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Research Article

Genetic diversity and geographical differentiation of cultivated six-rowed naked barley landraces from the Qinghai-Tibet plateau of China detected by SSR analysis

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Abstract

Cultivated six-rowed naked barley (*Hordeum vulgare* ssp. *hexastichon* var. *nudum* Hsü) is the oldest cultivated barley in China. We used 35 simple sequence repeat (SSR) markers selected from seven barley linkage groups to study the genetic diversity, geographical differentiation and evolutionary relationships among 65 *H. vulgare* ssp. *hexastichon* landrace accessions collected from the Qinghai-Tibet plateau of China, 25 accessions from Tibet (TB), 20 from Qinghai (QH) and 20 from Ganzi (GZ) prefecture in Sichuan province. At the 35 SSR loci we identified 248 alleles among the 65 accessions, 119 (47.98%) of the alleles being common alleles. We also found that the TB accessions possessed 47 private alleles, about 1.5 times more than the 31 private alleles found in the QH accessions and about 5 times more than 9 private alleles found in the GZ accessions. Generally, the TB accessions showed significantly higher genetic diversity than either the QH or GZ accessions whereas no significant difference in genetic diversity was found between the QH and GZ accessions. Partitioning analysis of genetic diversity showed that about 81% of the total variation was due to within-subgroup diversity and about 19% was clearly accounted for by geographical differentiation among the three subgroups. The distributions of alleles for most loci (71.4%) were significantly different among the three subgroups and geographical differentiation could be found according to the distribution of SSR alleles. Cluster analysis indicated that most of the accessions could be clustered into groups which basically coincided with their geographical distribution. These results suggest that Tibet might be a center of genetic diversity for cultivated barley, the cultivated six-rowed naked barley on the Qinghai-Tibet plateau of China may have evolved in Tibet and spread to Qinghai and then to Ganzi prefecture of Sichuan province.

Key words: Barley, genetic diversity, Qinghai-Tibet plateau, geographical differentiation, simple sequence repeat marker.

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Introduction

Barley (*Hordeum vulgare* L.) is one of the oldest cultivated crops in the world and studying genetic diversity and evolutionary relationships in barley is important for the effective conservation and utilization of barley genetic resources. Early studies suggested that there were two original centers of cultivated barley, one in the Fertile Crescent (Ancient Egypt, the Levant and Mesopotamia) as indicated by the widespread dispersion of *Hordeum spontaneum* Koch, the nearest wild ancestor of two- and six-rowed cultivated barley, in this region (Harlan, 1976) and another in the Tibetan region of China on the Qinghai-Tibet plateau ('the roof of the world') supported by the discovery of *Hordeum agriocrithon* Åberg, a six-rowed wild barley considered an ancestor of two- and six-rowed cultivated barley (Vavilov, 1926; Åberg, 1938; Brücher and Åberg, 1950).

Many studies have demonstrated that Tibetan wild barley populations were clearly different from the Fertile Crescent wild barley in respect to their distribution, ecology, morphology, archaeology, cytogenetics and isozyme complement (Xu, 1975, 1982; Zhou, 1981; Shao, 1982; Yao, 1982). This supports the hypothesis of separate evolutionary systems leading to Tibetan two-rowed wild barley becoming the ultimate progenitor of Chinese cultivated

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barley, Tibetan six-rowed wild barley being an intermediate form in the processes of transforming two-rowed wild barley to six-rowed cultivated barley (Xu, 1982). Naked barley (*Hordeum vulgare* var. *nudum*), also called Qingke, is a major food used to produce 'Zanba' by ethnic Zangs inhabiting the Qinghai-Tibet plateau of China. Cultivated six-rowed naked barley (*H. vulgare* ssp. *hexastichon* var. *nudum* Hsü) is the earliest cultivated barley in China (Fu *et al.*, 2000; Xu and Feng, 2001). Evidently, study on genetic diversity and geographical differentiation of cultivated six-rowed naked barley landraces from the Qinghai-Tibet plateau will be useful in understanding the evolutionary relationship of barley.

At present, most studies on the genetic diversity and evolution of cultivated barley from the Qinghai-Tibet plateau of China have dealt with morphology (Xu, 1986), botanical classification (Xu, 1982), cytogenetics (Yao, 1982; Shao, 1986) and isozymes (Shao, 1986; Dai and Zhang, 1989; Zhang *et al.*, 1992a, 1994; Sun *et al.*, 1995). However, some researchers have used DNA molecular markers, including restriction fragment length polymorphisms (RFLP) (Zhang *et al.*, 1992b, 1994), random amplified polymorphic DNA (RAPD) (Hong *et al.*, 2001) and ribosomal DNA spacer-length techniques (Li *et al.*, 2003), although, except for Tibetan landraces, cultivated six-rowed naked barley landraces from the Qinghai-Tibet plateau have rarely been included in such studies. Simple sequence repeat (SSR), or microsatellite, analysis possess a number of advantages over other forms of genetic analysis, including a high level of polymorphisms, locus specificity, codominance, reproducibility, random distribution throughout the genome and is also methodologically simple (Saghai-Maroof *et al.*, 1994; Feng *et al.*, 2002). Techniques based on SSR technology are useful in evaluating and characterizing genetic diversity, phylogenetic development and evolution as well elucidating the relationships within and between species and populations of members of the genus *Hordeum* (Saghai-Maroof *et al.*, 1994; Russell *et al.*, 1997; Davila *et al.*, 1998; De Bustos *et al.*, 1999; Fernández *et al.*, 2002; Zhang *et al.*, 2002; Feng *et al.*, 2003).

In the research described in this paper we used SSR markers covering the seven barley SSR linkage groups (Liu *et al.*, 1996) to investigate the genetic diversity and geographical differentiation of 65 cultivated six-rowed naked barley landraces collected from the Qinghai-Tibet plateau of China.

Materials and Methods

Plant materials

In this study we used 65 landrace accessions (Table 1) of the cultivated six-rowed naked barley *H. vulgare* L. ssp. *hexastichon* var. *nudum* Hsü (hereafter denominated as *nudum* barley) from different geographical locations on the Qinghai-Tibet plateau of China, of which 25 accessions were from Tibet (TB), 20 from Qinghai (QH) and 20 from Ganzi (GZ) prefecture in Sichuan province. Seeds of the different accessions were kindly provided by the following people: TB accessions by Mr. QIANG Xiao-Lin (Institute of Agricultural Sciences, Tibetan Academy of Agricultural & livestock Sciences); QH accessions by Prof. SUN Li-Jun (Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences); and GZ accessions by Mr. YANG Kai-Jun (Ganzi Institute of Agricultural Sciences in Sichuan province).

Genomic DNA extraction

The cetyltrimethylammonium bromide (CTAB) method (Stein *et al*., 2001) was used to extract total DNA from about 300 mg of young leaf-tissue of each accession. The quality of the DNA was checked using agarose-gel electrophoresis and the DNA concentration estimated spectrophotometrically and the solution diluted with distilled water to a final working DNA concentration of 20 ng μL^{-1}.

PCR amplification, electrophoresis and silver staining

We selected 35 simple sequence repeats (SSRs) (Table 2), five from each chromosome, from the genetic maps described by Liu *et al.* (1996). The primers were synthesized by a commercial company (AuGCT Biotechnology, Beijing, China). The polymerase chain reaction (PCR) was carried out in a final volume of 15 μL containing 2 μL of the 20 ng μL^{-1} genomic DNA solution described above (template DNA), 1.5 μL of 10xPCR buffer containing 15 mM Mg^{2+}, 1.5 μL of a 2.5 mM dNTP mixture, 0.5 units of rTaq DNA polymerase (TaKaRa Biotechnology, Dalian, China) and 1 μL of a 2 μM solution of the forward and reverse primers. Depending on the primer pair used, DNA amplifications were performed in a thermocycler using one of the following five PCR protocols: (1) A touchdown PCR reaction consisting of 18 cycles of a 94 °C denaturing step for 1 min and a 72 °C for 1 min extension, followed by annealing for 30 s with the temperatures decreasing by 1 °C every two cycles from 64 °C to 55 °C. The PCR reaction continued for 30 additional cycles at 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min. The reaction ended with a 5-min extension at 72 °C. (2) A similar touchdown procedure to the above protocol except that the annealing temperatures were decreased from 69 °C to 60 °C for 18 cycles, at which temperature the reaction continued for 20 additional cycles. (3) A normal PCR protocol consisting of one cycle of 94 °C for 3 min, 55 °C for 2 min and 72 °C for 1.5 min, followed by 30 cycles at 94 °C for 1 min, 55 °C for 2 min and 72 °C for 1.5 min. (4) Denaturing for 5 min at 95 °C followed by 42 cycles at 92 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min, and ending with a final 10-min elongation at 72 °C. (5) 35 reaction cycles of 96 °C for 1 min, 60 °C for

Table 1 - List and origin of cultivated six-rowed naked barley (*nudum* barley) landraces collected from the Qinghai-Tibet plateau of China used in this study.

Tibetan autonomous region (coded as TB in the text)			Qinghai province (coded as QH in the text)			Ganzi prefecture of Sichuan province (coded as GZ in the text)		
Code	National code	County of origin	Code	National code	County of origin	Code	National code	County of origin
107	ZDM4998	Milin	132	ZDM8088	Yushu	152	ZDM3829	Danba
108	ZDM5627	Naidong	133	ZDM8093	Yushu	153	ZDM3830	Yajiang
109	ZDM5855	Qiongjie	134	ZDM8096	Nangqian	154	ZDM3834	Kangding
110	ZDM5688	Nielamu	135	ZDM8098	Nangqian	155	ZDM3843	Yajiang
111	ZDM5997	Chayu	136	ZDM8115	Chengduo	156	ZDM3837	Kangding
112	ZDM5139	Zuogong	137	ZDM8181	Maqin	157	ZDM3847	Kangding
113	ZDM6019	Milin	138	ZDM8183	Maqin	158	ZDM3835	Ganzi
114	ZDM5927	Dazi	139	ZDM8135	Chengduo	159	ZDM3844	Ganzi
115	ZDM5941	Sajia	140	ZDM8196	Chengduo	160	ZDM3841	Batang
116	ZDM5950	Xietongmen	141	ZDM8200	Chengduo	161	ZDM3854	Batang
117	ZDM5964	Longzi	142	ZDM8212	Nangqian	162	ZDM3857	Batang
118	ZDM5901	Dazi	143	ZDM8139	Yushu	163	ZDM3863	Batang
119	ZDM5720	Sangri	144	ZDM8142	Banma	164	ZDM3859	Litang
120	ZDM5752	Ritu	145	ZDM8110	Yushu	165	ZDM3862	Daocheng
121	ZDM5844	Gongga	146	ZDM8160	Banma	166	ZDM3853	Xiangcheng
122	ZDM5874	Bianba	147	ZDM8112	Yushu	167	ZDM3880	Daofu
123	ZDM5693	Qusong	148	ZDM8144	Yushu	168	ZDM3839	Xinlong
124	ZDM5753	Duilongdeqing	149	ZDM8184	Yushu	169	ZDM3848	Derong
125	ZDM5781	Rikaze	150	ZDM8201	Yushu	170	ZDM3867	Shiqu
126	ZDM5696	Chaya	151	ZDM8223	Yushu	171	ZDM3882	Jiulong
127	ZDM6148	Lang						
128	ZDM5751	Longzi						
129	ZDM5999	Luolong						
130	ZDM5883	Chaya						
131	ZDM5914	Dazi						

1 min and 72 °C for 2 min, followed by a final extension for 10 min at 72 °C.

After PCR amplification 5 μL of 98% (v/v) formamide electrophoresis loading buffer containing 0.25% (w/v) bromophenol blue and 0.25% (w/v) xylene cyanole FF were added to each reaction mixture. The PCR products were denatured and separated on 6% denaturing polyacrylamide gel with 8 M urea and 1xTBE buffer running at a constant power of 60 W for one hour. A 50 to 1031 bp DNA ladder (Gene Ruler^{TM} 50bp DNA ladder, MBI Ferments) was used as a size standard and the DNA fragments were silver stained as described in Bassam *et al.* (1991).

Data analysis

The amplified DNA fragments of each SSR locus were assessed based on electrophoretic mobility using the Qbasic procedure of Rickwood *et al.* (1982) and the SSR profiles were scored for the presence (1) or absence (0) of clear bands. Genetic similarities were estimated using the DICE coefficient, 2a/(2a+b+c), where 'a' refers to alleles shared between two accessions and 'b' and 'c' to alleles present in either one of the two accessions compared (Rohlf, 1993). Similarity matrix cluster analysis was used to reveal associations among accessions based on the unweighted pair group method with arithmetic averages (UPGMA) implemented using the NTSYS-pc program (Rohlf, 1993).

Genetic diversity (H) was calculated with $H = 1 - \Sigma p_i^2$ in which p_i is the frequency of the i^{th} allele of the locus (Nei, 1973). For each locus, the frequencies of each allele in the entire sample were calculated as the expected allele frequencies, and the distribution of allelic frequencies among the three subgroups was tested using the Chi-square test (Rong *et al*., 1993). The genetic diversity of the entire sample (H_T) was partitioned into components reflecting genetic distance between subgroups (D_{ST}) and genetic polymorphism within subgroups (H_S), with genetic differentiation between subgroups (G_{ST}) being calculated as G_{ST} = 1 -

Table 2 - Primer sequences, PCR conditions, repeats and chromosomal locations of simple sequence repeat (SSR) markers of barley used in this study.

SSR	Primer sequences (5' → 3')		Chr.	Repeats	PCR[a]
HVM20	CTCCACGAATCTCTGCACAA	CACCGCCTCCTCTTTCAC	1H	$(AG)_{19}$	1
HVM43	GGATTTTCTCAAGAACACTT	GCGTGAGTGCATAACATT	1H	$(CA)_9$	1
HVM63	CGCGCAAGCATGAATACTC	ACTCACAAGTGGCGCGTAC	1H	$(GA)_9$	1
HVM64	GATGTGAAGGCTGCCTG	ACACGCCCTATTACCCAGTG	1H	$(GA)_4(GT)_7(CT)_2(GT)_4(GA)_8$	1
HVM70	CCGCCGATGACCTTCTC	ACCCACGACCTATGGCAC	1H	$(CA)_8$	3
HVCSG	CACTTGCCTACCTCGATATAGTTTGC	GTGGATTCCATGCATGCAATATGTGG	2H	$(CA)_4$, $(C)_7$	4
HVBKASI	ATTGGCGTGACCGATATTTATGTTCA	CAAAACTGCAGCTAAGCAGGGGAACA	2H	$(C)_{10}$, $(A)_{11}$	4
HVM23	TCGGTGAAGAAATACGAGGC	TCTTTGTGACCTACCGGTCC	2H	$(GA)_9$	2
HVM26	GGCTATCACATTTGGTACCATC	GCATGTGTAGGTGTTGGTGG	2H	$(CA)_{11}$	2
HVM36	TCCAGCCGAACAATTTCTTG	AGTACTCCGACACCACGTCC	2H	$(GA)_{13}$	1
HVM33	ATATTAAAAAAGGTGGAAAGCC	CACGCCCTCTCCCTAGAT	3H	$(CA)_7$	1
HVM27	GGTCGGTTCCCGGTAGTG	TCCTGATCCAGAGCCACC	3H	$(GA)_{14}$	1
HVM60	CAATGATGCGGTGAACTTTG	CCTCGGATCTATGGGTCCTT	3H	$(AG)_{11}$, $(GA)_{14}$	1
HVM62	TCGCGACCAGACGAGAAG	AGCTAGCCGACGACGCAC	3H	$(GA)_{11}$	1
HVM44	AAATCTCAGGTTCGTGGGCA	CCACGGAGACCACCTCACTT	3H	$(GA)_8$	1
HVM40	CGATTCCCCTTTTCCCAC	ATTCTCCGCCGTCCACTC	4H	$(GA)_6(GA)_4(GA)_7$	1
HVM3	ACACCTTCCCAGGACAATCCATTG	AGCACGCAGAGCACCGAAAAAGTC	4H	$(AT)_{29}$	3
HVM68	AGGACCGGATGTTCATAACG	CAAATCTTCCAGCGAGGCT	4H	$(GA)_{22}$	1
HVM67	GTCGGGCTCCATTGCTCT	CCGGTACCCAGTGACGAC	4H	$(GA)_{11}$	1
WMS6	CGTATCACCTCCTAGCTAAACTAG	AGCCTTATCATGACCCTACCTT	4H	$(GA)_{40}$	5
HVDHN7	TTAGGGCTACGGTTCAGATGTT	ACGTTGTTCTTCGCTGCTG	5H	$(AAC)_5$	4
HVDHN9	CATGGACAAGATCAAGGAGAAG	CCCATTATTTATCTGTAGGAACGC	5H	$(AC)_6$	4
HVLEU	TTGGAAGTGTACAGCAATGGAG	TGAAAGGCCCCACAAGATAG	5H	$(ATTT)_4$	4
HVM6	CATGAATGAATGATTGGTTTTG	CGCATCCGTATGTATGAGTAA	5H	$(GA)_9$	1
HVM30	AGTGGGGAATGAGAGAATGG	TGCTTGTGGGTCATCACAC	5H	$(CA)_8$	2
HVM14	CGATCAAGGACATTTGGGTAAT	AACTCTTCGGGTTCAACCAATA	6H	$(CA)_{11}$	1
HVM34	ACCATGTTGCGTGTTGCTT	CGGTTCGAAATCGAGTGG	6H	$(GA)_{10}$	2
HVM65	AGACATCCAAAAAATGAACCA	TGGTAACTTGTCCCCCAAAG	6H	$(GA)_{10}$	1
HVM22	TTTTGGGGGATGCCTACATA	TTTCAAATGGTTGGATTGGA	6H	$(AC)_{13}$	1
HVM74	AGGAAGTCATTGCGTGAG	TGATCAAGAATGATAACATGG	6H	$(GA)_{13}$	3
HVM49	CTCTATAGGCACGAAAAATTCC	TTGCACATATCTCTCTCTGTCACA	7H	$(CA)_{12}$	1
HVCMA	GCCTCGGTTTGGACATATAAAG	GTAAAGCAAATGTTGAGCAACG	7H	$(AT)_9$	4
HVM5	AACGACGTCGCCACACAC	AGGAACGAAGGGAGTATTAAGCAG	7H	$(GT)_6$, $(AT)_{16}$	3
HVM4	AGAGCAACTACCAGTCCAATGGCA	GTCGAAGGAGAAGCGGCCCTGGTA	7H	$(AT)_9$	3
HVM51	TCTAAATTACCTTCCCAGCCA	AAGCAGACATGTAGGAGGTCA	7H	$(GA)_3(GGGA)_3$, $(GA)_8$	1

[a]The numbers represent one of the five PCR conditions described in the materials and methods section.

Hs/H_T (Nei, 1973). The comparisons of genetic diversity were carried out using the Z-test (Zhang and Allard, 1986; Zhang *et al.*, 1992).

Results

Allelic variation of SSRs

Total alleles, common alleles and the number of private alleles are shown in Table 3. A total of 248 alleles were detected at the 35 SSR loci, with an average of 7.09 alleles per locus in the entire sample. The number of alleles varied from 16 at the *HVM68* locus to 2 each at the *HVM3*, *HVM44* and *HVM49* loci. Seven of the 35 loci showed more than 10 alleles per locus. The alleles for the 35 loci were distributed among the three geographical location accession subgroups (TB, QH and GZ) as follows: 193 alleles for TB, 180 for QH and 152 for GZ. No allele was detected at the *HVM44* locus in the QH accessions and *HVM14* locus in the GZ accessions and only one allele each was detected for the *HVM34*, *HVM49* and *HVM60* loci in the TB accessions, the *HVM64* locus in the QH accessions and the *HVM23*, *HVM49* and *HVM64* loci in the GZ accessions. The average number of alleles per locus and standard deviation (SD) of the three subgroups were as follows: TB (5.51 ± 3.17) > QH (5.14 ± 2.88) > GZ (4.34 ± 2.72). Of the 248 alleles, 119 (47.98%) were common to the three subgroups (common alleles) and the average number of common alleles was 3.40 ± 2.66, with the highest number of

Table 3 - Allele sizes in base-pairs (bp), total number of alleles and number of common and private simple sequence repeat (SSR) for three subgroups of cultivated six-rowed naked barley (*nudum* barley) collected from Tibet (TB), Qinghai (QH) and Ganzi (GZ) in the Qinghai-Tibet plateau of China.

Chromo-some	SSR	Allele size (bp)	Total alleles				Common alleles				Private alleles		
			TB	QH	GZ	Total	TB&QH	TB&GZ	QH&GZ	Total	TB	QH	GZ
1H	*HVM20*	137-232	4	6	5	8	3	3	3	2	0	2	1
	HVM43	109-294	11	4	4	11	4	4	3	3	6	0	0
	HVM63	121-249	10	3	2	10	3	2	2	2	7	0	0
	HVM64	119-233	8	1	1	8	7	1	0	0	6	0	0
	HVM70	123-381	4	4	4	4	4	4	4	4	0	0	0
2H	*HVM23*	387-700	3	5	1	5	3	1	1	1	0	2	0
	HVM26	195-458	7	7	7	7	7	7	7	7	0	0	0
	HVM36	101-165	6	9	3	10	5	2	3	2	1	3	0
	HVCSG	196-396	7	6	6	7	6	6	5	5	0	0	0
	HVBKASI	324-361	3	4	4	4	3	3	4	3	0	0	0
3H	*HVM27*	189-319	12	12	12	12	12	12	12	12	0	0	0
	HVM33	245-336	6	7	6	9	6	4	4	4	0	1	2
	HVM44	196-235	2	0	2	2	0	2	0	0	0	0	0
	HVM60	150-691	1	6	7	8	0	0	6	0	1	0	1
	HVM62	242-484	5	5	8	8	5	5	5	5	0	0	3
4H	*HVM3*	443-466	2	2	2	2	2	2	2	2	0	0	0
	HVM40	150-214	6	11	7	11	6	6	7	6	0	3	0
	HVM67	161-185	4	6	4	6	4	4	4	4	0	2	0
	HVM68	193-278	13	13	10	16	10	10	10	10	3	3	0
	WMS6	310-362	7	3	3	7	3	3	3	3	4	0	0
5H	*HVM6*	184-429	5	2	2	5	2	2	2	2	3	0	0
	HVM30	155-254	2	5	4	7	2	3	3	2	0	2	1
	HVDHN7	185-390	9	7	9	9	7	9	7	7	0	0	0
	HVDHN9	154-353	3	3	3	3	3	3	3	3	0	0	0
	HVLEU	283-574	3	3	2	4	2	2	2	2	1	1	0
6H	*HVM14*	104-233	9	4	0	12	1	0	0	0	8	3	0
	HVM22	333-392	9	3	3	9	3	3	3	3	6	0	0
	HVM34	747-874	1	3	2	3	1	1	2	1	0	2	0
	HVM65	132-256	7	6	3	7	6	3	3	3	1	0	0
	HVM74	293-674	3	7	5	8	3	3	4	3	0	3	1
7H	*HVM4*	233-426	5	5	5	5	5	5	5	5	0	0	0
	HVM5	292-530	4	6	4	6	3	3	4	4	0	1	0
	HVM49	103-143	1	2	1	2	1	1	1	1	0	1	0
	HVM51	106-192	5	5	5	5	5	5	5	5	0	0	0
	HVCMA	135-234	6	5	6	8	3	6	3	3	0	2	0
	Mean		5.51	5.14	4.34	7.09	4.00	3.71	3.77	3.40	1.34	0.89	0.26
	SD		3.17	2.88	2.72	3.21	2.61	2.70	2.59	2.66	2.40	1.16	0.66

common alleles (12) being detected at the *HVM27* locus while no common alleles were detected at the *HVM14*, *HVM44*, *HVM60* and *HVM64* loci. The number of alleles to specific private alleles per locus varied significantly among the three geographical subgroups with an average of 1.34 in TB, 0.89 in QH and 0.26 in GZ.

Comparison of genetic diversity

Table 4 presents the statistics relating to the genetic variation found at each locus. The average genetic diversity for the entire sample (H_T) was 0.6594 whereas the mean value for the three geographical subgroups was as follows: TB = 0.6172; QH = 0.4993; and GZ = 0.4766. There was a large variation in genetic diversity among the loci, the lowest diversity (0.1719) occurring at the *HVM70* locus and the highest (0.9862) at the *HVM14* locus. Genetic diversity in excess of 0.90 was found in eight loci in the TB subgroup, six in the QH subgroup and five in the GZ subgroup. Table 4 also shows that significant genetic diversity between the any two arbitrary-selected subgroups strongly depended on the loci involved. Genetic diversity in 10 of the 35 loci was in the order TB < QH, with only the *HVM74* showing no difference between TB and QH, while most loci (66.7%) showed a significant difference in the order TB > QH. We also found that 16 loci showed significantly larger genetic diversity in the TB and QH subgroups than in the GZ subgroup while only seven loci displayed obviously lower genetic diversity in the TB and QH subgroups than in the GZ subgroup. Similarly, 13 loci in the QH subgroup showed significantly larger genetic diversity than in the GZ subgroup whereas 11 loci in the QH subgroup presented significantly less genetic diversity than in the GZ subgroup. No difference was observed at the *HVM70* locus in respect of the QH and GZ subgroups. Generally, the genetic diversity among the three subgroups was in the order TB > QH > GZ and, on average, genetic diversity in the TB subgroup accessions was significantly larger than in either the QH or GZ subgroups whereas there was no significant difference between the amount of genetic diversity in the QH and GZ subgroup accessions.

Geographical differentiation and distribution of allelic frequencies

The total genetic diversity can be divided into within- and between-populations components, and in our case the proportion of each component varied from locus to locus. The genetic variation between subpopulations (G_{ST}) reflects the geographical differentiation of samples, the amount of differentiation among the three subgroups varying from 0.27% at the *HVM26* locus to 55.52% at the *HVM43* locus with an average of 18.58% and only five loci showing a G_{ST} value of less than 5%. The Chi-square test for the distribution of the allelic frequencies of the 35 loci among the three subgroups (Table 4) showed that two loci (*HVM4* and *HVDHN7*) displayed significant differences ($p < 0.05$) among the three subgroups and 23 loci highly

Table 4 - Genetic diversity, geographical differentiation (G_{ST}) and allele frequency distribution (χ^2) for the 35 simple sequence repeat (SSR) loci among cultivated six-rowed naked barley (*nudum* barley) collected from Tibet (TB), Qinghai (QH) and Ganzi (GZ) in the Qinghai-Tibet plateau of China.

Chromosome	SSR	Genetic diversity				Difference			G_{ST} (%)	χ^2
		TB	QH	GZ	H_T	TB-QH	TB-GZ	QH-GZ		
1H	*HVM20*	0.5968	0.6575	0.6100	0.6871	-0.0607	-0.0132	0.0475	9.84	25.98**
	HVM43	0.2752	0.0200	0.6175	0.6790	0.2552**	-0.3423**	-0.5975**	55.52	108.96**
	HVM63	0.2112	0.3050	0.5650	0.5297	-0.0938	-0.3538**	-0.2600**	34.13	48.75**
	HVM64	0.9920	0.9775	0.9975	0.8394	0.0145	-0.0055	-0.0200	2.68	87.89**
	HVM70	0	0.2775	0.2775	0.1719	0.2775**	-0.2775**	0	0.64	1.04
2H	*HVM23*	0.3824	0.4575	0.8775	0.6625	-0.0751	-0.4951**	-0.4200**	15.79	35.26**
	HVM26	0.2944	0.2775	0.1900	0.2578	0.0169	0.1044*	0.0875	0.27	0.93
	HVM36	0.3984	0.1650	0.3575	0.4457	0.2334*	0.0409	-0.1925*	29.55	46.11**
	HVCSG	0.2576	0.4225	0.5425	0.4309	-0.1649*	-0.2849**	-0.1200*	8.10	16.99
	HVBKASI	0.9968	0.1450	0.9700	0.8671	0.8518**	0.0268	-0.8250**	16.22	45.50**
3H	*HVM27*	0.5680	0.1475	0.1075	0.4811	0.4205**	0.4605**	0.0400	38.29	46.02**
	HVM33	0.7968	0.5625	0.7700	0.7807	0.2343**	0.0268	-0.2075**	8.22	34.42**
	HVM44	0.0784	0.9800	0.0975	0.5644	-0.9016**	-0.0191	0.8825**	35.91	38.32**
	HVM60	0.9936	0.6700	0.8400	0.9352	0.3236**	0.1536*	-0.1700*	9.45	51.89**
	HVM62	0.4016	0.3900	0.0475	0.6263	0.0116	0.3541**	0.3425**	44.39	49.76**
4H	*HVM3*	0.7296	0.6375	0.6750	0.7560	0.0921	0.0546	-0.0375	9.46	15.44**
	HVM40	0.9936	0.3059	0.1784	0.9508	0.6877**	0.8152**	0.1275*	34.67	51.60**
	HVM67	0.7984	0.7125	0.2525	0.6875	0.0859	0.5459**	0.4600**	12.15	58.03**
	HVM68	0.2464	0.1400	0.1000	0.2982	0.1064	0.1464*	0.0400	43.46	55.68**
	WMS6	0.9408	0.0925	0.9325	0.7946	0.8483**	0.0083	-0.8400**	14.78	37.02**
5H	*HVM6*	0.6448	0.9800	0.8000	0.9448	-0.3352**	-0.1552*	0.1800**	15.78	31.91**
	HVM30	0.8400	0.6325	0.0775	0.6001	0.2075**	0.7625**	0.5550**	9.77	20.13
	HVDHN7	0.3808	0.5125	0.6750	0.6345	-0.1317*	-0.2942**	-0.1625*	9.56	26.97*
	HVDHN9	0.8976	0.9600	0.8400	0.9039	-0.0624	0.0576	0.1200*	0.53	1.64
	HVLEU	0.8400	0.1700	0.0950	0.8610	0.6700**	0.7450**	0.0750	53.01	42.39**
6H	*HVM14*	0.9230	0.9900	0.9000	0.9862	-0.0670	0.0230	0.0900	5.04	30.43
	HVM22	0.8681	0.7523	0.2800	0.7112	0.1158*	0.5881**	0.4723**	8.39	16.36
	HVM34	0.6400	0.6350	0.4550	0.6143	0.0050	0.1850*	0.1800**	5.34	3.14
	HVM65	0.6368	0.9500	0.2300	0.7195	-0.3132**	0.4068**	0.7200**	15.50	95.60**
	HVM74	0.6400	0.6400	0.3625	0.6400	0	0.2775**	0.2775**	13.34	30.79**
7H	*HVM4*	0.3312	0.0750	0.0825	0.2583	0.2562**	0.2487**	-0.0075	31.90	17.63*
	HVM5	0.9168	0.5025	0.1325	0.8598	0.4143**	0.7843**	0.3700**	36.26	35.97**
	HVM49	0.9744	0.4950	0.9975	0.9179	0.4794**	-0.0231	-0.5025**	9.14	31.24**
	HVM51	0.5392	0.4200	0.2550	0.4620	0.1192*	0.2842**	0.1650*	10.15	7.12
	HVCMA	0.5760	0.4175	0.4925	0.5177	0.1585*	0.0835	-0.0750	3.13	22.11
	Mean	0.6172	0.4993	0.4766	0.6594	0.1179**	0.1406**	0.0227	18.58	
	SD	0.2934	0.2956	0.3226	0.2166				15.78	

*Significant by the Z-test at $p < 0.05$; **Significant by the Z-test at $p < 0.01$.

significant differences ($p < 0.01$), whereas ten loci showed no clear differences in their distribution among the three subgroups. These results clearly show that there exists significantly geographical differentiation among the three subgroups.

Cluster analysis

In order to reveal genetic relationships of 65 accessions of cultivated six-rowed naked barley (*nudum* barley) landraces from the Qinghai-Tibet plateau of China, the genetic similarity coefficients between accessions were calculated and a dendrogram was constructed depicting the relationships between the accessions (Figure 1). At a genetic similarity level of about 0.76 the accessions were clearly clustered into two large groups (Cluster I and cluster II) with all the TB accessions being located in cluster I while, except for accession QH132, the QH and GZ accessions were located in cluster II. At a genetic similarity of about 0.79 the accessions were clustered in five large groups (A, B, C, D and E, Figure 1), all the TB accessions being clustered in groups A, B and C while 13 of the 20 QH accessions and all 20 GZ accessions were clustered together in group D, with the four accessions from Chengduo county and the two accessions from Maqin county (both counties in Qinghai province, *i.e.* QH accessions) being separately clustered in group E (Figure 1). In group D the QH and GZ accessions were clustered into their own separate groups at a genetic similarity level of 0.8 (Figure 1). These results clearly reveal the geographical differentiation of *nudum* barley landraces in the Qinghai-Tibet plateau and their genetic relationship.

Discussion

In this study, we investigated the genetic variation among 65 nudum barley landraces at 35 SSR loci in the barley genome. There exists large genetic variation within and among the three geographical subgroups TB, QH and GZ. The genetic diversity was significantly higher in *nudum* barley from the TB region than it from either the QH or GZ regions while there was no significant difference in terms of genetic diversity between the QH and GZ barleys. The total number of alleles and their standard deviations (Table 3) were in the order TB > QH > GZ, while the average genetic distance followed the order TB (0.2071) > QH (0.2063) > GZ (0.1640). In addition, 18.58% of the total variation accounted for by differentiation among the three subgroups. These results appear to be in basic agreement with the Chi-square test on the distribution of allele frequencies (Table 4). The number of common and private alleles (Table 3) also reflected a certain geographical differentiation among the three subgroups, possibly due to the different geographical and ecological factors in the different regions of the Qinghai-Tibet plateau. As mentioned above, all the Tibetan accessions were clustered into one group (Cluster I) at a genetic similarity level of about 0.76 whereas the QH and GZ accessions (except the QH 132) were grouped in cluster II.

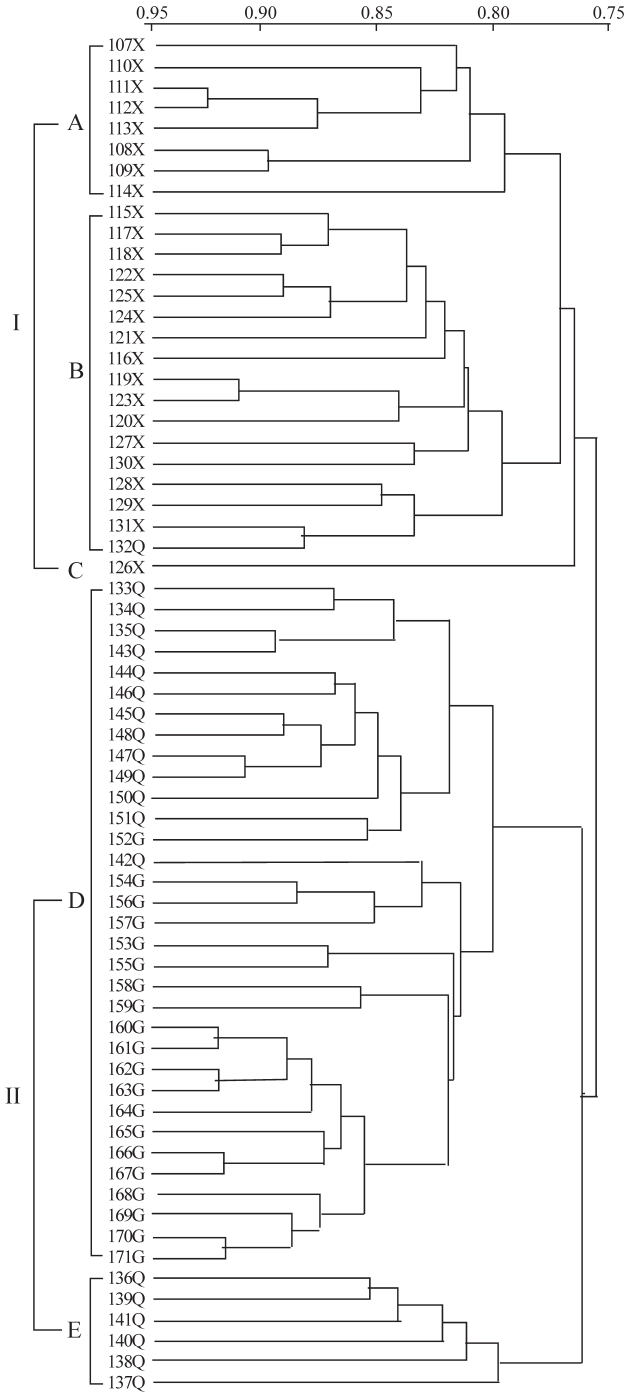

Figure 1 - Unweighted pair group method with averages (UPGMA) genetic similarity dendrogram of 65 cultivated six-rowed naked barley landraces from the Qinghai-Tibet plateau of China. Key: X = Tibet; Q = Qinghai; and G = Ganzi. See materials and methods for more details.

Overall, our *nudum* barley data suggests that Tibet might be an original center of evolution of cultivated six-rowed naked barley which then spread to Qinghai and Ganzi prefecture in Sichuan province. Vavilov (1955)

pointed out that the Middle-Western mountains of China were an original center of cultivated six-rowed naked barley. Previous morphological, ecological, distributional, archaeological and isozyme studies as well as genetics have shown that *H. spontaneum* in Tibet was the ultimate ancestor of Chinese cultivated barley whereas *H. agriocrithon* was an intermediate form in the transformation from *H. spontaneum* to cultivated barley (Xu, 1982).

Recently, our results on Tibetan wild barley using SSR markers also indicated that the Shannan region in Tibet might be the original center of Tibetan two-rowed wild barley (Feng *et al.*, 2003) and *Hordeum lagunculiforme* in Tibet an intermediate form in the transformation from *H. spontaneum* to *H. agriocrithon* (unpublished data). Dai and Zhang (1989) studied the genetic diversity of six isozyme loci in cultivated barley from different agro-geographical regions in Tibet and found that the degree of genetic diversity was significantly higher in Tibetan cultivated barley than Ethiopia barley using the same 6 isozyme loci (Zhang *et al.*, 1992a). Li *et al*. (2003) also supported the notion of Tibet being a center of genetic diversity for cultivated barley.

In conclusion, our SSR analysis of the 65 accessions of *H. v*. *hexastichon* var. *nudum* Hsü six-rowed naked barley from the Qinghai-Tibet plateau showed that the *nudum* barley landraces in Tibet possessed generally higher genetic diversity than those in Qinghai and Ganzi, with about 19% of the genetic diversity being accounted for by geographical differentiation among the three subgroups. This suggests that Tibet might be a center of genetic diversity of cultivated barley and that the cultivated six-rowed naked barley on the Qinghai-Tibet plateau of China might have evolved in Tibet and spread to Qinghai and then to Ganzi prefecture of Sichuan province.

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