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ORIGINAL PAPER

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Genetic relationships among South-East Turkey wild barley populations and sampling strategies of *Hordeum spontaneum*

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Abstract To assess the genetic diversity and the genetic structure of Turkish wild barley (*Hordeum spontaneum* Tell.) populations, 76 genotypes from ten ecologically and geographically different locations were analyzed by means of amplified fragment length polymorphism (AFLP) markers. Five primer combinations produced 187 scorable bands, of which 117 (62.6%) were polymorphic. Several population-specific and genotype-specific bands were identified, which differentiate populations or genotypes. Genetic distance, determined by Nei's distance coefficient, varied from 0.07 to 0.21 with an average of 0.13. In the UPGMA dendrogram based on Nei genetic distances, the *Hordeum spontaneum* populations were separated into two major clusters. Genetic diversity was larger among (68%) than within (32%) populations. Eight AFLP bands were strongly correlated to the altitude of the collecting site, while no clear trend was detected between geographical origin and genetic diversity. Our results strongly suggest the need for a change in *Hordeum spontaneum* sampling strategy: more populations, rather then more individuals within population, should be sampled to appraise and safeguard genetic diversity in the wild barley gene pool.

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Introduction

Wild relatives of crop plants are important gene sources for plant-breeding programs and in recent years they have received increasing attention. Wild barley (*Hordeum spontaneum* Tell.), the progenitor of cultivated barley, has no biological isolation barriers with the cultivated form, and hybrids between the two are present in nature (Harlan 1976). This makes *H. spontaneum* available for immediate use in barley breeding, in support of the improvement of agronomic characters such as disease resistance, drought, and salt tolerance. Wild barley is distributed throughout the Near East, with a range stretching from western Turkey eastward into southwest Asia including Afghanistan (Harlan and Zohary 1966). However, its primary habitats only occur in the area known as the Fertile Crescent covering Israel, Turkey, Lebanon, Jordan, Syria, Iraq, and Iran. Biotic and abiotic features of this region have led, in several plant species, to the accumulation of a wide range of diversity, both within and among populations (Zohary and Hopf 1993).

The use of biochemical and molecular markers represents a useful tool for the study of genetic diversity and phylogenetic relationship at different levels of taxa classification. The evolutionary relationships among and within wild barleys have been investigated using morphological and molecular markers such as isoenzymes (Nevo et al. 1986a, 1986b, 1986c, 1986d), RAPDs (Dawson et al. 1993; Reddy and Soliman 1997; Tanyolac 2003), ISSRs (Tanyolac 2003), RFLPs (Graner et al. 1994), and AFLPs (Pakniyat et al. 1997; Turpeinen et al. 2003).

Amplified fragment length polymorphism (AFLP) is highly reliable for the assessment of genetic variation among and within populations. The AFLP technique is a polymerase chain reaction (PCR)-based assay (Vos et al. 1995) that has the capacity to detect a higher

number of loci per assay, and thus a higher rate of polymorphism than RFLPs or RAPDs (Powel et al. 1996). AFLP analysis has been applied to genetic mapping (Becker et al. 1995; Alonso-Blanco et al. 1998; Castiglioni et al. 1998), to phylogenetic relationships studies (Pakniyat et al. 1997), to genetic diversity assessment (Turpeinen et al. 2003), and to the elucidation of the domestication process in cultivated crops (Badr et al. 2000; Heun et al. 1997; Ozkan et al. 2002).

Genetic diversity of *Hordeum spontaneum* from southeastern Turkey has not been studied extensively, even though this region is a wild barley primary habitat. The few reports available include only 11 populations of wild barley from Southeast Turkey (Nevo et al. 1986d, 1998). Recently Tanyolac (2003) has reported on the genetic diversity in *Hordeum spontaneum* sampled from the western coastal region of Turkey, a secondary habitat.

We sampled in Southeast Turkey ten natural populations of *Hordeum spontaneum* distributed across its ecological range, in order to evaluate their genetic variation. Of particular interest were (1) to investigate the existing levels of genetic diversity; and (2) to obtain information based on genetic diversity among and within populations, to provide insight and facilitate the conservation management of *Hordeum spontaneum*, at least for the area considered.

Materials and methods

Plant material

Seventy-six genotypes from ten populations of *Hordeum spontaneum* sampled from Southeast Turkey were analyzed (Table 1, Fig. 1). The samples were originally collected in 1997 and 2000. In each population, approximately 50 spikes were sampled from different plants and each plant was considered when separated at least by 1 m interval from others collected. Single seeds from randomly collected spikes were used. Each population was represented by eight individuals, except Hatay3 (seven individuals) and Kilis 2 (five individuals).

DNA extraction and AFLP reaction

After washing and drying, the leaf samples from each individual were frozen in liquid nitrogen and stored at −70°C. DNA was extracted by the CTAB method of Doyle (1991) with minor modifications. The extracted DNA concentration was estimated by comparing band intensity with λ_{DNA} of known concentrations, after 0.8% agarose gel electrophoresis and ethidium bromide staining. The DNA was diluted to 25 ng/µl for PCR reactions. The AFLP amplification was performed according to Vos et al. (1995) with minor modifications, using five primers combinations (Table 2).

About 3 µl of the mixture were loaded onto a 4.5% (w/v) polyacrylamide denaturing gel with 0.5×TBE buffer, with a pre-run electrophoresis at 60 V for 30 min and a run at 60 V until the loading dye reached the bottom of the gel. The gels were dried at 80°C for 3 h; autoradiographic analysis was made by exposing the gels for 2 days in an Hyperfilm-MP (Amersham, England) instrument.

Band scoring and data analysis

The AFLP bands were scored for presence (1) or absence (0); only clear-cut bands were considered. The correlations between longitudes, latitudes or altitudes and population frequencies of different AFLP markers were assessed.

Genetic distances between pairwise combinations of lines and between populations were computed, following Nei (1972). Percentage of polymorphic loci and Nei's (1973) gene diversity index (*He*) were calculated and relative degree of genetic diversity (G_{ST}) was estimated for each AFLP locus and populations according to Nei (1973), using the POPGENE program, version 1.31 (Yeh et al. 1997). NTSYS-pc (version 2.1, Exeter software, Setauket, USA; Rohlf 2000) was used for dendrogram construction (UPGMA), principal coordinates (PCo) analysis and for the calculation of cophenetic correlation coefficients. For the UPGMA tree built from Nei genetic distances (1972) between populations, bootstrapping (500 repetitions) was performed with PAUP 4.0b (Swofford 1998).

Table 1 Code, number of samples, collection site, geographical coordinates and altitude of ten wild barley (*Hordeum spontaneum*) populations from Sout-East Turkey

Code	Samples n°	Location	Year	Latitude	Longitude	Altitude
ADIYAMAN1	8	Adiyaman, Bebek village	1987	37°31′	38°15′	621
ADIYAMAN2	8	Adiyaman, Bardakci village	1987	37°26′	38°17′	—
GAZIANTEP1	8	Gaziantep, Nizip Belis dam	2000	37°00′	37°45′	526
HATAY1	8	Hatay, Belen Kici village	2000	36°30′	36°14′	1320
HATAY2	8	Hatay, Kirakhan Kocacavus village	2000	36°31′	36°24′	577
HATAY3	7	Hatay, Hassa Camizkisla village	2000	36°48′	36°31′	407
KILIS1	8	Kilis, Kazikli village	2000	36°49′	37°17′	633
KILIS2	5	Kilis, Corten village	2000	36°47′	37°15′	600
URFA1	8	Urfa, Ovacik Apaydin village	1987	37°30′	38°45′	707
URFA2	8	Urfa, Diyarbakir road, 28 km from Urfa	1987	37°35′	38°57′	599

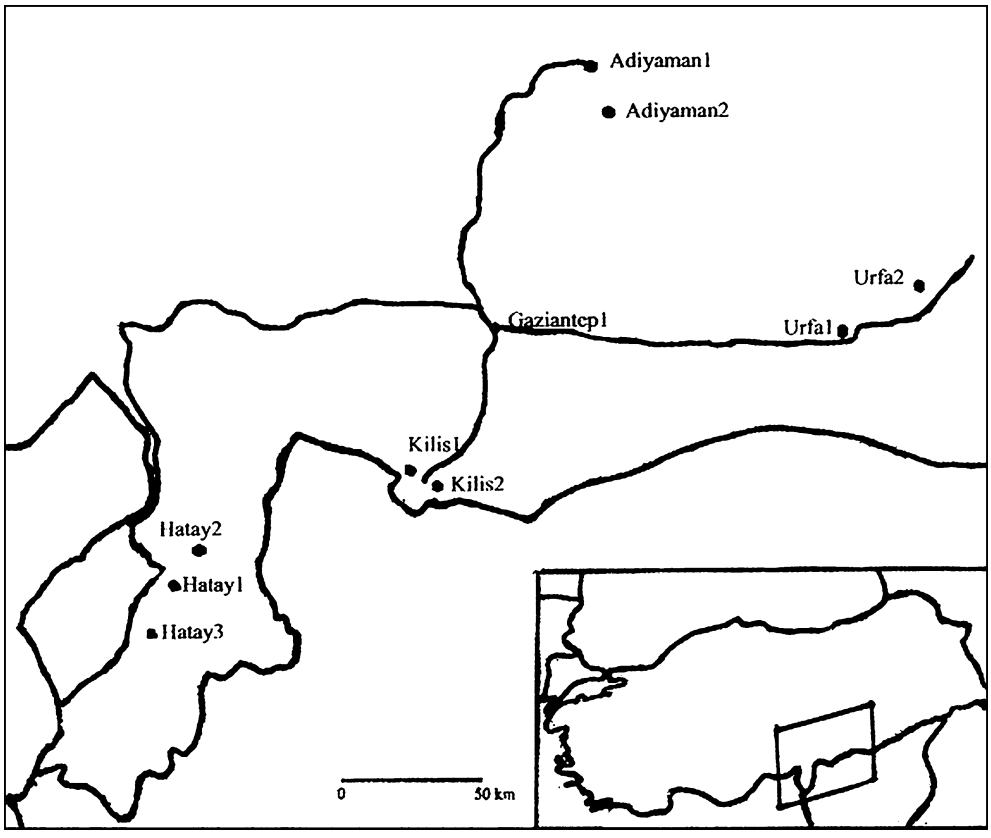

Fig. 1 Geographic distribution of sampling localities of wild barley in Turkey

Table 2 AFLP primers combinations, total number of bands, number and percentage of polymorphic bands detected in the ten populations

Primer combination	Total bands	Polymorphic bands	
	No.	No.	Percentage
E_{ACG}/M_{AGC}	39	28	71.8
E_{AGT}/M_{ACC}	44	26	59.8
E_{AGT}/M_{AAT}	16	7	43.8
E_{ACC}/M_{ACC}	42	25	59.5
E_{AGC}/M_{AGC}	46	31	67.4
Total	187	117	62.6
Average	37.4	23.4	

Analysis of molecular varianace (AMOVA) (Excoffier et al. 1992) was based on Arlequin version 2.000 (Schneider et al. 2000). The total variation was partitioned among individuals within populations, between populations within region, and between regions. Significant levels of variance components and F_{ST} were computed by non-parametric permutation procedures.

Correlation between interpopulation Nei genetic distances (1972) and geographic distances among collection sites was assessed by the Mantel test, as implemented in NTSYS-pc 2.1.

Results

AFLP polymorphisms

The AFLP fingerprinting of the 76 genotypes of wild barley (*Hordeum spontaneum* Thell.) with five primer combinations revealed 187 unambiguous amplified DNA fragments; 117 fragments were polymorphic (62.6 % of the total; Table 2). The E_{ACG}/M_{AGC} and E_{AGC}/M_{AGC} primer combinations revealed the highest level of polymorphism, 71.8 and 67.4 %, respectively (Table 2). Population-specific and genotype-specific bands were identified (data not shown). The five primer combinations did not detect polymorphisms among the individuals of the Adiyaman1 population (data not shown). This could influence the estimates of gene diversity and the AMOVA breakdown. Therefore all the analyses were repeated considering only the nine polymorphic populations. The results of this last analysis are however were very similar to those from all ten populations, and are not reported here.

Eight AFLP markers (E_{ACC}/M_{ACC}–30, E_{AGC}/M_{AGC}–33, E_{AGC}/M_{AGC}–5, E_{AGT}/M_{ACC}–18, E_{AGT}/M_{ACC}–37, E_{AGT}/M_{ACC}–41, E_{AGT}/M_{ACC}–44, E_{ACG}/M_{AGC}–23) were significantly correlated with the altitude

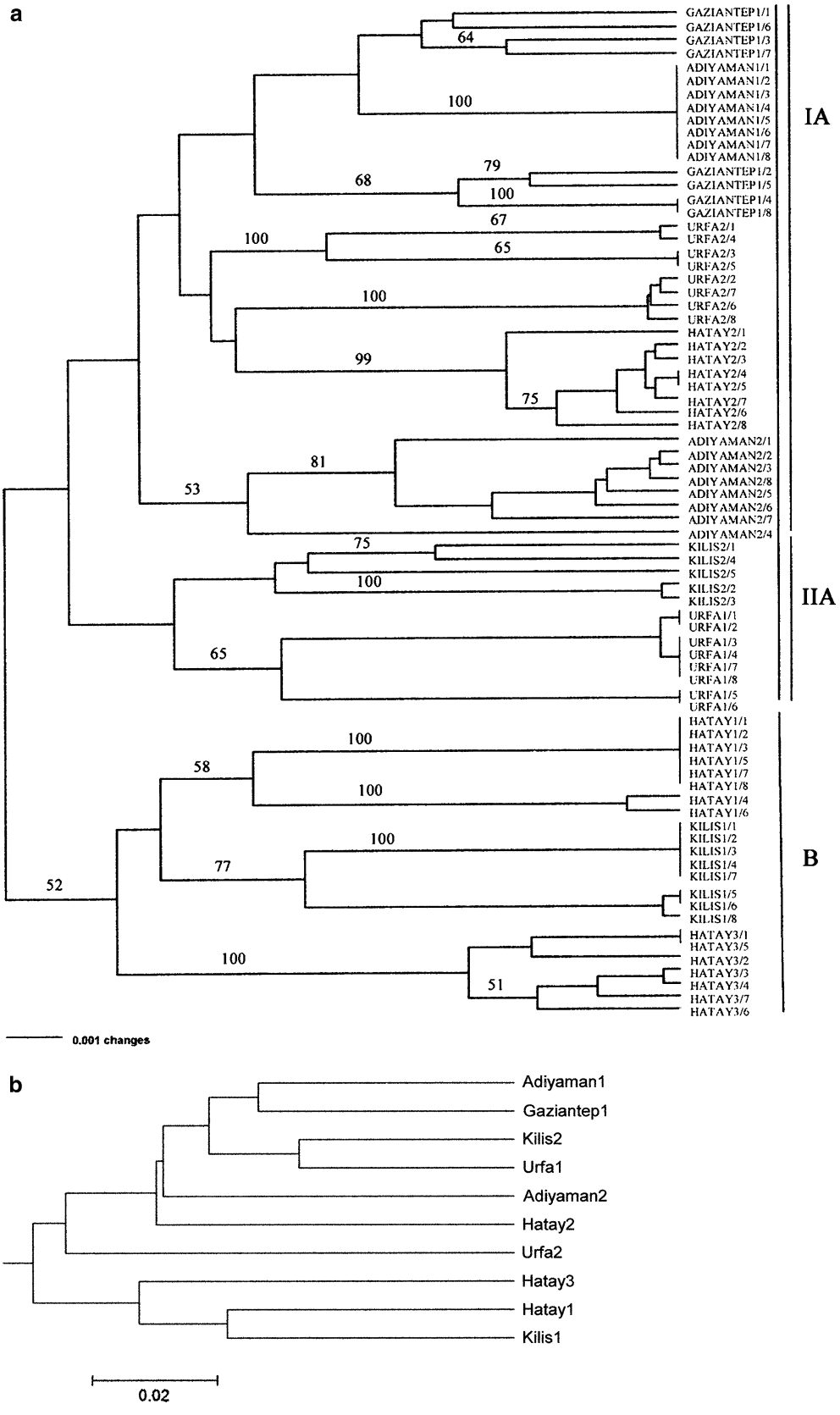

Fig. 2 Dendrogram representing the phylogenetic relationship among ten *Hordeum spontaneum* populations, revealed by UPGMA cluster analysis. The genetic distances were from Nei (1972). **a** Cluster based on AFLP fingerprinting data from single plants (76). **b** Cluster based on AFLP bands frequency from the populations (10)

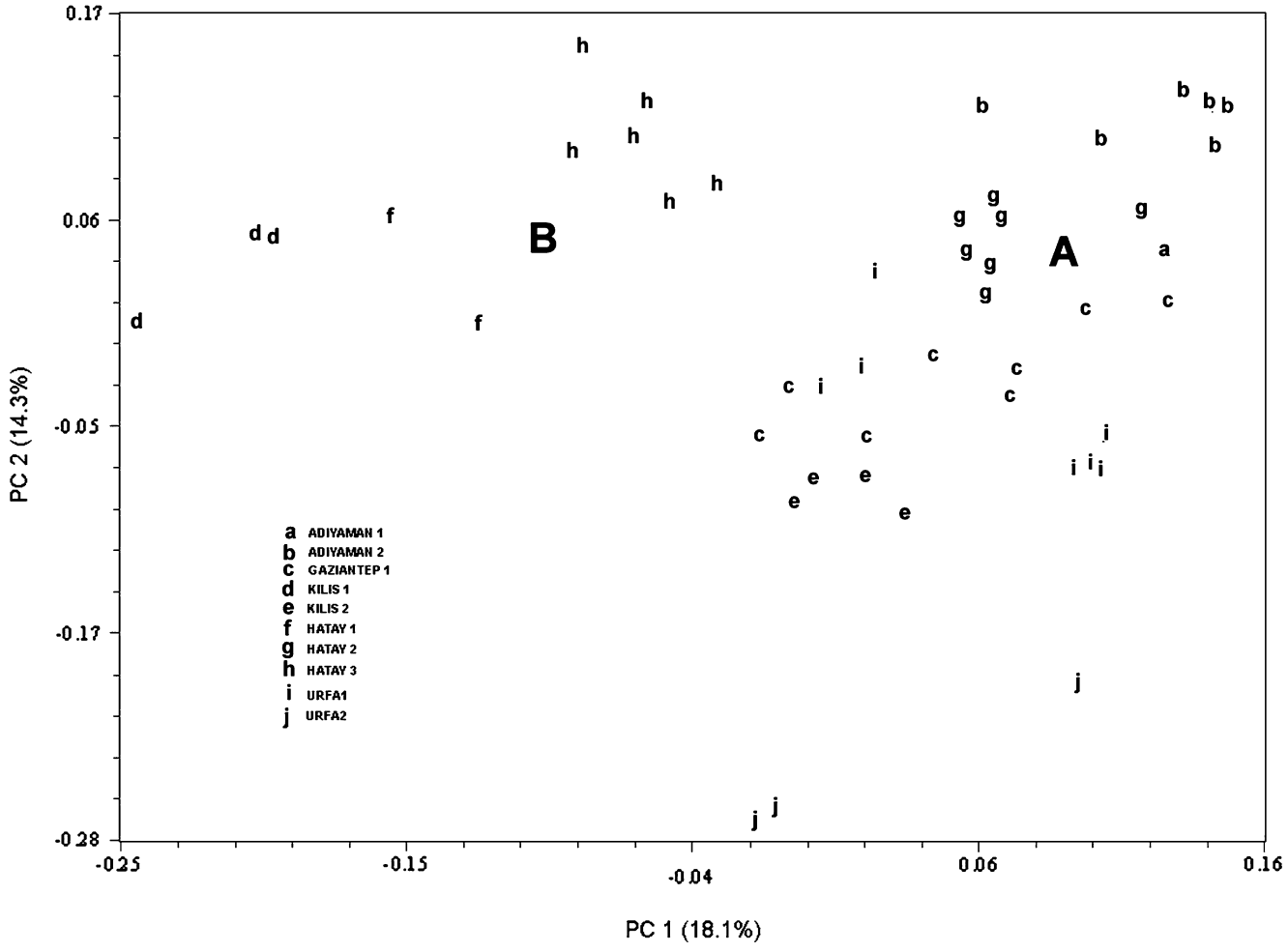

Fig. 3 Principal Coordinates (PCo) analysis of 76 *Hordeum spontaneum* samples from Sout-East Turkey. *Above* component 1 (PCo1) versus component 2 (PCo2); *below* component 3 (PCo3) versus component 4 (PCo4)

at which the populations were sampled. These markers were excluded from AMOVA, because they might be tightly linked to genes under selection, thus representing cases of non-neutral markers.

Samples analysis

The relationships among samples and populations are visualised in the UPGMA dendrograms generated from Nei (1972) genetic distances (Fig. 2a). The ten populations formed two major clusters: cluster A consisted of the Gaziantep1, Adiyaman1, Kilis2, Urfa1, Adiyaman2, Hatay2, and Urfa2 populations, while cluster B included the remaining three populations (Hatay1, Kilis1, and Hatay3). Within cluster A, two subgroups were evident, one containing the five populations, Gaziantep1, Adiyaman1, Hatay2, Urfa2, and Adiyaman2, while the other included Kilis2 and Urfa1. Different samples of each population clustered together, hinting that

Table 3 Genetic distances (Nei 72) among the ten populations

Populations	ADIYAMAN1	ADIYAMAN2	GAZIANTEP1	HATAY1	HATAY2	HATAY3	KILIS1	KILIS2	URFA1
ADIYAMAN2	0.10								
GAZIENTEP2	0.08	0.13							
HATAY1	0.16	0.16	0.15						
HATAY2	0.13	0.12	0.11	0.14					
HATAY3	0.12	0.14	0.14	0.11	0.15				
KILIS1	0.19	0.19	0.15	0.09	0.18	0.13			
KILIS2	0.11	0.12	0.10	0.12	0.13	0.15	0.11		
URFA1	0.10	0.09	0.08	0.14	0.09	0.12	0.13	0.07	
URFA2	0.14	0.19	0.14	0.18	0.18	0.18	0.21	0.10	0.11

Mean genetic distance: 0.13

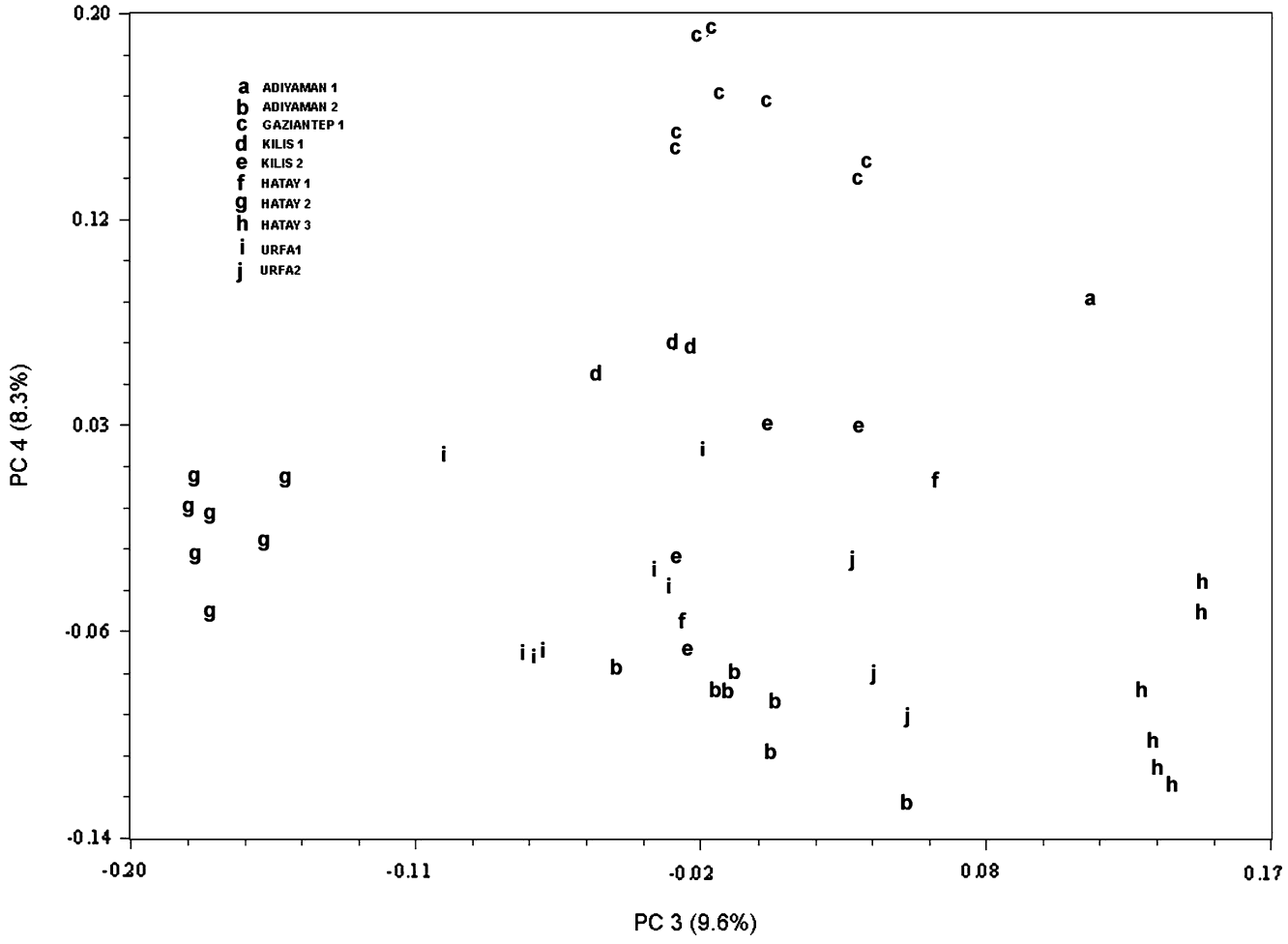

Fig. 3 (Contd.)

interpopulation differences were more significant compared to intrapopulation ones. In general, grouping based on genetic parameters was not related to the geographic origin of sampling regions.

The PCo analysis (Fig. 3) supported the results of clustering by UPGMA. The first PCo (18.1% of variation) separated clearly the populations into groups A and B, while the second PCo (14.3%) separates Urfa 2 from the other group A populations. PCo also indicates that samples from the same population are genetically much closer than samples from different populations.

Populations analysis

Population frequencies of AFLP bands (presented as additional material) are reminiscent of the Archipelago distribution of Nevo et al (1986 a) because some alleles that are overall rare, are concentrated in high frequency in a few populations.

A genetic distance matrix, based on the population frequencies, made possible to determine the relationships between the ten populations with a second approach. Genetic distance values ranged from 0.21 between Urfa2 and Kilis1 to 0.07 between Kilis2 and Urfa1 (Table 3). The UPGMA clustering of the populations (Fig. 2b) supported the results based on individual samples. The two clusters corresponding to the A and B groups of Fig. 2 provide further evidence. It was also clear from the PCo analysis that Urfa2 was genetically very different from the other populations.

The mean values of gene diversities (*He*) were calculated for each population (data not shown). Average genetic diversity varied between 0.080 ($P\% = 0.20$) in Adiyaman 1 and 0.00 ($P\% = 0.00$) in Adiyaman2. The mean gene diversity measured over all the loci and populations was equal to 0.156.

The relative degree of gene diversity within and between populations for each primer combination, calculated according to Nei (1973) is presented in Table 4. The proportion of diversity between and within populations indicated that on average 68% of the variation (Gst) was between populations and only 32% within populations (1−Gst). This proportion was fairly constant for all AFLP primer combinations.

The partitioning of the genotypic variance among samples within accessions, among accessions within geographic regions, and between geographic regions was

Table 4 Relative degree of gene diversity within and between ten *Hordeum spontaneum* populations from South-East Turkey, calculated according to Nei (1973)

Primer combination	Genediversity	
	Between(G_{ST})	Within(1-G_{ST})
E_{ACC}/M_{ACC}	0.659	0.341
E_{ACG}/M_{AGC}	0.656	0.344
E_{AGC}/M_{AGC}	0.662	0.339
E_{AGT}/M_{AAT}	0.737	0.263
E_{AGT}/M_{ACC}	0.719	0.281
All	0.677	0.323

assessed by AMOVA. The components of AMOVA were highly significant for within populations and between populations/within regions (Table 5): 34.9% of the genetic variation was within samples, while the within-populations component was 65.1% (57.6% among populations within regions and 7.5% between regions). When the analysis excluded the monomorphic Adiyaman1 populations similar results were obtained: 37.6% (significant) among samples, 57.4% (significant) among populations within regions and 5.0% (not significant) between regions.

The correlation between interpopulation Nei (1972) genetic distances and geographic distances computed by the Mantel test revealed absence of association: $r = 0.19$ for ten populations and 0.16 for nine populations (without Adiyaman1).

Discussion

AFLP polymorphisms

One advantage of AFLP-based DNA fingerprinting is its potential in revealing large genetic polymorphisms with a nearly complete coverage of the whole genome (Badr et al. 2000; Heun et al. 1997). In our study, five AFLP primer combinations were sufficient to disclose a proper number of polymorphic DNA fragments in support of all comparisons and of their statistical evaluation Recently, Turpeinen et al. (2003) used AFLP to investigate the population structure of *Hordeum spontaneum* in Israel: their polymorphism level was higher than the one we observed, probably because of diverse primer combinations. The populations used in our study showed different banding profiles (data not presented), and the differences were consistent among primer combinations. In this sense, genotype identification should be possible even with only one or two AFLP primer combinations, a situation which is not so easily achieved with other molecular marker techniques.

Genetic Diversity

He (Nei 1973) values were taken as a measure of genetic diversity. *He* is the average probability that two random gametes from the populations carry different alleles at a particular locus and correspond to the expected heterozygosity. In panmictic populations, *He* is the expected heterozygosity (Nevo et al. 1986d). All the populations tested, except Adiyaman1 ($He = 0.00$), had moderate levels of polymorphism: *He* varied from 0.000 to 0.020, while the overall mean genetic diversity (all loci and all populations) was 0.156. Using isozyme analysis, Nevo et al. (1986d) found that the mean gene diversity in 11 populations sampled from Southeast Turkey was 0.098. In this study, genetic diversity was similar to values previously reported for Israel (Nevo et al. 1986a, 1986b) and Iran (Nevo et al. 1986c).

To compare the ten populations, genetic distances were calculated following Nei (1972). The mean genetic distance observed was 0.132, with values ranging from 0.07 to 0.21. Similar results were observed by Nevo et al. (1986d), who reported that genetic distance value, based on allozyme analyses, in 11 populations of *H. spontaneum* sampled from Southeast Turkey ranged from 0.031 to 0.288. The broad range suggests that the wild barley samples from Southeast Turkey represent genetically diverse populations.

Distribution of genetic diversity

Cluster analysis classified the ten populations into two groups, one with seven and the other with three populations. Values of genetic distance estimates highlighted a sharp genetic differentiation over short geographic distances. Interestingly, geographically close population can be genetically highly distant whereas geographically distant population can, in cases, more genetically similar. For instance, the populations from Kilis1 and Urfa2 showed the highest genetic distance, whereas Kilis2 and

Table 5 Analysis of molecular variance (AMOVA) for 76 *Hordeum spontaneum* samples belonging to ten different populations within five geographic regions from Sout-East Turkey

Level of variation	df	Variance components		Fixation index	*P*
		Absolute	Percentage		
Among regions	4	1.148	7.5	Fct = 0.075	> 0.100
Among populations	5	8.810	57.6	Fsc = 0.623	< 0.001
Within populations	66	5.330	34.9	Fst = 0.651	< 0.001

Levels of significance are based on 1,000 iterations

Urfa1 had the lowest genetic distance. The significant correlation observed between altitude of sampling locations and the population frequency of eigth bands (see Supplementary material) suggests that some AFLP fragments might be the result of selection for specific adaptation to high-altitude environments. Several studies (Nevo et al. 1986a, 1986b, 1986c, 1986d; Turpeinen et al. 2001) accordingly indicated that wild barley genetic diversity is not randomly distributed, but it may be associated with specific ecogeographical factors. However, recent results (Morell et al. 2003) suggest that the situation is far more complex and that the genepool of this wild species is really "a mosaic of different histories generated by different evolutionary processes".

Wild barley is a self-pollinating species with very low levels of outbreeding (Brown et al. 1978). Seed dispersal is by far the primary method of relocation from one environment to another; once a suitable environment is reached, migrants can found new populations and persist even without crossing to local germplasm. This justifies the existence, over short distances, of striking genetic differences. Given sufficient time to such a process, very different populations will be colonizing even in geographically contiguous areas. This case seems to fit the results presented in this study.

In fact, the analysis of the relative degree of gene differentiation and AMOVA showed that diversity is greater between wild barley populations than within them and that genetic variation is not correlated to geographic distances among different collecting areas. This result was in part expected, given the high selfing rate of the species which limits, consequently, the gene exchange, and is consistent with Chalmers et al. (1992) who, using isozymes, reported greater diversity between populations than within them. On the contrary other studies on wild barley, observed a higher degree of genetic diversity within, populations rather than between them (Dawson et al. 1993; Baum et al. 1997; Turpeinen et al. 2001, 2003). The discrepancy might be explained by differences in the distribution of genetic diversity in the geographic areas of the Fertile Crescent where wild barley has its primary habitas, pointing to the limitations of our findings for the Souteast Turkey, and use of the diverse sampling strategy.

Conclusions

The assessment of genetic diversity is a component in germplasm characterization and conservation as well, as in establishing an effective use of wild germplasm in breeding. An understanding of the distribution of genetic variation within and between wild barley populations is thus a key for efficient germplasm preservation, characterization and utilization by barley breeders. *Hordeum spontaneum* within the entire Near East Fertile Crescent is highly polymorphic for molecular markers (Pakniyat et al., 1997, Castiglioni et al. 1998) and samples from Israel display a broad variation also for agrononomic characters such as earliness, biomass, yield (Nevo et al. 1984), and disease resistance (Moseman et al. 1983, 1990). Agronomic traits assessment on Turkish wild barley populations is missing, highlighting the necessity for screening this species in the primary Turkish gene pool for disease resistance and other economically important traits. Based on our results, a change in *Hordeum spontaneum* sampling strategy in Turkey and in general in the Southeastern Fertile Crescent is a priority: more populations with less individuals, rather then more individuals within populations, should be collected to appraise and safeguard the genetic diversity of wild barley gene pool. This strategy, was already proposed 20 years ago by Nevo et al (1986a) but scarcely employed in germplasm collection expeditions (Volis et al, 2001; Baek et al, 2003). The strategy increases the probability of sampling alleles that, although overall rare, are present in high frequency in a few scattered populations as a consequence of adaptative selection, and therefore boosts the chance of detecting useful alleles for stress and disease resistance.

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