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Population and multilocus isozyme structures in a barley landrace

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Abstract

Isozyme data were used to assess genetic diversity within and among a subdivided population of the salt-tolerant Batini barley landrace. Population diversity and its components were estimated on the basis of 12 isozymes scored on 450 single plants representing seven subpopulations. Two principal components, based on mean gene diversity, Shannon's diversity index, percentage polymorphic loci, genetic identity and genetic distance among subpopulations accounted for 91.7% of total variation and separated the subpopulations into four distinct groups. Contributions to total diversity by individual subpopulations were partitioned into intra- and inter-population components. The level of population differentiation indicates that a large percentage of total genetic diversity was apportioned within subpopulations. The presence of valuable genetic diversity in this landrace was confirmed.

Keywords: barley landrace; diversity; isozymes; multilocus association; Oman

Introduction

Traditional farmers, particularly those cultivating in marginal regions and stress-prone environments, have serendipitously maintained a wealth of genetic variation within crop landraces. Understanding the relationships among and within these landraces may have important implications in the design of programmes for conservation of such genetic diversity.

A high level of phenotypic variation has been documented in the Batini barley landrace from Oman (Jaradat *et al.*, 2004a) and substantial within-population variation was found for phenological and yield-related traits under salinity stress (Jaradat *et al.*, 2004b). Populations of this and other landraces (Doebley, 1989) must have been subjected to strong selective pressures which have resulted in modifications to their genetic structure.

Primitive cultivated barley landraces are held by some to be a more attractive genetic resource to barley breeders than wild barley because of their closer affinity with the modern cultivars of barley which are adapted to improved agricultural practices (Teshome *et al.*, 2001). These landraces require additional attention because of the accelerated rate of their extinction resulting from the modernization of farming systems and the socio-economic incentives associated with their substitution by modern cultivars (Brush, 1994).

Knowledge of the amount and distribution of genetic variability within and among subpopulations of a landrace is crucial when selecting germplasm to be included in a breeding programme (Teshome *et al.*, 2001). In addition, such knowledge is helpful to geneticists in managing plant genetic resources and it provides information for designing sampling protocols (Liu *et al.*, 1999, 2000).

Genetic diversity of isozymes, especially of esterases, has been intensively examined in cultivated barley. However, there is a paucity of isozyme diversity studies in barley landraces, especially those from Oman. Landraces from Jordan harbour a greater amount of genetic diversity than those from Turkey, and much of the

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diversity is due to the variability within, rather than between landrace populations of different peasant farmers (Jana and Pietrzak, 1988).

Most authors (e.g. Jana and Pietrzak, 1988; Parzies *et al.*, 2004) have concluded that there is considerable local differentiation in barley landrace populations from the major centres of diversity of *Hordeum*, and that the evolutionary processes occurring in heterogeneous populations of barley are quite complex. The objective of this present study was to assess genetic diversity on the basis of 12 isozymes scored on 450 single plants representing seven subpopulations in a population of the Batini barley landrace.

Material and methods

The original samples were acquired by the International Center for Biosaline Agriculture (ICBA) in 1999 from the Batinah region of Oman and comprised seven bulked samples collected from farmers' fields along the road from Sohar (24°20′N, 56°40′E) to Liwa (24°31′N, 56°36′E) (Jaradat *et al.*, 2004a). Initial seed multiplication and plant selection, on the basis of readily observable plant and spike morphological and phenological traits during the 1999–2000 cropping season, resulted in the selection of 3191 single spikes representing seven distinct populations (Jaradat *et al.*, 2004a). The collection was multiplied (spike to row) at the experimental station of ICBA (25°13′N, 55°17′E), Dubai, United Arab Emirates. Random spike samples (range 50–100 per subpopulation, based on subpopulation size) were collected at maturity to form a germplasm collection for genetic diversity studies. In all, 450 plants representing seven subpopulations of the Batini barley landrace were included in this study.

Based on seed availability, 70–80 seeds per subpopulation were germinated on vermiculite at 20°C and 12 h illumination per day, and enzyme extracts were made from the plumule of a minimum of 50 1-week-old seedlings per subpopulation. This sample size is required to be 95% certain of recovering a minimum of one copy of an allele that occurs in the subpopulation with frequency of 5% (Brown and Marshall, 1995). The methodology of enzyme extraction and electrophoretic assays followed those of Linde-Laursen *et al.* (1987). Scoring of electrophoretically discernible enzyme zones followed the procedure described by Brown (1983), Linde-Laursen *et al.* (1987) and modifications suggested by Jana and Pietrzak (1988). The classification developed by Marshall and Brown (1975) was adopted to classify each allele, based on its frequency in each subpopulation and the number of subpopulations in which it appeared.

Population genetic parameters calculated for each locus, the landrace as a whole and for each subpopulation were: number of genotypes, number of effective alleles per locus (*Ae*), mean gene diversity (*h*), Shannon's diversity index (*I*) and percentage polymorphic loci (*P-0.05*). Genetic diversity, defined as the frequency of heterozygous individuals that is expected under Hardy–Weinberg equilibrium, was divided within and among subpopulation levels (Nei, 1973). Total (H_T), within-subpopulations (H_S) and among-subpopulations (D_{ST}) genetic diversity, and the genetic differentiation coefficient (G_{ST}), which may be regarded as a multiallelic variant of Wright's F_{ST} originally formulated for loci with two alleles (Berg and Hamrick, 1997), were estimated for each isozyme system and subpopulation. Subpopulation-level estimates of genetic diversity were obtained by averaging H_T, H_S, D_{ST} and G_{ST} across all polymorphic loci. A quantitative decomposition of the contribution of each population to total diversity as suggested by Petit *et al.* (1998) and Finkeldey and Murillo (1999) was performed by calculating the same genetic diversity statistics for all subpopulations, except the subpopulation in question, thus splitting it into two additive components. One is due to the diversity of the subpopulation (C_S), and the other is due to its uniqueness, i.e. its divergence from other subpopulations (C_{ST}). Following the notation used by Nei (1973) for a population G composed of n subpopulations, each of relative size $c_j \ldots c_l$, total gene diversity due to variation within subpopulations (Hs) is estimated as $\Sigma_j c_j H(j)$ and the diversity due to differentiation among subpopulations (D_{ST}) is estimated as $\Sigma_j c_j \Sigma_l c_l$ d(j,l), where the term d(j,l) is the minimum genetic distance between the two subpopulations j and l (Nei, 1987, p. 219). Therefore, the relative contributions of subpopulation j to the gene diversity within subpopulations, $C_S(j)$, the genetic differentiation among subpopulations, $C_{ST}(j)$, and the total gene diversity, $C_T(j)$, are computed as: $C_S(j) = Hs(j)/H_S$, $C_{ST}(j) = D_{ST}(j)/D_{ST}$ and $C_T(j) = H_T(j)/H_T$.

The contribution to total, inter- and intra-population diversity by four population genetic parameters estimated on the whole germplasm collection (i.e. *Ae*, *h*, *I* and *P-0.05*) was quantified using partial least square (PLS) regression, which is a linear regression method that forms components (factors, or latent variables) as new independent variables (explanatory variables, or predictors) in a regression model (StatSoft Inc., 2003). The components in PLS are determined by both the response variable (e.g. C_S) and the predictor variables (i.e. *Ae*, *h*, *I* and *P-0.05*). A regression model from PLS can be expected to have a smaller number of components without an appreciably smaller R^2 value.

Nei's genetic identity (I_N) and genetic distance (D_N) were calculated for each pairwise combination of subpopulations (Nei, 1987), then populations were clustered via the unweighted pair group method with arithmetic average

(UPGMA) procedure using NTSYSpc (Rohlf, 2000). Estimates of gene flow, as the number of migrants per generation, N_m, were based on G_{ST} values for each subpopulation. A fixation index (F_{ST}) for each locus and subpopulation was calculated (Hartl and Clark, 1989) and its deviation from zero was tested using a χ^2 test. Log-linear analysis of multiple loci was employed to test (i) whether the isozyme markers showing high levels of diversity are independently distributed in subpopulations, and (ii) if associated, whether these associations differ significantly between subpopulations. The most parsimonious models with the best goodness-of-fit to the frequency tables of the observed isozyme data sets were obtained by model selection procedures based on the partitioning of likelihood-ratio statistics (G^2) (Agresti, 1990). The simplest model which fitted the data most satisfactorily was considered the 'best-fitting model' for a given combination of loci under consideration and then was interpreted for multilocus associations (Agresti, 1990). Finally, the non-linear iterative partial least squares (NIPALS) algorithm (StatSoft Inc., 2003), a combined implementation of principal component analysis (PCA) and PLS, was used to extract systematic variation and relationships in two data sets; the first included *Ae*, *h*, *I* and *P-0.05* for all seven subpopulations and the second included Wright's fixation indices for the 12 isozyme systems.

Several software packages (Labate, 2000) were used to estimate various diversity measures and genetic distances, infer population structure, test for multilocus equilibrium, and test polymorphic loci for evidence of selective neutrality. The software package STATISTICA Release 7 (StatSoft Inc., 2003) was used to perform PCA and develop log-linear models (Agresti, 1990) for groups of isozyme loci.

Results

Thirty-six alleles were observed at the 12 loci, of which eight were rare, 13 common widespread, nine common sporadic and six common localized. No rare localized alleles were detected. A total of 276 allele combinations were identified, of which 206 were represented by only one plant.

Allelic frequencies at the loci varied considerably among subpopulations (data not presented). As a result, a wide range of diversity indices was observed among individual loci. Average H_T (0.388) was dominated, in decreasing order, by the high diversity estimates for *Est*-2, *Pgd*-3, *Est*-5 and *Est*-4. However, esterases as a group accounted for 49.0% of total isozyme variation in this study (Table 1). Similarly, these loci differed significantly in the level of differentiation ($G_{ST} = 0.23$), with *Pgd*-2 and *Est*-5 being the most and least differentiated loci, respectively.

Genetic diversity parameters

Estimates *Ae*, *h*, *I*, *P-0.05*, D_N and I_N showed few similarities and a wide range of differences among subpopulations (Table 2). Batini-4 displayed the highest number of effective alleles (2.13), and Batini-3 the lowest, representing 39% more and 17% less, respectively, than the population average. Gene diversity (0.192 ± 0.2)

Table 1. Polymorphic loci of five isozyme systems, allele classification, total diversity (H_T) and level of differentiation (G_{ST}) for seven subpopulations of the Batini barley landrace from Oman

			Common alleles				
Isozyme system	Locus	Rare alleles[a]	Widespread	Sporadic	Localized	H_T[b]	G_{ST}
Aconitate hydratase	*Aco*-1	1	2			0.186 fe	0.254
	Aco-2	1		1		0.153 e	0.219
Acid phosphatase	*Acp*-3			1	1	0.416 d	0.259
Esterase	*Est*-1	1	1		1	0.520 b	0.235
	Est-2		2	2	1	0.605 a	0.206
	Est-4	1	1	2		0.457 c	0.212
	Est-5	1	1	2		0.490 bc	0.180
Phosphoglucose dehydrogenase	*Pgd*-1		1		1	0.194 f	0.242
	Pgd-2	1	1			0.322 e	0.297
	Pgd-3		1		1	0.534 b	0.189
Phosphoglucose isomerase	*Pgi*-1	1	2		1	0.386 d	0.251
	Pgi-2	1	1	1		0.405 d	0.196
Total		8	13	9	6		
Mean						0.388	0.229

[a] Allele classification according to Marshall and Brown (1975).
[b] Mean values within the column followed by the same letter do not differ significantly, $P = 0.05$.

Table 2. Estimates of effective number of isozyme alleles per locus (*Ae*), mean gene diversity index (*h*), mean Shannon diversity index (*I*), percentage polymorphic loci (*P-0.05*) in seven subpopulations, and mean genetic distance (D_N) and mean genetic identity (I_N) between each subpopulation and the remaining six subpopulations of the Batini barley landrace from Oman

Subpopulation	*Ae*	*h*	*I*	*P-0.05*	Mean D_N	Mean I_N
Batini-1	1.33	0.207	0.364	52.63	0.061	0.945
Batini-2	2.06	0.205	0.380	57.89	0.043	0.938
Batini-3	1.28	0.164	0.287	52.11	0.131	0.925
Batini-4	2.13	0.183	0.321	47.37	0.049	0.924
Batini-5	1.40	0.205	0.267	55.60	0.091	0.902
Batini-6	1.40	0.210	0.359	42.63	0.085	0.913
Batini-7	1.32	0.171	0.301	36.84	0.103	0.878
Mean (SD)	1.53 (0.04)	0.192 (0.019)	0.326 (0.043)	49.3 (7.5)	0.081 (0.032)	0.918 (0.023)

estimates ranged from 0.16 (Batini-3) to 0.21 (Batini-6), with a difference exceeding twice the standard deviation of the mean. However, *Ae* and *h* were positively and significantly correlated ($r = 0.84$, $P = 0.05$).

I ranged from 0.267 (Batini-5) to 0.38 (Batini-2); the difference between these estimates slightly exceeds twice the value of the standard deviation of the mean. *I* was significantly correlated with *Ae* ($r = 0.70$, $P = 0.05$) and *h* ($r = 0.95$, $P = 0.05$). Relatively high values for *P-0.05* (>50.0%) were observed for four subpopulations; the lowest for Batini-7 has below mean values for *h* and *I*. *P*-0.05 was significantly ($P = 0.05$) correlated with *Ae*, *h* and *I* ($r = 0.71$, 0.90 and 0.92, respectively). Genetic identity and the absolute measure of gene differentiation (i.e. minimum genetic distance among subpopulations) values (Table 2) indicate a relatively high level of genetic similarity (>0.90), except for Batini-7.

Distances among subpopulations

A two-dimensional biplot based on multivariate analysis (Fig. 1) using all genetic diversity attributes listed in Table 1 (except *Ae* due to its different scale) accounted for 91.7% of total variation and separated genetic diversity attributes and subpopulations into distinct groups. *P-0.05* and D_N were the most discriminating attributes among subpopulations along the first axis, whereas *I* in addition to *P-0.05* and D_N were most discriminating on the second axis. Associations among genetic diversity attributes and subpopulations, based on the covariance matrix and as depicted in the biplot, showed that subpopulations were separated largely along the first principal component (PC1) (accounting for 63.5% of total variation) based on multivariate differences among their *P-0.05*, *I*, I_N and *h* estimates (in decreasing order), on the one hand, and D_N estimates, on the other. The second principal component (PC2) (accounting for 28.2% of total variation) separated subpopulations based on multivariate differences of their *P-0.05* and D_N estimates, on the one hand, and *I*, I_N and *h* estimates (in decreasing order), on the other. However, when UPGMA was used to cluster these subpopulations based on Nei's dissimilarity matrix, they were separated into three and five clusters at the 33 and 67% of maximum distance (Fig. 2). Batini-3 and Batini-7 were the most distant, whereas Batini-2 and Batini-4 were closest to one another.

Isozyme fixation indices

Sixty-three per cent of Wright's fixation indices (average $F_{ST} = 0.42 \pm 0.08$; Table 3) for all 12 isozymes were significantly higher than zero (ranging from 28.7% for *Pgi*-1 and *Pgd*-3 to 85.7% for *Est*-1, *Est*-5, *Pgi*-2 and *Pgd*-1). Of these, 41.5% belong to the esterases. Almost one-third (28.6%) of the fixation indices were above 0.5. The loci differed in the number of fixation indices that are significantly higher than zero (range two to six). *Aco*-2 has both the lowest and highest fixation indices (Batini-1 and Batini-7, respectively).

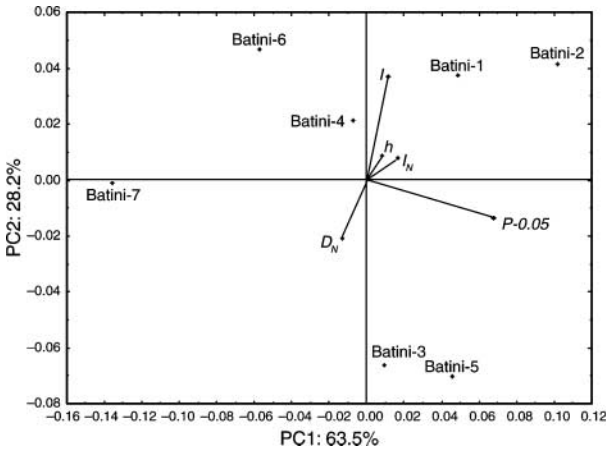

Fig. 1. Two-dimensional plot of seven subpopulations of the Batini barley landrace from Oman based on their mean gene diversity index (*h*), mean Shannon diversity index (*I*), percentage polymorphic loci (*P-0.05*), mean genetic distance (D_N) and mean genetic identity (I_N).

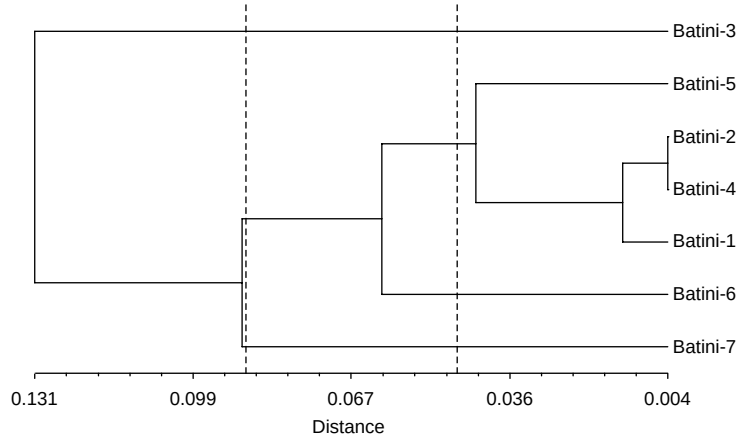

Fig. 2. UPGMA clustering of isozyme data based on Nei's genetic distances (dissimilarity) among seven subpopulations of the Batini barley landrace from Oman (dashed lines represent 33 and 67% of maximum distance).

Subpopulations differed in the number of fixation indices over all isozyme systems that are significantly higher than zero. Batini-6 showed the highest (11) followed by Batini-5 and Batini-7 (10 each), Batini-4 (eight), Batini-1 and Batini-3 (five each) and finally by Batini-2 (four significant fixation indices).

Two principal components (Fig. 3), based on the covariance matrix of fixation indices of the 12 isozymes, accounted for 82.4% of total variation. PC1 accounted for 66.0% of total variation and separated *Est*-1 and *Pgd*-2 from the remaining isozymes. PC2 accounted for 16.4% of total variation and separated *Pgd*-2, *Pgd*-3, *Pgi*-1, *Est*-4 and *Est*-5 from the remaining isozymes. Five isozymes (*Aco*-1 > *Aco*-2 > *Est*-4 > *Est*-2 > *Pgdi*-2) loaded highly positive on PC1; however, only one isozyme (*Est*-1) loaded highly negative on the same PC. On the other hand, four (*Est*-1 > *Pgdi*-2 > *Aco*-1 > *Est*-2) and two (*Acp*-3 > *Est*-4) isozymes loaded highly positive and negative, respectively, on PC2.

The two-dimensional plot (Fig. 3) suggests an association between one or more subpopulations with one or more isozyme loci. Batini-1 and Batini-2 were associated with *Est*-1, Batini-3 with *Pgd*-2, Batini-6 with six isozymes, and Batini-4, Batini-5 and Batini-7 were associated with five isozymes.

Partitioning of total genetic diversity

H_T, G_{ST} and N_m estimates reflect the characteristics of a predominantly inbreeding species (Table 4). The total genetic diversity ranged from 12.57% above (Batini-6) to 15% below (Batini-3) the population mean (0.1925). G_{ST} estimates, although negatively, but not significantly,

Table 3. Wright's fixation indices (F_{ST}) and χ^2 test for 12 isozyme loci scored on 450 plants in seven subpopulations of the Batini barley landrace from Oman

		F_{ST} and χ^2 test for subpopulations						
Isozyme system	Loci	Batini-1	Batini-2	Batini-3	Batini-4	Batini-5	Batini-6	Batini-7
Aconitate hydratase	*Aco*-1	0.211	0.165	0.063	0.679***	0.827***	0.319*	0.852***
	Aco-2	0.048	0.273	0.056	0.481**	0.538***	0.432*	0.879***
Acid phosphatase	*Acp*-3	0.337*	0.117	0.253	0.298	0.425**	0.822***	0.501**
Esterase	*Est*-1	0.665***	0.679***	0.467**	0.592**	0.351*	0.285	0.508**
	Est-2	0.302	0.247	0.559***	0.843***	0.751***	0.357*	0.893***
	Est-4	0.180	0.192	0.435**	0.703***	0.394*	0.806***	0.819***
	Est-5	0.389*	0.315*	0.123	0.674***	0.332*	0.518**	0.486**
Phosphoglucose dehydrogenase	*Pgd*-1	0.328*	0.283	0.315*	0.570***	0.648***	0.408*	0.430*
	Pgd-2	0.229	0.470**	0.388*	0.274	0.290	0.419*	0.283
	Pgd-3	0.197	0.124	0.149	0.224	0.433*	0.341*	0.247
Phosphoglucose isomerase	*Pgi*-1	0.054	0.138	0.077	0.281	0.292	0.428*	0.471**
	Pgi-2	0.367*	0.430*	0.067	0.615***	0.713***	0.382*	0.811***

*, **, ***Significant at $P = 0.05$, 0.01 and 0.001, respectively.

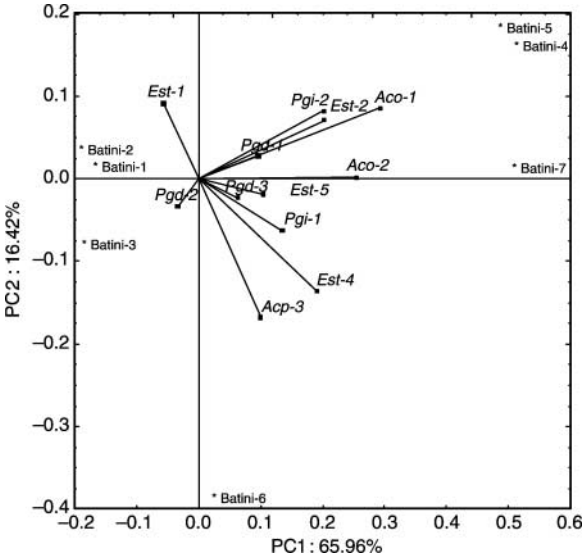

Fig. 3. Two-dimensional plot of 12 isozymes and seven subpopulations based on Wright's fixation indices for the 12 isozyme loci scored on 450 plants representing seven subpopulations of the Batini barley landrace from Oman.

correlated with H_T ($r = -0.55$, $P = 0.08$), ranged from 47.3% above (Batini-3) to 21.9% below (Batini-7) the population mean (0.185). N_m estimates (1.34–2.96) indicate a much higher level of gene flow among subpopulations than has been reported for an inbreeding species.

Contribution to total diversity (C_T), to intra-population diversity (C_S) and to inter-population diversity (C_{ST}), based on a diversity analysis of a subdivided population, indicates that three (Batini-1, Batini-5 and Batini-6) subpopulations contributed positively, whereas the remaining four subpopulations contributed negatively to total diversity (Table 4). However, the contribution to intra- and inter-population diversity differed among subpopulations. Two subpopulations contributed negatively to total diversity due to low inter-population (Batini-2 and Batini-7) or low intra-population diversity (Batini-3 and Batini-4) or to both (Batini-7).

PLS regression was used to predict the contribution to total, inter- and intra-population diversity using four population genetic parameters estimated on the whole germplasm collection (i.e. *Ae*, *h*, *I* and *P-0.05*). Two, two and three components (predictors) were needed to account for >90.0% of variation (R_2^y) in C_T, C_S and C_{ST}, respectively (Table 5). However, these components accounted for >93.0% of variation in the predictor variables (R_2^x). Subpopulations with high A_e, *h* and low *P-0.05* are expected to contribute more to the intra-population component, whereas those with high *P-0.05* and low *I* are expected to contribute more to the inter-population component of total diversity.

Table 4. Total genetic diversity (H_T), genetic differentiation (G_{ST}), estimate of gene flow (N_m) contribution to: total diversity (C_T), to intra-population diversity (C_S) and to inter-population diversity (C_{ST}), based on 12 isozyme systems, by seven subpopulations of the Batini barley landrace from Oman

Subpopulation	H_T	G_{ST}	N_m	C_T	C_S	C_{ST}
Batini-1	0.2048	0.1491	2.85	0.0154	0.0239	−0.006
Batini-2	0.2045	0.1615	2.59	−0.0011	0.0051	−0.007
Batini-3	0.1640	0.2723	1.34	−0.0275	−0.035	0.0075
Batini-4	0.1838	0.2133	1.84	−0.0089	−0.014	0.0058
Batini-5	0.2042	0.1952	2.06	0.0238	0.0142	0.0095
Batini-6	0.2167	0.1575	2.67	0.0227	0.0191	0.0034
Batini-7	0.1696	0.1443	2.96	−0.0159	−0.006	−0.009
Mean	0.1925	0.1847	2.33	0.0000	0.0000	0.0000

Table 5. Prediction of contribution to inter- and intra-population diversity components based on four population genetic parameters (*Ae*, *h*, *I* and *P-0.05*) in seven subpopulations of the Batini barley landrace from Oman, using partial least square (PLS) regression

Variable	Number of components	R_2^y	R_2^x	Predictors (*x*), PLS scaled regression coefficients			
				Ae	*h*	*I*	*P-0.05*
C_T	2	0.92	0.96	0.209	0.207	0.172	0.165
C_S	2	0.92	0.93	0.276	0.563	0.236	−0.763
C_{ST}	3	0.90	0.93	0.046	0.453	−3.241	2.003

Table 6. Non-random associations of alleles at loci of three groups (G1, G2 and G3) of isozyme systems detected by estimated U-terms and their standardized values in log-linear models, for seven subpopulations of the Batini barley landrace from Oman

	G1		G2		G3	
U-terms	Estimated	Standardized	Estimated	Standardized	Estimated	Standardized
U12	0.65	4.20***	0.35	2.15*	0.12	1.24
U13	0.38	2.40*	0.40	2.66**	0.69	6.35****
U23	0.23	1.98*	0.43	2.86**	0.32	3.69***
U123	0.56	3.96***	0.37	2.58**	0.30	3.58***
U14	0.78	8.92****				
U24	0.92	11.23****				
U34	0.57	3.96***				
U124	0.26	2.11*				
U134	0.25	2.58**				

G1: (1) *Est*-1, (2) *Est*-2, (3) *Est*-4, (4) *Est*-5; G2: (1) *Aco*-1, (2) *Aco*-2, (3) *Pgd*-3; G3: (1) *Acp*-3, (2) *Pgd*-1, (3) *Pgd*-2.
*, **, ***, ****Observed genotypic frequencies deviated from independence at significance levels of $P = 0.05$, 0.01, 0.001 and 0.0001, respectively.

Log-linear analysis

Preliminary analysis grouped 10 of the isozyme loci into three groups for log-linear analyses. The first group included *Est*-1, *Est*-2, *Est*-4 and *Est*-5. The second group was composed of *Aco*-1, *Aco*-2 and *Pgd*-3, whereas the third included *Acp*-3, *Pgd*-1 and *Pgd*-2. Pronounced non-random and significant associations were detected among two- and three-way contingency tables except the U12 in the third group (Table 6). However, no four-way associations were found in the first group with four isozymes.

The highest, highly significant, and two- and three-way associations were found among isozymes in the first group. The standardized U24 (*Est*-2/*Est*-5), followed by U14 (*Est*-1/*Est*-5) were highest. The three-way association U123 (*Est*-1/*Est*-2/*Est*-4) was highly significant ($P = 0.001$) when compared with U134 ($P = 0.01$) or U124 ($P = 0.05$) in the same group. The two- and three-way associations in the third group, except for U12, were highly significant and were intermediate between the first and second group.

Discussion

Indigenous farming communities in the Batinah region of Oman have contributed to the evolution and *in situ* conservation of the Batini barley landrace. One objective of a series of studies (Jaradat *et al*., 2004a, b) was to determine how farmers in this region manage on farm diversity and how to guard and enhance their capacity to conserve agricultural biodiversity. The relatively high percentage of the 276 allele combinations represented by a single plant confirms earlier conclusions (Bernardo *et al*., 1997) that no single genotype in a landrace is represented by more than a few plants, and that perhaps every plant in the population differs genotypically from other plants.

There is growing evidence (Nevo, 2001) that isozyme variation may not be selectively neutral at certain loci and may not accurately reflect population subdivision. Nevertheless, knowledge of the amount and distribution of genetic variability within this landrace is vital to plant breeders selecting germplasm to be included in breeding programmes (Yu *et al*., 2001). Also, it is helpful to geneticists managing its genetic resources and provides information for designing sampling protocols (Teshome *et al*., 2001).

On-farm populations of this and other barley landraces (e.g. Ethiopia, Syria, Tibet) have the capacity to support more rare alleles and multilocus genotypes (Dai and Zhang, 1989; Demissie and Bjørnstad, 1997; Parzies *et al*., 2004) than accessions conserved *ex situ*. This study identified a relatively lower (22.2%) portion of rare alleles in a small number of accessions in this landrace as compared to higher proportions of rare alleles (28.0–38.5%), albeit in much larger landrace collections (Liu *et al*., 1999, 2000). The inclusion of such rare, locally distributed alleles will be very dependent on stochastic effects and such alleles will only be included if the sample size is very large. Nevertheless, this landrace harboured a large number of rare alleles in a relatively small population size.

Distances among subpopulations, whether inferred from PCA of genetic parameters (Fig. 1), or isozyme fixation indices (Fig. 3), or based on Nei's minimum genetic distance (Fig. 2) confirm earlier results (Jaradat *et al*., 2004a) based on multiple quantitative and qualitative traits. PCA provided variable (diversity parameters, Fig. 1; isozyme

fixation indices, Fig. 3) independence and balanced weighting of traits, which led to an effective contribution of different traits on the basis of their respective variation. Aggregation of subpopulations in Figs 1 and 3 reflects genetic distances and reveals genetic similarities among these subpopulations. Moreover, data reduction achieved through PCA clarified the relationships between diversity parameters (Fig. 1) and between isozyme systems (Fig. 3) and divided total variance in the original two sets of traits into two uncorrelated new variables. The traits used in each PCA were of the same scale; therefore, the variance–covariance matrix was used and allowed absolute differences among subpopulations or among isozyme loci to be delineated (Mohammadi and Parsana, 2003).

A large portion of isozyme diversity (77.1%) was maintained within subpopulations; however, when only esterases were considered, this value reached 79.2%. On the other hand, the level of population differentiation indicated that 81.5% of the total variation was apportioned within subpopulations. This high value, typical of most barley landraces (e.g. Teshome *et al.*, 2001), may reflect adaptation to strong environmental dissimilarities or high levels of genetic drift maintained by restricted geneflow between populations (Demissie and Bjørnstad, 1997). A major concern to genebank managers and germplasm curators during the mass-propagation of the heterogeneous populations of this landrace is the high rate of loss of genetic diversity under the more favourable irrigated conditions (Nevo, 2001) in the Batinah region as compared to the slower rate under moisture-limiting dryland conditions.

Except for a few cases (e.g. Zhang *et al.*, 1994; Liu *et al.*, 1999), larger values of population differentiation were reported for barley landraces, as compared to modern cultivars. The seemingly very small level of population differentiation in these cases (e.g. barley landraces from Tibet, Bhutan and Nepal) is in agreement with the agricultural history of cultivated barley in these countries. Barley cultivation radiated from a nuclear region in these countries in the recent past, and not much natural or artificial selection has been practised during the migration and subsequent cultivation. The high population differentiation values estimated for seed, spike (Jaradat *et al.*, 2004a) and isozyme traits in the Batini landrace support the suggestion that barley was introduced into Oman through trade with cultures of old Mesopotamia (Willcox and Tengberg, 1995). Moreover, according to Zohary and Hopf (1993), this landrace must have undergone evolutionary modification as a result of selection and adaptation to the harsh environment of the Batinah region.

Subpopulations with high A_e and h, and low *P-0.05*, contributed more to the intra-population component, whereas those with high *P-0.05* and low *I* contributed more to the inter-population component of total diversity. These estimates were successfully used to predict subpopulation contribution to inter- and intra-population diversity (Petit *et al.* 1998). Two subpopulations contributed negatively to total diversity either due to lower than average C_{ST} (Batini-3) or C_S (Batini-7). The estimate of gene flow based on G_{ST} was high among these subpopulations ($N_m = 2.33$) which is high enough to prevent divergence resulting from genetic drift (Parzies *et al.*, 2004). However, this is not in agreement with the high level of gene differentiation in spite of the relatively high average distance among subpopulations. Nevertheless, N_m was much lower than the average value (4.92) reported for 12 populations of a Syrian barley landrace collected from different geographical regions (Parzies *et al.*, 2004). These high values may only be explained on the basis of seed exchange among farmers (Yu *et al.*, 2001; Parzies *et al.*, 2004).

Following the suggestions of Petit *et al.* (1998) and Finkeldey and Murillo (1999), this study examined the contribution of each subpopulation to total diversity. This procedure allowed diversity and differentiation to be measured separately, and their combined effect on the contribution of a subpopulation to diversity to be determined. Some contributions to diversity were negative, either because the diversity of the subpopulation is lower than the mean diversity or because the subpopulation is not very divergent. Relative contributions of subpopulations to total gene diversity can be useful in designing a conservation strategy of this landrace (Petit *et al.*, 1998).

Few comparable studies on multilocus structure of landrace populations are available in the literature (Brown and Feldman, 1981; Li and Rutger, 2000). Although the underlying evolutionary processes in landraces might be far from clear (Li and Rutger, 2000), the genetic structure and levels of genetic diversity identified in this and other landraces may arise from selection, genetic drift, or fragmentation of the population alone or in combination. In the meantime, diversity levels of landrace populations may change rapidly due to anthropogenic and environmental factors (Brush, 1994; Ribeiro-Caravalho *et al.*, 2004). Nevertheless, these structures are more likely to be retained under field conditions (Dai and Zhang, 1989; Li and Rutger, 2000) through selection, isolation and lack of migration, and restrictions on out-crossing and genetic recombination. The multilocus association did not appear to be different from one subpopulation to another; similar results have been reported even at a sub-regional level in Tibet (Zhang *et al.*, 1994). These results suggest that selection on multilocus gene complexes may be largely responsible for the maintenance of the extensive isozyme variation within this barley landrace in particular, and perhaps in *Hordeum* spp. in general.

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