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Altitudinal Variation in *Pinus brutia* TEN. and its Implication in Genetic Conservation and Seed Transfers in Southern Turkey

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Abstract

Seed collections along 2 elevational transects, 1 in the central part and the other on the western edge of the Antalya basin were made to study within- and between- population variation for growth characteristics of *Pinus brutia* TEN. Four common garden experiments were established at different elevations along the western transect. In addition, seed samples from 4 populations along a third elevational transect on the far eastern edge of the basin were collected to perform isoenzyme analyses. Population parameters for 6-year height growth showed that middle elevation populations have better performance and better uniformity than lower- and higher-elevation populations within each of the 4 test sites. They also exhibit higher adaptational plasticity and higher stability under varying environmental conditions. Furthermore, isoenzyme analyses indicated that middle-elevation populations have a higher heterozygosity level and higher numbers of alleles per locus. Our results based on growth and isoenzyme analyses indicate that middle-elevation populations of *P. brutia* present higher genetic variability for *in situ* conservation, and they can serve much wider elevational zones for afforestation and reforestation purposes than the lower and higher elevation populations. Therefore, they should be given high priority in forest tree breeding and selection activities in the region.

Key words: *Pinus brutia*, altitudinal variation, genetic conservation, isozyme analysis, adaptation.

FDC: 165.52; 181.2; 165.3; 232.314; 174.7 *Pinus brutia*; (560).

Introduction

Pinus brutia TEN. is an important forest tree species in Turkey for various economic and ecological reasons. It occupies 3.1 million ha of forest land, which constitutes 15% of the total forest areas in the country (NEYISCI, 1987). About 88% of *P. brutia* forests are located in southern and western Anatolia, mainly in the mountains facing the Mediterranean and Aegean Seas. It grows from sea level up to 1200 m, occasionally to 1400

m elevation in the Taurus Mountains along the Mediterranean. Within its altitudinal and horizontal distribution range, *P. brutia* exhibits considerable variation in various form and growth characteristics (SELIK, 1958; ARBEZ, 1974; ISIK, 1986; ISIK *et al.*, 1987).

In Turkey, 296.000 ha. of forest land were planted with *P. brutia* between 1985 and 1991; yearly average plantations being near 42000 hectares. In terms of plantation areas established in Turkey, areas planted with *P. brutia* constitute about 37% of the total area planted annually (GUNAY and TACENUR, 1993). A large proportion of the seeds used for these plantations are collected from 61 seed stands situated at different localities within the natural range of the species. A relatively small proportion of the seeds are obtained from 48 seed orchards, all of which are of first generation; many have not yet reached commercial stage in seed production. In the Antalya region alone there are 11 seed stands and 9 seed orchards (94.6 ha), as of 1995.

Because of this large planting programme and the high economic importance of the species it can be argued that *Pinus brutia* is the single forest tree species in Turkish forestry that deserves most attention for future selection, breeding and gene conservation purposes. Several questions remains to be answered on genetic architecture across the altitudinal and horizontal distribution range of the species in order to define seed transfer zones, and to determine genetically superior populations for future selection and breeding. Designation of appropriate and adequate areas of seed stands, establishment of seed orchards and determination of seed transfer zones all require additional information on the genetics and adaptability of the species. The purpose of the present study is to make a contribution along these lines. A specific purpose is to illustrate altitudinal variation of certain growth and isoenzyme characteristics of samples from natural populations grown around the Antalya basin in southern Turkey.

Materials and Methods

Genetic Material

Three elevational transects (Tr) – Tr1 in the eastern, Tr2 in the central and Tr3 in the western Antalya basin were taken (Table 1-A). Along the Tr1, seeds from 4 natural populations at different elevations were collected and kept separate by populations. These populations have also been used as seed stands in the region. Isoenzyme analyses reported in this study were based on seeds derived from Tr1. We studied 23 loci belonging to 14 enzyme systems.

In addition to transect 1, a total of 6 populations were sampled from transects 2 and 3, each transect including 3 populations, and each population 10 parent trees. Corresponding populations from each transect are located at similar altitudes (Table 1-A). Open-pollinated seeds were collected from total of 60 parent trees in transects 2 and 3 and kept separate by parent tree. These seeds were first grown in a local nursery, and then planted in 4 common-garden test sites located at different altitudes along the transect 3 in February 1979 (Table 1-B). There were 6 populations, 10 families per population, and 30 half-sibs per family within each test site. Hence, each test site contained 1800 test plants represented by 60 half-sib families, excluding border trees. Total heights (\bar{X}_{ijk}) of trees derived from these open-pollinated, half-sib families at age 6 years were measured and evaluated. Population means at each test site ($=\bar{X}_{ij}$) were calculated, and they were used as the basis for growth parameters in this study. Additional information on the test sites, families and populations can be found in ISIK *et al.* (1987).

Biostatistical Methods

We applied the following equations to estimate various population parameters:

$$\text{Overall Population Mean: } \bar{X}_i = \frac{1}{e} \sum_j \bar{X}_{ij} \quad \text{Eqn. 1}$$

$$\text{Environmental variance: } S_{xi}^2 = \frac{\sum_j (\bar{X}_{ij} - \bar{X}_i)^2}{e - 1} \quad \text{Eqn. 2}$$

$$\text{Mean Coefficient of Variation: } CV_w = \frac{1}{e} \sum_j \left[\frac{S_{100}}{\bar{X}_{ij}} \right] \quad \text{Eqn. 3}$$

where, $S = \sqrt{S^2}$ = Standard deviation of height of population i at site j ; and,

$e = 4$ = Environments (or Test sites);

$$S^2 = \frac{1}{(n_{ij} - 1)} \sum_j (\bar{X}_{ijk} - \bar{X}_{ij})^2$$

$$\text{Response index: } b_j = \frac{\sum_j (\bar{X}_j - \bar{X}_{..})(\bar{X}_{ij} - \bar{X}_i)}{(\bar{X}_j - \bar{X}_{..})^2} \quad \text{Eqn. 4}$$

Combined stability and performance index:

$$CSPi5 = \sum \frac{|r_{ij} - Op|}{e} \quad \text{Eqn. 5}$$

CSPi5 as a stability measure combines stability and performance of a genotype simultaneously into 1 single parameter (HUEHN, 1996).

where: X_{ijk} = Total ht of k th tree of population i at site j .

$i = 1, 2, \dots, p$, $p = 6$; and $j = 1, 2, \dots, e$, $e = 4$;

$p = 6$ = Populations;

$k = 1, 2, \dots, n$, initial $n = 30$;

$n_{ij} = n$ = Number of trees of population i at site j .

\bar{X}_{ij} = Phenotypic value (population mean) of population i at site j .

\bar{X}_i = Overall Population mean (Overall mean of population i).

\bar{X}_j = Environmental value of site j (= Mean of test site j).

$\bar{X}_{..}$ = Overall mean for species.

r_{ij} = Rank of population i at test site j .

Op = Rank of the population that have optimum value of the desired character (In our case $Op = 6$, since it is the rank of the fastest growing population M). [According to non-parametric method suggested by NASSER *et al.* (1994) and HUEHN (1996), highest rank number is given to the best performer, i.e. 6 for M].

Equations 1 and 3 were formulated from SOKAL and ROHLF (1995). Eqn. 2 was taken from BECKER and LEON (1986), and

Table 1. – Information on locations of *Pinus brutia* populations and the common garden test sites used in the study.

Tr	Name	Abr.	Elv., m.	Lat., N	Lon., E
A: Populations used in ecological genetic studies					
1	Kargi	Kr	350	36° 36'	31° 58'
	Guzelbag	G	650	36° 45'	31° 58'
	Urlupelit	U	850	37° 15'	31° 45'
	Eskibag	E	1000	36° 42'	32° 10'
2	Sarilar	S	92	36° 48'	31° 26'
	Murtbeli	M	490	37° 01'	31° 24'
	Kapan	K	933	37° 06'	31° 24'
3	Doyran	D	61	36° 52'	30° 32'
	Buk	B	480	36° 58'	30° 36'
	Hacibekar	H	1033	37° 19'	30° 11'
B: Common garden test sites established to include six populations from trs 2 and 3					
3	Kepez	Kp	90	36° 55'	30° 36'
	Duzlercami	Dz	350	36° 58'	30° 32'
	Buk	Bk	500	36° 59'	30° 26'
	Yenicedere	Yn	850	37° 01'	30° 25'

*) Tr: Elevational Transects (1: Eastern-, 2: Central-, and 3: Western regions of the Antalya basin)

Table 2. – Heights (\bar{X}_{ij} , cm), ranks (R) and % of “successful” families (Fm) of *P. brutia* populations from 2 different transects (Tr), planted at 4 test sites.

Tr	P	Test Sites												Overall	
		Kepez			Duzlercami			Buk			Yenicedere				
		\bar{X}_{ij}	R	Fm	\bar{X}_{ij}	R	Fm	\bar{X}_{ij}	R	Fm	\bar{X}_{ij}	R	Fm	\bar{X}_i	r_i
2	S	142.8 ^b	3	6.7	148.6 ^c	3	0.0	100.3 ^b	5	6.7	76.9 ^{ab}	3	13.3	117.1	3
	M	158.4 ^a	6	53.3	181.2 ^a	6	60.0	112.6 ^a	6	40.0	83.8 ^a	6	33.3	133.8	6
	K	146.6 ^b	5	20.0	164.0 ^b	5	26.7	99.3 ^b	4	26.7	81.6 ^{ab}	5	26.7	122.9	5
3	D	138.9 ^{bc}	2	13.3	148.7 ^c	2	0.0	97.7 ^b	2	13.3	72.8 ^b	1	6.7	114.9	2
	B	143.7 ^b	4	6.7	154.9 ^{bc}	4	13.3	99.2 ^b	3	13.3	79.2 ^{ab}	4	20.0	119.2	4
	H	133.1 ^c	1	0.0	146.8 ^c	1	0.0	90.6 ^c	1	0.0	74.0 ^b	2	0.0	111.1	1
$\bar{X}_{.j}$		143.9 ^b	100.0		157.4 ^a	100.0		99.8 ^c	100.0		78.0 ^d	100.0		$\bar{X}_{..}$:119.8	

*) S: Sarilar, M: Murtbeli, K: Kapan, D: Doyran, B: Buk, H: Hacibekar.

\bar{X}_{ij} : Population means with the same letters within a test site are not significantly different (DUNCAN's MRT, 5%).

\bar{X}_j : Site means with the same letters are not significantly different (DUNCAN's MRT, 5%).

\bar{X} : Overall mean for the species.

R: Height rank of a population in a given test site (Tallest population was given the highest rank)

Fm: Proportion of families that are included within the top 25% of all the families within each test site.

\bar{X}_i and r_i : Overall means-and rank of means-, respectively, of *i*th population.

eqn. 5 from HUEHN (1996). Response index in eqn. 4 was developed by FINLAY and WILKINSON (1963). We used SAS/STAT (1987) and our programs for biostatistical evaluations.

Isoenzyme data obtained from populations on the Tr1 were evaluated in the following way: When the frequency of the most common allele of *i*th locus is $\leq 95\%$, then, that locus is considered polymorphic (an index which is called 95% criterion). Polymorphism level (P, %) of a given population is calculated as the proportion of polymorphic loci to the total numbers of loci studied. Heterozygosity level (H) for each population was found by BIOSYS program (version 1.7) by SWOFFORD and SALENDER (1981). Further details on the techniques and methods of isoenzyme analyses related to this study are described in KARA (1996).

Results and Discussion

Mean heights at age 6 years growth (\bar{X}_{ij}) of each population are presented by test sites in Table 2. An earlier evaluation showed that there were significant differences among populations (*p*), among the families within populations [*f*(*p*)]; and also significant population x environment and families in population x environment interactions. Estimated components of variance from the common garden test sites indicated that about 4% of variation is due to differences among populations, 3% due to families within populations, and 92% due to within family variation (ISIK *et al.*, 1987). In this study, the emphasis will mainly be on the patterns of altitudinal variation in various growth parameters and diversity indices.

When total height growth is considered, middle (B and M) and upper-middle (K) elevation populations generally exhibited better growth than higher and lower elevation populations (Fig. 1-A). M from Murtbeli in transect 2 (el. 490 m) was the most successful group at all 4 test sites. K from Kapan in transect 2 (el. 932 m) was the second best grower at 3 test sites (Table 2). B, another middle elevation group from Buk (el. 480 m), was the third best grower, again at 3 test sites. Relative differences among the populations decreased as the elevation of the test sites increased (Fig. 1-B).

The best-grown 25% of the families (i.e., 15 best-grown = “successful” families out of 60) were determined within each

test site (Table 2). At the low altitude Kepez test site, for example, 53.3% of the best-grown families came from M (mid-elevation). K (upper-middle elevation) contributed 20%, D (low elevation) 13%, B (mid-elevation) and S (low elevation), each only 6.7% to the best-grown families at Kepez (Fig. 2). At the Duzlercami test site (mid-elevation), the contributions of middle- and upper-middle elevation populations were more conspicuous: i.e., M is the highest with 60%, K is the second with 26.7% and B is the third, with 13.3% contributions to the “successful” families. Lower elevation (D and S) and higher elevation (H) populations were not represented at all in the “successful” group at Duzlercami. Similarly, populations M, K and B were represented with much higher numbers among the top 25% best performing families both at Yenicedere and Buk test sites (Fig. 2).

Overall means of each population over all environments provides a comparative measure of performance of individual populations. We calculated these values (eqn. 1) and plotted against elevations of origin by transect (Fig. 3-A). Middle elevation populations from the both transects (Trs. 2 and 3) exhibited better performances than either the lower or the higher elevation populations of the respective transects. In addition, there were major differences between transects in height growth, with much better growth from populations in transect 2. The reason for this is not clearly known.

Environmental variance (eqn. 2) provides a measure of uniformity of populations across the environments. A population that has the lowest S^2_{xi} value would have the highest uniformity across the environments (Table 3). When the environments under consideration are similar, such a uniformity among trees is a desirable characteristics from the operational forestry viewpoint. However, as it is the case in most forest lands in Turkey, the forest environments are not homogeneous, and they do change both in space and time even within short distances. It is then desirable that populations to be planted on such heterogeneous environments should exhibit certain degree of plasticity to better fit to changing environmental conditions. Populations that have ability to exploit such heterogeneous environments could be considered to have higher adaptational plasticity. In the present study, we observ-

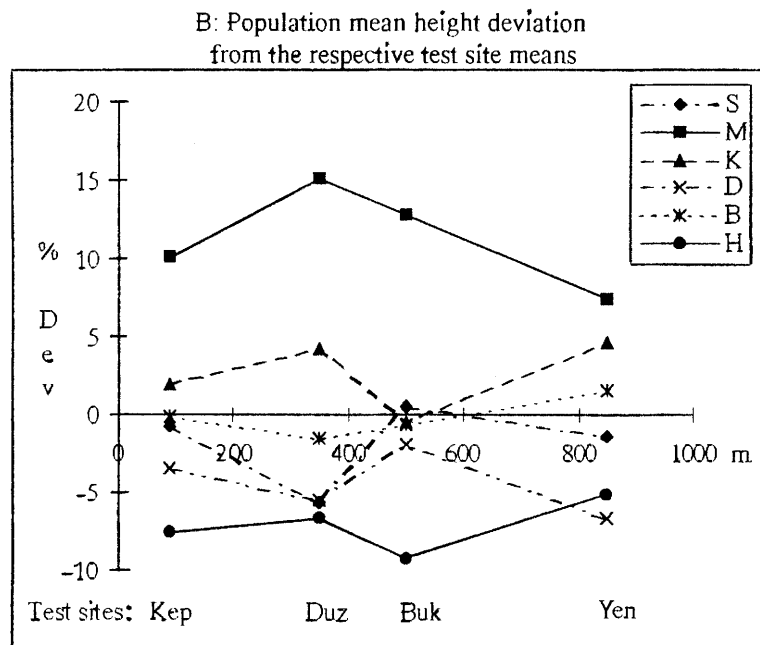
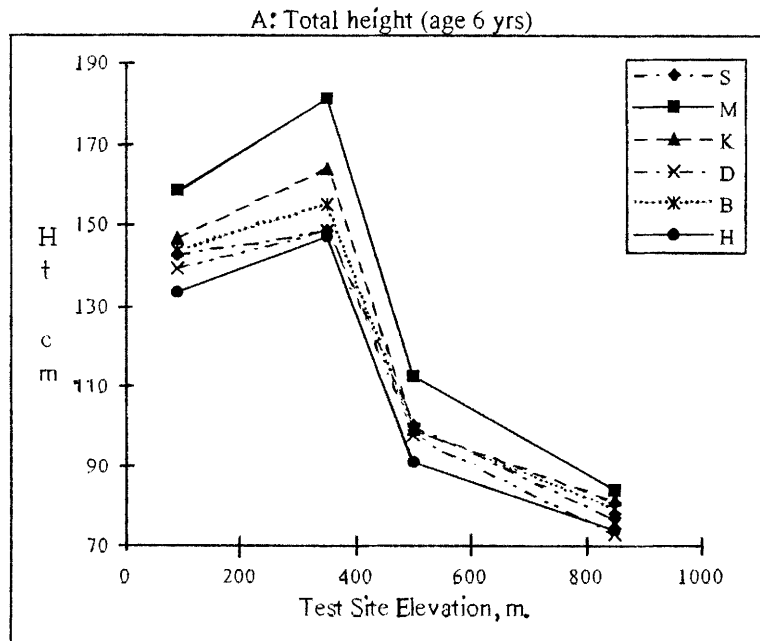


Fig. 1. - Comparisons of mean heights (age 6 years) of 6 *Pinus brutia* populations at 4 test sites.

ed that mid-elevation populations (M, B) within each transect, and upper-middle elevation population K, from Tr2 - as reflected in their relatively high environmental variances - have higher adaptational plasticity than the lower and higher elevation populations (Fig. 3-B).

FINLAY and WILKINSON (1963) developed an index that measures adaptation and sensitivity of a given genotype to environments. Using this approach, a linear regression of phenotypic value (i.e., X_{ij}) on the environmental value (i.e. site mean, \bar{X}_j) was calculated (eqn. 4) for each population. The regression coefficient (b_i or slope) thus obtained will show the "response" or "sensitivity" to changing environments (Table 3). A population would have average response if $b_i=1.0$ (= the

expected b_i value); it would be sensitive (or responsive) to site changes if $b_i > 1.0$. The population would be less sensitive when $0 < b_i < 1.0$. Mid-elevation populations within each transect (M from Tr 2 and B from Tr 3) had higher b_i values, and therefore, they were the most sensitive populations to changing environments among the members of their respective transects (Fig. 3-C). In other words, they had greater responses to changes in environmental quality. This property of mid-elevation populations, combined with their relatively better growth performance, make these populations more adaptable than the other populations. In addition, population K from upper-middle elevations, also had a high b_i value in addition to its high performance, which distinguishes it also as a relatively adaptable population. Significant positive correlations between

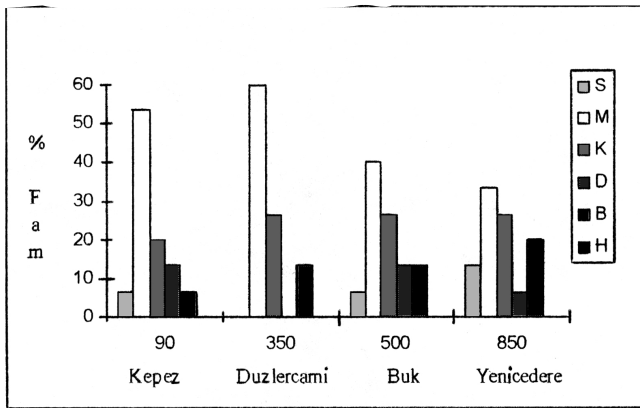


Fig. 2. – Proportion of the most successful families from each population included within the top 25% of the families planted at each test site.

overall-phenotypic-values and environmental variance, and also between overall-phenotypic-values and overall rank order (in both cases $r = 0.96$, $P < 0.01$) indicate that better growth performance associates with higher adaptational plasticity and better response to environmental changes. There were also very high positive correlations between environmental variance and response index, b_i ($r = 0.99$, $P < 0.001$).

Coefficient of determination (r^2), as proposed by PINTHUS (1973), is also a stability related statistics obtained by regression approach as b_i above. Index b_i may also be used as a stability parameter as long as the coefficient of determination and the overall performance of a genotype are also taken into consideration (FINLAY and WILKINSON, 1963; WESTCOTT, 1986; MCKEAND *et al.*, 1990). That is, a genotype would have average stability if $b_i \approx 1.0$, and if r^2 are high. In the present study, r^2 values were quite high for all populations (Table 3). B was the best performer of its transect, and at the same time had b_i close to 1.0, i.e. average stability. K is also considered to have average stability on these criteria. On the other hand, – although it exhibited the highest growth performance, and at the same time had high r^2 value – M showed a high b_i response index. Considering the b_i value by itself, M with $b_i = 1.18$ could have been detected as an unstable population, which implies high degree of GxE (genotype x environment) interaction (SHELBOURNE, 1972). On this criterion alone, M would have been regarded as an undesirable population. However, this is not the case as the following arguments indicate. First, several studies report that b_i , by itself, is not a good measure of stability (BREESSE, 1969; POWELL *et al.*, 1986; BECKER and LEÓN, 1988). Second, HUEHN (1996) argues that, a plant or animal breeder is not interested in GxE interaction *per se*, but (s)he is interested in whether the best genotype in any one environment is still the best in another environment. As seen in table 2, population M was the best performer, and constantly had the similar rank of 6 at all environments (Fig. 1). In other words, M was "very stable" population, since it is the best grower and had the constant rank of 6 at all environments. Further, according to the rank-based stability parameter developed by NASSAR *et al.* (1994), M has the smallest CSPi5, and therefore, is the most stable genotype (Table 3). On this criterion, the order of populations from the most to the least desirable levels were: M, K, B, S, D and H (Fig. 4-A). In our study, populations that had better growth also had better stability (*i.e.*, smaller CSPi5 value) ($r = -0.96$, $P < 0.01$). M showed above-average heights at all sites, which indicates that it has general adaptability. At

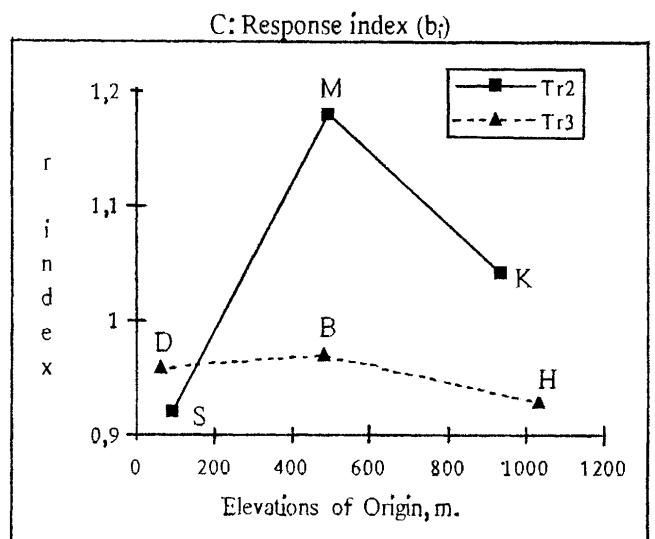
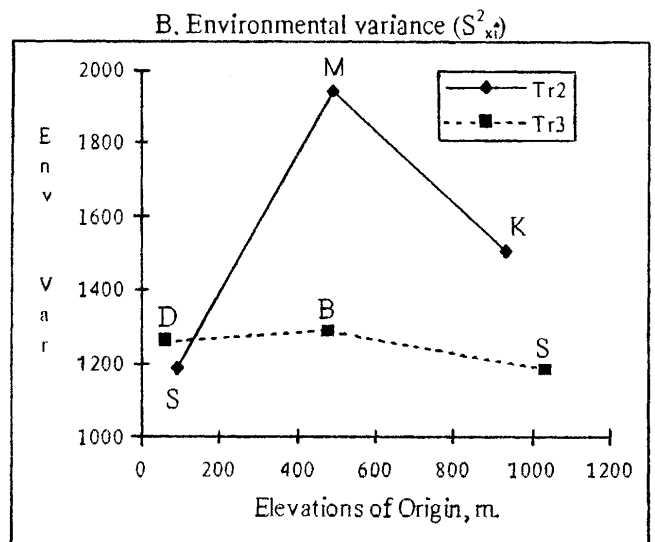
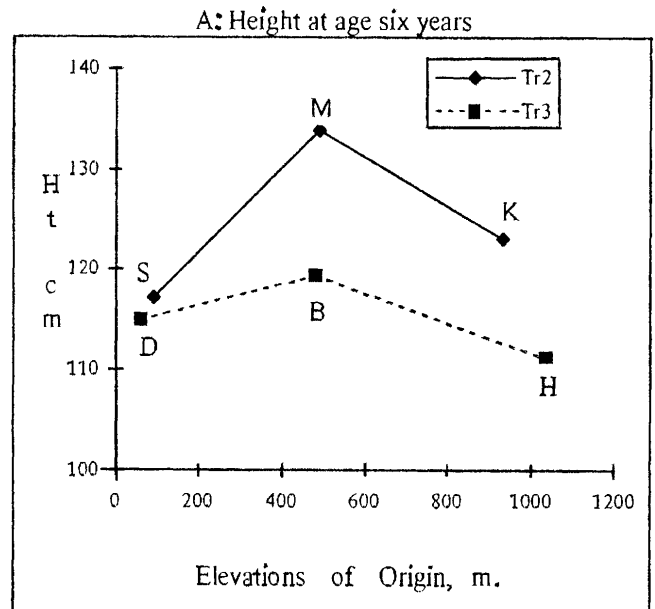


Fig. 3. – Three growth-related adaptability parameters plotted against elevations of origin of *Pinus brutia* populations coming from 2 elevational transects (Tr).

Table 3. – Six adaptability [†]) parameters of *Pinus brutia* populations from different elevational zones.

Tr*	Pop*	\bar{X}_i	S^2_{xi}	b_i	r^2	CSP _i 5	CVw
2	S	117.1	1184	0.92	0.988	2.75	30.0
	M	133.8	1942	1.18	0.993	0.00	27.7
	K	122.9	1504	1.04	0.994	1.25	28.8
3	D	114.5	1262	0.96	0.995	4.00	29.3
	B	119.2	1292	0.97	0.999	2.25	28.5
	H	111.1	1185	0.93	0.997	4.75	27.9

* See table 1 for additional information on transects and the populations

[†]) r^2 : Coefficient of determination

b_i : Response index

\bar{X}_i : Overall population mean

S^2_{xi} : Environmental variance

CSP_i 5: Combined stability and performance index

CVw: Mean coefficient of variation

the same time, it is responsive to environmental changes with greater specificity of adaptability to productive environments, which implies better response to intensive silvicultural manipulations.

Uniformity of genotypes within a given management unit (i.e., on a unit of land under similar treatments and similar

environmental conditions) is a valuable characteristic, especially during cultural manipulations, and for harvesting and marketing. As a measure of within-site uniformity for each population, we first calculated the CV of each population within each test site; and then calculated the mean CV (notated as CVw) for each population (eqn. 3). CVw compares overall relative variability levels of different populations having different means. A genotype with low CVw is less variable (or more uniform) than those with higher CVw values. In the present study, we found that M had the lowest CVw value (Table 3). In other words, on the average, M is the most uniform population within a given test site. H, B and K were also relatively uniform populations within the test sites (Fig. 4-B).

In addition to the growth-related morphological data presented above, we also obtained molecular data from populations of the Tr1 (Table 4). Results based on isoenzyme analyses showed that both average allele numbers per locus and heterozygosity level were relatively higher among the middle and upper-middle elevation populations than the lower and higher elevation populations (Fig. 5). Moreover, polymorphism levels of middle and higher elevation populations were larger than in the lower elevation (Kargi) population.

Conclusion

Pinus brutia grows from sea level up to 1200 m, occasionally to 1400 m on the Taurus Mountains. The results of our study based on both growth and isoenzyme analyses suggest that middle elevation populations (from approximately between 400 m and 900 m from sea level) perform better, represent higher genetic variability and have greater adaptability than the populations from much lower and/or higher elevations. Early observations at age 6 also indicated that mid-elevation populations are generally of much better stem straightness than those from lower elevations. Therefore, the mid-elevation populations can serve much wider elevational zones for afforestation and reforestation purposes, and should be given a high priority in forest tree breeding, selection and for *in situ* conservation activities in the region.

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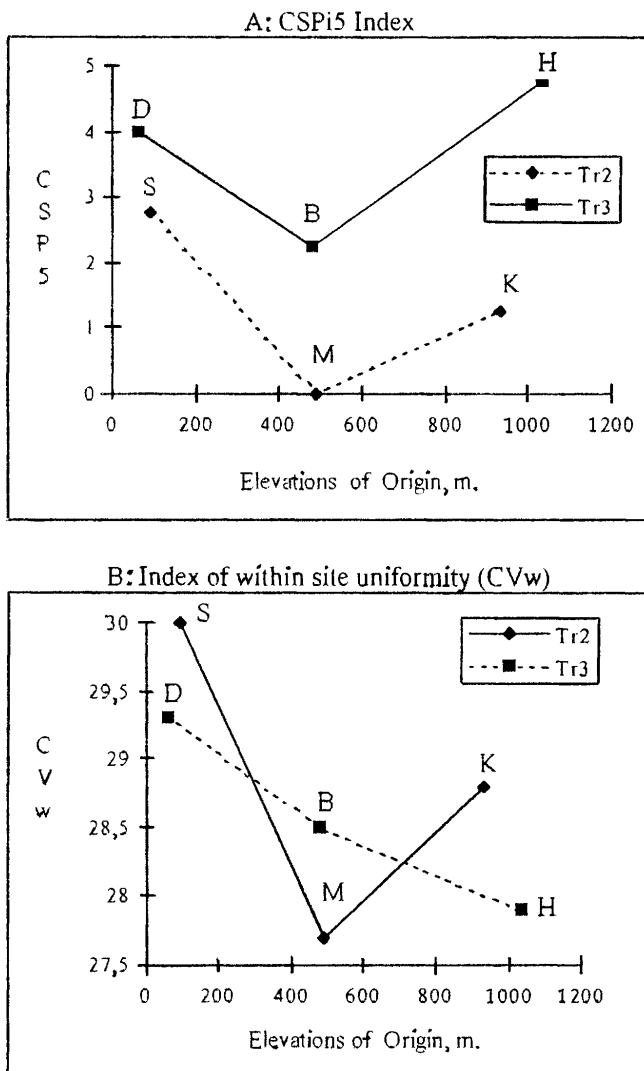


Fig. 4. – Stability (Fig. A) and uniformity (Fig. B) indices plotted against elevations of origin of *Pinus brutia* populations coming from 2 elevational transects (Tr).

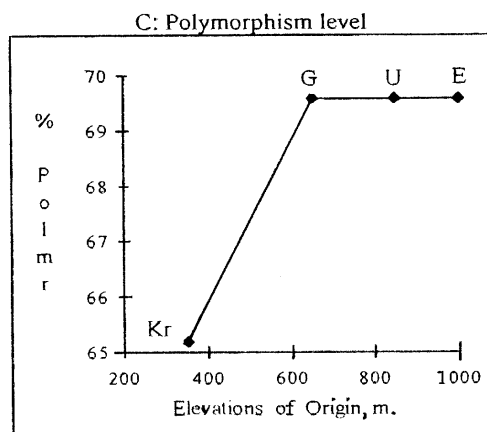
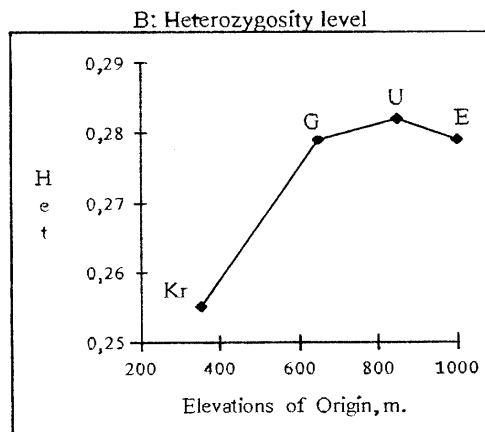
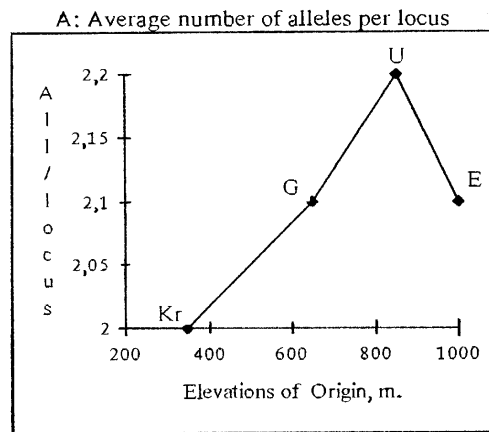


Fig. 5. – Three within-population diversity indices (based on isoenzyme analyses on 23 loci) plotted against elevations of origin of 4 populations coming from the 1st elevational transect.

Table 4. – Three diversity parameters of *Pinus brutia* populations from different elevational zones.

Tr*	Pop*	AA/L†	H**	P (%)†
1	Kr	2.0	0.255	65.2
	G	2.1	0.279	69.6
	U	2.2	0.282	69.6
	E	2.1	0.279	69.6

* See table 1 for additional information on transects and the populations

†AA/L: Average allele per locus

** H: Heterozygosity level (expected)

†P: Polymorphism level (%)

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Literature

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Genetic Diversity and Structure of Natural Populations of *Pinus thunbergii* in Korea

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Abstract

Thirteen natural populations of *Pinus thunbergii* were investigated by starch-gel electrophoresis. For 27 loci from 17 enzyme systems, the percentage of polymorphic loci (P ; 99% level), the number of alleles per locus (A), the observed (H_o) and expected (H_e) heterozygosities and the effective number of alleles (A_e) were 71.3%, 2.2, 0.214, 0.212 and 1.443, respectively. 13 natural populations of *P. thunbergii* seemed to be in equilibrium of HARDY-WEINBERG expectation. Of the total variability, more than 95% was within populations. The mean value of NEI’s (1978) unbiased genetic distance ($D = 0.008$) confirmed that the variation among populations was low. Weak correlation between genetic distance and geographic distance

was found. Cluster analysis showed that the populations sampled from the east-coast region, except Samcheok, were distinguished from other populations in the west-coast and south-coast region.

Key words: *Pinus thunbergii*, natural populations, allozymes, genetic variation.

FDC: 165.3; 165.5; 174.7 *Pinus thunbergii*; (519.5).

Introduction

As its Korean name ‘Haesong’ means the maritime pine, *Pinus thunbergii* PARL. grows along the coastlines in South Korea. It also occurs along the coasts of three main islands of Japan (MIROV, 1967). As we consider its natural range, *P. thunbergii* seems to have a good resistance to salt and wind, consequently it has been partially planted for windbreak along the sea coasts in South Korea. It is generally known that the western boundary line of natural distribution reaches to Namyang, Kyunggi province (37°20’ N. L.) and the eastern boundary line to Uljin, Kyungpook province (37° N. L.) (LEE, 1989).

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