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Grain protein variability among populations of wild barley (*Hordeum spontaneum* C. Koch.) from Jordan

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Summary. Protein content, kernel weight, and genetic diversity in the storage protein hordein, encoded by the *Hor1* and *Hor2* loci, were assessed in 12 populations of wild barley (*Hordeum spontaneum* C. Koch.) collected from central, peripheral, and marginal areas of its distribution in Jordan. Protein content ranged from 106.3 to 239.1 g kg^{-1}, and kernel weight ranged from 21.17 to 31.8 mg. Populations with high protein content and heavy kernels have been identified. Electrophoretic analysis of the storage protein hordein showed that the two hordein loci, *Hor1* and *Hor2*, are highly polymorphic, having 34 and 38 alleles, respectively. Polymorphism (H_e) was highest in central populations (H_e *Hor1* = 0.859, H_e *Hor2* = 0.782), intermediate in peripheral populations (H_e *Hor1* = 0.566, H_e *Hor2* = 0.509), and lowest in marginal populations (H_e *Hor1* = 0.392, H_e *Hor2* = 0.349). Geographical distances between populations were not indicative of Nei's genetic similarity (NI). NI values averaged 0.209 and ranged from 0.0 to 0.83, supporting the hypothesis of an island population model for the species. The high proportion of allelic diversity, apportioned among populations for *Hor1* (0.584) and *Hor2* (0.495) loci, indicates that these natural populations are a rich reserve of genetic variability for protein. This variability is readily exploitable in breeding.

Key words: Wild barley – *Hordeum spontaneum* – Hordein – Polymorphism – Jordan

Introduction

Kernel storage proteins of cultivated barley (*Hordeum vulgare* L.) and its immediate progenitor (*Hordeum spontaneum* C. Koch.) have been studied extensively (see Shewry et al. 1987, for a review). Hordein, which is the major storage protein in barley, accounts for 35–50% of total protein in the seed. Hordein consists of three major groups of polypeptides called B, C, and D. Genetic analysis (Doll and Brown 1979; Sozinov et al. 1987) showed that hordein B and C are controlled by the single structural loci *Hor2* and *Hor1*, respectively. Polymorphism of B and C hordein polypeptides have been reported for both cultivated (Shewry et al. 1987) and wild barley (Doll and Brown 1979; Nevo et al. 1983). Faulks and coworkers (cf. Shewry et al. 1987) showed the presence of between 8 and 17 major polypeptides at the *Hor2* locus in each of eight barley cultivars, with a total of 47 different polypeptides. Doll and Brown (1979) reported a minimum of 33 and 38 alleles at the *Hor1* and *Hor2* loci, respectively, in 51 populations of *Hordeum spontaneum* from Israel. A nonrandom (Sozinov et al. 1987) and partially adaptive (Nevo et al. 1983) distribution of *Hor1* and *Hor2* alleles, their combinations and frequencies have been detected in wild and cultivated barley. Corke and Atsmon (1990), in recent review of *Hordeum spontaneum*, concluded that the wild species may still be a source of traits related to protein content and its improvement in cultivated barley. Recently, barley landraces and populations of *Hordeum spontaneum*, collected in Jordan, have been evaluated for morphometric traits (Jaradat et al. 1987) and isozyme systems (Jana et al. 1987a, b). No information is available on the variability of storage proteins and their hordein component in these collections. This paper reports on the geographical variation of kernel storage proteins and their B and C hordein components, in 12 populations of *Hordeum spontaneum* collected from different agroecological regions in Jordan.

Materials and methods

Individual spikes of *Hordeum spontaneum* were collected at random from 12 populations during May–June, 1988, across the range of distribution of the species in Jordan (Fig. 1). Collection sites and their major ecogeographical parameters are presented in Table 1. These populations follow north–south and high–low rainfall transects. In each collection site, single spikes were collected from 50–60 plants, at least 1 m apart. The whole collection was planted in a spike-to-row nursery during the 1988–1989 cropping season at J.U.S.T. Experimental Station (32.50 °N, 36.00 °E). Total rainfall was 148 mm. At maturity, and before spike disarticulation, seed samples were collected from individual plants and used in the study. Mean kernel weight was determined for three replicates, each of 250 seeds per population. Total protein determination was carried out by a standard microkjldahl method on 40 mg of flour. Polyacrylamide gel electrophoresis (SDS-PAGE) of hordein polypeptides was carried out on 20 seeds per population, following the method described by Doll and Anderson (1981) with its modifications (Nevo et al. 1983). Allelic forms at the two multigenic loci, *Hor 1* and *Hor 2*, were scored separately, using the DENSITY function of the computer program SYGRAPH (Wilkinson 1988). Finally, the diversity statistic H_e was estimated for *Hor 1* and *Hor 2* loci (Nei 1973).

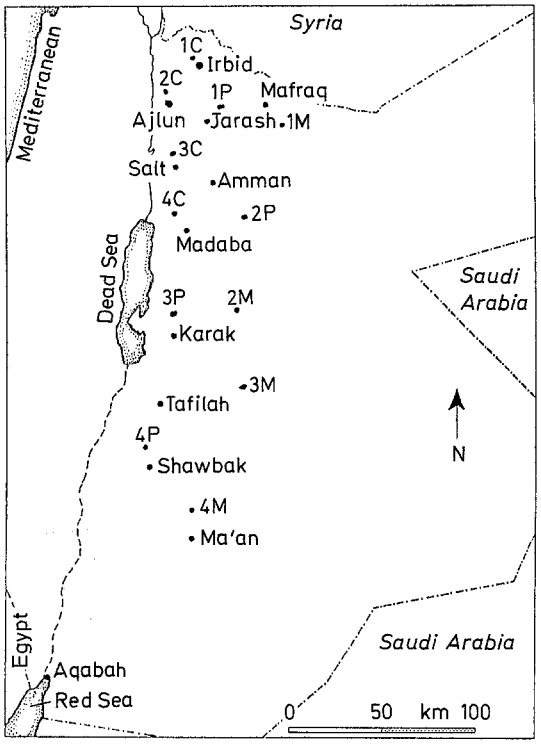

Fig. 1. Map of Jordan showing collection sites of wild barley. C: Central populations. P: Peripheral populations. M: Marginal populations

Results

Protein content averaged 157.1 g kg^{-1} for the whole collection. It was highest (239.1 g kg^{-1}) in accessions collected from the mesic parts of the country (Table 1), and lowest (106.3 g kg^{-1}) in accessions collected from the xeric parts. A strong positive correlation ($r=0.475$; $P<0.001$) was found between protein content and latitude of collection site. Increasing latitude (i.e., south-north transect) of collection sites implies increased rainfall and improved edaphic conditions (National Atlas of Jordan 1984).

Both *Hor 1* and *Hor 2* varied considerably in the number and mobility of their component bands. A total of 34 and 38 different phenotypes has been observed at the *Hor 1* and *Hor 2* loci, respectively. Frequency of different alleles at the *Hor 1* and *Hor 2* loci ranged from 0.11 to

Table 1. Geographical information, protein content, kernel weight, number of alleles, and diversity estimates at the *Hor 1* and *Hor 2* loci, for 12 populations of wild barley collected from different agroecological regions in Jordan

Group	Population	Location	Long. (Decimal)	Lat. (Decimal)	Alt. m	Soil type*	Rain mm	Ta** °C	Tj °C	Protein g kg^{-1}	Kernel wt. mg	No. alleles *Hor 1*	No. alleles *Hor 2*	H_e *Hor 1*	H_e *Hor 2*
Central	1	Irbid	35.37	32.50	600	1	400	26	10	17.49^{c***}	28.04^{b}	11.0	11.5	0.810	0.790
	2	Ajlun	35.60	32.50	1,000	1	600	24	8	17.49^{c}	29.49^{ab}	16.0	12.0	0.916	0.792
	3	Salt	35.60	32.00	900	1	500	24	8	19.12^{b}	28.81^{b}	12.0	11.0	0.870	0.760
	4	Madaba I	35.62	31.75	600	1	350	26	8	23.91^{a}	31.85^{a}	12.0	9.0	0.840	0.750
Peripheral	1	Jarash	35.90	32.10	750	2	300	26	8	15.45^{d}	26.99^{c}	8.5	7.0	0.540	0.511
	2	Madaba II	35.90	31.60	600	2	250	26	8	14.95^{d}	30.28^{a}	8.5	5.5	0.560	0.497
	3	Karak I	35.60	31.10	900	1	300	24	8	15.94^{d}	26.61^{c}	7.5	7.0	0.570	0.565
	4	Shawbak	35.30	30.30	1,200	2	250	20	4	15.22^{d}	27.65^{bc}	8.0	6.5	0.575	0.463
Marginal	1	Mafraq	36.25	32.40	600	3	200	26	8	10.63^{g}	21.17^{d}	4.5	4.5	0.378	0.365
	2	Karak II	36.10	31.20	900	4	150	26	8	12.41^{f}	26.10^{c}	5.0	3.5	0.397	0.356
	3	Qatranah	35.90	31.00	900	4	100	26	8	14.25^{e}	22.53^{d}	4.0	3.5	0.407	0.365
	4	Ma'an	35.55	30.50	1,300	4	150	28	6	15.09^{d}	29.79^{a}	4.5	3.0	0.412	0.353

* Soil type 1: red Mediterranean soils; 2: yellow Mediterranean soil; 3: regosols; 4: grey desert soil
** Ta: mean temperature in August, Tj: mean temperature in January;
*** Means within each column followed by the same letter do not differ significantly (DNMRT = 0.05)

Table 2. Analysis of variance and polymorphism for *Hor 1* and *Hor 2* diversity indices (H_e) for each of central, peripheral, and marginal populations of wild barley from Jordan

Group	Variable	Among pop. MS	Within pop. MS	*P*	H_e	H_e (*Hor 1 Hor 2*)	H_e (*Hor 1 + Hor 2*)	QT
Central	*Hor 1*	0.0130	0.0039	0.043	0.859			
	Hor 2	0.0125	0.0002	0.051	0.782			
						0.720	0.891	0.220
Peripheral	*Hor 1*	0.0004	0.0132	0.868	0.566			
	Hor 2	0.0083	0.0060	0.242	0.509			
						0.462	0.627	0.513
Marginal	*Hor 1*	0.0027	0.0011	0.092	0.392			
	Hor 2	0.0017	0.0030	0.652	0.349			
						0.324	0.340	0.920

22.16% (data not presented). According to Nevo et al. (1983), 14 and 12 phenotypes in *Hor 1* and *Hor 2*, respectively, were classified as common, while 20 phenotypes in *Hor 1* and 26 phenotypes in *Hor 2* were classified as rare. These frequencies suggest that 58.8% of *Hor 1* phenotypes and 68.2% of *Hor 2* phenotypes were rare, as compared to 53.3% of each of *Hor 1* and *Hor 2* phenotypes reported by Nevo et al. (1983).

A wide range of polymorphic levels characterizes the hordein subunits in these populations, as indicated either by number of alleles per locus per population (range: 3–16) or by H_e estimates (range: 0.353–0.916) (Table 1).

Polymorphism, for both hordein loci, was lowest in populations collected in the marginal areas of distribution. H_e in marginal populations for *Hor 1* was 0.392, while it was 0.349 for *Hor 2*. Peripheral populations displayed intermediate levels of polymorphism for *Hor 1* ($H_e = 0.566$) and *Hor 2* ($H_e = 0.509$). Populations collected in the center of distribution of the species in Jordan were characterized by the highest polymorphic levels for *Hor 1* ($H_e = 0.859$) and *Hor 2* ($H_e = 0.782$) (Table 2).

A decreasing trend was found for the statistics H_e (*Hor 1 Hor 2*) and H_e (*Hor 1 + Hor 2*) with increased aridity (Table 2). This was reflected in the coefficient of association (Q_T) values for central (0.220), peripheral (0.513), and marginal (0.920) populations.

Analysis of variance for *Hor 1* and *Hor 2* diversity indices (H_e) revealed significant differences among central, but not peripheral, populations for both hordein loci (Table 2).

Total allelic diversity (H_t) for each of *Hor 1* and *Hor 2* loci was subdivided into its components, i.e., mean allelic diversity within (H_s) and among (D_{st}) populations (Nei 1973). In addition allelic diversity among populations, as a percentage of total allelic diversity (G_{st}), mean number of alleles per polymorphic locus (A_p), and the proportion of total number of alleles found within each population (P_a) were calculated (Table 3). On the average, 59.3% of total allelic diversity exists among populations, and 40.7% within populations.

Table 3. Apportionment of genetic variation in *Hor 1* and *Hor 2* loci for 12 populations of wild barley from Jordan

Locus	Alleles	Sample	H_t[a]	H_s	D_{st}	G_{st}	A_p	P_a
Hor 1	34	240	0.941	0.375	0.584	0.620	8.9	0.23
Hor 2	38	240	0.875	0.380	0.495	0.566	6.3	0.31

[a] See text for abbreviations

The normalized identity of both hordein loci, between each pair of populations (Nei 1972), was used to calculate coefficients of hordein genetic similarity (i.e., Nei's identity index, NI). NI averaged 0.209 and ranged from 0.0 to 0.83. A few populations had NI values greater than 0.50 (e.g., Ajlun and Salt; Madaba II and Karak II), however, most NI values were very low (Table 4).

Discussion

The high protein content in populations of *Hordeum spontaneum* found in this study (range of 106.3 to 239.1 g kg^{-1}) is 3.5–45.5% higher than the average protein content reported for cultivated barley (Newman and McGuire 1985). This is comparable to protein values reported for *Hordeum spontaneum* by Ahokas (1982) and Nevo et al. (1985), who found relatively high protein content 158.0 g kg^{-1}) in *Hordeum spontaneum* grown under low fertility conditions. It is postulated that the species might have a higher nitrogen harvest index which, in view of the small kernel weight (Table 1), could explain its high protein content. Nevertheless, populations combining both high protein content and heavy kernels identified in this study confirm earlier findings (Ahokas 1982; Nevo et al. 1985) and suggest the possibility of combining both traits in cultivated barley.

The statistics H_e (*Hor 1 Hor 2*) and H_e (*Hor 1 + Hor 2*) (Table 2) (Doll and Brown 1979) reflect a high diversity level of hordein phenotypes, especially when compared

Table 4. Coefficients of Nei's genetic similarity (NI) based on two hordein loci, between 12 populations of wild barley from Jordan

Location	1	2	3	4	5	6	7	8	9	10	11	12
1 Irbid	0	0.29	0.0	0.0	0.3	0.45	0.0	0.3	0.0	0.0	0.09	0.0
2 Ajlun			0.2	0.05	0.83	0.02	0.0	0.05	0.0	0.0	0.20	0.0
3 Jarash				0.38	0.24	0.43	0.0	0.47	0.03	0.08	0.0	0.0
4 Mafraq					0.0	0.20	0.45	0.38	0.52	0.34	0.07	0.35
5 Salt						0.06	0.0	0.27	0.0	0.0	0.15	0.0
6 Madaba I							0.31	0.43	0.19	0.20	0.0	0.1
7 Madaba II								0.05	0.71	0.53	0.37	0.21
8 Karak I									0.24	0.63	0.55	0.32
9 Karak II										0.48	0.32	0.25
10 Qatranah											0.17	0.37
11 Shawbak												0.20
12 Ma'an												

with hordein diversity at a country (Doll and Brown 1979) or microgeographical (Nevo et al. 1983) level. Furthermore, the diversity statistics found in this study are much higher in magnitude than those reported for isozyme systems in cultivated (Britting and Goodman 1989) and wild barley from Jordan (Jana et al. 1987a), Israel (Brown et al. 1978), the Fertile Crescent, and in F_{22} of the spring barley composite cross XXI (Jana et al. 1987b). However, significant intrapopulational differenes have been detected within central, but not peripheral or marginal populations (Table 2). This sharply contrasts with the high diversity of high-molecular-weight glutenin subunits in marginal steppe populations of wild emmer from Israel (Nevo and Payne 1987).

Significant differences in allelic diversity due to geographical conditions are well documented for wild (Nevo et al. 1986; Nevo and Payne 1987), but not domesticated (Margiotta et al. 1988), members of the Triticeae. Obviously, natural selection and ecological factors (Nevo and Payne 1987) play an important role in differentiating among the wild species, while anthropic factors are more important in differentiating among domesticated ones (Spagnolleti-Zeuli et al. 1984; Margiotta et al. 1988).

The values of H_e estimates and coefficients of association (Q_T), i.e., the standardized portion of the gametic diversity, which is diversity at both *Hor1* and *Hor2* loci simultaneously for central, peripheral, and marginal populations, reflect a high level of diversity of the hordein phenotypes. A decreasing trend of association was observed between the two hordein loci, as indicated by the coefficients of association, Q_T. Marginal populations exhibited the highest values, while central ones had the lowest. However, Q_T values in these populations (Table 2) are much higher than that reported for the Atlas composite cross XXI (0.13) (Doll and Brown 1979). The high gametic association between both hordein loci in the wild species, especially in marginal and peripheral populations, may arise either from historical effects of fluctuating population size (Doll and Brown 1979; Levi and Feldman 1988), and restricted recombination due to moderate linkage and predominant self-fertilization (Tsuchiya 1987), or from epistatic selection (Doll and Brown 1979).

Average allelic diversity (Table 3) among (59.7%) and within (40.7%) populations of *Hordeum spontaneum* in this study reflect a relatively high allelic differentiation among these populations, and are higher than the value reported for isozymatic alleles, where 49.0% of total allelic diversity was apportioned among populations of *Hordeum spontaneum* (Schonewald-Cox et al. 1983). This contrasts with a very low G_{st} (0.036) value reported for cultivated barley (Britting and Goodman 1989). These results argue strongly for the island genetic population model, similar to that proposed for wild emmer (Nevo and Payne 1987), and call for collecting more populations, rather than more plants within populations, of the wild species in primary and secondary centers of diversity.

Coefficients of genetic similarity (Table 4) indicate that most populations displayed alternative alleles at both hordein loci. Similarly, populations of wild emmer (Nevo and Payne 1987) showed high genetic distances, as measured by differentiation of allozyme and HMW glutenins genes. In contrast, NI values, based on 12 different enzyme systems for populations of *Triticum monococcum* var. *boeoticum*, ranged from 0.894 to 1.0, and from 0.898 to 1.0 in populations of *Triticum urartu* (Smith-Heurta et al. 1989). This indicates genetically very uniform populations of both species as compared to *Hordeum spontaneum* populations.

It can be seen (Fig. 1 and Table 4) that geographical distances are not indicative of genetic similarities between these populations, and that migration (Nevo and Payne 1987) is not a major factor in population genetic differentiation of these proteins. Although the adaptive value of the wide variation of seed storage proteins in members of the Triticeae has not been totally accounted for (Levi and Feldman 1988), the variability found for these proteins, in this and other studies (Doll and Brown 1979; Nevo et al. 1983; Nevo and Payne 1987), may be

due to their ability to withstand random mutation caused by alterations in the structural genes coding for them (Soave and Salamini 1983; Kreis et al. 1983) without selective advantage (Corke and Atsmon 1990).

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