Characterization of genetic diversity in core collection accessions of wild barley, *Hordeum vulgare* ssp. *spontaneum*

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Genetic variability in the 143 core accessions of wild barley, *Hordeum vulgare* ssp. *spontaneum*, was assessed by allozyme analysis. A total of 34 alleles were detected at ten isozyme loci. All loci were polymorphic except *Pgd-1*, which was monomorphic. *Est-2* and *Est-4* were the most diverse loci, with genetic diversity values of 0.747 and 0.686, respectively. The comparison of the results with those of previous studies indicates that all alleles occurring in cultivated and wild barley are observed in this set of the wild Barley Core Collection. Only one allele (*Pgd-1 Tj*) was absent. It is noteworthy that one new allele at the *Ndh-2* locus and another new allele at *Aco-2* locus were first detected in the present study. Nine of the 34 alleles were rare and detected only in one to four accessions. The genetic similarities among the 143 accessions ranged from 0.18 to 1.00. Data analysis based on clustering and principal coordinate analysis showed that a high level of genetic variability exists in this set of core accessions, and indicated that some duplication probably exists in this set core based on the present study.

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Wild relatives of crop plants have often been considered to be important sources for obtaining genes of special interest in plant breeding programs. Wild barley *Hordeum vulgare* ssp. *spontaneum* (C. Koch) Thell., the progenitor of cultivated barley, forms part of the primary genepool of barley (BOTHMER et al. 1992). The value of wild species as a genetic resource for crop improvement depends on the amount of genetic variability they contain relative to that of the crop plant as particular, valuable genes. Reliable characterization of a wide range of traits is an essential step towards a fuller utilization of the wild genetic resources in plant improvement

Germplasm collections were originally set up to preserve the genetic diversity of crop species and their wild relatives. Today, however, better access to and use of the genetic resources in collections have become an important issue. Germplasm collections have grown markedly in size. The size of many large germplasm collections may be an obstacle for full exploitation, evaluation and utilization (HOLDEN 1984). As an example, the actual number of unique accessions of barley is impossible to state since the figure includes duplications and accessions without adequate passport data (BOTHMER 1996). The genetic diversity in the huge world collection of barley has not been adequately evaluated. It is also impractical to characterize all collected accessions in detail. This task could be more easily fulfilled by the use of sub-sets of the whole collection, called active working collections by HARLEN (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 minimum of repetitiveness, ideally conserving at least 70 % of the alleles in the whole collection (BROWN 1989).

There is a growing interest in the development of core collections (HODGKIN 1990). Core collections have been developed for several germplasm collections, such as okra (*Abelmoschus esculentus* Moneh.) (HAMON and VAN SLOTEN 1989), perennial *Glycine* ssp. (BROWN et al. 1987), winter wheat (*Triticum aestivum* L.) (MACKAY 1986, 1989), peanut (*Arachis hypogae* L.) (HOLBROOK et al. 1993), annual *Medicago* species (DIWAN et al. 1994), lentil (*Lens culinaris* Medikus) (ERSKINE and MUEHLBAUER 1991), barley (*Hordeum vulgare* s.l.) (HINTUM 1994; HINTUM et al. 1995), and *Elymus* species (SALOMON et al. 1997).

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*, which can serve as 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 1993). Once the core accessions are

Table 1. *The accessions of Hordeum vulgare ssp. spontaneum used for this study*

No.	Origin	No.	Origin	No.	Origin	No.	Origin	No.	Origin	No.	Origin
180001	SYR	180109	PAL	180508	PAL	180887	SYR	181176	IRN	181381	JOR
180006	SYR	180117	PAL:	180533	PAL	180897	SYR	181178	IRN	181387	JOR
180007	JOR	180131	PAL	180554	PAL	180902	SYR	181180	IRN	181399	JOR
180013	JOR	180148	PAL:	180573	PAL	180907	SYR	181182	IRN	181405	JOR
180014	JOR	180172	PAL	180593	PAL	180913	SYR	181184	IRN	181412	JOR
180018	JOR	180182	SUN	180631	PAL	180918	SYR	181186	IRN	181418	JOR
180029	SYR	180199	PAL	180687	PAL	180923	SYR	181216	JOR	181424	JOR
180035	UNK	180207	TKM	180715	PAL	180927	PAL	181228	TUR	181430	JOR
180044	AFG	180209	TKM	180743	PAL	180973	PAL	181238	SYR	181436	JOR
180046	IRQ	180211	TKM	180771	PAL	180982	PAL	181243	PAK	181442	JOR
180047	AFG	180213	TKM	180789	JOR	180994	PAL	181268	JOR	181466	JOR
180049	AFG	180215	TKM	180802	SYR	181059	PAL	181277	CYP	181469	SYR
180051	AFG	180231	PAL	180812	SYR	181094	PAL	181301	SYR	181475	SYR
180052	IRN	180244	PAL	180817	SYR	181150	PAL	181305	SYR	181481	SYR
180063	TUR	180260	PAL	180827	SYR	181154	IRN	181315	TKM	181492	TKM
180065	TUR	180277	PAL	180832	SYR	181156	IRN	181317	RUS	181496	TKM
180068	PAL	180303	PAL	180837	SYR	181158	IRN	181323	SYR	181498	UZB
180069	IRQ	180329	PAL	180842	SYR	181160	IRN	181324	LBY	181500	TJK
180070	TUR	180353	PAL	180847	SYR	181162	IRN	181326	LBY		
180072	IRN	180372	PAL	180857	SYR	181164	IRN	181328	LBY		
180079	PAL	180389	PAL	180862	SYR	181166	IRN	181331	RUS		
180084	PAK	180410	PAL	180867	SYR	181168	IRN	181338	SUN		
180092	PAL	180430	PAL	180872	SYR	181170	IRN	181351	CHN		
180095	PAL	180452	PAL	180877	SYR	181172	IRN	181365	CHN		
180102	PAL	180473	PAL	180882	SYR	181174	IRN	181373	CHN		

selected, the next concern is what level of genetic diversity exists in this core set, and how representative it is.

In this paper, we survey the genetic diversity of the *Hordeum vulgare* ssp. *spontaneum* 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 in which genetic diversity and allelic distribution been evaluated (Liu et al. 1999, 2000, 2001).

MATERIALS AND METHODS

Accession numbers and origins of the 143 *Hordeum vulgare* ssp. *spontaneum* accessions used in the present study are presented in Table 1. These accessions were kindly provided by Dr. A. A. Jaradat from ICACDA.

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

There have been a number of studies of allozyme variation in cultivated barley (*Hordeum vulgare*) with various objectives. In all of our gels we included a sample of the cultivar Atlas so that comparison of alleles between *Hordeum vulgare* ssp. *spontaneum* and cultivated barley can be made.

RESULTS

Genetic diversity for the total sample

A total of 34 alleles were observed at the ten isozyme loci in the 143 accessions of *Hordeum vulgare* ssp. *spontanum*. All allelic distributions are shown in Table 2. The *Pgd-1* was the single monomorphic locus among the studied loci, and genetic variation was not found in any of the samples. All other loci were polymorphic. Esterases showed abundant genetic variability. Six alleles were detected at the *Est-2* locus, two alleles (*Est-2*, *Sp* and *Pl*) were not observed in the core accessions of cultivated barley. Four alleles were observed at *Est-1*, *Est-4* and *Est-5*, and all alleles were also observed in cultivated barley (Liu et al. 1999, 2000, 2001). The alleles *Gpi-1 X*, *Aco-1 Si*, *Aco-2 C*, *Ndh-2 A* and *B* detected in this study have not been recorded for cultivated barley core collection accessions. Out of the 34 alleles, nine were rare, and only found in one to four accessions, with the distribution frequency of < 0.030 (Table 2). The gene diversity values are shown in Fig. 1. The highest genetic diversity was detected at the *Est-2* locus ($H = 0.747$), followed by *Est-4* (0.686), *Est-5* (0.516), and *Aco-1* (0.391).

Table 2. *Allele frequency at each locus and distribution of rare alleles*

Locus	*Est-1*				*Est-2*			*Est-4*				*Est-5*			
Allele	*Pr*	*Al**	*Ca*	*Af*	*Dr*	*Fr*	*Sp*	*Pl*	*Un*	*ne*	*Nz*	*Su*	*At*	*ne**	*Mi*	*Pi*	*Ri*	*od*
Frequency	0.042	0.014	0.818	0.126	0.189	0.371	0.259	0.056	0.077	0.063	0.343	0.329	0.296	0.021	0.056	0.664	0.154	0.126
Accession with rare allele		180065 180231												180001 180533 181331				

Locus	*Gpi-1*			*Pgd-1*	*Pgd-2*		*Aco-1*			*Aco-2*			*Ndh-2*			
Allele	*X**	*Be*	*Gu*	*Ak*	Ps	Tn*	Fn	Ge	Si	A*	B	C*	A*	B*	C	D*
Frequency	0.021	0.923	0.056	1	0.986	0.014	0.119	0.762	0.119	0.021	0.958	0.021	0.028	0.014	0.937	0.021
Accession with of rare allele	180001					180001				180172		180743	181160	181324		180771
	180244					180277				180231		180832	181186	181326		180907
	181326									180452		181430	181238			181268
													181498			

* : rare allele

Distribution of rare alleles

Of the four alleles detected at the *Est-1* locus, the *Al* allele was rare and only detected in accessions 180065 and 180231. A null allele at *Est-4* (*ne*) was only observed in three accessions (180001, 180533 and 181331). *Gpi-1* locus possessed three alleles (*X*, *Be* and *Gu*), of which the *Gpi-1 X* allele was rare and only found in three accessions (180001, 180244 and 181326). Only two accessions, 180001 and 180277 showed *Pgd-2 Tn* allele. Three alleles (*A*, *B* and *C*) were found at *Aco-2*, of which, two alleles (*A* and *C*) were rare. Four alleles were observed at the *Ndh-2* locus, three (*A*, *B* and *D*) of which were rare. The distribution of the rare alleles is summarised in the Table 2.

Cluster and principal coordinate analysis

Cluster analysis was used to reveal the association between the accessions. The genetic similarity was calculated from the electorphortic data by UPGMA cluster analysis based on Jacarrd's similarity coefficient. All 34 alleles were included in the statistical analysis. The similarity coefficient ranged from 0.18 to 1.00. Cluster analysis placed the 143 accessions into many small groups (Fig. 2). The clustering was unable to reduce the data into a few groups. Accession 180001 from Syria was the most diverse one, followed by accession 181326 from Libya and accession 180771 from Palestine. Principal coordinate analysis (PCoA) was also performed based on the similarity coefficient. The PCoA displayed an enormous genetic variability among the core accessions in general. Associations among 85 different genotypes revealed by PCoA are presented in Fig. 3. The first (PC1) and the second (PC2) principal coordinate accounted only for 13.3 % and 11.0 % of the total variation, respectively. The first 85 axes explain almost all of the variance.

DISCUSSION

Isozyme systems have been extensively utilised in monitoring genetic changes in experimental barley populations (CLEGG et al. 1972), in assessment of genetic diversity in wild ssp. *spontaneum* (BROWN et al. 1978; NEVO et al. 1979; JARADAT 1992) and cultivated barley (JANA and PIETRZAK 1988), in elucidating multilocus structure in both taxa (DAI and ZHANG 1989; ZHANG et al. 1993) and in characterising genetic diversity in the cultivated barley core collection (LIU et al. 1999, 2000, 2001). Especially esterases have been extensively examined (JARADAT 1992; KONISHI 1994; BERNARDO et al. 1997). In a survey of isozyme variation in about 3,000 accessions

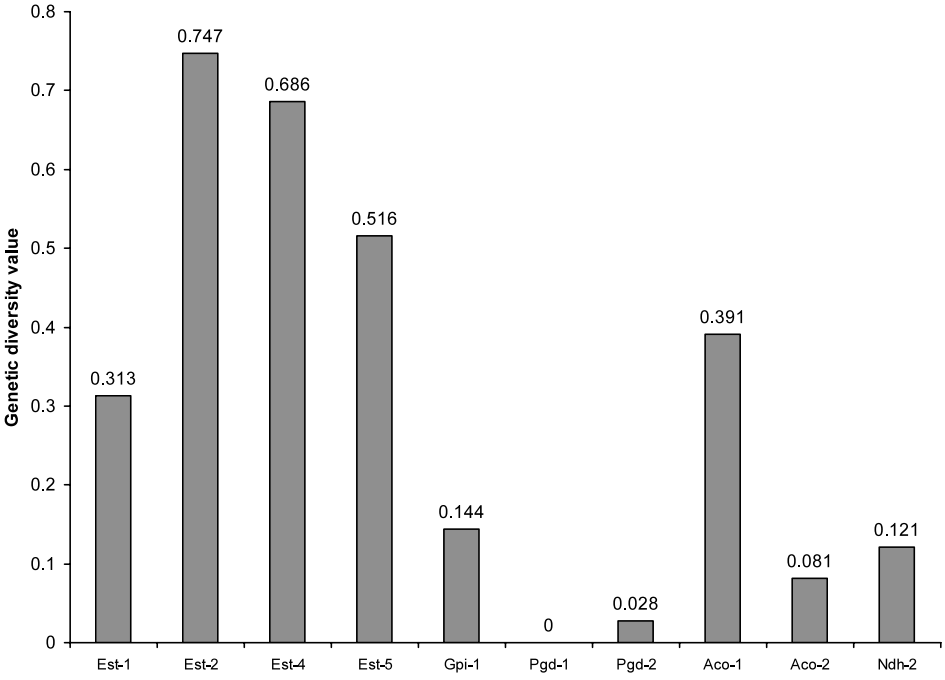

Fig. 1. Distribution of genetic diversity values of ten isozyme loci in the 143 core accessions of *H. vulgare* ssp. *spontaneum*.

of cultivated barley together with 180 strains of wild barley, ssp. *spontaneum*, KONISHI (1994) detected three alleles (*Al*, *Ca* and *Af*) at *Est-1* locus, six alleles (*Dr*, *Fr*, *Sp*, *Pl*, *Un* and *ne*) at *Est-2* locus, three alleles (*Nz*, *Su* and *At*) at *Est-4* locus. All theses alleles at the *Est* loci were also detected in present study. It is noticeable that we also observed the *Pr* allele at *Est-1* and the *ne* allele at *Est-4* in ssp. *spontaneum*, whereas KONISHI (1994) could only find these two alleles in cultivated barley. The gene diversity per locus for Esterases followed the same trend reported by other; i.e., *Est-2* > *Est-4* > *Est-5* > *Est-1* workers (NEVO et al. 1986, JARADAT 1992).

For phosphogluconate dehydrogenase, two loci, *Pgd-1* and *Pgd-2*, have been reported earlier (BROWN and MUNDAY 1982; BROWN et al. 1989), and allelic variation was found at both loci in wild barley (BROWN et al. 1978; BROWN and MUNDAY 1982; KONISHI and YOSHIMI 1993; ZHANG et al. 1993). In this set core accessions of ssp. *spontaneum*, we observed two alleles at *Pgd-2* locus with the *Ps* allele being the most common one, which corresponds well with previous reports. However, the *Tj* allele at the *Pgd-1* locus could not be detected in this set of accessions. The purpose of a core collection is to assemble accessions that will maximise the number of alleles. Since the *Tj* allele is rare in both cultivated and wild barley, it is important to conserve this rare allele from extinction. We suggest that the accessions possessing the *Tj* allele should be added to the barley core collection.

NIELSEN and JOHANSEN (1986) observed three alleles (*X*, *Gu* and *Be*) at the *Gpi-1* locus in cultivated barley, and HINTUM et al. (1995) detected three alleles at *Gpi-1* and *Ndh-2* from a comparative analysis of four European cultivated barley collections, respectively. BROWN et al. (1978) reported three alleles at *Gpi-1* locus in *H. spontaneum*. In this set of the ssp. *spontaneum* Core Collection, all three alleles of *Gpi-1* locus were observed. For the *Ndh-2* locus, four alleles were found including the three alleles reported in cultivated barley by previous researchers (HINTUM et al. 1995) and one new allele.

Previously, we reported three alleles at *Aco-1* and two alleles at *Aco-2* in the cultivated barley core set (LIU et al. 1999, 2000). NIELSEN and JOHANSEN (1986) reported three alleles at *Aco-1* and HINTUM et al. (1995) observed two alleles at the *Aco-2* locus in cultivated barley. In the present study, three alleles at *Aco-1* were detected. It is noticeable for the *Aco-2* locus that a new allele was detected in this set of ssp. *spontaneum* accessions beside the two previous reported ones.

The genetic similarities calculated for all pairwise comparisons among the 143 core accessions of *H. spontaneum* ranged from 0.18 to 1.00. In comparison, the similarity coefficient based on isozyme data in the 151 American core accessions ranged from 0.20 to 1.00, and in the 79 European accessions of the Barley Core Collection ranged from 0.40 to 1.00 (LIU et al. 2000, 2001). JARADAT (1992) reported the coefficients of Nei's genetic similarity based on four esterase loci

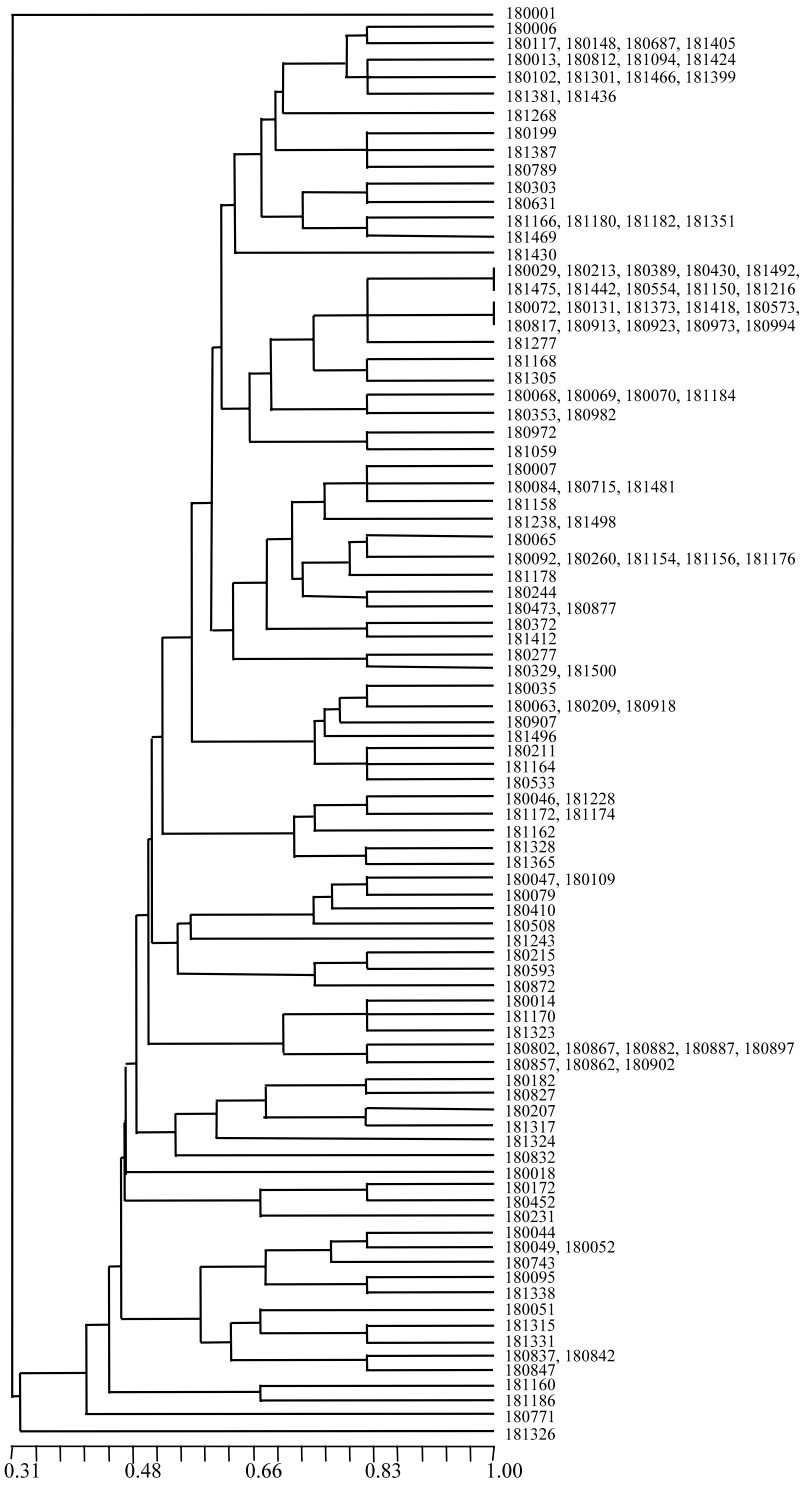

Fig. 2. Dendrogram for the 143 core accessions of *H. vulgare* ssp. *spontaneum*.

in natural populations of *H. spontaneum* from Jordan was from 0.23 to 0.87. 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 low genetic similarity value (0.18) obtained in present study indicated that this set core accession of wild barley is more diverse. The PCoA showed that 85 accessions

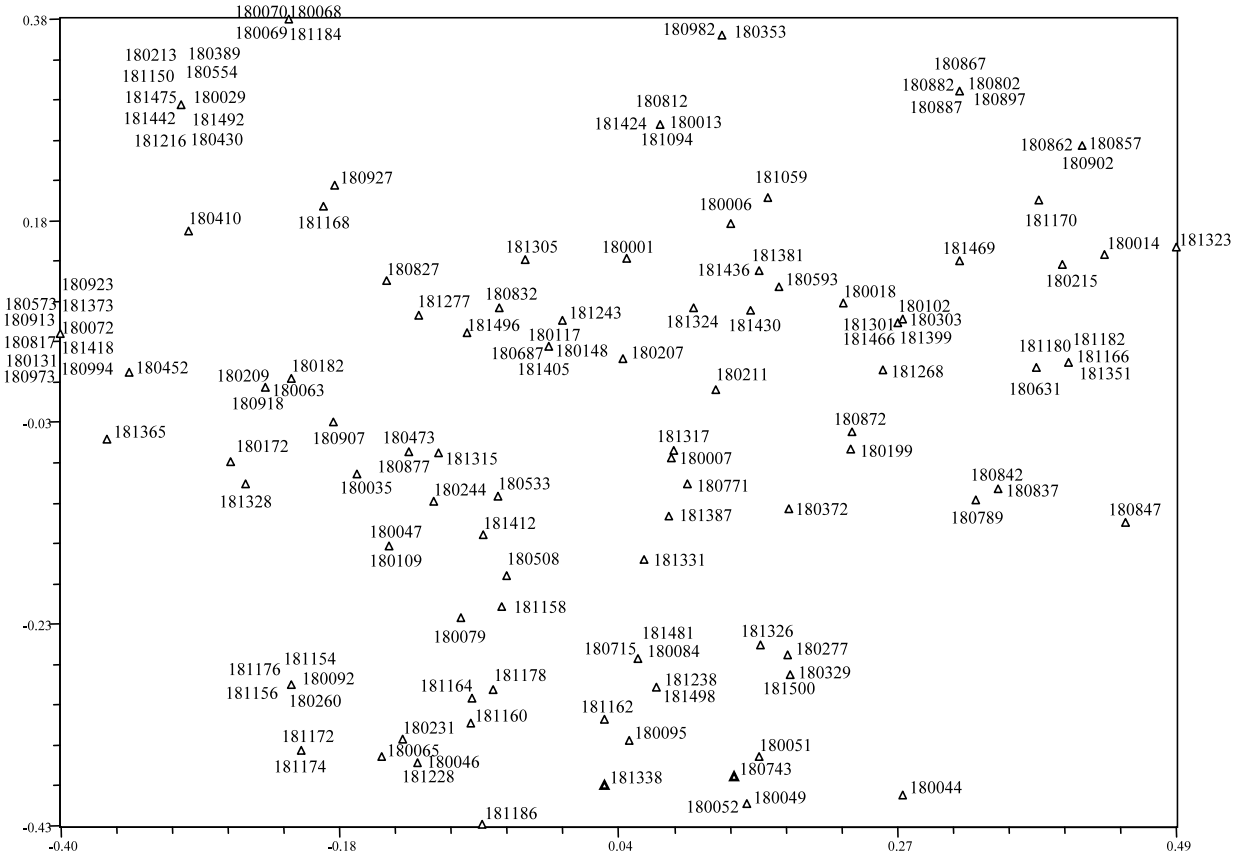

Fig. 3. Associations between 143 core accessions of *H. vulgare* ssp. *spontaneum* on the basis of the first two principal coordinates (PC1, PC2) from a principal coordinate analysis.

were well scattered. Data analysis, i.e. clustering and principal coordinate analysis, also revealed that the enormous genetic variability exist among the core accessions in general.

However, in spite of a high level of genetic diversity existing in this core set, the PCoA analysis revealed that 85 axes explained 100 % of diversity. The results indicate that genetic diversity was not detected in about one-third accessions at these loci. This implies that some accessions of this core set were probable duplication based on isozyme data. To confirm this, it is clear need further analysis of genetic diversity of this set of barley core accessions using DNA markers. Allozyme have been successfully utilised in barley and other crops to characterise the genetic variation in numerous taxonomic and population genetic studies (see DOEBLEY 1989 for review). The technique is an available, rapid and economic for the characterisation of genetic variation. However, compared to the DNA markers, the disadvantage is only small numbers of marker loci available, which provided only poor coverage of genome.

REFERENCES

Bernardo A, Luque A, Cuadrado A, Negro A, Jouve N and Soler C, (1997). The assessment of genetic variation in Spanish primitive cultivars of barley, Hordeum vulgare L., by a combination of isozymes and hordeins. Genet. Res. Crop. Evol 44: 217–226.

Bothmer von R, (1996). Conservation and use of wild relatives of barley. Proceedings of V IOC and VII IBGS., p. 120–127.

Bothmer von R, Fischbeck G, Hintum van Th, Hodgkin T and Knüpffer H, (1990). The Barley Core Collection, report of the BCC Working Group. Weihenstephan, 26 September (1990). Mimeographed.

Bothmer von R, Seberg O and Jacobsen N, (1992). Genetic resources in the Triticeae. Hereditas 116: 141–150.

Brown AHD, (1989). The case for core collection. In: The use of plant genetic resources (eds ADH Brown et al.). Cambridge University Press, Cambridge, UK, p. 136–156.

Brown ADH and Munday J, (1982). Population-genetic structure and optimal sampling of land races of barley from Iran. Genetica 58: 85–96.

Brown ADH, Nevo E, Zohary D and Dagan O, (1978). Genetic variation in natural populations of wild barley (Hordeum spontaneum). Genetica 49: 97–108.

Brown AHD, Grace JP and Speer SS, (1987). Designation of a core collection of perennial Glycine. Soybean Genet. Newsl. 14: 59–67.

Brown AHD, Lawrence GJ, Jenkin M, Douglass J and Gregory E, (1989). Linkage drag in backcross breeding in barley. J. Heredity 80: 234–239.

Clegg MT, Allard RW and Kahler AL, (1972). Is the gene the unit of selection? Evidence from two experimental plant populations. Proc. Natl. Acad. Sci. USA 69: 2474–2478.

Dai X and Zhang Q, (1989). Genetic diversity of six isozyme loci in cultivated barley of Tibet. Theor. Appl. Genet. 78: 281–286.

Diwan N, Bauchan GR and McIntosh MS, (1994). A core collection for the United States annual Medicago germplasm collection. Crop Sci. 34: 279–285.

Doebley J, (1989). Isozyme evidence and the evolution of crop plants. In: Isozyme in Plant Biology (eds DE Soltis and PS Soltis). Chapman and Hall, London, p. 165–191.

Erskine W and Muehlbauer FJ, (1991). Allozyme and morphplogical variability, outcrossing rate and core collection formation in lentil germplasm. Theor. Appl. Genet. 83: 119–125.

Frankel OH and Brown AHD, (1984). Plant genetic resources today: a critical appraisal. In: Crop genetic resources: conservation and evaluation (eds JHW Holden and JT Williams). George Allen and Unwin, Winchester, Massachusetts, p. 249–268.

Hamon S and van Sloten DH, (1989). Characterization and evaluation of okra. In: The use of plant genetic resources (eds ADH Brown, OH Frankel, DR Marshall and J Williams). Cambridge University Press, Cambridge, p. 173–196.

Harlen JR, (1972). Genetic resources in sorghum. In: Sorghum in the seventies (eds NGP Rao and LR House). Oxford and IBH, New Delhi, p. 1–13.

Hatz BG, Jahoor A and Fischbeck G, (1996). RFLP Polymorphism among European accessions of the Barley Core Collection. In V IOC & VII IBGS., p. 176–178.

Hintum TJL, (1994). Comparison of marker systems and construction of a core collection in a pedigree of European spring barley. Theor. Appl. Genet. 89: 991–997.

Hintum TJL, Bothmer von R and Visser DL, (1995). Sampling strategies for composing a core collection of cultivated barley (Hordeum vulgare s. lat.) collected in China. Hereditas 122: 7–17.

Hodgkin T, (1990). The core collection concept. In: Crop networks: searching for new concepts for collaborative genetic resources management (eds TJL van Hintum, L Frese and PM Perret). International Board for Plant Genetic Resources, Rome, Italy, p. 43–48.

Holbrook CC, Anderson WF and Pittman RN, (1993). Selection of a core collection from the U.S. germplasm collection of peanuts. Crop Sci. 33: 859–861.

Holden JHW, (1984). The second ten years. In: Crop genetic resources: conservation and evaluation (eds JHW Holden and J Williams). George Allen and Unwin, Winchester, Massachusetts, p. 277–285.

Jana S and Pietrzak LN, (1988). Comparative assessment of genetic diversity in wild and primitive cultivated barley in a center of diversity. Genetics 119: 981–990.

Jaradat AA, (1992). Genetic diversity of four esterase loci in natural populations of Hordeum spontaneum C. Koch from Jordan. Theor. Appl. Genet. 84: 725–729.

Knüpffer H and Hintum Th JL van, (1993). The Barley Core Collection- an international effort. In: Core Collections: improving the management and use of plant germplasm collections. Proceedings of an IBPGR/CGN/CENARGEN Workshop, 23–28 August 1992, Brasilia, Brazil.

Konishi T, (1994). Geographical diversity of isozyme genotypes in barley. Kyushu University Press.

Konishi T and Yoshimi R, (1993). Geographical distribution of isozyme alleles for phosphogluconate dehydrogenase in barley. Genome 36: 512–516.

Liu F, Bothmer von R and Salomon B, (1999). Genetic diversity among East Asian Accessions of the Barley Core Collection as revealed by six isozyme loci. Theor. Appl. Genet. 98: 1226–1233.

Liu F, Bothmer von R and Salomon B, (2000). Genetic diversity in European accessions of the Barley Core Collection as detected by isozyme electrophoresis. Genet. Res. Crop Evol. 47: 571–581.

Liu F, Sun GL, Salomon B and Bothmer von R, (2001). Distribution of allozymic alleles and genetic diversity in the American Barley Core Collection. Theor. Appl. Genet. 102: 606–615.

Mackay MC, (1986). Utilizing wheat genetic resources in Australia. In: Proc 5th Assembly Wheat Breed Soc Australia (ed. R McLean). Perth/Merredin, Australia, p. 56–61.

Mackay MC, (1989). Strategic planning for effective evaluation of plant germplasm. In: Wheat Genetic Resources: Meeting Diverse Needs (eds JP Strivastava and AB Damania). John Wiley & Sons Ltd, Chichester, England, p. 21–25.

Melchinger AE, Graner A, Singh M and Messmer MM, (1994). Relationships among European Barley Germplasm: I. Genetic diversity among winter and spring cultivars revealed by RFLPs. Crop Sci. 34: 1191–1199.

Nevo E, Zohary D, Brown ADH and Haber M, (1979). Genetic diversity and environmental associations of wild barley, Hordeum spontaneum in Israel. Evolution 33: 815–833.

Nevo E, Beiles A and Zohary D, (1986). Genetic resources of wild barley in the Near East: structure, evolution and application in breeding. Biol. J. Linnean Soc. 27: 355–380.

Nielsen G and Johansen HB, (1986). Proposal for the identification of barley variations based on the genotypes for 2 hordein and 39 isoenzyme loci of 47 reference varieties. Euphytica 35: 717–728.

Salomon B, Bothmer von R and Seberg O, (1997). A proposal for an Elymus Core Collection. Plant Genet. Resources Newsl. 111: 77–81.

Zhang Q, Sahgai-Maroof MA and Kleinhofs A, (1993). Comparative diversity analysis of RFLPs and isozymes within and among populations of Hordeum vulgare ssp. Spontaneum. Genetics 134: 909–916.