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First Analysis on Allozyme Variation in Cedar Species (Cedrus sp.)[1])[2])

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Summary

Isozyme variation among the four species of *Cedrus* genus was investigated in dormant vegetative buds of 25 trees of each species using starch gel electrophoresis. The most useful enzyme systems were LAP, MDH, 6PGD and PGI.

The results of this small study show that there is large heterozygosity in *Cedrus brevifolia, Cedrus libani* and *Cedrus atlantica.* In contrast, *Cedrus deodara* was fixed for the above studied enzymes. Moreover, it is shown that there are clear distinctions between *C. brevifolia, C. deodara* and the group of *C. atlantica-C. libani.*

Key words: Cedrus atlantica, Cedrus brevifolia, Cedrus libani, Cedrus deodara, isozyme variation, taxonomy.

Introduction

The genus *Cedrus,* according to a number of authors, includes four coniferous evergreen tree species, with geographically separated distributions. *Cedrus brevifolia* HENRY in Cyprus, *Cedrus atlantica* MANETTI in Algeria and Morocco, *Cedrus libani* A. RICH in Lebanon, Syria and Turkey, *Cedrus deodara* LOUDON in Afganistan and India (M'HIRIT, 1987; ARBEZ, 1987; DAVIS, 1965).

Cedrus has been successfully introduced in many countries outside of its natural distribution as ornamental and reafforestation species. According to TOTH (1980) and M'HIRIT (1987) it has, since the previous century, been introduced, to several European countries (France 1862, Italy 1866, Bulgaria 1890) and also into U. S. A. and Russia.

Due to the performance of its initial introductions, it soon became an important exotic species for Mediterranean and other countries with similar environmental conditions. In spite of the great interest in the genus, the limited information concerning the amount and pattern of its genetic variability, has been based mainly on provenance trials and studies of anatomical and morphological traits (FAO, 1989). On the other hand, in the best of our knowledge, there has been no publication, on enzyme system studies in the genus cedar.

Our objective was to determine the level of genetic variability among and within species, as well as the usefulness of isozymes for taxonomic classification of the genus cedar.

Material and Methods

Sample collection

Buds were collected from 25 randomly selected mature trees of each species and were stored in —20° C. The material for *C. brevifolia* came from a natural stand of cedar in Cyprus (Paphos forest), whereas that for the other three species came from a species and provenance plantation established in 1968, in the arboretum of Loutra Thermis — Thessaloniki.

Seeds were also used in order to carry out a segregation analysis for those trees of *Cedrus brevifolia,* which proved to be heterozygous for at least one locus from the bud-isozyme analysis.

Electrophoresis procedure

Four buds from each tree were homogenized with 0.3 ml vegetative extraction buffer pH 7 (CHELIAK and PITEL, 1984).

Megagametophytes dissected from germinated seeds (inhanced to germinate with cold stratification) with a radicle of 3 mm to 5 mm long, were homogenized separately in 0.4 ml seed extraction buffer, pH 7.6. The extraction buffer consisted of 0.1 M Tris, 3% (W/V) PVP-40, and 5 drops of b-mercaptoethanol. pH was adjusted to 7.6.

The homogenates were analyzed for: acid phosphatase (ACP E.C. 3.1.3.2.), aspartate aminotransferase (AAT or GOT E.C. 2.6.1.1.), diaphorase (DIA E.C. 1.6.4.3.), leucine aminopeptidase (LAP E.C. 3.4.11.1.), malate dehydrogenase (MDH E.C. 1.1.1.37.), peroxidase (PER E.C. 1.11.1.7.), 6-phosphogluconate dehydrogenase (6PGD E.C. 1.1.1.44.) and phosphoglucose isomerase (PGI E.C. 5.3.1.9.), in horizontal electrophoresis system with 11.5% (W/V) starch. Gels were prepared from a mixture (9:3 W/V) of MERK and CONNAUGHT starch.

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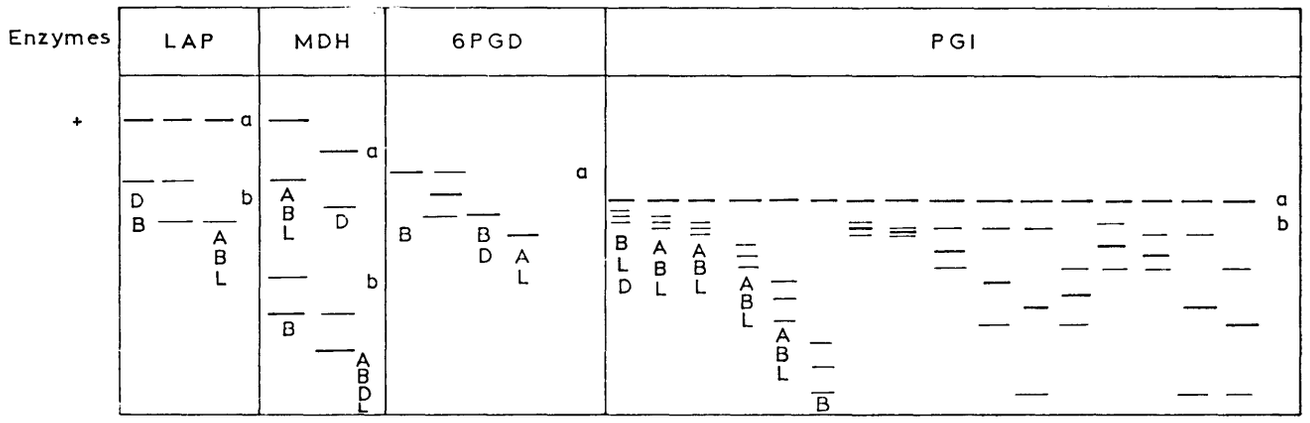

A: *Cedrus atlantica*
B: *Cedrus brevifolia*
D: *Cedrus deodara*
L: *Cedrus libani*

Figure 1. — Banding patterns observed in diploid tissues (buds) for four enzymes in *Cedrus sp.*.

Composition of gels, electrode buffers and staining recipes were similar to those of CHELIAK and PITEL (1984), except for the staining recipe of MDH, which was similar to that of CONCLE et al. (1982).

The alleles in each locus (allozymes) and the loci (isozymes) were numbered in decreasing order of anodal mobility.

Estimation of species heterozygosity (a measure of the genetic variation within the species) and the "genetic distance" (a measure of the differentiation between species) was based on the observed gene frequencies and was estimated according to NEI (1977, 1978). Cluster analysis, using the unweighted pair group method, was performed on the matrix of NEI's genetic distance.

Results

Six enzyme systems out of eight i. e. LAP, MDH, 6PGD, PGI, GOT, DIA, representing a number of 12 loci, were resolved with sufficient consistency and clarity. The isozyme banding patterns of LAP, MDH, 6PGD and PGI enzyme systems and the allele frequencies at each locus are shown in *figure 1* and *table 1* respectively. GOT and DIA enzyme systems appeared to be monomorphic in all species.

Leucine aminopeptidase (LAP)

Two zones of activity were observed for LAP which appeared to represent two distinct loci. The most anodal zone (LAPa) appeared to be monomorphic in all species. The cathodal zone (LAPb) produced two variants (allele b_1, b_2). In *C. brevifolia*, both b_1 and b_2 were found, while in *C. deodara* only b_1, and in *C. libani* and *C. atlantica* only b_2, were detected.

Malate dehydrogenase (MDH)

Two zones of activity were also observed for MDH. In both zones (locus) double-banded allozymes were present.

In the first locus (MDHa) 2 variants (a_1, a_2) were scored. Allele a_2 was species-specific to *Cedrus deodara* while a_1 was found in homozygous state in the other species.

In the cathodal locus (MDHb) again two alleles were detected. Allele b_2 was found in all species, but b_1 was found only in *C. brevifolia.*

6-Phosphogluconate dehydrogenase (6PGD)

6PGD behaved as a dimeric enzyme with a single locus and three variants (alleles a_1, a_2, a_3). Allele a_3 was found only in *Cedrus atlantica* and *Cedrus libani* at frequencies of 100%.

Phosphoglucose isomerase (PGI)

Two zones of activity were observed for PGI. The most anodal zone (loci: PGIa) was not resolved satisfactorily, but seemed to be monomorphic. The second one PGIb, appeared as a triple band isozyme with six variants (alleles b_1, b_2, b_3, b_4, b_5 and b_6). In the heterozygous phenotypes hybrid bands appeared, implying a possible dimeric

Table 1. — Allele frequencies of 5 isozyme loci in diploid tissue of *Cedrus sp.*.

Enzymes and Alleles / Species	LAP_b		MDH_a		MDH_b		6PGD			PGI_b					
	b_1	b_2	a_1	a_2	b_1	b_2	a_1	a_2	a_3	b_1	b_2	b_3	b_4	b_5	b_6
Cedrus atlantica	-	1.00	1.00	-	-	1.00	-	-	1.00	-	0.20	0.16	0.38	0.26	-
Cedrus brevifolia	0.82	0.18	1.00	-	0.04	0.96	0.58	0.42	-	0.10	0.20	0.20	0.24	0.10	0.16
Cedrus deodara	1.00	-	-	1.00	-	1.00	-	1.00	-	1.00	-	-	-	-	-
Cedrus libani	-	1.00	1.00	-	-	1.00	-	-	1.00	0.10	0.46	0.20	0.20	0.04	-

Table 2. — Genetic variability of five loci in *Cedrus* diploid tissue.

Species	Number of trees	Number of alleles	Observed Heterozygosity (H_o)	Expected Heterozygosity (H_e)	Chi-square test (X^2)	Degrees of Freedom
Cedrus atlantica	25	8	0.1280 ± 0.128	0.1474 ± 0.147	0.0026	4
Cedrus brevifolia	25	13	0.2400 ± 0.115	0.3420 ± 0.151	0.0304	4
Cedrus deodara	25	5	0.0000 ± 0.000	0.0000 ± 0.000	0.0000	4
Cedrus libani	25	9	0.1440 ± 0.144	0.1422 ± 0.142	0.0000	4

Table 3. — Unbiased genetic distances between *Cedrus sp.*.

	C. atlantica	C. libani	C. deodara
C. brevifolia	0.4749	0.4759	0.5672
C. atlantica		0.01554	1.5297
C. libani			1.4374

structure of the enzyme. Alleles b_1, b_2, b_3, b_4 and b_5 were found in *C. libani* whereas b_2, b_3, b_4 and b_5 in *C. atlantica*. *C. deodara* had only one allele b_1, while *C. brevifolia* had all of them. b_6 was species-specific to *C. brevifolia* with a frequency of 16%. The same isozyme banding patterns were found in both haploid and diploid tissues (megagametophytes) of *Cedrus brevifolia*, which were analysed to test Mendelian ratios. The Chi-square test ($X^2 = 0.32 < X^2_{0.05.49}$) showed that the segregation fitted the expected Mendelian ratio of 1:1.

In the case of MDH in haploid tissue, an additional locus with very low mobility also appeared, while the active loci were those found in the diploid tissue. It seems that in the genus *Cedrus* only three loci of MDH are present, corresponding to MDH_a, MDH_b and MDH_d (El-Kassaby, 1981), of with two appeared in the diploid tissue and three in the haploid. The lack of MDH_c locus and the heterodimeric band, which is the product of MDH_b and MDH_c, are lacking probably in *Cedrus*, as it was also detected in other conifers, e. g. *A. grandis*, *A. concolor* (El-Kassaby, 1981).

Genetic variation for each species of *Cedrus* was expressed through the expected heterozygosities, which were 0.3420 for *C. brevifolia*, 0.1474 for *C. atlantica*, 0.1422 for *C. libani* and 0 for *C. deodara* (*Table 2*). The Chi-square test showed that there were no significant differences between observed and expected heterozygosities.

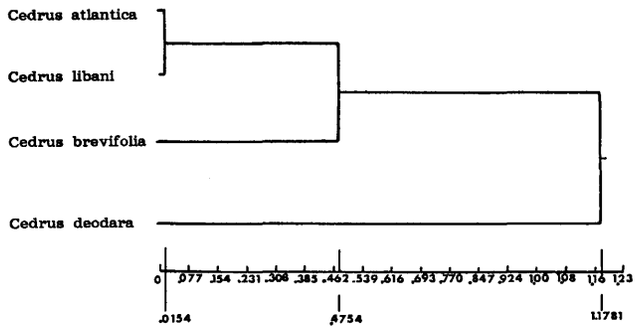

Figure 2. — Dendrogram of 4 *Cedrus* species, based on 5 polymorphic loci, constructed from the genetic distances estimated according to Nei's (1978) unbiased genetic distance.

The cluster analysis of unbiased genetic distances (*Table 3, Figure 2*) formed two distinct clusters: (a) *C. atlantica* and *C. libani*, with a very small genetic distance (0.0154), (b) *C. deodara*, with the largest genetic distance (0.1781). *C. brevifolia* occupied the place between the above 2 clusters but closer to the first one.

Discussion

Isozyme analysis of *Cedrus* diploid tissue has provided new information about the geographic distribution and the relative amounts of genetic variation in the genus. As was mentioned in the introduction, there has been no report based on isozyme data concerning the genus *Cedrus* yet, whereas the existing information is mainly based on morphological and anatomical traits.

The data of isozyme analysis raises doubts about the separation of *C. atlantica* and *C. libani* into 2 distinct species, since no distinguishing gene marker was detected to justify their taxonomic status. On the contrary, they have allele a_3 as a common gene marker in 6PGD, which distinguishes them from the other two species (*C. brevifolia* and *C. deodara*). The absence of allele b_1 in PGIb in *C. atlantica* may be due to sampling error or to a possible over-estimate of the b_2 allele, which was very close to b_1 (*Fig. 1*). In contrast, for MDHa, allele a_2 is a gene marker for *C. deodara* since it was not detected in the other species. *Cedrus brevifolia* has the b_6 allele in PGIb as a gene marker, which was not detected in the other species.

Concerning the dimeric structure of PGI and 6PGD enzyme systems the findings of the present work are in agreement with those of Neale and Adams (1981) for *Abies balsamea* and Adams and Joly (1980) for Loblolly Pine, Mitton et al. (1979) for Ponderosa Pine, Guries and Ledig (1978) for *Pinus rigida*, Scaltsoyiannes et al. (1990) for the Mediterranean species and provenances of *Abies*, Neale et al. (1984), Adams et al. (1990) and El-Kassaby et al. (1982) for Douglas fir. The multibanded pattern of PGIb allozymes was also noticed by Adams et al. (1990) in *Pseudotsuga menziesii* var. *menziesii* and *Scaltsoyiannes* et al. (unpublished) in Maroccan firs (*A. pinsapo* var. *marocana* and var. *tazaotana*).

The monomeric subunit structure of LAP enzyme system is in agreement with the findings for other conifers (Conkle, 1971; Adams and Joly, 1980; Millar, 1985).

The dendrogram (*Fig. 2*), constructed from the genetic distances estimated (*Table 3*), implies that *C. atlantica* is similar to *C. libani*. In contrast, there is a clear distinction between those two and the group *C. brevifolia* and *C. deodara*. The low differentiation between *C. libani* and *C. atlantica* was expected, since the break of physical connection between the 2 so-called species, due to the

desertification of North Africa is relatively recent, so there was not enough time for species differentiation. In the Med-Cecklist (GREUTER et al., 1984), *Cedrus atlantica* is listed as *Cedrus libani* subsp. *atlantica*, implying an uncertainty as to the existence of 2 separate species. The slight differentiation between those two species, could also be attributed to the origin of the material used in this study, which was collected from an arboretum where the trees may have been derived from only a few parental trees.

The heterozygosity (*Table 2*) shows that *C. brevifolia* has high variation (0.3420) while *C. deodara* has no variation at all. *C. libani* and *C. atlantica* are intermediate.

Generally, conifers exhibit high levels of heterozygosity. Up to now a notable exception to this rule was *Pinus resinosa* (FOWLER and MORRIS, 1977), probably as a result of a severe population restriction during the Pleistocene period. The fixation (zero heterozygosity) of *C. deodara* was unexpected, since the species occupies large areas, of about 500.000 ha (M'HIRIT, 1987) in Afganistan and the Himalayas. A possible explanation of this observation is the nature of the material used in the isozyme analysis. As noted above, samples of *C. deodara* were collected from an arboretum established with seeds of unknown origin. The lack of variation can be attributed to fixation of the donor source os the possible limited variation of the "European population".

Comparing the heterozygosity of the *Cedrus* species, excluding *C. deodara*, with that of other coniferous species, it appears that the heterozygosity of *C. brevifolia* is similar to that of *Abies sp.* (SCALTSOYIANNES, PANETSOS and ZARAGOTAS, 1990; SCALTSOYIANNES and PANETSOS, unpublished) while *Cedrus libani* and *Cedrus atlantica* have less heterozygosity.

This interpretation of enzyme variation of *Cedrus* sp. has important implications for future selection and improvement of the species. For *Cedrus deodara* more research is needed to solve the problem of its fixation. Research material should be derived from stands of its natural distribution. Further research on *C. atlantica* and *C. libani*, based on material from natural stands, will elucidate their taxonomic status.

Isozyme studies based on haploid tissue (endosperm) of *C. brevifolia* (unpublished) revealed the same pattern as the one presented in this study, coming from diploid tissue.

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Inheritance and Linkage of Some Allozymes in Taxus baccata L.

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Summary

Eleven enzyme systems coding for 22 loci were assayed in *Taxus baccata*. Mendelian inheritance was confirmed for allozymes at 11 loci by testing the fit of band-pattern segregation in macrogametophytes from heterozygous trees to the expected 1:1 ratio.