Genetica Vol. 49, 2/3: 97-108, 1978

GENETIC VARIATION IN NATURAL POPULATIONS OF WILD BARLEY (HORDEUM SPONTANEUM)

A.H.D. BROWN[1,2], E. NEVO[1], D. ZOHARY[3] & O. DAGAN[1]

[1] Institute of Evolution, University of Haifa, Israel
[2] Permanent Address: CSIRO, Division of Plant Industry, P.O. Box 1600, Canberra, Australia
[3] Laboratory of Genetics, Hebrew University of Jerusalem, Israel

Received April 3, 1978 / Accepted August 15, 1978

In order to evaluate the potential genetic resources of the wild relatives of crop plants, allozyme variation at 28 loci was determined for 28 Israel populations of *Hordeum spontaneum*, the progenitor of cultivated barley. Electrophoretic properties of these loci and their variants are described. The enzyme loci exhibited a great range of polymorphism, from one to fifteen alleles per locus being detected. The average probability that two gametes drawn randomly from this collection would differ genetically at a locus was 0.19. The evidence indicates that natural populations of this species represent very rich reserves of genetic variability.

The extent of differentiation between populations at each locus was compared with that shown by variation in spikelet morphology. The allozyme diversity was apportioned into 17% between regions, 32% between populations within regions, and 51% within populations. In contrast, spikelet variation occurred predominantly between populations. This indicated the species is highly differentiated in phenotype between regions, and presumably allozyme variation is involved in this differentiation. These results support field sampling and evaluation strategies which stress the sampling of more sites at the expense of reducing numbers per site.

Introduction

Over the last decade, there has been increasing concern about the dwindling level of genetic diversity in the plants bred and cultivated by man (see Frankel & Hawkes, 1975, and Harlan, 1975 for review and earlier references). This erosion of genetic resources has already led to an increased vulnerability of crops to disease (National Academy of Sciences, 1972), and continues to hamper breeding for increased adaptation. To remedy this situation, additional genetic variation has been sought from three kinds of sources: (1) collections of primitive or advanced cultivars already in hand, or collected and conserved before they vanish, (2) induced mutations, and (3) the evolutionary relatives and progenitors of crops. This last source in the case of barley, is the focus of this paper.

Harlan (1976) has reviewed the current usage in plant breeding of genetic variation from wild relatives of crops and concluded that it is rapidly accelerating. There is a strong case for a greatly increased enrichment of our germplasm collections by further collections of wild relatives, and for detailed study and evalution of these resources. In this connection, the protein variation revealed by electrophoresis is a powerful approach to measuring the genetic diversity in populations (Rick et al., 1977; Brown, 1978). We therefore surveyed the allozyme variation at several genetic loci in representative populations of wild barley, *Hordeum spontaneum*, in Israel, to ascertain the level of genetic variation within this species, and the nature of population genetic structure.

H. spontaneum was chosen for a variety of reasons. The species is the sole progenitor (see Zohary, 1969) of cultivated barley with which it is fully interfertile. It is a diploid, predominantly self pollinated, colonising annual, occurring abundantly in Israel and in a wide range of habitats (from mesic to xeric areas, from primary formations to secondary disturbed

sites). It is morphologically diverse, and ecotypic races are recognised. In addition, there are already a number of studies of electrophoretic variation in cultivated barley (references below). Although Israel itself represents only a very limited portion of the entire distribution of the species (Harlan & Zohary, 1966), it constitutes a model target area for one hypothetical plant collecting mission. Furthermore Israel encompasses a majority of habitats in which wild barley is typically found.

This paper reports the electrophoretic techniques employed to determine allozyme variation, and describes certain properties of the variants encountered. Analysis of the extent of differentiation between populations at each locus are presented, and compared with pattern of variation in spikelet morphology. Further studies of the multivariate structure of allozyme-environment relationships (Nevo et al., 1978b) and of the mating system (Brown et al., 1978) are to be reported elsewhere.

Materials and methods

Collections

Seeds of *H. spontaneum* were collected randomly as separate spikes from at least 50 mature plants at each of 28 locations. These locations were representative of the geographic and environmental range of the species in Israel. Table 1 lists the populations grouped into seven geographic regions, together with their altitude and the length of the cool growing season. The seasonal duration is arbitrarily measured by two parameters: the mean number of days per year on which the daily mean temperature is below 15°C and the mean number of rainy days per year. These climatic data are assembled from the detailed contour maps in Amiran et al. (1970). Further ecological details of the sampled sites are given in Nevo et al. (1978b).

The seven geographic regions are defined by broad similarities of topography, climate, and accessibility, and are intended to reflect an hierarchical strategy for the problem of sampling within the total target area. The regions are not intended to imply that climate is uniform within them, nor that similarities between regions do not occur. Thus Afiq is climatically more similar to region B; or it can be argued that Eizariya and Qilt comprise a separate Judean desert region. However we wished to keep the division into regions as limited as practicable and the number per region greater than two.

Great variation in the length of the growing season is evident in the two parameters relating to the length of favourable temperature and moisture regimes. The

Table 1

Sites of collection of *Hordeum spontaneum* in Israel

Locality		Altitude (m)	Season Duration Temp. (days)	Rain (days)
A				
1	Mt. Hermon	1530	150	55
2	Shifon	880	90	45
3	Afiq	330	80	40
B				
4	Tel Hay	400	90	60
5	Rosh Pinna	700	100	45
6	Gadot	100	80	42
7	Tabigha	0	70	45
C				
8	Zefat	800	150	60
9	Mt. Meron	1150	175	62
10	Maalot	500	125	55
11	Damon	430	100	55
D				
12	Shechem	400	125	45
13	Bar Giyyora	760	125	42
14	Talpiyyot	800	130	42
15	Eizariya	600	125	40
E				
16	Tel Shoquet	380	120	30
17	Bor Mashash	350	115	20
18	Revivim	320	115	18
19	Yeroham	490	115	18
20	Sede Boker	450	115	15
F				
21	Bet Shean	−120	70	42
22	Mechola	−150	70	40
23	Wadi Qilt	50	35	25
G				
24	Akhziv	10	90	55
25	Atlit	50	85	50
26	Caesarea	10	80	48
27	Herzliyya	25	75	48
28	Ashqelon	50	70	38

Regions: A. Golan Heights; B. Hule Valley and Kinneret; C. Galilee and Carmel Mountains; D. Central Mountain Ridge; E. Negev; F. Lower Jordan Valley; G. Meditteranean Coastal Plain.

two xeric steppe regions of collecting (as defined in Amiran et al., 1970) are E and F. In the Negev (E) the season is shortened predominantly by early cessation or erratic patterns of favourable moisture, whereas in the lower Jordan Valley (F) early hot temperature stress is evident. A more or less mesic Mediterranean climate prevails in the other five collecting regions with the Northern Galilee and Carmel mountains (region C) being the most temperate. These regions are classified as subhumid on the Thornthwaite moisture index (Amiran et al., 1970).

Sample preparation

After maturing for at least 3 months in cold storage, individuals seeds were dehulled, cut and germinated on filter paper in petri dishes. They were potted in 3 cm diameter plastic beakers in sand, watered with nutrient solution (0.03% of each of KNO_3, K_2HPO_4, KH_2PO_4, $(NH_4)_2SO_4$; 0.0005% $CaCl_2$, 0.0002% $MgCl_2$ and 0.0001% $MnCl_2$ and $ZnCl_2$), and grown in daylight at room temperature.

Leaf samples were cut at 10-14 days, 6-8 cm of the first leaf. Each sample was ground in 1 drop (0.05 ml) of 0.05 M pH 7.0 Na phosphate buffer, 0.1 M 2-mercaptoethanol. This yielded sufficient crude extract to saturate two 4 x 11 mm Whatman 3M paper wicks. In order to accommodate all the assays to be made on leaves, a second set of extracts was usually prepared a few days later. Thus each plant yielded four wicks of leaf extract for analysis. Two were run in buffer system M, and one each in buffer system B and H (Tab. 2). Each gel yielded four slices. The horizontal starch gel electrophoretic system was that of Yang (1971), using 12% Electrostarch (Hiller). In general, samples from 2 to 12 sites were included on one gel together with a standard reference sample.

Flooded root samples were then prepared by completely immersing each pot in half strength nutrient solution for 1-2 days. 5 cm of root tip were rinsed, dried and ground with 1-2 drops of 0.05 M pH 7.0 phosphate buffer containing 43 mM dithiothreitol.

Enzyme assays

The following are the enzyme assays used in this survey. Most are modifications of established procedures (e.g. Brewer and Sing, 1970, see for chemical abbreviations; Yang, 1971). All quantities are listed, as the amount required for about one millilitre of assay solution. The allocation of individual gel slices of the two kinds of tissues is given in Table 4 below.

Acid phosphatase E.C.3.1.3.2 (Acph)
For leaf samples on buffer system M, 1 ml distilled water, 0.4 mg Fast Garnett GBC salt, 0.2 mg α-naph-

Table 2

Electrophoretic systems used with electrostarch

	Buffers		Electrophoresis		
	Tray	Gel	Volt cm^{-1}	Duration	Watt cm^{-2} †
B.	0.375 M Borate 0.06 M NaOH	2.0 mM Citrate 11.5 mM Tris	9	½ h	< 0.04
			Sample inserts removed		
			14	c. 2¼ h	< 0.04
			until borate front migrates 8 cm beyond origin.		
H.	0.05 M Histidine HCL, adjust pH to 5.7 sodium citrate	1:13 dilution of tray buffer	6.7	5 h	< 0.03
M.	0.1 M Maleic acid	1:19 dilution of tray buffer			
	0.1 M Tris 10 mM Na_2 EDTA 10 mM $MgCl_2$ adjust pH to 7.4 with NaOH		10	4 h	< 0.053

B is a modified Kristjansson (1963) system, H is modified from Stuber et al. (1977) and M is from Yang (1971). †This quantity is amps x volts applied, divided by the horizontal surface area of the gel.

thyl acid phosphate, 0.2 mg β-naphthyl acid phosphate. For root samples, and leaf samples on buffer system B, the reagents are increased in concentration 2.5 fold.

Alcohol dehydrogenase E.C.1.1.1.1. (Adh)
1 ml 0.1 M pH 8.0 Tris-HCL, 0.08 ml ethanol, 0.3 mg NAD, 0.2 mg NBT, 0.05 mg PMS.

Aldolase E.C.4.1.2.13 (Ald)
1 ml 0.2 M pH 8.0 TrisHCL, 4 mg tetrasodium fructose 1.6 diphosphate, 1.2 units glyceraldehyde phosphate dehydrogenase, 0.3 mg NAD, 0.3 mg NBT, 0.1 mg PMS. Incubate at 37°C overnight.

Catalase E.C.1.11.1.6 (Cat)
Cover gel with 1% hydrogen peroxide solution for 60 seconds; empty substrate and rinse thoroughly twice (10 seconds) Cover with 1% potassium iodide solution, acidified by adding 1 drop of glacial acetic acid for 5 ml solution. Record white zones on appearance.

Esterase E.C.3.1.1.2 (Est)
1 ml 0.2 M pH 6.0 TrisHCL, 1.5 mg Fast Blue RR salt, 0.4 mg α-naphthyl acetate, 0.2 mg β-naphthyl acetate. The substrates are held in stock solutions of 1% in acetone.

Glutamate dehydrogenase E.C.1.4.1.2 (Gdh)
1 ml 0.1 M pH 8.0 Tris-HCL, 20 mg monosodium glutamate, 0.3 mg NAD, 0.2 mg NBT, 0.05 mg PMS.

Glutamate-oxaloacetate transaminase E.C.2.6.1.1 (Got)
1 ml distilled water, 0.73 mg α-Ketoglutaric acid, 2.66 mg L-aspartic acid, 10 mg PVP40, 1 mg EDTA acid. Adjust pH to 7.4 with disodium phosphate (about 22 mg). This substrate solution can be kept refrigerated as stock. 1 ml substrate solution, 1 mg Fast Blue BB, 0.002 mg pyridoxal 5 phosphate.

Malate dehydrogenase E.C.1.1.1.37 (Mdh)
0.9 ml 0.1 M pH 8.0 Tris-HCL, 0.1 ml 2.0 M pH 7.0 malate, 0.2 mg NAD, 0.2 mg NBT, 0.05 mg PMS.

NADH diaphorase E.C.1.6.4.3 (Nadhd)
1 ml 0.1 M pH 7.0 phosphate buffer, 0.05 mg phenolindo-2,6-dichlorophenol (0.1% solution), 0.15 mg NADH. Record white bands on appearance.

Peptidase E.C.3.4.13.11 (Pept)
1 ml 0.2 M pH 8.0 Tris-HCL, 0.1 mg $MnCl_2 .4H_2 0$, 1 mg leucyl-alanine, 0.2 mg L-amino acid oxidase (crude snake venom), 0.4 mg peroxidase, 0.4 mg 0-dianisidine diHCL. Stir vigorously until dissolved.

Phosphoenolpyruvate carboxylase E.C.4.1.1.31 (Pepc)
1 ml 0.1 M pH 7.0 NaOH-tricine, 6 mg $MgCl_2 .6H_2 0$, 5 mg PVP40, 1.2 mg phosphoenol pyruvate, 1.6 mg $NaHCO_3$. Incubate for 10 minutes at 37°C and add 3 mg Fast Blue BB dissolved in 0.1 ml water.

Glucosephosphate isomerase E.C.5.3.1.9 (Pgi)
1 ml 0.2 M pH 8.0 Tris-HCL, 2 mg $MgCl_2 .6H_2 0$, 0.5 mg fructose-6-phosphate, 0.8 units glucose 6 phosphate dehydrogenase, 0.25 mg NADP, 0.3 mg NBT, 0.1 mg PMS.

Phosphoglucomutase E.C.2.7.5.1 (Pgm)
1 ml 0.2 M pH 8.0 Tris-HCL, 13 mg glucose-1-phosphate, 0.01 mg glucose-1,6-diphosphate, 2 mg $MgCl_2 .5H_2 0$, 0.8 units glucose-6-phosphate dehydrogenase, 0.1 mg NADP, 0.1 mg NBT, 0.04 mg PMS.

Phosphogluconate dehydrogenase E.C.1.1.1.44 (6 Pgd)
1 ml 0.2 M pH 8.0 Tris-HCL, 2 mg $MgCl_2 .6H_2 0$, 1 mg trisodium 6 phosphogluconic acid, 0.2 mg NADP, 0.4 mg NBT, 0.1 mg PMS.

Tetrazolium oxidase E.C. unknown (To)
White zones appear on *Ald* when slice is left overnight. The zones also appear on the *Mdh* assay.

General protein E.C.4.1.1.39? (Gp)
1 ml of methanol:water:acetic acid (5:5:1), 2 mg Coomassie brilliant blue R. Destain background in methanol, water, acetic acid solution.

The following assays were developed late in the screening of *H. spontaneum* populations. Only limited data is available on them in this material. However they are now routinely assayed on our barley samples.

Indophenol oxidase E.C. unknown (Ipo)
1 ml 0.1 M pH 7.0 phosphate buffer, 1 mg N,N dimethylphenylene diamine, 1.5 mg α-naphthol predissolved in 0.04 ml acetone. The assay is conducted on flooded root samples using buffer system M.

Leucine aminopeptidase E.C.3.4.11.1 (Lap)
1 ml pH 6.5 phosphate buffer solution [10 mg Na_2HPO_4, 5 mg PVP40, 0.4 mg $MgCl_2.6H_20$, titrated to pH 6.5 using Na H_2PO_4 (about 8 mg)], 1 mg Fast Black K salt, 0.3 mg L leucyl β-naphthylamide HCl predissolved in 0.06 ml dimethyl formamide. The assay is conducted on leaf samples using buffer system H.

All assays were fixed in 50% ethanol except CAT, IPO, NADHD and GP*. In the case of GDH, this fixative revealed zones of ADH activity which proved to be a useful confirmative routine. The assays CAT, IPO and NADH are ephemeral and must be recorded and photographed on appearance. ACPH-3, PGM (leaf), PGI readily overstain and should be fixed within 30 minutes. Normally, 50 ml of assay solution was used per gel slice (150 mm x 80 mm x 1.5 mm). For 6 PGD only 10 ml were required. Since PGM zones migrated further than 28 mm and the PGI zone less than 28 mm, 25 ml of solution was adequate to assay the relevant gel pieces for these activities. There is a zone of PGI activity more anodal 30 + mm. This zone was shown to be determined by *6 Pgd-1* by genetic analysis.

Sampling

A random seedling from each progeny array was assayed to determine its genotype using the methods described. The object of the routine was to determine all loci on each plant. However, for various technical reasons, the data are not complete. During the course of the survey, assays were improved and new ones added. We generally assumed it was more efficient to include improvements as they arose, and to assay additional families or sites rather than materials partially scored.

Spikelet variation

Spikelet characters were also measured on collected spikes to index some phenotypic (racial) differences between populations from the sites. In particular, the total length of the spikelet, the length of the longest sterile glume, and of the caryopsis, and the weight of the enclosed caryopsis were measured on each of ten random families from each site (except Shifon and Gadot). The standard errors for the site means were determined after an analysis of variance between and within sites.

* Roman capitals are used here to denote the protein and italics to denote the genetic locus.

Results

Spikelet characters

Table 3 summarises the morphological data as the means of the four spikelet characters for each sampled population of wild barley. The components of variation are shown as the percentage composition of the total, found between regions, between sites (populations) within regions and between spikes (families) within sites. A statistically significant amount of variation occurred between sites (R + P) in all cases, the different regions accounting for about half the variation in three of the characters. The largest spikelets were found in the upper Jordan Valley (3-7), whereas the smallest and most delicate were from steppic areas (15-23). The montane (8-14) and coastal (24-28) are intermediate. This variation observed in natural populations in all likelihood has both genetic and environmental sources; intercrosses between all entries would be required to analyse these components. It is noteworthy that reproductive characters (here spikelet dimensions) are generally considered to be among the least variable characteristics of grasses and hence are important taxonomically. These results therefore indicate that wild barley in Israel is highly variable morphologically.

Allozyme variants

Various properties of each locus studied are given in Table 4. The multiple loci in any one enzyme system are numbered from the most anodally migrating zone first – except for the esterases where the designation of Hvid & Nielsen (1977) is now followed. Previously (Brown, et al, 1978) we had recorded *Est-1* as *Est-A*, *Est-2* as *Est-B*, *Est-4* as *Est-C* and *Est-5* as *Est-D*. The A,B,C,D, notation is that of Kahler and Allard (1970). First is listed the tissue in which locus is scored ('Present') and where it is lacking ('Absent'). Several of the loci can be studied either in fresh leaves (L) or flooded roots (R), for example *Acph-1,-2, Cat, Est-1,-4* etc. The isozymes specified by both *Adh* loci were present only after induction in flooded roots, although ADH-2 occurs constitutively in dry seed,

and weakly in the very young (< 6 days) primary leaf. Both ADH-1 and ADH-2 are apparently present constitutively in immature endosperm (Nilson and Hermelin, 1966). The fact that roots lacked the activity of ACPH-3, EST-2 and 5 was useful because in leaves, their zones of activity frequently obscured the activity of ACPH-2, EST- 1 and 4.

Next the buffer system in which the loci were scorable is listed according to the gel buffers defined in Table 2. Where two systems are listed, the first is the preferred one. We found late in the survey that ACPH-3 is best resolved in system B (Kahler et al., in prep.), but most of our scores are from system M. Resolution for EST-5 proved more difficult to obtain in Electrostarch than in Connaught starch in Canberra using a different concentration (3.2 mM) of citric acid. Thus variation at these two loci is likely to be under-estimated. The *Pept* loci were the most striking examples of the dependence of optimal detection on buffer system. All three variants at these loci, were clearly apparent in system M, but undetected in system B, despite its sharper resolution of the bands.

Table 3
Site means and variance component proportions for four spikelet metric characters

Site		Length (mm)			Weight
		Spikelet	Sterile Glume	Grain	(mg) Seed
1	Mt. Hermon	160	19	13	32
2	Shifon	–	–	–	–
3	Afiq	166	23	15	44
4	Tel Hay	201	20	15	42
5	Rosh Pinna	223	17	15	42
6	Gadot	–	–	–	–
7	Tabigha	220	26	16	46
8	Zefat	145	17	14	31
9	Mt. Meron	142	16	13	31
10	Maalot	187	19	14	42
11	Damon	169	19	13	36
12	Shechem	164	18	13	33
13	Bar Giyyora	166	16	14	42
14	Talpiyyot	200	22	14	40
15	Eizariya	105	26	13	26
16	Tel Shoqet	142	20	13	26
17	Bor Mashash	134	21	11	27
18	Revivim	129	20	11	25
19	Yeroham	143	19	10	20
20	Sede Boker	113	18	10	18
21	Bet Shean	151	14	9	15
22	Mechola	144	13	9	20
23	Wadi Qilt	112	25	14	26
24	Akhziv	197	20	14	27
25	Atlit	169	21	15	47
26	Caesarea	178	20	16	33
27	Herzliyya	159	18	13	33
28	Ashqelon	157	15	12	36
s.e. site mean		6	0.7	0.3	2
% variation for each component					
Regions (R)		56	0	51	44
Sites (P)		28	70	31	22
Spikes (F)		18	30	18	34

The number of alleles found at each locus excluding 'nulls' is then given. Nulls were recorded rather infrequently at *Est-5*, *Got-2* and *Gp*. In some cases, further assays of these plants indicated that the lack of activity had been a technical problem. The remaining cases have yet to be checked by genetic analysis. The most polyallelic locus detected in wild barley in Israel was *Est-2*. Although fifteen alleles were identified, the data of Allard et al. (1971) indicate that this is only a fraction of the total alleles at this locus in the wild and cultivated barley species as a whole. Polyallelic loci in plants are already known for a peroxidase locus in wild and cultivated tomato species (Rick & Fobes, 1976), and β-glucosidase in maize (Stuber et al., 1977). The main (most conspicuous) band determined by each allele of the *Est-2* complex showed an alteration in the order of mobility in the two different buffer systems. The alleles are designated a,b,..., o in decreasing order of mobility in the borate (B) system. In the histidine buffer (H) the order of decreasing mobility is:(b,c,d) > (e,f,g,m,) > (a,h,n) > l > (i,j) > k > o where alleles of indistinguishable mobility in this system are bracketed. The alleles out of sequence are a,l,m and n, which was especially useful in distinguishing alleles k and l.

The next most polyallelic locus was *Acph-2*. Mobility differences between adjacent alleles were in this case quite small. They were checked by comparisons of samples from replicate plants from spikes previously classified and ordered for ACPH-2 mobility. Separations were repeatable although insufficiently spaced to allow the distinction of two bands in a mixed

Table 4
Electrophoretic properties and variants at various genetic loci in wild barley in Israel

Locus	Tissues Present	Tissues Absent	Buffer System	Number of Alleles	Location of Modal Allele	Number of seg. families	HB	Resolution
Acph-1	L		B,M	2	24(M)	0		S,S
-2	R		M	9	11	5	1	S
-3	L	R	B,M	4	4(M)	6	0	V,SB
Adh-1	R	L	M,B	3	25	5	1	S,V
-2	R	L	M,B	3	15	2	1	S,V
Ald	L	R	M	1	28	–	–	S
Cat	L		B	2	20	1	3	S
Est-1	R		B	6	25	2	0	S
-2	L	R	B,H	15	32	20	0	V,S
-4	L		B	7	42	17	0	D
-5	L	R	H,B	5	60(B)	3	0	S,V
Gdh	R		B	3	19	0		S
Got-1	L		B	2	38	0		S
-2	L		B	3	32	1	1	S
Mdh-1	L		H	?	26	0	1	S
-2	L		H	4	15	0		D
Nadhd-1	L		M	4	40	7	0	S
-2	L		M	3	35	0		S
Pept-1	L		M	3	36	1	0	S
-2	L		M	2	20	3	0	S
Pepc	L	R	M	1	23	–	–	SB
Pgi	L		M	3	11	1		S
Pgm	R		M	3	40	2	0	S
6 Pgd-1	L		M	3	30	1	1	S
-2	L		M	3	12	0		S
To-1	L		M	1	50	–	–	S
-2	L		M	2	23	0		S
Gp	L	R	M	3	17	4?		S

Abbreviations: L = leaf, R = flooded root; B = borate, H = histidine, M = maleate; HB = the number of heteroallelic multimeric or 'hybrid' bands in heterozygotes; S = single sharp band, SB = single broad band, D = double band, V = variable multiplicity of bands.

sample. It is clearly impossible to intercross all allelic occurrences in all populations to check on genetic identity and differences. Such technical difficulties in the identification of all the genetic variants at a locus by electrophoresis are widely recognised (Marshall & Brown, 1975). Furthermore, two alleles determining proteins of similar mobility are more likely than average to be closely related evolutionarily. Therefore measures of genetic distance which give equal weight to all allele differences irrespective of mobility differences, and which place undue emphasis on the difference between finding zero and one copy of an allele (compared with the difference between one and two copies etc.) are particularly susceptible to bias when these technical problems exist.

The location on the gel of the activity of the most frequent allele at each locus in Israel is specified as the distance in millimetres from the origin towards the anode. The figure refers to the first buffer system unless otherwise indicated in brackets.

The number of naturally occurring families which segregated at these loci is shown in the next column of Table 4. These were uncovered when further samples of four seeds per family were assayed from spikes in which the first seed proved to be heterozygous (Brown et al., 1978). In all loci except *Gp* the segregation patterns followed Mendelian expectations in this limited number of cases. Further genetic studies of the variants are planned, after appropriate crosses and the necessary seed increases have been made. In the case of *Gp*, four families were found in which the intrafamily variation was much greater than usual. It is likely that the general protein stain is detecting fraction I protein, ribulose diphosphate carboxylase (E.C.4.1.1.39), because Fraction I protein is the major soluble leaf protein. In our samples, GP was not in the roots. One chlorotic seedling was obtained from a spike at Ashqelon. Of the 19 leaf allozyme activities tested, it lacked only ACPH-3 and GP indicating an association between these two zones and chloroplast presence or photosynthesis. We tentatively assume that the unexpected null variant for *Gp* is either an insoluble, or a less concentrated, or a cathodically migrating variant. In tobacco (Kung, 1976) and probably also in barley (Criddle et al., 1970; Strobaek & Gibbons, 1976), Fraction I protein is composed of two subunits; the large subunit is coded by chloroplast DNA and the other by nuclear DNA. The intrafamily variation for *Gp* would indicate that this variation is nuclear in origin. However, until a genetic analysis is complete, assignment of variation in this system to the nucleus must remain tentative. Nevertheless, variation in *Gp* is still part of the total variation detected in our survey, whether nuclear or chloroplastic in origin.

The number of heteroallelic multimeric zones, or hybrid bands formed from the products of two different alleles at a single locus in heterozygotes, where known, is shown in Table 4 in the column labelled HB. The two *Adh* loci also specify heterolocus multimeric bands (three extra bands in double heterozygotes, two in single heterozygoes, and one in double homozygotes) as has been found in at least four other diploid plant species (see Marshall et al., 1974 for references). The resolution of enzymes from homozygotes in our system is classified into four types: S

Table 5

Isozyme studies in barley (cf. Note added in proof)

Purpose	Authors	Common systems in which analogies are apparent
Tissue differences	Upadhya & Yee (1968)	*Acph, Cat, Est*
Mildew infection	Sako & Stahmann (1972)	*Gdh, 6 Pgd*
Varietal variation and identification	Nilson & Hemelin (1966)	*Adh*
	Nielsen & Frydenberg (1972)	*Est*
	Almgard & Landegren (1974)	*Acph, Adh, Est, Got, Lap, Pept, Pgm, 6 Pgd*
	Bassiri (1976)	*Acph, Est*
	Griffin (1976)	*Est*
Genetic analysis	Kahler & Allard (1970), Hvid & Nielsen (1977)	*Est*
	Kahler et al. (1978)	*Acph-3, Got, 6 Pgd*
Biochemical properties	Edwards (1975), Hvid & Nielsen (1977)	*Est*

signifies that a single discrete band (< 2mm width) was frequently or invariably seen; SB, a single broad zone of activity (2-4 mm); D, two more-or-less equally intense bands; and V signifies a variable number of 'shadow' bands or conformers. The mobility of each conformer usually bears a constant relationship with that of the main band in their variation between samples. They are more frequently encountered on the discontinuous borate (B) system, or for dehydrogenases in high activity (PGM in leaves, ADH).

There have been a number of studies of isozyme variation in cultivated barley (*Hordeum vulgare*) with various objectives. In many of our gels we included a sample of the cultivars Atlas or Clipper so that tentative analogies could be drawn with published analyses. The studies which employed similar tissues of barley, and enzyme assays as ours, and their purpose are listed in Table 5. In each case, the enzyme systems with zones of activity which are analogous to our results are listed. Our gel patterns were most readily reconciled with the studies of Kahler & Allard (1970), Almgard & Landegren (1974) and Hvid & Nielsen (1977) since they identified genetic variants for many of the zones and employed similar electrophoretic techniques.

Population differentiation

The number of alleles distinguished at each locus (Tab. 4) demonstrates that the loci range from highly variable (*Est-2*) to invariant (*Ald, Pepc, To-1*). The frequencies of the individual alleles at each locus in each population are presented elsewhere (Nevo et al., 1978b) in the context of a detailed discussion of allelic and environmental variation. Here we wish to consider the hierarchical pattern of distribution of allo-

Table 6

Diversity analysis between regions, and between and within populations at the 25 polymorphic loci

Locus	Total	Proportion between Regions within Israel	Populations within regions	Spikes within populations
Est-2	85.8	18	31	51
Acph-2	71.9	17	29	54
Est-4	69.8	32	18	50
Acph-3	66.0	5	38	57
Nadh-1	53.7	14	34	52
Est-5	52.6	6	44	50
Pept-2	25.8	10	61	29
Adh-1	24.2	47	20	33
Pgm	18.3	27	50	23
Pgi	12.7	14	20	65
Est-1	12.5	10	22	68
Adh-2	11.0	41	37	22
Mdh-2	8.3	5	19	76
Gdh	7.0	6	8	86
Gp	5.1	10	39	51
Got-1	3.4	15	33	52
Got-2	3.2	3	6	91
To-2	2.6	6	30	64
6 Pgd-2	2.3	3	11	86
Pept-1	2.2	5	13	82
Acph-1	1.4	4	18	78
6 Pgd-1	0.6	3	1	96
Mdh-1	0.6	1	6	93
Cat	0.4	1	2	97
Nadhd-2	0.4	1	2	97
Overall	19.4	17	32	51

zyme variation and its implications for sampling strategies and physiological testing.

Table 6 summarises the variation observed at each locus according to Nei's diversity statistics (Nei, 1973) which are analogous to Simpson's (1949) index of species diversity. The total diversity in Israel is the average percentage probability that two gametes randomly chosen from a composite of equal numbers of all sampled populations, will differ electrophoretically at that locus. It is computed from the average allele frequencies. The loci in Table 6 are listed in order of decreasing diversity, the three monomorphic loci being omitted.

This genic diversity can be analysed into its components – the average level between spikes within populations, that between populations within regions, and that between regions. These proportions are all expressed in Table 6 as a percentage of the total diversity and thus the sum of the two between populations components is analogous to Wright's F_{ST} (Nei, 1973). Using the values for the total diversity, the loci can be divided into four groups: 6 highly polymorphic loci, 6 moderately polymorphic loci, 13 weakly polymorphic loci and 3 invariant loci. In the first highly polymorphic group, about half the diversity occurred within local populations. The differentiation between regions was less striking than that within regions except for *Est-4*. The next group of loci was more heterogeneous. Two loci (*Pgi* and *Est-1*) showed most diversity within sites. The loci *Pept-2* and *Pgm* showed strong population differentiation within regions, whereas for *Adh-1* and *Adh-2*, the component between regions was highest. Variation at the *Pgm* locus also showed substantial differentiation between regions. For the third group of loci, the limited amount of variation was much less differentiated between populations and regions.

The average diversity within Israel, over all the loci studied was 19.4%. If the hierarchical diversity estimates are averaged over all loci before apportionment, and then the percentage components of diversity computed, they are 51% within populations, 32% between populations within regions and 17% between regions. In comparison, the averages over loci of the percentage components in Table 6 are 64%, 24% and 12% respectively. This comparison indicates that the more variable loci have a high degree of population differentiation.

Discussion and conclusions

Based upon this survey of 28 protein loci, natural populations of wild barley (*H. spontaneum*) in Israel represent a particularly rich source of genetic variation. An hypothetical, randomly mated, composite of all the populations so far examined would yield a bulk with an average heterozygosity per locus about 20%. The estimate would probably increase if more populations were studied. This figure can be compared with the diversity of 6.7% found for these same loci in Composite Cross XXI of cultivated barley at the seventeenth generation after synthesis (Nevo et al., 1978a).

From recent studies based on multiple electrophoretic, gel sieving or heat denaturation procedures, it is clear that genetic diversity is underestimated by a single starch gel routine, such as ours (see Johnson, 1977 for review). However, the more intensive procedures do carry with them the operational disadvantage of a reduction in sample size (in loci, individuals, and/or populations surveyed) per unit of experimental effort. While the extent of the bias from adopting simple procedures is still not generally clear, the available evidence indicates that it reduces rather than spuriously magnifies estimates of population differentiation.

A striking feature of the genetic variation detected is the significant degree of population differentiation. Individual populations differ not only in their overall diversity (from 0 to 18%, see Nevo et al., 1978a), but also in the kinds of alleles present (Table 6, see Nevo et al., 1978b). For example, at the *Pgm* locus, the *Pgm a* allele is essentially confined to one extreme of the range – Mount Hermon; the *Pgm c* allele is common in the Negev region. Both *Adh* loci are also highly differentiated. In particular the *Adh-1 c* allele is common or fixed in the Hule Valley region. Further analysis of the allozyme-environment relationships will be presented elsewhere (Neve et al., 1978b).

It is interesting to compare the component analysis of overall diversity (Tab. 6) with the analogous treatment of variation in spikelet metric characters (Tab. 3). For allozyme variation the order of contribution to diversity is

within population > within regions > between regions

whereas for the spikelet characters the order is essentially reversed. This difference tends to argue against

historical effects as the main source of the broad scale genetic differentiation observed for allozymes, since such stochastic effects would presumably bias neutral marker loci to higher levels of population differentiation, on average. The difference could arise if the regional morphological variation was to a large extent environmentally induced. However this is an unlikely explanation because ecotypic races in wild barley are well recognised and are stable in common garden experiments (Zohary, unpub.). A second possibility is that it is a scalar effect. This arises because the overall diversity measure is bounded above by 100%, whereas the variance components are not bounded. However this explanation seems insufficient to explain such a large discrepancy. A third possibility is that the two types of characters are sampling different sets of loci. A final possibility concerns the problem of phenotypic expression of a given allelic difference. This proposal assumes that if allele A_1 is substituted by another sympatric allele A_2 the effect on the phenotype may be say 10 units. If however A_1 is substituted by A_3, an allele from an entirely different region, the phenotypic effect may be 100 units. Thus an approximately equivalent substitution on the diversity index scale, becomes a radically different substitution on the phenotypic scale. As the diversity analysis is essentially based on allelic identity (Nei, 1973), all differences are treated equivalently, and there is no measure of the degree of genetic or phenotypic difference between two particular alleles. If this last explanation accounts for the discrepancy, it may be inferred that it would be easier experimentally to demonstrate an effect on phenotype for a strongly differentiated locus (e.g. *Adh-1,-2, Pgm*) than for another which as a similar level of diversity but with less differentiation (*Pgi, Est-1*).

The intensity of population and regional differentiation found for allozyme variants in *H. spontaneum* has important implications in the optimising of sampling strategies. The optimal sampling strategy can be defined as that which captures the maximum amount of useful genetic variation from the target area within the limitations of time and resources. Of all the alleles detected in our survey, about one quarter achieved appreciable population frequencies (> 0.10) in only one of the seven regions, and a further sixth in two regions (Brown, 1978). Thus a substantial fraction of variants would remain undetected if very much larger samples had been taken from fewer regions. These data provide evidence for the existence in plants of a large fraction of alleles which are locally common, but rare on a species basis. This class is of key importance for optimising strategies which emphasise larger numbers of sample sites instead of greater numbers per site (Marshall & Brown, 1975b).

Finally, these results together with those of others (especially Rick et al., 1977; see Brown (1978) for review) demonstrate the utility of electrophoretic screening as one important method of evaluating the genetic resources in the wild relatives of crop plants. In the quest for new genetic resources of crops it is likely that such screening will play an increasing role. This will not only assist in the formulation of optimal sampling strategies, but also contribute to the biological understanding of genetic structure of populations.

One of us (A.H.D.B.) is indebted to University of Haifa, Hebrew University of Jerusalem, and Stanford University for travel grants which made possible an extended visit to Israel. The research was supported by a grant from the United States-Israel Binational Science Foundation to D.Z. and E.N. We thank Drs H. Doll, G.F. Moran, A.J. Pryor and J.A. Thomson for comments on the manuscript.

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Note added in proof:

References to add to Table 5 include

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