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This paper has been contributed in honor of Azaria Alon on the occasion of his 90th birthday.

From mesic to arid environments: Morphological and genetic divergence in *Asphodelus aestivus* Brot. populations

YAEL SAMOCHA,[a] GALINA SHKLAR,[b] LEONID KOROL,[B] AND MARCELO STERNBERG[a,*]
[a]*Department of Plant Sciences, Tel Aviv University, Tel Aviv 69978, Israel*
[b]*Institute of Field Crops, Agricultural Research Organization, The Volcani Center, Bet Dagan, 50250, Israel*

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ABSTRACT

We studied the effects of different environmental conditions on morphological and genetic variations in the Mediterranean geophyte *Asphodelus aestivus* in Israel. The study included six populations, ranging from mesic Mediterranean to arid ecosystems. The results showed important differential morphological traits in one population of the Mediterranean ecosystem (M1). They included longer leaves, longer inflorescences-stalks, and larger number of flower buds than those found among the other populations. We used RAPD analysis to get a genetic profile for all studied populations: 30 loci revealed by the use of 11 primers were studied on 30 plants from each study site. No unique allele was found. Nevertheless, the MI population was always different from the others in several molecular markers, and even created a different clade on the phylogenetic tree. Abiotic factors were considered to have caused the differentiation of the M1 population. A contingency χ^2 test for heterogeneity of RAPD frequencies across all locations revealed significant differences in 17 RAPD loci (57%). The estimated genetic diversity within populations was 0.287 according to Nei's gene diversity and 0.432 according to Shannon's genetic diversity index. Differentiation among six populations of the RAPD loci (G_{ST}) ranged from 0.014 to 0.354 with a mean of 0.096. Principal coordinate analysis indicated the presence of genetically distinct sub-regional groups. Genetic and geographical distances among *A. aestivus* populations along the environmental gradient were found to be correlated, with the exception of the M1 population. In the present study, levels of gene variability within populations were higher than those between populations. Nevertheless, if we discard the unique M1 population, a clear genetic pattern linked to aridity and geographical distance was discerned.

Keywords: aridity, desert, genotype, geophyte, Mediterranean, plant traits

INTRODUCTION

The genus *Asphodelus* (Liliaceae) includes tall striking annuals and perennials, distributed among five sections and 16 species (Lifante and Aguinagalde, 1996). *Asphodelus aestivus* Brot. is a common Mediterranean geophyte, with a short rhizome surrounded by tubers that enable it to persist in a dormant form through the dry warm summer (Pantis et al., 1994). *A. aestivus* grows all over the Mediterranean basin and is dominant in overgrazed and fire-degraded areas; it is generally unpalatable to grazers and possesses underground structures that are not affected by fire (Pantis and Margaris, 1988). In Israel, which is characterized by a mild and rainy winter and a prolonged, rainless, and hot summer, *A. aestivus* is distributed from the upper Galilee (mesic Mediterranean, 780 mm mean annual rainfall)

*Author to whom correspondence should be addressed.
E-mail: MarceloS@tauex.tau.ac.il

in the north to the central Negev (arid, 90 mm mean annual rainfall) in the south (Feinbrun-Dothan, 1986; Feinbrun-Dothan and Danin, 1998). Such a steep aridity gradient, in addition to being the source of genetic differences, might be the cause of spatial differentiation in morphological traits among plant populations (Nevo, 1988). The species has survived the hazardous combination of climatic fluctuations and prehistoric and historic detrimental human interventions, in the form of agro-pastoral activity, in a very wide array of ecological niches in the region (Pantis and Margaris, 1988; Pantis et al., 1994). Determination of the population genetic structure in this region provides opportunities for understanding evolutionary processes, i.e., speciation, over small spatial scales. Moreover, climatic gradients, such as that found in Israel, offer an ideal framework for studying morphological and genetic variation of plant species (Petru et al., 2006).

Natural selection might be driven by edaphic and climatic conditions, and can operate on both coding and non-coding sequences (Gupta et al., 2002). Studies often reveal genetic patterns that correspond to environmental characteristics such as edaphic conditions (Owuor et al., 1999; Li et al., 2000), water factors (Turpeinen et al., 2001; Fahima et al., 2002), temperature (DiMichele and Powers, 1982; Barnes and Laurie-Ahlberg, 1986; Reyes et al., 2003), altitude (Fahima et al., 2002), and general characteristics of the geographic region (Schiller et al., 2003). In Israel, which has widely differing rainfall amounts over a short geographic distance, genetic differences between populations have also been revealed (Flavell et al., 1986; Chalmers et al., 1992; Gupta et al., 2002; Karanth et al., 2004). Ecotypic differentiation related to aridity gradient has also been found in several annual plants, with regard to phenological traits such as flowering time (Aronson et al., 1992; Piano et al., 1996; Del Pozo et al., 2002; Petru et al., 2006), morphological traits such as plant size (Aronson et al., 1992), and reproductive traits (Aronson et al., 1993; Norman et al., 1998). However, despite the importance of Eastern Mediterranean ecosystems in the biodiversity of the Mediterranean basin (Auerbach and Shmida, 1987; Myers et al., 2000), our literature review revealed no studies that analyzed the genetic structure of natural plant populations of *A. aestivus*. In this case, neutral markers are useful for estimating the relative evolutionary importance of genetic factors such as mutation rates, gene flow, and genetic drift. Molecular markers are the most powerful tools for genetic identification. Allozymes have been useful for distinguishing genets in many plant species (Ellstrand and Roose, 1987; Hamrick et al., 1992). However, much variation still remains undetected by the use of allozymes, although they are codominant and provide a means of estimating the level of genetic variation. Random amplified polymorphic DNA (RAPD) analysis is a polymerase chain reaction (PCR)-based marker method that increases the number of markers without limit (Williams et al., 1990) and is, therefore, a good alternative to the use of allozymes for distinguishing genets. Moreover, RAPD can be used to estimate population genetic parameters (Lynch and Milligan, 1994).

In this study we posed the following question: Are RAPD and morphological profiles of given populations consistent with environmental conditions, such as aridity? The aim of the present study was to examine both phenotypic morphological traits and genetic differences among populations of *A. aestivus* along an environmental gradient in Israel. *A. aestivus* was chosen to examine this relationship because of its significance in the Eastern Mediterranean flora, as it is commonly found in open shrubland and grassland communities. The morphological study, coupled with genetic analysis of these populations, provided an opportunity to create a profile of the studied populations and to characterize the differing populations.

MATERIALS AND METHODS

The study sites

Six populations of *A. aestivus*, located across most of the geographical range of the species' distribution in Israel, were surveyed for phenotypic variation. Two groups, mesic and xeric, were chosen to represent the different climatic regions. The mesic group comprised the MM, M1, and M2 populations (mesic Mediterranean and Mediterranean ecosystem types, respectively) and the xeric group consisted of SA1, SA2, and AR populations (semiarid and arid ecosystem types, respectively, Fig. 1). These sites represented different abiotic conditions such as rainfall (from mesic Mediterranean through Mediterranean and semiarid to arid), and edaphic and altitude variations (Table 1). Population size was determined using visual estimations by counting the number of isolated groups of plants in a 10 × 10 m area.

Plant collection and morphological sampling

In summer 2003, tubers of 20 *A. aestivus* plants from the Mediterranean (M1 and M2) and semiarid (SA2) populations were collected and transplanted to the Botanical Garden of Tel Aviv University. The tubers were weighed and the number of roots per tuber was counted. The tubers of each population were buried in autumn before the onset of the rainfall season in a plot with local soil (mixture of terra rossa and kurkar soil). All plants were

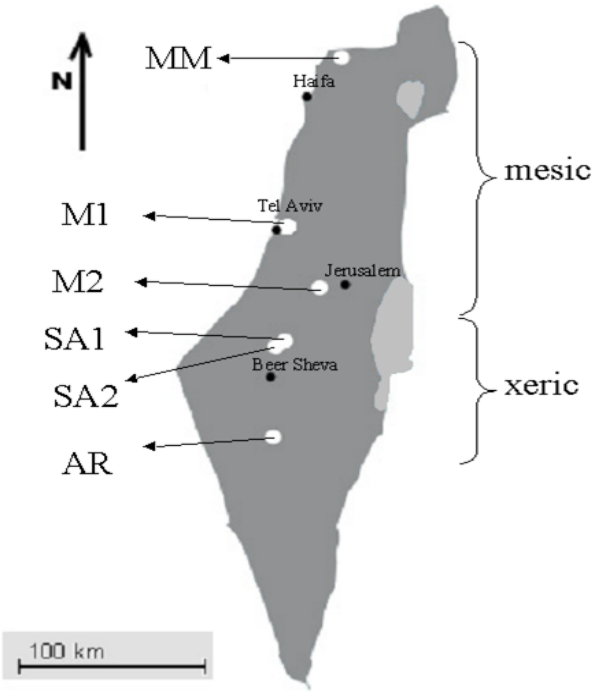

Fig. 1. Study sites of *Asphodelus aestivus* populations along the aridity gradient as follows: AR (Sde Boqer), SA1 (Lahav), SA2 (Lehavim), M1 (Ramat Aviv), M2 (Matta), and MM (Kabri).

exposed to similar climatic conditions (Mediterranean climate type). Tubers from remaining populations were not collected.

In winter 2003–2004, individuals within each population were randomly selected in the field from widely-spaced plant groups in order to minimize the chances of sampling individuals from the same genet. In each study site, 30 individuals of *A. aestivus* were sampled, i.e., a total of 180 plants for the whole field-sampling operation. In each sampling event the following morphological plant traits were monitored for each individual: (a) lengths of three random leaves, (b) inflorescence length, and (c) the total number of flower buds per inflorescence.

DNA extraction and RAPD analysis

In each population, undamaged leaf material was sampled from 30 different *A. aestivus* plants (180 individuals in total). The leaves were kept in plastic bags, in a container cooled with ice until they reached the laboratory, after which the leaf material was stored at –32 ºC pending DNA extraction. Before the extraction, the leaves were cleaned with 95% ethanol and ground in liquid nitrogen with a mortar and pestle. The genomic DNA was extracted and cleaned with the GenElute plant genomic DNA miniprep kit (Sigma, USA). DNA con-

Table 1
Geographic and physical characterization of the studied *Asphodelus aestivus* sites

Study site and location	Ecosystem type	Geographical regions	Elevation (m asl)	Bedrock	Mean annual rainfall	Population size	Clone size (mean ± SE)	
							Leaves per clone	Ramets per clone
Kabri (MM) N 33º0′ E 35º9′	Mesic Mediterranean	Western Galilee	500	Hard limestone	780	~3000	103.72 ± 14.67	10.23 ± 1.68
Ramat Aviv (M1) N 32º7′ E 34º48′	Mediter-ranean	Coastal plain	40	Kurkar (calcareous sandstone)	600	~500	101.56 ± 26.56	7.50 ± 2.18
Matta (M2) N 31º42′ E 35º3′	Mediterranean	Judean Hills	620	Hard limestone	540	~3000	24.31 ± 3.18	1.96 ± 0.36
Lahav (SA1) N 31º23′ E 34º54′	Semiarid	Northern Negev	590	Hard limestone	300	~3000	107.54 ± 12.67	12.00 ± 1.53
Lehavim (SA2) N 31º20′ E 34º45′	Semiarid	Northern Negev	280	Limestone and soft chalk	270	~3000	101.92 ± 20.87	9.42 ± 2.28
Sede Boker (AR) N 30º52′ E 34º46′	Arid	Mount Negev	470	Hard limestone	90	~1000	57.11 ± 9.24	4.93 ± 0.84

centrations were determined on 0.8% agarose gel and compared with a High DNA Mass Ladder (Invitrogen, USA) as weight markers. The analysis was performed with eleven 10-bp primers: OPA–09, OPA–10, OPA–11, OPA–16, OPB–04, OPB–09, OPB–11, OPB–12, OPB–13, OPB–14, and OPB–20 (Operon Technologies, USA). These primers were chosen out of 40 available primers (Operon Technologies kits A and B) because of their suitability to produce clear readable polymorphic loci (bands) and as a minimum, 3 repeated tests were provided for all samples.

All 40 primers were tested with six DNA samples from four populations. The 11 chosen primers that were used for the analysis generated 30 polymorphic loci (1–4 fragments for each primer), ranging in size from 200 to 2,750 bp. Amplifications were carried out in 15-µl volumes, each containing 1 µl of template DNA (≈40 ng), 10.45 µl of double-distilled sterile water, 1.5 µl of 2 mM $MgCl_2$, 1.5 µl of 10× buffer (Bio-Consult, Israel), 1.2 µl of 2.5 mM dNTP, 0.6 µl of primer at 3 pmol/µl, and 0.25 units (0.25 µl) of Taq polymerase (Norgen, USA). Polymerase chain reactions (PCR) were performed in a model 3300-B.M. thermal cycler (Biometra, Germany) with the program: 3 min at 94 ºC, followed by 45 cycles of 30 s at 94 ºC, 30 s at 36 ºC, 1 min at 72 ºC, followed by a final elongation step of 7 min at 72 ºC. The samples were kept at 4 ºC pending analysis. The amplification products were loaded on 1.8% agarose gel (with TBE as running buffer) and were electrophoresed. The gels contained 5 µl of ethidium-bromide per 100 ml TBE in order to enable them to be visualized and photographed under UV light. Molecular sizes of the RAPD products were estimated by comparison with molecular weight markers (pGEM, Promega, USA).

Data analysis

Analysis of variance (ANOVA) was used to analyze morphological differences among normally distributed variables of *A. aestivus* populations (normality was assessed with the Shapiro–Wilk W test). Variables that did not distribute normally were analyzed with the Kruskal–Wallis test. Post-hoc examination (in the case of significant differences) was performed with the Tukey–Kramer HSD test (in cases of normally distributed variables) and by Wilcoxon rank sum test (in cases of not normally distributed variables) by means of the JMP 5.01 software (SAS Institute, USA).

Each PCR product was assumed to represent a single locus. The RAPD-PCR fragments were analyzed as genetic markers under the following assumptions: (1) RAPD markers represent homologous loci and segregate in a Mendelian fashion; (2) the frequencies of occurrence of genotypes at RAPD loci follow Hardy–Weinberg proportions; (3) RAPD fragments behave as diploid, dominant markers, with alleles being either recessive "band-absent" or dominant "band-present" alleles. All calculations were performed only on polymorphic markers, i.e., those that were polymorphic across the whole data set. Photographs of ethidium-bromide-stained agarose gels were used to score the results of electrophoresis of RAPD-PCR fragments. Molecular diversity within each population was assessed by calculating the percentage of polymorphic fragments (P%), the Shannon diversity index (*I*), Nei's gene diversity (*h*); the number of observed alleles (na) and the effective number of alleles (ne) were calculated with POPGENE software, version 1.32 (Yeh et al., 1997). Gene diversity (*h*) equivalent to the expected heterozygosity (H_E) was estimated according to Nei (1973) and Shannon (Shannon and Weaver, 1949). Genetic diversity within each population was calculated for each locus and then averaged over all loci and populations. Estimates of *h* and *I* were obtained by averaging across loci. Genetic differentiation and within-population diversity were calculated according to Nei (1978). Estimates of genetic differentiation between populations were calculated with POPGENE. Corresponding estimates of gene flow (Nm), i.e., the average effective number of migrants exchanged between populations in each generation, were calculated according to McDermott and McDonald, 1993.

Genetic differentiation between populations was assessed by partitioning the genetic variance (Weir and Cockerham, 1984) by means of the ARLEQUIN software, version 2.00 (Schneider et al., 2000). A set of statistics was calculated: Φ-statistics, employing differences in allele frequencies only (analogous to F-statistics or Θ: Weir and Cockerham, 1984), and accounting for variance in size between pairs of alleles (Excoffier et al., 1992). Principal coordinate analysis was used to ordinate the relationship among individuals and populations by means of the Multi-Variate Statistical Package (MVSP) (Kovach, 1999). Pair-wise genetic distances between populations (Nei, 1978) were calculated from allele frequencies by using POPGENE. Mantel's test also was evaluated by using ARLEQUIN 2.0 software with 10,000 random permutations. The results were subjected to UPGMA clustering analysis. The phylogenetic inference package PHYLIP 3.5 (Felsenstein, 1993) was used to determine phylogenetic relations among the populations and to construct phylogenetic trees. We also used the Mantel permutation test to determine whether the genetic distances between populations correlated significantly with the corresponding measured geographical distances between the populations.

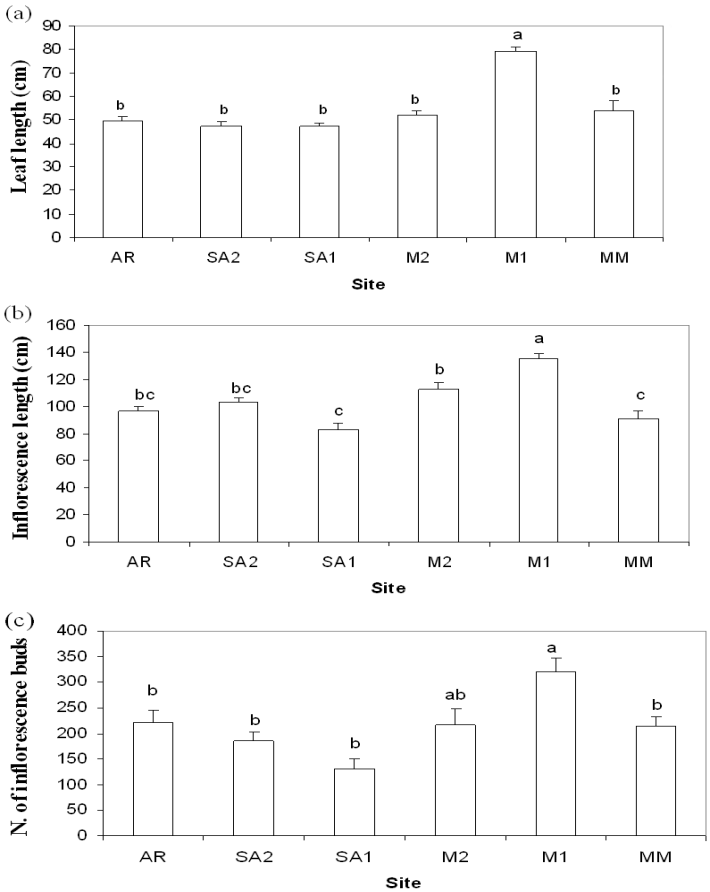

Fig. 2. Differences in length of leaf (a) (by Kruskal–Wallis test) inflorescences length (b) (by ANOVA test) and number of inflorescence buds (by ANOVA test) among populations of *Asphodelus aestivus* in Israel. Study sites are: AR (Sde Boqer), SA1 (Lahav), SA2 (Lehavim), M1 (Ramat Aviv), M2 (Matta) and MM (Kabri). Different letters indicate significant differences ($p > 0.001$) among sites according to Wilcoxon rank sum test (for leaf length) and Tukey's HSD test (for inflorescence length and number of inflorescence buds).

RESULTS

Morphological differences between populations

Leaf measurements showed significant differences ($p < 0.001$, by Kruskal–Wallis test) between the M1 population (with longer leaves) and all other populations (Fig. 2a). Similar patterns were noted for inflorescence length (Fig. 2b). The populations of MM and SA1 had significantly ($p < 0.001$, by ANOVA test) shorter inflorescences than that of M2. The populations of AR and SA2 were characterized by intermediate values. The number of inflorescence buds was also highest in the inflorescences from M1 (by ANOVA test) followed by intermediate value of the M2 population (Fig. 2c). The Botanical Garden-transplanted plants confirmed the results obtained from the field measurements. The M1 population showed significantly higher values for leaf length ($p < 0.05$, tested by ANOVA) and inflorescence length ($p < 0.01$, by Kruskal–Wallis test), while no difference was found in the number of flower buds among the populations ($p = 0.0501$, by Kruskal–Wallis test). These results are not likely to be attributed to differences in the morphological characteristics of the tubers brought from the different locations. No significant differences in the tubers' properties (tubers' weight and number of roots per tuber) were found between the different sources, which were examined before planting in the Botanical Garden ($p = 0.708$, $p = 0.391$, respectively, by Kruskal–Wallis test).

RAPD patterns and polymorphism

Out of the total number of RAPD loci that were produced by the use of the 11 chosen primers, only 30 polymorphic bands were chosen. The RAPD primers

Table 2
RAPD loci and allele frequency of each locus in all six studied populations

Sample no.	Primer name	Size (bp) of fragment	MM	M1	M2	SA1	SA2	AR	SD*
1	OPA-09	1150	0.000	0.107	0.103	0.206	0.069	0.000	0.077
2	OPA-09	600	0.931	0.785	0.758	0.931	0.862	0.896	0.073
3	OPA-09	200	0.414	0.714	0.413	0.620	0.517	0.448	0.123
4	OPA-10**	1000	0.897	0.586	0.821	0.607	0.482	0.344	0.206
5	OPA-10	670	0.069	0.000	0.000	0.035	0.000	0.000	0.029
6	OPA-10	580	0.172	0.620	0.392	0.392	0.275	0.275	0.154
7	OPA-10**	500	0.207	0.000	0.000	0.035	0.000	0.000	0.082
8	OPA-11**	1600	0.483	0.482	0.758	0.586	0.758	0.785	0.142
9	OPA-11	1100	0.241	0.000	0.206	0.241	0.172	0.000	0.114
10	OPA-11**	700	1.000	0.931	0.827	0.896	0.827	0.750	0.088
11	OPA-16**	2500	0.896	0.620	0.379	0.620	0.379	0.285	0.226
12	OPA-16	1200	0.759	0.310	0.689	0.655	0.517	0.714	0.167
13	OPA-16**	1160	0.965	0.758	1.000	1.000	0.965	0.607	0.162
14	OPB-04**	1140	0.620	0.344	0.793	0.827	0.827	0.724	0.186
15	OPB-09**	2750	0.571	0.758	0.392	0.517	0.344	0.310	0.168
16	OPB-09	1190	0.000	0.310	0.321	0.172	0.172	0.103	0.122
17	OPB-11**	1160	0.357	0.655	0.862	0.275	0.551	0.551	0.210
18	OPB-11**	1150	0.000	0.344	0.275	0.206	0.586	0.724	0.262
19	OPB-11**	1140	1.000	1.000	0.931	0.965	1.000	1.000	0.028
20	OPB-11**	520	0.571	0.241	0.069	0.206	0.275	0.137	0.173
21	OPB-12**	700	0.551	0.724	0.000	0.172	0.103	0.172	0.284
22	OPB-12**	680	0.793	0.965	0.931	0.689	0.758	0.931	0.112
23	OPB-13	2750	0.000	0.000	0.034	0.137	0.034	0.172	0.073
24	OPB-13	1140	0.310	0.275	0.241	0.206	0.103	0.172	0.074
25	OPB-13**	580	0.379	0.586	0.310	0.310	0.724	0.379	0.168
26	OPB-14	1500	0.689	0.206	0.448	0.517	0.642	0.413	0.174
27	OPB-14	1400	0.724	0.551	0.551	0.620	0.857	0.724	0.119
28	OPB-14	300	0.448	0.724	0.344	0.448	0.357	0.241	0.164
29	OPB-20**	700	0.379	0.000	0.000	0.142	0.103	0.000	0.148
30	OPB-20**	670	0.103	0.896	0.137	0.428	0.069	0.069	0.329

*—SD, standard deviation of allele frequency among population. **—Significant heterogeneity of RAPD frequencies.

Table 3
Genetic diversity indices and approximate population size of the six populations of *A. aestivus* studied

Population	Sample size	Npol	P%	na	ne	*h*	*I*
MM	30	24	80	1.80	1.485	0.285	0.425
M1	30	24	80	1.80	1.546	0.309	0.454
M2	30	25	83	1.83	1.467	0.277	0.417
SA1	30	29	97	1.97	1.503	0.303	0.463
SA2	30	27	90	1.90	1.493	0.287	0.433
AR	30	24	80	1.80	1.449	0.264	0.400
Mean		25	85	1.85	1.490	0.287	0.432

Npol–Number of polymorphic RAPD loci, P%—Percentage of polymorphic loci, na—Observed number of alleles, ne—Effective number of alleles, *h*—Nei's gene diversity, *I*—Shannon's diversity index.

varied in their power to detect diversity within populations: each primer amplified 1–4 clear and reproducible RAPDs. The contingency χ^2 tests of RAPD frequency heterogeneity between populations revealed significant differences in 17 RAPD loci (57%) across populations. Table 2 presents the average allele frequency for each locus in all six populations. The average number of effective alleles (Ne) for this species was 1.537 ± 0.323. Gene diversity within populations was also measured for each locus; the mean genetic diversity was calculated as the average of all RAPDs. The highest Hs value was 0.471 for locus OPB-04_{1140}; the lowest Hs value—of only 0.0175—was for locus OPA-10_{670}. The average genetic diversity was 0.318 ± 0.156 according to Nei's gene diversity, or 0.480 ± 0.198 according to Shannon's genetic diversity index. Differentiation of the RAPD loci among the six populations (G_{ST}) ranged from 0.014 in the OPB-13_{1140} to 0.354 in the OPB-20_{670}.

Intra-populational genetic diversity

Parameters involving differentiation among populations are presented in Table 3. The observed number of alleles (Na) was highest in the SA1 population and the effective

number of alleles (Ne) was highest in the M1 population (1.546). The percentage of polymorphic loci (P%), the Nei gene diversity (*h*), and Shannon's diversity index (*I*) for each population are also shown in Table 3. The frequency of the RAPD alleles in the populations ranged from 0 to 95.5%. Populations were similarly polymorphic, so that the percentage of polymorphism ranged from 80 to 97% (with a mean of 85%). On the basis of 95% confidence limits, the diversity *(h)* within the population of M1 was significantly higher than the average of the AR population. Shannon's diversity index (*I*) differed significantly between the SA1 and the AR populations: those of the SA1 population were highest, and those of the AR population were lowest.

Hierarchical analyses

The two chosen climatic groups, mesic (MM, M1, and M2 populations) and xeric (SA1, SA2, and AR populations), were used for the hierarchical analysis. Of the variations in RAPDs, 97% occurred within populations, with only 2% attributable to groups and 1% to the variation among populations within groups.

Among-population genetic differentiation

The single locations of RAPD null alleles in loci OBP-09_{1190}, OPB-01_{1150}, and OPB-12_{700} were found in populations MM and M2 (Table 2). Thus, only six (20%) out of 30 RAPD markers were not detected in three populations or less, and only three (10%) were confined to a single location. Two null alleles (OPB-09_{1190} and OPB-11_{1150}) were absent in populations MM and one (OPB-12_{700}) in M2, respectively; however they were always detected in other populations. The estimated overall population differentiation was calculated as G_{ST}(analogous to Fst). The average proportion of genetic diversity stemming from the differences between populations was 0.0966, which indicated that most of the gene variability occurred within populations. The corresponding average value of gene flow (Nm) was 4.68. G_{ST} for the mesic group was 0.104, with a corresponding Nm of 4.431; that for the xeric group was 0.049, with Nm of 9.679.

The relationship among populations was summarized by means of principal coordinat analysis. Axes 1 and 2 extracted 9.4 and 8.0% of the variance, respectively, and 17.4% of the total variation was described by the first two axes (Fig. 3). This analysis revealed similarity among the M2, SA2, and AR populations, as defined on the basis of geographic distance. On the other hand, the semiarid population (SA1) was differentiated from this group, although it was clustered together with the Mediterranean (M2) population (Fig. 4). Interestingly, the M2 population was more similar to that of the arid region, despite its location in the Mediterranean region. Population M1 was quite different from all the other analyzed populations.

Genetic distance analysis

Nei's genetic distances between populations were estimated from allele frequencies. Figure 4 shows the phylogenetic relationships among the studied populations of *A. aestivus*, based on the UPGMA method.

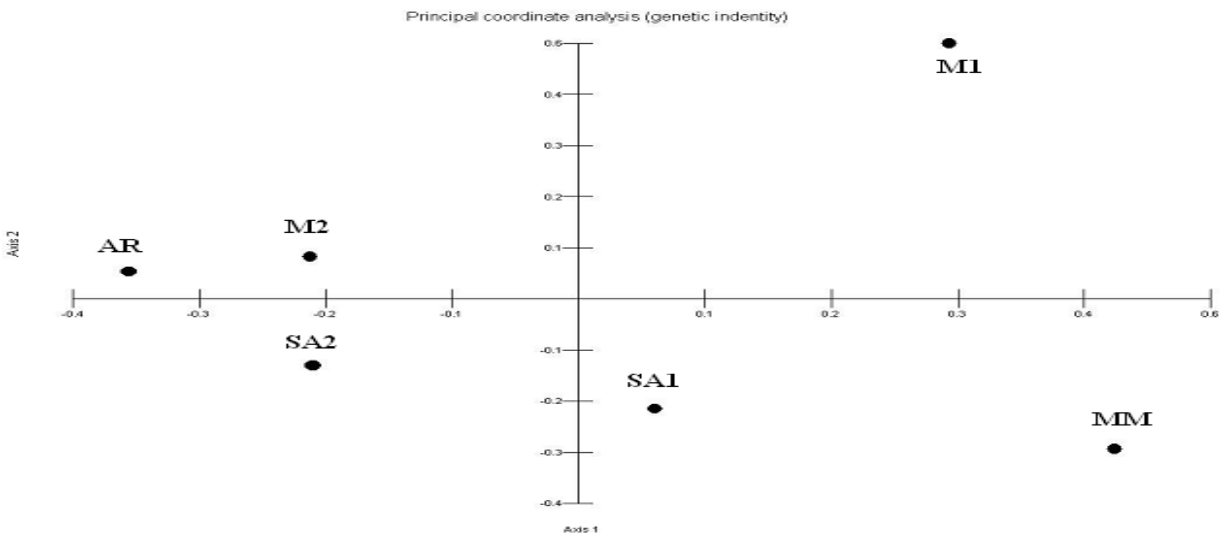

Fig. 3. Principal coordinate analyses of six populations of *Asphodelus aestivus* based on RAPDs frequencies. Study sites are: AR (Sde Boqer), SA1 (Lahav), SA2 (Lehavim), M1 (Ramat Aviv), M2 (Matta), and MM (Kabri).

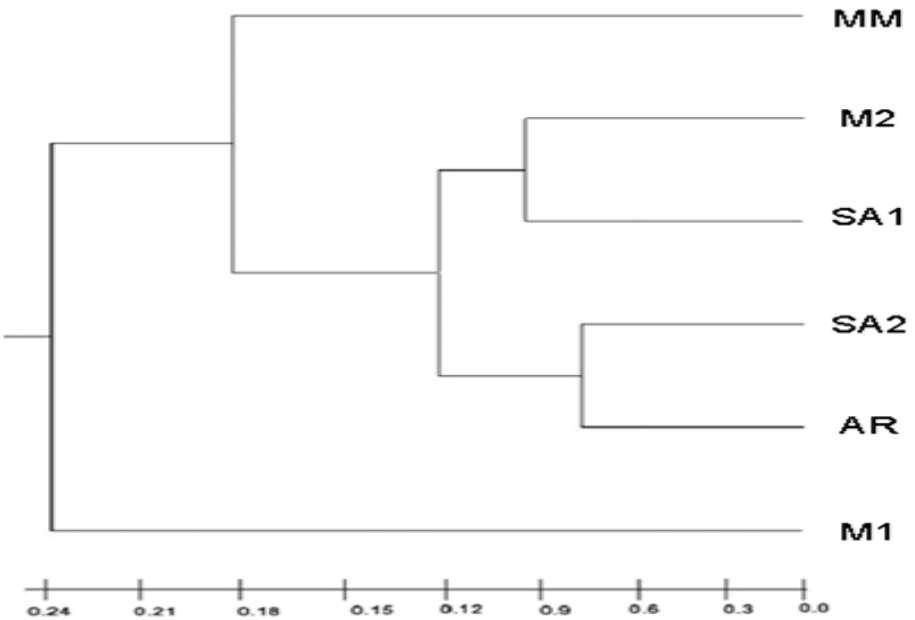

Fig. 4. UPGMA dendrogram of six geographic populations of *Asphodelus aestivus* based on Nei's genetic distances representing mesic and xeric climatic regions. Study sites are: AR (Sde Boqer), SA1 (Lahav), SA2 (Lehavim), M1 (Ramat Aviv), M2 (Matta), and MM (Kabri).

The distance analysis shows a high degree of genetic divergence between the population of M1 and all the other populations that are located in different clusters. The clade of the other populations showed great similarities between SA2 and AR and between M2 and SA1. The geographically close populations of SA1 and SA2 did not cluster, whereas the most northerly population, MM, created a different substitution in the clade. The UPGMA phenograms, based on Nei's (1978) unbiased genetic distances between all pair-wise combinations of populations, revealed differences in genetic structures of *A. aestivus*. On the basis of allele frequencies, *A. aestivus* appeared to constitute two different units, with two major clusters (Fig. 4) and two group populations. Genetic structures of M1 and MM differ from populations that cover areas with mean annual rainfall less than 540 mm. Moreover, the way populations of *A. aestivus* tended to cluster was independent of their geographical origin, and there was no significant association between genetic and geographical distances, as shown by Mantel's test ($r = 0.114$; $p = 0.362$). However, if we omit the M1 population from the analysis, the remaining populations tend to cluster according to aridity and geographical location, with significant association between genetic and geographical distance ($r = 0.771$; $p = 0.018$).

DISCUSSION

Intra-population gene diversity

The intra-population genetic diversity, which was studied within six populations by means of RAPD analysis, displayed within-population average diversity of 0.432 for Shannon's index (*I*) and 0.287 for Nei's expected heterozygosity (*h*), and the mean proportion of polymorphic loci (P%) was 85%. Unlike the findings of other studies—that diversity within populations increased with aridity (Nevo et al., 1994a; Ben-Shlomo et al., 1996)—our present results revealed no such pattern, and the diversity was quite homogeneous. The fact that adult plants of *A. aestivus* have the capability for vegetative growth (clone formation), can account for our finding of the same RAPD phenotype among several individuals sampled within a given population. Hence, all the populations we studied contained more than one genotype.

Differentiation among populations: The unique M1 population

Low genetic differentiation was detected among *A. aestivus* populations in Israel. The value of G_{ST} in our study was 0.096, which is lower than that reported for outcrossing species and for those with an autogamous breeding

system (Lifante and Aguinagalde, 1996; Nybom and Bartish, 2000). The highly variable and unpredictable desert environment may select genotypes suitable for local adaptation that can promote genetic divergence. Some specifically regional adaptive processes can give rise to quantitatively detectable traits. However, unlike studies that correlate between aridity and morphological characteristics (Kutrup et al., 2006), in the present study, none of the variations in the studied morphological traits were found to be consistent with the aridity: They all indicated that the Mediterranean population, M1, was different from the other populations along the gradient. The transplant experiment at the Botanical Garden strengthened the results obtained from field measurements. The morphological characteristics that differ in the M1 population were not found to be related to the aridity gradient. This fact might suggest that differentiation was the cause of the special characteristics revealed in this M1 population. The position of this M1 population in the principal coordinate analysis and the creation of a different clade in the UPGMA dendrogram, strengthen the hypothesis that the M1 population is on the verge of differentiation. If gene flow is overcome by habitat-specific natural selection, the result might be ecotypic differentiation. Differences in abiotic conditions could be the main driving factor behind the selection process that caused the differences observed when examining *A. aestivus* populations. It is important to consider the different bedrocks associated with the M1 ("kurkar", local name for calcareous sandstone) and the SA2 (limestone and chalk) populations. There are numerous examples of genetic changes within populations of plant species that were caused by differing soil types (Snaydon and Davis, 1982; Mopper et al., 1991; Nevo et al., 1994b; Linhart, 1998; Li et al., 2000). Population divergence in response to changes in environmental conditions is obviously complex, but the different bedrock and the consequently different soil may account for the variations of the M1 population. An additional factor that differentiates the M1 population from the others is related to its lower elevation (Table 1). Such environmental variation has also been found to be correlated with genetic variation (Fahima et al., 2002), and this may apply in our case. One possible reason for genetic differentiation is the accumulation of mutations in a separated population. Although mutation rates are unknown, somatic mutations may play an important role within the populations of *A. aestivus*.

Fluctuation in a population size may lead to a situation in which the population passes through a "bottleneck", in which only a few individuals survive, and later expands again under more favorable conditions. The M1 population is a small population (Table 1) that might have been subjected to various stress conditions due to anthropogenic influences (on account of the proximity to agricultural fields and urban environment). As a result, the selection process might have played an important role in shaping the population size and genetic structure. The frequencies of the genes in a small population may differ from those in the parental population, and high frequencies of rare alleles can occur. Although no unique allele was found in the M1 population, this population was always different from the other populations, both in genetic and in morphological traits (both in field and Botanical Garden transplanting experiments). A contingency χ^2 test for heterogeneity of RAPD frequencies across all locations revealed significant heterogeneity in frequencies in 17 RAPDs. Although vegetative reproduction usually causes low genetic variability within population, high levels of gene variability within the M1 population might be preserved due to this species' ability to reproduce vegetatively (M1 is characterized by large clones). The unique population of M1, which was distinct both genetically and morphologically, emphasized the clear connection between RAPD loci and morphological traits.

Differentiation among populations: Environmental factors and genetic traits

The lower genetic diversity in the xeric group is attributed to the shorter geographic distances between populations, which enables more gene flow. In fact, pair-wise G_{ST} values among populations were very low, and this suggests that the G_{ST} estimates were almost insensitive to mutations and migrations affecting the allele frequencies in the investigated populations. Surveys of genetic variations of plant species in Israel have shown clinal variations that appear to be related to environmental factors such as aridity and temperature (Reyes et al., 2003). Within the clade created by the UPGMA dendrogram, the oldest population is the mesic-Mediterranean (MM), followed by the split between the Mediterranean and the semiarid populations (M2 and SA1, respectively). The split between the other semiarid population (SA2) and the arid one (AR) formed the youngest populations. This interpretation of the UPGMA dendrogram might not be the only explanation, as the genetic distance between populations is affected not only by colonization history, but also by other factors such as founder effect, migration, local adaptation, or random genetic drift. Nevertheless, similar north–south gradients (from oldest to youngest) have also been found in other populations in Israel, such as the blind subterranean mole rat, *Spalax ehrenbergi* "super-species" (sensu Nevo et al., 1994a; Ben-Shlomo et al., 1996). This suggests a connection between environmental factors and the various stages of evolutionary divergence, as well as differential colonization times. However, the absence of significant

genetic subdivision among populations of *A. aestivus* shows that there is no considerable divergence within the species in two geographically separated sets of populations (xeric and mesic). Our morphometric data also support this point of view, and it is obvious that evolutionary processes and adaptation to an arid environmental could not induce incipient species formation (Templeton, 1986). Similar results have also been described for *Senecio glaucus*, which showed no genetical differentiation among populations due to ecological aridity factors (Comes et al., 1999).

Principal coordinate analysis of the populations showed a genetic divergence among populations within this species, although the geographical separations between some populations were short. Nevertheless, genetic and geographical distances were correlated (except from the M1 population), and a few clusters of populations corresponded to geographical position. Moreover, a hierarchical genetic structure among the populations was also found. The shortness of both the geographical and genetic distances between the populations in the second group (the xeric climate region) suggests that prevention of isolation by facilitation of gene flow is a simple function of geographical distance. Since the interpretation of RAPD data is sometimes compromised, further corroboration by other techniques will be valuable in the future. In the present case, neighboring populations are expected to be closely related, but the two semiarid populations SA1 and SA2, which are located relatively close to each other, did not show much resemblance, as they were not clustered in the same sub-clade. Low levels of gene flow between those two populations might be attributed to the ability of *A. aestivus* to reproduce vegetatively (both population are characterized by large clones, Table 1). Furthermore, the fact that these two populations have evolved on different bedrock (Table 1) might cause the geographical distance effect to be negligible. Examples of such a correlation between geographical distance and genetic diversity on the regional scale but not on the local scale have already been reported (see Müller-Schärer and Fisher, 2001; Haldimann et al., 2003). This is consistent with the claim that distinct populations are often characterized by obvious ecotypes (Briggs and Walters, 1997). Although our results do not reveal any formation of different ecotypes, they clearly reveal a hierarchical genetic pattern that is consistent with aridity, excluding the unique M1 population.

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REFERENCES

Aronson, J., Kigel, J., Shmida, A., Klein, J. 1992. Adaptive phenology of desert and Mediterraenan populations of annual plants grown with and without water-stress. Oecologia 89: 17–26.

Aronson, J., Kigel, J., Shmida, A. 1993. Reproductive allocation strategies in desert and Mediterranean populations of annual plants grown with and without water-stress. Oecologia 93: 336–342.

Auerbach, M., Shmida, A. 1987. Spatial scale and the determinants of plant species richness. Trends Ecol. Evolut. 2: 238–242.

Barnes, P.T., Laurie-Ahlberg, C.C. 1986. Genetic variability of flight metabolism in *Drosophila melanogaster*. III. Effect of G_{PDH} allozymes and environmental temperature on power output. Genetics 112: 267–294.

Ben-Shlomo, R., Fahima, T., Nevo, E. 1996. Random amplified polymorphic DNA of the *Spalax ehrenbergi* superspecies in Israel. Isr. J. Zool. 42: 317–326.

Briggs, D., Walters, S.M. 1997. Plant variation and evolution. Cambridge University Press, Cambridge, UK.

Chalmers, K.J., Waugh, R., Watters, J., Forster, B.P., Nevo, E., Abbott, R.J., Powell, W. 1992. Grain isozyme and ribosomal DNA variability in *Hordeum spontaneum* populations from Israel. Theor. Appl. Genet. 84: 313–322.

Comes, H.P., Abbott, R.J. 1999. Population genetic structure and gene flow across arid versus mesic environments: a comparative study of two parapatric *Senecio* species from the near east. Evolution 53: 36–54.

Del Pozo, A., Ovalle, C., Aronson, J., Avendano, J. 2002. Ecotypic differentiation in *Medicago polymorpha* L. along an environmental gradient in central Chile. I. Phenology, biomass production and reproductive patterns. Plant. Ecol. 159: 119–130.

DiMichele, L., Powers, D.A. 1982. Physiological basis for swimming endurance differences between LDH-B genotypes of *Fundulus heteroclitus*. Science 216: 1014–1016.

Ellstrand, N.C., Roose, M.L. 1987. Patterns of genotypic diversity in clonal plant species. Am. J. Bot. 74: 123–131.

Excoffier, L., Smouse, P.E., Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479–491.

Fahima, T., Röder, M.S., Wendehake, K., Kirtzhner, V.M., Nevo, E. 2002. Microsatellite polymorphism in natural populations of wild emmer wheat, *Triticum dicoccoides*, in Israel. Theor. Appl. Genet. 104: 17–29.

Feinbrun-Dothan, N. 1986. Flora Palaestina, 4. The Israel Academy of Sciences and Humanities, Jerusalem, Israel, p. 21.

Feinbrun-Dothan, N., Danin, A. 1998. Analytical flora of Israel. Cana Publishing House, Jerusalem, Israel, p. 769.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package) Ver. 3.5c. Department of Genetics, University of Washington, Seattle, WA. Focus 12: 13–15.

Flavell, R.B., O'Dell, M., Sharp, P., Nevo, E., Beiles, A. 1986. Variation in intergenic spacer of ribosomal DNA of wild wheat, *Triticum dicoccoides*, in Israel. Mol. Biol. Evol. 3: 547–558.

Gupta, P.K., Sharma, P.K., Balyan, H.S., Roy, J.K., Sharma, S., Beharav, A., Nevo, E. 2002. Polymorphism at rDNA loci in barley and its relation with climatic variables. Theor. Appl. Genet. 104: 473–481.

Haldimann, P., Steinger, T., Müller-Schärer, H. 2003. Low genetic differentiation among seasonal cohorts in *Senecio vulgaris* as revealed by amplified fragment length polymorphism analysis. Mol. Ecol. 12: 2541–2551.

Hamrick, J.L., Godt, M.J.W., Sherman-Broyler, S.L. 1992. Factor influencing levels of genetic diversity in woody plant species. New Forest 6: 95–124.

Karanth, K.P., Avivi, A., Beharav, A., Nevo, E. 2004. Microsatellite diversity in populations of blind subterranean mole rats (*Spalax ehrenbergi* superspecies) in Israel: speciation and adaptation. Biol. J. Linn. Soc. 83: 229–241.

Kovach, W. 1999. Multi-Variate Statistical Package 3.11b. Kovach Computing Services, Aberystwyth, Wales, UK.

Kutrup, B., Bulbul, U., Yilmaz, N. 2006. Effect of the ecological conditions on morphological variations of the green toad, *Bufo viridis*, in Turkey. Ecol. Res. 21: 208–214.

Li, Y.C., Fahima, T., Korol, A.B., Peng, J., Roder, M.S., Kirzhner, V., Beiles, A., Nevo, E. 2000. Microsatellite diversity correlated with ecological-edaphic and genetic factors in three micro-sites of wild emmer wheat in North Israel. Mol. Biol. Evol. 17: 851–862.

Lifante, Z.D., Aguinagalde, I. 1996. The use of Random Amplified Polymorphic DNA (RAPD) markers for the study of taxonomical relationships among species of *Asphodelus* sect. *Verinea* (Asphodelaceae). Am. J. Bot. 83: 949–953.

Linhart, Y.B. 1998. Intrapopulation differentiation in annual plants. III. The contrasting effect of intra- and interspecific competition. Evolution 45: 1047–1064.

Lynch, M., Milligan, B.G. 1994. Analysis of population genetic structure with RAPD markers. Mol. Ecol. 3: 91–99.

McDermott, J.M., McDonald, B.A. 1993. Gene flow in plant pathosystems. Annu. Rev. Phytopathol. 31: 353–373.

Mopper, S., Mitton, J.B., Whtham, T.G., Cobb, N.S., Christensen, K.M. 1991. Genetic differentiation and heterozygosity in pinyon pine associated with resistance to herbivory and environmental stress. Evolution 45: 989–999.

Müller-Schärer, H., Fisher, M. 2001. Genetic structure of the annual weed *Senecio vulgaris* in relation to habitat type and population size. Mol. Ecol. 10: 17–28.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., Kent, J. 2000. Biodiversity hotspots for conservation priorities. Nature 403: 583–590.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 70: 3321–3323.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 283–290.

Nevo, E. 1988. Natural selection in action: the interface of ecology and genetics in adaptation and speciation at the molecular and organismal level. In: Yom-Tov, Y., Tchernov, E., eds. The Zoogeography of Israel. Dr W. Junk Publisher, Dordrecht.

Nevo, E., Filippucci, M.G., Beiles, A. 1994a. Genetic polymorphisms in subterranean mammals (*Spalax ehrenbergi* superspecies) in the Near East revisited: patterns and theory. Heredity 72: 465–487.

Nevo, E., Krugman, T., Beiles, A. 1994b. Edaphic natural selection of allozyme polymorphisms in *Aegilops peregrine* at a Galilee microsite in Israel. Heredity 72: 109–112.

Norman, H.C., Smith, F.P., Cocks, P.S., Nutt, B.J. 1998. Reproductive strategies in Mediterranean annual clovers: germination and hardseededness. Aust. J. Agric. Res. 49: 973–982.

Nybom, H., Bartish, I.V. 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspect. Plant. Ecol. Evol. Syst. 3: 93–114.

Owuor, E.D., Fahima, T., Beharav, A., Korol, A., Nevo, E. 1999. RAPD divergence caused by microsite edaphic selection in wild barley. Genetica 105: 177–192.

Pantis, J.D., Margaris, N.S. 1988. Can systems dominated by *Asphodelus* be considered as semi-deserts? Int. J. Biometeorol. 32: 87–91.

Pantis, J.D., Sgardelis, S.P., Stamou, G.P. 1994. *Asphodelus aestivus*, an example of synchronization with climate periodicity. Int. J. Biometeorol. 38: 28–32.

Petru, M., Tielboerger, K., Belkin, R., Sternberg, M., Jeltsch, F. 2006. Life history variation in an annual plant under two opposing selective forces along a steep climatic gradient. Ecography 29: 66–74.

Piano, E., Pecetti, L., Carroni, A.M. 1996. Climatic adaptation in subterranean clover populations. Euphytica 92: 39–44.

Reyes, A., Nevo, E., Saccone, C. 2003. DNA sequence variation in the mitochondrial control region of subterranean mole rats, *Spalax ehrenbergi* superspecies, in Israel. Mol. Biol. Evol. 20: 622–632.

Schiller, G., Shklar, G., Korol, L. 2003. Genetic diversity assessment by random amplified polymorphic DNA of oaks in Israel. 1. Tabor oak (*Quercus aegilops* L. ssp. *ithaburensis* [Decne] Boiss.). Isr. J. Plant Sci. 51: 1–10.

Schneider, S., Roessli, D., Excoeffier, L. 2000. Arlequin ver. 2.0: A software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.

Shannon, C.E., Weaver, W. 1949. The mathematical theory of communication. University of Illinois Press, Urbana, Illinois.

Snaydon, R.W., Davies, T.M. 1982. Rapid divergence of plant

populations in response to recent changes in soil conditions. Evolution 36: 289–297.

Templeton, A.R. 1986. Coadaptation and outbreeding depression. In: Soule, M.E., ed. Conservation biology: the science of scarcity and diversity. Sinauer Associates, Sunderland, MA, USA, pp. 105–116.

Turpeinen, T., Tenhola, T., Manninen, O., Nevo, E. 2001. Microsatellite diversity associated with ecological factors in *Hordeum spontaneum* populations in Israel. Mol. Ecol. 10: 1577–1591.

Weir, B.S., Cockerham, C.C. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38: 1358–1370.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18: 6531–6535.

Yeh, F.C., Yang, R.C., Boyle, T.B.J., Ye, Z.H., Mao, J.X. 1997. POPGEN Ver. 1.32. The user-friendly software for population genetic analysis. Molecular Biology and Bio-technology Center, University of Alberta, Alberta, Canada.