RELATIONS BETWEEN NATIVE ISRAELI AND JORDANIAN ALEPPO PINE (*PINUS HALEPENSIS* MILL.) BASED ON ALLOZYME ANALYSIS: A NOTE[1]

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ABSTRACT

Horizontal starch gel electrophoresis of enzymes extracted from the megagametophytes of germinated Aleppo pine (*Pinus halepensis* Mill.) seeds showed that alleles which are unique to the East Mediterranean group, *i.e.*, *Cat–2* allele two and *Aap–1* allele two, appear also in the Jordanian populations. Mean expected heterozygosity of the Israeli and the Jordanian Aleppo pine groups was 0.143 and 0.140 respectively. Mean genetic identity between these two groups of Aleppo pine populations is 0.991±0.001 in comparison with the mean genetic identity among the eight Israeli Aleppo pine relict populations (0.994±0.005), or between the Israeli and other circum Mediterranean Aleppo pine populations (0.977±0.015). Mean H_S was 0.143 and mean H_T was 0.149, mean G_{ST}, *i.e.*, proportion of total diversity among populations was relatively low – 0.05. Mean genetic distance was 0.006 which is similar to the genetic distance among native Israeli populations. The results show that most of the genetic diversity in *P. halepensis* lies within populations and only a little among the two groups of populations namely the Israeli and Jordanian group. These results confirm a previous hypothesis about the genetic identity of *Pinus halepensis* populations in Israel and Jordan.

Key words: *Pinus halepensis* Mill., genetic diversity, isoenzymes

INTRODUCTION

Aleppo pine (*Pinus halepensis* Mill.) forests are widely distributed around the Mediterranean sea (CRITCHFIELD & LITTLE 1966). Present area of distribution is the result of geomorphological and climatic changes in the Tertiary and Quaternary (NAHAL 1962; PANETSOS 1981) and probably, to some extent, also due to human activity (SCHILLER & BRUNORI 1992; SCHILLER & MENDEL 1995).

Allozyme analysis provided evidence for the subdivision of circum Mediterranean populations of Aleppo pine into two groups: (1) West Mediterranean and, (2) East Mediterranean group (SCHILLER *et al.* 1985). GRUNWALD *et al.* (1986) concluded that the genetic parameters which distinguish the East Mediterranean group, *i.e.*, the Israeli relict populations, from all the others circum Mediterranean populations, are probably identical also for the relict populations in Jordan in Lebanon and Syria. Since there is no reason to expect that native Aleppo pine growing in Israel and Jordan should differ much among themselves, hence the Jordan valley is too narrow to be a genetic barrier.

The establishment of peace between Israel and Jordan enabled us to receive seed material to examine the hypothesis by GRUNWALD *et al.* (1986). Therefore, the objective of the present work was to estimate the genetic similarity or distance between the largest relict population of *Pinus halepensis* Mill. in Israel and Jordan.

MATERIAL AND METHODS

Seed Materials

Wind-pollinated seeds were collected from 172 trees growing in five different stands within the native *P. halepensis* forest on the Mt. Carmel range (35°00' E, 32°43' N) which is the largest among the eight relicts of *P. halepensis* in Israel. Two bulked seed lots of native Jordanian *P. halepensis* stands (ZOHARY 1973) were received via the seed and nursery unit of the forest department of the Jewish National Fund (JNF); one

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Table 1 Enzyme names, abbreviations of the names and electrophoretic systems

Enzyme system	Abbreviation	EC No	Buffer System
Aconitase	ACO	4.2.1.3	III
Alanine aminopeptidase	AAP	3.4.11.2	I
Alcohol dehydrogenase	ADH	1.1.1.1	I
Catalase	CAT	1.11.1.6	II
Glutamate dehydrogenase	GDH	1.4.1.3	II
Glutamic-oxaloacetic transaminase	GOT	2.6.1.1	II
Isocitric dehydrogenase	IDH	1.1.1.42	III
Leucine aminopeptidase	LAP	3.4.11.1	I
Malate dehydrogenase	MDH	1.1.1.37	IV
Menadione reductase	MNR	1.6.99.2	I
Phosphoglucomutase	PGM	2.7.5.1	I
Phosphoglucose isomerase	PGI	5.3.1.9	IV
6-Phosphogluconate dehydrogenase	6PGD	1.1.1.44	III
Superoxide dismutase	SOD	1.15.1.1	II

seed lot was from trees growing in the area of Jerash (35°54' E, 32°17' N) and the second from the area of Ajlun (35°46' E, 32°20' N). Seeds were stored in a refrigerator at +5 °C until analysis.

Electrophoresis

Seeds were germinated on moistened filter paper, Whatman No. 3 in Petri dishes at +20 °C.

Extraction of enzymes and horizontal starch gel electrophoresis were performed according to the laboratory manual by CONKLE *et al.* (1982). Analyses were performed using eight haploid megagametophytes per tree in Israeli populations, or 76 individual megagametophytes from the bulked seed material of the Jordanian populations.

The maternal tissue was homogenized in a grinding plate (KELLEY & ADAMS 1977) together with 75 µl of 0.2 M phosphate buffer pH 7.5, 0.1% Triton X–100, 1% BSA, 0.1% b-mercaptoethanol, for all enzyme systems. Four different electrophoresis buffer systems were used to analyze 14 enzyme systems (Table 1):

System I: Gel buffer: 0.02 M tris, 0.02 M boric acid, 0.002 M EDTA pH 8.4. Electrode buffer: 0.2 M tris, 0.2 M boric acid, 0.004 M EDTA pH 8.4 (WENDEL & PARKS 1982).

System II: Gel buffer: 0.01M tris, 0.005 M citric acid pH 8.8 Electrode buffer: 0.05 M NaOH, 0.3 M boric acid pH 8.0 (CONKLE *et al.* 1982).

System III: Gel buffer: 0.002 M citric acid, adjusted with N-(3-aminopropyl) morpholine to pH 6.1 Electrode buffer: 0.04 M citric acid, adjusted with morpholine to pH 6.1 (CONKLE *et al.* 1982).

System IV: Gel buffer: 0.002 M citric acid, adjusted with morpholine to pH 8.3. Electrode buffer: 0.04 M citric acid, adjusted with morpholine to pH 8.3 (CONKLE *et al.* 1982).

After electrophoresis the gels were sliced and stained for each enzyme system according to CONKLE *et al.* (1982). The 14 enzyme systems were stained and 25 putative loci were resolved.

Statistics

The IBM PC version 1.7 of the BIOSYS–1 computer program for the analysis of allelic variation in genetics (SWOFFORD & SELANDER 1981) was used to calculate parameters of intra- and interpopulation genetic diversity: mean sample size per locus, mean number of alleles per locus, percentage of polymorphic loci, mean heterozygosity expected from Hardy-Weinberg proportions, estimation of genetic differentiation and genetic distances. The data of allele frequencies were also used to calculate the proportion of total diversity among populations (G_{ST}) (NEI 1973, 1978), the total genetic diversity (H_T) and within populations diversity (H_S); G_{ST} was determined according to HAMRICK and GODT (1989) as $G_{ST} = (H_T - H_S)/ H_T$. The G_{ST} values were calculated for each polymorphic locus and then averaged over all loci.

RESULTS

Twenty five loci were resolved of them 15 (60%) were polymorphic in at least one of the stands. Allelic frequencies of 14 polymorphic loci (excluding *Cat–2*) is shown in Table 2. In contrast to the Israeli and the Jerash populations, the Ajlun population had only 11 polymorphic loci, the loci *Lap, Mdh–2* and *Mdh–3*, were monomorphic; and the third allele in the *Mnr* locus did not exist. Level of allozyme variation within the seven stands and within the Israeli and the Jorda-

Table 2 Alleleic frequencies of 14 isoenzyme loci in seven stands of *Pinus halepensis* in Israel and Jordan (isozyme abbreviations as in table 1; *n* – number of observations)

Locus	Allele	Carmel 1 $n = 30$	Carmel 2 $n = 23$	Car.mel 3 $n = 46$	Carmel 4 $n = 28$	Carmel 5 $n = 45$	Jerash $n = 20$	Ajlun $n = 56$
Aap-1	1	0.350	0.304	0.326	0.393	0.239	0.393	0.393
	2	0.650	0.696	0.674	0.607	0.761	0.607	0.607
Aco	1	0.883	0.935	0.815	0.768	0.935	0.804	0.714
	2	0.117	0.065	0.185	0.232	0.065	0.196	0.286
Adh-2	1	0.883	0.783	0.859	0.929	0.815	0.784	0.852
	2	0.117	0.217	0.141	0.071	0.185	0.216	0.148
Got-3	1	0.900	0.913	0.663	0.607	0.543	0.579	0.571
	2	0.100	0.087	0.337	0.393	0.457	0.421	0.429
Idh-1	1	1.000	0.957	1.000	1.000	0.804	0.887	0.955
	2	0.000	0.043	0.000	0.000	0.196	0.113	0.045
Lap	1	0.883	0.891	0.793	0.929	0.913	0.932	1.000
	2	0.117	0.109	0.207	0.071	0.087	0.068	0.000
Mdh-2	1	0.033	0.087	0.087	0.036	0.026	0.021	0.000
	2	0.967	0.913	0.913	0.964	0.974	0.979	1.000
Mdh-3	1	0.800	0.674	0.707	0.714	0.763	0.769	1.000
	2	0.200	0.326	0.293	0.286	0.237	0.231	0.000
Mdh-4	1	0.300	0.174	0.228	0.179	0.092	0.201	0.266
	2	0.700	0.826	0.772	0.821	0.908	0.799	0.734
Mnr-1	1	0.083	0.196	0.109	0.196	0.044	0.143	0.222
	2	0.583	0.478	0.500	0.625	0.467	0.394	0.778
	3	0.334	0.326	0.391	0.179	0.489	0.463	0.000
Pgi-2	1	0.833	0.848	1.000	1.000	1.000	1.000	1.000
	2	0.167	0.152	0.000	0.000	0.000	0.000	0.000
Pgm-2	1	0.283	0.152	0.098	0.232	0.043	0.263	0.089
	2	0.717	0.848	0.902	0.768	0.957	0.737	0.911
6Pgd-2	1	1.000	1.000	1.000	1.000	0.946	1.000	1.000
	2	0.000	0.000	0.000	0.000	0.054	0.000	0.000
6Pgd-3	1	1.000	1.000	0.967	1.000	0.989	0.997	0.982
	2	0.000	0.000	0.022	0.000	0.000	0.003	0.018
	3	0.000	0.000	0.011	0.000	0.011	0.000	0.000

Table 3 Genetic diversity within the seven stands of *Pinus halepensis* and in the Israeli and Jordanian groups

Population	N	L	A	A_p	P	H_e
Carmel 1	30	25	1.522	2.091	0.478	0.145
Carmel 2	23	25	1.565	2.083	0.522	0.149
Carmel 3	46	25	1.565	2.182	0.478	0.154
Carmel 4	28	25	1.478	2.100	0.435	0.143
Carmel 5	45	25	1.609	2.077	0.565	0.136
Israeli group	**172** [1]	**25**	**1.548**	**2.106**	**0.496**	**0.145**
Jerash	20	25	1.565	2.083	0.522	0.164
Ajlun	56	25	1.391	2.000	0.391	0.116
Jordanian group	**76** [2]	**25**	**1.478**	**2.041**	**0.456**	**0.140**
Overall mean	35	25	1.528	2.088	0.484	0.144
s.e.	5		0.028	0.020	0.022	0.006

N – number of individual trees or megagametophyts analyzed; L – number of loci sampled, A – number of alleles per locus; A_p – number of alleles per polymorphic locus, P – polymorphic loci; H_e – expected heterozygosity. [1] No. of trees analyzed; [2] No. of megagametophytes analyzed.

Table 4 Genetic diversity statistics of polymorphic loci (unbiased for sample size and population number)

Locus	H_t	H_s	D_{st}	G_{st}
Aap	0.451	0.452	0.000	0.000
Aco	0.276	0.266	0.010	0.036
Adh–2	0.265	0.263	0.002	0.006
Got–3	0.441	0.397	0.043	0.098
Idh–1	0.109	0.099	0.009	0.085
Lap	0.172	0.166	0.005	0.030
Mdh–2	0.080	0.079	0.001	0.012
Mdh–3	0.352	0.334	0.018	0.051
Mdh–4	0.328	0.324	0.004	0.012
Mnr	0.591	0.550	0.041	0.070
Pgi–2	0.089	0.078	0.011	0.123
Pgm–2	0.279	0.266	0.014	0.048
6Pgd–2	0.015	0.015	0.001	0.039
6Pgd–3	0.018	0.018	0.000	0.000

H_t – genetic diversity within populations; H_s – total genetic diversity; D_{st} – ; G_{st} – proportion of total diversity among populations

nian groups is relatively similar (Table 3). Average number of alleles per polymorphic locus (A_p) and percentage of polymorphic loci (P) was 2.106 and 0.496 in the Israeli group and 2.041 and 0.456 in the Jordanian group, respectively. Expected heterozygosity was higher in the Israeli group 0.145, than in the Jordanian group 0.140, respectively. Over all mean expected heterozygosity was 0.144±0.006; the level variation of genetic diversity within the Israeli group is smaller than in the Jordanian group. Diversity statistics of polymorphic loci (Table 4) shows that the genetic diversity within populations was higher than the diversity among populations.

Nei's genetic identity or distances among the seven stands and between the Israeli and Jordanian Aleppo pine groups (Table 5) shows that the genetic distance between the seven stands is very small, *i.e.*, they are virtually identical. The mean value of Nei's genetic identity for Israel was 0.994, with a range between 0.985 to 0.999. For Jordan the mean genetic identity was 0.988. Genetic distance between the two groups was very small 0.006. The range of genetic distance within groups was from 0.001 to 0.015. The smallest genetic distance between pairs was 0.001 (stand 1 and stand 2 on Mt. Carmel; stand 3 on Mt. Carmel and Jerash); the greatest genetic distance was 0.020 (stand 2 on Mt. Carmel and Ajlun). Values of genetic variation (Table 6) show that the total genetic diversity (H_T), including the monomorphic loci, of the seven populations was 0.143 (s.e., 0.005), the mean genetic diversity (H_S) within populations was 0.143 (s.e., 0.005) and the mean proportion of total diversity due to differences between populations (G_{ST}) was 0.043, *i.e.*, 4.3%.H_T, H_S and G_{ST} of the Jordanian group were lower than those of the Israeli group.

DISCUSSION

According to SCHILLER *et al.* (1985) allele frequencies within the two loci *Aap–1* and *Cat–2* distinguish between the East Mediterranean and the West Mediterranean group of *P. halepensis* native populations. In our study, no significant difference was found between

Table 5 Nei's genetic identities (above) or distances (below) and means of identities (right) and distances (below) among the seven populations and between the Israeli and Jordanian *Pinus halepensis* groups

Population	Carmel 1	Carmel 2	Carmel 3	Carmel 4	Carmel 5	Jerash	Ajlun	Mean
Carmel 1		0.999	0.994	0.994	0.985	0.993	0.985	0.992
Carmel 2	0.001		0.996	0.993	0.990	0.994	0.981	0.992
Carmel 3	0.006	0.004		0.998	0.995	0.999	0.987	0.995
Carmel 4	0.006	0.007	0.002		0.991	0.998	0.994	0.995
Carmel 5	0.015	0.010	0.005	0.009		0.997	0.983	0.990
Jerash	0.007	0.006	0.001	0.002	0.003		0.988	0.995
Ajlun	0.015	0.020	0.013	0.006	0.018	0.012		0.986
Mean	0.008	0.008	0.005	0.005	0.010	0.005	0.004	

Group	Mean	Identity range		Mean	Distance range	
Israel	0.993	0.985	0.999	0.006	0.001	0.015
Jordan	0.988		0.988	0.012		0.012

Table 6 Values of genetic variation of *Pinus halepensis* and of geographic groups

Populations	H_t	H_s	D_{st}	G_{st}
All seven stands	0.149	0.143	0.006	0.043
Israeli group	0.149	0.143	0.006	0.043
Jordan group	0.142	0.137	0.005	0.035

the Mt. Carmel and Jordanian Aleppo pine native populations in the allele frequencies in these two loci. This enables us to conclude that the Jordanian native Aleppo pine is part of the East Mediterranean group. However, the results show that there is a small difference in numbers of polymorphic loci and allele frequencies between Israeli and Jordanian native populations mainly due to differences occurring in the Ajlun population in the *Lap, Mdh–2* and *Mdh–3* loci. Comparison of genetic characteristics between the Israeli and Jordanian populations shows a high level of genetic similarity or genetic identity between this two groups. The genetic identity between the two groups of populations was 0.991±0.001; this in comparison with the genetic identity within the native Israeli group among the five stands on Mt. Carmel which was 0.994 ±0.005. Similar identity measure was also found among all the eight native relict populations in Israel of 0.994±0.005 (GRUNWALD *et al.* 1986); genetic identity with other circum Mediterranean populations was found to be only 0.977±0.015 (SCHILLER *et al.* 1985). Nevertheless, small differences were found; in three out of the 15 polymorphic loci values of H_T were less than 0.10, showing that the common allele at these loci has a frequency higher 0.95. According to HAMRICK *et al.* (1992) and EDWARDS and HAMRICK (1995), the fact that allele frequencies are nearly fixed in only a few enzyme systems is typical of many conifer species.

Similarity in allele frequencies at the *Cat–2, Aco* and *Mdh–4* locus was used by GRUNWALD *et al.* (1986) to divide the native Israeli eight relict populations into three different geographic assemblages of Galilee, Mt. Carmel and Samaria and Judea. Our present study shows that the allele frequencies of *Cat–2* and *Aco* in the Jordanian stands are similar to those of the Samaria and Judea group, and less so to the Mt. Carmel or the Galilee group. This results are logic from the geographical point of view as the aerial distance between populations of the Samaria and Judea group and the populations of the Jordanian group are less then 60 km.

To conclude, the high similarity between larger parts of Cis- and Trans Jordan landscapes (ZOHARY 1962 1973); and because of the very high resemblance of the insect fauna in the pine forests on both sides of the Jordan, which where both geographically separated from the forests in Lebanon and Syria (GOAS 1995; MENDEL *et al.* 1994) strengthen the assumption resulted from allozyme analysis, that *P. halepensis* in Cis- and Trans Jordan are relicts of a single ancestor population, established on both sides of the rift valley probably during the Pleistocene (HOROWITZ 1992), arriving from North Africa (SCHILLER *et al.* 1985). As the result of migration to particular habitats followed by isolation that population has split into local races corresponding to the various relict occurrences.

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