TABOR OAK IN ISRAEL, GENETIC DIVERSITY WITHIN AND BETWEEN POPULATIONS [1]

Leonid Korol [1], Galina Shklar & Gabriel Schiller

* Contribution number 127/2002 of the
Department of Agronomy and Natural Resources, Agricultural Research Organization, The Volcani Center,
P.O.Box 6, Bet Dagan 50250, Israel.
[1] Author to whom correspondence should be addressed.

ABSTRACT

Tabor oak (*Quercus ithaburensis* [Desc.]) is a deciduous and thermophilous species growing at altitudes between 50 to 500 m a.s.l. along the eastern shores of the Mediterranean Sea. It is one of many Mediterranean oaks of which we lack the genetic knowledge necessary for genetic conservation and forest management. We hypothesized that as the result of extensive destruction and fragmentation of the forest area, as a result of human activities, and the existence of a geo-climatic cline, differences in genetic diversity and structure among 16 *Q. ithaburensis* relict populations in Israel are inevitable. The specific objective of this study was to acquire knowledge on within- and between-populations genetic diversity by means of molecular DNA markers based on PCR methods (RAPD). The results show that total genetic variation (H_t) was 0.4142, Gene diversity within populations (H_s) ranged from 0.329 to 0.396, with an average of 0.3619; and differentiation among populations (G_{st}) ranged from 0.0754 to 0.1056, with an average 0.1263. UPGMA analysis based on genetic distances revealed three main clusters of populations that are coherent with geographic regions of the country: 1 – the Golan Heights and Upper Galilee group, 2 – The Lower Galilee group, 3 – The Mt. Carmel, Samaria and Coastal Plain group. These results support our hypothesis on genetic differentiation of populations according to site geo-climatic conditions.

Key words: *Quercus ithaburensis*, genetic diversity, differentiation, Israel

INTRODUCTION

The Tabor oak (*Quercus aegilops* L. ssp. *ithaburensis* [Decaisne]) is a deciduous and thermophilous species growing at altitudes between 50 to 500 m a.s.l., rarely up to 1000 m (ZOHARY 1973; KAPLAN 1984), in countries bordering the eastern shores of the Mediterranean Sea. Its area of distribution extends from latitude 37°30'N in Turkey (*i.e.*, the coastal plain bounded by Mersin – Adana – Iskandereun) via Syria, Lebanon and Jordan, to latitude 32° 00' N in Israel (the coastal plain north of Tel Aviv); and between longitudes 34° 30' E and 37° 00' E (AVI-SHAI 1967).

Since humans settled in the Middle-East, waves of land colonization have strongly contributed to the very extensive destruction and fragmentation of the Mediterranean sclerophyllous broad-leaf forests in the area (*e.g.*, EIG 1933; ZOHARY 1962, 1973; ALONI & ORSHAN 1972; KARSCHON 1982). This process

has created island-like scattered small populations that, because of geographic features probably cannot exchange genetic material; and this creates the danger of genetic drift. On the other hand, there is no doubt that since antiquity Tabor oak was also planted as a supplementary food source, because of its very large and sweet acorns (ELIAV 1985). In spite of its importance as a dominant tree species in Israel's landscape, *Q. ithaburensis* is among the many Mediterranean oaks of which we lack the genetic knowledge needed as the basis for genetic conservation (SCHIRONE & SPADA 2000) and forest management.

We hypothesized that as the result of the strong fragmentation of the forest area and the existence of a geo-climatic cline, differences in genetic diversity and structure among *Q. ithaburensis* relict populations are inevitable. The specific objective of this study was to acquire knowledge on genetic diversity within and between-populations by means of

molecular DNA markers based on PCR methods (RAPD).

MATERIAL AND METHODS

Plant material

The geographical properties of the 16 Q. ithaburensis populations in Israel, that are assumed to be natural relics of a more coherent prehistoric area of distribution, are presented in Table 1. In each population, separate samples of young leaf material that had not been affected by insects or fungi were taken from about 50 randomly selected trees; the distance between sampled trees was usually some tens of meters. A total of 789 trees were sampled, the leaf material was stored at –20 °C pending DNA extraction.

DNA extraction and RAPD procedure

Leaves collected from each tree were ground in liquid nitrogen with a handle-driven pestle and homogenized in an extraction buffer (100 mM Tris, 1.4 M NaCl, 20 mM EDTA, 2 % CTAB, 2 % PVP-40, 0.2 % 2-mercaptoethanol, pH 8.5). Total genomic DNA was extracted according to DOYLE and DOYLE (1990), with minor modification. The homogenate was incubated at 60 °C for 40 min and then extracted in an equal volume of chloroform for 30 min. After centrifugation at 12,000 rpm for 10 min, the aqueous phase with the DNA was precipitated in isopropanol and washed in 70 % ethanol. DNA was dried and re-suspended in 10 mM Tris, 1mM EDTA, pH 8.0. The samples from this procedure were incubated for 30 min at 37 °C with RNase (10 mg·ml⁻¹). The DNA concentration of each

sample was measured by spectrophotometric assay at 260 nm.

The best conditions for amplification of all primers were obtained in a total volume of 15 µl containing: 1.0 unit Taq-polymerase; 50 mM Tris-HCl, pH 9.1; 20 ng DNA; 3.5 mM MgCl₂; 200 µM dNTP (Sigma); 150 µg·ml⁻¹ BSA; and 5 pmol primer (Operon kits A, B, D). The amplification parameters were: denaturation step at 95 °C for 2 min, followed by 44 cycles of 94 °C for 1 min, 37 °C for 1 min and 72 °C for 2 min, followed by the last step of 72 °C for 5 min. The amplification products were loaded on 1.8 % agarose gel (TBE was used as a running buffer) and were electrophoresed. The gels were stained with 0.5 µg·l⁻¹ ethidium bromide for 30 min (SAMBROOK et al. 1989). The molecular sizes of the RAPD products were estimated by comparison with molecular weight markers (pGEM, Promega).

Data analysis

Intra- and inter-population genetic diversity was calculated by means of the POPGENE software, version 1.32 (YEH et al. 1997). Shannon's diversity index was calculated to estimate the degree of genetic variation according to LEWONTIN (1972). Single-locus analyses of the genotype were done to estimate heterozygosity (NEI 1973), and G^2 tests were used to determine the heterogeneity of RAPD frequencies across populations. Intrapopulation diversity, G_{st} was calculated according to NEI (1978). Differentiation between populations was analyzed by the component diversity between population (NEI 1977). Multiple comparisons of differences between populations and groups of populations were done by means of ANOVA (SigmaStat statisti-
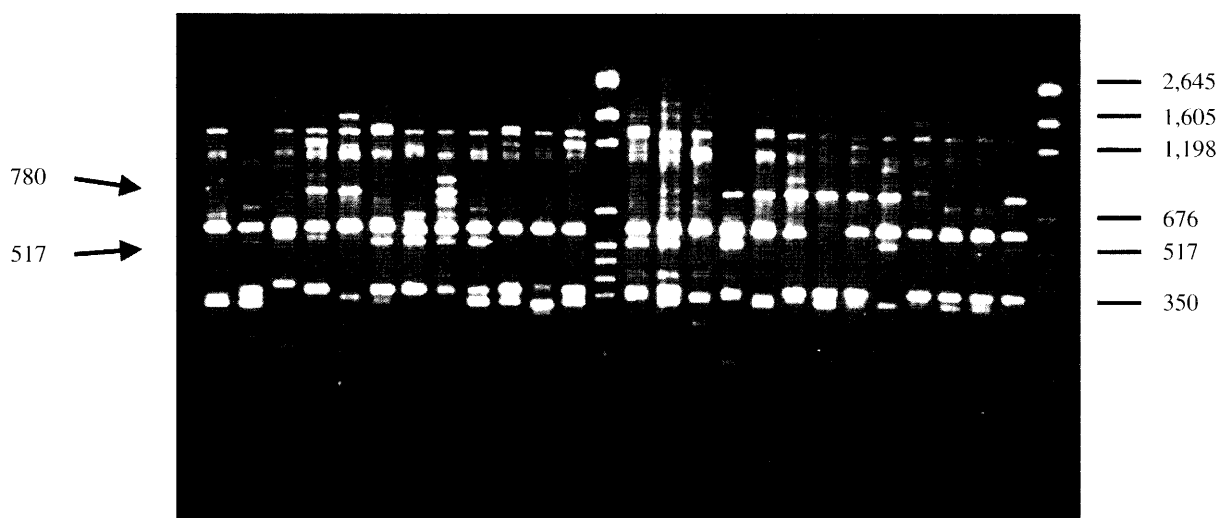

Figure 1. RAPD profiles amplified with OPD-20 primer (Operon). Right lines are the DNA molecular weight markers (pGEM, Promega). Left lines are molecular weight of scored polymorphic fragments. Molecular sizes are shown in bp.

Table 1. Geographic location, soil and bedrock and annual rainfall at the populations sampled.

Population			Latitute	Longitude	Altitude (m)	Bedrock formation	Soil type	Annual rainfall (mm)
A. The Golan Heights and the Upper Galilee								
1	Ein Zivan	EZ	2785	2240	950	Basalt	Basaltic lithosol	988
2	Wadi Metzer	WM	2385	2155	250	Chalk and marl covered with nari	Brown Rendzina	450
3	Yehudiya forest	YF	2628	2145	250	Basalt	Basaltic lithosol	584
4	Horshat-Tal	HT	2925	2100	100	Travertine	Brown rendzina & alluvium	809
B. The Lower Galilee								
5	Kachal	Ka	2560	1980	200	Lime stone and chalk	Brown rendzina	492
6	Bet Keshet	BK	2355	1875	150	Marl covered by nari, chalk and limestone	Grey rendzina	536
7	Ha Movil Junction	HJ	2425	1782	215		Brown rendzina	582
8	Waldheim	Wa	2385	1650	200	Marl covered by nari	Brown rendzina	648
9	Ramat - Johanan	RJ	2445	1645	175	Chalk	Brown rendzina	566
						Marl covered by nari		
C. Mt. Carmel								
10	Ha' Muchraka	Mu	2310	1585	350	Limestone	Terra Rosa	687
11	Bat Shlomo	BS	2238	1510	150	Marl covered by nari	Light Rendzina	661
D. Samaria								
12	Alona forest	AF	2175	1488	75	Chalk	Brown rendzina	581
13	Hirbet Zekress	HZ	2067	1508	50	Calcareous sandstone	Hamra	603
14	Eiron forest	EF	2100	1558	125	Limestone with nari	Terra Rosa	685
E. The Coastal Plain								
15	Hedera North	HN	2070	1410	50	Calcareous sandstone	Hamra	603
16	Ilanot - Kadima	Il-K	1890	1420	75	Calcareous sandstone	Hamra	621

Table 2. List of RAPD primers used, their sequence and number of fragments.

Primer name	No. of total bands (T)	No. of polymorphic bands (P)	Sequence (5' –3')
OPA01	7	2	CAGGCCCTTC
OPA05	3	2	AGGGGTCTTG
OPA12	9	3	TCGGCGCTCG
OPA15	8	5	TTCCGAACCC
OPA17	10	7	GACCGCTTGT
OPA18	6	2	AGGTGACCGT
OPA19	7	4	CAAACGTCGG
OPA20	4	4	GTTGCGATCC
OPB01	5	3	GTTTCGCTCC
OPB02	4	3	TGATCCCTGG
OPB04	5	4	GGACTGGCGT
OPB05	6	4	TGCGCCCTTC
OPB14	4	3	TCCGCTCTGG
OPB16	4	2	TTTGCCCGGA
OPB20	4	2	GGACCCTTAC
OPD03	7	6	GTCGCCGTCA
OPD06	8	5	ACCTGAACGG
OPD12	5	2	CACCGTATCC
OPD17	6	5	TTTCCCACGG
OPD20	4	3	ACCCGGTCAC

cal software, SPSS Inc.). Genetic distances between populations were computed by considering every locus, according to NEI (1978). Cluster analyses were performed with the unweighted pair group algorithm (UPGMA).

RESULTS

Variation of RAPD markers within population

Twenty of the 60 primers tested showed clear polymorphic banding patterns. These 20 random primers, which were chosen for analysis, generated 72 RAPD fragments that ranged in size from 300 to 2000 bp (Figure 1). Each random primer was represented by 2 to 7 scored RAPD fragments (Table 2) with a mean number of 3.5. The 16 populations were examined by RAPD, and all 72 loci scored were polymorphic within the species, although some loci were monomorphic in some of the populations. The G^2-test for heterogeneity of RAPD frequencies across populations showed that four of the 72 RAPD markers (OPA15-4, OPA18-1, OPA20-4 and OPB20-1) were heterogeneous across all populations. The frequency of some markers in the populations ranged from 6 to 96.9 %. The populations were similar to one another in their polymorphism, which ranged from 91.7 % in the Horshat-Tal population to 100 % in Ha'Movil Junction population (Table 3). Gene diversity within populations (H_s) was

calculated for each primer, and the mean H_s values were calculated as the averages of all RAPD markers (Table 3). The highest H_s value was obtained in the Ha'Movil Junction population, and the lowest, of only 0.323, in the Horshat-Tal population. The mean within-population genetic diversity (H_s) across the 16 populations was 0.362 (Table 3). RAPD primers varied in their power to detect diversity within populations: among the 16 populations, the differentiation (G_{st}) of the RAPD loci ranged from 0.0244 in the OPB20-1 to 0.4812 in the OPB14-3.

Differentiation among populations

Genetic diversity parameters based on allele frequencies are presented in Table 3, which shows that all the population showed very similar levels of variation. The average number of alleles (N_a) ranged from 1.917 in the Horshat-Tal population to 2.0 in the Ha'Movil Junction population; and Shannon's information index (I) ranged from 0.481 to 0.580, respectively, in these populations. The effective number of alleles (N_e) ranged between 1.554 at the Horshat-Tal population to 1.704 in the Ha'Movil Junction population.

UPGMA analysis based on genetic distances revealed three main clusters of populations (Figure 2): 1 – the Golan Heights and Upper Galilee group, 2 – The Lower Galilee group, 3 – The Mt. Carmel, Samaria and Coastal Plain group. Within the groups the proportions of total diversity among populations

Table 3. Genetic diversity estimates within populations of _Quercus ithaburensis_ (Desc.).

Population		Abbreviation	N_{pol}	$P\%$ 99	N_a	N_e	H_s	I
1	Ein Zivan	EZ	71	98.61	1.986	1.635	0.367	0.543
2	Kachal	Ka	70	97.22	1.971	1.669	0.380	0.558
3	Bet Keshet	BK	70	97.22	1.972	1.612	0.356	0.530
4	Alona Forest	AF	69	95.83	1.958	1.571	0.333	0.498
5	Hirbet Zekkes	HZ	69	95.83	1.958	1.646	0.370	0.545
6	Horshat Tal	HT	66	91.67	1.917	1.554	0.323	0.481
7	Wadi Metzer	WM	70	97.22	1.972	1.597	0.344	0.512
8	Yhudiya Forest	YF	70	97.22	1.972	1.662	0.376	0.551
9	Ha' Movil Junction	HJ	72	100.00	2.000	1.704	0.396	0.580
10	Bat Shlomo	BS	71	98.61	1.986	1.654	0.369	0.542
11	Waldheim	Wa	70	97.22	1.972	1.628	0.362	0.535
12	Ilanot-Kadima	Il-K	69	95.83	1.958	1.636	0.364	0.540
13	Ramat Johanan	RJ	68	94.44	1.944	1.635	0.363	0.535
14	Ha' Muchraka	Mu	70	97.22	1.972	1.638	0.360	0.530
15	Hadera North	HN	68	94.44	1.944	1.598	0.346	0.513
16	Eiron Forest	EF	70	97.22	1.972	1.690	0.387	0.564
	Mean		69.6	96.61	1.966	1.633	0.362	0.535

N_{pol} = number of polymorphic loci,
$P\%99$ = percentage of polymorphic loci,
N_a = average number of alleles,
N_e = effective number of alleles [CROW AND KIMURA (1964)],
H_s = NEI's (1973) gene diversity expressed as heterozygosity,
I = Shannon's information index [LEWONTIN (1972)].

Table 4. Differentiation between groups in _Quercus ithaburensis_.

Group	Abbrev.	N_{pol}	$P\%_{99}$	H_t	H_s	G_{st}	Nm
Golan Heights und Upper Galilee group	GR 1	69	96.18	0.3939	0.3523	0.1056	4.2354
Lower Galilee group	GR 2	70	97.22	0.4091	0.3717	0.0914	4.9705
Mt. Carmel, Samaria and Coastal Plain group	GR 3	70	96.66	0.3937	0.3640	0.0754	6.1320
Across all populations		69.6	96.61	0.4142	0.3619	0.1263	3.4600

N_{pol} = number of polymorphic loci,
$P\%_{99}$ = percentage of polymorphic loci
H_s = gene diversity within population,
H_t = total gene diversity,
G_{st} = component diversity between population $(H_t-H_s)/H_s$ (NEI 1977),
Nm = estimate of gene flow from G_{st} or G_{cs}. e.g., $Nm = 0.5(1 - G_{st})/G_{st}$; see McDERMOTT & McDONALD (1993).

(G_{st}) decrease from the Golan Heights in the north to the Coastal Plain area in the south (_i.e._, from GR1 to GR3 (Table 4)). The major part of total genetic variation (H_t = 0.4142) was expressed within populations (H_s = 0.3619), only 12.63 % of the total diversity was found between populations (G_{st} = 0.1263).

Three of the 20 RAPD loci, namely OPA15-2, OPB04-1 and OPB14-3, appeared to be area specific

(Figure 3). There were significant differences among populations growing in different areas, in the frequencies and presence of bands. Differences between the Golan Heights group and the Samaria – Coastal plain group in the frequencies of OPA-15-2 were significant ($P = 0.005$); for OPB04-1, $P = 0.004$ and for OPB14-3, $P = 0.003$.
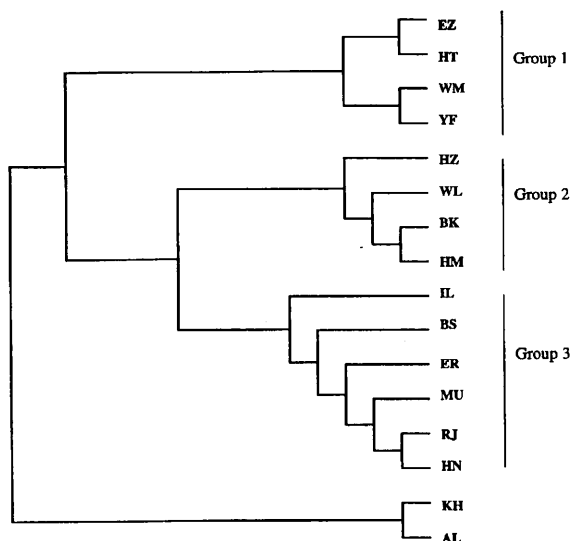
Figure 2. UPGMA dendrogram based on genetic distance values (NEI 1978) between *Quercus ithaburensis* populations.
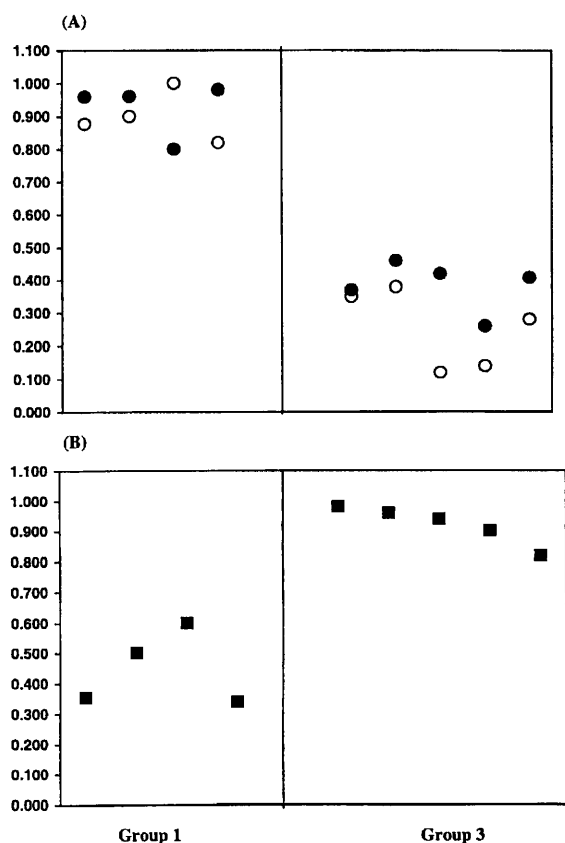

Figure 3. Frequencies of RAPD markers in group 1 and group 3 populations of *Quercus ithaburensis*. (A) Frequencies of OPB14-3 (o), and OPA15-2. (●); (B) Freuencies of OPB04-1 (■). Group 3 without the Hirbet Zerkess population.

DISCUSSION

RAPD markers have been shown to be a powerful tool for analyzing genetic diversity in *Q. ithaburensis*. The large numbers of polymorphic loci (72) and of trees sampled within populations (50), to avoid sampling errors for low levels of differentiation, allow a good estimation of the genetic variation within and between populations: information that is needed in breeding and genetic resource conservation (DIAZ *et al.* 2001).

UPGMA analysis, based on genetic distances between populations, has revealed three main clusterings of populations, that correspond with three geographic regions of the country. These results support our hypothesis on genetic differentia tion of populations according to site geo-climatic conditions. Nevertheless, three out of the 16 populations analyzed did not conform with our premises: the Kachal and Alona forest populations formed a separate cluster (Figure 2), and the Hirbet Zerkess population, which is located in the Coastal Plain, clustered within the Lower Galilee group. Analysis of three site-specific RAPD markers indicated that the frequencies of the presence of these markers correlated with the results of the UPGMA analysis. The frequencies of these RAPD markers form an easily used means to define to which group a population is related. The dominating proportion of appearances taken by the RAPD marker OPB14-3 in the Hirbet Zerkess population was similar to its share of the marker distribution in the Lower Galilee group of populations. In the light of these results it seems plausible to suggest that the source of this population (possible artificial) is located in Lower Galilee.

Geo-climatic parameters, the founder effect, and levels of genetic flow are among the parameters that define the degree of differentiation between populations. The genetic structure of populations could also be changed drastically as a result of human activity as is the case in the eastern Mediterranean region. Nevertheless, in spite of the relatively short distances between populations (an average of 50 km), three clearly distinct regional groups of populations could be identified. These findings have implications for decisions on *in-situ* and *ex-situ* genetic conservation, and for forest management planning and practices. Population size and level of a genetic flow are very critical parameters, therefore, small populations, such as the Ilanot-Kadima population, should be enlarged as much as possible by the planting of local material.

Small populations are subject to a high risk of

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genetic drift and increased inbreeding, that can result in fixation and loss of rare alleles. Determination of the density of particular allelic configurations within small-spatial-scale populations of forest trees can be considered as the first step in the identification of loci involved in the microevolutionary processes (ALLARD 1975; LINHART et al. 1981; HAMRICK & GODT 1989). Furthermore, analysis of the variations among major environmental parameters and in the frequencies of multilocus genotypes could be a useful approach to the study of the selective forces involved in microevolu-tionary processes.

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