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Ecological-genomic diversity of microsatellites in wild barley, Hordeum spontaneum, populations in Jordan

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Abstract We analyzed the ecological-genomic diversity of microsatellites of wild barley, *Hordeum spontaneum* (C. Koch) Thell., at 18 loci in 306 individuals of 16 populations from Jordan across a southward transect of increasing aridity. The 18 microsatellites revealed a total of 249 alleles, with an average of 13.8 alleles per locus (range 3–29), with *nonrandom* distribution. The proportion of polymorphic loci per population averaged 0.91 (range 0.83–1.00); gene diversity, *He*, averaged 0.512 (range 0.38–0.651). We compared the number of alleles of the 18 loci to those found in Israel populations by Turpeinen et al.. Out of the 280 alleles, 138 (49.3%) were unique (i.e. occurred in only one of the countries). The percentage of unique alleles in Jordan and Israel populations was 43.0% and 17.9%, respectively, suggesting that Jordan is an important center of origin and diversity of wild barley. Estimates of mean gene diversity were highest in the populations collected near the Golan Heights, such as Shuni North, Shuni South and Jarash. Sixty nine percent of the microsatellite variation was partitioned within populations and 31% between populations. Associations between ecogeographical values and gene diversity were established for eight microsatellite loci. The cluster produced by simple sequence repeat (SSR) data is mostly coincidence with the result of the dendrogram of the *Spalax ehrenbergi* superspecies of subterranean mole rats in Jordan based on allozyme gene loci. The major soil type in the wild barley habitat of each ecological group was different. Stepwise multiple regression analysis indicated that the variance of gene diversity was explained by altitude (R^2 = 0.362**). These observations suggest that microsatellites are at least partly adaptive and subject to natural selection. Electronic Supplementary Material is available if you access this article at http://dx.doi.org/10.1007/s00122-002-1029-7. On that page (frame on the left side), a link takes you directly to the supplementary material.

Keywords Genetic diversity · Microsatellite markers · Wild barley · *Hordeum spontaneum* · Natural selection

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Introduction

Cultivated barley, *Hordeum vulgare* L., is one of the principal cereals in world crop production and is cultivated in all temperate areas of the world (von Bothmer et al. 1995). Wild barley, *Hordeum spontaneum* (Nevo 1992), has been considered a progenitor of cultivated barley and is distributed throughout the Near East from Turkey to southwest Asia (Harlan and Zohary 1966). Several studies have been conducted at the allozyme (literature cited in Turpeinen et al. 2001) and DNA level (Turpeinen et al. 2001 and literature cited therein) analyzing genetic diversity in wild barley. These studies at the macro- and micro-scales identified adaptive divergence of genetic diversity at the protein and DNA levels. Significant correlations between microsatellites and ecogeographical variables were identified in several microsites in northern Israel in wild emmer wheat (*Triticum dicoccoides* Schweinf.), indicating adaptation to variable and stressful environments, at both the macro- and microscales, suggesting the operation of natural selection on microsatellites (Li et al. 2000a, b, c, 2002; Nevo et al. 2002). Similar results have been obtained for wild barley in Israel (Turpeinen et al. 2001).

Microsatellites, also known as simple sequence repeats (SSRs), are short (1–6 bp long) tandemly repeat-

Table 1 Geographical and climatological data for 16 populations of *H. spontaneum* from Jordan[a]

Population	Site of origin[b]	Lt	Ln	Al	Rn	Ta	Tj	Tav	Td	Soil	Bioclimate[c]
1	Shuni North	32.60	35.63	50	370	29	14.4	21.7	14.6	Marl	5
2	Irbid	32.55	35.85	585	433	22.3	11.4	16.85	10.9	Black Rendzina	2
5	Mafrak	32.25	36.20	695	164	25.3	11.2	18.25	14.1	Loess	6
6	Zarqa	32.08	36.10	610	148	26.1	10.4	18.25	15.7	Rendzina	4
7	Amman	31.90	35.92	750	260	22.2	10.4	16.3	11.8	Terra rossa	2
8	Madaba	31.68	35.83	790	368	21.9	9.8	15.85	12.1	Terra rossa	2
9	Mount Nebo	31.72	35.70	802	300	24.4	10.2	17.3	14.2	Rendzina	2
11	Wadi Arnon	31.45	35.85	500	200	24	13.4	18.7	10.6	Desert soil	8
13	Karak	31.15	35.72	1,150	370	23.8	9.5	16.65	14.3	Rendzina	2
15	Wadi Hassa South	30.93	35.73	300	170	21.4	8.1	14.75	13.3	Desert soil	4
16	Tafila	30.80	35.62	600	420	19	5.4	12.2	13.6	Desert soil	2
17	Dana	30.62	35.60	1,500	220	18.5	4.7	11.6	13.8	Desert loess	2
18	Wadi Mussa North	30.37	35.53	1,650	240	26.5	11.3	18.9	15.2	Desert soil	4
20	Shuna South	31.88	35.67	–120	215	23.2	10.8	17	12.4	Alluvial terrace	5
23	Jarash	32.27	35.87	630	385	20.7	9.2	14.95	11.5	Terra rossa	2
25	Qalat Ajlun	32.32	35.78	1,000	600	20.5	10.6	15.55	9.9	Terra rossa	1

[a] Symbols of variables: geographical: Lt = latitude (decimals), Ln = longitude (decimals), Al = altitude (m); water availability: Rn = mean annual rainfall (mm); temperature: Ta = mean hottest month temperature (°C), Tj = mean coldest month temperature (°C), Tav = mean of Ta plus Tj, Td = seasonal temperature difference; edaphic: Soil = soil type

[b] See locations in Fig. 1

[c] Bioclimatic region according to Long (1957, see explanation in Introduction)

ing DNA sequences and highly polymorphic as a result of frequent variation in the number of times the core sequence is repeated. The observed polymorphism may originate from unequal crossing-over or from slipped strand mispairing during replication. They are ubiquitous, found both in *coding* and *noncoding* regions in eukaryotic genomes and show high allelic variation (literature cited in Turpeinen et al. 2001). SSRs have been used extensively as DNA markers for identifying cultivars or accessions, or for genetic mapping and in population genetics and ecology studies (Turpeinen et al. 2001 and literature cited therein). The ubiquitous occurrence of SSRs has often indicated a functional significance. Some models of simple repeat evolution, in the absence of close linkage to a selected locus, are often referred to as "genetic hitchhiking" models and are regarded as selectively neutral (literature cited in Turpeinen et al. 2001). By contrast, research conducted at the Institute of Evolution on wild emmer wheat (Li et al. 2000a, b, c, 2002) and wild barley (Turpeinen et al. 2001), suggest that SSRs are also subjected to natural selection. A review summarizing this work was published recently (Li et al. 2002). Likewise, the SSR variation was compared with allozyme and RAPD variation in a recent book on wild emmer wheat (Nevo et al. 2002).

The current study was conducted to: (1) reveal for the first time the gene structure and diversity of microsatellites in wild barley, *H. spontaneum*, from populations across Jordan, (2) compare SSR diversity in wild barley in Jordan and Israel, and (3) elucidate the evolutionary significance of the pattern of the largely *noncoding* SSRs across the ecogeographical spectrum and evaluate the forces driving their evolution.

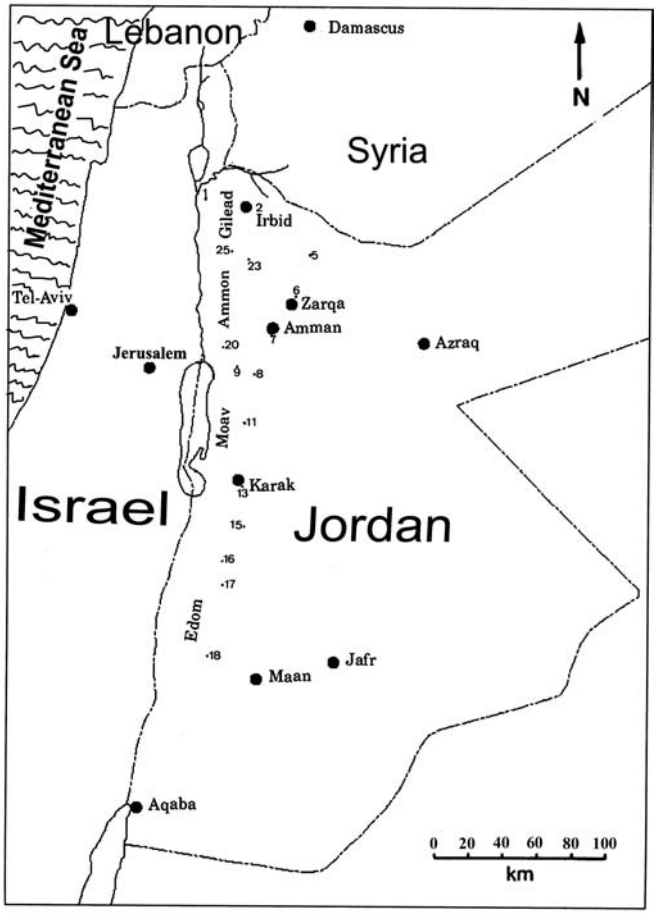

Fig. 1 Geographic distribution of 16 populations of *H. spontaneum* from Jordan: *1* Shuni North, *2* Irbid, *5* Mafrak, *6* Zarqa, *7* Amman, *8* Madaba, *9* Mt. Nebo, *11* Wadi Arnon, *13* Karak, *15* Wadi Hassa South, *16* Tafila, *17* Dana, *18* Wadi Mussa North, *20* Shuna South, *23* Jarash, *25* Qalat Ajlun

Table 2 Primer sequences, repeat motif, predicted size in base pairs (bp), allele size ranges (bp), and chromosomal location of 18 microsatellite primers

Name	Primer sequences (5′-3′)	Repeated motif	PCR[a]	Predicted size	Allele size range	Chromosomal location[b]
HVM5	AACGACCTCGCCCACAC AGGAACGAAGGGAGTATTAAGCAG	$(GT)_6$,$(AT)_{16}$	WMS 56.4	202	162–224	1(7H)
BMS64	CTGCAGGTTTCAGGAAGG AGATGCCCGCAAAGAGTT	$(AC)_{21}$	WMS 61.7	155	139–195	1(7H)
HVM36	TCCAGCCGAACAAGAGTT AGTACTCCGACACCACGTCC	$(GA)_{13}$	WMS 55.0	114	106–130	2(2H)
HVBKASI	ATTGGCGTGACCGATATTTATGTTCA CAAAACTGCAGCTAAGCAGGGGAACA	$(C)_{10}$	WMS 61.7	197	176–204	2(2H)
HVCSG	CACTTGCCTACCTCGATATAGTTTGC GTGGATTCCATGCATGCAATATGTGG	$(CA)_4(C)_{17}$	WMS 50.4	196	192–204	2(2H)
HVM9	CTTCGACACCATCACCCAG ACCAAAATCGCCATCGAACAT	$(TCT)_5$	Morgante (1994)	221	218–230	3(3H)
HVM62	TCGCGACCAGACGAGAAC AGCTAGCCGACGACGCAC	$(GA)_{11}$	WMS 51.9	251	223–259	3(3H)
HVM60	CAATGATGCGGTGAACTTTG AGTTCCCTGACCCGATGTC	$(AG)_{11}$ $(GA)_{14}$	WMS 50.0	115	81–121	3(3H)
HVM40	CGATTCCCCTTTTCCCAC ATTCTCCGCCGTCCACTC	$(GA)_6(GT)_4(GA)_7$	WMS 55.0	160	132–172	4(4H)
HVM68	AGGACCGGATGTTCATAACG CAAATCTTCCAGCGAGGTC	$(GA)_{22}$	WMS 61.7	204	172–228	4(4H)
HVM67	GTCGGGCTCCATTGCTCT CCGGTACCCAGTGACGAC	$(GA)_{11}$	WMS 61.7	116	104–124	4(4H)
HVM20	CTCCACGAATCTCTGACAA CACCGCCTCCTCTTTCAV	$(GA)_{19}$	WMS 60.0	151	129–155	5(1H)
HVM43	GGATTTTCTCAAGAACACTT GCGTGAGTGCATAACATT	$(CA)_9$	Liu (1996)	239	225–243	5(1H)
BMS90	ACATCAACCCTCCTGCTC CCGCACATAGTGGTTACATC	$(AC)_{20}$	WMS 62.0	221	193–237	5(1H)
HVM14	CGATCAAGGACATTTGGGTAAT AACTCTCGGGTTCAACCAATA	$(CA)_{11}$	WMS 48.0	158	146–150	6(6H)
HVLEU	TTGGAAGTGTACAGCAATGGAC TGAAAGGCCCCCACAAGATAG	$(ATTT)_4$	WMS 61.7	166	165–170	7(5H)
HVM6	CATGGATGAATGATTGGTTTTG CGCGTCCGTATGTGTATGAGTAA	$(GA)_9$	WMS 50.0	175	171–187	7(5H)
HVDHN9	CATGGACAAGATCAAGGAGAAG CCATTATTTATCTGTAGGAACGC	$(AC)_6$	WMS 56.4	128	128–144	7(5H)

[a] Annealing temperature (·) and PCR method
[b] Chromosome numbering follows barley nomenclature with the homologous chromosome group given in parenthesis (IBGS 1996)

Materials and methods

Plant material

Seeds from 306 genotypes from 16 populations of *H. spontaneum*, collected by Nevo in May 1996, from different mesic and xeric sites across the ecological spectrum of wild barley in Jordan, were used for microsatellite analysis. In each population 13 to 20 genotypes were analyzed. The sampling sites are given in Fig. 1, and the ecogeography and climatic profile for each of the sampling sites is documented in Table 1, based on the data of Nevo et al. (2000), Zohary (1973) and Al-Eisawi (1985). We considered environmental variables in the following categories: **geography**, including longitude, latitude, and altitude; **water availability**, means of annual rainfall; **temperature**, including mean January and August temperatures and seasonal temperature difference, **edaphic variables**, including the following soil types, marl, black rendzina, loess, terra rossa, rendzina, desert soil and alluvial terrace; **bioclimate region**, classified by Long's (1957) criteria. Biogeographically, Jordan consists of three major phytogeographical regions (*Atlas of Israel* 1970; Zohary 1973; Al-Eisawi 1985). These involve the *Mediterranean* (sub-humid), *Irano-Turanian* (semiarid), and *Saharo-Arabian* regions. Long (1957) divided Jordan into eight bioclimatic regions: (1) *Sub-humid Mediterranean*, warm and cool varieties; (2) *Semi-arid Mediterranean*, warm variety; (3) *Semi-arid Mediterranean*, cool variety; (4) *Arid Mediterranean*, cool variety; (5) *Arid Mediterranean*, warm variety; (6) *Arid Mediterranean*, very warm variety; (7) *Saharan Mediterranean*, cool variety; and (8) *Saharan Mediterranean*, warm variety.

DNA extraction and polymerase chain reaction

Eighteen microsatellite markers employed in this study are described in Table 2. Seeds were germinated in Petri dishes at room temperature after 3 days breaking dormancy treatment in a 4 °C chamber. Genomic DNA was extracted from fresh leaves of 10-day old seedlings using the plant genomic DNA isolation reagent DNAzolES (Molecular Research Center, Incorporated, Cincinnati, Ohio, USA), with some modification. The frozen leaves were pulverized in liquid nitrogen with a mortar and pestle. The frozen, fine leaf powder was transferred to 2-ml centrifuge tubes containing 0.7 ml of DNAzolES solution and 70 ng of Rnase A. This mixture was incubated for 5 min with occasional shaking and the addition of chloroform/isoamyl alcohol (24:1,v/v). The resulting mixture was stored at room temperature for 5 min and then centrifuged at 12,000 g for 10 min. The aqueous phase was trans-

Table 3 Comparison of the number of alleles of 18 shared SSR loci in Jordan and Israel populations (unique alleles in parenthesis)

Locus	Jordan[a]	Israel[b]	Total	Jordan (5000 randomized populations)			*p* value[c]	Significant
				Min.	Max.	Mean		
BMS64	29(13)	16	29	15	28	22.28	0.0002	***
BMS90	20(8)	15(3)	23	11	20	16.38	0.0888	
HVDHN9	4(2)	2	4	2	4	3.84	0	***
HVBKASI	18(11)	7	18	12	18	16.02	0	***
HVCSG	11(6)	7(2)	13	5	11	8.31	0.0466	*
HVLEU	3(3)	3(3)	6	2	3	2.30	0.7008	
HVM14	3(3)	7(7)	10	2	3	2.31	1	
HVM20	11(4)	8(1)	12	6	11	8.98	0.0582	
HVM36	12(3)	11(2)	14	7	12	10.19	0.6196	
HVM40	16(2)	15(1)	17	9	16	12.79	0.9306	
HVM43	10(5)	6(1)	11	6	10	8.47	0	***
HVM5	28(14)	16(2)	30	13	26	20.08	0.0048	**
HVM6	9(1)	9(1)	10	3	9	6.90	0.9618	
HVM60	19(10)	10(1)	20	12	19	16.58	0	***
HVM62	18(8)	14(4)	22	9	18	14.29	0.2736	
HVM67	10(3)	7	10	6	10	9.08	0.0002	***
HVM68	23(9)	17(3)	26	14	23	19.44	0.015	*
HVM9	5(2)	3	5	4	5	4.32	0	***
Total	249(107)	173(31)	280	183	222	202.54	0	***
Average	13.80	9.61	15.56	10.17	12.33	11.25	0	***

[a] This study

[b] From Turpeinen et al. (2001), but sample sizes are different. In Jordan the number of tested populations was 16 with 306 genotypes. In Israel (Turpeinen et al. 2001), the number of tested populations was 10 with 94 genotypes. Therefore we conducted standardization by randomly choosing the same number of populations and genotypes in Jordan as in Israel. The standardized analysis appears on the right hand side of the table

[c] Comparison between the mean number of alleles from the Jordanian randomized population against the number of alleles from the real population of Israel

ferred to fresh 1.5-ml tubes. The DNA was precipitated by adding 650 µl of 100% ethanol and centrifuged at 5,000 g for 5 min. Extracted DNA pellets were washed first with DNAzolES-ethanol solution and then with 95% ethanol, respectively. After the ethanol was removed, the DNA pellets were dissolved in 100 µl of 8 mM NaOH by overnight incubation at room temperature, and the pH was adjusted to 8.0 by adding 11.5 µl of 0.1 M HEPES.

PCR analysis

The PCR reaction was performed in a 25-µl volume using a Perkin-Elmer 9,600 thermocycler. The reaction mixture contained 250 nM of each primer, 0.2 mM of each dNTP, 1.5 mM of $MgCl_2$, 1 U of *Taq*-polymerase and 50 ng of template DNA. After 3 min of denaturalization at 94 °C, 45 cycles were performed with 1 min at 94 °C, 1 min annealing at 48 °C to 61.7 °C (depending on the individual microsatellite primer), 2 min extension at 72 °C and a final extension step of 10 min at 72 °C. In case of HVM9 and HVM43, we used the protocol of Morgante et al. (1994) and Liu et al. (1996) (Table 2). Fragment analysis was carried out on an Automatic Laser Fluorescence (ALF) Sequencer (Pharmacia) using a short gel cassette. Denaturing gel (0.35 mm thick) with 6% polyacrylamide was prepared using ReproGelTM (Amersham Pharmacia Biotech). The gels were run in 0.75 × TBE buffer at 800 V, 40 mA, and 50 W with 2 mW laser power and a sampling interval of 0.84 s. In each lane, fragments with known sizes were included as standards. Fragment sizes were calculated with a Fragment Analyzer Version 1.02 (Pharmacia) computer program by comparing internal size standards, and different alleles were detected by lane-matching analysis.

Data analysis

Allele frequencies, genetic diversity, and a homogeneity test of allele frequencies (χ^2-test) were calculated with Popgene version 1.31 program (Yeh et al. 1997). We used haploid analysis because the level of heterozygosity is less than 0.5%. An estimate of the gene diversity index (*He*), the proportion of polymorphic loci (*P*), and the mean number of alleles per locus (*A*) were calculated for each locus and population according to Nei (1973): $H_e = 1 - \sum Pi^2$ where Pi is the ith allele frequency. *Genetic partition* among and within populations was calculated by *Gst* analysis according to Nei (1973). *Unbiased genetic distances among populations* were calculated according to the distance of Nei (1978). The distance matrix was used to reveal associations between populations based on the unweighted pair group method with arithmetic averages (UPGMA) and with bootstrapping 5,000 times. *Variance analyses* were conducted using SAS software (SAS Institute 1996). *Discriminant analysis* procedures (SAS Institute 1996) were carried out as follows: (1) the SAS procedure PROC STEPDISC, using the STEPWISE option, was employed to choose alleles with the best differentiating factors among the 16 populations and among the five ecogeographic regions; (2) the SAS procedure PROC NEIGHBOR, which is suitable for classification when the classes have radically non-normal distribution, was used for correct classification of plants into their respective populations or regions, based on 6–15 loci chosen by PROC STEPDISC; (3) the SAS procedure PROC CANDISC was used to generate a graphic display of the first three canonical discriminant functions. Spearman rank correlation coefficients were used to assess differences in genetic indices and climatic variables in 16 populations. *Multiple Regression (MR) analysis* was conducted to test the best predictors of *P* and *He* in the 16 populations using these genetic indices as dependent variables and the ecogeographic factors as independent variables (SAS 1996).

Spatial autocorrelation analysis is a statistical approach for quantifying spatial relations among a univariate data set. The analysis shows the autocorrelation coefficients as a function of distance between the pairs of localities being considered (Sokal and Oden 1978a, b). The analyses were conducted by the SAAP program release 2.3 (Sokal and Wartenberg 1983). We computed the Moran's I autocorrelation coefficient from allele frequencies of 30

Table 4 Mean number of alleles per locus (*A*), proportion of polymorphic loci (*P*) –5%, mean SSR gene diversity, and unique alleles from the 16 Jordanian populations of wild barley, *H. spontaneum*

Population	Sample size (N)	*A*	*P*	*He*[a]	Unique alleles
1. Shuni North	18	4.833 b	0.94	0.622 a[b]	4
2. Irbid	20	4.222 b	1.00	0.481 ef	5
5. Mafrak	13	3.278 c	0.94	0.475 ef	3
6. Zarqa	20	3.278 c	0.89	0.527 bcde	2
7. Amman	16	3.222 c	0.89	0.382 g	1
8. Madaba	20	3.167 cd	0.83	0.410 fg	5
9. Mount Nebo	19	3.278 c	0.89	0.512 cde	4
11. Wadi Arnon	20	4.611 b	0.89	0.575 abcd	4
13. Karak	20	4.278 b	0.94	0.523 bcde	1
15. Wadi Hassa South	20	4.556 b	0.89	0.529 bcde	6
16. Tafila	20	4.611 b	0.94	0.586 abc	2
17. Dana	20	2.444 d	0.89	0.380 g	0
18. Wadi Mussa North	20	3.444 c	0.94	0.491 def	6
20. Shuna South	20	4.389 b	0.94	0.607 ab	5
23. Jarash	20	5.611 a	0.94	0.651 a	12
25. Qalat Ajlun	20	3.222 c	0.84	0.438 efg	2
Average		3.698	0.91	0.512	3.9
Range		2.444–5.611	0.83–1	0.38–0.651	0–12

[a] According to Nei (1973)
[b] Statistic diffrences presented by different letters using the Waller- Duncan K-ratio *t*-test (SAS institute 1996) at $p < 0.05$

relatively common alleles (belonging to 17 loci) across the entire geographical range of *H. spontaneum* in Jordan, including the 16 populations. The space was partitioned into four distance classes (0–47 km; 47–82 km; 82–142 km; 142–250 km), so that each class contained equal numbers (30) of locality pairs.

Results

Distribution of alleles at polymorphic SSR loci

Eighteen microsatellite primers previously documented (Turpeinen et al. 2001 and literature cited therein) were used (Table 2). All 18 microsatellite loci examined were polymorphic when considered over all populations. A total of 249 amplified fragments were detected over all loci (Table 3). The largest number of alleles was detected for the BMS64 locus (29 alleles) and the lowest number of alleles was in the HVLEU and HVM14 SSR loci (three alleles each). Out of 249 alleles at the 18-shared loci, 62 alleles (24.9%) were unique (i.e. occurred in only one of the populations). All the populations, except the Dana population, had 1–12 unique alleles (Table 4). The largest number of unique alleles (12) was detected in the Jarash population. All the primer pairs except HVDHN9 had 1–10 unique alleles. The largest number of unique alleles (10) was found in HVM5 primer pairs. Detailed information on allele frequencies is ocumented in an Appendix as additional electronic submitted material.

We compared the number of alleles of the 18 loci to those found in Israel populations by Turpeinen et al. (2001) (Table 3). Out of 280 alleles at the 18-shared loci, 138 (49.3%) were *unique* (i.e. occurred in only one of the countries). Out of these 138 alleles, 107 occurred in Jordan and 31 in Israel, and the percentages of *unique* alleles in each of the countries were 43.0% and 17.9%, respectively. In 13 loci (72.2%), from the 18, the mean number of alleles (from 5,000 randomized data sets) was higher for the Jordanian randomized population than the real population of Israel. The total proportion (10/18 = 55.6%) of loci showing significant differences far exceeded the 5% level expected by chance (Binomial test, 202.54 against 173, $p < 0.001$). The average, as well as the total number of alleles from all 18 loci, were significantly higher for the Jordanian randomized population than for the Israeli real population (11.25 against 9.61, $p < 0.001$).

SSR variation in fragment size

The allele size ranges for all populations at 18 loci are presented in Table 2. The largest difference in fragment size was observed for the compound locus HVM5, with fragments ranging from 162 to 224 bp. The repeat at locus HVM5 is dinucleotide, (GT) and (AT), yielding a 62-bp difference among individuals. The second largest difference (56 bp) was observed for loci BMS64 and HVM68.

SSR genetic diversity

A summary of the genetic diversity data of the 16 populations from Jordan is given in Table 4. For each population we calculated the mean number of alleles per locus (*A*), the proportion of the polymorphic loci (*P*), the mean gene diversity (*He*) (Nei 1973) and the unique alleles in the population. The average values were: $A = 3.698$ (range 2.444–5.611); $P = 0.91$ (range 0.83–1.00); $He = 0.512$ (range 0.380–0.651). The means of *A* and *He* were highest in the Jarash population ($A = 5.611$; $He = 0.651$) and second highest at Shuni North population ($A = 4.833$; $He = 0.622$). Lowest values were estimated for Dana ($A = 2.444$; $He = 0.380$), Madaba ($A = 3.167$; $He = 0.410$) and Amman ($A = 3.222$; $He = 0.382$). The level of poly-

Table 5 Partitioning of genetic diversity of *H. spontaneum* within and between 16 populations from Jordan based on 18 polymorphic microsatellite loci (Nei 1987)

Locus	H_T	H_S	D_{ST}	G_{ST}
BMS64	0.92	0.68	0.24	0.26
BMS90	0.89	0.68	0.21	0.24
HVDHN9	0.17	0.14	0.04	0.20
HVBKASI	0.92	0.68	0.23	0.25
HVCSG	0.76	0.65	0.11	0.14
HVLEU	0.49	0.09	0.40	0.81
HVM14	0.46	0.20	0.25	0.55
HVM20	0.78	0.46	0.32	0.41
HVM36	0.77	0.53	0.23	0.30
HVM40	0.88	0.62	0.27	0.30
HVM43	0.72	0.51	0.21	0.29
HVM5	0.84	0.62	0.21	0.26
HVM6	0.68	0.50	0.18	0.27
HVM60	0.89	0.61	0.28	0.31
HVM62	0.84	0.60	0.25	0.29
HVM67	0.76	0.53	0.23	0.30
HVM68	0.91	0.62	0.30	0.32
HVM9	0.70	0.49	0.21	0.30
Mean	0.74	0.51	0.23	0.31

morphism was not significantly different across the populations ($\chi^2_{(15)} = 6.95$, $p = 0.959$). The highest values of *A*, *P* and *He* were obtained in the lower altitude, xeric populations, collected near the Golan Heights, such as Shuni North, Shuni South and Jarash, compared to the populations from the middle and southern more mesic part of Jordan.

Genetic differentiation (G_{ST}) within and between populations

The total gene diversity (H_T) of populations can be portioned into: the mean gene diversity within the populations (H_s), the average gene diversity among populations ($D_{ST} = H_T - H_s$) and the gene diversity between the populations, relative to H_T ($G_{ST} = D_{ST}/H_T$) (Nei 1973). The overall distribution of variability showed that loci differ in their capacity to detect total observed diversity (H_T). The most variable loci were BMS64 and HVBKASI (0.92) and the least variable was HVDHN9 (0.17). The proportion of diversity among and within population statistics indicated that, on average, 31% (G_{ST}) of the variation was among populations and 69% ($1 - G_{ST}$) was within populations (Table 5). To test the significance of these results, a permutation test was applied, by producing 5,000 randomized data sets by random permutations of the genotypes within the various populations. The G_{ST} obtained from the randomized data sets averaged 0.050, ranging from 0.042 to 0.121, i.e. G_{ST} was very low for all 5,000 randomized data sets when compared to that of the real data ($p < 0.001$). Therefore, we suggest that the partition of SSR genetic diversity in the present study showed strong differentiation across the 16 populations.

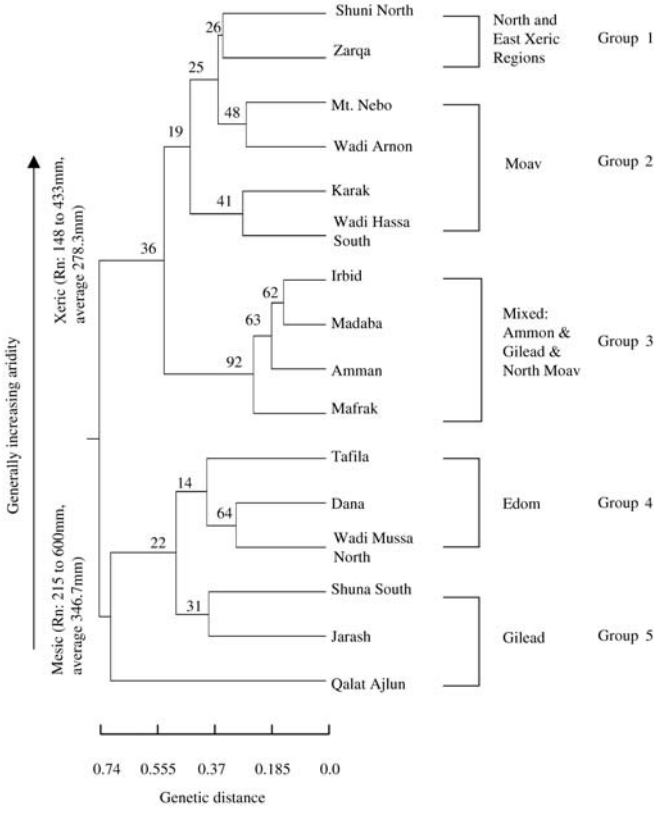

Fig. 2 Unweighted pair group method with an arithmetic averages (UPGMA) phenogram constructed from D distances illustrating the clustering of 16 *H. spontaneum* populations from Jordan (Nei et al. 1983). The mesic regions are, on average, more humid than the xeric regions. The *numbers* on the branches are values from bootstrap analysis with 5,000 replications

Genetic distance and Cluster analysis

The genetic distances (*D*) among the 16 populations were estimated by unbiased genetic distance (Nei 1978, Table 6). The largest genetic distance ($D = 1.48$) among the 16 populations was found between Wadi Mussa North and Madaba. The smallest genetic distance ($D = 0.15$) was observed between Irbid and Madaba populations. To represent the relationships between populations, cluster analysis (UPGMA) was used to generate a phenogram (Nei et al. 1983) based on *D* values (Fig. 2). The Cluster divided into five groups. Group 1 was composed of the populations from north and east xeric regions. Group 2 was comprised of the populations from Moav. Group 3 involved the populations from Ammon, except one population from Irbid and one (Madaba) from north Moav. Group 4 consisted of the populations from Edom. Group 5 included populations from Gilead. Also, major soil types in wild barley habitats of each group were different, such as marl and rendzina, rendzina, terra rossa and loess, desert soil and terra rossa, respectively. The cladogram splits into "xeric" (1–3) and "mesic" (4, 5) sub-branches suggesting a major climatic split.

Table 6 Genetic distance (Nei 1978) matrix values determined across 18 loci in 16 populations of *H. spontaneum* from Jordan. Mean = 0.696, range = 0.15–1.48

Population	Shuni North	Irbid	Mafrak	Zarqa	Amman	Madaba	Mount Nebo	Wadi Arnon	Karak	Wadi Hassa South	Tafila	Dana	Wadi Mussa North	Shuna South	Jarash	Qalat Ajlun
Shuni North																
Irbid	0.36															
Mafrak	0.38	0.23														
Zarqa	0.37	0.47	0.55													
Amman	0.45	0.21	0.24	0.62												
Madaba	0.53	0.15	0.31	0.54	0.19											
Mount Nebo	0.39	0.57	0.51	0.40	0.66	0.65										
Wadi Arnon	0.38	0.40	0.48	0.40	0.52	0.46	0.28									
Karak	0.50	0.69	0.78	0.62	0.94	1.00	0.52	0.37								
Wadi Hassa South	0.60	0.71	0.63	0.62	0.86	0.91	0.45	0.37	0.30							
Tafila	0.69	0.95	0.91	0.80	1.09	1.05	0.62	0.56	0.63	0.33						
Dana	0.89	1.07	0.98	1.26	1.14	1.46	1.30	0.91	0.90	0.61	0.42					
Wadi Mussa North	0.93	1.13	0.81	1.17	1.22	1.48	0.92	0.81	0.61	0.43	0.46	0.33				
Shuna South	0.57	0.99	0.86	0.79	1.10	1.15	0.75	0.70	0.64	0.64	0.44	0.63	0.40			
Jarash	0.51	0.76	0.64	0.57	0.75	0.82	0.60	0.60	0.73	0.79	0.50	0.69	0.68	0.42		
Qalat Ajlun	0.61	0.91	1.08	0.69	1.09	0.98	0.81	0.82	1.12	1.10	0.71	1.13	1.00	0.80	0.49	

Ecogeographical correlates

Spearman rank correlations

We calculated Spearman rank correlations between the average of genetic indices (*He*, *A*) and *He* of each of the 18 microsatellite loci and ecogeoraphical variables (Table 7). We recorded eight variables for all 16 populations; three geographical variables: Latitude (Lt), Longitude (Ln) and Altitude (Al); five climatic variables: Rainfall (Rn), mean August temperature (Ta), mean January temperature (Tj), mean Ta and Tj (Tav) and seasonal temperature difference (Td). The mean number of alleles per locus (*A*, $r_s = –0.613$; $p = 0.0116$) and the mean SSR diversity (*He*, $r_s = –0.647$; $p = 0.0067$) were negatively correlated with altitude. The correlation matrix between *He* in 18 microsatellite loci and geographic variables contained 160 correlations, 11 (6.88%) of them were significant ($p < 0.05$). Six loci (BMS64, HVBKASI, HVCSG, HVM62, HVM67 and HVM68) were significant, and negatively correlated with altitude (which correlates with temperature) ($r_s = –0.647$; $p = 0.007$). HVM36 and HVM6 loci were positively correlated with temperature and water availability factors, i.e. latitude ($r_s = 0.583$; $p = 0.0179$), rainfall ($r_s = 0.582$; $p = 0.018$) and mean January temperature ($r_s = 0.510$; $p = 0.0436$).

Multiple regression analysis

Tests of the best predictors of *He* and representative allele frequencies at the 18 polymorphic loci in the 16 Jordanian populations were conducted by stepwise multiple regression (MR) analysis, using these characters as dependent variables, and geographic and climatic factors as independent variables. The variances in the frequencies of nine loci were explained significantly (at $p < 0.05$) by 1 to 3 ecogeographical variables (Table 8). Altitude, mean hottest month temperature, rainfall, latitude and longitude factors singly or in combination, explained a significant proportion of the variation in polymorphism of SSRs. Altitude (correlated with both temperature and rainfall) significantly explained the mean *He* (over all loci) and *A* ($R^2 = 0.362$, 0.377 of their variance, respectively, $p < 0.05$).

Discriminant analysis

The discriminant analysis was based on multilocus data involving 66 alleles belonging to 18 loci. The criterion for including alleles in the test was the frequency of at least 0.5 in one of the 16 populations. Discriminant analysis was performed and the STEPDISC procedure chose the suitable alleles as the best differentiating factors. The summary of the results is presented in Table 9 and Fig. 3. The levels of correct classification of individual genotypes into their respective populations by differentiating alleles were (no. of selected alleles, in parenthesis) 41.8% (6), 58.2% (9), 66.3% (12) and 75.8% (15). These results indicate that SSR markers can be used to differentiate between *H. spontaneum* genotypes and to classify them according to their respective

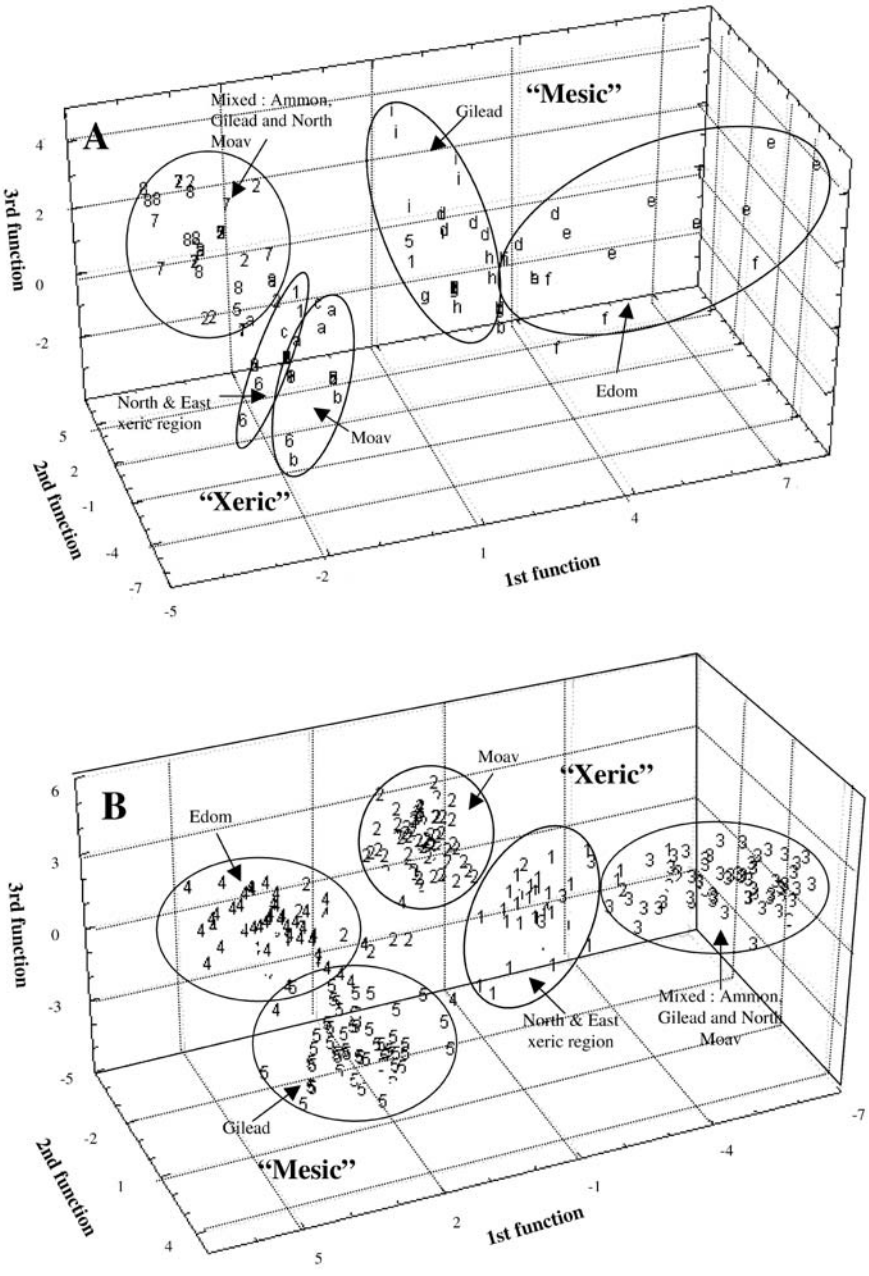

Fig. 3A, B Plot of canonical discriminant functions 1, 2 and 3 based on the best 15 discriminating SSR alleles. Figure **A** displays the pattern of 16 populations. Symbols: *1* = Shuni North, *2* = Irbid, *5* = Mafrak, *6* = Zarka, *7* = Amman, *8* = Madaba, *9* = Mt. Nebo, *a* = Wadi Arnon, *b* = Karak, *c* = Wadi Hassa South, *d* = Tafila, *e* = Dana, *f* = Wadi Mussa North, *g* = Shuna South, *h* = Jarash, *i* = Qalat Ajlun. The proportion of the eigenvalues of functions 1, 2 and 3, from the sum of the eigenvalues of the 15 discriminant functions, are 37.3%, 15.7% and 13.1%, respectively. Figure **B** displays the pattern of five regions. Symbols: *1* = North and East xeric region, *2* = Moav, *3* = mixed population of Ammon, Gilead and Moav, *4* = Edom, *5* = Gilead. The proportion of the eigenvalues of functions 1, 2 and 3, from the sum of the eigenvalues of the three discriminant functions, are 53.6%, 23.0% and 16.3%, respectively

populations, based on multilocus analysis (Fig. 3A). This figure shows that the genotypes of most of the populations from the same region clustered together. The population from Irbid separated from the Gilead population and combined with populations from the Ammon region by the first three canonical discriminant functions. The separation is probably due to the difference of ecogeographic conditions, such as soil type and bioclimate, etc. The levels of correct classification of individual genotypes into their respective ecogeographic regions by differentiating alleles were 74.8% (6), 85.9% (9), 90.8% (12) and 94.4% (15) (Fig. 3B) (percent correctly classified, expected by chance, without variable selection: 100/5 = 20%).

Spatial autocorrelation

The spatial autocorrelation results are presented in Table 10. The average autocorrelation coefficient over all tested alleles decreased consistently with an increase in the distance (km) class. The average coefficient was positive (0.069) in the low-order short distance class (0–47 km), with 0.136 deviation from the expected value (–0.067). The average coefficient of the 2nd distance class (47–82 km) was –0.015; however, this value was higher (deviation of 0.052) than the expected value. According to Duncan's multiple range test, these two average autocorrelation coefficients were significantly higher ($p < 0.05$) than that of the 3rd distance class (82–142 km), which averaged –0.140, and that of the

Table 7 Spearman rank correlations (upper) and probabilities (lower) between genetic indices in 18 microsatellite loci and 8 ecogeographical variables in 16 populations of *H. spontaneum* from Jordan. Abbreviations see Table 1

Genetic Indices	Locus	Lt	Ln	Al	Rn	Ta	Tj	Tav	Td
A	Mean	–	–	–0.613* 0.0116	–[a]	–	–	–	–
He	BMS64	–	–	–0.536* 0.0323	–	–	–	–	–
	BMS90	–	–	–	–	–	–	–	–
	HVDHN9	–	–	–	–	–	–	–	–
	HVBKASI	–	–	–0.692** 0.0030	–	–	–	–	–
	HVCSG	–	–	–0.580* 0.0184	–	–	–	–	–
	HVLEU	–	–	–	–	–	–	–	–
	HVM14	–	–	–	–	–	–	–	–
	HVM20	–	–	–0.490@ 0.0538	–	0.446@ 0.0832	–	–	–
	HVM36	–	–	–	0.582* 0.0180	–	–	–	–
	HVM40	–	–	–	–	–	–	–	–
	HVM43	–	–	–0.475@ 0.0633	–	–	–	–	–
	HVM5	–	–	–	–	–	–	–	–
	HVM6	0.583* 0.0179	0.494@ 0.0515	–	–	–	0.510* 0.0436	–	–
	HVM60	–	–	–	–	–	–	–	–
	HVM62	–	–	–0.554* 0.0259	–	–	–	–	–
	HVM67	–	–	–0.648** 0.0066	–	–	–	–	–
	HVM68	–	–	–0.612* 0.0119	–	–	–	–	–
	HVM9	–	–	–	–	–	–	–	–
	Mean	–	–	–0.647** 0.0067	–	–	–	–	–

Level of significance: ** = $p < 0.01$; * = $p < 0.05$; @ = $p < 0.1$
[a]–: non-significant values

high-order distance class (142–250 km), which demonstrated the lowest average (–0.176) with 0.105 deviation from the expected value. (*1*) *Low-order short distance* (*0–47 km*) *autocorrelations*. Positive deviation from the expected value (–0.067) was displayed by 22 (73.3%) of the 30 SSR alleles, seven (23.3%) of them being significant ($p < 0.05$). This proportion of significant loci exceeds the 5% level expected by chance (binomial test, $p < 0.01$). Negative deviation was displayed by only eight (26.7%) alleles, none of them significant. (*2*) *Second order distance* (*47–82 km*) *autocorrelations*. Only 3 (10%) of the 30 autocorrelations were significant ($p < 0.05$). This proportion (of significant loci) is not statistically significant (binomial test, $p = 0.376$). Positive deviation from the expected value (–0.067) was displayed by 20 (66.7%) of the 30 SSR alleles. (*3*) *Third order distance* (*82–142 km*) *autocorrelations*. Only ten (33.3%) of the 30 autocorrelations displayed positive deviation from the expected value (–0.067), whereas negative deviation was displayed by 20 (66.7%) alleles, two of them significant. (*4*) *High-order distance* (*142–250 km*) *autocorrelations*. Negative deviation from the expected value was 19 (63.3%) SSR alleles, six (20.0%) of them were significant ($p < 0.05$). This proportion of significant loci exceeds the 5% level expected by chance (binomial test, $p < 0.01$). However, note that in each distance class of the autocorrelation analysis, positive and negative deviations from the expected value were obtained (Table 10). Thus, there was no similar pattern across all loci. Therefore, we suggest that geographic distance cannot explain the genetic diversity pattern alone, but might be explained by a mixture of geography and ecology. Our genetic distance results showed a significant positive association with geographic distance ($r_s = 0.407$, $p < 0.001$), confirmed by the averages of the autocorrelation results.

Discussion

SSR polymorphism of wild barley, *H. spontaneum*, populations from Jordan

The main findings of this study are: all 18 microsatellite loci examined were polymorphic when all populations were considered. A total of 249 amplified fragments were

Table 8 Coefficients of multiple regressions (R square) of genetic indices and microsatellite band frequencies as dependent variables and eight ecographical variables as the independent variables in 16 populations of *H. spontaneum* from Jordan. Abbreviations see Table 1

Genetic indices	Locus	Stepwise model by ecogeographical variables		
A	Mean	Al 0.377*		
He	BMS64	Al 0.327*		
	BMS90			
	HVDHN9			
	HVBKASI	Al 0.282*	Al Ta 0.43*	
	HVCSG	Al 0.224@		
	HVLEU	Td 0.148ns	Al Td 0.286ns	
	HVM14	Tav 0.186@		
	HVM20	Al 0.294*	Al Ta 0.459*	
	HVM36	Rn 0.301*	Al Rn 0.414*	Lt Al Rn 0.579*
	HVM40	Td 0.204@		
	HVM43	Rn 0.209@	Al Rn 0.355@	Lt Al Rn 0.545*
	HVM5	Al 0.192@	Ln Al 0.391*	
	HVM6	Tj 0.302*		
	HVM60			
	HVM62	Al 0.176ns		
	HVM67	Al 0.287*		
	HVM68	Ln 0.198@	Ln Al 0.415*	Ln Al Ta 0.512*
	HVM9			
	Mean	Al 0.362*		

Level of significance:
** = $p < 0.01$;
* = $p < 0.05$;
@ = $p < 0.10$;
ns = $p > 0.10$

detected over all loci. The populations from Madaba and Qalat Ajlun were fixed to one allele in three loci, six populations (Wadi Hassa South, Zaqra, Amman, Wadi Arnon, Mount Nebo and Dana) were fixed for one allele in two loci and eight populations were fixed for one allele in one locus. Out of 249 alleles at the 18-shared loci, 62 alleles (24.9%) were unique. All the populations except the Dana population had 1–12 unique alleles.

The nature of microsatellite polymorphism

The dinucleotide repeats are reported to be most common and polymorphic among plants (Lagercrantz et al. 1993; Morgante and Olivieri 1993; Li et al. 2002). In our case, all except two loci (HVM14 and HVDHN9) of simple or compound dinucleotide repeats produced more alleles than three (HVM9) or four (HVLEU) nucleotide repeats. The most-polymorphic locus was BMS64 (AC_{21}) with 29 alleles. The locus with one nucleotide repeat (HVBKASI) produced a moderate (18) level of alleles. The observed polymorphism could be traced to variation in the repeat length of the microsatellite region except for HVLEU. In HVLEU, 43.5% of the observed sizes were not in accordance with the expected size reported in the literature (166-bp) for cultivated barley. This may be attributed to insertion/deletion events in the flanking sequences of microsatellites (Ortí et al. 1997; Huang et al. 1998; Turpeinen et al. 2001).

SSR gene diversity of the wild barley comparison between Jordan and Israel

We compared the number of alleles of the 18-shared SSR loci in the Jordan and Israel populations (Table 3). Of the total of 280 alleles at 18-shared loci, 249 (88.9%) were observed in the Jordan population, but only 173 (61.8%) were observed in Israel populations. The number of unique alleles in Jordanian populations (107) was much higher than those of Israeli populations (31). The average of mean gene diversity indices (Nei 1973), *He* in Jordanian populations (0.512), was higher than that of Israeli populations (0.467). The range of *He* of Jordanian populations (0.38–0.65, see Table 4)) was higher than those of Israeli populations (0.01–0.61) also. The gene diversity index of the northern part of Jordanian populations near the Golan Heights, such as Shuni north (0.62), Shuni south (0.61) and Jarash (0.65), are higher than those of central (Moav) or south Jordanian (Edom) populations. Accord-

Table 9 Summary table of 15 chosen loci obtained by stepwise discriminant analysis, based on the allele frequencies of 18 polymorphic loci among 16 populations and 5 regions of *H. spontaneum* from Jordan

Locus	Number in	Partial R^2	*F* Statistic	Prob > *F*	Wilks' lambda
Populations					
1. HVLEU-165	1	0.8315	95.417	0.0001	0.16848
2. HVM5-222	2	0.6787	40.706	0.0001	0.05413
3. HVM68-208	3	0.5420	22.724	0.0001	0.02479
4. HVM68-206	4	0.5244	21.093	0.0001	0.01179
5. HVM14-146	5	0.4772	17.404	0.0001	0.00616
6. HVM36-120	6	0.4258	14.089	0.0001	0.00354
7. HVM68-196	7	0.4465	15.273	0.0001	0.00196
8. HVM6-171	8	0.4155	13.411	0.0001	0.00115
9. BMS64-157	9	0.4050	12.796	0.0001	0.00068
10. BMS64-143	10	0.3829	11.624	0.0001	0.00042
11. HVM40-150	11	0.3751	11.205	0.0001	0.00026
12. BMS64-189	12	0.3548	10.227	0.0001	0.00017
13. HVM9-218	13	0.3470	9.850	0.0001	0.00011
14. HVM43-241	14	0.3263	8.943	0.0001	0.00007
15. HVM67-116	15	0.3225	8.757	0.0001	0.00005
Regions					
1. HVLEU-165	1	0.8183	338.874	0.0001	0.18171
2. HVM5-222	2	0.4643	64.996	0.0001	0.09735
3. HVM43-235	3	0.2822	29.39	0.0001	0.06987
4. HVM40-152	4	0.2466	24.39	0.0001	0.05264
5. HVM9-227	5	0.1732	15.552	0.0001	0.04352
6. HVM36-120	6	0.1684	14.99	0.0001	0.03619
7. HVM68-192	7	0.2193	20.716	0.0001	0.02826
8. HUBKASI-199	8	0.1623	14.242	0.0001	0.02367
9. HVM14-148	9	0.1409	12.009	0.0001	0.02034
10. HVM20-145	10	0.1571	13.604	0.0001	0.01714
11. HVM5-164	11	0.1186	9.786	0.0001	0.01511
12. HVM36-108	12	0.119	9.792	0.0001	0.01331
13. HVM68-194	13	0.1192	9.78	0.0001	0.01172
14. HVM6-171	14	0.0994	7.949	0.0001	0.01056
15. HVM67-110	15	0.0839	6.567	0.0001	0.00967

ing to these data, we assumed that Jordan is closer to the primary center of origin of wild barley, *H. spontaneum*, than Israel, or that the center of wild barley diversity may be in the grasslands of the Golan Heights.

Pattern of population structure in *H. spontaneum*

Partitioning of genetic variability by means of gene diversity statistics (Nei 1987) indicated that, on average, 69% of SSR diversity was distributed within the *H. spontaneum* populations and 31% between populations. This is consistent with findings from other studies indicating that considerable genetic diversity is partitioned within, rather than between, wild barley populations (Nevo et al. 1979, 1986a; Dawson et al. 1993; Baum et al. 1997; Turpeinen et al. 2001). By contrast, Dawson et al. (1993) reported converse findings when the number of populations was increased. Greater diversity between populations occurred rather, than within, them and was also reported by Chalmers et al. (1992) with isozymes. Zhang et al. (1993) with isozymes demonstrated, a larger amount of within-population diversity, whereas with AFLP, they resolved higher between-population diversity. From these observations, it appears that the diversity within- and between-populations can be affected by the sample size and the marker system applied. In wild emmer wheat the interpopulation diversity is much higher than the intrapopulation diversity, resulting in an "archipelago" population genetic structure (Nevo and Beiles 1989; Nevo et al. 2002).

Genetic distances (*D*) are in accordance with the ecological geographical pattern (Table 6). The wild barley populations of Jordan are clustered ecogeographically (Fig. 2). The first split in the cladogram is between "mesic" (Gilead, Edom) and "xeric" (north and east xeric regions, Moav, and a mixed group of Gilead and Ammon with 148 to 433 mm of annual rainfall). In each of these main branches there is another split between Edom (240–420 mm) and Gilead (433–600 mm) populations. The major soil types of mesic regions are desert soil and terra rossa, and that of the xeric region is rendzina. Therefore, the clustering represents climatic and edaphic divergence. The wild barley cluster is mostly coincidental with the result of the dendrogram of the *Spalax ehrenbergi* superspecies in Jordan based on allozyme gene loci (Nevo et al. 2000). From these observations, we assumed that the clustering to different groups is due to different ecogeographic conditions, subject to natural selection.

Spatial autocorrelation

Autocorrelation analysis tests whether the observed values (here, allele frequencies) at one locality are signifi-

Table 10 Spatial autocorrelation analysis of 30 allele frequencies belonging to 17 loci of 16 *H. spontaneum* populations from Jordan. Moran's I coefficients for each allele in four distance classes (30 locality pairs for each class) are given. The expected I is –0.067 for classes 1–3 and –0.071 for class 4

Locus	Allele	Distance (km)			
		0–47	47–82	82–142	142–250
BMS64	151	0.08	0.01	–0.49**	0.1
HVM6	173	–0.02	–0.14	–0.13	0
HVM6	175	0.46***	0.1	–0.56**	–0.28
BMS90	207	0.08	–0.06	0.06	–0.33
HVM40	142	0.05	–0.05	–0.12	–0.11
HVM40	158	–0.05	–0.23	–0.09	0.09
HVBKASI	179	–0.12	0.05	–0.18	–0.05
HVM62	239	0.04	0.15	–0.16	–0.3
HVM62	143	–0.09	–0.17	–0.02	0.01
HVM62	145	0.19	–0.21	–0.01	–0.22
HVM20	133	0.51***	0.31*	–0.17	–0.85***
HVM20	149	0.18	–0.29	0.03	–0.18
HVM20	153	0.1	0.17	–0.13	–0.37*
HVM9	221	–0.12	–0.01	–0.02	–0.11
HVM9	224	–0.1	–0.07	–0.13	0.03
HVM9	227	0.02	–0.08	–0.05	–0.14
HVM67	106	–0.06	0.1	–0.08	–0.24
HVM67	112	–0.2	0.04	–0.04	–0.06
HVM68	182	0.03	–0.19	–0.16	0.07
HVLEU	165	–0.12	0.04	–0.06	–0.16
HVM5	200	0	–0.16	–0.15	0
HVDHN9	128	–0.14	–0.01	–0.15	0.01
HVM14	146	0.53***	0.3*	–0.22	–0.84***
HVCSG	196	0.02	0.13	–0.34	–0.09
HVCSG	198	0.27*	–0.4*	–0.21	0.07
HVCSG	200	0.3*	–0.14	0.07	–0.47**
HVM36	108	–0.27	0.13	–0.04	–0.07
HVM36	110	0.27*	0.14	–0.31	–0.34*
HVM43	235	0.26*	0.03	–0.15	–0.38*
HVM43	237	–0.04	0.05	–0.2	–0.08
Mean		0.069	–0.015	–0.14	–0.176

*, **, ***, significance (textitp < 0.05, $p < 0.01$ and $p < 0.001$) autocorrelation

cantly correlated with those of neighboring localities. The autocorrelation coefficients and their significance are a function of the distance between pairs of localities (Sokal and Oden 1978a, b). The results of the average autocorrelation coefficients among all tested alleles supported the above suggestion. The highest positive autocorrelation average was for the low-order short distance, while the highest negative autocorrelation was with the high-order large distance. The autocorrelation analysis in Jordan reflects the fact that geography is intimately intermixed with ecology, implicating gradients of increasing altitude, both southward and eastward. Thus interpopulation divergence associates with ecogeographical heterogeneity involving climatic (temperature and water availability factors) and edaphic factors. This is also evident in the cluster analysis (Fig. 2) and the discriminant analysis (Fig. 3).

Natural selection on microsatellite diversity

Application of microsatellite loci as molecular markers in population genetics is often assumed to be neutral (Shriver et al. 1993; Valdes et al. 1993; Di Roenzo et al. 1994; Awadalla and Ritland 1997). However, some studies have led to the suggestion that natural selection may affect the level of microsatellite variation (Harding et al. 1992; Stephan and Cho 1994; Garza et al. 1995; Innan et al. 1997; Li et al. 2000a, b, c, 2002; Nevo et al. 2002). In the study of the selfing plant, *Arabidopsis thaliana L.*, Innan et al. (1997) suggested that there is no reason to reject any form of balancing selection on microsatellites, particularly with those microsatellites involved in an important function such as gene regulation. Although a general function for microsatellite loci has not yet been documented, certain effects have been observed for some microsatellites that could indicate their regulatory functional role in selection at particular loci (reviewed in Kashi et al. 1997; King and Soller 1999; Wren et al. 2000; Trifonov 2002). Previous studies have shown that $(TC)_n$, $(GA)_n$, and $(TA)_n$ control the transcriptional activity of the Ultrabithorax, hsp26 and actin5C genes in *Drosophila*. The $(TG)_n$ repeat modulates the transcription activity of the prolactin gene in rats (reviewed in Kashi et al. 1997). Saghai Maroof et al. (1994) studied the change of the composition of four microsatellite allele frequencies in two generations (F_8 and F_{53}) of composite cross material from *H. vulgare* and *H. spontaneum*, and explained the observed changes in allele frequencies as natural selection operating on chromosomal segments in which the marker loci reside. Several studies in wild barley (Nevo et al. 1986b; Ahokas and Naskali 1990; Chalmers et al. 1992; Dawson et al. 1993; Pakniyat et al. 1997; Nevo 1998; Turpeinen et al. 1999; Kalendar et al. 2000) have indicated that genetic diversity is not randomly distributed but is associated with certain ecogeographical factors, primarily climatic (temperature and water availability) and edaphic. Recently, Turpeinen et al. (2001) showed associations between ecogeographical variables (water availability, temperature and geography) and microsatellites. Similarly, Li et al. (2000a–c, 2002) and Fahima et al. (2002) have shown nonrandom SSR allele distribution in wild emmer wheat, locally and regionally. We reviewed the partly adaptive nature of SSRs in Li et al. (2002).

In the current study the genetic diversity within low-altitude populations stressed by drought and high temperature was the highest. Increasing diversity toward low altitude was also revealed in our Spearman rank correlation test, in which negative correlations were typically found with water factors and positive correlations with temperature factors. These results are in agreement with the SSR study of wild barley in Israel (Turpeinen et al. 2001). Furthermore, the stepwise multiple regression analysis revealed eight loci (BMS64, HVBKASI, HVM20, HVM36, HVM43, HVM5, HVM67 and HVM68) whose variation was significantly explained by altitude, i.e. associated with temperature and water factors.

Origins of wild barley

The Fertile Crescent is considered to be a primary center of diversity and origin of wild barley (Nevo 1992; Badr

et al. 2000; Lev-Yadun et al. 2000). Primary centers of diversity are usually characterized by high within- and between-population diversities, which were also found in this study. For the future, in situ conservation of populations in the primary center of diversity should be advanced by a dynamic approach to keep the level and composition of genetic diversity as high as possible (Nevo 1998) and for safeguarding these precious genetic resources for crop improvement. Considering this, it must be recognized that the determinants that generate and maintain genetic diversity in nature can be both stochastic and deterministic. The question is the relative importance of these factors in the evolutionary process and in biodiversity differentiation. Our data suggest that natural selection of SSRs, at least partially, is a determining force of diversity at the *noncoding* as well as the *coding* genomic regions, and that the SSR variation may be critical for regulating gene expression. Critical tests are essential, however, to unravel the direct function of SSRs in dynamic evolution.

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