Identification of *Pinus brutia* TEN., *P. halepensis* MILL. and Their Putative Hybrids

By K. PANETSOS[1]), A. SCALTSOYIANNES[1]), F. A. ARAVANOPOULOS[1]), K. DOUNAVI[2]) and A. DEMETRAKOPOULOS[1])

(Received 17th March 1997)

Abstract

Identification of *Pinus brutia* TEN., *P. halepensis* MILL. and their putative interspecific natural hybrids was studied in Rhodes island, Greece by using morphological, anatomical and isoenzymatic markers. Twentytwo morphological and anatomical characteristics and 3 loci (GDH-A, LAP-A, IDH-A) were used for identification of the above species and their hybrids. Among the 22 tested morphological and anatomical characteristics, 19 (all except three: peduncle, seed width and teeth per centimeter) presented higher mean values in trees regarded as *P. brutia*, while 14 (all except eight: needle thickness, stomata rows, teeth per centimeter, resin ducts, cone width, seed width, cone length, and length of seed plus wing) presented intermediate mean values in trees regarded as interspecific natural hybrids. The three tested loci, were proved to be useful markers for the discrimination of both pine species and their interspecific hybrids. An agreement among the clusters of the morphological analysis and the species characterization by the biochemical analysis, was noticed.

Key words: identification, *Pinus brutia*, *Pinus halepensis*, natural hybrids, morphological-anatomical-isoenzymatic markers.

FDC: 165.71; 165.3; 164; 174.7 *Pinus brutia*; 174.7 *Pinus halepensis*; (495).

Introduction

Aleppo pine (*Pinus halepensis* MILL.) and Brutia pine (*Pinus brutia* TEN.), form a distinct group within the Eurasian hard pines and their combined geographic distribution reflects their prominence among low elevation Mediterranean forest species. *P. halepensis* has a wide distribution ranging from southern Europe and Morocco in the West, to Syria in the Southeast and mainland Greece in the North-East of the Mediterranean basin (PANETSOS, 1981). *P. brutia* presents a more restricted distribution ranging from Iraq in the East, to Greece in the West, in particular being present in Thrace and in the islands of the eastern Aegean sea including Crete. Isolated occurrences of one species inside the range of the other have been attributed to human interference's (PANETSOS, 1981). As it can be appreciated, the eastern limits of *P. halepensis* and the western limits of *P. brutia* come close in Greece. The two species, besides spatial isolation, have developed other kinds of barriers, such as prevention of fertilization when *P. halepensis* is the female parent, reduced embryo viability in hybrid seeds, low temperature tolerance, as well as differences in blooming time. They should be therefore considered, as two well established pine species (PANETSOS, 1986).

However, artificial hybridization has revealed that the two species can be successfully crossed, and even that natural hybrids are formed in areas where the two species come in contact, due to human interference (PANETSOS, 1975; MOULALIS et al., 1976).

In Rhodes island in particular, *P. halepensis* is considered to have been introduced about one hundred years ago (PANETSOS, 1975), at the northwest of the island. Initially, *P. halepensis* and *P. brutia* were separated by a broad belt of natural *Cupressus sempervirens* stands, but due to human activity this zone ceased to exist. Several intermediate forms have been observed in a broad area located between the two species and the existence of hybridization and active introgression among the two species has been verified by an analysis of morphological variation (PANETSOS, 1975). In this investigation, the pine populations of Rhodes island are being studied employing two approaches, morphological and enzymatic. These approaches are being compared in order to appreciate their relative efficiencies in assessing the patterns of hybrid introgression.

Materials and Methods

The sampled areas were: the Apolakia area of the old *P. halepensis* introduction, the Profitis Elias area, considered as a pure *P. brutia* stand, and an area located between the two above mentioned sites which is considered to be the area of the putative hybrids (PANETSOS, 1975). Within each area, care was taken to sample individuals which phenotypically corresponded to *P. brutia*, *P. halepensis* and their hybrids respectively.

A total of 112 individuals were sampled, 28 of which came from the Apolakia area, 25 from the Profitis Elias area and 59 from the intermediate zone. Trees were chosen to be at least

Table 1. – Morphological and anatomical characteristics measured in *Pinus brutia*, *P. halepensis* and their putative hybrids.

No	Character	Unit of measurement
1	Angle between cone axis and bearing branch	°
2	Peduncle length	mm
3	Cone length	cm
4	Cone width	cm
5	Needle length	cm
6	Needle width	mm
7	Needle thickness	mm
8	Sheath length	mm
9	Number of teeth per cm	–
10	Number of stomata rows on dorsal face of needle	–
11	Number of stomata rows on convex face of needle	–
12	Number of stomata per cm on dorsal face of needle	–
13	Number of stomata rows on convex face of needle	–
14	Number of resin ducts in the upper side of the needle	–
15	Number of resin ducts in the lower side of the needle	–
16	Seed length	mm
17	Seed width	mm
18	Seed thickness	mm
19	Length of seed and Wing	mm
20	Total number of stomata (both sides) per cm	–
21	Total number of stomata per needle	–
22	Total number of resin ducts	–

[1]) Laboratory of Forest Genetics and Plant Breeding, Department of Forestry and Natural Environment, Aristotle University of Thessaloniki, Thessaloniki 54006, Greece

[2]) Mediterranean Agronomic Institute of Chania, Alsyllion Agrokepion, 73100 Chania, Greece

Table 2. – Descriptive statistics of morphological and anatomical characteristics in *P. brutia*, *P. halepensis* and their putative hybrids.

Profitis Elias Area				
Variable	**Mean**	**Std. Error**	**Std. Dev.**	**CV**
Angle	110.539	1.64	15.458	**13.984**
Peduncle	0.562	0.023	0.218	**38.969**
Cone Length	7.4	0.11	1.029	**13.908**
Cone Width	4.109	0.044	0.414	**10.087**
Needle Length	13.606	0.23	2.118	**15.567**
Needle Width	0.112	0.001	0.012	**11.518**
Needle Thickness	0.068	0.0008	0.0075	**11.031**
Sheath Length	0.83	0.017	0.162	**19.556**
Rows Of Stomata Dorsal	8.98	0.14	1.339	**14.918**
Rows Of Stomata Convex	3.624	0.071	0.678	**18.724**
Teeth Per Cm	32.376	2.55	5.266	**16.265**
Resin Ducts Dorsal	5.936	0.092	0.889	**14.983**
Resin Ducts Convex	3.904	0.078	0.754	**19.323**
Total Resin Ducts	9.84	0.14	1.373	**13.956**
Stomata / Cm / Row Dorsal	86.952	0.748	7.192	**8.271**
Total Stomata /Cm/ Dorsal	780.424	14.124	132.968	**17.038**
Stomata /Cm/Row Convex	95.63	0.866	8.273	**8.651**
Total Stomata /Cm Convex	346.289	7.44	71.028	**20.511**
Total Stomata / Cm	1126.63	19.14	182.732	**16.219**
Total Stomata /Needle	15421.96	405.57	3876.28	**25.134**
Seed Length	0.78	0.007	0.06	**7.792**
Seed Width	0.0463	0.005	0.047	**10.193**
Seed + Wing Length	2.543	0.03	0.279	**10.972**
Seed Thickness	0.353	0.004	0.038	**10.813**

Apolakia Area				
Variable	**Mean**	**Std Error**	**Std Dev**	**CV**
Angle	48.88	2.555	23.273	47.61
Peduncle	1.444	0.045	0.457	31.658
Cone Length	7.159	0.026	1.297	18.118
Cone Width	3.341	0.042	0.442	13.242
Needle Length	9.203	0.2	2.041	22.183
Needle Width	0.095	0.005	0.055	57.638
Needle Thickness	0.058	0.001	0.008	13.866
Sheath Length	0.465	0.013	0.134	28.942
Rows Of Stomata Dorsal	6.648	0.124	1.197	18.005
Rows Of Stomata Convex	2.52	0.06	0.691	27.451
Teeth Per Cm	56.103	1.01	10.387	18.515
Resin Ducts Dorsal	4.67	0.078	0.81	17.347
Resin Ducts Convex	2.155	0.047	0.492	22.838
Total Resin Ducts	6.827	0.104	1.074	15.73
Stomata / Cm / Row Dorsal	80.358	0.701	7.228	8.994
Total Stomata /Cm/ Dorsal	535.062	10.924	112.741	21.07
Stomata /Cm/Row Convex	87.71	1.2	8.152	9.294
Total Stomata /Cm Convex	222.586	6.831	70.499	31.672
Total Stomata / Cm	757.648	16.428	169.541	22.377
Total Stomata /Needle	7189.94	308.74	3186.13	44.313
Seed Length	0.61	0.007	0.073	11.992
Seed Width	0.351	0.004	0.039	11.238
Seed + Wing Length	2.433	0.033	0.342	14.07
Seed Thickness	0.234	0.003	0.003	14.348

Intermediate Zone Area				
Variable	**Mean**	**Std. Error**	**Std. Dev.**	**CV**
Angle	103.653	1.812	26.298	25.371
Peduncle	0.729	0.021	0.307	42.205
Cone Length	6.589	0.078	1.136	17.244
Cone Width	3.544	0.031	0.46	12.992
Needle Length	12.11	0.188	2.774	22.906
Needle Width	0.103	0.001	0.014	13.647
Needle Thickness	0.062	0.0005	0.0082	13.235
Sheath Length	0.673	0.011	0.17	25.316
Rows Of Stomata Dorsal	8.08	0.095	1.397	17.291
Rows Of Stomata Convex	3.425	0.112	1.644	47.988
Teeth Per Cm	36.561	0.619	9.13	24.972
Resin Ducts Dorsal	5.496	0.069	9.13	24.972
Resin Ducts Convex	3.401	0.062	0.904	26.587
Total Resin Ducts	8.898	0.114	1.678	18.865
Stomata / Cm / Row Dorsal	83.495	0.558	8.22	9.845
Total Stomata /Cm/ Dorsal	676.707	10.19	144.501	21.353
Stomata /Cm/Row Convex	90.567	0.6	8.84	9.761
Total Stomata /Cm Convex	312.049	11.28	166.323	53.3
Total Stomata / Cm	988.96	17.16	252.753	25.55
Total Stomata /Needle	12188.17	313.57	4619.35	37.9
Seed Length	0.675	0.006	0.084	12.442
Seed Width	0.394	0.004	0.054	13.894
Seed + Wing Length	2.263	0.025	0.368	16.287
Seed Thickness	0.297	0.003	0.047	15.787

50 m apart so as to avoid sampling from related individuals. Five vigorous branches were collected from each tree within the center of the crown of trees more than twenty years old. Ten separate measurements were made concerning 22 morphological and anatomical characteristics which are presented in *table 1*. Three additional composite variables that were introduced by summation of individual variables are also presented in *table 1*. Statistical analysis of the data was carried out using SAS software (SAS, 1985). Univariate statistics of the morphological data were computed. Data normality was investigated in the 22 characters measured by using the SHAPIRO-WILKS statistic. In the cases where deviations from normality were detected, the logarithmic transformation was successfully employed. Correlation between characters were not very high and PEARSON's r did not exceed 0.85. In order to reduce the dimension of the space while classifying individuals, a principal component analysis (PIMENTEL, 1979) was performed to give a hierarchy of the most discriminant traits.

Subsequently Euclidean distances between individuals were computed from a matrix of the "n" first principal components, the number of which was judged by the percent of variation explained and the deflection point of a plot of eigenvalues. In general, the Euclidean distance between individuals i an j based upon p variables is:

$$d_{ij} = \left(\sum_{k=1}^{P} (\times_{ik} - X_{jk})^2\right)^{1/2}$$

(PIMENTEL, 1979). The dissimilarity matrix formed was then subjected to a hierarchical cluster analysis, by using the average linkage clustering method. In particular, the unweighted pair group algorithm using arithmetic averages (UPGMA) was employed (PIMENTEL, 1979). A hierarchical tree based on the generated average distances was produced, by using the STATISTICA® software package.

In order to enhance and verify the above morphological analysis, a subset of the sampled populations was used for isoenzyme analysis. Three enzyme systems were employed: GDH (E.C. 1.4.1.2.), IDH (E.C. 1.1.1.42), and LAP (E.C. 3.4.11.1.). The above enzyme systems were chosen because they have been proved to provide species specific gene markers between the two species. This finding, originally reported by CONKLE *et al.* (1988) and used by KOROL *et al.* (1995) to identify potential F_1 hybrids in an artificial plantation, was also detected in this study. In particular, in the GDH enzyme system the A1 and A2 alleles were specific to *P. brutia*, while

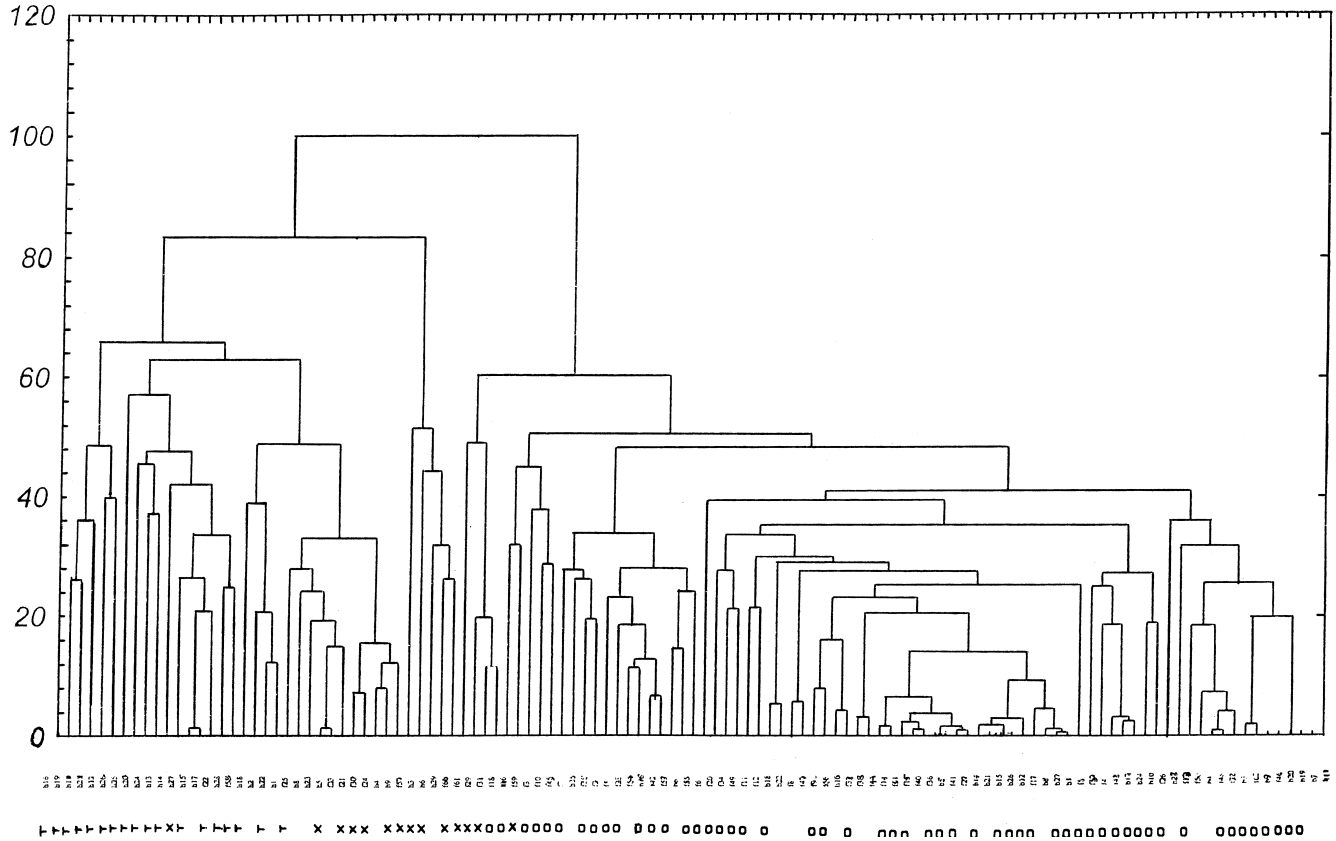

Figure 1. – Hierarchical tree of *Pinus* individuals based on the *UPGMA* clustering procedure of morphological data with superimposed species and hybrid designations by biochemical markers (O: *P. brutia*, X: hybrid, T: *P. halepensis*.

Table 3. – Eigenvalues and percent of trace calculated from the principal component analysis of *Pinus* morphological data.

Component	Eigenvalue	Percent of Trace	Cumulative
PC1	6.17	0.41	0.41
PC2	1.66	0.11	0.52
PC3	1.42	0.09	0.62
PC4	1.01	0.07	0.68
PC5	0.96	0.06	0.75
PC6	0.77	0.05	0.80
PC7	0.50	0.03	0.83
PC8	0.50	0.03	0.86
PC9	0.46	0.03	0.89
PC10	0.40	0.02	0.91
PC11	0.33	0.02	0.93
PC12	0.32	0.02	0.95

the A3 allele was specific to *P. halepensis*. In the IDH enzyme system the A2 and A3 alleles were specific to *P. brutia* while the A1 allele to *P. halepensis*. In the LAP enzyme system, the A1 allele was characteristic of *P. brutia* and the A2 allele of *P. halepensis.*

Electrophoresis was carried out on haploid endosperms. Seeds were stratified at 4 °C for one week. They were subsequently placed on moist filter paper in Petri dishes in a seed germinator provided with a 16 h photoperiod. Germination (radicle length of 1 mm to 2 mm) normally occurred within a week. Endosperms (megagametophytes) were dissected from germinated seeds and were homogenized individually with a pestle and mortar by adding the 0.20 M phosphate extraction buffer of CONKLE *et al.* (1982). The homogenates were analyzed for the enzyme systems mentioned above in a horizontal electrophoresis system with 10.5% Sigma starch. Buffer systems, gel composition, electrode buffers and staining recipes were prepared according to CHELIAK and PITEL (1984) except for the staining recipe for LAP which was prepared according to CONKLE *et al.* (1982). Gels were run under refrigeration (4 °C) and constant amperage (60 mA) with the exception that voltage was not allowed to exceed 320 V. The loci and alleles at each locus were numbered in decreasing order of anodal mobility. Seven endosperms per tree were used in order to reveal the underlying multilocus genotype with a sufficient statistical confidence.

Results and Discussion

The mean values, standard deviations and coefficients of variation of the morphological variables are presented in *table 2*. As it can be appreciated from this table, the trees from the Profitis Elias area (considered as *P. brutia*), presented higher mean values in their morphological characteristics when compared to the trees of the Apolakia area (considered as *P. halepensis*). This was evident for all characteristics but tree: peduncle length, seed width, and teeth per centimeter. The trees from the area between Profitis Elias and Apolakia, which are considered as putative hybrids, presented intermediate mean values in 14 out of 22 morphological measured characteristics; in four characteristics (needle thickness, stomata rows of the convex side of the needle, teeth per centimeter, and resin ducts of the convex side of the needle) the mean values were closer to the values of the Profitis Elias population. In two characteristics (cone width, and seed width) the mean values were closer to the Apolakia population and in two others (cone length, and length of seed and wing) the mean values of the individuals from the intermediate zone were lower than the mean values of either the Profitis Elias or the

Table 4. – Genotypes and designation of *Pinus* individuals inferred by 3 species-specific gene markers.

Code number of sampled individuals	Species - specific gene markers			
	GDH	IDH	LAP	Designation
b 1	A1A1	A2A2	A1A1	P. brutia (O)
3	A1A1	A2A2	A1A1	P. brutia (O)
5	A2A2	A2A2	A1A1	P. brutia (O)
9	A2A2	A2A2	A1A1	P. brutia (O)
10	A1A1	A2A2	A1A1	P. brutia (O)
12	A2A2	A2A2	A1A1	P. brutia (O)
13	A2A2	A2A2	A1A1	P. brutia (O)
14	A2A2	A2A2	A1A1	P. brutia (O)
15	A2A2	A2A2	A1A1	P. brutia (O)
17	A2A2	A2A2	A1A1	P. brutia (O)
18	A2A2	A2A2	A1A1	P. brutia (O)
19	A2A2	A2A2	A1A1	P. brutia (O)
20	A2A2	A2A2	A1A1	P. brutia (O)
24	A2A2	A2A2	A1A1	P. brutia (O)
26	A1A2	A2A2	A1A1	P. brutia (O)
27	A2A2	A2A2	A1A1	P. brutia (O)
f 1	A2A2	A2A2	A1A1	P. brutia (O)
2	A2A2	A2A2	A1A1	P. brutia (O)
3	A2A2	A2A2	A1A1	P. brutia (O)
4	A2A2	A2A2	A1A1	P. brutia (O)
5	A2A2	A2A2	A1A1	P. brutia (O)
6	A1A1	A2A2	A1A1	P. brutia (O)

*) b: Profitis Elias
h: Apolakia
f: Intermediate Zone

42	A1A2	A2A2	A1A1	P. brutia (O)
43	A2A2	A2A2	A1A1	P. brutia (O)
45	A1A2	A2A2	A1A1	P. brutia (O)
46	A1A1	A2A2	A1A1	P. brutia (O)
48	A1A1	A2A2	A1A1	P. brutia (O)
49	A1A2	A2A2	A1A1	P. brutia (O)
51	A1A2	A2A2	A1A1	P. brutia (O)
53	A2A2	A2A2	A1A1	P. brutia (O)
55	A2A2	A2A2	A1A1	P. brutia (O)
56	A2A2	A2A2	A1A1	P. brutia (O)
57	A1A2	A1A2	A1A2	Hybrid (X)
58	A3A3	A1A1	A2A2	P. halepensis (T)
59	A1A1	A1A2	A2A2	Hybrid (X)
60	A3A3	A1A2	A2A2	Hybrid (X)
61	A3A3	A1A2	A1A2	Hybrid (X)
62	A1A2	A2A2	A1A1	P. brutia (O)
h 3	A2A3	A1A2	A1A2	Hybrid (X)
4	A1A3	A1A2	A1A1	Hybrid (X)
5	A2A3	A1A2	A1A2	Hybrid (X)
6	A2A3	A1A2	A1A2	Hybrid (X)
9	A3A3	A1A2	A1A2	Hybrid (X)
11	A3A3	A1A1	A2A2	P. halepensis (T)
12	A3A3	A1A1	A2A2	P. halepensis (T)
13	A3A3	A1A1	A2A2	P. halepensis (T)
14	A3A3	A1A1	A2A2	P. halepensis (T)
15	A3A3	A1A1	A2A2	P. halepensis (T)
16	A3A3	A1A1	A2A2	P. halepensis (T)

Cont.

Cont.

7	A2A2	A2A2	A1A1	P. brutia (O)
9	A2A2	A2A3	A1A1	P. brutia (O)
10	A2A2	A2A2	A1A1	P. brutia (O)
11	A1A2	A2A2	A1A1	P. brutia (O)
13	A1A2	A2A2	A1A1	P. brutia (O)
14	A2A2	A2A2	A1A1	P. brutia (O)
15	A1A2	A2A3	A1A1	P. brutia (O)
16	A2A2	A2A2	A1A1	P. brutia (O)
17	A2A2	A2A2	A1A1	P. brutia (O)
18	A2A2	A2A2	A1A1	P. brutia (O)
20	A1A2	A2A2	A1A1	P. brutia (O)
21	A2A3	A1A1	A2A2	Hybrid (X)
22	A3A3	A1A1	A2A2	P. halepensis (T)
24	A2A3	A2A2	A1A2	Hybrid (X)
25	A3A3	A1A1	A2A2	P. halepensis (T)
26	A2A2	A2A2	A1A1	P. brutia (O)
28	A1A2	A2A2	A1A1	P. brutia (O)
29	A2A2	A2A2	A1A2	Hybrid (X)
30	A2A3	A1A1	A1A2	Hybrid (X)
31	A2A3	A1A2	A1A1	Hybrid (X)
32	A1A2	A2A2	A1A1	P. brutia (O)
33	A1A2	A2A2	A1A1	P. brutia (O)
34	A2A2	A2A2	A1A1	P. brutia (O)
35	A2A2	A2A2	A1A1	P. brutia (O)
36	A2A2	A2A2	A1A1	P. brutia (O)
37	A2A2	A2A2	A1A1	P. brutia (O)
41	A2A2	A2A2	A1A1	P. brutia (O)

18	A3A3	A1A1	A2A2	P. halepensis (T)
19	A3A3	A1A1	A2A2	P. halepensis (T)
20	A3A3	A1A1	A2A2	P. halepensis (T)
21	A3A3	A1A1	A2A2	P. halepensis (T)
22	A3A3	A1A1	A2A2	P. halepensis (T)
24	A3A3	A1A1	A2A2	P. halepensis (T)
25	A3A3	A1A1	A2A2	P. halepensis (T)
26	A3A3	A1A1	A2A2	P. halepensis (T)
27	A3A3	A1A1	A1A2	Hybrid (X)
28	A3A3	A1A1	A2A2	P. halepensis (T)

Apolakia populations. A possible explanation for the above results would be that the putative hybrid population (intermediate sampling zone) consisted of a mixture of individuals resulting from F_1 or advanced generations and backcrosses.

At this point, it should be noted that some morphological variables presented high values for the coefficient of variation (CV), in the samples of the populations from the intermediate zone and the Apolakia areas. This outcome can be attributed to the finding that, as it was proved by the morphological multivariate analysis (see *Fig. 1*) as well as the biochemical analysis (see results and discussion below), these populations contain mixtures of *P. brutia*, *P. halepensis* individuals and individuals of hybrid origin. The presence of individuals from different species within each group, resulted in higher variability in morphological characters.

The results of the principal components analysis of morphological and anatomical variables are presented in *table 3*. The

first 12 principal components accounting for 95% of the total variability are shown. According to these results more than half of the total variability (60%) is explained in low principal space, in particular by the first three axes. All components were bipolar. The first component was characterized by high loadings in the eigenvector matrix in cone and needle characters, such as sheath length, the number of rows of stomata, cone length, cone width needle length and needle width. The second component characterized by a high eigenvector value of the total number of stomata per cm. The third component was constituted mainly by seed variables, in particular seed length, seed width and length of seed and wing.

Based on the above results and the deflection point of a plot of eigenvalues it was judged to employ the matrix of the first seven principal components, accounting for 83% of the total variability for the calculation of Eucledian distances among individuals and the construction of a hierarchical dendrogram (*Fig. 1*). As it can be observed in *figure 1*, two distinct classes exist. The larger cluster (individuals h16 to f61) was considered as representing mainly the *P. brutia* trees and consisted of all trees from the Profitis Elias area and a large number of individuals from the intermediate zone of sampling. The second cluster (individuals f29 to b11) was considered as representing mainly the *P. halepensis* trees and incorporated individuals from the Apolakia area as well as individuals from the intermediate zone.

The fact that a large number of individuals from the intermediate zone area appeared sequentially in the left branches of the *P. brutia* cluster and the right branches of the *P. halepensis* cluster, is in agreement with the results of PANETSOS (1975), who showed that hybridization and active introgression occurs in the vicinity of the area where the two species come in contact.

The results of the biochemical analysis are presented in *table 4*. Electrophoretic phenotypes are schematized in *figure 2*. As it can be seen in this table, all individuals from the Profitis Elias area were designated as *P. brutia* since their multilocus genotypes only contain allelic characteristics to *P. brutia*. On the contrary, in the Apolakia area, not all individuals were designated as *P. halepensis*; some were proven to be hybrids according to the above presented explanation of their occurrence. In the intermediate zone, all potential cases occurred: individuals were determined as *P. brutia*, *P. halepensis* or of hybrid origin.

In order to compare the morphological and biochemical analyses, we superimposed the biochemical results on the cluster analysis dendrogram (*Fig. 1*). There was an agreement among the clusters of the morphological analysis and the species characterization from the isoenzyme analysis. High concordance between morphological and biochemical characteristics in a study where pure species and their putative interspecific hybrids were evaluated, has also been observed by YEH *et al*. (1986) in two *Picea* species and their hybrids. Similar results have been observed in two other conifers, in particular *Larix laricina* (DICKINSON *et al*., 1988) and *Picea abies* (LAGERCRANZ and RYMAN, 1990). Similar patterns of variation between morphological and alloenzyme data sets have been observed in a number of other studies as well (MICHEVICH and JOHNSON, 1976).

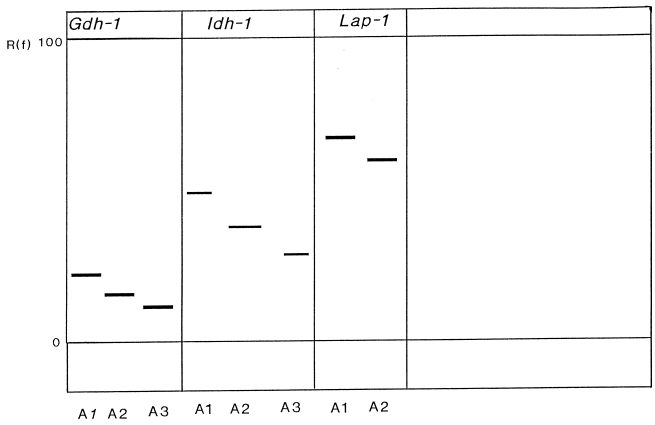

Figure 2. – Banding patterns of zymogram and assignment of alleles in the enzyme systems studied in *Pinus* individuals.

Conclusion

When comparing the morphological data and the isoenzyme data approaches in the study of natural hybridization and introgression, the effectiveness of the isoenzyme analysis, especially when informative gene markers are available, is evident. The use of both approaches in the study of putative hybrids and potential introgression can be essential in clarifying relationships between and among species and populations. The morphological analysis allowed the visualization of the interspecific hybridization and introgression while the isoenzymatic analysis provided similar results but with less data "noise" due to environmental variation. The isoenzymatic analysis achieved to show the existence of backcrosses in the hybrid swarm population, and in this respect, it elucidated in a more clarifying way the phenomenon and the dynamics of introgression.

It is clear that the study of introgressive populations requires large sample sizes and a high number of species-specific gene markers in order to reveal in elucidating detail the magnitude and patterns of introgression in general, and in the case of Rhodes island in particular.

Acknowledgements

This paper is dedicated to our graduate student HASSAN HAJAJE who passed away unexpectedly last year. This work has been supported by EU funding under the AIR-CT93-0803 project. Parts of this work are portions of an M.Sc. Thesis project that K. DOUNAVI has been conducting in the Mediterranean Agronomic Institute of Chania.

Literature

CONKLE, M. I., HODGKISS, P. D., NUNNALY, L. B. and HUNTER, S. C.: Starch gel electrophoresis of conifer seeds: A laboratory manual. Gen. Tech. Rep. PSW-64, USDA Forest Service, Pacific SW, Forest and Range Experimental Station, Berkeley, USA (1982). — CONKLE, M. I., SCHILLER and GRUMWALD, C.: Electrophoretic analysis of diversity and plylogeny of *Pinus brutia* and closely related taxa. Systematic Botany **13**: 411–424 (1988). — DICKINSON, I., KNOWLES, P. and PARKER, W. H.: Data set congruence in Northern Ontario Tamarach (*Larix laricina, Pinaceae*). Systematic Botany **13**: 442–455 (1988). — KOROL, L., MADMONY, A., KIOV, Y. and SCHILLER, G.: *Pinus halepensis* x *Pinus brutia* hybrids. Identification using morphological and biochemical traits. Silvae Genetica **44**: 186–190 (1985). — LAGERCVANTZ, V. and RYMAN: Genetic structure of Norway spruce (*Picea abies*): concordance of morphological and allozyme variation. Evolution **44**: 38–53 (1990). — MICKEVICH, M. F. and JOHNSON, M. S.: Congruence between morphological and allozyme data in evolutionary inference and character evolution. Systematic Zoology, 260–270 (1976). — PANETSOS, K. P.: Natural hybridization between *Pinus brutia* and *P. halepensis* in Greece. Silvae Genetica **24** (5–6): 163–168 (1975). — PANETSOS, K. P.: Monograph of *Pinus halepensis* (MILL.) and *P. brutia* (TEN.). Annales Forestales **9**: 39–77 (1981). — PANETSOS, K. P.: Genetics and breeding in the group *halepensis* Forêt Méditerranéenne **8**: 5–12 (1990). — PIMENTEL, R. A.: Morphometrics: the multivariate analysis of biological data. Kendule/ Hunt Publ. Co., Dubuque, Iowa. 276 pp. (1979). — SAS Insatiate: SAS user's guide: statistics. SAS Insure Publ., Cary, North Carolina, 958 pp. (1985). — YEH, F. C. and ARNOTT, J. F.: Electrophoretic and morphological differentiation of *Picea sitchensis*, *Picea glauca* and their hybrids. Canadian Journal of Forest Research **16**: 791–798 (1986).