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Grain isozyme and ribosomal DNA variability in *Hordeum spontaneum* populations from Israel

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Summary. Grain isozyme and ribosomal DNA (rDNA) variability was examined in *Hordeum spontaneum* populations sampled from 27 geographical sites in Israel. Considerable phenotypic variability was observed with variants of ADH1, EST3, EST10, BMY1 and WSP detected, which are not available in the *H. vulgare* gene pool. Seven new rDNA phenotypes were detected in the *H. spontaneum* populations. Shannon's index of diversity was used to partition the total phenotypic variation into between and within population components. Most of the variation occurred between *H. spontaneum* populations. The distribution of both grain isozyme and rDNA phenotypes was non-random and correlated with a range of ecogeographical factors. In particular, the G phenotype of BMY1 was restricted to the Negev Desert and Dead Sea regions of Israel. Over 78% of the variation in the frequency of this particular phenotype could be explained by the number of rainy days per year and mean temperature in January. This suggests that variation at this locus or at loci linked to it may be of adaptive significance and of value in the introgression of genes controlling abiotic stress tolerance from *H. spontaneum* into the *H. vulgare* gene pool.

Key words: *Hordeum* – Grain – Isozymes – Ribosomal DNA – Genetic adaptation

Introduction

The potential of wild plant species as genetic resources for enhancing the germ plasm of crops is well established (Frankel and Bennett 1970; Nevo 1986). Wild species are important sources of genetic adaptations to extreme environments and of disease and pest resistance genes not possessed by their cultivated relatives. Genetical analysis of population structure and diversity within these wild relatives is an important step towards exploiting these resources efficiently in breeding programmes.

Hordeum spontaneum, the wild ancestor of cultivated barley (Harlan 1979) exhibits considerable variability, particularly in the Near East Fertile Crescent. A number of studies have described the structure and genetical basis of diversity in *H. spontaneum* in Israel (Nevo et al. 1979, 1986; Nevo 1991) and have emphasised the extensive isozyme variability present in these populations. Importantly, the patterns of variation appear, at least in part, to be predictable both ecologically and climatically. These studies have also indicated that the geographical distribution of isozyme variation within *H. spontaneum* may be of adaptive significance.

Modern plant breeding practices have reduced the range of genetic variability amongst cultivars of many crops, including barley, and recent studies of isozyme variability in *H. vulgare* cultivars have confirmed this trend (Thompson et al. 1990; Forster et al. 1991). The availability of molecular techniques has improved the precision with which genetic variation in cultivated and natural plant populations can be examined, and analysis of ribosomal DNA (rDNA) variation has been particularly informative. rDNA is organised into tandem repeat units, with each repeat containing a highly conserved transcription unit and a more variable intergenic spacer (IGS) region. Changes in the rDNA IGS composition have been observed in *H. vulgare* (Saghai-Maroof et al. 1984), *Triticum dicoccoides* (Flavell et al. 1986), *Zea mays* (Rocheforde et al. 1990) and *Drosophila melanogaster* (Cluster et al. 1987) following natural or artificial selection.

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Isozyme and protein loci have been shown to be linked to genes controlling spring/winter growth habit in barley cultivars (Forster and Ellis 1991) and with a range of quantitatively controlled traits in barley doubled haploids (Powell et al. 1990). Associations between alleles at the rDNA loci and a range of agronomically important characters have also been detected (Powell et al. 1991). These studies have prompted us to examine the spectrum of molecular variability in natural populations of *H. spontaneum* sampled from its entire ecological range in Israel. Saghai-Maroof et al. (1990) have also examined ribosomal DNA variability in wild and cultivated barley, but the present study has included an analysis of five mature endosperm proteins together with rDNA IGS variability, thus allowing us to compare and relate protein and rDNA variability in *H. spontaneum* to a range of ecogeographical factors. Information on the geographical distribution of isozyme and rDNA phenotypes is discussed in relation to abiotic stress tolerance in barley.

Materials and methods

Plant material

A total of 135 accessions of *H. spontaneum* were sampled from 27 sites in Israel, and 5 accessions per population were examined for grain protein and rDNA variability. The specific geographical locations and the ecological and climatic background for each of the sampling sites have been given previously (Nevo et al. 1979). The distribution of sampling sites is given in Fig. 1. The ecogeographical variates are as follows: (a) geographical: longitude (Lon, in decimals), latitude (Lat, in decimals), altitude (Alt, in meters); (b) temperature: mean annual temperature [Tm (°C)], mean August temperature [Ta (°C)], mean January temperature [Tj (°C)], mean seasonal temperature difference [Td (°C)], day-night temperature difference [Tdd (°C)], mean number of tropical days (Trd), mean number of Sharav days, i.e., hot and dry days (Sw); (c) water availability: mean annual rainfall [Rn (mm)], mean number of rainy days (Rd), mean annual humidity [Huan (%)], mean humidity at 1400 hours [Hu14 (%)], mean number of dew nights in summer (Dw), Thornthwaite's moisture index (Th), mean annual evaporation [Ev (cm)], mean interannual variability of rainfall [Rv (%)], mean relative variability of rainfall [Rr (%)]; (d) edaphic: soil type (So), 2 [rendzina (=Rnz)], 3 [alluvium (=Alu), 4 [sandy loam (=SLo)], 6 [loess (=Loe)].

Protein extraction

β-amylase and grain esterase. Portions of endosperm (20 mg) from mature dry grains were handmilled and mixed with 50 µl distilled water. Samples were left to extract for 1 h and then centrifuged briefly prior to loading onto the gel.

Water-soluble protein and alcohol dehydrogenase. Portions of endosperm (40 mg) from mature dry grains were handmilled and mixed with 70 µl of 10 m*M* dithiothreitol (DTT) with sucrose added at a concentration of 200 mg/ml. The samples were left to extract for 1 h, then centrifuged briefly prior to loading onto the gel.

Isoelectric focusing

Ultra-thin polyacrylamide gels (220 × 120 × 0.1 mm) were prepared containing 5% v/v ampholines. The ampholytes mixtures used for each protein marker system are given in Table 1, together with the protein symbols and gene loci. The gels were first prefocused for 500 volt hours (Vh), and then sample extracts were placed on the gel surface 1 cm from the cathode using 5 × 10 mm paper wicks and run for a further 500 Vh after which

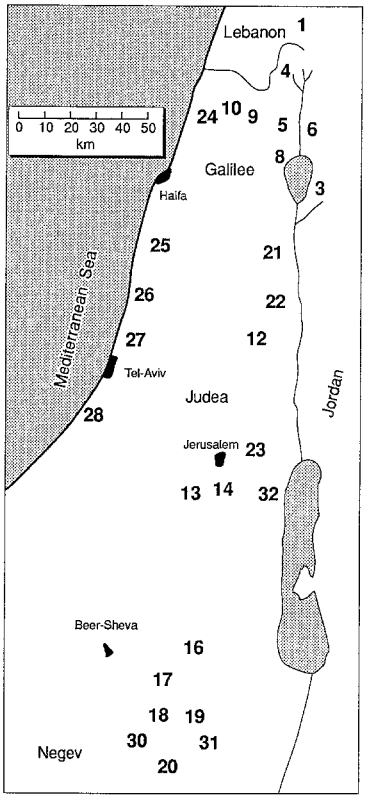

Fig. 1. Geographic distribution of sampling localities for *H. spontaneum* in Israel. 1 Mt. Hermon; 3 Afiq; 4 Tel Hay; 5 Rosh Pinna; 6 Gadot; 8 Zefat; 9 Mt. Meron; 10 Maalot; 12 Shechem; 13 Bar Giyyora; 14 Talpiyyot; 16 Tel Shoket; 17 Bor Mashash; 18 Revivim; 19 Yeroham; 20 Sede Boqer; 21 Bet Shean; 22 Mehola; 23 Wadi Qilt; 24 Akhziv; 25 Atlit; 26 Caesarea; 27 Herzliyya; 28 Ashquelon; 30 Avedat; 31 Ha-Machtesh; 32 Ein Zukim

Table 1. Proteins, loci and ampholyte mixtures used for isoelectricfocusing

Protein symbol	EC number	Gene symbol	Chromosome location[a]	Ampholyte 5% mixture v/v
EST10	EC 3.1.1	*Est10*	3H	pH (4–6.5) (4.2–4.9) (4.5–5.4) 1:1:1
EST3	EC 3.1.1	*Est3*	7H	pH (4–6.5) (4.2–4.9) (4.5–5.4) 1:1:1
BMY1	EC 3.2.1.2	*Bmy1*	4H	pH (4–6.5)
ADH1	EC 1.1.1.1	*Adh1*	4H	pH (4–6.5) (4.2–4.9) (4.5–5.4) 1:1:1
WSP2, 3		*Wsp2*	5H	pH (4–6.5) (4.2–4.9) (4.5–5.4) 1:1:1
		Wsp3	4H	

[a] Chromosome nomenclature is based on homoeology in the *Triticeae*

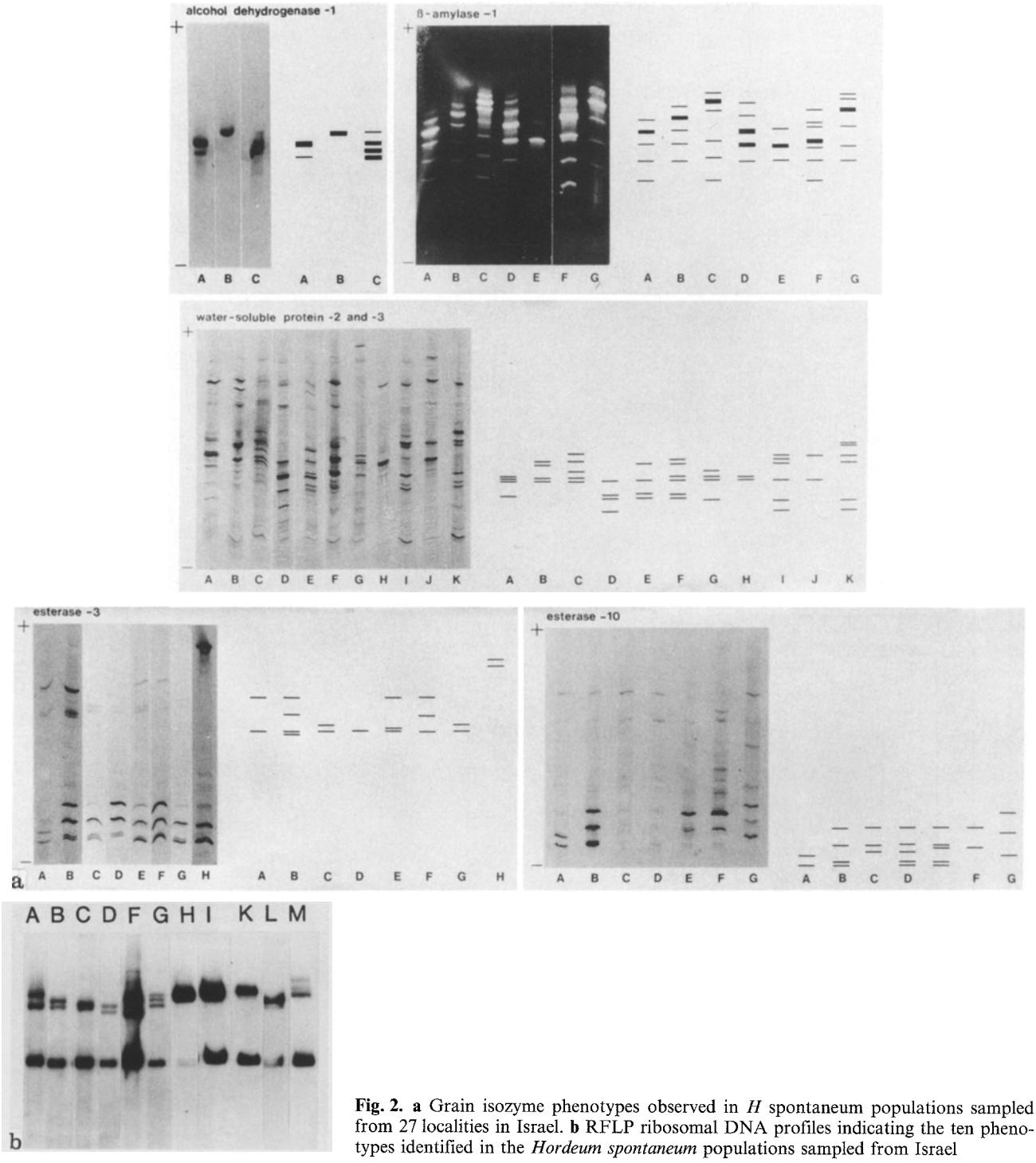

Fig. 2. **a** Grain isozyme phenotypes observed in *H* spontaneum populations sampled from 27 localities in Israel. **b** RFLP ribosomal DNA profiles indicating the ten phenotypes identified in the *Hordeum spontaneum* populations sampled from Israel

the paper wicks were removed. Proteins were separated for a further 2500 Vh. The power applied to the gel was 1 W/cm length (with a maximum voltage of 3000 V).

Staining procedures

Grain esterases (EST3 and EST10). Gels were incubated in a solution of 50 mg α-naphtyl acetate and 100 mg Fast Blue RR salt dissolved in 2 ml dimethyl sulfoxide and made up to 100 ml with 1 *M* $NaHPO_4$ (pH 7.6) for 30 min at 37 °C and then destained in 7% acetic acid. The EST3 isozymes studied here were extracted from grains, they have similar pIs to the EST3 of leaves but have different patterns in IEF gels. We therefore regard them as being distinct although both loci are located on chromosome 7H (Nielsen and Hejgaard 1986; Hvid and Nielsen 1977).

β-amylase (BMY1). Gels were immersed in 30 g/l soluble starch for 10 min, drained and washed to remove superficial starch and

Table 2. Phenotypic frequencies and diversity estimates (H_0) for the six marker systems analysed from 27 geographical locations in Israel

Marker	Population																										
		1	3	4	5	6	8	9	10	12	13	14	16	17	18	19	20	21	22	23	24	25	26	27	28	30	31	32
ADH1	A			0.2							0.4																	
	B	1.0	1.0	0.8	1.0	1.0	1.0	0.4	1.0	1.0	0.6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	C							0.6																				
	H_0			0.722				0.971			0.971																	
WSP2,3	A		0.8		1.0	0.4	0.2		0.8	0.6		1.0	0.6	0.2	1.0			1.0	1.0		0.4	0.2	0.4	1.0	0.8	1		0.8
	B					0.2	0.6				0.4										0.6	0.2			0.2			
	D																											
	E		0.2			0.4																						
	F			1.0							1.0												0.6					
	G	1.0														1.0											0.2	0.2
	H													0.8			1.0			1.0							0.8	
	I								0.2													0.6						
	J						0.2	1.0																				
	K									0.4																		
	H_0		0.722			1.520	1.370		0.722	0.971			0.971	0.722							0.971	1.370	0.971		0.722		0.722	0.722
EST3	A	0.8	0.5	1.0	1.0		0.8	1.0	1.0	1.0		0.2	0.4	0.6	1.0	1.0		0.2	0.4	0.5	0.6	0.8	0.8	0.8	0.5	1.0	1.0	0.4
	B										0.4																	0.6
	C		0.5			0.2					0.6		0.2							0.25	0.2				0.25			
	D											0.8		0.2				0.6						0.2	0.25			
	E	0.2					0.2						0.4	0.2				0.2		0.25	0.2	0.2	0.2					
	F						0.8																					
	G																		0.6									
	H_0	0.722	1.000			0.722	0.72				0.971	0.722	1.520	1.370				1.370	0.971	1.500	1.370	0.722	0.722	0.722	1.500			0.971
EST10	B	1.0	1.0	0.4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8	1.0	1.0	1.0	1.0	1.0	0.8	1.0	1.0	1.0	0.2	1.0	1.0		1.0	
	C			0.6																								
	E																						0.8					
	F												0.2						0.2									
	G																									1.0		1.0
	H_0			0.971									0.722						0.722				0.722					
BMY1	A				1.0	0.4	1.0	0.6		0.8																		
	B	1.0	1.0	0.4		0.6				0.2	0.6	1.0			0.6	1.0	1.0	0.4	0.2	1.0	0.8	0.8	0.4					
	C														0.4			0.6	0.8		0.2	0.2	0.6					
	D			0.6							0.2																	
	E							0.2																				
	F							0.2	0.2																			
	G								0.8		0.2		1.0	1.0		1.0										1.0	1.0	1.0
	H_0			0.971		0.971		1.370	0.722	0.722	1.370			0.971			0.971	0.722		0.722	0.722	0.971						
rDNA	A		1.0	0.25			0.2					0.67						0.5	0.33			0.6	0.5	0.4	0.5			1.0
	B			0.25	1.0		0.4	1.0				0.33	0.5	0.67		1.0	1.0		0.33		0.5			0.6	0.25	1.0	1.0	
	C			0.5					1.0		1.0		0.3										0.25		0.3			
	D									1.0													0.25					
	F						0.2																					
	G						0.2							0.33				0.5										
	I																		0.33									
	K																				0.5							
	L																					0.2						
	M																					0.2						
	H_0			1.500			1.920					0.918	1.500	0.918				1.000	1.370		1.000	0.971	1.500	0.971	1.500			

then stained with a 3% stock iodine (65 g/l I_2, 1.95 g/l KI) and 1% acetic acid. The gels were developed in the iodine solution for a further 10 min

Water-soluble protein (WSP). Gels were placed in a solution of 34.6 g sulphosalicylic acid and 115 g trichloroacetic acid made up to 1 l for 15 min. They were then immersed in a Coomassie Blue R solution (0.46 g Coomassie Blue R dissolved in 400 ml of 25% absolute ethanol with 7% acetic acid and heated to 70 °C) for 10 min and subsequently destained in 25% absolute ethanol with acetic acid.

Alcohol dehydrogenase (ADH). Gels were placed in a solution containing 30 mg NAD, 1 ml absolute alcohol, 20 mg MTT, 5 mg PMS and 100 ml 0.05 *M* TRIS pH 8.6 until dark blue bands developed (30 min).

DNA procedures

Total genomic DNA was isolated by a modification of the procedure of Saghi-Maroof et al. (1984). DNA was digested with the restriction enzyme *Sac*I. Digestion was carried out in a total volume of 100 µl using 10 µg DNA, 25 units of enzyme and 1 × standard digestion buffer (0.33 *M* TRIS-acetate, pH 7.8, 0.065 *M* potassium acetate, 10 m*M* magnesium acetate, 4 m*M* spermidine, 0.5 m*M* DTT) overnight at 37 °C. Digested DNA was precipitated by the addition of 0.1 vol. 3 *M* Na acetate and 2 vol. 100% absolute ethanol, washed in 70% absolute ethanol, dried and re-dissolved in TE. Restriction fragments were fractionated in 1% agarose gels and transferred to Hybond N^+ nylon membrane (Amersham) using the alkaline blotting procedure of Reed and Mann (1985). Prehybridisation and hybridisation conditions were those described by Maniatis et al. (1982). Hybridising fragments were visualised following autoradiography at −70 °C with intensifying screens for 12 h.

The genomic clone pBG35 which contains one entire flax rDNA repeat unit was used as the hybridisation probe (Goldsborough and Cullis 1981). Radioactive probes were prepared by oligolabelling using α[^{32}P]dCTP (3000 Ci/m*M*) and the entire plasmid as template (Feinberg and Vogelstein 1984).

Results

The phenotypes observed in the *H. spontaneum* populations for the five endosperm protein systems are given in Fig. 2a together with a selection of the rDNA RFLP profiles (Fig. 2b). Phenotypic data on protein variation in European cultivars (*H. vulgare*) for EST3, EST10, WSP1 and BMY1 have been published previously (Thompson et al. 1990). Several new isozyme and protein phenotypes were detected in *H. spontaneum* that had not been previously observed in the cultivated barley gene pool. For example, three phenotypes were observed for ADH1, which is monomorphic in cultivated barley. Similarly, seven WSP1 phenotypes, five EST3, four EST10, and three BMY1 phenotypes were detected in *H. spontaneum* that were not present in the *H. vulgare* cultivars examined by Thompson et al. (1990). A total of ten rDNA phenotypes were detected in the *H. spontaneum* populations, whereas only three rDNA phenotypes have been detected in the *H. vulgare* gene pool (K. J. Chalmers unpublished).

Table 3. Partitioning of the genetic diversity between and within populations of the six marker systems using Shannon's diversity index

	H_{pop} H. spontaneum	H_{ssp} H. spontaneum	H_{pop}/H_{ssp} H. spontaneum	$(H_{ssp}-H_{pop})/H_{sp}$ H. spontaneum
WSP2, 3	0.469	2.420	0.194	0.806
ADH1	0.102	0.310	0.329	0.671
EST3	0.691	1.692	0.408	0.592
EST10	0.143	0.695	0.206	0.794
BMY1	0.418	1.842	0.227	0.773
rDNA	0.800	2.689	0.298	0.702
Mean	0.437	1.608	0.277	0.723

Phenotypic frequencies for the six marker systems sampled from 27 geographical locations in Israel are given in Table 2. Estimates of diversity (H_0) within populations were calculated using Shannon's information measure. $H_0 = -\sum pi \log_2 P_i$, where P_i is the phenotypic frequency (King and Schaal 1989). The results show that for each system examined there are marked differences in phenotype frequencies between populations. In each case a proportion of the populations are monomorphic, while the remainder vary in degree of polymorphisms. For example, for ADH1 two phenotypes which are unique to wild barley were restricted to three sites: Tel Hay (phenotype C), Mt Meron (C) and Bar Giyyora (A), while populations at other sites were monomorphic for the phenotype found in *H. vulgare*. Considerable variability both within and between populations was observed for the rDNA phenotypes, with six populations exhibiting three or more distinct rDNA profiles.

Shannon's index of phenotypic diversity (King and Schaal 1989) was used to quantify the levels of polymorphism detected and to partition this variability into between and within population components (Table 3). H_{pop} provides a measure of the average diversity within populations. ADH1 and EST10 show relatively low levels of polymorphism, while in contrast, rDNA and EST3 exhibit relatively high levels of diversity within populations. The greatest levels of diversity within *H. spontaneum* (H_{ssp}) are found for rDNA and WSP2,3. An examination of the proportion of diversity present within populations (H_{pop}/H_{ssp}) and between populations ($H_{ssp}-H_{pop}/H_{ssp}$) indicates that on average most of the phenotypic variation occurs between *H. spontaneum* populations.

Correlation coefficients were calculated between estimates of genetic diversity (H_0) for the six marker systems and a range of ecogeographical variables (Table 4). Thirteen of the 114 correlation coefficients computed were significant. In particular, ADH1 diversity was negatively correlated with soil type (So; $r = -0.498$; $P < 0.010$),

Table 4. Correlation coefficients (r) between genetic diversity (H_0) and ecogeographical variables in *H. spontaneum* for six marker systems in the 27 sites sampled

Variable	ADH1	BMY1	EST3	EST10	WSP2,3	rDNA
Lon	0.43	0.065	0.079	0.009	0.039	0.009
Lat	0.255	0.034	0.034	0.015	0.306	0.269
Alt	0.395	−0.109	−0.423*	−0.172	−0.269	−0.378
Tm	−0.384	0.011	0.395*	0.118	0.135	0.194
Ta	−0.269*	−0.065	0.477*	0.080	0.051	0.102
Tj	−0.296	0.093	0.519**	0.084	0.228	0.334
Td	0.127	−0.242	−0.189	0.092	−0.326	−0.268
Tdd	−0.371	−0.168	0.049	0.082	−0.104	−0.160
Rn	0.335	0.124	−0.118	−0.065	0.012	0.173
Rd	0.377	0.298	0.035	0.035	0.117	0.349
Hu14	0.096	0.329	0.202	−0.027	0.298	0.465*
Huan	0.076	0.324	0.186	−0.004	0.330	0.449*
Dw	−0.095	0.036	−0.240	−0.107	0.166	−0.203
Sw	0.283	−0.364	−0.200	−0.107	−0.455*	−0.213
Th	0.597	0.229	−0.316	−0.050	0.162	0.079
Trd	−0.165**	−0.133	0.293	0.127	−0.110	−0.121
Ev	−0.089	−0.386*	−0.040	−0.009	−0.297	−0.433*
So	−0.498**	−0.041	0.164	0.131	0.222	−0.103
Pl	−0.297	0.405*	0.206	0.040	0.153	−0.176

Level of significance: * $P < 0.05$, ** $P < 0.01$
Lon, Longitude (in decimals); Lat, latitude (in decimals); Alt, altitude (m); TM, mean annual temperature (°C); Ta, mean August temperature (°C); Tj, mean January temperature (°C); Td, mean seasonal temperature difference; Tdd, day-night temperature difference; Trd, mean number of tropical days; Rn, mean annual rainfall (mm); Rd, mean number of rainy days; Hu14, mean humidity at 1400 hours (%); Huan, mean annual humidity (%); Dw, mean number of dew days in summer; Th, Thornthwaites moisture index; Ev, mean annual evaporation (cm); So, soil type; Pl, plant community

Table 5. Coefficients of multiple regression (R^2) of dependent variable H_0 and independent ecogeographical variables for 27 populations of *H. spontaneum* in Israel

	Stepwise model							
	X_1	X_2	X_3	X_4	R_1^2	R_2^2	R_3^2	R_4^2
Constant								
(H_0) ADH1	So	Lon	Alt	Trd	20.1*	23.8*	26.1*	29.9*
(H_0) BMY1	Ev	Pl	So	Trd	5.9	8.2	25.6*	30.7*
(H_0) WSP2,3	Ev	So	Lat	Pl	15.0*	19.2*	36.5**	38.4**
(H_0) EST3	Tj				22.3*			
(H_0) rDNA	Alt	Rd	Lat	Rn	23.5*	25.9*	28.8*	33.1 *

Level of significance: * $P<0.05$, ** $P<0.01$
Abbreviations: see Table 4 and text

Table 6. Coefficients of multiple regression (R^2) for dependent phenotypic frequencies and variable ecogeographical variables in 27 populations of *H. spontaneum* in Israel

	Stepwise model									
	X_1	X_2	X_3	X_4	X_5	R_1^2	R_2^2	R_3^2	R_4^2	R_5^2
Constant										
BMY1, A	Rd	Tj	Alt	Dw	Pl	10.8*	21.8*	33.4*	39.8**	46.1**
BMY1, B	Lat	So	Ev	Rn	Alt	25.0*	32.6**	35.8**	39.2**	48.8**
BMY1, G	Rd	Tj	Dw	Tdd	Th	63.6***	78.4***	83.2***	88.8***	89.5***
rDNA-A	Tj	Pl	Ev	Huan	Rn	19.6*	40.2**	44.9**	59.9**	63.3**
rDNA-B	Huan	Ev	Ev	Trd	Lon	31.6**	51.6**	65.1***	77.3***	82.9***

Level of significance: * $P<0.05$, ** $P<0.01$, *** $P<0.001$
Abbreviations: see Table 4 and text

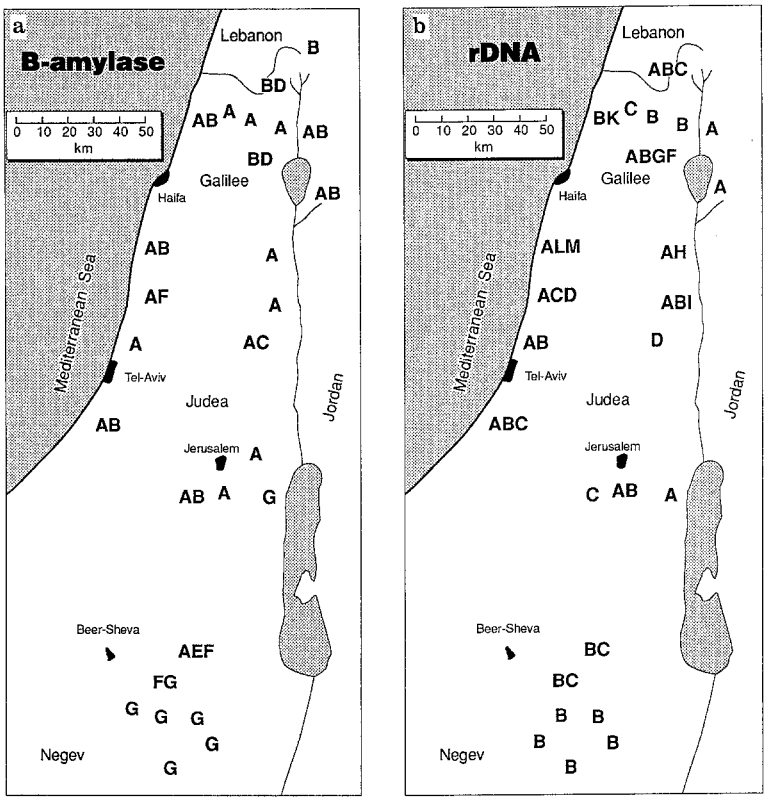

Fig. 3. **a** The distribution of β-amylase phenotypes in Israel. For geographical location see Fig. 1. **b** The distribution of rDNA phenotypes in Israel

while genetic diversity for BMY1 was negatively correlated with mean annual evaporation (Ev; $r = -0.386$) and positively correlated with plant community (Pl; $r = 0.405$; $P < 0.01$). Only for EST10 was there no evidence of a correlation between the level of genetic diversity within a population and one or more ecogeographical variables.

To extend the analysis further, a stepwise multiple regression analysis was performed using Shannon's information measure (H_0) as the dependent variable and the ecogeographical factors as the independent variables. The results (Table 5) indicate that a combination of ecogeographical factors can account for 29.9%, 30.7% 22.3%, 38.4% and 33.1% of the variation observed respectively for ADH1, BMY1, EST3, WSP and rDNA. The relationship between specific phenotypic frequencies and environmental factors was also examined (Table 6) for marker phenotypes with a frequency greater than 10%, i.e. the A, B and G phenotypes for BMY1 and the A and B phenotypes for rDNA. It was found that the mean number of rainy days (Rd) and the mean temperature in January (Tj) significantly influence the occurrence of the A and G phenotypes of BMY1. Thus, 78.4% of the variation in the frequency of the BMY1 G phenotype can be explained by Rd and Tj. These two factors are also responsible for a significant portion of the variation in the frequency of phenotype A. In contrast, over 25.0% of the variation in the frequency of phenotype B could be explained by latitude. These results indicate that the individual phenotypes of BMY1 observed in *H. spontaneum* populations are associated with certain geographical regions where specific environmental regimes predominate. The geographical distribution of the BMY1 phenotypes is presented in Fig. 3a. The distribution of the A and G phenotypes are quite distinctive, with phenotype G being restricted to the Negev desert and the Dead Sea regions.

Multiple regression analysis of the A and B rDNA phenotypic frequencies indicate that 63.3% of the distribution of phenotype A and 82.9% of the distribution of phenotype B could be accounted for by five environmental factors. The distribution of rDNA phenotypes is given in Fig. 3b and highlights the predominance of B phenotypes in the Negev desert region of Israel. These data demonstrate that the occurrence of certain rDNA and BMY1 phenotypes in Israel can be predicted from a limited number of ecological and environmental factors.

Discussion and conclusions

Several previous studies have examined isozyme variation in *H. spontaneum* from Israel (Nevo et al. 1979; Nevo et al. 1981) and the Near East (Nevo et al. 1986) but the one reported here is the first examination of the level of polymorphism for endosperm-derived isozymes in *H. spontaneum*. Several new phenotypes were observed that have not been detected in the *H.* vulgare gene pool

(Thompson et al. 1990). For EST3, EST10 and BMY1 these novel phenotypes correspond to new alleles. For ADH1, three phenotypes were found with a distribution in good agreement to that found for ADH1 phenotypes based on leaf extracts (Nevo et al. 1979). The common phenotype 'B' is equivalent to 'b' in Nevo et al. (1979); the rare phenotype 'A' (equivalent to Nevo et al.'s 'a') was found in population 13 (Bar Giyyora), and by Nevo et al. (1979) in the neighbouring population, 14 (Talpiyyot); similarly the 'C' phenotype has a similar distribution to Neo et al.'s 'c' phenotype. For WSP it is not possible to identify specific alleles since more than one locus is involved in the control of this marker system (Forster et al. 1991). Judged over all marker systems, a greater proportion of phenotypic diversity, as measured by Shannon's index of diversity, was found between the populations of *H. spontaneum*.

Both the overall level of diversity and the frequency of certain electrophoretic phenotypes were significantly correlated with a number of ecological or environmental factors. Similar findings have been reported for isozymes (Nevo et al. 1979) and hordein polymorphisms (Nevo et al. 1983) in *H. spontaneum*. These examples serve to illustrate the importance of wild relatives of cultivated barley as a source of useful genetic variability. Modern plant breeding practices have reduced the range of genetic variability available in the cultivated *H. vulgare* gene pool, and *H. spontaneum* germ-plasm represents an important source of genetic variability that can be introduced into barley cultivars through conventional hybridisation and recombination. An improved understanding of the genetic structure of a population can facilitate the exploitation of wild relatives of crop plants such as *H. spontaneum*. In the present study an examination of the geographical distribution of the *Bmy1* alleles revealed a replacement of the A phenotype by the G phenotype in the more arid regions of Israel. Furthermore, regression analysis showed that over 63% of the variation in the frequency of the G phenotype can be accounted for by the mean number of rainy days (Rd) per year. Recent studies have demonstrated that biochemical markers, including *Bmy1* on chromosome 4H, are linked to genetical factors controlling time to ear emergence in barley (Hackett et al. 1992). Further genetical studies are now required to elucidate the underlying mechanism for the restricted distribution of the G phenotype in the *H. spontaneum* populations studied.

Considerable rDNA IGS variability was observed both within and between *H. spontaneum* populations, and much of the variation between populations was correlated with a combination of ecological and climatic variables (Table 6). A previous study by Flavell et al. (1986) of variation in the IGS of rDNA of tetraploid wheat (*T. dicoccoides*) originating from 12 sites in Israel has also shown significant correlation between spacer length variation and a range of environmental variables. A general question arising from these studies is the role of selection in generating and maintaining the extensive heterogeneity between and within populations. There are at least two possibilities: selection is operating directly upon the rDNA or, alternatively, the rDNA loci are linked to adaptively important traits.

In a study of 54 generations of a *H. vulgare* composite cross population, Saghai-Maroof et al. (1984) observed that specific classes of spacer length variants (slv) that were originally present at a low frequency at the *Rrn2* locus (6H) became dominant in later generations, whereas the originally predominant class decreased markedly and others disappeared entirely. In addition, the *Rrn1* locus (5H) became fixed for a specific slv. They concluded that the enduring phenotypes were strongly favoured over all other rDNA alleles by natural selection under the environmental conditions in which the populations were grown. However, as the barley composite cross population studied was generated from a wide genetic base with diverse parents from contrasting geographical regions, changes in the observed slv frequencies may have been due to the loss of unadapted genotypes.

Further evidence that selection may act directly on the rDNA loci or genes linked to such loci in barley comes from recent work by Powell et al. (1991). Doubled haploids generated from reciprocal F_1 hybrids which were heterozygous for alleles at the *Rrn2* locus were used to assess the effect of this locus on several quantitative traits. Variation at the *Rrn2* locus was shown to be responsible for a significant proportion of the genetic variation for yield, thousand-grain weight and water sensitivity. This would indicate that the *Rrn2* locus is associated with traits that will influence reproductive advantage in polymorphic populations, and hence the observed frequencies of the slvs.

In a study of rDNa variability in *H. spontaneum* similar in kind to that of the present investigation, Saghai-Maroof et al. (1990) demonstrated that genetic diversity and genotypic frequencies among populations sampled from Israel and Iran were also significantly correlated with ecogeographical factors, suggesting that alleles and genotypes marked by the slvs differ in adaptive properties. They concluded that the most common slv behaved as a well-adapted wild-type allele, while other locally frequent slvs acted to enhance adaptedness to locally specialised habitats. Slvs detected at low frequencies and in the presence of the adapted phenotypes were considered to be subvital or semi-lethal, and maintained as part of a compound allele along with a favoured slv. A genetic analysis of the inheritance of slvs in barley (Allard et al. 1990) has shown that homozygotes for rare slvs frequently occur in the F_2 at a frequency significantly lower than expected. From this it was concluded that rare slvs have adverse effects on reproductive capacity and/or viability

under glasshouse conditions and possibly in natural populations also.

Allard et al. (1990) have favoured the possibility that selection acts directly on the rDNA loci in barley stemming from the sequence variability in the transcription units associated with the slvs. They have concluded that natural selection acting directly on the rDNA alleles of *Rrn1* and *Rrn2* plays a major role in the development and maintenance of the observed patterns of molecular and genetic organisation of rDNA variability in both wild and cultivated barley. It can not be ruled out, however, that traits governed by genes associated or linked to the rRNA loci may be responsible for the observed differences in reproductive advantage as suggested by Powell et al. (1991). Allard et al. (1990) have suggested that one possible way to resolve the dilemma is to sequence the slvs and transcription units of a selected set of alleles and then attempt to relate sequence data to differences in selective advantage.

In conclusion, we have demonstrated that both grain protein and rDNA phenotypes are distributed in a non-random manner in *H. spontaneum* populations sampled from Israel. The restricted distribution of the BMY1 G phenotype, which is largely confined to the Negev desert region of Israel, suggests that variation at this locus or associated loci may be of adaptive significance for abiotic stresses in barley. Consequently, allelic variation at the *Bmy1* locus is likely to be a useful indirect marker for abiotic stresses in barley. Since *H. spontaneum* is easily crossed with *H. vulgare,* the phenotypic variation detected in *H. spontaneum* can be readily transferred into the cultivated gene pool for evaluation by barley breeders.

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