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Genetic Variation in Wood Specific Gravity of Half-sib Families of *Pinus nigra* subsp. *pallasiana* Tested at the Juvenile Stage: Implications for Early Selection

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Summary

Seeds from 7 populations (total of 281 half-sib families, *progeny test*) and 35 seed stands (*provenance test*) representing natural range of Anatolian black pine (*Pinus nigra* subsp. *pallasiana*) were sown in a forest nursery in Ankara in 1990 and raised until age 3. Stem wood specific gravity (WSG) of all seedlings was determined at age of 3. The results of this study indicated that WSG did not vary significantly neither among the 7 populations (ranging from 0.41 to 0.42) nor among 35 seed stands (ranging from 0.37 to 0.46). Differences between half sib families for WSG were, however, statistically significant. Estimated family heritablity was moderately high (0.38). Genetic correlations between seedling growth traits and WSG were low, but consistently negative. The families with better height and diameter growth had lower WSG values. Also families with late budset and budburst dates in 1991 had lower WSG values. Seedlings originating from northern latitudes had lower WSG than those from southern latitudes. From the results of the study, it seems that early selection of families for WSG (indirect selection for WSG at mature age) would be possible and substantial genetic gain in WSG could be achieved if the selection based on a multi-trait index selection by giving appropriate weights to WSG and other traits. Further implications of early selection for WSG in Anatolian black pine are also discussed in the paper.

Key words: Pinus nigra subsp. *pallasiana,* wood specific gravity, genetic variation, genetic correlation.

Introduction

The Anatolian black pine (*Pinus nigra* Arnold subspecies *pallasiana* (Lamb.) Holmb.) is an important timber species, occurring naturally as a widespread mid-elevation species (ranging from 250 to 1550 m) in Toros, western and northern Anatolian Mountains of Turkey (*Figure 1*). Anatolian black pine forests cover more than 2 million hectares. It is the first species for afforestation of the high Anatolian steppes (KAYA and TEMERIT, 1994). Anatolian black pine has, therefore, great importance in Turkish forestry (KAYA and TEMERIT, 1994; KOSKI and ANTOLA, 1993).

To date, 88 seed stands and 55 clonal seed orchards have been established by Turkish Ministry of Forestry for Anatolian black pine. Large areas in Turkey are also reforested or afforested with the species, but there is lack of genetic studies which will help to manage and improve the genetic resources of the species. Tree improvement program concerning the species is still in its beginning. Clonal seed orchards and seed stands have been established to provide needed seeds for reforestation and afforestation activities. The first generation progeny tests were established to allow the selection of best parents and roguing of clonal seed orchards.

Since long rotations are used in forestry, early evaluation of genotypes for adaptive and volume traits are an important component of tree improvement strategy (BRIDGEWATER and MCKEAND, 1997; LAMBETH *et al.,* 1982; LOWE and VAN BUIJTENEN, 1989).

Wood density in pines shows considerable genetic, environmental and age related variation (ZOBEL and VAN BUIJTENEN, 1989). Wood density is strongly influenced by the amount of early and latewood produced late in the season. Generally early wood has low density while late wood has higher density (ZOBEL and VAN BUIJTENEN, 1989). Significant genetic variation in early and late wood proportions has been reported in Douglas-fir families (VARGAS-HERNANDEZ and ADAMS, 1994) and in Norway Spruce and Douglas-fir provenances (VARGAS-HERNANDEZ and ADAMS, 1994; SKROPPA and DIETRICHSON, 1999). Also, strong genetic correlations between growth traits in juvenile ages (ages 2–7) and wood density in mature age (age 29) were reported in Norway spruce (SKROPPA and DIETRICHSON, 1999). These correlations were negative and good predictors of wood density at age 29. Diameter growth in pines in juvenile ages is mainly made up of early wood and very little late wood. Thus, overall wood density which is not affected as much as late wood by environmental conditions and assuming that it is made up mainly by early wood could be used to select families

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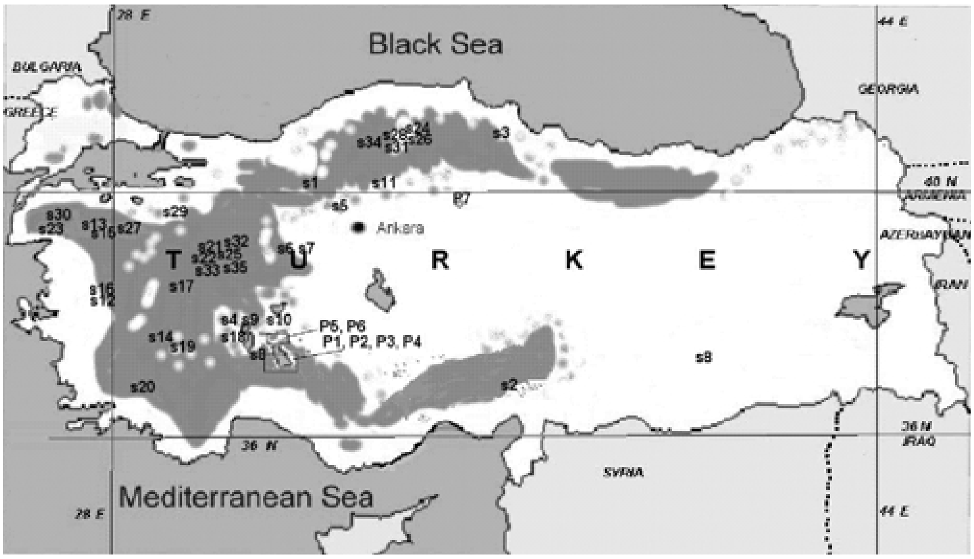

Figure 1. – Natural distribution of *Pinus nigra* Arnold subsp. *pallasiana* in Turkey. The locations of populations were indicated as P1 through P7 while seed stands were labeled as S1 to S35.

or provenances with high wood densities at the early ages, given that WSG at juvenile age and mature age of families and provenances are strongly correlated.

Genetic structure and patterns of genetic variation in adaptive seedling traits (KAYA and TEMERIT, 1994) and proteins (KAYA *et al.,* 2001) were studied in different populations of the species. Both seedling and isozyme studies indicated that there is a large genetic variation within populations. In the previous study (KAYA and TEMERIT, 1994) using the same materials from Anatolian black pine, we have investigated the genetic variation in adaptive seedling traits in populations sampled from marginal and optimum habitats in the interior Toros mountains. The results indicate that large genetic variation within populations exists for adaptive traits. But, to date, there has not been any study dealing WSG in Anatolian black pine.

Considering that there is no study to date on genetic control of wood density in Anatolian black pine, wood density of seedlings from the same materials as well as from 35 seed stands representing the natural range of the species in Turkey at the age of three was studied in the present study; (i) to test if there is genetic variation among families, populations or provenances of Anatolian black pine in overall wood density of seedlings at early ages, (ii) to test if there is any correlations between adaptive seedling traits and seedling wood density.

Material and Methods

Six populations from the interior Toros Mountains and one population from northern central Turkey were sampled (*Figure 1, Table IA*). Geographic and topographic descriptions of the populations were given in details elsewhere (KAYA and TEMERIT, 1994). The north central Turkey population in the study is a small and isolated one which is about 264 ha and has a national park status currently. Open pollinated seeds from parent trees were sown in three nursery beds in Kızılcahamam Forest Nursery (70 km NE of Ankara, latitude 32° 38'N, longitude 31° 26'E and elevation 1100m) in April 1990 (*progeny test*). Total of 281 families (45 families per population except for Taşlıca-2:31 families and Çamlık-2:25 families) were allocated to five seedling row plots in a randomized complete block design with 3 replications. Also, bulk seeds from 35 seed stands representing the natural range of Anatolian black pine (*Table*

Table I. – A) List of populations and number of half sib families per population. B) Geographic information on sampled Anatolian black pine seed stands and mean wood specific gravity (WSG) values.

A)

Codes	Populations	Number of half-sib families
P1	Fadara-1 (Beyşehir)	45
P2	Fadara-2 (Beyşehir)	45
P3	İslibucak (Beyşehir)	45
P4	İslibucak (Beyşehir)	45
P5	Taşlıca-1 (Ilgın)	45
P6	Taşlıca-2 (Ilgın)	31
P7	Çamlık (Yozgat)	25

Table I. – Continued.

B)

Codes	Seed Stands	Latitude	Longitude	Altitude	Aspect	WSG (mean ±SD)
S1	Mengen (Bolu)	41^{0}.00'N	32^{0}.00'E	950m	NW	0.40±0.04
S2	Adana	37.70	35.70	1200	E	0.46±0.07
S3	Dere (Çarşamba)	39.80	29.50	1400	NE	0.41±0.04
S4	Ahırdağı-1(Afyon)	38.70	30.00	1400	N	0.43±0.03
S5	Kızılcahamam	41.50	32.50	1300	N	0.38±0.02
S6	Pazar (Eskişehir)					0.41±0.02
S7	Seed orchard (Eskişehir)	39.30	29.20	1500	N	0.43±0.03
S8	Eğridir (Isparta)	37.80	30.10	1230	SE	0.40±0.02
S9	Çataloluk (Afyon)	38.70	30.20	1510	NE	0.44±0.07
S10	Ahırdağı-2 (Afyon)	38.70	30.00	1350	NE	0.51±0.04
S11	Çildağı-Çerkes	40.80	32.70	1260	N	0.41±0.08
S12	Ovacık-Bayındır	38.30	27.70	850	NE	0.44±0.07
S13	Gölcük-Alaçam	39.50	29.50	1050	E	0.41±0.04
S14	Sapacaboğazı (Denizli)	27.70	29.50	1050	E	0.41±0.02
S15	Korucu (Balıkesir)	39.30	27.30	800	N	0.45±0.06
S16	Güneşli-Gördes	39.20	28.50	1200	N	0.39±0.05
S17	Oturak-Çatak (Uşak)	38.80	30.00	1450	SE	0.40±0.04
S18	Çataloluk-Sincali (Afyon)	38.70	30.20	1510	NE	0.42±0.04
S19	Karatepe-Gökkaya	37.70	29.00	1250	SE	0.38±0.04
S20	Elkindağı-Yılanlı	37.20	28.50	1100	E	0.46±0.03
S21	Alasöğüt-Simav	39.20	28.70	1300	N	0.38+0.04
S22	Fındıklı-Tavşanlı	39.50	29.20	1500	N	0.44±0.03
S23	Çinarlıhan-Edremit	39.70	26.80	1225	SW	0.39±0.05
S24	Karadere (Kastamonu)	41.20	34.00	1275	E	0.40±0.06
S25	Domaniç (Kütahya)	39.80	29.50	1400	NE	0.40±0.05
S26	Sorgun-Daday	41.20	34.00	1120	N	0.40±0.04
S27	Değirmeneğrek-Alaçam	39.50	28.50	1200	S	0.40±0.04
S28	Kiraz-Daday	41.20	34.00	1120	N	0.40±0.03
S29	Burhandağ-M.Kemalpaşa	39.80	28.70	1000	S	0.42±0.03
S30	Kapıdağ-Edremit	39.70	26.80	1225	SW	0.44±0.08
S31	Elekdağ-Boyabat	41.50	34.50	1300	E	0.37±0.04
S32	İkizoluk-Tavşanlı	39.50	29.20	1500	N	0.39±0.03
S33	Balıköy-Emet	39.50	29.70	1100	N	0.39±0.03
S34	Boruklu-Boyabat	41 29	34 39	595	S	0.41±0.04
S35	Kocagüvez-Simav	39.30	29.00	1400	N	0.39±0.04

IB and *Figure 1*) in Turkey were obtained from the Forest Trees and Seeds Improvement Research Directorates, Ankara in the fall of 1990 and planted in Kızılcahamam Forest Nursery in 5 seedling- row plots with three replications (*provenance test*).

A total of 8 seedling traits dealing with the growth and adaptation were recorded (*Table 2*) and a description of trait data collection was given in an earlier study (KAYA and TEMERIT, 1994). Adaptive seedling traits and growth data for 1990 and 1991 growing seasons were utilized from the previous study (KAYA and TEMERIT, 1994). In 1992 growing season, height growth (HT92) was recorded for seedlings in October.

At the age of three, data on wood related traits (wood specific gravity and the proportion of dry wood over total wood) were collected in addition to adaptive seedling traits. Five centimeter stem disks from all seedlings (3 years old) were obtained in the fall of 1992. Stem disks were placed in a freezer, debarked and cut 1 cm in length. Then, they were soaked in distilled water. Saturated stem pieces were placed in a desiccator (alternative vacuum was applied for about 2 days). These stem samples were weighed at maximum moisture content (green weight) and at air moisture content (after storage of stem samples for 1 day room temperature) and oven-dried stem samples (at 102 ± 3 °C for 2 days) were reweighed to obtain wood-weight at 0% moisture content. The specific gravity of each sampled stem was determined by the maximum moisture content method (SMITH, 1954; VURDU, 1979) as follows:

$$\textbf{\textit{Wood Specific Gravity (WSG)}} = \frac{1}{\dfrac{Mn - Mo}{Mo} + \dfrac{1}{G_{so}}}$$

Where $\boldsymbol{Mn}$ = the weight of water saturated wood (in grams), $\boldsymbol{Mo}$ = weight of oven-dry wood sample (in grams), $\boldsymbol{G_{so}}$ = 1.53 is a constant used as a specific gravity of the non-porous cellulose cell wall.

For the progeny test (in which families were involved), analysis of traits was based on plot means (means of five seedlings) because of seedling mortalities in family- row- plots. There were three plots with no seedlings in family-row-plots. As the experimental design was unbalanced due to missing plots, analysis of variance (ANOVA) for all traits was carried out by using a generalized least square procedure (GLM) of SAS (SAS Inst., 1988), according to the following statistical model.

$$\boldsymbol{Z_{jkl} = \mu + B_k + P_{(j)} + F_{(l)j} + e_{jkl}}$$

Where $\boldsymbol{\mu}$ is the experimental mean, $\boldsymbol{Z}_{jkl}$ is the mean performance of the l th family in the j th population in k th replication. $\boldsymbol{B}_k$ = the fixed effect of k th replication [$\boldsymbol{E}(\boldsymbol{B}_k) = 0$, Var($\boldsymbol{B}_k$) = $\boldsymbol{K}^2_k$]; $\boldsymbol{P}_j$ = Fixed effect of j th population [$\boldsymbol{E}(\boldsymbol{P}_j) = 0$, Var($\boldsymbol{P}_j$) = $\boldsymbol{K}^2_j$]; $\boldsymbol{F}_{(l)j}$ = random effect of l th family in j th population [$E(F_{(l)j})$ = 0; Var ($\boldsymbol{F}_{(l)j}$) = $\boldsymbol{\sigma}^2_{(l)j}$]; e_{jkl} = experimental error [$\boldsymbol{E}(\boldsymbol{e}_{jkl}) = 0$, Var ($\boldsymbol{e}_{jkl}$) = $\boldsymbol{\sigma}^2_e$].

For the provenance test, analysis of variance based on the following linear model.

$$\boldsymbol{Z_{jkl} = \mu + B_k + S_{(j)} + e_{jkl}}$$

Where $\boldsymbol{\mu}$ is the experimental mean, $\boldsymbol{Z}_{jkl}$ is the performance of l th seedling in j th seed stand (provenance) in k th replication. $\boldsymbol{B}_k$ = the random effect of k th replication [$\boldsymbol{E}(\boldsymbol{B}_k) = 0$, Var($\boldsymbol{B}_k$) = $\boldsymbol{\sigma}^2_k$]; S_j = Random effect of j th seed stand [$\boldsymbol{E}(\boldsymbol{S}_j) = 0$, Var($\boldsymbol{S}_j$) = $\boldsymbol{\sigma}^2_j$]; $\boldsymbol{e}_{jkl}$ = experimental error [$\boldsymbol{E}(\boldsymbol{e}_{jkl}) = 0$, Var($\boldsymbol{e}_{jkl}$) = $\boldsymbol{\sigma}^2_e$].

In the progeny test, components of variance and covariance at the population and families within population levels were estimated according to the expectations from the above model of analysis of variances (FALCONER, 1981; KAYA *et al.,* 1989; NAMKOONG, 1979).

Family heritabilities (h^2_{fx}) were estimated by using he following equation:

$$\boldsymbol{h^2_{fx} = \sigma^2_{(fx)} / (\sigma^2_{(fx)} + \sigma^2_e / r)}$$

where $\sigma^2_{(fx)}$ is the family component of total variance for the trait, r is the number of replications and equals to 3 and σ^2_e is the error variance.

Genetic correlations were estimated from the component of variance and covariance (FALCONER, 1981) and substituted into the standard equation for the product moment correlation coefficient.

$$\textbf{\textit{Genetic correlation}}\ (\boldsymbol{R}_{g(x,y)}) = \frac{Cov_{f(x,y)}}{\sqrt{\boldsymbol{\sigma}^2_{f(x)}\,\boldsymbol{\sigma}^2_{f(y)}}}$$

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

The phenotypic correlations between traits x and y were calculated from family mean squares and mean cross products for the traits (KAYA *et al.,* 1989). The standard errors of genetic and phenotypic correlations were calculated according to BECKER (1992).

In the provenance test, Pearson correlations using *PROC CORR* procedure of SAS statistical package (SAS Inst. Inc., 1988) were estimated between wood specific gravity and adaptive traits and topographic variables.

Results

Since the pattern of genetic variation for adaptive seedling traits in 1990 and 1991 from half-sib material was reported and discussed elsewhere (KAYA and TEMERIT, 1994), only the results on genetic variation in wood specific gravity and adaptive seedling traits recorded in 1992 in half sib families and in 1990, 1991 and 1992 growing seasons in provenance material will be emphasized and discussed here.

Pattern of genetic variation

Progeny tests (half-sib families)

There was significant variation at both population and family levels in all adaptive seedling traits. Most of the variation was observed between families within populations. It ranged from 12.6% in BB91 to 23.7% in COT (*Table 3A*). The population component of total variation was significant, but made up a very little portion of total variation in adaptive seedling traits, ranging from 1.6% to 4.7%. The implications of these results were discussed in details elsewhere (KAYA and TEMERIT, 1994). For wood specific gravity, results were similar, that is, there was no significant variation among populations, but families within population varied significantly. The component of variation due to families was 11.3%. Accordingly, population means for WSG did not show much variation, ranging from 0.41 in Fadara, Taşlıca-2 and İslibucak-2 to 0.42 in Fadara-2 population. On the other hand, the range of variation of WSG between families within population was great for all studied populations (*Table 4*). Heritability estimates for both adaptive traits and WSG were moderately high ranging from 0.28 in BB91 to 0.50 in COT traits. The family heritability estimate for WSG was 0.38 which is considerably high (*Table 3A*).

Table 2. – Description of studied traits.

Codes for traits	Definitions of traits	Units of traits
COT	Number of cotyledons	Number
BS90	Date of bud set in 1990	days from January1, 1990
HT90	Height increment in 1990	Mm
BB91	Date of bud burst in 1991	days from January 1991
BS91	Date of bud set in 1991	days from January 1991
HT91	Total height increment in 1990+1991	Mm
DIAM91	Total diameter increment in 1990+1991	Mm
HT92	Height increment in 1992	Mm
WSG	Wood specific gravity	coefficient

Table 3. – A) Progeny test: Analysis of variance (mean squares, component of variance as a % of total variance (VC)) and family heritabilities for adaptive and growth seedling traits and wood specific gravity (WSG). B) Provenance test: Analysis of variance for adaptive and growth seedling traits and WSG.

A)

Traits[1]	Replications df=2	Populations df=6	Variance component due to populations	Families/ Populations df=274	Variance component due to families	Error df=554	Variance component due to error	Mean	Family heritability h^2_f
COT	0.400	1.260**	1.6	0.541**	23.7	0.277	74.7	8.25	0.51
BS90	1364.15	97.42**	1.9	37.95**	23.5	19.57	74.6	265.81	0.49
HT90	2277.58	203.60**	3.1	56.11**	22.0	29.88	74.9	24.99	0.47
BB91	78.11	18.21**	1.6	7.28**	12.6	5.06	85.8	115.84	0.28
BS91	2013.37	827.34**	2.8	238.65**	20.1	134.29	77.1	261.99	0.44
HT91	98556.14	40470.9**	2.1	14703.2**	20.9	8131.5	77.0	549.14	0.45
DIAM91	178.22	43.02**	4.7	8.37**	19.8	4.71	75.5	11.57	0.44
HT92	66518.90	13504.4**	3.2	3610.9**	20.9	1985.3	75.9	220.18	0.45
WSG	0.0192	0.00085ns	0	0.0016**	11.3	0.0011	88.7	0.415	0.38

B)

Traits[1]	Replications df=2	Seed Stands df=34	Error df=236	Mean
COT	1.30	2.71**	1.01	7.98
BS90	411.55	328.87ns	278.39	266.6
HT90	169.62	162.32*	114.74	30.02
BB91	15.47	10.01ns	12.62	119.2
BS91	266.19	866.58*	467.52	260.7
HT91	7275.03	19607.23ns	17241.37	571.8
DIAM91	7.12	32.99*	17.61	14.6
HT92	174.30	60.50ns	55.89	600.2
WSG	0.0157	0.006ns	0.0097	0.414

ns: not statistically significant; * significant at $P < 0.05$; ** significant at $P < 0.01$; [1] See *Table 2* for definition of codes for traits.

Provenance (seed stand) test

The pattern of variation in wood specific gravity in the provenance test indicated that wood specific gravity did not vary significantly among seed stands. The variation in few of the adaptive seedling traits was found to be significant such as COT, HT90, BS91 and DIAM91 (*Table 3B*). Nevertheless, the range of WSG among provenances was wide- varying from 0.37 in Elekdağ (Boyabat) to 0.51 in Ahırdağı-2 (Afyon) (*Table 1B*).

Correlations between WSG and adaptive seedling traits

Progeny test: Genetic correlations

Since the magnitude and sign of estimated genetic and phenotypic correlations were similar, here only genetic correlations will be reported. Genetic correlations between WSG and adaptive seedling traits revealed that families with a better height and diameter growth in a given year had lower wood specific gravity values. For instance, genetic correlation between WSG and growth traits varied from –0.24 (between HT92 and WSG) to –0.34 (between WSG and COT). The genetic correlations between WSG and phenological traits were weak and positive in 1991, suggesting that families with late bud burst or late bud set in the growing season had higher wood specific gravity value (*Table 5*).

Provenance test: Pearson correlation

From the provenance test, seedlings with better height growth had lower WSG values (Pearson correlation, –0.17). Also, latitude of seed stands and wood specific gravity of seedlings were significantly and negatively correlated (Pearson correlation coefficient, –0.13) (*Table 6*).

Discussion

Neither populations (half-sib materials) nor seed stands varied significantly in wood specific gravity. The wood specific gravity of seedlings at early ages is assumed to be determined by mostly juvenile woods (JAYAWICKKRAMA *et al.,* 1997). The shoot growth of seedlings in early ages are produced by predetermined growth (or first flush) (KAYA and TEMERIT, 1994) and the wood produced by shoot growth pattern is considered to be a juvenile wood. Provenance differences in WSG in loblolly pine (JAYAWICKKRAMA *et al.,* 1997) are explained by the amount of late wood production differences. Although we did not test, but

Table 4. – Population means and ranges for the adaptive seedling and wood specific gravity traits.

	Populations						
Traits[1]	Fadara-1	Fadara-2	İslibucak-1	İslibucak-2	Taşlica-1	Taşlica-2	Çamlik
COT	8.31 *(6-10)*	8.28 *(6.7-9.8)*	8.35 *(7-10)*	8.22 *(6-10)*	8.28 *(7-10)*	8.23 *(6-10)*	7.99 *(6-9)*
BS90	265.5 *(254-280)*	265.18 *(256-277)*	266.60 *(256-278)*	265.30 *(256-277)*	267.15 *(256-280)*	266.61 *(205-277)*	264.42 *(258-284)*
HT90	25.64 *(7.8-50.2)*	26.87 *(8.0-42.0)*	25.35 *(9.3-45.0)*	24.21 *(10.0-42.0)*	25.31 *(10.0-50.5)*	23.15 *(8.0-38.3)*	23.82 *(10.5-44.5)*
BB91	115.14 *(89-123)*	115.82 *(114-124)*	115.82 *(114-123)*	115.81 *(114-121)*	116.17 *(114-124)*	116.12 *(114-124)*	116.36 *(114-127)*
BS91	263.6 *(204-325)*	260.7 *(247-323)*	265.3 *(252-312)*	261.3 *(254-322)*	259.1 *(254-289)*	263.6 *(254-325)*	257.2 *(254-318)*
HT91	543.8 *(261-854.8)*	569.9 *(350.3-876.7)*	552.8 *(328.17-842.5)*	533.8 *(365-773)*	577.4 *(340-774.5)*	556.6 *(217-786.7)*	536.2 *(289.5-830.5)*
DIAM91	11.8 *(3.7-21.0)*	12.2 *(6.0-20.5)*	12.1 *(5.7-19.7)*	11.4 *(6.5-17.7)*	11.6 *(6.2-19.2)*	10.8 *(5.1-16.0)*	10.6 *(5.7-17.0)*
HT92	224.7 *(106.6-337.5)*	229.8 *(95-356.2)*	231.4 *(93.3-373.3)*	220.2 *(133.3-348.0)*	212.6 *(90-310)*	203.6 *(60-318)*	211.0 *(90-390)*
WSG	0.41 *(0.23-0.54)*	0.42 *(0.32-0.58)*	0.415 *(0.28-0.59)*	0.41 *(0.31-0.54)*	0.416 *(0.24-0.48)*	0.41 *(0.33-0.68)*	0.417 *(0.30-0.77)*

[1] See *Table 2* for definition of codes for traits.

Table 5. – Genetic and phenotypic correlations between adaptive seedling traits and wood specific gravity in progeny test. Standard errors of estimates are in parenthesis.

Traits	Genetic correlations	Phenotypic correlations
	WSG	WSG
COT	-0.34(0.15)	-0.11(0.11)
BS90	-0.12(0.07)	-0.01(0.07)
HT90	-0.32(0.14)	-0.24(0.07)
BB91	0.20(0.20)	-0.05(0.08)
BS91	0.25(0.15)	0.13(0.08)
HT91	-0.26(0.14)	-0.25(0.07)
DIAM91	-0.32(0.15)	-0.26(0.07)
HT92	-0.24(0.15)	-0.23(0.07)

Table 6. – Pearson correlation between WSG and other seedling traits and topographic variables calculated in provenance test.

Traits	WSG
Cot	-0.05 [a]ns
BS91	0.06 ns
HT90	0.004 ns
BB91	-0.01 ns
BS92	0.02 ns
HT91	-0.11 ns
DIAM92	-0.06 ns
HT92	-0.17*
LATITUDE	-0.13*
LONGITUDE	-0.05 ns
ALTITUDE	-0.004 ns

[a] Sample size = 182, * Significant at $p < 0.05$

it would be worthwhile to test if late wood production in Anatolian black pine seedlings at the provenance level is not significant at juvenile ages so that provenance differences for WSG at early ages in Anatolian black pine could not be expressed. Furthermore, seed stands were represented with 15 seedlings in the experiment (5 seedlings x 3 replication = 15 seedlings). For some seed stands, the sample size was lower than this due to mortalities occurred during the course of the experiment. Thus, sample size to estimate wood specific gravity for seed stands seems to be insufficient.

There were significant population and seed stand differences for height growth in three growing season. These differences were particularly pronounced at both population and family within population levels in progeny test. Although both height and diameter growth varied significantly among populations in progeny test and seed stands in provenance test in three consecutive years, differences for density of wood were not significant neither among populations nor seed stands, indicating that population and seed stand differences for growth traits may be due to the amount of predetermined growth which produces only juvenile wood.

However, family differences within populations for WSG was significant and made up a considerable portion of the total variation. Estimated family heritability for WSG is moderate allthough it is not as high as the heritability estimates for WSG of mature woods of pines. Nevertheless, significant family differences for WSG of juvenile wood were reported in other species such as Douglas-fir (Vargas-Hernandez and Adams, 1994), Norway spruce (Skroppa and Dietrichson, 1999) and loblolly pine (Jayawickkrama *et al.,* 1997). These kinds of differences were also observed for ponderosa pine in older trees (Koch and Fins, 2000).

Considering the magnitude of family heritability for WSG, early selection of families with high WSG values in Anatolian black pine could yield rapid improvement for this trait in juvenile wood as early as at 3 years old. Since selection based the WSG of juvenile wood is an indirect selection of WSG at the mature ages (rotation age), strong genetic correlation between juvenile WSG and mature WSG as well as high heritabilities for WSG at the juvenile and mature ages would require to maximize the genetic gain per time. After evaluating data in numerous species of *Pinaceae,* Lambeth (1980) suggested that early selection in loblolly pine could be made as early as at the age of 6, given the rotation age is about 40 (southern pines). If the WSG data is collected from the seedlings by the age of 6 in Anatolian black pine and juvenile-mature correlation gets improved for WSG as materials get older, considerable genetic gain could be achieved per rotation time. However, moderate family heritability values for WSG should be interpreted with caution. Based on the isozyme data, the earlier study by Kaya *et al.* (2001) revealed that magnitude of genetic diversity showed different pattern in the populations of the Bolkar Mountains vs. Kazdağı. In the Kazdağı populations, the excess of homozygotes was observed while the excess of heterozygotes was present in the Bolkar Mountain populations. It is obvious that considerable amount of inbreeding occurs in the populations from Kazdağı. Thus, high family heritability estimates may not be expected in such populations as in the case of the current study. Furthermore, if the precautions are not taken to prevent inbreeding, heritability, in turn, genetic gains could be lowered further in the future generations of breeding populations of Anatolian black pine dealing with WSG.

From the genetic correlations between WSG and adaptive seedling traits, it appears that selection of families with high WSG will cause an indirect and negative selection on annual height and diameter increments. An early selection of families with higher WSG may include the families with less height growth and diameter in turn less volume production. The negative correlation between WSG and height growth of families was also observed in later ages in other species (Jayawickkrama *et al.,* 1997; Skroppa and Dietrichson, 1999). Furthermore, selection of families with higher WSG may indirectly select the families with later budset date during growing season (longer growing seasons) since genetic correlation between WSG and BS91 was positive (Rg = 0.25). The families with higher WSG values will be also the ones with later bud set dates and are likely to suffer from early frost damages.

Although provenance (seed stand) or progeny (populations) differences were not significant for WSG in this study, the presence of significant negative correlation between WSG and height growth as well as latitude of seed stands at provenance level suggests that provenance differences for WSG may be expressed in later ages when the late wood production makes up considerable portion of annual wood production. The studies which were with WSG of older materials reported that trees (populations) originating from more northern latitudes had lower WSG value than those originating from more southern latitudes (Jayawickkrama *et al.,* 1997, Skroppa and Dietrichson, 1999).

WSG is not a simple wood characteristic. It is a combination of characteristics such as type of wood, cell size, cell wall ticknes, each of which has strong inheritance pattern. Considering the nature of WSG trait, moderate family heritability estimate and negative genetic correlations between WSG and growth traits in the current study, and expected changes in type of wood production in the mature trees, early selection for WSG in Anatolian black pine (i.e., indirect selection for WSG at mature age) should involve multi-trait index selection. To have maximum economic value with the improvement of WSG, a multi-trait based-selection index should be developed by giving appropriate weight to each trait according to its relative economic importance, its heritability, and genetic and phenotypic correlations between traits. Such index selection for WSG will provide the flexibility to breeders during culling of families (provenances) in later ages when late wood production is pronounced and provenance differences for WSG may be expressed better.

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Molecular Differentiation of Pine Species using a Primer Pair

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Summary

The identification of informative PCR-based markers is difficult in species with large, complex genomes such as conifers. We have isolated and characterized few microsatellite DNA markers from *Pinus resinosa*. The designed pairs resolved DNA variants showing consistent but complex multilocus patterns. A primer pair that allows the unambiguous differentiation of pine species was identified. This primer pair generated nine alleles which were highly informative. In fact, eight of the nine loci identified were polymorphic in trees from different populations. Each of the seven pine species examined had unique one or two locus genotypes, but each also had at least one common allele with *P. resinosa* from which the microsatellite clones were derived. The data set generated with a marker such as this can be of use in phylogenetic studies and the identification of interspecies hybrids.

Key words: Pinus resinosa, Simple sequence repeat, allelic frequency, DNA fingerprinting.

Introduction

The genus *Pinus* includes more than 100 species, more than any other gymnosperm. Rational management and usage of forests should be combined with measures aimed to preserve the biological diversity. This would be facilitated by a good understanding of taxonomy and phylogenetic relationships of pine species. The systematic of the genus Pinus has been extensively studied and has been repeatedly reconstructed. The first classifications were based on morphology of cones, position of resin ducts in the needle, shape of seed wings, intercrossing abilities of the species, pollen characteristics, anatomy of the seeds and needles, and resin compound. A classification which encompasses all well argumented views from other classifications was given by LITTLE and CRISTCHFIELD (1969). This has been for more than 25 years the most widely accepted classification of the genus *Pinus.* The authors summarized the worldwide distribution of the pines (CRISTFIELD and LITTLE, 1966) and presented a detailed intrageneric classification of the genus (LITTLE and CRISTCHFIELD, 1969). With the event of molecular techniques, this classification has been refined.

Comparative studies of chloroplast (cp) DNA variation have shed new light on the phylogenetic structure within the genus *Pinus.* There has been a surge of studies carried out on chloroplast, nuclear, and mitochondrial DNA in recent years, aimed at inferring phylogenetic relationships at intrageneric or higher taxonomic levels (BREMER, 1991; STRAUSS and DOERKSEN, 1990; WANG and SZMIDT, 1993). Phylogenetic analysis of plastid DNA restriction site and rearrangement mutations by KRUPLIN et al. (1996) suggested a number of major revisions to tax-

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