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Genetic Differentiation Among and Within Natural and Planted *Cupressus sempervirens* L. Eastern Mediterranean Populations[1])

By L. KOROL[2]), N. KARA[3]), K. ISIK[3]) and G. SCHILLER[2])[4])

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Abstract

The aim of the study was to extend our knowledge of the geographic patterns of genetic variation of *Cupressus sempervirens* L., and to try to relate the earliest plantations of *C. sempervirens* in Israel to its ultimate seed origins. Seeds of 22 populations, most of them of *C. sempervirens* var. *horizontalis*, were used for the analysis of the genetic diversity within and among East Mediterranean populations. Bulk seed lots from 13 populations, and single-tree seed collections, representing about 30 trees from each of the populations from Cyprus, Syria and Turkey, and from 267 trees from 20 Israeli plantations were available. Horizontal starch gel electrophoresis was used to resolve allele patterns in 22 loci encoding 13 enzyme systems. The mean number of alleles per locus was 1.7; the over all percentage of polymorphic loci was 41.7% (S.E. ± 1.1). The over all mean observed heterozygosity and the expected heterozygosity, *i.e.*, genetic diversity within populations – were 0.149 (S.E. ± 0.021) and 0.181 (S.E. ± 0.03), respectively; and the over all mean total genetic diversity among the natural populations was 0.192 (S.E. ± 0.032). The fixation indices, F_{it}, F_{is}, F_{st} and G_{st} for each polymorphic locus, over all the populations, are 0.180, 0.333, 0.187 and 0.049, respectively. The phylogenetic tree enabled us to define three main groups: a north-eastern Mediterranean group which includes the populations from central Mediterranean Turkey, Syria and Iran; a low-altitude east Aegean group which includes the populations of Kos, 2 Turkish population on the shores of the Aegean Sea together with populations on the island of Samos; a third group which includes populations from Crete, Cyprus, Rhodes, Jordan and plantations in Israel. The results imply that plantations of *Cupressus sempervirens* in Israel probably originated from seed material imported from Crete, Cyprus, Rhodes and Jordan.

Key words: Cupressus sempervirens, genetic diversity, populations structure, polymorphism, isoenzymes.

FDC: 165.3; 174.7 *Cupressus sempervirens;* (4-015).

Introduction

Natural relict stands of *Cupressus sempervirens* L. (Italian cypress or Mediterranean cypress) are growing in Iran, Syria, Jordan, Lebanon and Libya and on the Aegean islands, Kos, Samos and Rhodes, and there are larger forests of this species in Turkey, Cyprus and Crete (PAVARI, 1934; ZOHARY, 1973). This tree species grows under various Mediterranean climates (EMBERGER *et al*., 1963), it grows from sea level up to 2000 m or more, and on a variety of bedrock formations and soils types, and therefore, in a variety of plant associations (ZOHARY, 1973). Natural relicts and forests of *C. sempervirens* are composed of var. *horizontalis* (MILL.) GORDON, which grows spreading branches. Single specimens of var. *pyramidalis* NYMAN (var. *stricta* AITON), the erect form which has a columnar or narrowly conical habit occur rarely (BOLOTIN, 1964). The erect form was most probably selected for ornamental and short rotation planting (MAKKONEN, 1968), and spread all around the Mediterranean in ancient times. Unfortunately, no paleobotanical evidence is known which could support the division of this species into the 2 most widespread varieties and help us to identify the exact range of distribution in the past.

Due to the importance of *C. sempervirens* L. for afforestation, and for ornamental and windbreak plantings all around the Mediterranean region and in other parts of the world, where this species has been introduced, there is considerable interest in the geographic patterns of genetic variation. However, so far, quantitative information on the gene pool of natural relict stands of *C. sempervirens* L. is available only for several Greek populations (PAPAGEORGIOU *et al.,* 1994, 1995); information is lacking on the gene pool of other geographic regions where this species grows naturally.

Contrary to a previously published opinion (LIPHSCHITZ and BIGER, 1989), natural occurrences of this species, that could be used as seed sources, are not known to have existed in Cis Jordan during recent centuries, although this area is included within the phytogeographical range of *C. sempervirens* L. (ZOHARY, 1973). Consequently, the seed source and/or the origin of trees that were planted in the last 2 centuries in this area is most likely foreign.

Therefore, the aims of the present study were twofold: (i) to extend our knowledge of the geographic patterns of genetic

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[2]) Department of Agronomy and Natural Resources, Forestry Section, Agricultural Research Organization, the Volcani Center, P. O. Box 6, Bet Dagan, 50250 Israel.

[3]) Faculty of Arts and Sciences, Department of Biology, Akdeniz University, 07058 Antalya, Turkey.

[4]) To whom correspondence should be addressed.

variation of this species and, (ii) to relate *C. sempervirens* L. var. *horizontalis* and var. *pyramidalis* growing in several plantations in Israel to possible seed origins.

Material and Methods

Seed

Bulked seed material and single-tree seed collections from more then 30 trees per provenance from natural stands and plantations was obtained from different provenances (*Table 1*) with the help of the Forestry Departments of Greece and Cyprus. Prof. PANETSOS of the Laboratory of Forest Genetics, Aristotelian University of Thessaloniki, Greece, provided several seed collections from the Mediterranean Agronomic Institute at Chania, Crete. Dr. OSMAN TASKIN, of the Ministry of Forestry of Turkey, helped us to obtain seed collections from 3 natural relict populations in Turkey. In Israel, single-tree seed collections were obtained from 267 trees distributed among the oldest 20 plantations in the country; var. *horizontalis* and var. *pyramidalis* growing mixed in the 20 plantations. Furthermore, seeds were collected at the Mt. Scopus botanical garden, from trees which are known to have originated from the vicinity of Tadmor in Syria (Syrian provenance). Bulked seed material was obtained from Iran and from Jordan.

Table 1. – Geographic origin of *Cupressus sempervirens* L. seed samples.

Country	Provenance	Longitude	Latitude	Altitude	Crown form
		Relict forests			
Cyprus*	Phasouri	–	–	–	H
Cyprus*	Akamas	–	–	–	H
Cyprus*	Athalassa	–	–	–	H
Greece (Crete)	Askifu	–	–	800	H
Greece (Crete)	Apokoronu	24°12'	35°23'	273	H
Greece (Crete)	Anapoli	–	–	–	H
Greece (Crete)	Zourva	–	–	450	H
Greece (Kos)	Asphendio	27°13'	36°31'	–	H
Greece (Kos)	Kos-1	–	–	–	H
Greece (Rhodes)	Prophitis Ilias	27°54'	36°14'	–	H?
Greece (Rhodes)	Rhodes-1	–	–	–	H-P
Greece (Samos)	Samos-1	–	–	–	H
Greece (Samos)	Kosmadei	26°40'	37°46'	560	H
Iran	–	–	–	–	H
Jordan	–	Jerash	–	–	H
Syria*	Mt. Scopus**	–	–	–	H
Turkey*	Beskonak	31°36'	37°19'	600	H
Turkey*	Datca	27°35'	36°45'	250	H
Turkey*	Fethiye	29°15'	36°40'	600	H
		Plantations			
Greece (Peloponnese)	Mystras	22°12'	37°17'	–	H-P
Israel*	–	–	–	–	P
Israel*	–	–	–	–	H

*) Single tree seed collection; Crown form: H = var. *horizontalis*; P = var. *pyramidalis*.
**) A plantation growing in the Botanical garden on Mt Scopus, Israel, established from seeds collected at the vicinitiy of Tadmore in Syria (39°30' long. E., 35°00' Lat. N.) by the late Prof. MICHAEL ZOHARY.

Electrophoretic analysis

For the analyses the seeds were germinated on moistened Whatman N3 filter paper, in Petri dishes at 20 °C.

Horizontal starch gel electrophoresis was used to analyze allele frequency patterns in 22 loci encoding 13 enzyme systems. The haploid megagametophyte and the diploid perisperm were homogenized (RADDI *et al*., 1990; PAPAGEORGIOU *et al*., 1993) in a grinding plate (KELLEY and ADAMS, 1977) together with 35 µl of 0.2M phosphate buffer pH 7.5 (CONKLE *et al*., 1982), 0.1% Triton x-100, 1% BSA and 0.1% DTT. The liquid fraction from the macerated tissues was analyzed simultaneously in 4 gel buffer systems:

System I, Gel buffer: 0.02M tris, 0.02M boric acid, 0.002M EDTA, pH 8.4. Electrode buffer: 0.2M tris, 0.2M boric acid, 0.002M EDTA, pH 8.4. Enzyme systems analyzed: phosphoglucose isomerase (Pgi), phosphoglucomutase (Pgm), menadione reductase (Mnr).

System II, Gel buffer: 0.01M tris, 0.005M citric acid, pH 8.8. Electrode buffer: 0.05M NaOH, 0.3M boric acid pH 8.0. Enzyme systems analyzed: glutamate dehydrogenase (Gdh), glutamate-oxaloacetate (Got), glucose-6-phosphate dehydrogenase (G6pd), catalase (Cat), superoxide dismutase (Sod).

System III, Gel buffer 0.002M citric acid adjusted with morpholine [N-(3-aminopropyl)] to pH 6.1. Electrode buffer: 0.04M citric acid adjusted with morpholine [N-(3-aminopropyl)] to pH 6.1. Enzyme systems analyzed: aconitase (Aco), isocitric dehydrogenase (Idh), 6-phosphogluconate dehydrogenase (6Pgd), shikimate dehydrogenase (Skdh).

System IV, Gel buffer: 0.002M citric acid adjusted with morpholine to pH 8.3. Electrode buffer: 0.04M citric acid adjusted with morpholine to pH 8.3. Enzyme systems analyzed: malic dehydrogenase (Mdh), alcohol dehydrogenase (Adh).

Gels were sliced and stained for each enzyme system according to CONKLE *et al*. (1982).

Statistics

Calculations of parameters of intra- and interpopulation genetic diversity (mean sample size per locus, mean number of alleles per locus, percentage of loci that were polymorphic, mean heterozygosity expected from HARDY-WEINBERG proportions), estimation of genetic differentiation and genetic distances, clustering and construction of dendrograms, were done with the IBM PC version 1.7 of the BIOSYS-1 program (SWOFFORD and SELANDER, 1981); and by the GeneStat - PC version 3.31.

Results

Allele frequencies varied significantly among populations, geographic regions, and varieties, and between natural stands and plantations. Of the 13 enzyme systems analyzed, 3 (23%) were monomorphic: Cat, Sod and Skdh. Among the other 10 enzyme systems, 6 of the 18 loci identified were monomorphic. The enzyme systems and polymorphic loci were: Aco, Gdh, Got_3, G6pd, Idh_2, Mdh_3 and Mdh_4, Mnr, Pgi_2, Pgm_1, $6Pgd_1$ and $6Pgd_2$.

Allozyme variation within the populations analyzed is presented in *table 2*. The over all mean number of alleles per locus was 1.7 ± 0.02 (range from 1.5 to 1.9); the over all mean percentage of polymorphic loci was 41.7 ± 1.1 (range from 27.3 to 50.0); and the over all mean expected heterozygosity of natural populations (excluding plantations) was 0.181 ± 0.021 (range from 0.134 to 0.210). *Table 2* also shows that the observed and/or expected heterozygosity of planted var. *horizontalis* and *pyramidalis* in Israel are relatively high in comparison with the observed and/or expected heterozygosity of planted population at Mystras on the Peloponnese and in comparison with the natural stands in the various geographic regions. Observed and/or expected heterozygosity of *C. sempervirens* growing on the islands of Crete, Samos and Kos is relatively low in comparison with that of the populations

in Turkey, Syria and Cyprus. The observed and expected heterozygosity was highest in the Beskonak population in the central Mediterranean Taurus Mountains, it declined in progressing from there to Syria, Jordan and Iran to the southeast and to the west.

Table 2. – Allozyme variation within 22 populations of *C. sempervirens* L..

Country	Population	N	A	P%	Ho	He
Relict forests						
Cyprus	Phasouri	123.3	1.8 (0.2)	40.9	0.116 (.034)	0.206 (.056)
Cyprus	Akamas	132.3	1.7 (0.2)	40.9	0.109 (.036)	0.176 (.050)
Cyprus	Athalassa	125.8	1.7 (0.2)	40.9	0.133 (.041)	0.182 (.050)
Greece (Crete)	Askifu	26.0	1.6 (0.2)	45.5	0.140 (.052)	0.170 (.045)
Greece (Crete)	Apokoronu	105.6	1.7 (0.2)	40.9	0.167 (.053)	0.170 (.045)
Greece (Crete)	Anapoli	80.8	1.7 (0.2)	40.9	0.154 (.047)	0.179 (.049)
Greece (Crete)	Zourva	36.8	1.6 (0.2)	40.9	0.130 (.047)	0.134 (.041)
Greece (Kos)	Asphendio	116.8	1.6 (0.2)	27.3	0.174 (.063)	0.150 (.048)
Greece (Kos)	Kos-1	24.0	1.5 (0.1)	31.8	0.139 (.045)	0.162 (.051)
Greece (Rhodes)	Prop. Ilias	179.3	1.8 (0.2)	40.9	0.164 (.048)	0.195 (.052)
Greece (Rhodes)	Rhodes-1	87.8	1.7 (0.2)	45.5	0.163 (.051)	0.205 (.052)
Greece (Samos)	Samos-1	66.3	1.7 (0.2)	45.5	0.147 (.053)	0.186 (.047)
Greece (Samos)	Kosmadei	144.3	1.7 (0.2)	40.9	0.147 (.050)	0.163 (.045)
Iran	Iran	119.1	1.8 (0.2)	45.5	0.137 (.046)	0.165 (.047)
Jordan	Jeresh	138.6	1.6 (0.2)	36.4	0.148 (.047)	0.195 (.056)
Syria	Mt. Scopus	18.0	1.7 (0.2)	45.5	0.174 (.049)	0.200 (.051)
Turkey	Beskonak	28.0	1.8 (0.2)	45.5	0.201 (.054)	0.210 (.054)
Turkey	Datca	21.9	1.7 (0.2)	45.5	0.157 (.045)	0.205 (.051)
Turky	Fethiye	25.2	1.9 (0.2)	45.5	0.152 (.048)	0.191 (.052)
Plantations						
Greece (Peloponnese)						
	Mystras	177.5	1.6 (0.2)	36.4	0.088 (.039)	0.119 (.043)
Israel	(var. pyr.)*	135.9	1.8 (0.2)	50.0	0.166 (.041)	0.235 (.055)
Israel	(var. hor.)*	66.9	1.7 (0.2)	45.5	0.171 (.046)	0.236 (.058)

*) pyr. = var. *pyramidalis*; hor. = var. *horizontalis*;
N = Mean sample size per locus; A = Mean number of alleles per locus; P% = Percentage of polymorphic loci; Ho = Observed heterozygosity; He = Expected heterozygosity.
Numbers in parantheses = ± SE

Table 3. – Genetic diversity among populations and regions of *C. sempervirens* L..

Country and Regions	Number of Populations	Ht	Hs	Gst
Cyprus	3	0.202 (0.055)	0.199 (0.052)	0.058
Greece				
Crete	4	0.196 (0.048)	0.165 (0.042)	0.158
Kos	2	0.184 (0.049)	0.154 (0.046)	0.162
Peloponnese	1	0.119 (0.043)	0.119 (0.043)	---
Rhodes	2	0.205 (0.052)	0.199 (0.051)	0.032
Samos	2	0.188 (0.047)	0.180 (0.044)	0.045
Iran	1	0.158 (0.044)	0.158 (0.044)	---
Israel	2	0.237 (0.057)	0.234 (0.056)	0.012
Jordan	1	0.194(0.055)	0.194 (0.055)	---
Syria	1	0.200(0.051)	0.200 (0.051)	---
Turkey	3	0.213 (0.055)	0.197 (0.047)	0.150
Mean		0.192	0.181	0.056
S.D.		0.032	0.031	0.067

H_t = Total genetic diversity in all populations of a region.
H_s = Intrapopulation genetic diversity.
G_{st} = Proportion of genetic diversity residing among populations within a region. Numbers in parentheses = S.E.

Parameters of genetic diversity are shown in *table 3*. Total genetic diversity (H_t) among all the natural populations was highest in Turkey (0.231) and lowest in Iran (0.158). The highest total diversity was found in the Israeli planted population (0.237). Intrapopulation genetic diversity (H_s) in natural populations was highest (0.200) in the population originated from the vicinity of Tadmor in central Syria which grows at Mt. Scopus in Jerusalem, and the lowest (0.154) was found in those on the island of Kos. In planted populations, intrapopulation genetic diversity (H_s) was highest in Israeli plantations (0.234) and lowest in the Mystras population (0.119). The proportion of the total diversity among populations (G_{St}) could be measured only for geographic areas represented by more than one population; it was high in Turkey, Kos and Crete, and much lower in Cyprus, Rhodes, Samos and Israel; its mean value was only 0.056.

A dendrogram, based on the genetic distances among populations under investigation, is present in *figure1*. Three main groups were distinguished: at the bottom (in the figure) consists of 6 populations; 2 from the West Mediterranean coastal region of Turkey (Datcha and Fethiye), the populations on the islands of Kos (Kos-1, Asphendio) and 2 populations from Crete. The 2 populations of Samos (Samos-1, Kosmadei) separated very early from the second branch, near its root but they are closer to the lower branch. The second main branch contains the three populations from Cyprus, two from Rhodes, that from Jordan, 2 from Crete and the 2 assembled populations from Israel, var. *horizontalis* and var. *pyramidalis*. The third branch includes the Iranian and Syrian populations and the Turkish one from Beskonak and the plantation at Mystras.

Table 4 presents the F statistics, F_{it}, F_{is}, F_{st} for each polymorphic locus, over all populations. The degree of inbreeding within populations (F_{is}) ranges from –0.368 at the

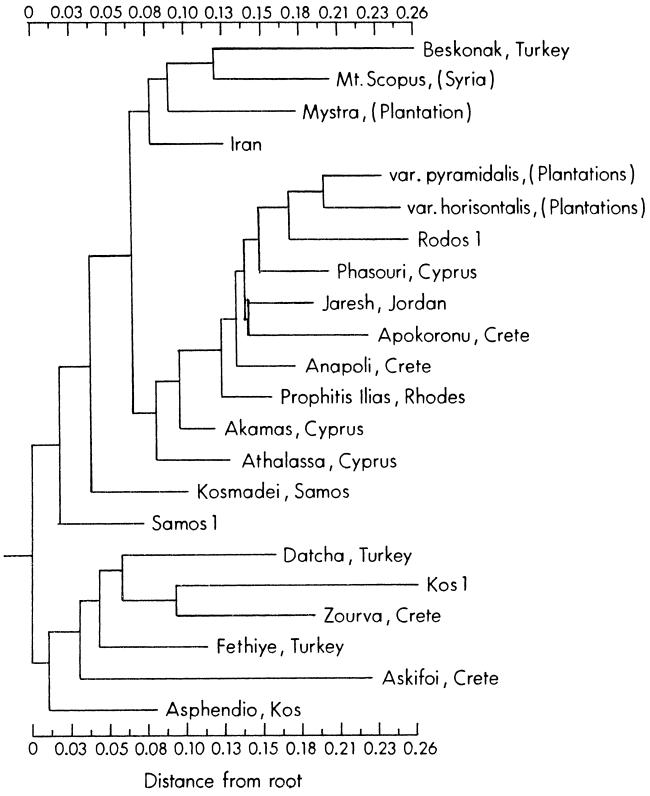

Figure 1. – Phylogenetic tree for *Cupressus sempervirens* L. produced with the WAGNER distance procedure.

Mdh_3 locus to 0.570 at the Gdh locus. The weighted mean over all loci and populations is 0.180, *i.e.*, there is a deficiency of 18% in heterozygotes relative to HARDY-WEINBERG expectations. Variance among populations, in allele frequencies (F_{st}) ranged from 0.045 at the $6Pgd_1$ locus to 0.400 at the Aco locus; the mean F_{st} was 0.187. The average apparent value of F (F_{it}) was 0.333, *i.e.*, heterozygosity shows a deficiency of 33%.

Table 4. – Summary of F-statistics at all polymorphic loci over all populations in *C. sempervirens* L..

Locus	Fis	Fst	Fit
PGI_2	-0.106	0.080	-0.016
PGM_1	-0.120	0.159	0.058
MNR	0.262	0.232	0.434
GOT_3	0.413	0.146	0.499
G6PD	0.340	0.115	0.416
MDH_3-	-0.368	0.242	-0.038
MDH_4	0.068	0.100	0.161
GDH	0.570	0.142	0.631
IDH_2	0.254	0.247	0.439
$6PGD_1$	0.067	0.045	0.109
$6PGD_2$	0.226	0.109	0.310
ACO	0.311	0.400	0.586
Mean	0.180	0.187	0.333

F_{is} = measure of the degree of inbreeding within populations.
F_{st} = measure of the degree of allelic frequencies among populations.
F_{it} = measure of the apparent value of F.
– (negative) = excess of heterozygotes; = (positive) = deficit in heterozygtes.

The fixation index (f) for each polymorphic locus in each of the populations examined was calculated, and the results show that no one of the populations is similar to any other in excess or deficiency of heterozygotes in each one of the loci.

Discussion

Cupressus sempervirens L. has been heavily used for building since ancient times, because of its straight bole and the physical properties of its wood; it was much valued because of its moth-repellant chemical properties and therefore used to make storage chests for clothing (MAKKONEN, 1968).

The small wingless seed, on the one hand, and the ecological changes in sites caused by clear cutting and/or burning and grazing, on the other hand, made the regeneration of *Cupressus* in the destroyed forests very difficult (ZEIDE, 1977). This is probably one of the main reasons for the drastic reduction of the forest area of this species to several disjunct relict populations scattered within the former range of distribution, as can be deduced from the studies on prehistoric wood remains of this species in Israel (LEV-YADUN and WEINSTEIN-EVRON, 1993).

The phenomenon of relatively high heterozygosity in *Cupressus sempervirens* L., which can not be explained by the different theories about the relations between life history or areal and mod of distribution (PAPAGEORGIOU, 1995), might be resolved by the phenomenon of a special type of cone serotiny in this species; serotinous cones stay alive within trees canopy for at least 20 years (LEV-YADUN, 1995). This phenomenon might possibly be one of the ways of transfer, throughout the millennia and in spite of catastrophic events, of the high genetic diversity that has been maintained in the species in spite of the very disjunct areal of distribution and relatively small populations that probably prevented gene exchange. The capability to grow under very adverse environmental conditions prevailing within the area of distribution, extending over about 30 degrees of longitude and 15 degrees of latitude might probably be the result of this high genetic diversity.

Relations among the several populations analyzed, as shown in the phylogenetic tree, are of interest; some of the populations analyzed by PAPAGEORGIOU *et al*. (1994) and also in the present study are similarly separated on the dendrograms, as in the case of Anapoli and Askifu populations from West Crete, or of Samos and Samos-r (= Samos-1). The occurrence of the Beskonak, Syrian and Iranian populations on the same branch may point to genetic relationships among these populations, and to the existence of a north-eastern Mediterranean group with some similarity in its genetic constitution. The phylogenetic location of the low-altitude western Turkish populations together with the low-altitude populations of the Islands of Kos and Samos, may also point to the existence of a low-altitude east Aegean group; the separation of the Rhodes and Cyprus populations together with 2 populations from Crete on a different branch of the phylogenetic tree cannot easily be explained.

The position of var. *pyramidalis* and var. *horizontalis* growing in plantation in Israel are of interest as they are both on the same branch with the populations from Cyprus, Rhodes and Jordan; this may point to the possibility that seeds were imported from these areas and used for planting of several plantations of *Cupressus* in the area of Cis-Jordan, in as much as *C. sempervirens* var. *pyramidalis* occurs on the island of Rhodes and Cyprus since the distant past. The 2 varieties of *C. sempervirens* L. growing in Israel today do not differ in their percentage of polymorphic loci, mean number of alleles per total number of loci, mean number of alleles per polymorphic locus and mean expected heterozygosity per polymorphic locus; the genetic distance between the 2 varieties of *C. sempervirens* L. in Israel is very small, only 0.007 (SCHILLER and KOROL, 1997).

Stile, drawing conclusions about the natural genetic diversity and range-wide genetic relationships of this cypress tree is very difficult because of human activity. The existence of *Cupressus sempervirence* L. forests in Toscana, Italy (PAVARI, 1934), is an evidence to the transfer of seed and/or seedlings in historical times. The occurrence of *C. sempervirens* var. *pyramidalis* all around the Mediterranean basin is too an evidence of the transfer by man of seed or seedling material from the East to the West, as the erect form of this species has first been "published" on old Persian well paintings and tapestry describing Eden. Variation in canopy form in forest trees species extending over large areas is a known phenomenon and is partly the result of selection due to snow pressure (KLOTZLI, 1975; SCHMIDT, 1943). According to PAVARI (1934) cited in BOLOTIN (1964) there is reason to believe that differences in canopy form within *Cupressus sempervirens* L. are due to climatic conditions; the columnar (*pyramidalis*) form is the more ancient one which has been developed under cold (continental ?) climatic conditions whereas the spread branches (horizontalis) form is more recent and has been developed under the influence of hot (Mediterranean ?) climatic

conditions. Man has transferred forest tree seeds or seedlings around the Mediterranean, as has been established for *Pinus halepensis* and which is known to have happened in the case of *Pinus pinea*. Using isoenzyme analysis, it was found that *Pinus halepensis* growing in Umbria, Italy, have several alleles that are characteristic of *Pinus halepensis* growing in Israel and Jordan; historical evidence supports the hypothesis that seeds of the East Mediterranean group of *Pinus halepensis* were transferred to and planted in Umbria, Italy (SCHILLER *et al*., 1985; SCHILLER and BRUNORI, 1992). *Pinus pinea* (stone pine; "*Pinus domestico*") is thought to originate in the Iberian peninsula, but because its seeds are edible this tree was transferred around the Mediterranean probably in Roman times (MIROV, 1967).

The highest values of observed and expected heterozygosity and total genetic diversity among *C. sempervirens* var. *horizontalis* recorded for the Turkish populations suggest that these populations might be relicts of the center of origin of this variety. On the other hand, the high diversity among the Israeli *Cupressus sempervirens*, indicates that these plantations might have come into being from several different origins.

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Genetic Diversity in *Pinus brutia* TEN.: Altitudinal Variation[1])

By N. KARA[2])[4]), L. KOROL[3]), K. ISIK[2]) and G. SCHILLER[3])[5])

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Abstract

The aim of this study was to describe the genetic structure of *Pinus brutia* TEN. subsp. *brutia* (NAHAL, 1983) growing at different elevations in the Taurus Mountains in Turkey, and to use these data to define seed collection and transfer zones.

Isoenzyme analysis was performed on the maternal tissue of seeds to investigate the relationship between allele frequencies and altitude of populations occurring over a narrow geographic region in the vicinity of Antalya, in southern Turkey. Twenty-three loci encoding 14 enzyme systems were analyzed and 17 of these loci (69.6%), encoding 10 enzyme systems, were found to be polymorphic (69.6%). The mean genetic diversity within populations (H_s) was 0.263 and the mean total genetic diversity (H_t), 0.278, therefore, the proportion of total diversity among populations (G_{st}) was only 0.053; the mean degree of inbreeding within populations (F_{is}) was 0.167. Deficiency of heterozygotes was found in the Mnr-1, Mdh-4, 6Pgd-2 and Mpi loci. The results indicate that most of the genetic diversity in *P. brutia* is within populations. Significant relations were found between allele frequencies and altitude in Mdh-1, Mdh-4 and Skdh-1, Aco and Gdh enzyme systems. These results support earlier

[1]) Contribution from the Agricultural Research Organization, the Volcani Center, Bet Dagan 50250, Israel. No. 1784-E, 1995 series.

[2]) Department of Biology, Faculty of Arts and Sciences, Akdeniz University, Antalya, 07058 Turkey.

[3]) Department of Agronomy and Natural Resources, Forestry Section, Agricultural Research Organization, the Volcani Center, P. O. Box 6, Bet Dagan 50250, Israel.

[4]) In partial fulfillment of the requirements for M. Sc. degree at the Akdeniz University, Antalya, 07058 Turkey.

[5]) To whom correspondence should be sent.