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Distribution of allozymic alleles and genetic diversity in the American Barley Core Collection

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Abstract A survey of allozymic alleles and genetic diversity was made for 151 accessions of the American Barley Core Collection. A total of 25 alleles at ten loci were observed. Two loci were monomorphic. The average diversity index for individual loci ranged between 0.026 and 0.649. Most significant differences in allelic frequency and genetic diversity value were found between spring and winter barley. Spring barley showed a greatly higher average diversity than winter barley (t=2.124, P=0.071). The smallest differences in allelic frequencies and diversity values were observed between the two geographical regions, North and South America. Rare alleles were detected only in a few accessions. Seven rare alleles were associated with spring barley. The genetic similarities among the 151 accessions ranged from 0.20 to 1.00, which showed that a high level of genetic variability exists in this set of core accessions. Cluster analysis and principal coordinate analysis did not give clear-cut separation of different types of barley, but most of the winter barley accessions were closely associated.

Keywords *Hordeum vulgare* · Barley · Genetic diversity · Isozymic variation · Core collection

Introduction

Barley, as one of the major crops, has attracted the special attention of scientists for collection and conservation. There exists around 485000 accessions of wild and cultivated barleys stored in gene banks (Bothmer 1996). Nevertheless, the size of many large germplasm collections may be an obstacle for their full exploitation, evaluation and utilization (Holden 1984). This task could be more easily fulfilled by the use of sub-sets of the whole collection, called active working collections by Harlan (1972) and core collections by Frankel and Brown (1984). A core collection should include a maximum of the genetic variation contained in the whole collection, with a minimum of repetitiveness, ideally conserving at least 70% of the alleles in the whole collection (Brown 1989a). The Barley Core Collection (BCC) is a selected and limited set of accessions, optimally representing the genetic diversity of cultivated barley (*Hordeum vulgare* L. *s.l.*) and wild species of *Hordeum*, and providing well-known genetic standards (Bothmer et al. 1990). The size of the BCC should not exceed 2000 accessions, in order to keep it manageable (Knüpffer and Hintum 1995).

In order to maximize the diversity, the selection of core entries should be based on available data on geographic origin, genetic characteristics, and traits of possible value to breeders and other users. Brown (1989b) proposed that stratified sampling from groups of accessions, in logarithmic or absolute proportion to the group size, is the best strategy for the establishment of a core collection. Hintum et al. (1995) compared several sampling methods for the selection of core entries in cultivated barley from China, suggesting that a stratification sampling based on collection sites would give the best result and contain the largest number of alleles in the core collection.

Once the core accessions are selected, the next concern is what level of genetic diversity exists in this core set, and how representative it is. For the conservation of genetic resources by the development of core collections, as well as for breeding purposes, it is necessary to estimate the magnitude of genetic variation. In the current project, we have earlier estimated the isozyme diversity and allelic distribution in the Asian and European parts of the International Barley Core Collection (Liu et al. 1999, 2000). In the present paper, we survey the genetic diversity of the American accessions of the Barley Core Collection by isozyme electrophoresis. The objective is to provide valuable information for the further development of an optimal core collection in barley. This study forms a part of a more general survey of the entire Barley Core Collection.

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Table 1 The American accessions of the barley core collection (S=spring, W=winter)

No.	*Accession*	Spike row	Habit	Hull	Use	Origin
10	*23, 47, 59, 75, 106, 112, 114, 121, 122, 128*	2	S	Covered	Feed	North America
2	*12, 68*	2	S	Covered	Feed	South/Central America
1	*29*	2	S	Covered	Feed	Introduced
6	*1, 38, 73, 90, 102, 104*	2	S	Covered	Malt	North America
6	*41, 60, 61, 95, 97, 72*	2	S	Covered	Malt	South/Central America
2	*19, 116*	2	S	Covered	Malt	Introduced
3	*5, 43, 139*	2	S	Covered		North America
8	*3, 8, 9, 13, 20, 70, 131, 138*	2	S	Covered		South/Central America
3	*36, 49, 130*	2	S	Hulless	Feed	North America
2	*37, 94*	2	S	Hulless		South/Central America
32	*11, 14, 16, 17, 18, 22, 27, 28, 42, 46, 52, 66, 76, 77, 80, 83, 87, 88, 89, 92, 96, 101, 109, 111, 115, 118, 133, 140, 141, 142, 143, 145*	6	S	Covered	Feed	North America
13	*6, 7, 10, 34, 39, 40, 56, 57, 62, 67, 71, 78, 119*	6	S	Covered	Feed	South/Central America
13	*4, 15, 21, 50, 63, 74, 85, 93, 103, 105, 124, 125, 132*	6	S	Covered	Malt	North America
3	*81, 91, 98*	6	S	Covered	Malt	South/Central America
1	*110*	6	S	Covered	Malt	Introduced
1	*107*	6	S	Covered		North America
12	*26, 44, 48, 55, 64, 65, 100, 120, 123, 126, 134, 148*	6	S	Covered		South/Central America
2	*35, 69*	6	S	Hulless	Feed	North America
1	*127*	6	S	Hulless	Feed	South/Central America
1	*58*	6	S	Hulless		North America
12	*2, 25, 30, 31, 32, 45, 82, 99, 113, 136, 149, 151*	6	S	Hulless		South/Central America
14	*24, 33, 51, 53, 79, 84, 86, 117, 129, 135, 137, 144, 147, 150*	6	W	Covered	Feed	North America
1	*146*	6	W	Covered	Feed	Introduced
1	*108*	6	W	Covered		North America
1	*54*	6		Covered		South/Central America

Materials and methods

The set of the American part of the International Barley Core Collection used in the present study consists of 151 accessions (Table 1), mainly originating from the Americas, and kindly provided by Dr. Harold Bochelman, USDA, Aberdeen, Idaho. A detailed list of the material is available form the authors on request.

The methods for electrophoresis, staining procedures, enzyme nomenclature, and statistical treatments of the data have previously been described in detail by Liu et al. (1999, 2000).

Results

Allelic distribution and genetic diversity for the total sample

Five isozyme systems were analysed with ten loci showing clear banding patterns: esterase (*Est-1, Est-2, Est-4,* and *Est-5*), glucose phosphate isomerase (*Gpi-1*), phosphogluconate dehydrogenase (*Pgd-1* and *Pgd-2*), aconitate hydratase (*Aco-1* and *Aco-2*) and NADH dehydrogenase (*Ndh-2*). The number of alleles observed at each locus ranged from one (*Gpi-1* and *Pgd-1*) to four (*Est-2, Est-4* and *Est-5*). A total of 25 alleles were identified in the 151 accessions investigated. The distribution frequencies of all alleles are presented in Table 2. The *Est-2 Dr, Un, ne; Est-4 Nz, ne; Est-5 Mi, od,* and *Ndh-2 D* were rare alleles, and detected only in a few accessions (Table 3). The *Gpi-1* and *Pgd-1* loci were monomorphic. Genetic diversity values (0.000–0.649) are presented in Fig. 1.

Allelic distribution and genetic diversity in various barley groups

Two-rowed vs six-rowed barley

This set of the American Barley Core Collection contained 43 two-rowed and 108 six-rowed barley accessions. In total, 23 and 25 alleles were detected in the two-rowed and six-rowed barley, respectively. The allelic frequency in the two-rowed and six-rowed barleys was different for some alleles. For example, the *Est-1 Pr* allele had a much higher frequency in the two-rowed than in the six-rowed barley. The *Est-2 Un* allele and *Ndh-2 D* allele were only found in the six-rowed barley. Seven out of the ten loci were polymorphic in the two-rowed barley, and eight out of ten loci were polymorphic in the six-rowed barley. The largest diversity was found at the *Est-1* locus, followed by the *Est-4* locus for both types of barley

Table 2 Distribution of allelic frequencies in 151 accessions of the American Barley Core Collection

Loci	Alleles	Two-rowed	Six-rowed	Spring	Winter	Covered	Hulless	Malt	Feed	North	South	Total accessions
Est-1	*Pr*	0.512	0.167	0.256	0.031	0.254	0.285	0.258	0.215	0.194	0.348	0.265
	Al	0.140	0.334	0.218	0.813	0.315	0.095	0.323	0.291	0.306	0.234	0.278
	Ca	0.348	0.491	0.496	0	0.431	0.667	0.355	0.468	0.469	0.426	0.457
Est-2	*Dr*	0.047	0.009	0.026	0	0.015	0.095	0.032	0.025	0.031	0	0.020
	Fr	0.930	0.870	0.910	0.813	0.954	0.571	0.968	0.899	0.918	0.787	0.887
	Un	0	0.111	0.075	0.125	0.031	0.381	0	0.051	0.041	0.170	0.079
	ne	0.023	0.009	0.008	0.063	0.008	0	0	0.025	0.010	0	0.013
Est-4	*Nz*	0.023	0.111	0.098	0	0.015	0.429	0	0.051	0.041	0.191	0.086
	Su	0.605	0.472	0.474	0.875	0.531	0.381	0.581	0.544	0.541	0.404	0.510
	At	0.349	0.398	0.436	0.125	0.438	0.190	0.355	0.392	0.357	0.383	0.384
	ne	0.023	0.019	0.015	0	0.023	0	0.032	0.013	0.020	0.021	0.020
Est-5	*Mi*	0.023	0.009	0.015	0	0.015	0	0.032	0	0	0.043	0.013
	Pi	0.837	0.796	0.797	0.875	0.792	0.905	0.839	0.772	0.776	0.851	0.808
	Ri	0.116	0.157	0.150	0.125	0.154	0.095	0.096	0.177	0.173	0.106	0.146
	od	0.023	0.037	0.038	0	0.038	0	0.032	0.051	0.051	0	0.033
Gpi-1	*Gu*	0	0	0	0	0	0	0	0	0	0	0
	Be	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgd-1	*Tj*	0	0	0	0	0	0	0	0	0	0	0
	Ak	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Pgd-2	*Tn*	0.442	0.222	0.331	0.188	0.269	0.381	0.226	0.304	0.296	0.234	0.285
	Ps	0.558	0.778	0.669	0.813	0.731	0.619	0.774	0.696	0.704	0.766	0.715
Aco-1	*Si*	0	0	0	0	0	0	0	0	0	0	0
	Ge	0.814	0.704	0.722	0.875	0.754	0.381	0.258	0.759	0.786	0.766	0.735
	Fn	0.186	0.296	0.278	0.125	0.246	0.619	0.742	0.241	0.214	0.234	0.265
Aco-2	*A*	0.070	0.241	0.211	0	0.192	0.190	0.258	0.190	0.235	0.106	0.192
	B	0.930	0.759	0.789	1.000	0.808	0.810	0.742	0.810	0.765	0.894	0.808
Ndh-2	*C*	1.000	0.981	1.000	0.938	0.992	1.000	1.000	1.000	0.990	0.979	0.987
	D	0	0.019	0	0.063	0.008	0	0	0	0.010	0.021	0.013
Total accessions		43	108	133	16	130	21	31	79	98	47	151

Table 3 The rare alleles in the American accessions of the Barley Core Collection (accession nos. refer to Table 1)

Allele	Accession no.												
Est-2 Dr	49	27	73										
Est-2 Un	2	30	32	45	53	69	76	92	100	136	149	151	
Est-2 ne	68	150											
Est-4 Nz	2	30	32	45	49	65	68	69	76	100	136	149	151
Est-4 ne	73	126	128										
Est-5 Mi	55	60											
Est-5 od	42	43	46	75	125								
Ndh-2 D	58	134											

(Fig. 2a). The six-rowed barley showed a slightly higher average diversity value than the two-rowed barley (0.370 versus 0.306; t=1.668, P=0.139).

Spring vs winter barley

In the material studied, 149 of the barley accessions were known to be of spring (133) or winter (16) types. The allelic frequency was found to be highly different in the two types for some alleles. Seven alleles (*Est-1 Ca; Est-2 Dr; Est-4 Nz, ne; Est-5 Mi, od* and *Aco-2 A*), were present in spring barley but absent in winter barley, whereas one allele (*Ndh-2 D*) was observed in winter barley but not found in spring barley. In addition to the loci monomorphic for all 151 accessions (*Gpi-1* and *Pgd-1*), the locus *Ndh-2* was monomorphic in spring barley and the *Aco-2* locus was monomorphic in winter barley. Genetic diversity values for the different loci are shown in Fig. 2b. The average diversity values for spring and winter barley were 0.363 and 0.217, respectively. The spring barley had a significantly higher diversity than the winter barley (t=2.124, P=0.071).

Covered vs hulless barley

The set of 151 accessions consisted of 130 covered and 21 hulless barleys. Twenty five alleles were detected in

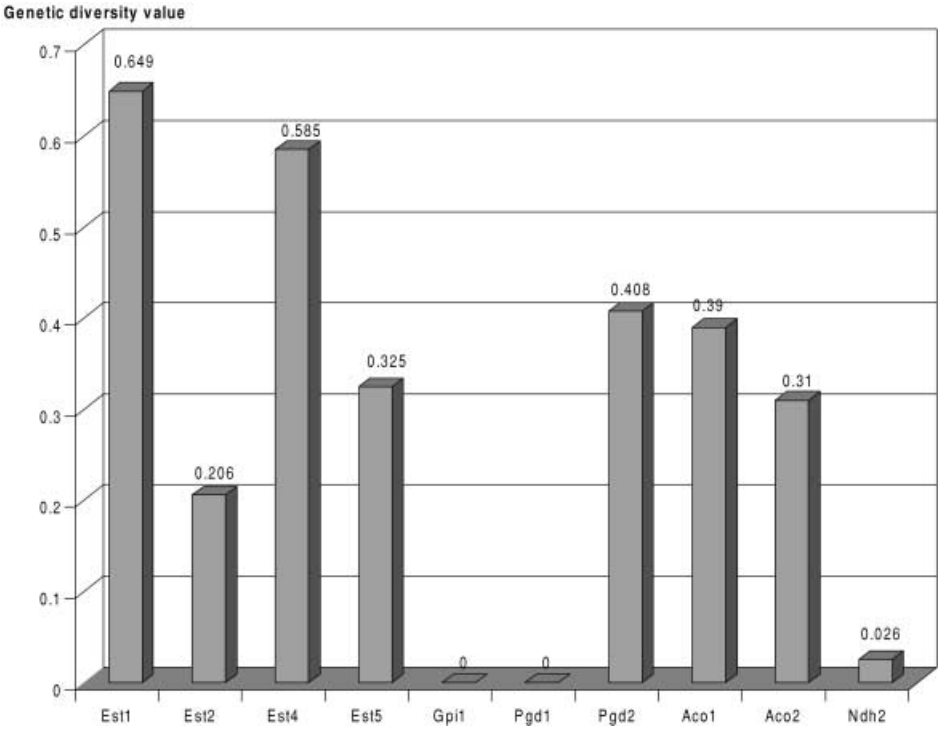

Fig. 1 Distribution of genetic diversity values of ten isozyme loci in the 151 American accessions of the Barley Core Collection

covered barley, and 20 alleles were observed in hulless barley. The frequencies of some alleles were found to be different between covered and hulless barley (Table 2). For example, the *Est-2 Un* and *Est-4 Nz* displayed a much higher frequency in hulless than in covered barley. The *Est-2 ne*, *Est-4 ne*, *Est-5 Mi*, *od* and *Ndh-2 D* were only detected in covered barley. The genetic diversity values differed in the two types and are shown in Fig. 2c. The locus *Ndh-2* was monomorphic in hulless barley. The average diversity values for covered and hulless barley were 0.338 and 0.380, respectively (t=0.623, P=0.553).

Malt vs feed barley

Among the accessions, 110 were provided with information in respect of their use. Hence, the analysis included 31 accessions of malt barley and 79 accessions of feed barley. The frequencies for most of the alleles were similar between the two types. However for the *Aco-1* locus, *Aco-1 Ge* is the dominant allele in feed barley, whereas *Aco-1 Fn* is the most common allele in malt barley. The *Est-2 Un* and ne alleles were only detected in feed barley. The diversity values are shown in Fig. 2d. The average diversity values for feed barley and malt barley were 0.357 and 0.337 respectively (t=0.771, P=0.553).

North vs South/Central American barley

In all, 145 of the accessions are of American origin. The frequencies of the most-frequent alleles at ten loci were almost the same between the two geographical groups (Table 2). Differences were found for some rare alleles. The *Est-2 Dr*, *ne* and Est-5 *od* alleles were detected only in North American barley, and the *Est-5 Mi* allele was only observed in South American barley. Diversity values for the two groups are compared in Fig. 3. The average diversity for the North American accession (0.360) was similar to that (0.357) from South America (t=0.069, P=0.947).

Cluster and principal coordinate analysis

Cluster analysis was used to reveal the association between the accessions. The genetic similarity was revealed by UPGMA cluster analysis based on Dice's (1945) similarity coefficient calculated from the allozymic data. All 25 alleles were included in the statistical analysis. The similarity coefficient ranged from 0.20 to 1.00. With the exception of cultivar 'Harrington' (73), the cluster analysis (Fig. 4) separated the 151 accessions into two major clusters. Cluster A contained three cultivars and nine landraces, eight of which originated from Peru. Cluster B comprised 138 accessions, divided into many sub-clusters. Fourteen out of sixteen winter barleys were put into one sub-cluster (I). The cluster analysis could not show any clear separation of the two-rowed and six-rowed, malt and feed, covered and hulless accessions. Associations in the studied material revealed by PCoA are presented in Fig. 5. The first (PCo1) and the second (PCo2) principal coordinates accounted for 18% and 13% of the total variation,

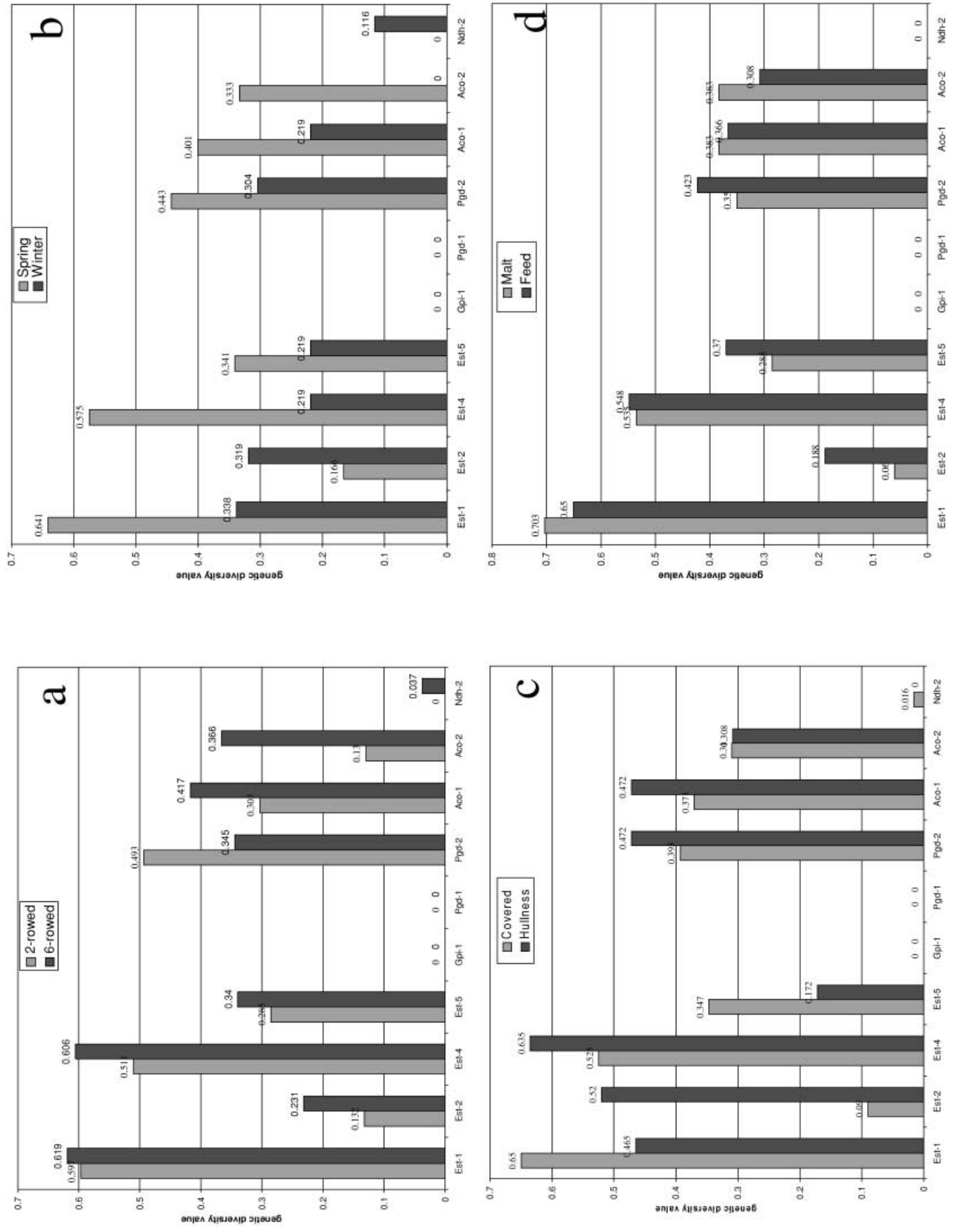

Fig. 2 Distribution of genetic diversity values in various barley groups for ten isozyme loci

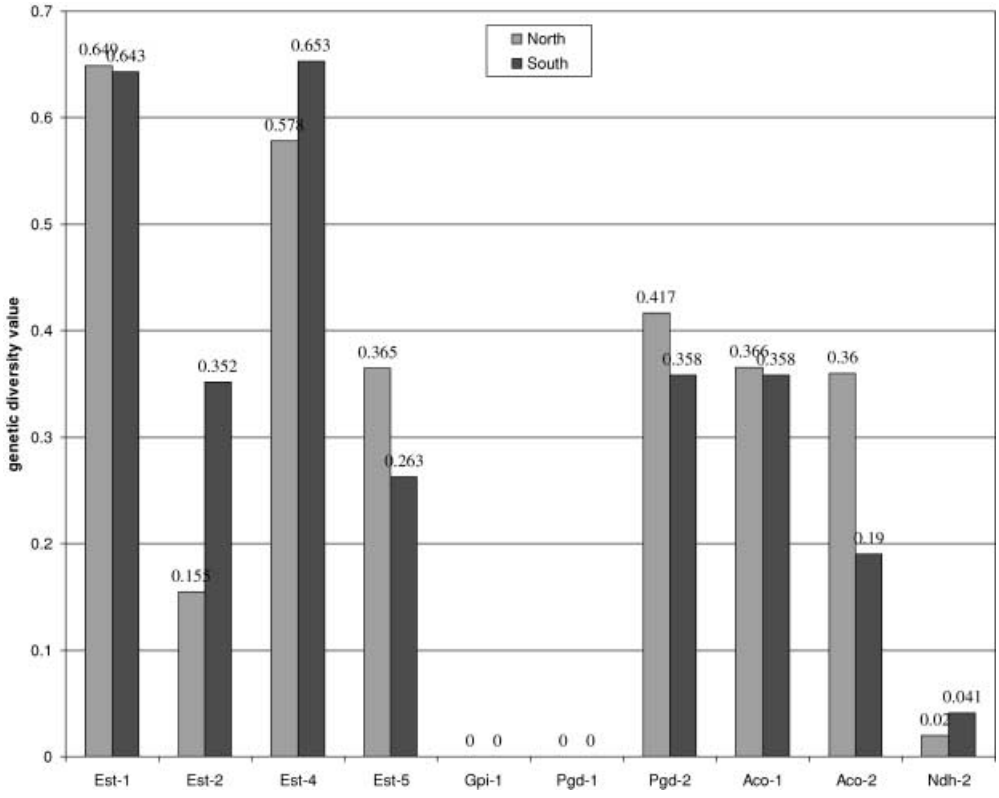

Fig. 3 The distribution of genetic diversity values in North and South American barleys at ten isozyme loci

respectively. The PCo1 separated all winter barleys but one into quadrants III and IV. The spring barley accessions were spread more widely, especially with regard to PCo2.

Discussion

Genetic diversity of isozymes, especially esterases, have been intensively examined in cultivated barley (Kahler and Allard 1970; Nielsen and Frydenberg 1971 1972; Nielsen and Johansen 1986; Linde-Laursen et al. 1987; Dai 1989; Konishi 1994; Bernardo et al. 1997). At least ten loci for esterase isozymes are known in different tissues, and some of them are highly polyallelic (Hvid and Nielsen 1977; Brown 1983). Kahler and Allard (1981) examined the pattern of genetic variation among four esterase loci in a worldwide collection of barley. Five alleles at *Est-1*, seven alleles at *Est-2*, four alleles at *Est-3* and six alleles at *Est-4* were detected in North American barley. Three alleles at *Est-1*, *Est-2*, *Est-3* and *Est-4* were found in Central American barley. For South American barley, three alleles at *Est-1*, *Est-2* and *Est-3*, and four alleles at *Est-4* were reported. According to the esterase nomenclature proposed by Hvid and Nielsen (1977), the *Est-1*, *Est-2*, *Est-4* and *Est-5* in our study correspond to Kahler and Allard's *Est-1*, *Est-2*, *Est-3* and *Est-4*, respectively. A standard barley variety of known banding pattern ('Atlas') included in Kahler and Allard's study (1981) was also included as a standard in each gel in our study, which makes a direct comparison possible. The results from the present study and those of previous ones indicate that most of the esterase alleles occurring in North and South American barleys are also present in this set of the American Barley Core Collection. Two alleles, *Est-1 Af* and *Est-5 Ag*, were absent in the North American barley of this core set. Two alleles, *Est-5 Ag* and *od*, were not found in the South American accessions of this core set. The purpose of a core collection is to assemble accessions that will maximise the number of alleles. We suggest that varieties possessing the lacking alleles should be added to the Barley Core Collection.

The present set of the Barley Core Collection was divided into five groups according to spike characters (two-rowed and six- rowed, covered and hulless), growth habit (winter and spring type), geographical origin (North and South America) and utilization (feed and malt). Saghai Maroof et al. (1994) analysed a set of 182 barley cultivars representing six different countries for restriction fragment length polymorphisms (RFLPs). Their results indicate that genetic differentiation among different geographical groups was more profound than between spring and winter types, and between two- and six-rowed types. A later study (Saghai Maroof et al. 1995) showed that levels of diversity were about equal in spring and winter groups and also in the groups with two- and six- rowed spikes. However, significant differences of allelic frequencies were detected between the group pairs. In the present study, genetic diversities and allele distributions for five groupings were examined. Our results show that most significant differences in al-

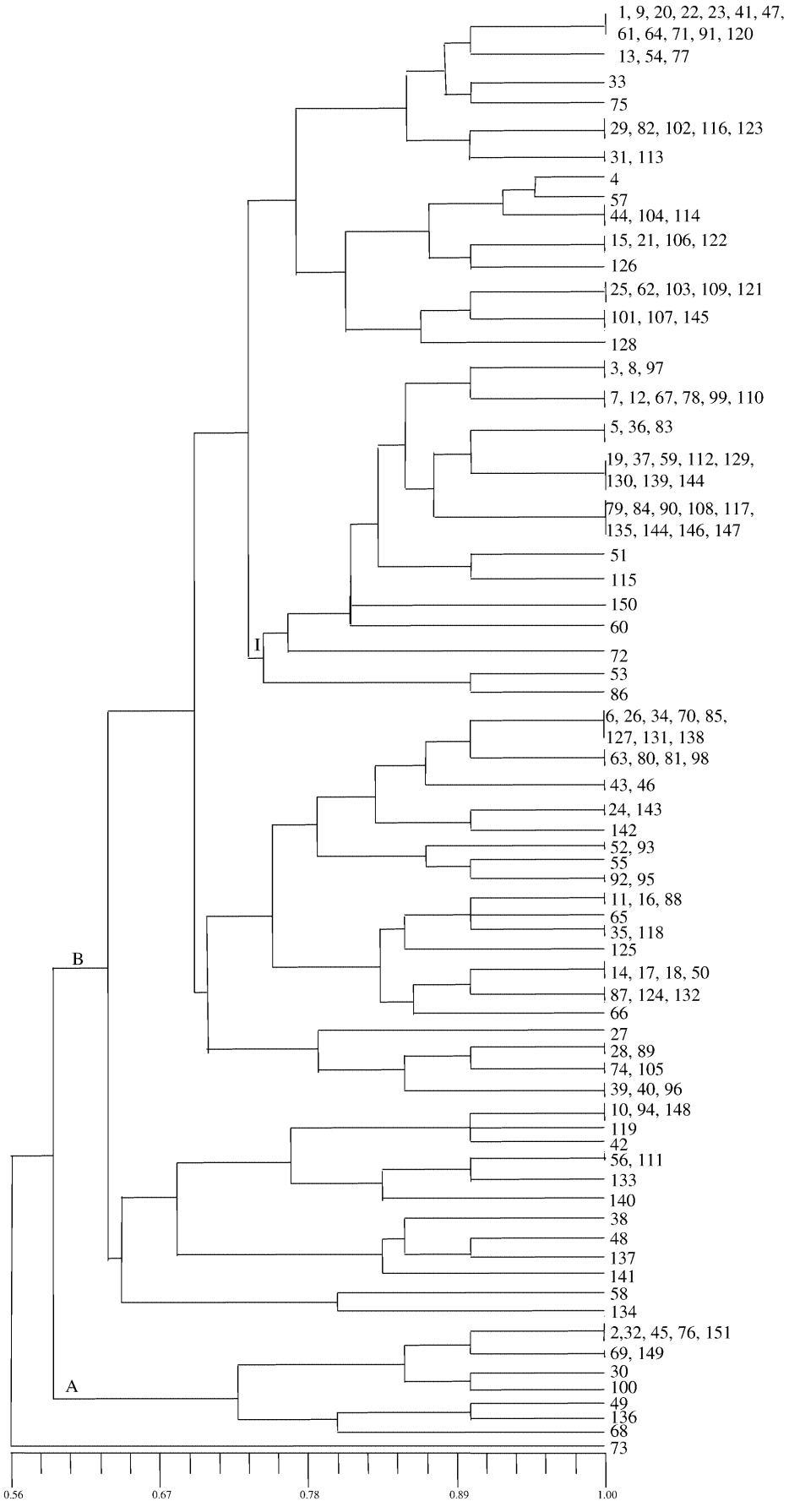

Fig. 4 Dendrogram for 151 accessions of the American Barley Core Collection based on a cluster analysis (UPGMA) of Dice similarity coefficients. The number before the dash is the accession no., refer to Table 1

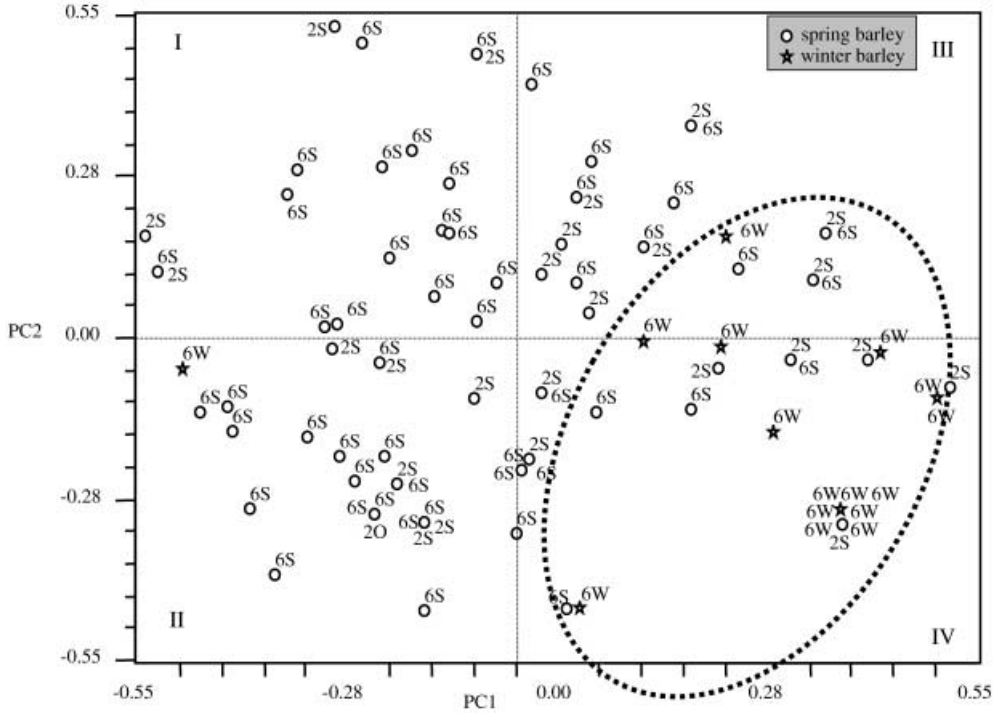

Fig. 5 Associations between accessions of the American Barley Core Collection on the basis of the first two principal coordinates (PC1, PC2) from a principal coordinate analysis of the Dice similarity coefficient. All winter barleys but one are distributed in the ring area

lelic frequency and genetic diversity values were found between spring and winter barley, thus contradictory to the results by Saghai Maroof et al. (1994). The allelic frequencies and diversity values for the two geographical regions, North and South America, were least differentiated, possibly reflecting an earlier introduction and an exchange of material among the two areas. Spring barley showed a significiantly higher average diversity than winter barley (t=2.124, P=0.071). Seven rare alleles were associated with spring barley. This is the opposite situation to what we found for the European part of the Barley Core Collection in which winter barley showed a higher average diversity value than spring barley. Furthermore, most rare alleles were detected in six-rowed winter barley (Liu et al. 2000). This may be caused by a sampling effect associated with the small numbers and narrow regions involved. Only 16 accessions of winter barleys from USA and Canada were included in the Core set.

Eight out of the twenty five alleles in the present study were rare. Five rare alleles were detected only in two or three accessions. The rare alleles *Est-2 Un* and *Est-4 Nz* were detected in 12 and 13 accessions, respectively. Most accessions containing *Est-2 Un* and *Est-4 Nz* alleles were landraces from Peru. Only two cultivars, 'Dicktoo' (53) and 'Godiva' (69), contain the *Est-2 Un* allele. Four cultivars ('Condor', 'Galeras', 'Gobernadora' and 'Godiva') displayed the *Est-4 Nz* allele.

A study of 48 barley cultivars for RFLPs showed that genetic similarities ranged from 0.64 to 0.93 (Melchinger et al. 1994). The genetic similarity based on RFLP data between all possible pairs of lines (217 accessions) ranged from 0.43 to 0.99 (Hatz et al. 1996). The similarity coefficient based on isozyme data in the 79 European accessions of the Barley Core Collection ranged from 0.40 to 1.00 (Liu et al. 2000). The genetic similarities calculated for all pairwise comparisons among the 151 American core accessions ranged from 0.20 to 1.00, showing that a high level of genetic variability exists in the American BCC set.

Furthermore, the principal coordinate analysis of genetic-similarity estimates based on RFLPs resulted in a separate grouping of winter and spring cultivars (Melchinger et al. 1994). Two-rowed winter types were intermediate between the spring and six-rowed winter types. Similar results were observed by Russell et al. (1997) using RFLPs, AFLPs, RAPDs and SSRs, where principal coordinate analysis (PCoA) clearly separated the spring from the winter types using RFLP and AFLP data, with the two-rowed winter types forming an intermediate group. Cluster analysis based on the kinship coefficient or Roger's distance for 27 Canadian spring lines separated two-rowed and six-rowed lines into distinct groups (Tinker et al. 1993). We attempted a further classification of the materials by cluster and principal coordinate analysis based on isozyme data. The cluster and principal coordinate analysis based on ten isozyme loci could not clearly separate the spring cultivars from the winter accessions, nor the two-rowed from the six-rowed barley. However, the cluster analysis put 14 out of the 16 accessions of winter barley into one sub-cluster (I). A factor affecting genetic diversity assayed by different marker techniques is the number of markers or probes used in an analysis (Smith et al. 1992) and the kind of information provided by each type of marker (Sun et al. 1999). Isozyme variation on-

ly reflects differences in protein-coding sequences and, hence, only in a small fraction of all mutational events (Clegg 1989). Moreover, only 25 alleles from ten loci can be clearly detected and used for cluster analysis in the present study. This is probably the major reason for the non-clear separation of different types of barley. Principal coordinate analysis (PCoA) was conducted based on the similarity matrix. This multivariate approach was chosen to complement the cluster-analysis information, because cluster analysis has a higher resolution for the analysis of closely related populations, while PCoA is more informative regarding distances among major groups. PCoA revealed that the PCo1 separated all the winter barleys but one accession into quadrants III and IV.

In spite of a high level of genetic diversity existing in this core set, the PCoA analysis revealed that 64 axes explained 100% of the diversity. The results indicate that genetic diversity was not detected in more than half the number of accessions at these loci. The isozyme technique is an available, rapid and economic tool for the estimation of genetic variation. However, the isozyme technique is a disadvantage for further classification in narrow-based elite breeding material because of the small numbers of available marker loci compared to the DNA markers in high-density genetic marker maps. Further analysis of the genetic diversity of this set of barley core accessions using more isozyme markers or DNA markers will certainly merit attention.

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