\sigma_{f(y)}^2}}$$
[4]

where $r_{g(x,y)}$ = estimated genetic correlation between traits x and y, $\sigma_{f(x)}^2$ = estimated components of variance of families within populations for trait $x, \sigma_{f(y)}^2$ = estimated components of variance of families within populations for trait y and $Cov_{f(x,y)}$ = estimated component of covariance of families within populations between traits x and y, estimated from covariance analysis.

Standard errors of genetic correlations were calculated according to FALCONER (1989):

$$\sigma_{(r_{x})} = 1 - r_{g}^{2} \sqrt{\left[\frac{\sigma_{(h_{x}^{2})} - \sigma_{(h_{y}^{2})}}{h_{x}^{2} + h_{y}^{2}}\right]}$$
[5]

where, $\sigma_{r(g)}$ is the standard error of genetic correlation between traits x and y, r_g^2 is the square of genetic correlation between traits x and y, σ_{h^2} is the standard error of heritability for trait x, $\sigma_{h_2^2}^{n_x}$ is the standard error of heritability for trait y, h_x is the heritability for trait x, h_y^2 is the heritability for trait y. $\sigma_{(h_{c}^{2})}$ is estimated by the following formula:

$$\sigma_{(h_x^2)} = \sqrt{\frac{\frac{2}{k^2} \left(\frac{MS_f}{df_f + 2}\right) + \left(\frac{MS_e}{df_e + 2}\right)}{MS_f}}$$
[6]

where, k stands for harmonic mean number of individuals per family, df_c stands for degrees of freedom of families within populations, df_a stands for degrees of freedom of error, MS_t stands for mean squares of family, MS_e stands for mean squares of error.

days

Family heritabilities $(h_{f(x)})^2$ were estimated from components of variance by using the following formula;

$$h_{f(x)}^{2} = \frac{\sigma_{f(x)}^{2}}{(\sigma_{e}^{2}/r) + \sigma_{f(x)}^{2}}$$
[7]

where, $\sigma_{f(x)}^2$ is the family component of total variance for trait x, $r = 4, \sigma_e^2$ is the error variance (Kaya & Temerit 1994).

Discriminant analysis was performed to discriminate between populations. When more than two populations are involved, two-dimensional plots are often used to separate the different populations. Based on the studied traits, canonical discriminant function analysis was carried out by using the "PROC CAN DISC" procedure of SAS.

The biological distances (Mahalonobis distance) among populations were calculated by using the following equation (KRZANOWSKI & MAR-

RIOTT 1994):

$$D_{ilj}^2 = (\bar{X}_i - \bar{X}_j) COV^{-1} (\bar{X}_i - \bar{X}_j)$$
 [8]

where, $D_{(ij)}^2$ stands for squared distance between populations *i* and *j*, \bar{X}_i stands for mean of the population *i* for the trait *x*, \bar{X}_j stands for mean of the population *j* for the trait *x*, COV stands for covariance matrix for the population *i* and *j* for the trait *x*.

RESULTS

Pattern and magnitude of genetic variation

Growth traits

The differences among populations for most of growth traits were statistically significant, except for number of branches (BRANUM) (Table 3). The component of variance due to populations was generally lower than the components due to families within population in most traits. It varied from 3.8 % in HT1 to 5.7 % in COT. The family component of total variance was ranged from 5.8 % in HT1 to 10.9 % in D2 (Table 4).

Mean number of cotyledons was higher in Çan population which had the highest average cotyledon number (6.0) than in other studied populations (Table 3). Eybekli populations had the highest number of BUDNUM while Kapıdağ had the lowest BUDNUM. Regarding height growth trait, Kapıdağ population had the families with the lowest mean height growth in the first year (HT1; 4.6 cm) while Gürgendağ had the highest one (5.2 cm) (Table 3). In the second year, Çan population had the highest mean height growth (27.6 cm) and Gürgendağ had the greatest diameter growth while Kapıdağ population had the lowest height (21.7 cm) and diameter (1.5 mm).

Survival traits

Both populations and families within populations for survival (SURV) and environmental stress (ESTR) traits significantly varied (Table 4). The portion of total variance due to populations ranged from 2.4 % in SURV to 3.2 % in ESTR. The family component of total variance was much higher than population component and varied from 14. 1 % in ESTR to 20.7 % in SURV. Both Çan and Gürgendağ populations had the low survival (40 %) whereas Eybekli and Kapıdağ populations had high survival (50 %). Relative to other populations, Çan was the one which slightly more suffered from the environmental stresses (2.6) (Table 3).

Phenological traits

For phenology traits, populations did not vary

Traits	Mean and standard deviations of populations						
	Eybekli	Çan	Gürgendağ	Kapıdağ			
Growth traits							
COT	5.7 a	6.0 b	5.6 a	5.6 a			
BRANUM	2.5 a 1.3 a	2.1 b 1.3 a	1.9 c 1.3 a	1.8 c 1.3 a			
HT1 HT2	5.0 a 25.6 a	4.8 ab 27.6 b	5.2 c 25.3 a	4.6 ab 21.8 c			
D2	1.6 a	1.58 a	1.7 b	1.5 c			
Survival traits							
SURV	0.5 a	0.4 b	0.4 b	0.5 a			
ESTK	2.3 a	2.0 0	2.4 a	2.4 a			
Phenological traits							
BS1	231.0 a	233.6 a	236.5 a	236.0 a			
BS2 BS3	118.8 a 266.2 a	114.3 b 268.6 a	117.6 a 267.8 a	116.4 a 268.8 a			

Table 3. Means and standard deviations of traits in populations.

Trait	Replica- tion df=3	Popu df	lation = 3	Families tion df =	s / popula- = 118–122	E_1 df = 2	rror 201–370	Mean	h_f^2
	MS	MS	VC(%)	MS	VC(%)	MS	VC(%)		
Growth trait	S								
СОТ	0.797	4.494	5.7**	0.73	8.4*	0.543	85.9	5.72	0.26±0.14
BUDNUM	9.958	3.401	4.7**	0.687	7.9 ns	0.532	87.4	2.04	0.23 ± 0.14
BRANUM	30.54	0.038	0. ns	0.573	10.0*	0.421	90.0	1.26	0.20 ± 0.14
HT1	40.35	1.996	3.8**	0.456	5.8 ns	0.377	90.4	4.91	0.17±0.14
HT2	4.427	3.117	3.8**	0.767	9.3*	0.571	87.0	20.1	0.26 ± 0.14
D2	13.63	3.636	4.2*	0.93	10.9*	0.694	84.9	1.58	0.25±0.14
Survival trai	ts			_					
SURV	0.709	0.419	2.4*	0.148	20.7**	0.072	76.9	0.43	0.51±0.13
ESTR	6.868	2.827	3.2*	0.913	14.1*	0.626	85.2	2.40	0.31±0.16
Phenologica	l traits								
BS1	114.1	0.221	0.6 ns	0.133	0. ns	0.142	99.1	235.9	с
BS2	108.8	0.163	0. ns	0.174	10.4*	0.126	89.4	116.8	0.28 ± 0.14
BS3	21.15	0.555	0. ns	0.664	4.7 ns	0.559	95.3	267.8	0.16±0.15

Table 4. Results of the analysis of variance, experimental means and family heritabilities (h^2) for examined traits.

MS – mean square; VC(%) – variance component in %.

^{a)} degrees of freedom varied depending on traits and number of missing plants,

^{b)} please, refer to Table 2 for codes and definition of traits,

^{c)} family heritability could not be estimated due to lack of family variance,

ns – not significant; * – significant at p < 0.05 level; ** – significant at p < 0.01 level.

significantly for both the timing of bud set and bud burst. Families within populations showed significant variation for only timing of the bud burst. The component of variance due to families within population made up 10.4 % of the total variance (Tables 3 and 4).

Family heritabilities (h_i^2)

Due to low survival of seedlings in family plots of all populations, the estimation of family heritabilities were conducted though individual tree heritability would be more appropriate. Family heritabilities for those growth traits with significant family variance components varied from 0.20 for BRANUM to 0.26 for COT and D2 while they were moderately high for survival traits, ranging from 0.31 –0.50. For phenological traits, only significant family component variance was observed in BB2 and estimated family heritability was 0.28 (Table 4).

Genetic and phenotypic correlations between studied traits

Since, the values of genetic and phenotypic correlations between traits were generally in the same direction in sign and similar magnitudes, thus, here, only the results on genetic correlations between traits have been provided (Table 5). However, the standard errors of estimated genetic correlations were high due to low number of families involved in three of studied four populations.

There were positive and low to moderate genetic correlations between number of cotyledons and growth traits (ranged from 0.12 between COT and BRNUM to 0.69 between COT and D2 (Table 5). Thus, the families with high number of cotyledons had more height and diameter growth in the first two years, indicating maternal effect on early growth of seedlings. Genetic correlations between BUDNUM and height and diameter growth traits were again moderately and strongly correlated and varied from 0.41 between BUDNUM and HT1 to 1.21 between BUDNUM and D2 traits (Table 5). There were also strong and positive genetic correlations between height growth of the families in the

			Growt	h traits			Surviva	l traits		Phenological	traits
	COT	BUDNUM	BRANUM	HT1	HT2	D2	SURV	ESTR	BSI	BB2	BS2
Growth trait	S										
СОТ		0.25±0.43	0.12 ± 0.38	0.21 ± 0.48	0.40 ± 0.40	0.69 ± 0.44	0.09 ± 0.26	0.29 ± 0.36	٩	-0.37 ± 0.40	-0.63 ± 0.68
BUDNUM	0.06 ± 0.13		-0.07 ± 0.41	0.41 ± 0.50	1.12 ± 0.35	1.21 ± 0.54	-0.30 ± 0.28	-0.24 ± 0.37	٩	$-0.32\pm0.$	0.01 ± 0.60
BRANUM	0.09 ± 0.13	-0.11 ± 0.13		0.40 ± 0.48	-0.31 ± 0.37	-0.22 ± 0.41	-0.20 ± 0.27	0.14 ± 0.35	٩	0.07±0.	-0.20 ± 0.56
НТІ	0.03 ± 0.13	0.19±0.13	0.12 ± 0.13		0.80 ± 0.48	1.19 ± 0.59	-0.02 ± 0.24	-0.48 ± 0.44	٩	-0.06±0.	-0.36 ± 0.70
HT2	0.12 ± 0.13	-0.50 ± 0.11	-0.19 ± 0.12	0.28 ± 0.12		1.23 ± 0.41	0.05 ± 0.26	-0.16 ± 0.35	٩	-0.71±0.	-0.03 ± 0.56
D2	0.18 ± 0.14	-0.43 ± 0.13	-0.05 ± 0.14	0.35 ± 0.13	0.46±0.12		-0.31 ± 0.20	-0.39 ± 0.36	ام	-0.52±0.	-0.04 ± 0.58
Survival trait	ts										
SURV	-0.02 ± 0.23	-0.13 ± 0.26	0.09±0.25	-0.04 ± 0.25	-0.10 ± 0.25	-0.18 ± 0.30		0.17 ± 0.35	٩	0.12±0.	0.11 ± 0.36
ESTR	0.07 ± 0.12	-0.24 ± 0.37	0.14 ± 0.35	-0.47 ± 0.40	0.16 ± 0.35	-0.39 ± 0.30	-0.07 ± 0.24		٩	0.13±0.	0.61±0.54
Phenological	traits										
BSI	0.01 ± 0.14	-0.12±0.14	0.18±0.14	0.07±0.14	-0.06 ± 0.14	-0.16 ± 0.15	0.05 ± 0.28	0.03 ± 0.14		م ا	٩
BB2	-0.04 ± 0.13	-0.32 ± 0.39	0.03 ± 0.12	-0.01 ± 0.11	-0.71 ± 0.34	-0.29 ± 0.37	0.12 ± 0.25	0.13 ± 0.34	-0.05 ± 0.14		0.55 ± 0.60
BS2	-0.04 ± 0.14	-0.02 ± 0.14	-0.02 ± 0.13	-0.02 ± 0.13	-0.03 ± 0.56	-0.40 ± 0.58	0.09±0.27	0.19±0.13	0.24 ± 0.15	0.09 ± 0.13	
^a Please refer t ^b not estimatec	o Table 2 for c I due to lack o	codes and defi f variance am	inition of trait ong families w	s. vithin populat	ions						

Table 5. Genetic (above diagonal) and phenotypic (below diagonal) correlations among traits and their standard errors.

52

ÇIÇEK, F.F. ET AL.: GENETIC STRUCTURE OF FOUR KAZDAĞI FIR (ABIES EQUITROJANI) POPULATIONS

Since there was a positive, but a low genetic correlation between SURV and ESTR (0.17), only the genetic correlation between ESTR and growth traits will be given here. ESTR was weakly and mostly negatively correlated with growth traits (ranging from 0.14 between ESTR and BRANUM to -0.39 between D2 and ESTR) (Table 5), indicating that those families with more growth suffered less from environmental stresses. Genetic correlations between ESTR and phenological traits were positive and ranged from 0.13 between ESTR and BB2 to 0.61 between ESTR and D2. suggesting that those families burst bud late in the spring and set winter buds later were the ones which suffered more from environmental stresses in Ankara (Table 5).

Genetic correlations between BS1 and other studied traits could not be estimated since there was lack of family variance in this trait. However, positive and moderately high genetic correlation between BB2 and BS2 was estimated ($r_g = 0.55$). The families with late bud-burst date set also their buds later at the end of the second growing season.

Genetic correlations between phenological traits (BS2 and BB2) and growth traits were either very low or negative and ranged from -0.15 between BS2 and D2 to -0.80 between HT1 and BS2 (Table 5). The families with less growth set their winter buds late and burst buds also late. There were also moderate and negative genetic correlations between COT and BB2 (-0.37) as well as between COT and BS2 (-0.63), indicating that families with less cotyledon numbers.

Differentiation of populations

Based on adaptive seedling trait data, results of pairwise squared distance analysis showed that the smallest distance was between Kapidağ and Gürgendağ populations (0.592). Among studied populations, the distance between Çan and Kapıdağ was the greatest (1.339). Çan appeared to be highly differentiated from the remaining populations (Table 6). Çan was also the most distantly located population from the core area of Kazdağı fir. Although Çan was found to be the most divergent, when the results were plotted using the first two canonical discriminant functions, there was not any clear separation or grouping of other Kazdağı fir populations (Figure 4).

DISCUSSION

The results of the present study indicated that there was substantial genetic variation at both population and families within population levels for adaptive seedling traits. The magnitude of variance components due to populations was very low for the studied traits. Studies with other conifers in Turkey, such as Anatolian black pine and Turkish red pine reported similar results that most of the variation in adaptive seedling traits was attributable to families within populations (KAYA & TEMERIT 1994, KAYA & IŞIK 1997, GÜLBABA 1998). Considering the narrow range of the species, observation of significant population differences for most adaptive traits suggests that population differences in most of the studied traits might be the result of evolution of a number of traits together (JOLY et al. 1989). The isolated populations of the species seem to begin differentiation due to mosaics of climatic and topographic conditions coupled with limited-gene flow between core populations and remaining isolated ones.

The Çan population had the lowest survival ratio among the studies populations. This population was also the most suffered one from both frost and summer drought damages in the nursery environments in Ankara. The Çan population is located in the lowest elevation and has the smallest population size as compared to other populations. It is also the most distantly located one from the core population of Gürgendağ. This population

	Eybekli	Çan	Gürgendağ	Kapıdağ	
Evbekli	_	0.928	0.736	0.599	
Çan		_	0.934	1.339	
Gürgendağ			_	0.592	
Kapidağ					

Table 6. Mahlanobis distances among populations.



Figure 4. Plot of populations based on two caninical discriminant functions. Numbers represent the populations: 1 = Eybekli, 2 = Çan, 3 = Gürgendağ, 4 = Kapıdağ.

may be adapted to its microenvironment, thus it may not be buffered against extreme conditions like frost of Ankara. Its high environmental sensitivity values calculated for the adaptive seedling traits (ÇIÇEK 2000), being low elevation population and having the lowest annual precipitation (1,108 mm in Çan vs. 1,486 mm in Kapıdağ) point out that set of adaptive traits were selected and characterized the population. This population may be avoided as a seed sources for afforestation programs dealing with high elevation where late frost damages are frequent.

Heritability is a character of a particular population under particular environmental conditions (FALCONER 1989) and may change over years. So, the results were specific to four Kazdağı fir populations grown under two-year observations. Estimated heritabilities were generally low and did not exceed 0.51 (for SURV) because of low survival and high environmental variation in carried out experiments. Nevertheless, magnitude of family heritabilities for growth traits in Kazdağı fir suggests that considerable genetic improvement could be made on the basis of best performing families that is, selection within populations if early selection is planned to be practiced.

There was a lack of significant family variance component for bud set timing in the first year (BS1) and budburst timing in the second year (BB2) so that family heritabilities for BB2 and BS2 traits could not be estimated. Although significant family variance component was estimated for the budset timing in the second year, the estimated heriatbilitiy was low. These results suggest that either phenological traits were subjected to strong selection in the past or narrow range of Kazdağı fir was insufficient to maintain genetic diversity at the family level for the growing season length. Furthermore, the observation of low-heritability estimates for most traits could be also attributed to the nature of experiments (early ages and statistical power). Thus, family heritability estimates for a slow growing-shade tolerant species such as Kazdağı-fir can be expected to improve in later ages and well-conducted field experiments (O'NEILL *et al.* 2000)

The directions of genetic and phenotypic correlations were generally the same. In most cases, genetic correlations were higher than phenotypic ones. However, estimated genetic correlations had large associated standard errors associated so genetic correlations between seedling-traits should be interpreted with caution. Most of the growth traits correlated moderately and positively with the number of cotyledons at the family level. This means that the families with high number of cotyledons had more growth, in turn, larger seedling size after the first two growing seasons; in other words, maternal effects on seedlings growth were apparent and had an effect on the seedling growth performances in the early ages in Kazdagi fir. Similar results were also reported on *Pinus nigra* subsp. pallasiana and Pinus brutia species (KAYA & Temerit 1994, Doğan 1997, Kaya & İşik 1997, Gülbaba 1998).

Environmental stress (ESTR) trait was weakly and negatively (in most cases) correlated with growth traits, suggesting that the families with more growth had less damages from environmental stresses. Also, the families with late budburst and budset dates suffered the most from environmental stresses in Ankara nursery conditions. Damages to seedlings occurred due to summer heat as well drought and early frost for late budburst families and late budset families, respectively. Both frost and summer drought caused retardation in the growth of seedlings.

The families with late bud-burst date set also their buds later at the end of the second growing season. The families with later budset and bud burst dates had less growth in the second growing season. Selection of families based on growth traits should be practiced with care, since growth traits were, in general, negatively correlated with phenology traits. Selection based on growth traits *e.g.* height or diameter may result in selection of the families with early bud burst that are under the risk of late frost damages. When Kazdağı fir seed sources are used in plantations other than in its natural range, it would be advantageous to choose seed sources and families with early bud set dates to avoid early frost or summer drought damages.

Although significant amount of variation among populations in Kazdağı fir for adaptive seedling traits were detected in the present study, it was notable that the populations were not clearly distinguishable from each other, and only Çan population was found to be relatively distant. This is not surprising since all four populations are within the gene flow distance of the species. However, Çan population is relatively isolated with low elevation and annual precipitation. Here, not latitude as in the case of Douglas-fir (CAMPBELL & SORENSEN 1973) but altitude, topographic and micro-climatic variation may affect the expression of seedling traits and may have selected different sets of adaptive traits compared to the core populations. Small population size and isolation from core populations do not seem to have any effect on magnitude of genetic diversity in Çan population. This was evident from the previous study based on isozyme markers. GÜLBABA et al. (1998) found that estimated fixation indexes for Can and Kapidağ populations were nearly zero. This means that the possibility of inbreeding is not substantial. Another study (KAYA et al. 2005) on Turkish firs based on molecular markers (Randomly Amplified DNA polymorphism and Chloroplast-Simple Sequence Repeats) indicated that Can population has been significantly differentiated from the other Abies equirojani seed sources and other Turkish firs species. By considering that the natural range of narrow-endemic species of Kazdağı fir is restricted to a 3600 ha area, the existence of high genetic variation in adaptive seedling traits could be explained by occurrence of substantial gene flow between isolated and core populations of the species (KESSELI 1992, LEDIG 1988).

In order to conserve and enrich the genetic diversity, and carry this potential to the future, maximum diversity should be saved. However, it is obvious that every natural population can not be

protected as a whole (LEDIG 1998). It is much more convenient to preserve representative, and extreme or unusual populations (LEDIG 1988). In the present study, Gürgendağ population is located in the core area of the species distribution and biologically closer to both Kapıdağ and Eybekli populations. This result might be due to the central location of this population, thus its having the optimal distribution of the Kazdağı fir and sharing adaptive gene complexes, which were conserved over the years. It could be proposed that one of the in situ reserves could be located in Gürgendağ to capture most of the genetic diversity. In their isozyme study, VELIOĞLU et al. (1999) proposed three Gene Management Zones (GMZ) (in situ reserves) for Pinus nigra subsp pallasiana which forms mixed natural stands with Kazdağı fir and one of these is located in Gürgendağ. Thus, a GMZ in Gürgendağ could preserve both species. GÜLBABA et al. (1998) also proposed a GMZ to be located at Gürgendağ, based on the results of isoenzyme study.

For the security of *in situ* conservation of Kazdağı fir, it could be suggested that GMZs should be located in at least two populations. Çan might be proposed for a second GMZ, since it possesses high heterozygosity (GÜLBABA *et al.* 1998) and it is biologically the most distant population as compared to others. Gürgendağ populations located in the Nature conservation area and part of it has been reserved as seed collection stand and Eybekli population was designate as gene conservation forest, but there is no conservation status associated with Çan population yet.

ACKNOWLEDGEMENT

This study was financially supported by the Turkish Ministry of Environment and Forestry as a part of "*In situ* Conservation of Plant Genetic Diversity" project which was funded by the World Bank acting as Trustee of grant funds from Participants of the Global Environment Facility (GEF) and the Middle East Technical University (Project #: AFP-99-06-02-25).

REFERENCES

- AKMAN, Y. 1995: Türkiye Orman Vejetasyonu, A.Ü. Fen Fak. Botanik A. B. D. Ankara.
- ATA, C. 1975: Kazdağı göknarının (A. equitrojani Ascher et Sinten) Türkiye'deki yayılışı ve silvikültürel özellikleri. Orman Fak. Dergisi, Seri A, Cilt 24, Sayı 11:165–219.
- AYTUG, B. 1959: Türkiye göknar (Abies Tourn.) türleri

© ARBORA PUBLISHERS

üzerinde morfolojik esaslar ve anatomik araştırmalar. İ. Ü. Orman Fak. Dergisi 9(2): 165–197.

- BROWN, A.H.D.1992: Human impact on plant gene pools and sampling for their conservation. *Oikos* 63: 109-118.
- CAMPBELL, R.K. & SORENSEN, F.C.: Cold-acclimation in seedling Douglas-fir related to phenology and provenance. *Ecology* **54**(5): 1148–1151.
- CICEK, F.F. 2000: Genetic Variation in Adaptive Seedling Traits of Natural Populations of Abies equitrojani Aschers. et Sint. from Kazdağı. Unpublished doctoral dissertation, Middle East Technical University, Department of Biological Sciences, Ankara, 144p.
- DOGAN, B. 1997: Patterns of genetic variation within and between natural Turkish red pine (*Pinus brutia* Ten.) Populations in Dalaman Çayı watershed. Ministry of Forestry, Ege Forestry Research Institute, İzmir: Publ. No. 9, Turkey.
- FALCONER, D.S. 1989: Introduction to Quantitative Genetics, England: Longman Scientific & Technical.
- FRANKEL, O. H. & SOULÉ, M. E. 1981: Conservation and Evolution. Cambridge University Press.
- GEMICI, Y., SEÇMEN, Ö., GÖRK, G., ACAR, İ. & ÖZEL, N. 1998: Plant Diversity on Kazdağları (Mt. Ida). *In*: Zencirci, N., Kaya, Z., Anikster, Y., & Adams, W. T. (eds), Proceedings of the International Symposium of *in situ* Conservation of Plant Genetic Diversity (pp. 281–288). Ankara: Central Research Institute for Field Crops, Turkey.
- GÜLBABA, A. G. 1998: Genetic variation in the natural black pine (*Pinus nigra* subsp. *pallasiana*) populations of Bolkar Mountains and designation of gene management zones. J.ournal of DOA (East. Mediterran. For. Res. Inst.) 4: 99–130.
- GÜLBABA, A.G., VELIOĞLU, E., ÖZER, A.S., DOĞAN, B., DOERKSEN, A.H. & ADAMS, W.T.1998: Population genetic structure of Kazdağı fir (*Abies equitrojani* Aschers et Sint.), a narrow endemic to Turkey: Implications for *in situ* conservation. *In*: Zencirci, N, Kaya, Z., Anikster, Y., & Adams, W. T. (eds), Proceedings of the International Symposium of *in situ* Conservation of Plant Genetic Diversity (pp. 271 -280). Ankara: Central Research Institute for Field Crops, Turkey.
- IŞIK K. 1999: Çevre Sorunları Biyolojik Eeritlilik ve Orman Gen Kaynaklarımız. TEMA Vakfı Yayınları, İstanbul.
- JOLY, R.J., ADAMS, W.T. & STAFFORD S.G.1989: Phenological and morphological responses of mesic and dry site sources of Coastal Douglas-fir to water deficit. *For. Sci.* 35(4): 987–1005.
- KAYA, Z., CAMPBELL, R.K. & ADAMS W.T. 1989: Correlated responses of height increment and components of increment in 2-year-old Douglas-fir seedlings. *Can. J. For. Res.* 19: 1124–1130.
- KAYA, Z. & TEMERIT, A. 1994: Genetic structure of marginally located *Pinus nigra* var. *pallasiana* populations in Central Turkey. *Silvae Genet.* 43(5–6): 272 –277.

- KAYA, Z. & IŞIK, F. 1997: The pattern of genetic variation in shoot growth of *Pinus brutia* Ten. populations sampled from the Toros Mountains in Turkey. *Silvae Genet.* 46(2–3): 73–81.
- KAYA, Z., KÜN, E. & GÜNER, A. 1997: National Plan for in situ Conservation of Plant Genetic Diversity in Turkey. Millî Eğitim Basımevi, İstanbul.
- KAYA, Z., SKAGGS, A. & NEALE, D.B., 2005: Genetic differentiation of *Abies equitrojani* populations from Kazdağı in Turkey and genetic relationship between Turkish firs belonging to the *Abies nordmanniana* complex. *Manuscript in prepration*.
- KAYACIK, H. 1965: Cins: Abies Miller. Orman ve Park Ağaçlarının Özel Sistematiği (Gymnospermae) (pp. 74–86). I. Cilt, İ.Ü. Orman Fak. Yayın no:1105.
- KESSELI, R.V. 1992: Population biology and conservation of rare plants. *In*: Jain, S. K., and Botsford, L. W. (eds.), Applied Population Biology, Kluwer Acad. Publ., Netherlands, 68–90.
- KRZANOWSKI, W.J. & MARRIOT, F.H.C. 1994: Multivariate Analysis, Part 1, Distributions, Ordination and Inference, Edward Arnold, Great Britain, pp. 221–223.
- LEDIG, F.T. 1988: The conservation of Diversity in Forest Trees. *BioScience* **38**(7): 471–479.
- LEDIG, F.T. 1998. Genetic diversity in tree species: with special preference to conservation in Turkey. *In*: Zencirci, N, Kaya, Z., Anikster, Y., & Adams, W. T. (eds): Proceedings of the International Symposium of *in situ* Conservation of Plant Genetic Diversity (pp. 231 –248). Ankara: Central Research Institute for Field Crops, Turkey.
- NEALE, D.B. 1998. Understanding the molecular basis of genetic diversity through genome mapping and DNA sequencing, *In*: Zencirci, N, Kaya, Z., Anikster, Y., & Adams, W. T.(Eds), Proceedings of the International Symposium of *in situ* Conservation of Plant Genetic Diversity (pp. 385–390). Ankara: Central Research Institute for Field Crops, Turkey.
- O'NEILL, G.A., AİTKEN, S.N. & ADAMS, W.T. 2000: Genetic selection for cold hardiness in coastal Douglas-fir seedlings and saplings. *Can. J. For. Res.* 30: 1799–1807.
- SAATÇIOGLU, F. 1969: Göknar (Abies Mill.) Türleri. Silvikültürün Biyolojik Esasları ve Prensipleri (pp. 252 –266). İ.Ü. Orman Fak. Yayınları, No:138.
- SAS Inst. Inc. 1988:SAS/STAT User's Guide, Release 6.03 Edition. Cary, NC, 1028p.
- SNEATH, P. & SOKAL, R.R. 1973: Numerical Taxanomy. W.H. Freeman and Co., San Francisco.
- VELIOĞLU, E., TOLUN, A.A., ÇENGEL, B. & KAYA, Z. 1999b: Isozyme variation in natural black pine (*Pin-us nigra* Arnold. subsp. *pallasina* (Lamb.) Holmboe) Populations Sampled from the Bolkar Mountains. Ministry of Forestry, Forest Tree Seeds and Tree Breeding Research Directorate, Publ. No: 11, Ankara, Turkey.
- VIDAKOVIĆ, M. 1991: Conifers Morphology and Variation. Zagreb: Grafički Zavod Hrvatske.
- ZOBEL, B. & TALBERT, J., 1984. Applied Forest Tree Improvement. Canada: John Wiley & Sons.