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T. Turpeinen · T. Vanhala · E. Nevo · E. Nissilä

AFLP genetic polymorphism in wild barley (Hordeum spontaneum) populations in Israel

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Abstract The genetic diversity produced by the amplified fragment length polymorphism (AFLP) method was studied in 94 genotypes of wild barley, *Hordeum spontaneum* (C. Koch) Thell., originating from ten ecologically and geographically different locations in Israel. Eight primer pairs produced 204 discernible loci of which 189 (93%) were polymorphic. Each genotype had a unique banding profile and the genetic similarity coefficient varied between 0.74 and 0.98. The phenogram generated from these similarities by the UPGMA method did not group genotypes strictly according to their geographical origin, which pattern was also seen in the principal coordinate (PCO) plot. Genetic diversity was larger within (69%) than among (31%) populations. Associations between ecogeographical variables and the mean gene diversity were found at one primer pair. The results are discussed and compared with data obtained by the simple sequence repeat (SSR) method.

Keywords *Hordeum spontaneum* · Wild barley · Amplified fragment length polymorphism · AFLP · Genetic diversity

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T. Turpeinen and T. Vanhala contributed equally to this work

T. Turpeinen (✉)
MTT Agrifood Research Finland, Plant Production Research, Crops and Biotechnology, FIN-31600 Jokioinen, Finland
e-mail: timo.turpeinen@mtt.fi
Tel.: +358 3-4188-2538, Fax: +358 3-4188-2496

T. Vanhala
Laboratory of Plant Breeding, Department of Plant Sciences, Wageningen University, P.O.Box 386, 6700 AJ Wageningen, Netherlands

E. Nevo
Institute of Evolution, University of Haifa, Mt. Carmel, Haifa 31905, Israel

E. Nissilä
Boreal Plant Breeding Ltd, FIN-31600 Jokioinen, Finland

Introduction

Genetic diversity, the basis of evolution by natural selection, is gravely threatened by the progenitors of cultivated plants, and its exploration, evaluation, conservation in situ and ex situ is imperative to guarantee sustainable development (Nevo 1998a). Wild barley is the progenitor of cultivated barley, *Hordeum vulgare* L., and therefore is a valuable genetic reservoir for barley breeding. Several studies have been conducted to establish the extent of variation in wild barley at the allozyme (Nevo et al. 1979, 1981, 1986; Chalmers et al. 1992; Nevo et al. 1997) and DNA level (Weining and Henry 1995; Baum et al. 1997; Owuor et al. 1997; Nevo et al. 1998; Turpeinen et al. 2001). These studies have shown macro- and micro-scale adaptive divergence of genetic diversity at the protein and DNA levels.

After the introduction of the polymerase chain reaction (PCR), different types of molecular genetic markers have become abundant. Many of these have become valuable tools for searching answers to problems of diversity in the coding and noncoding genome. In particular there is a lack of understanding of the role of noncoding sequences. Are they largely junk DNA or do they participate in regulation of the coding sequences?

AFLP (amplified fragment length polymorphism; Zabeau and Vos 1993) is a marker method which can detect polymorphism at many loci over the entire genome, simultaneously from both coding and noncoding sequences (Vos et al. 1995). It combines strategies of restriction fragment length polymorphism (RFLP) and PCR. Many aspects (i.e. no need for prior sequence information, a large number of polymorphisms and a small amount of genomic DNA required) have made AFLP a very attractive method to study DNA polymorphism in general. AFLP analysis has been applied to genetic mapping (Becker et al. 1995; Waugh et al. 1997; Van der Voort et al. 1997; Alonso-Blanco et al. 1998), phylogenetic relationships and genetic diversity (Donini et al. 1997; Pakniyat et al. 1997; Russell et al. 1997; Schut et al. 1997; Miyashita et al. 1999) studies. The ability of

Table 1 AFLP primers, their sequences, and the number of bands produced by eight combinations of primers

Primers/adapters	Sequences							
*Eco*RI adapter	5′-CTCGTAGACTGCGTACC-3′ 3′-CATCTGACGCATGGTTAA-5′							
*Mse*I adapter	5′-GACGATGAGTCCTGAG-3′ 3′-TACTCAGGACTCAT-5′							
E00	5′-GACTGCGTACCAATTCA-3′							
M00	5′-GATGAGTCCTGAGTAA-3′							
Primer	E32M61	E33M58	E35M48	E35M55	E38M54	E38M55	E39M61	E45M55
E00	AAC	AAG	ACA	ACA	ACT	ACT	AGA	ATG
M00	CTG	CGT	CAC	CGA	CCT	CGA	CTG	CGA
Number of bands produced	12	21	46	31	20	16	28	30

the AFLP method to differentiate individuals in a population has also made it useful for paternity analyses (Krauss 1999), gene-flow experiments and for Plant Variety registration (Law et al. 1998).

In the present study we have analysed the genetic diversity pattern in natural populations of wild barley, *H. spontaneum*, from ten different locations in Israel by using the AFLP method. The results were correlated to environmental variables, and compared to the data of a previous study from the same material produced by simple sequence repeats (SSRs).

Materials and methods

Plant material and DNA extraction

Ninety four genotypes from ten populations of *H. spontaneum* collected from different mesic and xeric sites across the ecological spectrum of wild barley in Israel were used for analysis. Each population contained between eight and ten genotypes. A map showing the site locations, as well as the ecogeography and climatic profile, was documented by Turpeinen et al. (2001). Plants were grown and DNA extracted as described by Turpeinen et al. (2001).

AFLP analysis

AFLP analyses with a minor modification followed the protocol according to Vos et al. (1995). Total DNA (100 ng) was double-digested with *Eco*RI (recognition sequence 5′-GAATTC-3′) and *Mse*I (TTAA). After ligating *Eco*RI and *Mse*I adaptors to the digested DNA, pre-amplification was conducted with an *Eco*RI primer (E00 + A as a selective nucleotide) and a *Mse*I primer (M00 + C as a selective nucleotide). The pre-amplification product was used as a template for selective amplification. The amplification was conducted using three selective nucleotides for both *Eco*RI and *Mse*I primers. Eight pairwise combinations of selective primers previously documented in *Hordeum vulgare* by Qi and Lindhout (1997) were employed (Table 1). All the *Eco*RI selective primers were labeled at the 5′ end with either a 700 or 800 nm infrared dye (IRD700 and IRD800). The amplification products were run on a sequencing gel connected to a LI-COR automated sequencer (Li-Cor Inc., Lincoln, Neb., U.S.A.). The gel image was visualized by the program Base ImagIR v. 4.1 (Li-Cor Inc., Lincoln, Neb., U.S.A.), and band scoring was done by using the program Cross Checker (Buntjer 1999). Bands with lengths between 50 and 500 base pairs were included for statistical analysis.

Data analysis

H. spontaneum plants were considered homozygous because of the high rate of selfing (Brown et al. 1978). The bands of AFLP gels were scored for present (1), absent (0) or as a missing observation (2), and we regarded each band as a locus. Scored bands were designated by labels using primer combination information as a prefix with an ascending order of band lengths from high to low. The genetic similarities (gs) were calculated according to Nei and Li (1979):

$$gs_{xy} = \frac{2N_{xy}}{N_x + N_y},$$

where N_{xy} is the number of bands present in both genotypes x and y, N_x is the number of bands present in genotype x, and N_y is the number of bands present in genotype y. Missing observations for a marker in genotype x and/or y were excluded from the calculation of gs_{xy}. A similarity matrix was used for the average linkage cluster analysis between the groups (UPGMA) using the method of Sneath and Sokal (1973) to construct a phenogram, and the same matrix was also used for principal coordinate (PCO) analysis in a multivariate statistical program (MVSP 2001). The gene diversity (H_e) and relative degree of genetic diversity (G_{ST}) were estimated for each locus and population according to Nei (1973). The mean gene diversity was calculated over all loci from each primer pair, and used in Spearman rank correlation analysis between the mean gene diversity and ecogeographical factors. In our previous paper (Turpeinen et al. 2001) we employed principal component analysis (PCA) to analyse ecogeographical data, and wild barley populations could be separated by three factors (water, temperature and geography). We used the principal component scores produced previously by PCA in this study for stepwise multiple regression analysis. The programs POPGENE (Yeh et al. 1997) and SPSS (SPSS 1998) were used to perform these analyses.

Results

The distribution of genotypes

Eight primer pairs produced a genotype x loci (bands) matrix of 19,176 observations with 300 missing cases comprising a total of 18,876 observations. The missing cases were due to failure in amplification. The distribution of number of observations within each primer pair between absence (0) and presence (1) of loci across all genotypes is presented in Fig. 1. The highest number of loci per genotype was detected with the E35M48 primer pair (46) and the lowest with E32M61 primer pair (12) (Table 1). The total of 204 loci was identified and 189 loci were polymorphic among the genotypes. The propor-

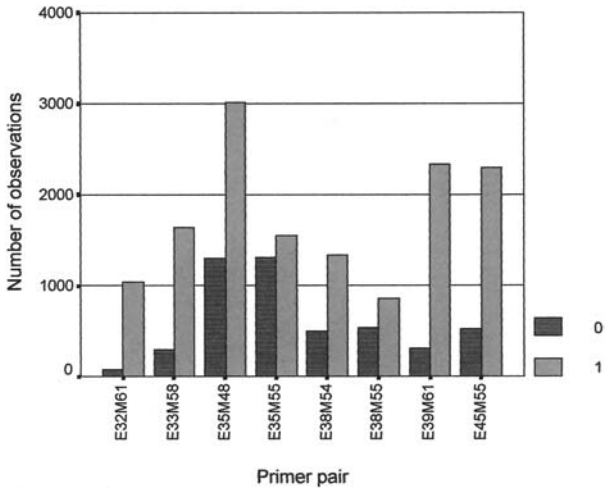

Fig. 1 The number of observations (y-axis) produced by eight AFLP primer pairs (x-axis) of 94 *H. spontaneum* genotypes

tion of polymorphic loci out of all loci was 0.93 and they were distributed within populations and primer pairs as shown in Table 2. Because of the missing values some loci and samples were omitted from the genetic similarity estimation, creating a 90 × 90 genotypes data matrix based on Nei and Li's formula. The range between genetic similarity coefficients varied from 0.74 to 0.98. Each genotype had a unique banding profile and the genotypes were clustered as seen in Fig. 2. When testing the resolving power of a single primer pair there were four primer pairs, which distinguished all genotypes separately (E35M48, E35M55, E39M61 and E45M55). The similarity matrix was further used for principal coordination analysis, and the plot of genotypes based on the first two axes is presented in Fig. 2. The first axis accounted for 11% and the second for 8% of the total variation. The genotypes belonging to populations from Mt. Meron and Mehola were grouped together, and genotypes from populations of Maalot and Revivim comprised almost uniform groups. For the rest of the populations the genotypes were more scattered inside the plot.

Fig. 2 The phenogram of 90 genotypes of ten populations of *H. spontaneum* in Israel based on Nei and Li's similarity matrix. Genotypes were clustered by the unweighted pair group method with the arithmetic averages (UPGMA) method. The genotypes are presented at the end of branches based on their population number: 1 = Mount Hermon, 9 = Mount Meron, 10 = Maalot, 11 = Damon, 14 = Talpiyyot, 18 = Revivim, 20 = Sede Boqer, 22 = Mehola, 23 = Wadi Qilt, 33 = Avedat

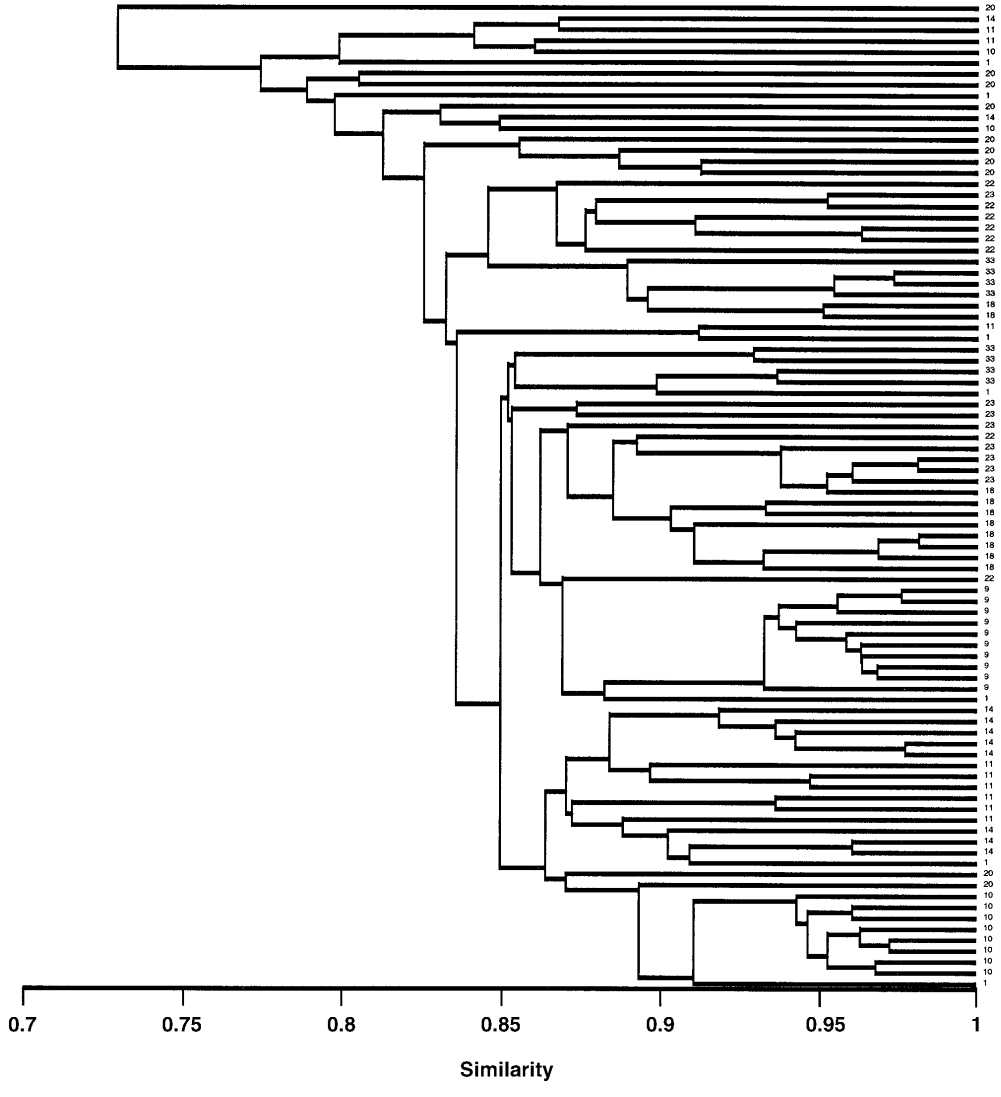

Table 2 Summary of genetic variation based on 204 AFLP loci of ten *H. spontaneum* populations

Population/ primer pair	E32M61			E33M58			E35M48			E35M55		
	P[a]	H_e[b]	N[c]	P	H_e	N	P	H_e	N	P	H_e	N
Mt. Hermon	0.17	0.03 (0.07)	9	0.33	0.11 (0.17)	8	0.59	0.22 (0.2)	10	0.55	0.17 (0.18)	8
Mt. Meron	0	0	10	0.29	0.11 (0.19)	10	0.24	0.07 (0.15)	10	0.42	0.14 (0.19)	10
Maalot	0.42	0.14 (0.17)	10	0.19	0.06 (0.14)	10	0.61	0.15 (0.14)	10	0.52	0.14 (0.15)	10
Damon	0.67	0.18 (0.16)	10	0.29	0.1 (0.18)	10	0.70	0.22 (0.18)	10	0.55	0.17 (0.18)	10
Talpiyyot	0.17	0.04 (0.10)	10	0.28	0.06 (0.13)	10	0.57	0.19 (0.19)	10	0.49	0.18 (0.21)	10
Revivim	0.08	0.01 (0.05)	10	0.33	0.1 (0.17)	10	0.54	0.19 (0.2)	10	0.74	0.27 (0.18)	10
Sede Boqer	0.75	0.26 (0.19)	10	0.29	0.1 (0.17)	10	0.78	0.27 (0.17)	10	0.84	0.29 (0.17)	10
Mehola	0.08	0.04 (0.14)	8	0.33	0.13 (0.2)	8	0.59	0.22 (0.2)	8	0.68	0.23 (0.19)	8
Wadi Qilt	0.08	0.02 (0.07)	7	0.33	0.09 (0.14)	8	0.46	0.14 (0.17)	8	0.81	0.28 (0.16)	8
Avedat	0.08	0.01 (0.06)	8	0.19	0.06 (0.14)	8	0.59	0.2 (0.19)	8	0.61	0.22 (0.19)	8
Mean	0.83	0.11 (0.1)	92	0.76	0.15 (0.18)	92	0.98	0.28 (0.15)	94	0.97	0.32 (0.14)	92
	E38M54			E38M55			E39M61			E45M55		
	P	H_e	N	P	H_e	N	P	H_e	N	P	H_e	N
Mt. Hermon	0.95	0.36 (0.12)	8	0.75	0.29 (0.19)	7	0.71	0.21 (0.17)	10	0.93	0.33 (0.15)	10
Mt. Meron	0.20	0.04 (0.09)	10	0.01	0.01 (0.04)	10	0.07	0.01 (0.04)	10	0.17	0.05 (0.13)	10
Maalot	0.85	0.15 (0.06)	10	0.50	0.15 (0.17)	9	0.36	0.08 (0.13)	10	0.77	0.19 (0.13)	10
Damon	0.95	0.4 (0.1)	10	0.87	0.34 (0.15)	10	0.68	0.23 (0.19)	10	0.73	0.23 (0.18)	10
Talpiyyot	0.90	0.26 (0.15)	10	0.94	0.36 (0.12)	10	0.32	0.11 (0.17)	10	0.57	0.14 (0.15)	10
Revivim	0.45	0.15 (0.19)	10	0.31	0.09 (0.15)	10	0.25	0.08 (0.15)	10	0.23	0.08 (0.16)	10
Sede Boqer	0.90	0.32 (0.14)	10	0.81	0.28 (0.17)	9	0.82	0.28 (0.17)	10	0.70	0.22 (0.17)	10
Mehola	0.55	0.16 (0.21)	8	0.31	0.1 (0.17)	7	0.14	0.06 (0.15)	8	0.37	0.12 (0.18)	8
Wadi Qilt	0.40	0.16 (0.21)	8	0.44	0.12 (0.15)	8	0.25	0.08 (0.16)	8	0.33	0.1 (0.15)	8
Avedat	0.35	0.12 (0.19)	8	0.50	0.17 (0.19)	8	0.21	0.08 (0.17)	8	0.50	0.16 (0.18)	8
Mean	1	0.31 (0.14)	91	1	0.28 (0.19)	88	0.82	0.17 (0.13)	94	0.97	0.23 (0.13)	8

	All[d]		
	P	H_e	N
Mt. Hermon	0.64	0.22 (0.19)	9
Mt. Meron	0.21	0.06 (0.14)	10
Maalot	0.54	0.13 (0.14)	10
Damon	0.67	0.23 (0.19)	10
Talpiyyot	0.52	0.17 (0.18)	10
Revivim	0.41	0.14 (0.18)	10
Sede Boqer	0.74	0.25 (0.18)	10
Mehola	0.42	0.15 (0.19)	8
Wadi Qilt	0.42	0.13 (0.17)	8
Avedat	0.42	0.15 (0.18)	8
Mean	0.93	0.24 (0.16)	93

[a] P = proportion of polymorphic loci
[b] H_e = gene diversity (Nei 1973). Standard deviations (SD) are in parenthesis
[c] N = mean sample size
[d] All = means across all loci

Gene diversity and genetic differentiation in populations

The χ^2-test showed significant differences at the 0.05 level for 117 loci between populations, indicating a non-random distribution of alleles. The mean gene diversity (H_e) estimates are summarized in Table 2. The highest gene diversity across all loci was observed in Sede Boqer (0.25) and the lowest for Mt. Meron (0.06). The gene diversity measured over all loci and populations was 0.24.

Total gene diversity of a subdivided population (H_T) can be analysed into the mean gene diversity in a population (H_S), the average between populations ($D_{ST} = H_T - H_S$) and the relative ($G_{ST} = D_{ST}/H_T$) degree of gene differentiation among subpopulations (Nei 1973). The proportion of diversity between and within population-statistics varied between loci having the highest G_{ST} (0.8) from E33M58 and the lowest (0.06) from E39M61. The mean G_{ST} over all loci indicated that, on average, 31% of the variation was between populations and 69% (1– G_{ST}) was within populations.

Correlations between the mean gene diversity and ecogeographical variables

We performed the Spearman rank correlation analysis between the mean gene diversity (H_e) produced by eight AFLP primer pairs from each population and 15 ecogeographical variables. In one primer pair (E35M55) the significant associations were established between H_e and the ecogeographical variables (Table 3). Also we ran a test for the best predictor of the mean H_e of eight primer pairs by stepwise multiple regression analysis, using H_e as a dependent variable and ecogeographical variables in the form of a principal component (pca) score as independent variables. At one primer pair (E35M55) PCA1 (water factors) explained significantly ($P = 0.017$) over 50% ($R^2 = 0.53$) of the variation in gene diversity.

Table 3 Spearman rank correlations (upper) and probabilities (lower) between gene diversity (H_e), overall loci in eight AFLP primer pair and 15 ecogeographic variables in ten populations of *H. spontaneum*. Symbols of variables: Pop = Population; Georaphical variables: Lon = longitude (decimals); Lat = latitude (decimals); Alt = altitude (m). Temperature variables: Tm = mean annual temperature (°C); Ta = mean August temperature (°C); Tj = mean January temperature (°C); Td = mean seasonal temperature difference (°C); Tdd = mean daily temperature difference (°C); Ev = mean annual evaporation; Water variables: Rn = mean annual rainfall (mm); Rd = mean number of rainy days; Hu14 = mean humidity at 14:00 (%); Huan = mean annual humidity (%); Dwsm = mean number of dewy nights in summer; (mm); Dummy soil variables: So = soil types: 1 Rendzina; 2 Terra Rossa; 3 Loess; 4 Alluvium. Soil types are in ascending order from light to heavy

Item	Lon	Lat	Alt	Tm	Ta	Tj	Td	Tdd	Rn	Rd	Hu14	Huan	Dwsm	Ev	So
E32M61	–0.049	–0.018	–0.220	0.025	0.037	0.142	–0.484	–0.114	–0.079	–0.021	0.184	0.162	0.235	–0.268	–0.238
	0.894	0.960	0.542	0.946	0.920	0.696	0.156	0.755	0.828	0.953	0.611	0.656	0.513	0.455	0.428
E33M58	0.530	0.461	–0.112	–0.013	0	–0.013	0.469	–0.190	0.287	0.363	–0.138	–0.175	–0.169	0.167	0.354
	0.115	0.180	0.758	0.972	1	0.972	0.171	0.598	0.422	0.303	0.704	0.629	0.641	0.645	0.316
E35M48	–0.117	–0.222	–0.123	0.081	0.121	0.081	–0.032	0.013	–0.283	–0.198	–0.152	–0.364	0.463	0.071	0.143
	0.748	0.538	0.735	0.824	0.740	0.824	0.930	0.972	0.428	0.584	0.676	0.302	0.178	0.845	0.694
E35M55	–0.384	–0.738*	–0.610	0.772**	0.844**	0.623	0.003	0.770**	–0.860**	–0.813**	–0.666*	–0.767**	–0.012	0.822**	0.771**
	0.273	0.015	0.061	0.009	0.002	0.054	0.993	0.009	0.001	0.004	0.036	0.01	0.973	0.004	0.009
E38M54	0.146	0.146	–0.073	–0.012	–0.049	0.154	–0.197	–0.352	0.109	0.226	0.407	0.164	0.238	–0.160	–0.141
	0.688	0.688	0.841	0.973	0.893	0.671	0.585	0.318	0.763	0.531	0.243	0.651	0.508	0.660	0.697
E38M55	–0.103	–0.079	0.321	–0.276	–0.299	–0.117	–0.323	–0.433	0.067	0.122	0.506	0.272	0.365	–0.398	–0.532
	0.777	0.829	0.365	0.440	0.402	0.748	0.362	0.211	0.855	0.738	0.136	0.448	0.300	0.255	0.144
E39M61	–0.294	–0.175	0.144	–0.139	–0.154	–0.063	–0.451	–0.214	–0.150	–0.060	0.384	0.121	0.612	–0.278	–0.285
	0.410	0.629	0.692	0.701	0.671	0.862	0.191	0.554	0.679	0.870	0.274	0.739	0.060	0.437	0.472
E45M55	0.042	0.152	0.273	–0.362	–0.390	–0.276	–0.133	–0.439	0.164	0.201	0.433	0.173	0.541	–0.434	–0.406
	0.907	0.676	0.446	0.304	0.265	0.440	0.714	0.204	0.651	0.578	0.211	0.633	0.106	0.210	0.244
Heall[a]	–0.201	–0.238	0.055	–0.043	–0.025	0.025	–0.239	–0.161	–0.220	–0.113	0.117	–0.134	0.529	–0.114	0.094
	0.577	0.508	0.880	0.906	0.946	0.946	0.506	0.657	0.542	0.756	0.748	0.713	0.116	0.754	0.795

*, **Significance at $P < 0.05$, 0.01, respectively

[a] Mean H_e across all loci

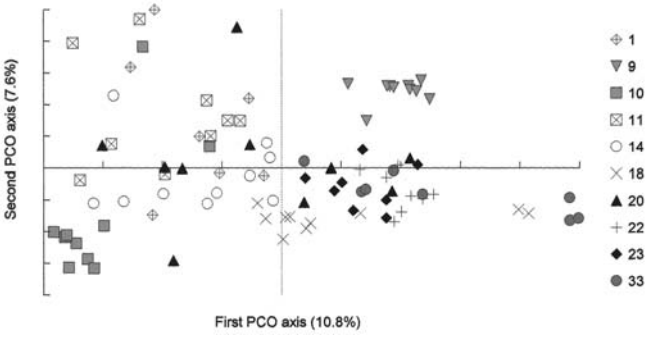

Fig. 3 The principal coordinate analysis (PCO) plot of 90 genotypes was based on Nei and Li's similarity matrice. The percentage variation explained by the axis is given between parentheses in the axis legend. For population names see Fig. 2

Discussion

Assessment of AFLP polymorphism

The major advantage of the AFLP-method has been its capacity for simultaneous identification of a large number of amplification products in the same lane, i.e. a high multiplex ratio. Our material primer pairs differed in their capacity to amplify bands, but the number of loci detected with even the less-sensitive primer pair (E32M61) was moderately high (12) compared to several other methods. The genotypes each had a different banding profile and therefore genotype identification was possible even with one AFLP primer pair combination. The level of polymorphism has not been as high with AFLPs as with some other techniques (Russell et al. 1997). When compared over all populations the polymorphism rate was high (93%) but within populations the rate was lower (21–74%). The disadvantage of the AFLP has been that it is mainly a dominant marker type. But since *H. spontaneum* has an estimated outcrossing rate of 1.6% (Brown et al. 1978), it was not regarded as a problem. Considering the technical ease and high information it produces, it is an advantageous method for carrying out population genetic studies in wild barley.

Genetic diversity pattern in wild barley

G_{ST} analysis overall loci showed that the degree of diversity is greater within than between populations. This is in contrast to expectations under conditions of limited migration between populations and the high selfing rate within the population. However, this is in agreement with other wild barley studies where a higher degree of genetic diversity is partitioned within, rather than between, populations (Nevo et al. 1979, 1986; Dawson et al. 1993; Baum et al. 1997; Turpeinen et al. 2001).

The results based on genotype information through hierarchical clustering and principal coordinate analyses are in accordance with the results of Gst-analysis. The genotypes were not clustered into distinct groups corresponding to their population, but were merely scattered along the phenogram and the PCO plot.

In a predominantly inbreeding organism the observation of high genetic diversity within populations should be taken into account when sample sizes of studies are considered. Low numbers of samples per population seem not to be enough to reveal the existing genetic differentiation structure of wild barley.

Comparison of gene diversity of AFLP and SSR, and associations with ecogeographical factors

Since the same genotypes have been analysed previously by simple sequence repeat (SSR) markers, straight comparisons were possible with the results of our present study. The populations for the highest and lowest gene diversity estimates were the same, Sede Boqer and Mount Meron, respectively. A comparison of gene diversity estimates of all populations between SSR and AFLP by the Pearson correlation analysis showed a significant correlation ($r = 0.65$, $P = 0.043$) between them. We were able to show associations between gene diversity and ecogeographical factors of some SSR loci, and we speculated on the possible adaptive role of the diversity pattern of SSR allele distribution in wild barley. Interestingly some SSR loci were in the same linkage group with dehydrin genes, which are identified as proteins induced by water deficits and may function against drought resistance. With AFLP, the significant association between gene diversity and ecogeographical factors was observed in one primer pair where negative correlation was linked to water factors and positive to temperature factors. Associations follow the trend where larger diversity could be traced from their more stressful environments, as have been reported in several other papers on wild barley (Nevo et al. 1979, 1981, 1986; Dawson et al. 1993; Baum et al. 1997; Nevo et al. 1997; Owuor et al. 1997; Pakniyat et al. 1997). Our result is in accordance with the niche-width variation hypothesis (Van Valen 1965) where species living in a spatio-temporally more heterogeneous environment are predicted to have larger genetic diversity. The niche-width is the proportion of the total multidimensional space of limiting resources used by a species. The relationships between environmental factors and genetic diversity are based on correlation and regression analyses. The use of these methods has the potential drawback that the obtained relationships, even though statistically significant, are spurious. The robustness of the patterns obtained could be estimated by comparing the results of SSR and AFLP with other similar studies. The accumulated data available from different species and genera support the pattern observed by SSR and AFLP (reviewed by Nevo 1998b).

What advantage could the larger genetic diversity offer for species living in a more heterogeneous environment? Environmental unpredictability selects for higher levels of genetic diversity, possibly because of their buffering effects in heterogeneous environments (Nevo 1988). The

spatial patterns and environmental correlates, and predictors of genetic variation of *H. spontaneum* in Israel, may indicate that genetic variation in wild barley populations is not only rich but also at least partly adaptively selected and predictable by the environment (Nevo 1998a). Consequently, conservation and utilization programs should optimise sampling strategies by following the ecological-genetic factors as effectively predictive guidelines.

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