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Molecular analysis of cultivated naked barley (*Hordeum vulgare* L.) from Qinghai-Tibet Plateau in China using SSR markers

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Abstract Naked barley is widely planted in Qinghai-Tibet Plateau in China and is essential for the daily life of Tibetans in those regions. In this study, the genetic diversity of 64 cultivated naked barley accessions from Qinghai-Tibet Plateau in China was analyzed using 30 mapped SSRs linked with the important traits of barley improvement. A total of 132 alleles were identified at 22 polymorphic SSR loci, with the number of each locus ranging from 2 to 15, the polymorphism information content (*PIC*) values ranging from 0.16 to 0.91, and with an average of 0.65. Of the selected SSRs, 13 SSR markers with high *PIC* value were highly efficient for the genetic analysis of Chinese barley. The accessions were divided into five main groups by cluster analysis and could be differentiated from each other. The genetic diversity in the Tibet accessions was slightly higher than in the Sichuan accessions. It is found that there were specific genes linked with the collecting sites. These results indicate the cultivated naked barley from Qinghai-Tibet Plateau in China are highly polymorphic and could be considered as an important resource bank for cultivated naked barley breeding in the future.

Keywords cultivated naked barley, genetic diversity, molecular marker, Qinghai-Tibet Plateau in China, SSR

Translated from *Acta Scientiarum Naturalium Universitatis Sunyatseni*, 46(2): 82–86 [译自: 中山大学学报(自然科学版)]

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1 Introduction

The assessment of genetic variation in germplasm arrays is essential for crop breeding, varietal control and registration, stock handling and efficient conservation and use of germplasm resources (Russell et al., 1997a; Dávila et al., 1998). Usually, for the germplasm identification, it has been attempted to use morphological and phenological characteristics, storage proteins, allozymes and molecular markers. But, morphological characters are often influenced by the environment, and difficult, ambiguous, time consuming and subjective. As the markers, both storage proteins and allozymes are limited by the amount of polymorphism. Recently, many types of molecular markers have been used to characterize germplasm, including random amplification of polymorphic DNA (RAPDs), restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), and simple sequence repeats (SSRs), etc..

SSRs (or microsatellites) are hypervariable DNA elements which consist usually of tandemly repeated mono-, di-, tri-, tetra- or penta-nucleotide motifs, present abundantly in genomes of most higher organisms (Tautz and Renz, 1984) and have a number of advantages, such as the high level of polymorphisms, locus specificity, co-dominance, reproducibility, ease of use through PCR (polymerase chain reaction), and random distribution throughout the genome (Weber, 1990; Wang et al., 1994; Powell et al., 1996a). These advantages make SSR an excellent molecular marker system for many types of genetic analyses in plant studies, including linkage mapping, germplasm surveys, cultivar identification and the purity control of varieties, biodiversity analyses, phylogenetic studies and other applications (Liu et al., 1996; Powell et al., 1996b; Barandalla et al., 2006). Update, SSRs have been successfully used for genetic analysis in several crops including sorghum (Smith et al., 2000; Djè et al., 2000), maize (Pejic et al., 1998; Senior et al., 1998), wheat (Prasad et al., 2000), rice (Akagi et al., 1996), soybean (Diwan and Cregan, 1997) and barley (Becker and Heun, 1995).

As an economically important cereal and well known for being grown in a wide range of environments, barley is also considered a model species for genetic analysis. Extensive genetic linkage maps, quantitative trait locus (QTL) information, and genetic diversity knowledge are currently available in barley (Heun et al., 1991; Kleinhofs et al., 1993; Becker and Heun, 1995; Liu et al., 1996; Pillen et al., 2000; Ramsay et al., 2000; Karakousis et al., 2003a). Genetic surveys at molecular marker loci in wild and cultivated barley have also been conducted using AFLPs (Russell et al., 1997a), RFLPs (Petersen et al., 1994; Russell et al., 1997a), RAPDs (Russell et al., 1997a), and SSRs (Saghai-Marooif et al., 1994; Russell et al., 1997a, 1997b; Struss and Plieske, 1998; Pillen et al., 2000; Ivandic et al., 2002). So far, a total of over 600 barley SSRs have been isolated which can be deployed in genetic studies and more than a half have been genetically mapped (Becker and Heun, 1995; Liu et al., 1996; Struss and Plieske, 1998; Ramsay et al., 2000). Furthermore, the association between some SSR markers and the important barley agronomic traits has been developed (Ivandic et al., 2002; Karakousis et al., 2003b). Therefore, the specific SSRs could be selected for barley genetic analysis.

According to the grain type, barley is classified into hulled barley and naked barley. Compared with hulled barley, naked barley is produced on a small scale and used as human food mainly because of its easiness in processing and edibility (Taketa et al., 2004). Recently, naked barley is increasingly attracting more attention as feed and as healthy food owing to its high nutritional value and abundance of dietary fiber (Bhatty 1986; Oscarsson et al., 1996; Newman et al., 1998). Qinghai-Tibet Plateau, located in the west of China and mainly covering Tibet, Qinghai Province and part of Sichuan Province, is a center of origin for six rowed cultivated barley (Zhang et al., 1994; Badr et al., 2000; Yin et al., 2003) and particularly rich in naked barley (Sun, 1999; Pan et al., 2007). Naked barley plays a very important role in Tibetan life, used as an essential food crop, the main animal feed, an important firewood and also made into other commercial products (Nimazhaxi, 1998; Pan et al., 2007). However, characterization of naked barley germplasms is very limited throughout the world, mainly due to the lack of naked barley germplasms.

In term of the rich naked barley resources from Qinghai-Tibet Plateau in China, it has only been done a large scale of phenotypic investigation (Ma and Li, 1997; Qiang et al., 1997) and isozymatic evaluation (Zhang et al., 1994). A few of studies on the hordein polymorphism of barley included some naked germplasms (Tang et al., 2002; Pan et al., 2006). However, a limited reports involved in molecular markers, such as RAPDs (Hou et al., 2005), SSRs (Feng et al., 2003) and ISSR (Hou et al., 2005) have been noted in the naked barley germplasm. Thus, further genetic study in the naked barley is import-

ant for the efficient use of the germplasm and the development of new varieties.

The primary objectives of this study were to evaluate the genetic diversity of the cultivated naked barley germplasms using genetically mapped SSRs which were associated with important traits. It will be helpful to choose high efficient SSR markers for barley genetic analysis in the future.

2 Materials and methods

2.1 Plant materials

Sixty-four accessions of cultivated six-rowed naked barley (*Hordeum vulgare* L.) from Qinghai-Tibet Plateau in China were selected. Among the materials, 45 accessions were collected from Tibet, which were provided by the Tibet Academy of Agricultural and Animal Husbandry Sciences, and 19 from Sichuan, which were provided by the Agricultural Institute of Aba Autonomous State, Sichuan. According to growth habits, 51 accessions were of spring types and 13 of winter types. The altitudes of the collected areas were from 1600 m to 3850 m above sea level (Table 1).

2.2 DNA isolation

Genomic DNA was extracted from fresh leaf collected from leaf positions 2–3 in the one-month-old plants grown in a greenhouse, according to a small-scale DNA isolation procedures described by Wang and Fang (1998) with some modifications.

2.3 SSR markers

Microsatellite markers used in this study were obtained from the published sequences of Sagahi- Marooif et al. (1994), Becker and Heun (1995), Liu et al. (1996), Struss and Plieske (1998) and Ramsay et al. (2000). A total of 30 primer pairs for SSRs were collected. Most of them were known for the map locations and identified as being linked within 10 cM of loci controlling the important traits for barley improvement, including brewing quality, disease resistance, abiotic stresses and physiological traits. Information on chromosome location and linked traits of the SSR markers are listed in Table 2.

2.4 PCR amplification

Two touchdown PCR protocols for SSR assay were used as described by Karakousis et al. (2003b) with some modifications. The medium annealing temperature protocol (T-55°C) included a pre-denature of 5 min at 94°C, 10 cycles of 30 s at 94°C, 30 s starting at 64°C and decreasing

Table 1 The 64 cultivated hulless cultivars collected from Qinghai-Tibet Plateau used in this study

collecting region	accession name	ordinal no.	growth habit	altitude/m
Tibet	Zangqing1	TZ01	spring	3700
Tibet	Zangqing21	TZ03	spring	3700
Tibet	Zangqing80	TZ04	spring	3700
Tibet	Zangqing85	TZ05	spring	3700
Tibet	Zangqing3179	TZ06	spring	3700
Tibet	Zangqing7239	TZ07	spring	3700
Tibet	Zangqing311	TZ08	spring	3700
Tibet	Zangqing148	TZ09	spring	3700
Tibet	Zangqing320	TZ10	spring	3700
Tibet	815078	TZ11	spring	3700
Tibet	940690	TZ12	spring	3700
Tibet	QB01	TZ13	spring	3850
Tibet	QB02	TZ14	spring	3850
Tibet	QB24	TZ16	spring	3850
Tibet	QB16	TZ17	spring	3850
Tibet	QB28	TZ19	spring	3850
Tibet	Ximala2	TZ20	spring	3600
Tibet	Ximala4	TZ21	spring	3600
Tibet	Ximala6	TZ22	spring	3600
Tibet	Ximala8	TZ23	spring	3600
Tibet	Ximala9	TZ24	spring	3600
Tibet	Ximala10	TZ25	spring	3600
Tibet	Ximala11	TZ26	spring	3600
Tibet	Ximala15	TZ27	spring	3600
Tibet	Ximala16	TZ28	spring	3600
Tibet	Ximala19	TZ29	spring	3600
Tibet	Ximala42	TZ30	spring	3600
Tibet	Lashagoumang	TZ32	spring	3700
Tibet	Dongqing1	TZ34	winter	3700
Tibet	Dongqing8	TZ35	winter	3700
Tibet	Dongqing11	TZ36	winter	3700
Tibet	Dongqing15	TZ37	winter	3700
Tibet	Dongqing16	TZ38	winter	3700
Tibet	96-97180	TZ39	winter	3700
Tibet	94-95-955	TZ40	spring	3700
Tibet	WB20	TZ42	winter	3600
Tibet	WB21	TZ43	winter	3600
Tibet	WBQ64-97	TZ44	winter	3600
Tibet	WB19-97	TZ45	winter	3600
Tibet	WB07-97	TZ46	winter	3600
Sichuan	Changmang	SZ47	spring	3600
Sichuan	Handiziqingke	SZ48	spring	3600
Sichuan	Litangbaishanshe	SZ50	spring	3300
Sichuan	Chunqingke	SZ51	spring	3300
Sichuan	Changtichuqingke	SZ53	spring	2300
Tibet	Pingbi14	SZ54	winter	3700
Tibet	Beiqing3	SZ55	spring	3700
Sichuan	Abasiqing	SZ56	spring	3300
Sichuan	Abashandan	SZ58	spring	3300
Sichuan	Jingchuan	SZ66	spring	2000
Sichuan	Tonghuamapeng	SZ67	spring	1600
Sichuan	Aqing5	SZ71	spring	3000
Sichuan	98029-1	SZ73	spring	3450
Sichuan	97039	SZ74	spring	3450
Sichuan	Tanbahei	SZ75	spring	3000
Sichuan	Zhongdianhei	SZ76	spring	3200
Sichuan	Qianning	SZ77	spring	2400
Tibet	Zangqing434	TZ78	spring	3700
Tibet	Zangqing447	TZ79	spring	3700
Sichuan	Kangqing3	SZ81	spring	3450
Sichuan	Kangqing6	SZ82	spring	3450

(Continued)

collecting region	accession name	ordinal no.	growth habit	altitude/m
Sichuan	97-9	SZ84	spring	3450
Sichuan	Kangqing7	SZ83	spring	3450
Tibet	Zangqing25	SZ87	spring	3700

1°C per cycle, 30 s at 72°C, followed by 28 cycles for 30 s at 94°C, 30 s at 55°C, 30 s at 72°C, with a final extension of 5 min at 72°C. The low stringency protocol (T-50°C) was identical to T-55°C, except that it started with an annealing temperature of 59°C and decreased to 50°C. PCR amplification protocols for each set of primers were shown in Table 2. Each PCR reaction was consisted of 25 µL volume, containing approximately 100 ng of template DNA, 10 × PCR buffer, 1 U of *Taq* DNA polymerase, 0.2 mmol·L⁻¹ dNTPs, and 10 pmol of each primer. In this study, all the PCR reagents were purchased from Sino-American Biotechnology Co.. The PCR amplifications were carried out using the Perkin-Elmer thermocycler (PE 9700) with 96-well PCR plates.

2.5 Separation of the PCR products and detection

One µL PCR reaction plus 1 µL loading buffer containing 0.25% (w/v) formamide, 0.25% (w/v) bromophenol blue, 40% (w/v) sucrose solution, were mixed for loading. PCR products were separated on 6% acrylamide gels (AA: BIS = 29:1) in 1 × TBE buffer running at 200 V for 3.0 h. The gels were stained using silver nitrate. The detailed procedures were as follows: the gel was firstly stained for 10 min in 0.1% (w/v) silver nitrate, then rinsed three times in deionized water and two times in developing solution (2.0 g·100 mL⁻¹ NaOH + 40 mg·100 mL⁻¹ Na₂CO₃ + 100 µL 37%–40% HCHO solution), the gel was rinsed in deionized water two times again before developed in developing solution for about 15 min until the bands become visible. The developing process was stopped finally in the stop solution. Fragment lengths were identified using the Quantity One 4.0 software package (BioRAD) by comparison with a 100-bp DNA ladder marker.

2.6 Data analysis

Allele frequencies and the polymorphism information content (*PI*C) were calculated using the POPGENE1.31 software (Yeh et al., 1999). The *PI*C value was calculated using the following formula: $PI C = 1 - \sum P_i^2$, where P_i is the frequency of the *i*th SSR allele (Nei, 1973). Jaccard's coefficient of similarity was calculated, and the lines were grouped by cluster analysis using the Unweighted pair-group method (UPGMA). A dendrogram was produced using the program NTSYSpc2.0 (Rohlf, 1998).

Table 2 Chromosome locations, linked traits and PCR procedures of SSRs

SSR	chromosome location	linked trait	PCR procedure	SSR	chromosome location	linked trait	PCR procedure
Ebmac0501	1HS	malt extract, DP**	T-50	HVM03	4HL	RcaA+, early maturity++	T-55
Bmag0125	2HL	Ha2**	T-55, T-50	GMS027	5HL	Ha4**	T-50
Bmag0692	2HS	malt extract**	T-50	GMS001	5HL	malt extract, DP**	T-50
HVM54	2HL	malt extract**, flowering time++	T-55	Bmac0096	5HS	leaf rust**	T-50
GMS003	2HS	boron tolerance**	T-50	HVDHN9	5H	mRNA for dehydrin-9###	T-50
Bmac0093	2HS	boron tolerance**	T-50	HVM11	6HL ##		T-55
Bmag0603	3HL	boron tolerance**	T-50	Bmag0359	7HL	naked grain**	T-55, T-50
HVM33	3HL	dwarfing gene uz**	T-55	Bmac0167	7HL	naked grain**	T-50
Bmag0006	3HL	Yd2**	T-55	GMS056	7H		T-50
Bmac0310	4HL	AL tolerance**	T-50	GMS046	7H		T-50
Ebmac0701	4HS	boron tolerance**	T-50	Bmag0206	7H	scald resistance**	T-50
HVRCABGA	4H		T-55	HvAMY2	7HL	DP**	T-50
HVRCABGB	4H		T-55	Hvwaxy4	7H	starch synthesisase**	T-50
HVM40	4HS	manganese efficiency**, flowering time++	T-55	HVM04	7H	starch synthase (Waxy) gene+	T-55
HVM67	4HL	DP**, flowering time++	T-55	HVCMA	7H	naked grain**, -amylase inhibitor###	T-55
							T-50

Note: ** Karakousis et al., 2003; + Saghai Maroof et al., 1994; ++ Ivandic et al., 2002; ## Liu et al., 1996; ### Becker and Heun, 1995.

3 Results

3.1 Allelic variation of SSRs

No amplification products for 5 SSR markers in the 30 SSRs selected were obtained after two attempts. Accordingly, 25 SSRs were assayed further in the 64 germplasm (Table 3). Among them, 22 SSR loci were polymorphic and 3 monomorphic, including HVRCABGA, HVRCABGB and Ebmac0501. The total number of alleles at the 22 polymorphic SSRs assayed in all 64 accessions was 132 alleles. The number of alleles at a locus ranged from 2 (HVDHN9 and Hvwaxy) to 15 (GMS027), with a mean of 6.0 alleles over all polymorphic loci. The *PIC* value of the SSR loci ranged from 0.16 (HVCMA) to 0.91 (GMS027), with an average of 0.65 per locus. Only HVCMA and Hvwaxy loci produced the *PIC* value lower than 0.50 and there were 13 SSR markers with a high average *PIC* value of 0.76, ranging from 0.60 to 0.91 per locus. The number of different microsatellite genotypes observed at a locus ranged from 2 to 11, with a mean of 4.73 over the 22 polymorphic loci. Thus, concerning the *PIC* value, GMS027 was the most-informative locus and HVCMA the least-informative for cultivar fingerprinting and differentiation. Based on the number of the genotypes observed, HVM33 had the strongest discriminating power with 11 genotypes observed.

Table 3 Results of SSR markers assayed in the tested naked barley accessions

SSR markers	no. of alleles	allele size	<i>PIC</i>	genotype
Bmag0603	4	113–138	0.57	2
Bmac0310	5	142–216	0.75	8
Bmag0206	10	254–320	0.86	7
Bmag0692	8	179–287	0.73	7
Ebmac0501	2	129–151	0.00	1
Ebmac0701	6	124–163	0.53	3
GMS001	10	130–171	0.78	5
GMS003	4	132–165	0.53	3
GMS046	4	144–185	0.74	7
HVM11	4	148–179	0.51	6
HVRCABGA	1	125	0.00	1
HVRCABGB	1	168	0.00	1
Hvwaxy4	2	164–173	0.48	3
Bmac0167	4	187–226	0.52	2
Bmac0096	10	155–215	0.70	5
Bmag0006	6	162–214	0.67	4
HVM03	6	172–211	0.79	3
HVM54	6	145–186	0.82	3
HVM40	4	144–181	0.69	3
HVM67	5	105–140	0.58	4
HVM33	9	155–210	0.86	11
Bmac0093	4	147–188	0.60	2
GMS027	15	137–188	0.91	10
HVCMA	4	114–134	0.16	4
HVDHN9	2	128–148	0.50	2
mean	6		0.65	
total	136			

3.2 Genetic difference between Sichuan and Tibet populations

The genetic difference between Sichuan and Tibet populations was investigated. A total of 124 alleles were observed in Tibet population with a mean of 5.6 alleles per locus, while a total of 121 alleles and an average of 5.1 alleles in Sichuan population. The *PIC* value was produced with the range from 0.19 to 0.91 in Tibet population and with the range from 0.09 to 0.88 in Sichuan population. On average, the *PIC* value was 0.65 and 0.63 in Tibet population and Sichuan population, respectively. The most and the least informative SSRs were identical, e.g. GMS027 and HVCMA, respectively (Table 4). Bmag0692, HVM11, Bmac0167, Bmac0096, HVM3, HVM67 and GMS027 produced specific genes in Tibet population, while Emac0701 and Bmag006 generated specific genes in Sichuan population. In general, the frequencies of most alleles exhibited the similar tendency in the two populations. There, however, were some exceptional cases. Some alleles at loci GMS46, HVM33 and HVM54 were present with a higher frequency in Tibet population while only present in several Sichuan accessions. On the contrary, some alleles at these three loci occurred in several Tibet accessions while presented in the majority of Sichuan accessions.

Table 4 *PIC* value and number of alleles in Tibetan and Sichuan populations

locus	<i>PIC</i> value		no. of alleles	
	Tibet	Sichuan	Tibet	Sichuan
Bmag0603	0.54	0.62	4	4
Bmac0310	0.76	0.74	5	4
Bmag0206	0.87	0.84	10	10
Bmag0692	0.75	0.65	8	6
Ebmac0701	0.50	0.59	2	6
GMS001	0.78	0.77	8	8
GMS003	0.52	0.55	4	4
GMS046	0.71	0.74	4	4
HVM11	0.52	0.49	4	3
Hvwaxy	0.49	0.43	2	2
Bmac0167	0.52	0.50	4	2
Bmac0096	0.73	0.63	10	6
Bmag0006	0.67	0.66	5	6
HVM03	0.79	0.75	6	4
HVM54	0.81	0.82	6	6
HVM40	0.71	0.62	4	4
HVM67	0.59	0.55	5	4
HVM33	0.84	0.87	9	9
Bmac0093	0.62	0.55	4	4
GMS027	0.92	0.88	15	12
HVCMA	0.19	0.09	3	2
HVDHN9	0.50	0.50	2	2
total			124	121
mean	0.66	0.63	5.64	5.10

3.3 Cluster analysis

Figure 1 shows the dendrogram derived from these similarity coefficients using the UPGMA clustering method. The accessions could be classified into five groups (referred to I, II, III, IV and V). The first group contained over a half of the accessions studied, including 23 Tibetan cultivars and 14 Sichuan cultivars. The second group contained 10 Tibetan cultivars and 2 Sichuan cultivars. The third group was composed of nine Tibetan cultivars and two Sichuan cultivars. The fourth group only included two Tibetan accessions and one Sichuan accessions. Accession TZ19 was distinct from other accessions and separately clustered into the group labeled V. In the cluster analysis, no two accessions had the same SSR patterns and all the accessions were differentiated individually.

4 Discussion

Microsatellite DNA or SSR markers, due to their hyper-variability, codominance, high reproducibility, and easiness of use, are ideal markers for a number of genetic analyses. Up to now, large numbers of SSRs are available for barley research programs (Ramsay et al., 2000). However, not all SSRs are of the same quality. They can differ in robustness, quality of the amplification products, the *PIC* value, and so on. For instance, the *PIC* values for a set of 15 highly informative SSR markers in the study of Struss and Plieske (1998) varied from 0.3 to 0.86, with 5–15 alleles in the same genotypes examined. In 58 Australian barley genotypes, Ebmac0501 displayed a high *PIC* value of 0.70 (Karakousis et al., 2003b), but showed no polymorphism in this study. Previously, it was found that Bmac0096 had only a *PIC* value of 0.08 in 96 elite breeding germplasm (Matus et al., 2002), whereas 0.69 in 58 Australian barley breeding genotypes. Karakousis et al. (2003b) found that high informative SSR markers for screening European genotypes could not always be useful for discriminating Australian genotypes. Therefore, it is essential to choose the most informative and robust SSR set in the genetic analysis for the specific populations. The number of alleles, the *PIC* value for a genetic marker and its normally coincides with the highest number of alleles, indicate the informativeness of this marker (Pillen et al., 2000; Matus et al., 2002; Barandalla et al., 2006). In our study, a set of 13 SSRs were of high quality, robust, highly informative with the high *PIC* value higher than 0.60, suggesting that they are useful for the molecular study on the cultivated naked barley in Qinghai-Tibet Plateau in China.

The number of alleles and *PIC* value for SSR marker system detected depended, in large part, on the diversity of germplasm sampled, which was reported as did Matus et al. (2002) and Barandalla et al. (2006). Lower number

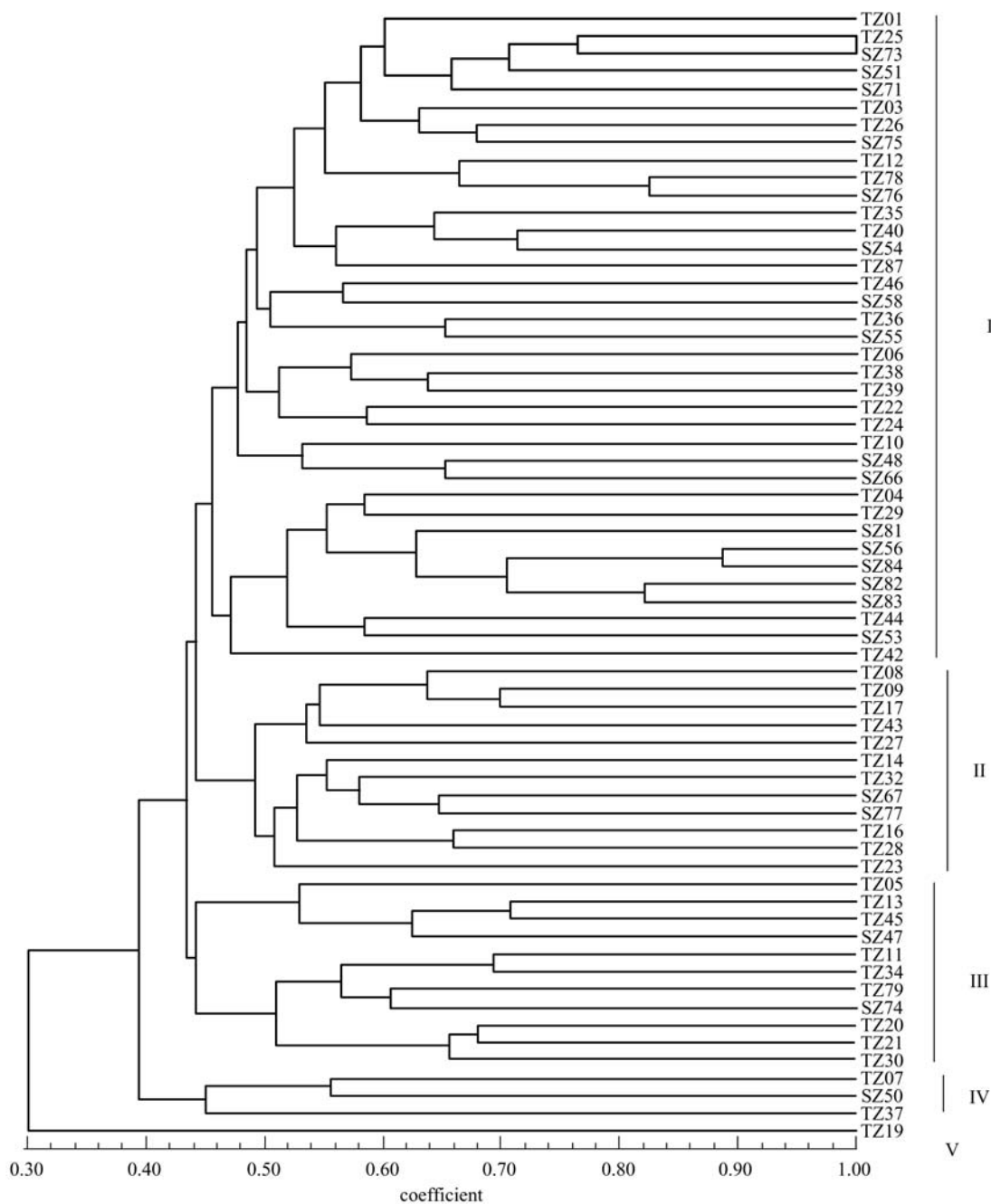


Fig. 1 Dendrogram based on SSR polymorphism

of alleles or *PIC* value for SSR marker was reported in different genotype groups assayed. The number of alleles for 15 SSRs averaged only 2.1 in 11 barley accessions (Becker and Heun, 1995). An average *PIC* value of 0.50 for the 11 microsatellites were found between 24 modern spring and winter barley cultivars (Russell et al., 1997b). A mean of 3.2 bands and an average *PIC* value of 0.38 per SSR were observed among a set of 28 barley cultivars and two wild-form accessions (Pillen et al., 2000). In other reports, the number of alleles or *PIC* value for SSR marker was much higher. An average of 11.3 alleles and an

average *PIC* value of 0.855 for sixteen SSRs were found in 27 accessions of wild barley (Dávila et al., 1999). Matus et al. (2002) observed an average of 16.3 alleles and an average *PIC* value of 0.74 in 147 genotypes. An average of 18 alleles for 4 SSRs was found in 104 accessions of subspecies *Vulgare* and 103 accessions of subspecies *spontaneum* (Saghai-Marooif et al., 1994). Ivandic et al. (2002) reported an average *PIC* value of 0.79 in 39 germplasm. Karakousis et al. (2003b) revealed an average of 6.19 alleles and the *PIC* value of 0.71 for 36 SSRs among 56 Australian barley breeding lines. In our study, the number

of alleles averaged 6.0 and the average *PIC* value was equal to 0.65 over all polymorphic loci in 64 accessions of naked barley germplasms. These data together indicate that a high level of genetic diversity based on SSR marker loci is present within the cultivated hulless barley from Qinghai-Tibet Plateau in China.

In our study, most of SSRs were linked with the important traits for barley improvement and a high level of polymorphisms was found at these loci. This result is very useful for barley improvement. For instance, Bmag0692, GMS001 and HVM54, related to malt quality, produced eight alleles and seven genotypes, nine alleles and five genotypes and six alleles and three genotypes among the accessions studied, respectively. Locus *Hvwaxy* was associated with starch character and generated 2 alleles and 3 genotypes. Bmag0206, linked with scald resistance, produced 10 alleles and 7 genotypes. Bmac0096, linked with leaf rust resistance, revealed 10 alleles and 7 genotypes. GMS027, linked with resistance of Ha2, produced 15 alleles and 10 genotypes (Tables 2, 3). This information could provide a basis for selection of parent combination or targeting gene discovery.

The dendrogram of cluster demonstrates all accessions were able to be differentiated. It reflects the SSR marker system powerful discrimination among the genotypes, which were also confirmed by Stuss and Plieske (1998), Matus et al. (2002), Pillien et al. (2000) and Dávila et al. (1999). It also indicates the cultivated naked barley from Qinghai-Tibet Plateau in China present a wide range of diversity, which is consistent with the previous results based on morphological characters (Ma et al., 2000) and storage proteins (Pan et al., 2007), and supports the hypothesis that Tibet-Plateau is the center of genetic diversity of the multi-rowed naked barley (Ma et al., 2000). Both the major accessions from Tibet and Sichuan were located in group II, although the average number of alleles and the *PIC* value per locus in Tibet population were slightly higher than those in Sichuan population and some rare alleles also present the specific-region character. This indicates that the classification does not relate to geographic origin, whereas maybe result from the intensive exchange of seed that often makes the accessions mis-alignment, resulting in the difficulty to clearly identify the real origin of cultivars or advanced landraces (Struss and Plieske, 1998).

In conclusion, the extent of genetic diversity was estimated in cultivated naked barley from Qinghai-Tibet Plateau in China using mapped SSRs which were associated with important traits. A high level of diversity is present and this cultivated naked barley population could be taken as a gene pool with large diversity, and could be applied in breeding of cereal crops. It also supports the notion that Qinghai-Tibet Plateau is the center of cultivated hulless barley. Meanwhile, it is confirmed that SSR marker has strong power of discrimination between the genotypes and is an ideal molecular tool for germplasm

assay. The set of microsatellites markers selected in this study is very useful for identification of Chinese barley germplasm. Of course, a higher precision for monitoring the extent of genetic diversity of the barley collection can be achieved by using more markers with an evenly distribution over the whole genome.

Acknowledgements This research was supported by the program of Chinese Academy of Sciences (No. KSCX2-SW-304), the West Light Foundation of the Chinese Academy of Sciences, Ministry of Science and Technology of China (No. 2006FY110700), and the Frontier Project of Chengdu Institute of Biology, CAS, China (No. CIB-2007LYQY-09).

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