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Genetic diversity among East Asian accessions of the barley core collection as revealed by six isozyme loci

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Abstract Studies of allelic variations at six isozyme loci revealed genetic diversity of 380 East Asian accessions of the Barley Core Collection. Genetic variation was found in both cultivars and landraces in different regions. Allelic variations at the *Aco-1* and *Aco-2* loci were detected for East Asian barley for the first time. Moreover, the *Aco-1* locus displayed the highest genetic diversity among the six loci assayed. Indian cultivars showed the highest diversity, followed by Korean and Chinese cultivars. Landraces from Bhutan and Nepal showed the lowest diversity. Cultivars had generally higher diversity than landraces within as well as among regions. The cluster analysis of genetic identity showed that all landraces from different countries can be placed in one group; the cultivars from Japan, India and Korea each form independent groups. *Gpi-1 Gu*, *Pgd-1 Tj*, *Aco-1 Si*, *Ndh-2 D* and *Aco-2 A* were rare alleles found in only a few accessions of 6-rowed barley. The *Pgd-2 Tn* allele was very rare in East Asian accessions.

Key words Barley · Genetic diversity · Core collection · Isozyme loci · Allelic variation

Introduction

Barley (*Hordeum vulgare* L.) is one of the oldest cultivated crops. It is also an economically important and genetically well-suited species. Presently, more than 320,000 barley accessions are held in gene banks throughout the world (FAO 1993), and the number is increasing. The huge number of accessions has intensified the problems of optimal conservation and utilisation; for example, what material to choose for plant breeding purposes from a germplasm collection, what material to include in the collection and what material to remove from the collection. The information currently available is usually not sufficient for users and curators. The concept of core collections was formulated (Frankel and Brown 1984) and further developed (Brown 1989a, b) as a means to increase the efficiency of utilisation and management. The core collection, with a minimum of repetitiveness, should represent as much of the diversity as possible in the crop. The selection of the core entries should be based on available data on geographic origin, genetic characteristics and traits of possible value for 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 establishment of a core collection. Several sampling methods for selecting core entries in cultivated barley from China were compared by Hintum et al. (1995). They suggested that a stratification sampling based on collection site would give the best result, with the largest number of alleles being included in the core collection.

The Barley Core Collection (BCC) is a selected and limited set of accessions that optimally representing the genetic diversity of cultivated barley (*Hordeum vulgare* L. *s.l.*) and wild species of *Hordeum* and provides well-known genetic standards (Bothmer et al. 1990). The size of the BCC should not exceed 2,000 accessions in order to keep it manageable (Knüpffer and Hintum 1993).

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 this paper, we survey the genetic diversity of East Asian accessions of the Barley Core

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Table 1 Number of accessions assayed with different characters from East Asian regions of the Barley Core Collection

Region	Category		Type of row			Caryopsis		Growth habit				No. of accessions
	Landrace	Cultivar	2-rowed	6-rowed	Irregular	Covered	Naked	Spring	Winter	Irregular	Unknown	
Bhutan	15	0	0	15	0	0	15	10	4	1	0	15
China	85	20	9	96	0	66	39	59	36	10	0	105
India	50	15	1	58	6	42	23	36	1	13	15	65
Japan	50	25	12	63	0	47	28	37	27	11	0	75
Korea	50	20	2	68	0	49	21	21	40	7	2	70
Nepal	50	0	0	50	0	32	18	23	1	26	0	50
Total no.	300	80	24	350	6	236	144	186	109	68	17	380

Collection using six isozyme loci and provide information for the further development of an optimal core collection in barley. This study is a part of a more general survey of the entire Barley Core Collection.

Materials and methods

Materials

The material consisted of 380 East Asian accessions of the Barley Core Collection, selected and obtained from the Barley Germplasm Centre of Okayama University, Kurashiki, Japan. The selected core originates from six countries of Eastern Asia and includes cultivars and lines selected by SSD from landraces. They represent different row types, different types of kernels and different growth habits. The accessions assayed with different characteristics are listed in Table 1.

Electrophoresis

Seeds were germinated on a perlite medium supplied with liquid fertiliser in a growth chamber kept at 20°C under 12 h of illumination per day. The first leaf of a 10-day-old seedling was used for isozyme analysis. The samples were homogenised after adding 100 µl of extraction buffer (TRIS-HCl, pH 7.2 and 0.05% β-mercaptoethanol) and some grains of fine sand, and then centrifuged for 4 min at 14,000 rpm. The supernatants were absorbed onto small pieces of filter paper (Wathman no. 4, 10×2 mm). The filter paper pieces were inserted into a slot of a 11% starch gel, 3.5 cm from the cathode end. The variety 'Atlas' was used as a standard and was included in each gel to aid in establishing migration distance of bands. The gel buffer was 0.005 *M* L-histidine (pH 8.0). The electrode buffer was 0.4 *M* trisodium-citrate-2H_2O (pH 8.0). The starch gel was placed between the two cooling plates in which cooling water was circulating at approximately 4°C. The electrophoresis was run at 300 V and 120 mA for 4.5–5 h.

Staining

At the end of the run, each gel was cut horizontally into four slices of 2 mm each. The slices were placed separately in plastic containers and stained independently for various enzyme systems. All enzymes were stained in the dark at 37°C except for aconitate hydratase, which was stained at 60°C. The staining solutions for each enzyme system were:

1) Aconitate hydratase (ACO, E.C. 4.2.1.3): 15 ml 0.2 *M* TRIS-HCl pH 8.6, 40 mg *Cis*-aconitric acid, 340 µl isocitrate dehydrogenase, 750 µl $MgCl_2$ (250 mg/ml), 900 µl NADP (5 mg/ml), 300 µl MTT (10 mg/ml), 120 µl PMS (5 mg/ml) and 18 ml agar (2%).
2) Glucose phosphate isomerase (GPI, E.C. 5.3.1.9): 15 ml 0.2 *M* TRIS-HCl buffer pH 8.0, 18 mg fructose-6-phosphate, 15 µl glucose-6-phosphate dehydrogenase, 450 µl $MgCl_2$ (250 mg/ml), 900 µl NADP (5 mg/ml), 450 µl MTT (10 mg/ml), 120 µl PMS (5 mg/ml) and 18 ml agar (2%).
3) Phosphogluconate dehydrogenase (PGD, E.C. 1.1.1.43): 15 ml 0.1 *M* TRIS-HCl pH 8.0, 30 mg gluconate-6-phosphate, 600 µl $MgCl_2$ (250 mg/ml), 120 µl NADP (5 mg/ml), 600 µl MTT (10 mg/ml), 24 µl PMS (5 mg/ml) and 18 ml agar (2%).
4) NADH dehydrogenase (NDH, E.C. 1.6.99.3): 15 ml 0.2 *M* TRIS-HCl pH 8.1, 25 mg NADH, 1 ml MTT (10 mg/ml), 3 drops of 2-6-dichlorophenol-indophenol (2 mg/ml) and 18 ml agar (2%).

Nomenclature

The enzyme nomenclature and abbreviations follow the Nomenclature Committee of the International Union of Biochemists (I.U.B. 1984). The designations of alleles at the *Gpi-1*, *Pgd-2* and *Aco-1* loci follow Nielsen and Johansen (1986) and at the *Pgd-1* locus, Konishi and Yoshimi (1993). In this study, the designations of the alleles at the *Aco-2* and *Ndh-2* loci have no previous references. In these cases, we used the capital letters *A* and *B* to indicate the observed alleles at the *Aco-2* locus, *C* and *D* to indicate the observed alleles at the *Ndh-2* locus. The letter order is according to band mobility towards the anode (Fig. 1).

Statistical analysis

Gene diversity (H) at each locus was calculated using the gene diversity index of Nei (1973):

$$H = 1 - \sum p_i^2$$

in which p_i is the frequency of the *i*th allele of the locus. Genetic divergence among cultivars and landraces of each country was also estimated using Nei's unbiased genetic distance, which accounts for sample size (Nei 1978). The resultant distance matrix was subjected to an UPGMA (unweighted pair group method with arithmetic average) cluster analysis (Sneath and Sokal 1973) to generate a dendrogram illustrating the relationships of cultivars and landraces of each country. Statistical analyses were performed using the BIOSYS-1 computer package (Swofford and Selander 1989).

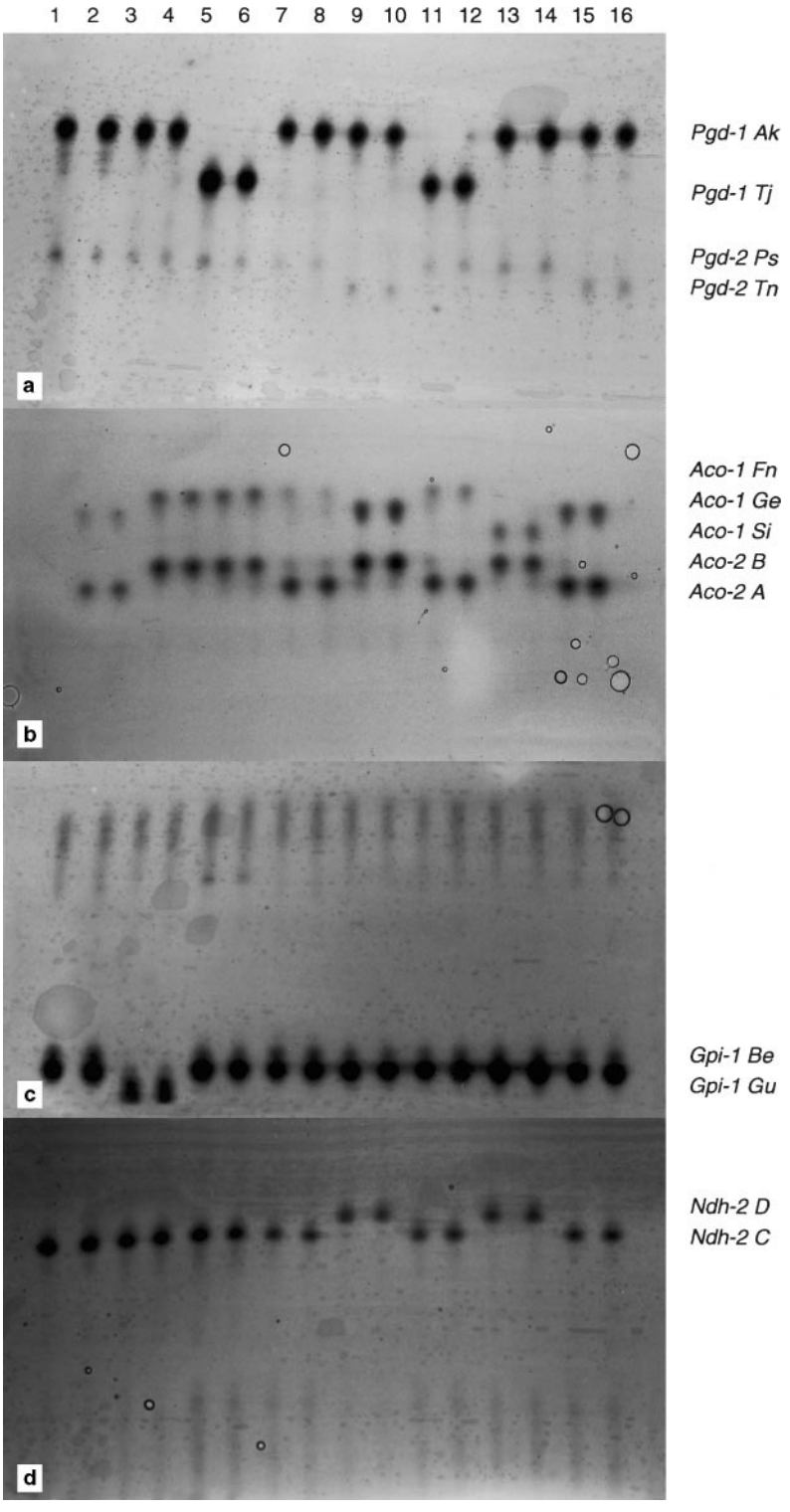

Fig. 1a–d Banding patterns of some rare alleles in East Asian accessions of the Barley Core Collection. **a** Phosphogluconate dehydrogenase, **b** aconitate hydratase, **c** glucose phosphate isomerase, **d** NADH dehydrogenase. *Lanes 1, 2* reference variety 'Atlas', *3, 4* Dras 2, *5, 6* K. 12, *7, 8* Dhumpu 1, *11, 12* 'Karan 16', *13, 14* 'Lakham', *15, 16* 'Jing Luo 2 hao' PL 172

Results

Allelic distribution

Four isozyme systems were analysed with six loci showing clear banding patterns: glucose phosphate isomerase (*Gpi-1*), phosphogluconate dehydrogenase (*Pgd-1* and *Pgd-2*), aconitate hydratase (*Aco-1* and *Aco-2*) and NADAH dehyrogenase (*Ndh-2*). Two alleles were observed at each locus, except for *Aco-1* that showed three alleles (Fig. 1). A total of 13 alleles were employed in the present study. The distribution frequencies of all alleles are presented in Table 2. *Gpi-1*,

Table 2 Geographical distribution frequency of the major alleles at six isozyme loci in East Asian accessions of the Barley Core Collection

Region		*Gpi-1*		*Pgd-1*		*Pgd-2*		*Aco-1*			*Aco-2*		*Ndh-2*		No. of accessions
		Gu	*Be*	*Tj*	*Ak*	*Tn*	*Ps*	*Si*	*Ge*	*Fn*	*A*	*B*	*B*	*C*	
Bhutan	landrace	0	1.000	0	1.000	0	1.000	0	0.200	0.800	0	1.000	1.000	0	15
China	landrace	0	1.000	0	1.000	0.012	0.988	0	0.329	0.671	0.024	0.976	1.000	0	85
	cultivar	0	1.000	0	1.000	0.100	0.900	0	0.350	0.650	0.050	0.950	0.950	0.050	20
India	landrace	0.020	0.980	0.020	0.980	0	1.000	0	0.300	0.700	0	1.000	1.000	0	50
	cultivar	0	1.000	0.067	0.933	0.200	0.800	0	0.333	0.667	0.267	0.733	0.933	0.067	15
Japan	landrace	0	1.000	0	1.000	0.040	0.960	0	0.360	0.640	0.020	0.980	1.000	0	50
	cultivar	0	1.000	0	1.000	0.080	0.920	0	0.560	0.440	0	1.000	1.000	0	25
Korea	landrace	0	1.000	0	1.000	0	1.000	0	0.460	0.540	0	1.000	1.000	0	50
	cultivar	0	1.000	0	1.000	0.350	0.650	0	0.500	0.500	0.053	0.947	1.000	0	20
Nepal	landrace	0	1.000	0	1.000	0	1.000	0.020	0.380	0.600	0	1.000	1.000	0	50

Table 3 Genetic diversity index (H) and average value at six isozyme loci in East Asian accessions of the Barley Core Collection

Region		*Gpi-1*	*Pgd-1*	*Pgd-2*	*Aco-1*	*Aco-2*	*Ndh-2*	Average
Bhutan	landrace	0	0	0	0.320	0	0	0.053
China	landrace	0	0	0.024	0.442	0.047	0	0.085
	cultivar	0	0	0.180	0.455	0.095	0.095	0.136
India	landrace	0.040	0.040	0	0.420	0	0	0.083
	cultivar	0	0.125	0.320	0.444	0.391	0.125	0.234
Japan	landrace	0	0	0.077	0.461	0.039	0	0.096
	cultivar	0	0	0.147	0.493	0	0	0.106
Korea	landrace	0	0	0	0.497	0	0	0.082
	cultivar	0	0	0.455	0.500	0.100	0	0.178
Nepal	landrace	0	0	0	0.495	0	0	0.056
Average		0.004	0.017	0.120	0.453	0.067	0.022	

Gu, *Pgd-1 Tj*, *Aco-1 Si*, *Ndh-2 D* and *Aco-2 A* were rare alleles and detected only in a few accessions. The *Pgd-2 Tn* allele had slightly higher distribution frequencies but was rare in Chinese and Japanese landraces and not found at all in landraces from Bhutan, India, Korea and Nepal. The other alleles were widely distributed in both landraces and cultivars in each region. The *Gpi-1 Be*, *Pgd-1 Ak* and *Ndh-2 C* alleles in particular showed very high distribution frequencies ($\geqslant$0.933).

Genetic diversity at six loci

All six loci were polymorphic, and genetic diversity value of each locus was calculated (Table 3). The *Aco-1* locus displayed the highest genetic diversity, with an average diversity index of 0.453, followed by the *Pgd-2* and *Aco-2* loci, with average genetic diversity indices of 0.120 and 0.067, respectively. The *Gpi-1*, *Pgd-1* and *Ndh-2* loci had a very low genetic diversity ($\leqslant$ 0.022). With respect to region, Indian cultivars had the highest diversity, with an average diversity index of 0.234, followed by Korean cultivars (0.178) and Chinese cultivars (0.136). The lowest genetic diversity was detected in landraces from Bhutan and Nepal. With respect to the cultivars and landraces, cultivars showed a higher genetic diversity than landraces within as well as among regions.

Comparison of five rare alleles

Gpi-1 Gu, *Pgd-1 Tj*, *Aco-1 Si*, *Ndh-2 D* and *Aco-2 A* were rare alleles and scarcely detected in East Asian accessions of the Barley Core Collection. The accessions carrying rare alleles are compared in Table 4. The *Gpi-1 Gu* and *Aco-1 Si* alleles were each detected in 1 accession, respectively. The *Gpi-1 Gu* allele (Fig. 1c) was detected in the Indian landrace Dras 2 (original no: OUI 382), which is a 6-rowed, naked spring barley. The *Aco-1 Si* allele (Fig. 1b) was detected in the Nepalese landrace Dhumpu 1 (OUN 647), which is a 6-rowed, covered barley with irregular growth habit. The *Pgd-1 Tj* allele (Fig. 1a) was found in 2 accessions, both originating from India, viz. the cultivar 'Lakham' and the landrace K. 12 (OUI 602). They had similar characteristics: 6-rowed and covered and possessing the same alleles at the six isozyme loci except for a difference at the *Aco-1* locus. 'Lakham' possessed the *Fn* allele at the

Table 4 Comparison of rare alleles in East Asian accessions of the Barley Core Collection

Allele	Name	Original no.	Origin	Category	Type of row	Caryopsis	Growth habit	Other alleles					
								Gpi-1	*Pgd-1*	*Pgd-2*	*Aco-1*	*Aco-2*	*Ndh-2*
Gpi-1 Gu	Dras 2	OUI 382	India	Landrace	6-rowed	Naked	Spring		*Ak*	*Ps*	*Fn*	*B*	*B*
Pgd-1 Tj	Lakham	133	India	Cultivar	6-rowed	Covered	Unknown	*Be*		*Ps*	*Fn*	*B*	*B*
	K.12	OUI 602	India	Landrace	6-rowed	Covered	Spring	*Be*		*Ps*	*Ge*	*B*	*B*
Aco-1 Si	Dhumpu 1	OUN 647	Nepal	Landrace	6-rowed	Covered	Illegible	*Be*	*Ak*	*Ps*		*B*	*B*
Ndh-2 C	Jing Luo 2 hao	ZDM 8254	China	Cultivar	6-rowed	Naked	Spring	*Be*	*Ak*	*Ps*	*Fn*	*A*	
	Karan 16	134	India	Cultivar	6-rowed	Naked	Unknown	*Be*	*Ak*	*Tn*	*Fn*	*A*	
Aco-2 A	Jing pi C627-6	ZDM 8251	China	Cultivar	6-rowed	Covered	Spring	*Be*	*Ak*	*Ps*	*Ge*		*B*
	Jin Luo 2 hao	ZDM 8254	China	Cultivar	6-rowed	Naked	Spring	*Be*	*Ak*	*Ps*	*Fn*		*C*
	Sanho	OUC 306	China	Landrace	6-rowed	Covered	Spring	*Be*	*Ak*	*Ps*	*Fn*		*B*
	BHS 169	127	India	Cultivar	6-rowed	Covered	Unknown	*Be*	*Ak*	*Ps*	*Fn*		*B*
	Sonu	128	India	Cultivar	6-rowed	Covered	Unknown	*Be*	*Ak*	*Ps*	*Fn*		*B*
	Karan 16	134	India	Cultivar	6-rowed	Naked	Unknown	*Be*	*Ak*	*Tn*	*Fn*		*C*
	PL 172	135	India	Cultivar	6-rowed	Covered	Unknown	*Be*	*Ak*	*Tn*	*Ge*		*B*
	Taiki Omugi	OUJ 605	Japan	Landrace	6-rowed	Naked	Spring	*Be*	*Ak*	*Ps*	*Ge*		*B*
	Saeolbori	263	Korea	Cultivar	6-rowed	Covered	Spring	*Be*	*Ak*	*Tn*	*Fn*		*B*

Table 5 Comparison of allelic distribution frequencies (%) at three loci with larger genetic diversity

Loci	Allele	Landrace	Cultivar	2-rowed	6-rowed	Covered	Naked	Spring	Winter
Pgd-2	*Tn*	1.0	20.0	20.8	3.4	6.4	1.4	5.9	2.8
	Ps	99.0	80.0	79.2	96.6	94.6	98.6	94.1	97.2
	Si	0.3	0	0	0.3	0.4	0	0	0
Aco-1	*Ge*	35.3	45.0	50.0	37.4	38.9	34.0	42.5	26.6
	Fn	64.3	55.0	50.0	62.3	60.6	66.0	57.5	73.4
Aco-2	*A*	0.7	8.7	0	2.6	2.5	2.1	2.7	0
	B	99.3	91.3	100.0	97.4	97.5	97.9	97.3	100.0
No. of accessions		300	80	24	350	236	144	186	109

Aco-1 locus, while K. 12 possessed the *Ge* allele. The *Ndh-2 D* allele (Fig. 1d) was found only in 2 accessions, one was the Chinese cultivar 'Jing Luo 2 hao' (ZDM 8254) and the other was the Indian cultivar 'Karan 16'. Both were characterised by being 6-rowed, naked and having the same alleles at the six isozyme loci except for the *Pgd-2* locus. At this locus, 'Jing Luo 2 hao' possessed the *Ps* allele, whereas 'Karan 16' possessed the *Tn* allele. The *Aco-2 A* allele was detected in 9 accessions, mainly Chinese and Indian cultivars. Out of 300 landraces, only 1 Chinese and 1 Japanese landrace carried the *Aco-2 A* allele. All accessions carrying the *Aco-2 A* allele were 6-rowed spring barley except for 4 Indian cultivars with unknown growth habit.

Comparison of the allelic distribution of three loci with large genetic diversity

The *Aco-1*, *Aco-2* and *Pgd-2* loci showed large genetic diversity. Allelic distribution of the three loci is compared in Table 5. Three alleles were detected at the *Aco-1* and two at the *Aco-2* locus. There are only a few reports of allelic variations at the *Aco-1* and *Aco-2* loci for East Asian barley (Konishi 1994). In the present study, the *Aco-1* locus showed the largest genetic diversity among the six loci assayed. *Aco-1 Si* was a rare allele (described above). The *Aco-1 Ge* and *Aco-1 Fn* alleles were widely distributed in both cultivars and landraces. The *Aco-1 Fn* allele showed slightly higher distribution frequencies than *Aco-1 Ge* in each compared character (Table 5), except for 2-rowed types where the *Fn* and *Ge* alleles showed equal frequencies. At the *Aco-2* locus, *Aco-2 A* was a rare allele, not found in winter types and in 2-rowed barley. Contrary to this, *Aco-2 B* was a common allele and detected frequently in 2- and 6-rowed types as well as in spring and winter barley. At the *Pgd-2* locus, two alleles were observed. The *Pgd-2 Tn* allele was rare in several character types, it was especially rare in landraces, and only 1 Chinese and 1 Japanese landrace carried this allele. The accessions carrying the *Pgd-2 Tn* allele were mainly 2-rowed

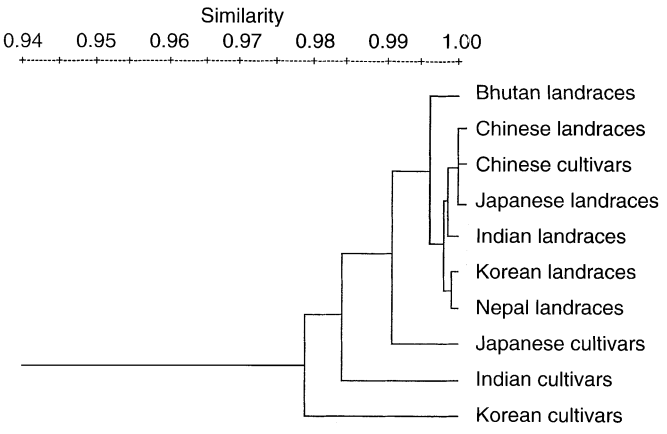

Fig. 2 Genetic identity dendrogram generated by UPGMA of Nei's (1978) unbiased genetic identity coefficients

barley, spring varieties, with covered kernels. On the other hand, the *Pgd-2 Ps* allele was frequently detected in East Asian barley.

Cluster analysis

Genetic identity values (I) were calculated for all pairs of landraces and cultivars of each country (I from 0.980 to 1.000). The cluster analysis of genetic identity showed that all landraces from different countries could be placed in one big group together with cultivars from China. Cultivars from Japan, India and Korea formed independent groups (Fig. 2).

Discussion

Generally, a core collection should be composed of 5–10% of the total number of accessions found in the gene banks and contain at least 70% of the alleles in the whole collection (Brown 1989a). For an optimal core collection, genetic diversity must be maximal, as measured by the number of alleles per locus, which means that the core entries should contain as many alleles as possible. Therefore, it is important to investigate the distribution of alleles in the selected accessions of a core collection, especially rare alleles. In this set of the Barley Core Collection, *Pgd-1 Tj*, *Gpi-1 Gu*, *Aco-1 Si*, *Ndh-2 D* and *Aco-2 A* were rare alleles. Allelic variation was originally not detected at the *Pgd-1* locus in cultivated barley (Kahler and Allard 1981; Brown and Munday 1982; Nielsen and Johansen 1986). However, allelic variation at this locus has been reported in wild barley (*H. vulgare* ssp. *spontaneum*) from Israel (Brown et al. 1978; Brown and Munday 1982). Konishi and Yoshimi (1993) were the first to find allelic variation at this locus in cultivated barley and designated the alleles as *Tj* and *Ak*. They found that only 2 accessions of Nepalese cultivated barley (Thomje and Tomlung) and an Indian landrace (K. 12) carried the *Tj* allele. In this set of the Barley Core Collection, the *Pgd-1 Tj* allele was detected in only 2 accessions, namely in the Indian landrace K. 12 and in the Indian cultivar 'Lakham'. We did not detect the *Tj* allele in any of the 50 accessions of the Nepalese landraces included in the Barley Core Collection. Hence, we suggest that at least one of the accessions Thomje or Tomlung possessing the rare allele *Tj* should be added to the Nepalese core accession.

Konishi (1994) investigated about 3,000 accessions of cultivated barley, mainly landraces collected from different regions of the Old World. He did not find any allelic variation at the *Aco-1* locus in accessions from the East Asian region; only European accessions and *H. vulgare* ssp. *spontaneum* showed small allelic variation at this locus. Nielsen and Johansen (1986) and Linde-Laursen et al. (1987) reported allelic variation at the *Aco-1* locus only for European barley, whereas Nielsen and Johansen (1986), Linde-Laursen et al. (1987) and Konishi (1994) found no allelic variation at the *Aco-2* locus. However, Hintum and Visser (1995) found allelic variation at the Aco-*1* and *Aco-2* loci in European barley by single gel slice staining. In the present study, allelic variation at the *Aco-1* and *Aco-2* loci was reported for East Asian barley for the first time. Furthermore, the *Aco-1* locus showed the largest genetic diversity among the six loci assayed. This is probably caused by differences in staining techniques. Nielsen and Johansen (1986), Linde-Laursen et al. (1987) and Konishi (1994) simultaneously stained ACO and PGD isozymes on the same gel slice, whereas we separately stained the ACO and PGD isozymes on different gel slices.

In this set of the Barley Core Collection, the *Gpi-1 Gu* and *Aco-1 Si* alleles were found in only 1 accession, respectively. Nielsen and Johansen (1986) reported 4 accessions carrying the *Gpi-1 Gu* allele and 15 accessions carrying the *Aco-1 Si* allele among 66 European spring and winter barley varieties. Linde-Laursen et al. (1987) investigated 59 related varieties or lines and one population of a European 2-rowed spring barley. They detected 8 accessions carrying the *Aco-1 Si* allele. Recently, we investigated 80 European accessions of the Barley Core Collection. The *Gpi-1 Gu* and *Aco-1 Si* alleles were detected in 2 accessions (unpublished data), respectively. It seems that the *Gpi-1 Gu* and *Aco-1 Si* alleles are more frequent in Occidental than in Oriental barleys. Linde-Laursen et al. (1987) reported that the *Aco-1 Si* allele was associated with the powdery mildew-resistance gene *Ml*-(*La*) from *H. distichum* var '*Laevigatum*' (Dros 1957). Although not all *Ml*-(*La*)-carrying varieties had the *Si* allele, all *Si*-carrying entries in his study and the comprehensive study of Nielsen and Johansen (1986) had *Ml*-(*La*). The *Si* allele

was previously apparently rare but has increased in frequency in modern varieties through the simultaneous selection of the powdery mildew-resistance gene *Ml-(La)*.

This set of the Barley Core Collection includes landraces from six countries and cultivars from four countries. Most accessions were landraces (300 landraces versus 80 cultivars), but the largest genetic diversity was detected among cultivars. Indian cultivars had the highest average diversity index, followed by Korean, Chinese and Japanese cultivars. *Ndh-2 D* was a rare allele and was detected only in 2 cultivars. The *Aco-2 A* and *Pgd-1 Tj* alleles were mainly found in cultivars: 3 out of 4 accessions possessing the *Pgd-1 Tj* allele were cultivars (including 2 Nepalese accessions reported by Konishi and Yoshimi 1993) and 7 out of 9 accessions carrying the *Aco-2 A* allele were cultivars. In general, the occurrence of higher variation in traditional cultivars or landraces than in modern cultivars is a common observation. For example, Allard (1992) noted that barley cultivars from California possessed 1.44 alleles per locus in comparison with the 2.75 alleles per locus found in landraces from the Middle East. In this study, our results showed that cultivars possessed higher genetic diversity than landraces in this set of the core collection. This may be due to artificial sampling effects, including subjective efforts, resulting in a genetic diversity pattern mirrored by the isozyme pattern, which is different in the core accessions.

Development of an effective core collection will be difficult without access to evaluation and characterisation data. Central to the problem of how to draw samples from large genetic resource collections is the purpose for which the collection is to be sampled. The purpose of the core collection is to improve the management and use of gene bank accessions. Diwan et al. (1995) compared 11 methods of assembling a core collection of the U.S. National collection of annual *Medicago* species. He found that core collections assembled with evaluation data and cluster analysis better represented the germplasm collection than core collections assembled based solely on passport data and random selection of accessions; the relative diversity and the logarithmic methods generated better core collections than the proportional method. Brown (1989b) suggested that stratified sampling would increase the efficiency with the right choice of sample sizes for each group. The determination of major groups may be based on taxonomy, geographical, qualitative and quantitative data or marker loci. Hintum et al. (1995) suggested that a stratification sampling based on collection site would give the best result and contain the larger number of alleles in the core collection. The best sampling strategy should provide a mechanism to ensure that the least-represented types are included. In such collections, morphological data are usually the principal descriptors that have been used to detail the accessions held. Recently, molecular indicators of genetic diversity have been used (Bernatsky and Tanksley 1989; Bonierbale et al. 1995; Hatz et al. 1997) in attempts to assemble representative samples in germplasm collections which would serve the dual purpose of conservation and improved access to genetic resources. Core collections provide a means for the application of more expensive characterisation activities on representative subsets than would be practical on large collections. The stability in a core collection permits the comparison of the data on a common set of genotypes over sites and years. However, total stability is not expected, since any core collection will suffer from imperfections. Duplication may occur that can be eliminated later, or variability that should be included in the core may subsequently be discovered or recognised. The representatives of the core will also depend on the methods used for characterisation. In the present study, 380 East Asian accessions of the Barley Core Collection contained almost all of the alleles for these isozyme loci which have been detected and reported in a large barley germplasm collection. From this point of view, the core seems to be representative, but some of the rare alleles (e.g. Nepalese accessions carrying the *Pgd-1 Tj* rare allele) should be included in this set of the Barley Core Collection.

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