Theor Appl Genet (1989) 78: 281–286

Genetic diversity of six isozyme loci in cultivated barley of Tibet

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Received February 10, 1989; Accepted March 29, 1989
Communicated by P. M. A. Tigerstedt

Summary. A random sample of 463 accessions of cultivated barley from the Tibet *Hordeum* germplasm collection was assayed electorphoretically for genetic diversity at six isozyme loci. Two loci (*Acp-1* and *Got-1*) were found to be monomorphic and extensive variation was detected at the remaining four loci (*Est-1, Est-2, Est-3* and *Est-4*). The allelic composition of Tibetan barley appeared to be distinct as compared to the results of previous studies of barleys from other parts of the world. Partitioning of genetic diversity showed that approximately 96% of the total variation was maintained at the within-subregion level and only about 4% was accounted for by differentiation among the eight subregions. Analysis of multilocus genotypes revealed non-random association of the alleles at the four loci, both in the entire sample and in all the subregions, although the four major multilocus genotypes did not show significant departure from the expectation based on complete random association. The possible causes for the establishment of these multilocus associations were discussed.

Key words: Population genetics – *Hordeum vulgare* – Isozyme – Single-locus genotype – Multilocus association

Introduction

The possible significance of Tibetan barley in the origin and evolution of cultivated barley has been a concern for over half a century (e.g. Åberg 1938; Fresleben 1943; Brücher and Åberg 1950; Takahashi 1955, 1964; Shao 1981; Xu 1982). In 1938, Åberg discovered a six-rowed wild barley, which was named *Hordeum agriocrithon* Åberg. (Åberg and Wiebe 1945), in a wheat seed lot collected from Taofu, Sichuan Province, in southwest China. This finding led to the hypothesis that this wild barley was one of the progenitors of cultivated barleys. Subsequently, Fresleben (1943), Brücher and Åberg (1950) and Schiemman (1951) discovered more seeds of six-rowed wild barley from seed samples of other cereals collected from central and southern Tibet, and isolated several distinctive varieties of *H. agriocrithon*. This finding led Brücher and Åberg (1950) to consider Tibet as one of the major gene centers of barley. Despite numerous speculations, little research has been done regarding the extent of genetic diversity present in Tibetan barleys, presumably because of the sparse representation of samples from this region in all the known world collections of *Hordeum* germplasm.

Recently, several expeditions were made to the Qinghai-Xizang highlands through the joint efforts of the Chinese National Academy of Science and the Chinese Academy of Agricultural Science; a large number of barley stocks was collected in these expeditions. It was revealed that barleys in Tibet are extremely variable: almost all the forms of cultivated barley that have been observed in other parts of the world also occur in Tibet (Xu 1982). In addition, two wild relatives, *H. spontaneum* Koch. and *H. agriocrithon*, have also been found to be widely distributed in the Qinghai-Xizang highlands and western Sichuan Province. There is extensive variation in a series of morphological characters such as naked versus covered seed, two- versus six-row, long versus short awn, presence versus absence of lateral spikelets, lemma color, seed color, seed weight, plant height and maturing date (X. Dai, unpublished data). Differences of chromosome banding pattern and isozymes have also been ob-

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served between cultivated species and their wild relatives (Yao 1982; Zhou et al. 1983).

In this paper, we report our survey of genetic diversity of cultivated barley from Tibet at six isozyme loci. Our main objective was to assess the extent and distribution pattern of genetic polymorphism in cultivated barley grown all over Tibet, as the first step toward a comprehensive investigation of the total genetic variation maintained in Tibetan barleys.

Materials and methods

Ecological background

Tibet (now spelt as Xizang), constituting 12.5% of the total area of China (Fig. 1), is located on the Qinghai-Xizang highlands which are known as the "roof of the world". The entire region consists of several of the highest mountain ranges in the world, with an average altitude above 5,000 m. For most of this region, the avarage annual temperature is around 0 °C, and the average temperature of the warmest month (July) is around 10 °C. Only in valleys of low elevation is the annual temperature substantially higher than 0 °C and the temperature of the warmest month near 15 °C.

Barley is widely distributed in Tibet, ranging from valleys with an altitude 2,200 m to plateaus and mountain slopes at 4,750 m above sea level, including locations with July temperatures below 10 °C, and less than 100 days with daily temperature of ≥ 5 °C. Most of the cultivated barley in Tibet is grown in areas with elevations about 4,000 m above sea level, and the majority of these places are very dry. In many areas, the annual precipitation is below 500 mm and relative humidity is under 50%. In the extreme case of western Tibet, the annual rainfall is only 68.9 mm and relative humidity is 32%. It is clear that the environmental conditions of these barley-growing areas represent the limits of distribution for most (if not all) crop species.

The region of Tibet is divided into eight agricultural-geographical subregions as shown in Fig. 1, according to ecological conditions (Chen et al. 1984). The subregions differ markedly in altitude, temperature, humidity, precipitation, vegetation and many other physical and biological factors which exert a profound influence on the agriculture of each region. There is also considerable heterogeneity in eco-geographical conditions within each subregion, which has led to notable differentiation in agriculture and crop types within subregions.

Sampling

The materials used in this study were 463 accessions of cultivated barley (*Hordeum vulgare* L.) taken at random from the *Hordeum* collection maintained at the Tibetan Institute of Agriculture in Lhasa, the capital city of Tibet Autonomous Region, China. Usually, single heads were collected from the fields, each head was planted in a single row for multiplication and seeds of the same row were harvested in bulk and maintained as an accession. In very rare cases, plants in the same row showed marked morphological differences, the seeds were harvested separately and maintained as different accessions. Altogether, 58 of the 72 counties with representatives of all the eight regions were included in this sample, which is believed to represent almost the entire ecological range of barley-growing areas in Tibet (Fig. 1). The number of accessions assayed from each subregion is listed in Table 1.

Electrophoretic assay

Each accession was assayed for six enzyme loci following the methods described by Kahler and Allard (1970) and Kahler et al. (1981). The enzymes assayed are coded for by genes of four esterase loci (*Est-1, Est-2, Est-3* and *Est-4*), one acid phosphatase locus (*Acp-1*) and one glutamate oxalate transaminase locus (*Got-1*). A standard variety "Atlas" with known banding pattern of all loci was inserted in the middle of each gel. Two seedlings were assayed for each accession, and the alleles were scored according to their mobility in the gel following the convention of Kahler and Allard (1970) and Kahler et al. (1981). Identical banding patterns were observed for paired seedlings of every accession.

Results

Allelic frequencies

The loci *Got-1* and *Acp-1* were found to be monomorphic for alleles with migration distances 2.3 cm (from origin)

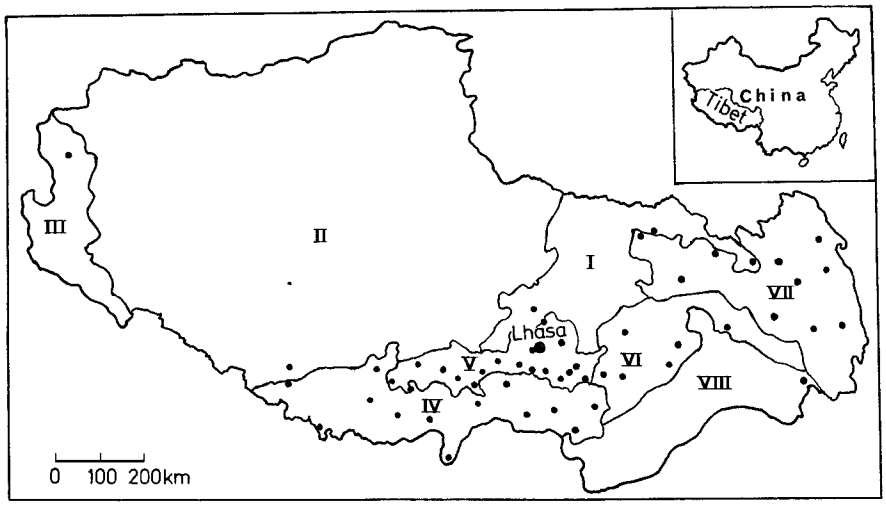

Fig. 1. Schematic diagram of the geographical location of Tibet in China (*upper right*), and distribution of barley samples included in the present study in the eight agricultural geographical subregions of Tibet. I, II, etc. are subregions, and *dots* represent counties from which barley stocks were collected and assayed

Table 1. Frequencies of all the alleles and standard errors of the major alleles (in parentheses) at four esterase loci in cultivated barley from eight subregions of Tibet

Subregion	*Est-1*			*Est-2*					*Est-3*			*Est-4*			*N*
	[a]0.2	1.0	1.8	1.6	2.3	2.7	3.4	3.9	4.4	4.9	5.4	6.4	6.6	null	
	[b]3	2	1	2	5	1	4	3	3	2	1	2	1	0	
1	0.909 (0.061)		0.091			0.909 (0.061)		0.091	0.091	0.091	0.818 (0.082)	0.727 (0.095)	0.273		11
2	1.000					1.000				0.333	0.667 (0.136)	0.500 (0.144)	0.500		6
3	1.000					1.000				1.000		1.000			1
4	[c]0.876 (0.025)	0.034	0.090	0.011	0.011	0.899 (0.022)	0.067	0.011	0.124	0.225	0.652 (0.036)	0.534 (0.037)	0.421	0.045	89
5	0.943 (0.010)	0.008	0.050		0.075	0.877 (0.014)	0.034	0.004	0.034	0.218	0.747 (0.019)	0.609 (0.021)	0.356	0.034	261
6	0.964 (0.025)		0.036		0.071	0.893 (0.041)	0.036		0.036	0.357	0.607 (0.065)	0.607 (0.065)	0.321	0.071	28
7	0.931 (0.024)	0.052	0.017		0.017	0.914 (0.026)	0.069		0.052	0.121	[d]0.828 (0.035)	[d]0.724 (0.041)	0.259	0.017	58
8	1.000					1.000				0.750	[d]0.250 (0.108)	[d]0.250 (0.108)	0.750		8
Unknown	1.000					1.000				1.000			1.000		1
Entire sample	0.931 (0.008)	0.017	0.052	0.002	0.051	0.897 (0.010)	0.043	0.006	0.054	0.227	0.719 (0.015)	0.604 (0.016)	0.362	0.035	463

[a] 0.2, 1.0, 1.8, etc. are migration distances (cm from the origin)
[b] 3, 2, 1, etc. are allele designations after recoding
[c,d] are significantly different from the allele frequency in the entire sample at probability levels 0.05, 0.01, respectively

Table 2. Genic diversity index (H) for four esterase loci among barleys from different subregions of Tibet

Subregion	*Est-1*	*Est-2*	*Est-3*	*Est-4*	Average
1	0.165	0.165	0.314	0.397	0.260
2	0	0	0.444	0.500	0.236
3	0	0	0	0	0
4	0.233	0.187	0.509	0.536	0.364
5	0.108	0.206	0.393	0.501	0.302
6	0.069	0.196	0.503	0.523	0.323
7	0.130	0.159	0.297	0.408	0.249
8	0	0	0.375	0.375	0.187
Entire H_T	0.130	0.191	0.428	0.502	0.313
Sample H_s	0.128	0.188	0.400	0.491	0.302
H_s/H_T	0.985	0.984	0.935	0.978	0.964

and 2.0 cm, respectively, both of which were the same as the variety Atlas. Consequently, data of these two loci were not included in subsequent analyses.

The occurrence and frequencies of alleles in each subregion and in the entire sample for the remaining four esterase loci are presented in Table 1. In the entire sample, three alleles were observed for *Est-1*, five for *Est-2*, three for *Est-3* and three for *Est-4*. At the *Est-1* and *Est-2* loci, there was one very frequent allele (≥ 0.90), and one of the other alleles was also observed at a substantial frequency (≥ 0.05). The frequencies of the major alleles at the *Est-3* and *Est-4* loci (0.609 and 0.719, respectively) were lower than those at the *Est-1* and *Est-2* loci. This pattern was similar in all subregions with relatively large samples (≥ 28) surveyed.

Genetic diversity and eco-geographical differentiation

Genetic diversity ($H = 1 - \sum p_i^2$, where p_i is the frequency of the i^{th} allele) at each locus was calculated for each subregion and for the entire sample (Table 2). *Est-4* had the highest diversity ($H = 0.502$) in the entire sample, followed by *Est-3* (0.428), *Est-2* (0.191) and *Est-1* (0.130). This was also true for all the subregions.

The level of diversity for a given region can be evaluated by calculating the average diversity index over loci ($\bar{H} = \sum H/n$, n = number of loci). Among regions with relative large sample sizes, region 4, a long and narrow area in southern Tibet, appeared to have the highest level of diversity ($H = 0.364$), followed by regions 6 (0.323) and 5 (0.302), whereas barley from region 7 in the northeast was the least variable (Table 2).

The total diversity of a large population (H_T) can be partitioned into components reflecting genetic distance between subpopulations (D_{ST}) and genetic polymorphism within subpopulations (H_S) as the following (Nei 1972), $H_T = H_S + D_{ST}$ where H_S is estimated by $\sum N_i H_i / N$ (N_i and H_i are, respectively, the sample size and diversity index of the i^{th} subregion; N is the size of the entire sample).

Table 3. Observed and expected numbers of multilocus genotypes in four subregions and in the entire sample of Tibetan barleys. Only those with observed or expected numbers $\geqslant 5$ in the entire sample are listed

Multilocus genotype	Subregion								Entire sample	
	4		5		6		7			
	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.	obs.	exp.
1111	0	3	1	5	0	0	0	1	1	9[b]
1112	0	2	0	3	0	0	0	0	0	6[a]
1431	3	0.04[b]	4	0.01[b]	1	<0.01[b]	0	0	8	1[b]
3110	3	2	5	6	1	1	1	1	9	10
3111	26	24	99	99	10	9	35	29	180	168
3112	25	19	66	58	4	5	10	10	110	101
3121	12	8	40	29[a]	7	5	5	4	67	53
3122	8	7	14	17	2	3	2	2	34	32
3131	1	5	0	5[a]	0	0	0	2	1	13[b]
3132	0	4[a]	0	3	0	0	0	1	0	8[b]
3411	0	2	0	4[a]	0	0	0	2	0	8[b]
3412	0	1	0	2	0	0	0	1	0	5[a]
3511	0	0.30	9	8	0	1	0	1	9	10
3512	1	0.02[b]	9	5	2	0.37[b]	1	0.20	13	6[b]
Others	10	12	14	17	1	4	4	4	31	33
N	89		261		28		58		463	
χ^2	289[b]		1,622[b]		110[b]		12.7		108[b]	

[a,b] observed and expected numbers are significantly different at probability levels 0.05, 0.01, respectively

The H_S values are also listed in Table 2. The within-region component of *Est-1* (0.128) accounted for 98.5% of total variation, followed by *Est-2* (98.4%), *Est-4* (97.8%) and *Est-3* (93.5%), with an average of 96.4%. Thus, within-accession diversity accounted for 93%–98% of the total genetic diversity existing at the four esterase loci, and only about 2%–7% of the total variation was due to differences in genetic composition among barleys from different subregions.

However, the small proportion accounted for by the genetic distance did not imply that this component was trivial. To see this, the standard error ($SE=\sqrt{p(1-p)-2N}$, where p is the allele frequency and N the number of individuals surveyed) for the frequency of the major allele at each locus was calculated for each subregion (Table 1). Assuming the allele frequencies in the entire sample represent those of all the barley grown in Tibet, differences in allele frequency between a subregion and the entire sample by 1.960 or 2.576 standard errors would be considered as statistically significant at probability levels 0.05 or 0.01, respectively. Thus, the frequency of major allele at *Est-1* in subregion 4 was significantly lower than that of the entire sample. Likewise, the major alleles of *Est-3* and *Est-4* in subregions 7 and 8 differed significantly from the entire sample. This indicated that significant differentiation of genetic compositions also took place among barleys from different subregions, although the proportion accounted for by this component was small as compared to within-subregion diversity.

Multilocus genotypes

For ease of description, we recoded the alleles of each locus following the designations used in most of the previous work (e.g. Weir et al. 1972, 1974; Kahler and Allard 1981), in order to be consistent with those studies. For example, at the locus *Est-1*, allele 1.8 was recoded as allele 1, allele 1.0 as allele 2 and allele 0.2 as allele 3. The designations of the recoded alleles are also given in Table 1, together with the migration distance.

Table 3 presents the multilocus genotypes with observed or expected numbers larger than or equal to 5 in the entire sample. Also listed are the observed and expected numbers for subregions with relatively large sample sizes (subregions 4–7). A χ^2 test showed highly significant non-random association of alleles at the four loci in three of the four subregions (subregions 4, 5 and 7) and in the entire sample. The behavior of individual multilocus genotypes can be examined by calculating the standardized residues [$SR=(obs-exp)/\sqrt{exp}$] resulting from the goodness-of-fit test. Asymptotically, a standardized residue follows a normal distribution with mean zero and unit variance (Dr. W. O. Johnson, personal communication). Thus, the absolute value of 1.960 or 2.576 for an SR indicates a statistically significant difference between the expected and observed numbers of a particular multilocus type at probability levels of 0.05 or 0.01, respectively.

It is clear from Table 3 that in the entire sample, there was not significant departure between the expected and observed numbers for the four major multilocus genotypes (3111, 3112, 3121 and 3122), although the observed numbers of these genotypes were slightly in excess. The observed numbers of another two genotypes (3110 and 3511), as well as those which are not listed in the table (others), were also very close to the expectation. Thus, the frequencies of these multilocus genotypes can simply be ascribed to the allele frequencies at individual loci. Similar non-significant departures between the observed and expected numbers of those genotypes were also observed in the four subregions.

The observed frequencies of two multilocus genotypes, 1431 and 3512, were significantly higher than expected in the entire sample and in almost all the subregions, whereas genotypes 1111 and 3131 were highly significant in deficiency. Furthermore, genotypes 1112, 3132, 3411 and 3412, which were expected to occur in measurable frequencies, were not observed in these 463 accessions assayed.

Discussion

Population diversity of these four esterase loci of barley has been a subject of extensive studies (see Allard 1988, for a recent review). All the alleles observed in the present study of Tibetan barley have also been found in electrophoretic assays of the USDA world barley collections (Kahler and Allard 1981; Q. Zhang, M. A. Saghai-Maroof, R. W. Allard, unpublished data). The frequencies of the most frequent alleles of *Est-2* (2.7) and *Est-4* (6.4) in the present survey corresponded well to those found in the USDA barley collection in which these two alleles were the most frequent ones of the respective loci, both in every regional sample and worldwide. The most frequent allele of *Est-3* (5.4) in the present study was found to be the most frequent in barleys grown in Ethiopia and middle South Asia (Afghanistan, India, Iran and Nepal), although it was not the most frequent allele of that locus worldwide, as deduced from the study of the USDA collection.

However, the finding that allele 0.2 of *Est-1* was at a very high frequency was different from that of barleys grown in other parts of the world where the frequency of this allele has never exceeded 0.5, although it was the most frequent allele of that locus in barley from southwest Asia (Cyprus, Iran, Israel, Jordan, Syria, Turkey and Yeman). Furthermore, *Acp-1* was monomorphic for allele 2.0 in Tibetan barley, whereas this locus was highly polymorphic in all the regions of the world (Q. Zhang, M. A. Saghai-Maroof, R. W. Allard, unpublished data). This suggests that the genetic compositions of Tibetan barleys are substantially different from that of barleys grown in other parts of the world. This is not surprising because the ecological conditions of Tibet are so distinctive that barley adapted to this region has to be distinctive in genetic constitution.

The results have also uncovered extensive genetic variation in cultivated barley of Tibet, about 96% of which was maintained at the within-subregion level. Subregion 5 of central Tibet is the oldest and most intensive agricultural area in Tibet, according to historical records (Chen et al. 1984). Barley in this area has been grown as the most important crop since very ancient times, and is assumed to contain a major portion of the genetic diversity existing in Tibetan barley. Our results revealed, however, that the level of genetic variation maintained in peripheral populations (e.g. subregions 4 and 6) might also contain comparable amounts of genetic variation. This suggested the need for more intensive collection of barley stocks from those peripheral populations for adequate conservation of existing genetic resources, as those regions were likely to be under-represented in the collections maintained in China, based on the extrapolation from the present data.

Multilocus associations of these four esterase loci in barley have been established as a feature in experimental populations and in the world barley collection (Allard 1988). Our present study has also established the pattern of multilocus association in Tibetan cultivated barley. The multilocus association did not appear to be very different from one subregion to another. The four major multilocus genotypes occurred in proportions close to expectation of random association among alleles of these four loci, although overall the observed numbers were slightly in excess as compared to the expected. Quite a few genotypes were in deficiency, and several genotypes, which were expected to occur at measurable frequencies within the limit of detection of the present sample sizes, were not observed.

It is possible that the absence of these genotypes has resulted from sampling errors. To examine this possibility, we tentatively view the occurrence of a specific genotyps as following a Poisson distribution with the mean equal to the expected number. Thus, it can easily be calculated that, with the expected number larger than 3, the probability for the event not to occur (i.e. observed zero) is less than 0.05. It can be seen from Table 3 that all the genotypes with zero observed values in the entire sample have as expected numbers ≥ 5. Thus, it is unlikely that the absence of those multilocus genotypes was due to sampling alone. Studies have been initiated to investigate possible relationships between alleles or genotypes and ecological factors which, we believe, may be helpful in identifying possible causes for the deficiency and excess of specific multilocus gentoypes.

Acknowledgements. This research was supported by a grant from the Chinese National Natural Science Foundation.

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