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Analysis of genetic diversity of hordein in wild close relatives of barley from Tibet

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Abstract We analyzed genetic diversity in the storage protein hordein encoded at *Hor-1, Hor-2* and *Hor-3* loci in seeds from 211 accessions of wild close relatives of barley, *Hordeum vulgare* ssp. *agriocrithon* and *H. vulgare* ssp. *spontaneum*. Altogether 32, 27 and 13 different phenotypes were found for *Hor-1, Hor-2* and *Hor-3*, respectively. A comparison of our results with those of previous studies indicates that Tibetan samples reflect the highest diverse level of hordein phenotypes when compared to samples from Israel and Jordan. This high degree of polymorphism supports the hypothesis that Tibet is one of the original centers of *H. vulgare* L.

Keywords Tibetan barley · *Hordeum vulgare* L. · Hordeins · Genetic diversity

Introduction

The role of Tibet in the origin and evolution of cultivated barley has been an outstanding issue for several decades. Initially, two distinct groups in world barley were suggested – an Oriental group and an Occidental group (Vavilov 1926; Freisleben 1940). Genetic analyses of ear rachis brittleness and powdery mildew resistance showed that there is genetic differentiation between these two barley groups (Takahashi 1955). Similar differentiation has also been observed in patterns of multilocus associations among enzyme loci (Kahler and Allard 1981; Zhang et al. 1990, 1992a). A study on the variation and diversity of rDNA alleles suggested that Tibet is the center of Oriental barley and Ethiopia the center of Occidental barley (Zhang et al. 1992b).

Since the end of the 1950s, it has been reported that there are 601 cultivated varieties and 428 wild close varieties in Tibet (Ma 2000). Among the 601 cultivated species, 490 varieties have been identified and named recently, of which 443 are Tibetan-specific. Among the 428 wild close varieties, 418 have been identified and named recently, of which 390 are Tibetan-specific. The barley samples collected have made it possible to study the genetic diversity of barley in this region. Preliminary analyses of morphological characters and allozymes have demonstrated that barley collected from Tibet is indeed highly variable (Dai and Zhang 1989; Zhang and Dai 1991) and that cultivated barley in Tibet is genetically at least as diverse as Ethiopian barley (Zhang et al. 1992b).

Hordeins are the major storage proteins of endosperm of the barley grain and account for 35–50% of the total protein content in the seed (Jaradat 1991). Components of the hordein fraction are classified into four groups of polypeptides – A, B, C and D – in order of decreasing mobility (Køie et al. 1976; Miflin and Shewry 1977; Field et al. 1982). The A polypeptides are not generally considered to be a storage fraction (Miflin and Shewry 1977). The B, C and D groups of polypeptides are coded for by three complex loci, designated *Hor-2*, *Hor-1* and *Hor-3*, respectively. Previous work has shown that *Hor-1* and *Hor-2* are located on the short arm of chromosome 5, while *Hor-3* is on the long arm (Blake et al. 1982; Shewry et al. 1983). Hordeins are largely tolerant to mutations and are selected neutrally (Nevo et al. 1983). They represent a multiple gene family, and their variability is higher than that of allozyme loci. Hordeins can be used as a genetic maker (Echart-Almeida and Cavalli-Molina 2000) and are essential in evolutionary studies (Nevo et al. 1983).

A number of studies have been performed on storage proteins of barley from Israel (Nevo et al. 1983), Jordan (Jaradat 1991), Ethiopia (Zemede 1989) and China (Zhan et al. 1991). However, only ten wild close accessions

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from China that cannot represent the wild close barley groups in Tibet were evaluated (Zhan et al. 1991). The wild close accessions from Tibet have never been examined with respect to hordeins.

We report here our analysis of the accessions from Tibetan wild close relatives of barley and subsequent evaluation of the diversities of B, C and D hordein subunits coded by barley groups [*Hordeum vulgare* ssp. *spontaneum* (HS) and *H. vulgare* ssp. *agriocrithon* (HA)] from Tibet. We also postulate possibilities for the origin and evolution of barley.

Materials and methods

Plant materials

Seeds of 211 accessions (200 accessions from Tibet and 11 accessions from nearby lands, Table 1), including 123 HS accessions and 88 HA accessions, were provided by the Institute of Crop Germplasm Resources, Chinese Academy of Agriculture Sciences. These accessions were selected randomly from 3,105 accessions registered in the Catalogue of genetic resources of wild close relatives of barley in China, which were collected between 1952 and 1985 in Tibet (Ma 2000). The collected area of altitudes of 1,600 m to 4,350 m is located on the Qinghai-Tibet Plateau in China. Chinese Spring (null, 7+8, 2+12) was used as a reference to the D hordein subunits of barley.

Protein extraction

The hordeins were extracted as described previously (Alvarez et al. 2001). Seeds crushed into a fine powder were used to extract the endosperm storage proteins. The monomeric prolamins were extracted with a 1.5 *M* dimethylformadie aqueous solution in a 1:5 ratio (mg/μl) and stored at –20 °C. The pellet was double-washed with 50% (v/v) 1-propanol at 60 °C for 30 min, with agitation every 10 min. Hordeins was extracted with 250 μl of a buffer containing 50% (v/v) 1-propanol, 80 m*M* Tris-HCl, pH 8.5, and 2% (w/v) dithiothreitol at 60 °C for 30 min. After centrifugation, 200 μl of the supernatant was transferred to a new tube, mixed with 3 μl 4-vinylpyridine and incubated for 30 min at 60 °C. The samples were precipitated with 1 ml of cold acetone. The dried pellet was solubilized in a buffer containing 625 m*M* Tris-HCl, pH 6.8, 2% (w/v) sodium dodecyl sulphate (SDS), 10% (v/v) glycerol, 0.02% (w/v) bromophenol blue, and 2% (w/v) dithiothreitol in a 1:5 ratio (mg/μl) to wholemeal.

Protein analyses

Hordeins were analyzed by SDS polyacrylamide gel electrophoresis (PAGE). D hordein subunits were separated on a 8% polyacrylamide gel with 4 *M* urea, whereas B and C subunits were analyzed on a 10% gel with 4 *M* urea. Gels were stained overnight with a 12% (w/v) trichloroacetic acid solution containing 5% (v/v) ethanol and 0.05% (w/v) Coomassie Brilliant Blue R-250, then destained and photographed.

Statistical analyses

The genetic diversity of the hordein phenotypes was evaluated using Shannon's information statistic (Bowman et al. 1971)

$$H = -\sum f_i \ln f_i$$

where f_i is the frequency of the ιth phenotype. For a large number of the samples consisting of individuals from two or more subgroups, the above diversity index was partitioned into an among-subgroups competent and a within-subgroups competent (Nei 1973). The partitioning was performed in this study using the two barley groups HA and HS.

Results

Variations in B, C and D hordein subunits in Tibetan barley groups

We used SDS-PAGE gel with 4 *M* urea to separate the hordein subunits in the barley seeds and found total of 27, 32 and 13 different patterns between the B, C and D hordein subunits, respectively.

As shown in Fig. 1A, B, C and D hordeins ran as separate groups each with distinct molecular-weight regions. The migration patterns of B and C hordein subunits in barley seeds are shown in Fig. 1B, while Fig. 1C shows a diagram of the 32 different patterns of C hordein subunits (diagram of B hordein subunits not presented) in the 211 accessions. These latter patterns are formed by one to six C subunits coded at *Hor-1* loci. Patterns with three or four bands are the most frequently present among the 32 accessions (ten and nine, respectively), and that with four bands the highest ratio (40.8% of total protein bands). The last two bands of pattern 1 are the most frequent bands (12 of 32) and have the highest ratio (74.9%). The most frequent patterns were pattern 1, 12, 13 and 25, which were found in 112 (53.1%) of the 211 accessions evaluated in this study. In contrast, other patterns such as those of accessions 2, 4 and 16 were only found in one accession. The remainder of the patterns were distributed between several accessions.

We further examined the migratory patterns of D hordeins (Fig. 2). One to three D hordein subunits formed a pattern produced by the *Hor-3* locus. Pattern 7, only one band, showed faster mobility than the others. In general, there are two closely conjointed bands in patterns 6, 8, 10, 12 and 13. Pattern 13 was the most frequent (164 of 211 accessions, 77.7%). In contrast, patterns 3, 4, 6, 7, 8, 10 and 11 were only found in one accession. The other patterns were distributed among several accessions.

The frequencies of each electrophoretic pattern in the two barley groups are shown in Tables 2, 3 and 4 for B, C and D hordein subunits, respectively. The most frequent patterns occurred at dominantly high frequencies at the *Hor-3* locus (frequency 0.777). Patterns 1, 2 and 3 at *Hor-2* and patterns 1, 12, 13 and 25 at *Hor-1* were common (frequencies >0.100), and the majority of the others were rare (frequencies <0.05). Although the relative frequencies of patterns at each locus differed between the two barley groups, the frequent patterns were similar between them at *Hor-2* and *Hor-3*, and two to three patterns were similar at *Hor-1*. On the other hand, several patterns were found only in one barley group and not in the other (Tables 2–4).

Table 1 Location of 200 accessions from Tibet only used in this study

Location	Accessions[a]		Altitude (m)
	Two-rowed barley	Six-rowed barley	
Lhasa City			**3,650–4,200**
Chengguan District			3,650
Nyemo Coun	0027 0981 0983 1484 1488	1939 2310	3,750–4,200
Doilungdeqen Coun.	1490 1491 1492	1045 1952	3,700–3,970
Lhunzhub Coun	1044	0642	4,000–4,100
Maizhokunggar Coun		0315 1043 1933	3,890
Qamdo Prefecture			**2,840–4,100**
Qamdo Coun.	0001 1428 1430	0242 0505 2245	3,170–3200
Lhorong Coun.		1024 1026 2268	3,250–3,640
Gonjo Coun.	0003	1013 1020 2258	2,840–3,660
Chagyab Coun.	0002 0973 0974 1447 1448 1449	0259 1027 2271	3,100–3,300
Zogang Coun.	0005 0006 0007 0976 1444 1445 1446	1035 1036 1868	3,300–3,980
Baxoi Coun.		0554	3,080–4,100
Markam Coun.	0012 0013 1450 1451 1452	1037 1038 2275	3,000–3,800
Nyingchi Prefecture			1,600–3,804
Nyingchi Coun.		1890	3,000–3,200
Bomi Coun.		1932 4182[b]	2,700–2,750
Gongbogyamda Coun.	0019 0020 0022 0978 0980	1042 1898	3,300–3,804
Mainling Coun.		1039 1891	2,970–3,450
Nangxian Coun.	0028 0029 0036 1460 1461	0316 0317 1893	3,200–3,720
Zayü Coun.		2287	1,600–2,328
Shannan Prefecture			**3,300–4,350**
Nedong Coun.	0194 1493 0996	1955	3,500–3,800
Gyaca Coun.	0040 0041 0045 1495 1496 1498 1504	1961	3,300–3,900
Qusum Coun.	0180 0182 0185 1511 1513	0437 1976	3,820–4,200
Sangri Coun.	0192 0193	0438 2008	3,830–3,870
Zhanang Coun.	1810 1811 1812		3,650
Gonggar Coun.		0804	3,500
Qonggyai Coun.	0195 0200 1545 1548 0997	0448 1989	3,500–3,900
Lhozhag Coun.	0140 0141 0146 1780 1789	2180	3,300–3,930
Comai Coun.	0106 0107 0108 1732 1733 1735	2136	3,600–4,200
Cona Coun.	0091 0093 0874 0995 1518 1520 4163	0354 1980	3,630–4,350
Lhünze Coun.	0058 0059 0870 0871 0872 0873 0986 0987 0990 1565	2011 2112	3,570–4,000
Xigaze Prefecture			**3,700–4,300**
Xigaze City	0210 0875 0876 1569 1814 1816 1822	2197	3,860
Gyangze Coun.		2212	3,980–4,040
Namling Coun.	0957 1819 1820	0961 1089 2200 2201 0965 0966	3,950
Tingri Coun.	0213 0215 0958 0959 0960 1823 1824 1826	0962 0963 0968 0969 0970 2202	3,800–4,300
Rinbung Coun.		0834 0964	3,700
Yadong Coun.		2216 4181[b]	4,200
Nagri Prefecture			**3,550–3,830**
Burang Coun.	0241 1010 1838 1840	2244 2517	3,710–3,830
Zanda Coun.	0221 0223 0232 0238 1829 1836	0479 0971 0972 2220 2510	3,550
n	**122**	**78**	**1,600–4,350**

[a] The serial number of the accession is the accession number: ZYM (Zhong Ye Mai, in Chinese)
[b] The serial number of the accession is the accession number: ZDM (Zhong Da Mai, in Chinese)

Diversity value between the two barley groups

The genetic diversity value of each locus was calculated for each of the two barley groups. The order for the value was HS < HA for the *Hor-2* locus, and HA < HS for the other two loci. When the average of the three loci was calculated, the value of HA was lower than that of HS (1.802 to 1.906, Table 5).

Partitioning of the diversity value showed that the proportion of genetic diversity accounted for by differentiation among groups was generally small. It varied from a low of 3.99% at the *Hor-2* locus to a high of 9.03% at the *Hor-3* locus, with an average of 6.65% (Table 5).

Discussion

In this study we analyzed genetic polymorphism in Tibetan wild close relatives of barley using hordein markers representing three loci in the barley genome. The samples of HS and HA used in this study contain an adequate representation of these two barley groups in

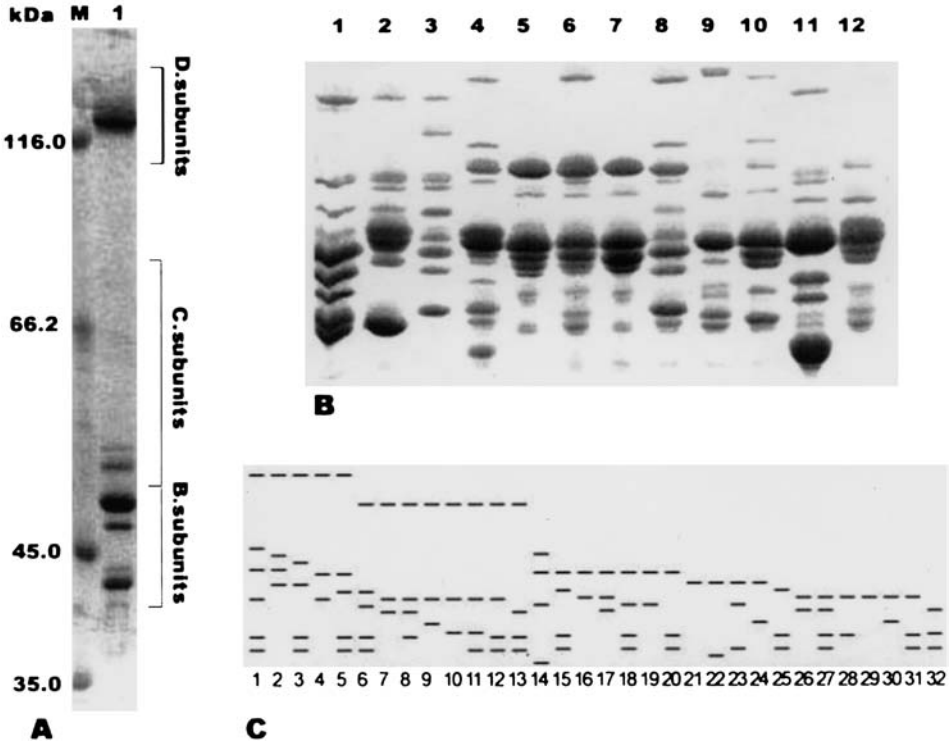

Fig. 1A–C Separation of B, C and D hordeins from Tibetan accessions. **A**. B, C and D hordeins ran as separate groups with distinct molecular-weight regions on a SDS polycrylamide gel, **B** part of the gel corresponding to the B and C hordeins, **C** diagram of all patterns found for the C hordeins. *M* Molecular-weight maker. The B subunit ran between 37 kDa and 50 kDa; C ran between 50 kDa and 70 kDa. D ran above 100 kDa. The experiments were performed to separate hordeins as described in Materials and methods; similar results were obtained

Table 2 Frequencies of patterns of B hordein subunits in Tibetan barley (*HS Hordeum vulgare* ssp. *spontaneum*, *HA H. vulgare* ssp. *agriocrithon*)

Patterns	HS	HA	Total
1	0.179	0.102	0.147
2	0.220	0.227	0.223
3	0.325	0.352	0.336
4	0.057	0.023	0.043
5	0.016	0.011	0.014
6	0.008	0.000	0.005
7	0.000	0.034	0.014
8	0.008	0.034	0.019
9	0.008	0.023	0.014
10	0.016	0.057	0.033
11	0.008	0.000	0.005
12	0.000	0.011	0.005
13	0.049	0.011	0.033
14	0.000	0.011	0.005
15	0.016	0.000	0.009
16	0.000	0.011	0.005
17	0.008	0.000	0.005
18	0.000	0.011	0.005
19	0.000	0.011	0.005
20	0.000	0.011	0.005
21	0.024	0.034	0.028
22	0.008	0.000	0.005
23	0.000	0.011	0.005
24	0.024	0.011	0.019
25	0.008	0.000	0.005
26	0.008	0.000	0.005
27	0.008	0.000	0.005
n	20	19	27

Table 3 Frequencies of patterns of C hordein subunits in Tibetan barley

Patterns	HS	HA	Total
1	0.114	0.205	0.152
2	0.000	0.011	0.005
3	0.065	0.023	0.047
4	0.024	0.000	0.014
5	0.024	0.080	0.047
6	0.000	0.045	0.019
7	0.033	0.000	0.019
8	0.008	0.023	0.014
9	0.081	0.011	0.052
10	0.000	0.023	0.009
11	0.033	0.023	0.028
12	0.114	0.102	0.109
13	0.228	0.068	0.161
14	0.008	0.000	0.005
15	0.049	0.068	0.057
16	0.000	0.011	0.005
17	0.016	0.011	0.014
18	0.000	0.011	0.005
19	0.000	0.023	0.009
20	0.000	0.011	0.005
21	0.008	0.000	0.005
22	0.000	0.011	0.005
23	0.016	0.034	0.024
24	0.008	0.000	0.005
25	0.057	0.182	0.109
26	0.016	0.000	0.009
27	0.033	0.011	0.024
28	0.008	0.000	0.005
29	0.000	0.011	0.005
30	0.016	0.000	0.009
31	0.024	0.000	0.014
32	0.016	0.000	0.009
n	23	23	32

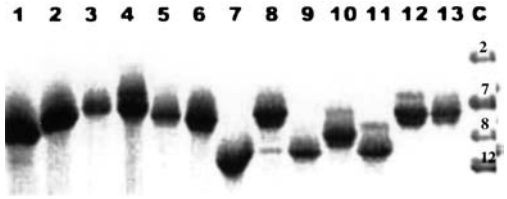

Fig. 2 Separation of the D hordeins from the Tibetan accessions. (*C* Chinese Spring)

Table 4 Frequencies of patterns of D hordein subunits in Tibetan barley

Patterns	HS	HA	Total
1	0.114	0.045	0.085
2	0.008	0.011	0.009
3	0.000	0.011	0.005
4	0.008	0.000	0.005
5	0.016	0.000	0.009
6	0.000	0.011	0.005
7	0.008	0.000	0.005
8	0.008	0.000	0.005
9	0.057	0.000	0.033
10	0.008	0.000	0.005
11	0.000	0.011	0.005
12	0.033	0.080	0.052
13	0.740	0.830	0.777
n	10	7	13

Table 5 Diversity value (*H*) of each locus in HS and HA from Tibet (H^a average genetic diversity)

Locus	HS	HA	Total	G_{ST} (%)
Sub B	2.051	2.127	2.176	3.99
Sub C	2.660	2.579	2.815	6.93
Sub D	1.007	0.700	0.939	9.03
H^a	1.906	1.802	1.977	6.65

Tibet, at least with respect to agri-geographical distribution.

A total of 32, 27 and 13 different hordein phenotypes were found at the *Hor-1, Hor-2* and *Hor-3* locus, respectively, in 211 accessions. Phenotypes of numbers 23 and 20 were seen at the *Hor-1* and *Hor-2* loci in the HS group from Tibet. These numbers are larger than those for phenotypes of 15 and 16 in a sample of 123 accessions of HS that represent the whole of Israel (Nevo et al. 1983). Similarly, these numbers are also larger than the number of phenotypes in a comparable sample size that represent 12 populations of HS collected from different agro-ecological regions in Jordan (Jaradat 1991). This demonstrates that the wild close relatives of barley in Tibet are as highly variable in their genetics as the cultivated barley in Tibet (Zhang et al. 1994).

Studies on genetic characteristics have been performed on the level and distribution of genetic variability in HS from Southwest Asia, mostly in Jordan and Israel (Nevo et al. 1983; Jaradat 1991; Saghai Maroof et al. 1984, 1990; Zhang et al. 1990, 1992b). It was hypothesized that this wild barley is the progenitor of the cultivated barley worldwide. Although HS from Southwest Asia is genetically highly diverse, it is difficult to explain the distribution of rDNA alleles (Zhang et al. 1992b): why does one explain why rDNA allele (allele 104) occur at a very high frequency in the Occidental barley groups but occur very rarely (frequency < 0.02) in this group. Also, it was not clear why the rDNA patterns of HS from Southwest Asia were more similar to those of barley in the Oriental region than to those of cultivated barley in its immediate surroundings.

The Oriental-Occidental differentiation of barley has been studied by analyzing morphological characters, disease resistance (Takahashi 1955), allozymes (Kahler and Allard 1981; Zhang et al. 1990, 1992a) and rDNA spacer-length variants (Zhang et al. 1992b). For example, the *0.2* allele at the *Est1* locus, which occur at a low frequency in Occidental barley, was detected at a predominantly high frequency in Tibetan barley (Zhang et al. 1990). The predominant allele *107* at the *Rrn2* locus in Tibet barley occurs at a low frequency in Occidental barley (Zhang et al. 1992b). In contrast, allele *104*, which is the most frequently occurring allele in the Occidental barley group, was hardly present in Tibetan barley (Zhang et al. 1992b). A profound differentiation at two genetic loci controlling rachis brittleness, a character that is under the immediate influence of domestication (Takahashi 1955), strongly suggests that the barley in Oriental and Occidental regions were domesticated independently of each other. This supports the hypothesis of a diphyletic origin of cultivated barley. The generation of one allele from another would involve a series of molecular events, including unequal crossing-over and the homogenization of large arrays of repeating units. If this is true, it would probably need thousands of years to accomplish unless there was very strong selection for such turnover (Zhang et al. 1994).

Using RFLP markers, Molina-Cano and Moralejo (1999) analyzed thirty-five populations from nine countries – Afghanistan, Crete (Greece), Cyprus, Iran, Iraq, Israel, Libya, Morocco and Turkey – and obtained results that suggest that Morocco is a center of origin of domesticated barley. However, this hypothesis was negated immediately by AFLP data (Badr et al. 2000) and RAPD data (Frank et al. 2001). In Morocco, spontaneous back mutation to wild type and crossing between wild barley and cultivated lines occurred, which gave rise to the local weedy forms of barley (Frank et al. 2001).

Barley as a crop grown in the Qinghai-Tibet Plateau (including the upper reaches of the Yellow River) had been in agriculture records 3,000 years B.C. (Xu 1982), indicating that barley in this region has an ancient history. Also, this region is geographically isolated from the rest of the world by several of the world's largest mountain ranges. Our study demonstrates a large amount of genetic variation in wild barley from Tibet, thereby, further supporting the hypothesis that the Qinghai-Tibet Plateau

and its vicinity are the center of origin for the cultivated barley in the Oriental region (Zhang et al. 1994).

Based on our findings, we hypothesize that the six-rowed wild barley of Tibet origin, HA, is the immediate progenitor of cultivated barley in Tibet, a conclusion in agreement with previous reports (Åberg 1940; Freisleben 1940; Takahashi 1955; Zhang et al. 1994). However, a difficulty with this speculation is that the amount of genetic variation detected in HA is not adequate for the genetic diversity of HV (*Hordeum vulgare* ssp. *vulgare*) in Tibet. One possible explanation for such a discrepancy is that a part of the genetic variation found in HV of Tibet was introgressed from the two-rowed wild barley, HS, which has been found to be widely distributed in the Tibet region (Zhang et al. 1994).

We did not include accessions of HV from Tibet and HS from Southwest Asia in the present study. To access the relative levels of genetic diversity among the barley group in Tibet, further studies using the three barley groups – HS, HA and HV – from Tibet are definitely needed. Studies on phylogenetic relationships among the barley groups and between HS from Tibet and Southwest Asia will also be needed.

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References

Åberg E (1940) The taxonomy and phylogeny of Hordeum L. sect. Cerealia Ands, with special reference to Tibetan barleys. Symb Bot Ups 4:1–156

Alvarez JB, Martín A, Martín LM (2001) Variation in the high-molecular-weight glutenin subunits coded at the *Glu-H^{ch}*1 locus in *Hordeum chilense*. Theor Appl Genet 102:134–137

Badr A, Müller K, Schäfer R, El Rabey H, Ibrahim HH, Pozi C, Rohde W, Salamini F (2000) On the origin and domestication history of barley (*Hordeum vulgare*). Mol Biol Evol 17:499–510

Blake TK, Ullrich SE, Nilan RA (1982) Mapping of the *Hor-3* locus encoding D hordein in barley. Theor Appl Genet 63:367–371

Bowman KD, Hutcheson K, Odum EP, Shenton LR (1971) Comments on the distribution of indices of diversity. Stat Ecol 3:315–359

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

Echart-Almeida C, Cavalli-Molina S (2000) Hordein variation in Brazilian barley varieties (*Hordeum vulgare* L.) and wild barley (*H. euclaston* Steud. and *H. stenostachys* Godr.). Genet Mol Biol 23:425–433

Field JM, Shewry PR, Miflin BJ (1982) The purification and characterization of homologous high molecular weight storage proteins from grain of wheat, rye and barley. Theor Appl Genet 62:329–336

Frank R, Blattner, Ana G, Badani Méndez (2001) RAPD data do not support a second center of barley domestication in Morocco. Genet Res Crop Evol 48:13–19

Freisleben R (1940) Die phylogenetische Bedeutung asiatischer Gersten. Der Züchter 12:257–272

Jaradat AA (1991) Grain protein variability among populations of wild barley (*Hordeum spontaneum* C. Koch) from Jordan. Theor Appl Genet 83:164–168

Kahler AL, Allard RW (1981) Worldwide patterns of genetic variation among four esterase loci in barley (*Hordeum vulgare* L.). Theor Appl Genet 59:101–111

Køie B, Ingversen J, Andersen AJ, Doll H, Eggum BO (1976) Composition and nutritional quality of barley protein. In: Evaluation of seed protein alterations by mutation breeding. IAEA, Vienna, pp 55–61

Ma Dequan (2000) Genetic resources of Tibetan barley in China. China Agriculture Press, Beijing, pp 216–254

Miflin BJ, Shewry PR (1977) Techniques for the separation of barley and maize seed proteins. EEC, Luxemburg

Molina-Cano JL, Moralejo M (1999) Further evidence supporting Morocco as a center of origin of barley. Theor Appl Genet 98:913–918

Nei M (1973) Analysis of diversity in subdivided populations. Proc Natl Acad Sci USA 70:3321–3323

Nevo E, Beiles A, Storch N (1983) Microgeographic edaphic differentiation in hordein polymorphism of wild barley. Theor Appl Genet 64:123–132

Saghai Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Saghai Maroof MA, Allard RW, Zhang Q (1990) Genetic diversity and ecogeographical differentiation among ribosomal DNA alleles in wild and cultivated barley. Proc Natl Acad Sci USA 87:8486–8490

Shewry PR, Finch RA, Parmar S, Franklin J, Miflin BJ (1983) Chromosomal location of *Hor-3*, a new locus governing storage proteins in barley. Heredity 50:179–189

Takahashi R (1955) The origin and evolution of cultivated barley. Adv Genet 7:227–266

Vavilov NI (1926) Studies on the origin of cultivated plants (Russian with English summary). Bull Appl Bot Genet Plant Breed USSR 16:1–248

Xu TW (1982) Origin and evolution of cultivated barley in China (in Chinese with English abstract). Acta Genet Sin 9:440–446

Zemede Asfaw (1989) Variation in hordein polypeptide pattern within Ethiopian barley, *Hordeum vulgare* L. (Poaceae). Hereditas 110:185–191

Zhan Xiaoyan, Yu Zhilong, Huang Peizhong (1991) Research of hordein polymorphism in barley from China (in Chinese). Acta Genet Sin 18:252–262

Zhang Q, Dai XK (1991) Patterns of diversity and geographical differentiation of isozymes in cultivated barley of Tibet. In: Munck L (ed) Barley genetics VI: Proc 6th Int Barley Genet Symp, Helsinborg, Sweden, pp 25–28

Zhang Qifa, Saghai Maroof MA, Allard RW (1990) Worldwide pattern of multilocus structure in barley determined by discrete log-linear multivariate analyses. Theor Appl Genet 80:121–128

Zhang Qifa, Dai Xiankai, Saghai Maroof MA (1992a) Comparative assessment of genetic variation at 6 isozyme loci in barley from two centers of diversity: Ethiopia and Tibet. Acta Genet Sin 19:236–243

Zhang Qifa, Saghai Maroof MA, Yang GP (1992b) Ribosomal DNA polymorphisms and the Oriental-Occidental genetic differentiation in cultivated barley. Theor Appl Genet 84:682–687

Zhang Qifa, Yang GP, Dai Xiankai, Sun JZ (1994) A comparative analysis of genetic polymorphism in wild and cultivated barley from Tibet using isozyme and ribosomal DNA markers. Genome 37:631–638