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Genetic diversity of four esterase loci in natural populations of *Hordeum spontaneum* C. Koch from Jordan

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Summary. The diversity of four esterase loci was studied electrophoretically in 690 individual spikes representing 12 populations of wild barley (*Hordeum spontaneum* C. Koch.) collected from central, peripheral and marginal regions of its distribution in Jordan. A minimum of 6, 10, 5 and 5 alleles were observed at the *Est-1, Est-2, Est-4* and *Est-5* loci, respectively. *Est-2* and *Est-4* were the most diverse loci ($H_e = 0.53 \pm 0.05$ and 0.46 ± 0.07, respectively). *Est-5* was intermediate ($H_e = 0.33 + 0.07$) and *Est-1* was the lowest ($H_e = 0.22 \pm 0.04$). Polymorphism was highest in the central populations ($H_e = 0.52 \pm 0.04$), followed by the peripheral ($H_e = 0.40 \pm 0.05$) and the marginal ($H_e = 0.22 \pm 0.05$) populations. Average allelic diversity between ($G_{st} = 0.49$) and within ($H_s = 0.51$) populations reflects a high allelic differentiation among these populations. Log-linear analyses revealed that four two-locus terms and two three-locus terms were significantly associated ($P < 0.05$). Geographical distances between populations were not significantly correlated with Nei's genetic similarity index ($r = 0.16$; $P < 0.19$). It is postulated that diversifying selection is a major factor in the population genetic differentiation of these esterase loci.

Key words: Wild barley – *Hordeum spontaneum* – Esterases – Diversity – Jordan

Introduction

Allozyme studies in plant species provide useful insights into the genetic structure of, and the evolutionary relationships among, wild, weedy and crop species (Hauptli and Jain 1984; Jana and Pietrzak 1988; Rebordinos and Perez de la vega 1989). Esterases (EC 3.1.1.–), among other isozyme systems, have been extensively utilized in monitoring genetic changes in experimental barley (*Hordeum vulgare* L.) populations (e.g., Clegg et al. 1972), in a comparative assessment of genetic diversity in wild (*Hordeum spontaneum* C. Koch.) and cultivated (*H. vulgare* L.) barley (Jana and Pietrzak 1988), and in elucidating multilocus structure in both species (Dai and Zhang 1989; Zhang et al. 1990).

Esterases were among the most differentiating loci between populations of *H. spontaneum* from the Fertile Crescent, as indicated by their average degree of gene diversity (Nevo et al. 1986). Moreover, substantial within-population variation, and the localization of certain esterase alleles to particular environments, have been found in these populations (Brown et al. 1978; Nevo et al. 1979).

Reliable characterization of a wide range of traits is an essential step towards a fuller utilization of the wild genetic resources in plant improvement. Nevo et al. (1979) emphasized the importance of sampling natural populations from their entire ecological range in order to represent, and then to evaluate, the structure of their genetic variation, both for population and genetic studies (Nevo et al. 1986) and for crop improvement (Stuber et al. 1982; Hauptli and Jain 1984). Recently (Jaradat, 1991), populations of *H. spontaneum* representing the entire ecological range of the species in Jordan have been characterized for kernel protein content and its B and C hordein components. This paper presents patterns of genetic variation, based on four esterase loci, in 12 populations of *H. spontaneum* collected from central, peripheral and marginal areas of the species' distribution in Jordan.

Materials and methods

Geographical and ecological information on the 12 populations of *H. spontaneum* analyzed in this study is presented in Jaradat

Table 1. Diversity indices (H), wild alleles (w.a.) and mean comparisons for four esterase loci among 12 populations of wild barley from Jordan

Group	Population	Locus								Mean
		Est-1		*Est-2*		*Est-4*		*Est-4*		
		w.a.	H	w.a.	H	w.a.	H	w.a.	H	
Central	1	e	0.25	f	0.80	g	0.34	b	0.50	0.47[b*]
	2	e	0.37	h	0.67	e	0.53	f	0.69	0.57[a]
	3	e	0.35	h	0.49	e	0.73	a	0.21	0.45[b]
	4	e	0.42	g	0.70	g	0.63	c	0.60	0.59[a]
	Mean		0.35[a]		0.67[a]		0.56[a]		0.50[a]	
Peripheral	1	e	0.23	f	0.37	g	0.48	c	0.04	0.28[b]
	2	e	0.26	g	0.62	b	0.50	c	0.38	0.44[a]
	3	3	0.18	g	0.55	e	0.13	c	0.73	0.40[a]
	4	e	0.32	f	0.67	e	0.62	c	0.33	0.49[a]
	Mean		0.25[b]		0.55[a]		0.43[a, b]		0.37[b]	
Marginal	1	e	0.00	f	0.15	g	0.50	a	0.25	0.23[b]
	2	e	0.00	g	0.49	e	0.08	c	0.00	0.14[c]
	3	e	0.08	h	0.50	b	0.62	c	0.06	0.32[a]
	4	e	0.00	g	0.40	e	0.15	c	0.32	0.22[b]
	Mean		0.02[c]		0.39[b]		0.34[b]		0.16[c]	

* Means within each group of genotypes, or within each esterase locus, followed by the same letter to not differ significantly (DNMRT = 0.05)

(1991). A total of 690 single spikes, representing the 12 populations collected from central, peripheral and marginal regions of the species' distribution in Jordan were used. Each spike was assayed for four enzyme loci following the methods described by Kahler and Allard (1970; 1981). The enzymes assayed are coded for by genes of the four esterase loci (*Est-1, Est-2, Est-4* and *Est-5*). The electrophoretic variants (i.e. alleles) were labelled alphabetically in order of decreasing mobilities of their allozymes, as suggested by Nevo et al. (1979; 1986). Allelic frequencies were calculated, and the wild-type was identified at each esterase locus and population (Kahler and Allard 1981). Total genetic diversity (H_t), average gene diversity within populations (H_s), average gene diversity between populations (D_{st}), and gene diversity between populations relative to total gene diversity (G_{st}) were calculated for each esterase locus following (Nei 1973).

The normalized genetic identity (I_N) between all 12 populations, over the four esterase loci, were calculated according to Nei (1972). Finally, the four esterase loci *Est-1, Est-2, Est-4* and *Est-5*, designated A, B, C, and D, respectively, were reduced to a diallelic state following the convention used by Weir et al. (1972), and then the pattern of multilocus structure in the whole collection was determined by discrete log-linear multivariate analyses following the methods described by Jana and Pietrzak (1988) and Zhang et al. (1990).

Results

The number of genotypes observed, and the most frequent one (considered to be the wild-type), at each esterase locus, are presented in Table 1. A total of 26 genotypes were identified, of which six were observed at the *Est-1* locus, ten at the *Est-2* locus and five at each of the *Est-4* and *Est-5* loci. *Est-1e, Est-2g, Est-4e* and *Est-5c* were the most frequent genotypes; they constituted 100, 42, 50 and 67%, respectively, of the genotypes at these loci.

Table 2. Apportionment of genetic variation in four esterase loci for 12 populations of wild barley from Jordan

Locus	Variation component[a]					
	H_t	H_s	D_{st}	G_{st}	A_p	P_a
Est-1	0.204	0.087	0.117	0.57	1.92	0.32
Est-2	0.534	0.298	0.236	0.44	3.67	0.37
Est-4	0.456	0.212	0.244	0.53	2.60	0.52
Est-5	0.329	0.197	0.132	0.40	2.50	0.50
Mean	0.381	0.198	0.183	0.49	2.67	0.43

[a] See text for abbreviations

Genotypic frequencies at individual loci varied considerably over populations (data not presented) and resulted in a great range of diversity indices for individual loci and populations (Table 1). On the average, *Est-2* and *Est-4* were the most diverse loci ($H = 0.53 \pm 0.05$, and 0.46 ± 0.07, respectively); *Est-1* was the lowest ($H = 0.204 \pm 0.04$), while *Est-5* was intermediate ($H = 0.33 \pm 0.07$). However, two marginal populations were monomorphic at the *Est-1* locus and one was monomorphic at both the *Est-1* and *Est-5* loci. Average diversity (H) for central, peripheral and marginal populations was calculated as the average diversity index over loci. Central populations appeared to have the highest level of diversity (0.52 ± 0.04), followed by peripheral populations (0.40 ± 0.05),

Table 3. Coefficients of Nei's genetic similarity (NI) (above the diagonal) and geographic distances in km (below the diagonal) based on four esterase loci, between 12 populations of wild barley from Jordan

Population		1	2	3	4	5	6	7	8	9	10	11	12
1	Irbid		0.69	0.76	0.47	0.60	0.52	0.74	0.62	0.36	0.77	0.61	0.76
2	Ajlun	20		0.76	0.47	0.38	0.35	0.47	0.58	0.27	0.54	0.44	0.50
3	Salt	45	35		0.58	0.40	0.45	0.58	0.48	0.30	0.62	0.34	0.61
4	Madaba I	72	44	36		0.36	0.39	0.36	0.37	0.28	0.47	0.23	0.50
5	Jarash	28	30	40	60		0.70	0.68	0.67	0.48	0.66	0.59	0.80
6	Madaba II	84	80	48	40	60		0.56	0.49	0.43	0.51	0.55	0.65
7	Karak I	140	120	85	60	115	45		0.66	0.41	0.77	0.60	0.78
8	Shawbak	220	185	150	120	180	130	75		0.30	0.68	0.52	0.72
9	Mafraq	40	44	60	80	40	52	110	180		0.30	0.37	0.51
10	Karak II	140	125	90	60	90	60	40	85	100		0.43	0.87
11	Qatranah	180	160	125	100	150	90	60	60	130	40		0.61
12	Ma'an	240	220	190	160	215	160	90	40	200	105	75	

whereas marginal populations were the least variable (0.22 ± 0.05) and differed significantly from both central and peripheral populations.

Total allelic diversity (H_t) for each esterase locus was subdivided into its components: average gene diversity within a population (H_s), average (D_{st}) and relative (G_{st}) degree of gene differentiation among subpopulations, for each of the four loci (Table 2). Average H_t over all four loci is 0.38; it ranged from 0.20 (*Est-1*) to 0.53 (*Est-2*). *Est-2* and *Est-4* were the most genetically differentiating loci among populations based on their D_{st} values.

On average, 49.0% of the total allelic diversity exists among populations, and 51.0% within populations (Table 2). The mean number of alleles per polymorphic locus (A_p) ranged from 1.92 in *Est-1* to 3.67 in *Est-2*, while the proportion of the total number of alleles found within each population (P_a) ranged from 0.32 to 0.50.

Log-linear analyses were performed after reducing each locus to a diallelic state following the convention used by Weir et al. (1972). Four two-locus terms and two three-locus terms were found to be significantly associated ($P < 0.05$). The model that best fits the data for the four esterase loci is as follows:

$$\ln f_{i,j,k,l} = u + u_{(A)(i)} + u_{(B)(j)} + u_{(C)(k)} + u_{(D)(l)} + u_{(AB)(ij)} + u_{(AC)(ik)} + u_{(BC)(jk)} + u_{(CD)(kl)} + {}_{(ABC)(ijk)} + u_{(BCD)(jkl)}$$

for all $i, j, k, l = 1, 2$.

This model indicates that the isozyme markers are not totally independently distributed in the populations of wild barley used in this study.

The normalized identity of all four esterase loci between each pair of populations (Nei 1972) was used to calculate the coefficients of esterase genetic similarity (i.e., Nei's identity index, NI) (Table 3, above the diagonal). NI averaged 0.53 and ranged from 0.23 to 0.87 (Table 3). However, it was lowest (0.52) among marginal populations and highest among peripheral (0.63) and central (0.62) populations. Geographic distances (Table 3, below the diagonal) among these populations were not indicative of genetic similarity ($r = 0.16$; $P < 0.19$).

Discussion

The number of esterase genotypes and the total diversity found in this study are comparable to those reported for *H. spontaneum* populations from individual countries in the Near East (Nevo et al. 1986), and for four eastern Mediterranean countries (Jana and Pietrzak 1988). Considering the number of populations and the number of spikes per population assayed for esterase loci, the present study based on 690 spikes representing 12 populations, revealed more genotypes per locus per population (6.5) than *H. spontaneum* from Turkey (4.5) or from Iran (4.75). However, Israeli populations of *H. spontaneum* revealed even more esterase genotypes (8.5) per locus per population (Nevo et al. 1986). On the other hand, the number of genotypes per locus in this study was higher than that reported for 3,000 barley varieties from the Old World (4.2) (Konishi 1989), and lower than the one (7.5) reported for land races from four eastern Mediterranean countries (Jana and Pietrzak 1988).

Total diversity per locus followed the same trend reported by other workers (e.g., Brown et al. 1978; Nevo et al. 1986); i.e., *Est-2* > *Est-4* > *Est-5* > *Est-1*.

Central populations showed a significantly higher level of diversity ($H = 0.52$) than peripheral ones ($H = 0.40$) which, in turn, were significantly more diverse than the marginal populations ($H = 0.22$). The same trend was found for two horden loci (Jaradat 1991) in the same populations. Additionally, the same trend can be deduced from data presented by Brown et al. (1978) and Nevo et al. (1986). However, significant intrapopulational differences have also been detected within central, peripheral and marginal populations (Table 1). These

differences can be attributed to macro- and micro-geographical factors (Nevo et al. 1986).

The localization of certain alleles, especially the *Est-2* and *Est-4* loci, to particular regions is supported by the findings of Brown et al. (1978) and Nevo et al. (1986). However, all four esterase loci were strongly polymorphic, displaying either shifts in the wild allele (e.g., *Est-2* and *Est-4*) or sharp geographic differentiation over short geographic distances (Table 1).

Average allelic diversity (Table 2) among populations for each esterase locus (G_{st}) ranged from 0.40 to 0.57. Average allelic diversity over loci between (0.49) and within (0.51) populations reflect a relatively high allelic differentiation among these populations. However, G_{st} values (Table 2) are lower than those reported for *H. spontaneum* in the Near East (Nevo et al. 1986) and higher than those deduced from data reported for cultivated barley (Dai and Zhang 1989; Britting and Goodman 1989). These results support earlier findings on hordein alleles (Jaradat 1991), and reflect the distinctive nature of *H. spontaneum* populations from diverse habitats in Jordan. Moreover, indirectly, these results, imply the existence of localized ecotypes each adapted to the particular environmental conditions of its habitat (Nevo et al. 1986). The total diversity observed at each esterase locus (Nei 1973) indicate that *Est-2* was highly polymorphic, *Est-4* and *Est-5* were intermediate, and *Est-1* was weakly polymorphic. The same trend was observed at the same loci by Brown et al. (1978) and Nevo et al. (1986).

The proposed log-linear model given earlier suggests strong, non-random association of alleles at these esterase loci similar to those reported for wild (Jana and Pietrzak 1988) and cultivated barley (Dai and Zhang 1989; Zhang et al. 1990). The complex multilocus associations that are held by the four two-locus interactions and by the two three-locus interactions support earlier findings by Jana and Pietrzak (1988) and Zhang et al. (1990). However, the associations AD (*Est-1, 4*), BD (*Est-2, 5*), and ACD (*Est-1, 4, 5*), reported for wild barley from four eastern Mediterranean countries, were not significant in the present study. On the other hand, the associations AB (*Est-1, 2*) and *AC* (*Est-1, 3*) found in cultivated barley from South West Asia (Zhang et al. 1990) were highly significant in the present study.

The coefficients of Nei's genetic similarity index (NI) (Table 3) were not significantly correlated with the geographic distances between these populations. Average NI (0.53) is higher in magnitude than that reported for two hordein loci (0.209) in the same populations (Jaradat 1991). Also, it is higher than the one (0.357) based on two glutenin loci between 11 populations of wild emmer from Israel (Nevo and Payne 1987) and contrasts with the very high NI values reported for *Triticum monococcum* var. *boeoticum* (range 0.894–1.0) and *Triticum urartu* (range 0.898–1.0) (Smith-Heurta et al. 1989). NI values (Table 3) suggest that the pattern of esterase genes in *H. spontaneum* from Jordan displayed a less distinctive genetic population differentiation than that of hordein subunit genes (Jaradat 1991). A similar conclusion was reported for HMW glutenin subunits and allozymes of wild emmer (Nevo et al. 1982).

The intra- and inter-populational diversity for esterases in *H. spontaneum* from Jordan supports earlier findings (Jaradat 1991) in that the species displays local and regional adaptive differentiation as a result of diversifying selection (Nevo et al. 1986) and that migration plays no, or very little, role in population genetic differentiation of these esterase loci.

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