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 (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 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 Monenh.) (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 Muelbauer 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 (Knüpf-fer and Hintum 1993). 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.

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. spontaneum. 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).
Genetic diversity of wild barley

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 electrophoretic data by UPGMA cluster analysis based on Jaccard’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

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

<table>
<thead>
<tr>
<th>Locus</th>
<th>Frequency</th>
<th>Accession with rare allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>Est-5</td>
<td>0.021</td>
<td>180001</td>
</tr>
<tr>
<td>Est-4</td>
<td>0.042</td>
<td>180001</td>
</tr>
<tr>
<td>Est-3</td>
<td>0.082</td>
<td>180001</td>
</tr>
<tr>
<td>Est-2</td>
<td>0.098</td>
<td>180001</td>
</tr>
<tr>
<td>Est-1</td>
<td>0.139</td>
<td>180001</td>
</tr>
<tr>
<td>Gpi-1</td>
<td>0.119</td>
<td>180001</td>
</tr>
<tr>
<td>Pgd-1</td>
<td>0.121</td>
<td>180001</td>
</tr>
<tr>
<td>Pgd-2</td>
<td>0.125</td>
<td>180001</td>
</tr>
</tbody>
</table>

* : rare allele
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 these 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 YOSHIKI 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 Ndhi-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 Ndhi-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.
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


