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Full Length Research Paper

Diversity analysis of the developed qingke (hulless barley) cultivars representing different growing regions of the Qinghai-Tibet Plateau in China using sequence-related amplified polymorphism (SRAP) markers

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Genetic diversity among 68 accessions of the developed qingke (hulless barley) cultivars from Sichuan, Gansu, Tibet, Qinghai and Yunnan provinces of the Qinghai-Tibet Plateau in China was evaluated by using a newly developed sequence-related amplified polymorphism (SRAP) marker system. The results showed that, 20 primer combinations produced a total of 350 clear bands with an average of 17.5 bands per primer pair, of which 153 bands (43.7%) were polymorphic. 324 allelic phenotypes were amplified with an average of 16.2 alleles per primer pair. The genetic diversity mean in Sichuan, Gansu, Tibet and Qinghai provinces was 0.6773, 0.5042, 0.7080 and 0.6816, respectively, while average genetic distance was 0.0418, 0.0657, 0.0605, 0.0921, respectively. The genetic differentiation among different regions ranged from 9.02 to 48.22% with an average of 18.77%. The 68 accessions were classified into four major groups by cluster analysis using Unweighted Pair Group Method with Arithmetic Mean (UPGMA), indicating that the significant relationships between the original regions of accessions were existed. Thus, it is suggested that SRAP could be used as an effective molecular marker in researching barley genetic diversity, and also narrow genetic basis and poor genetic diversity made it imperative to search elite hulless barley germplasm for breeding superior hulless barley.

Key words: Barley (*Hordeum vulgare* L.), qingke (hulless barley), genetic diversity, sequence-related amplified polymorphism (SRAP), genetic differentiation.

INTRODUCTION

Qingke, which is called hulless barley (*Hordeum vulgare* L. var. *nudum* Hook. F), is one of the major food crops in Qinghai-Tibetan regions of China. It is an excellent barley germplasm resources on the Qinghai-Tibet plateau of China. Hulless six-row barley (*H. vulgare* ssp. *hexastichon* var. *nudum* Hsü) was the earliest cultivated in China (Xu,1982; Xu and Feng, 2001). In recent years, qingke breeding and its exploitation have been extensively valued. Study of genetic diversity of qingke germplasm resources is very beneficial for the protection of qingke resources and the development of new qingke varieties as well as the life-level's improvement of Zang-nationality people in China (Yang et al., 2008).

Nowadays, more and more DNA markers are widely utilized in genetic diversity (Petersen et al., 1994; Baum et al., 1997; Struss and Plieske, 1998; Fernandez et al., 2002; Feng et al., 2006a, 2006b; Pan et al., 2008; Gong et al., 2009), germplasm analysis (Powell et al., 1996), and

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map construction (Graner et al., 1991; Liu et al., 1996) of barley. Few reports about qingke landraces and developed varieties were made using molecular markers (Feng et al., 2006b; Pan et al., 2008; Gong et al., 2009). Recently, Zhou et al. (2007) analyzed genetic diversity of hordein loci in developed qingke varieties from the plateau regions of Tibet and Sichuan province of China by acid-polyacrylamide gel electrophoresis (A-PAGE). It is very important to study genetic diversity of the developed qingke varieties from the Qinghai-Tibet plateau of China and develop efficient molecular markers for qingke varieties improvement.

Sequence-related amplified polymorphism (SRAP) markers developed by Li and Quiros (2001), was successfully applied in cultivar identification (Li et al., 2006), map construction (Li and Quiros, 2001; Lin et al., 2003), diversity evaluation (Ferriol et al., 2003; Budak et al., 2004; Sun et al., 2006), comparative genomics (Li et al., 2003), and gene location (Wang et al., 2004) of different plant species. In barley, we first reported the utilization of SRAP markers in the evaluation of genetic diversity among the developed qingke varieties from the plateau regions of Sichuan, China (Yang et al., 2008). It also verified the effectiveness of SRAP markers in genetic diversity evaluation of barley (Yang et al., 2008). In this work, genetic diversity analysis of 68 accessions of developed qingke varieties from the Qinghai-Tibet plateau regions including Sichuan, Gansu, Tibet, Qinghai and Yunnan provinces of China was further evaluated.

The objectives of the present study include: (1) To evaluate the levels of genetic diversity and differentiation of these accessions; (2) to further understand the genetic basis of these accessions, and to formulate appropriate strategies for the conservation and utilization of the developed qingke genetic resources.

MATERIALS AND METHODS

Plant materials

Seeds of sixty-eight developed qingke (hulless barley) varieties, including 25 collections from Sichuan province, six from Gansu province, seventeen from Tibet, eighteen from Qinghai province and two from Yunnan province, China, were collected from the Qinghai-Tibet Plateau regions of China (Table 1).

Genomic DNA extraction

Collected accession seeds from different listed locations (Table 1) were grown separately. The cetyl trimethyl ammonium bromide (CTAB) protocol (Sharp et al., 1988) was used to extract genomic DNA from about 100 mg of fresh young leaf-tissue of each accession. The quality and the concentration of the DNA were estimated by Beckman counter DU800 nucleic acid/protein analyzer (USA). The isolated genomic DNA was stored at -20°C for use.

Polymerase chain reaction (PCR) analysis

The SRAP primer sequences used in this study were designed according to Li and Quiros (2001), and listed in Table 2. Primers were produced by TakaRa Bio Co. (Dalian, China). The PCR amplification was carried out in a PTC-100^{TM} Thermo Cycler in a 15 µl volume containing 1.5 µl of 10 × buffer(Mg^{2+}), 0.2 mM of dNTP, and 0.25 µM of forward and reverse primers, 0.6 U of rTaq DNA polymerase (TaKaRa, China) basically according to the method described by Li and Quiros (2001). PCR reaction conditions were as follows: 95°C, 1 min; 94°C, 1 min, 35°C, 1 min, 72°C 1 min, 5 cycles; 94°C 1 min, 50°C 1 min, 72°C, 1 min, 35 cycles; 72°C, 10 min. The PCR products were separated on 6% denatured polyacrylamide gels using 1×TBE buffer. The gel was pre-run at 220 V constant voltage for 20 min before the samples were loaded, by using DYY-6C electrophoresis equipment (made in LiuYi Instrument Factory, Beijing, China). After loading samples, the gel was run at 450 V constant voltage for 1.5 to 2.0 h until the xylene cyanol reached the 2/3 of the gel towards the bottom. DL100 DNA marker (produced by Dalian TaKaRa, China) was used as a size standard. After electrophoresis, the gel was stained by $AgNO_3$ solution (Bassam et al., 1991).

Data analysis

Each SRAP primer pair was considered to be one genetic marker. The SRAP profiles were scored for the presence (1) or absence (0) of clear bands for each qingke genotype. The genetic diversity (H) at each primer pair was estimated with

$$H = 1 - \sum P_i^2$$

Where, P_i is the frequency of the i^{th} allele of the locus (Nei, 1973). The genetic diversity (H_T) of the entire sample ($H_T = H_S + D_{ST}$) can be partitioned into components, reflecting genetic distance among subgroups (D_{ST}) and genetic polymorphism within subgroups (H_S) (Nei, 1973), with differentiation among subgroups (G_{ST}) being calculated as (Nei, 1973)

$$G_{ST} = 1 - H_S / H_T$$

Genetic similarities (GSI) were estimated using the formula:

$$GS_{ij} = 2N_{ij} / (N_i + N_j)$$

Where, N_{ij} is the number of bands shared by accessions i and j, N_i and N_j are the number of bands from accessions i and j, respectively (Nei and Li, 1979). Genetic distance (GD) was calculated as

$$GD = 1 - GSI$$

Pair wise comparison of genetic diversity between subgroups was carried out according to Z-test method (Zhang and Allard, 1986; Zhang et al., 1992).

POPGENE ver.3.11 was employed to analyze the genetic distance among different geographical regions of barley (Excoffier and Heckel, 2006). Cluster analysis of the similarity matrix was used to reveal associations among accessions and among different geographical regions using the UPGMA method (unweighted pair group method with arithmetic averages), employing the NTSYS-pc (version 5.1) program (Rohlf, 1993).

RESULTS

Polymorphism and alleles of PCR products

Figure 1 shows amplification results of the primer

Table 1. List of names and origin of the qingke cultivars used in this study.

Accession code	Name	Origin (province)	Accession code	Name	Origin (province)
SC1	Kangqing 1	Sichuan, China	XZ35	Ximala 15	Tibet, China
SC2	Kangqing 2	Sichuan, China	XZ36	Ximala 19	Tibet, China
SC3	Kangqing 6	Sichuan, China	XZ37	Zangqing 25	Tibet, China
SC4	Kangqing 7	Sichuan, China	XZ38	Zangqing 80	Tibet, China
SC5	Bailiuleng	Sichuan, China	XZ39	Zangqing 148	Tibet, China
SC6	Emu 1	Sichuan, China	XZ40	Zangqing 311	Tibet, China
SC7	Gangtuoqingke	Sichuan, China	XZ41	Zangqing 320	Tibet, China
SC8	Heiqingke	Sichuan, China	XZ42	Zangqing 690	Tibet, China
SC9	Dongshenghei	Sichuan, China	XZ43	Zangqing 3179	Tibet, China
SC10	Danbaheiqingke	Sichuan, China	XZ44	Shanqing 7	Tibet, China
SC11	Qianninglvqingke	Sichuan, China	XZ45	Alidangdiqingke	Tibet, China
SC12	Minxianqingke	Sichuan, China	XZ46	Baidicunqingke	Tibet, China
SC13	98172-2	Sichuan, China	XZ47	QB23	Tibet, China
SC14	813	Sichuan, China	XZ48	Lasagoumang	Tibet, China
SC15	Songpanziqingke	Sichuan, China	QH49	Beiqing 1	Qinghai, China
SC16	Qianningqingke	Sichuan, China	QH50	Beiqing 2	Qinghai, China
SC17	Baiyuqingke	Sichuan, China	QH51	Beiqing 3	Qinghai, China
SC18	33-2-1-1	Sichuan, China	QH52	Beiqing 4	Qinghai, China
SC19	Ganziheimawei	Sichuan, China	QH53	Beiqing 5	Qinghai, China
SC20	603	Sichuan, China	QH54	Beiqing 6	Qinghai, China
SC21	Qipangou	Sichuan, China	QH55	Beiqing 7	Qinghai, China
SC22	Cunqingke	Sichuan, China	QH56	Beiqing 8	Qinghai, China
SC23	Kangqing 5	Sichuan, China	QH57	Kunlun 2	Qinghai, China
SC24	Aqing 4	Sichuan, China	QH58	Kunlun 3	Qinghai, China
SC25	Aqing 5	Sichuan, China	QH59	Kunlun 8	Qinghai, China
GS26	Ganqing 2	Gansu, China	QH60	Kunlun 10	Qinghai, China
GS27	Ganqing 3	Gansu, China	QH61	Kunlun 12	Qinghai, China
GS28	Ganqing 4	Gansu, China	QH62	Dongqing 1	Qinghai, China
GS29	9516	Gansu, China	QH63	Dongqing 2	Qinghai, China
GS30	Dulihuang	Gansu, China	QH64	Mennong 1	Qinghai, China
GS31	94-19-1	Gansu, China	QH65	Moduocha 1	Qinghai, China
XZ32	Zangqing 1	Tibet, China	QH66	Nanfan 3	Qinghai, China
XZ33	Zangqing 21	Tibet, China	YN67	Diqing 1	Yunnan, China
XZ34	Ximala 96	Tibet, China	YN68	Diqing 4	Yunnan, China

Table 2. List of primer sequences (forward and reverse) used for SRAP marker in this study.

Primer	Forward primer (5′→3′)	Primer	Reverse primer(5′→3′)
me5	T GAG TCC AAA CCGG AAG	em12	GA CTG CGT ACG AATT GTC
me6	T GAG TCC AAA CCGG TAA	em13	GA CTG CGT ACG AATT AGC
me7	T GAG TCC AAA CCGG TCC	em14	GA CTG CGT ACG AATT CAG
me8	T GAG TCC AAA CCGG TGC	em15	GA CTG CGT ACG AATT CTG
me9	T TCA GGG TGG CCGG ATG	em16	GA CTG CGT ACG AATT CGG
me10	T GGG GAC AAC CCGG CTT	em17	GA CTG CGT ACG AATT CCA
me11	C TGG CGA ACT CCGG ATG	em18	GA CTG CGT ACG AATT GGT

me12	G GTG AAC GCT CCGG AAG	em19	GA CTG CGT ACG AATT CCG

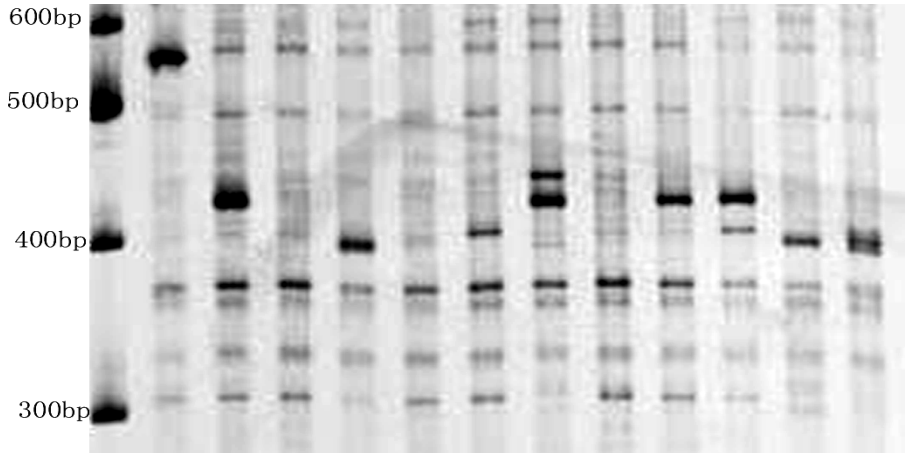

Figure 1. Amplification results of the primer combination "me8/em12" in some of the accessions.

Table 3. List of primer combination and NPB/TNB, genetic diversity per number of alleles, and genetic differentiation (G_{ST}) from 20 SRAP primer pairs among the developed qingke varieties.

Primer combination	NPB / TNB	Genetic diversity/No. of alleles					G_{ST} (%)
		SC	GS	XZ	QH	Entire sample	
me5/em15	6 / 20	0.8672 / 11	0.4444 / 2	0.6307 / 3	0.5741 / 4	0.8563 / 12	19.48
me5/em17	7 / 17	0.8928 / 13	0.2778 / 2	0.7612 / 7	0.8580 / 11	0.9412 / 26	15.69
me5/em18	5 / 11	0.2784 / 3	0.6111 / 3	0.6574 / 4	0.2901 / 3	0.5840 / 6	29.89
me5/em19	6 / 12	0.7008 / 5	0.6111 / 3	0.5536 / 3	0.1975 / 2	0.8067 / 9	35.85
me6/em13	7 / 20	0.8736 / 14	0.7778 / 5	0.8097 / 8	0.8395 / 10	0.9206 / 21	8.85
me6/em16	5 / 16	0.3488 / 5	0.5000 / 3	0.5952 / 4	0.5671 / 4	0.6143 / 7	21.2
me6/em18	11 / 16	0.8928 / 15	0.7778 / 5	0.7059 / 6	0.8364 / 8	0.9213 / 26	11.12
me7/em13	10 / 21	0.8576 / 10	0.4444 / 5	0.8097 / 7	0.7346 / 8	0.8958 / 21	23.21
me7/em14	7 / 17	0.6368 / 8	0.5000 / 3	0.8062 / 7	0.7840 / 7	0.8508 / 18	10.20
me7/em17	9 / 15	0.6976 / 7	0.1944 / 2	0.7474 / 6	0.9352 / 13	0.8306 / 21	48.22
me8/em12	8 / 22	0.5664 / 7	0.7222 / 4	0.8478 / 8	0.9136 / 14	0.0864 / 20	10.20
me8/em18	6 / 15	0.4064 / 5	0.2778 / 2	0.7128 / 6	0.8025 / 8	0.1975 / 14	31.94
me9/em13	4 / 18	0.2176 / 3	0.0000 / 1	0.3945 / 4	0.2778 / 2	0.7222 / 5	17.61
me9/em18	8 / 19	0.8544 / 10	0.6111 / 3	0.8927 / 12	0.7130 / 5	0.2870 / 21	11.66
me10/em14	15 / 25	0.8544 / 11	0.7778 / 5	0.6851 / 8	0.7716 / 4	0.9320 / 26	16.18
me10/em15	9 / 16	0.6784 / 7	0.5000 / 2	0.5813 / 3	0.7469 / 8	0.7208 / 13	9.02
me10/em19	7 / 18	0.7904 / 9	0.7778 / 5	0.7128 / 6	0.7654 / 7	0.9151 / 22	16.68
me11/em12	6 / 16	0.7520 / 5	0.2778 / 2	0.7336 / 4	0.6605 / 4	0.3395 / 10	21.33
me12/em12	7 / 18	0.6080 / 5	0.2778 / 2	0.6298 / 4	0.5679 / 3	0.4321 / 7	9.51
me12/em19	10 / 18	0.7712 / 7	0.7222 / 4	0.8927 / 12	0.7963 / 7	0.2037 / 19	7.60
Mean	7.7 / 17.5	0.6773 /8.0	0.5042 / 3.2	0.7080 /6.1	0.6816 / 6.6	0.6529 /16.2	18.77
SD	2.5 / 3.2	0.2129 /3.5	0.2284 /1.3	0.1234 /2.7	0.2113 /3.5	0.2897 /7.1	10.69

NPB, Number of polymorphic bands; TNB, total number of bands.

combination "me8/em12" in the 12 accessions. Twenty pairs of primers, which were formed between one of eight forward primers and one of eight reverse primers, generated a total of 350 clear fragments with an average of 17.5. Among these primer pairs, one hundred and fifty-three polymorphic bands were produced, with 43.7% of polymorphic percentage. Number of polymorphic bands (NPB) per primer pair ranged from 4 (me9/em13) to 15 (me10/em14) with an average of 7.65. The primer combination "me6/em18" generated 11 polymorphic bands,

which was the highest polymorphism (68.8%), and the primer combination "me9/em13" only showed 4 polymorphic bands with the polymorphic percentage of 22.2% (Table 3).

Table 4. List of primer pair combination and the number of common alleles / genetic diversity showing difference between the arbitrary pairs of the four subgroups (SC, GS, XZ and QH).

Primer pair	No. of common alleles / genetic diversity difference					
	SC-GS	SC-XZ	SC-QH	GS-XZ	GS-QH	XZ-QH
me5/em15	1 / 0.4228**	2 / 0.2305**	4 / 0.2931**	1 / -0.1922*	0 / -0.1296*	1 / 0.0626*
me5/em17	2 / 0.6150**	2 / 0.1316**	3 / 0.0348**	0 / -0.4835**	1 / -0.5802**	2 / -0.0968**
me5/em18	2 / -0.3327**	2 / -0.3790**	2 / -0.0117	3 / -0.0463	2 / 0.3210**	2 / 0.3673**
me5/em19	1 / 0.0897*	1 / 0.1472**	0 / 0.5033**	1 / 0.0575	0 / 0.4136**	1 / 0.3561**
me6/em13	4 / 0.0958**	4 / 0.0639**	4 / 0.0341**	1 / -0.0319	0 / -0.0617*	3 / -0.0298**
me6/em16	2 / -0.1512*	4 / -0.2464**	3 / -0.2129**	2 / -0.0952	2 / -0.0617	2 / 0.0334
me6/em18	2 / 0.1150**	2 / 0.1869**	3 / 0.0564**	0 / 0.0719*	1 / -0.0586*	0 / -0.1305**
me7/em13	2 / 0.4132**	3 / 0.0479**	0 / 0.1230**	2 / -0.3652**	0 / -0.2901**	0 / 0.0751**
me7/em14	1 / 0.1368*	4 / -0.1694**	3 / -0.1472**	2 / -0.3062**	0 / -0.2840**	1 / 0.0223
me7/em17	2 / 0.5032**	4 / -0.0498**	3 / -0.2376**	2 / -0.5530**	2 / -0.7407**	2 / -0.1878**
me8/em12	2 / -0.1558**	6 / -0.2814**	5 / -0.3472**	2 / -0.1255**	2 / -0.1914**	7 / -0.0658**
me8/em18	2 / 0.1286	3 / -0.3064**	2 / -0.3961**	2 / -0.4350**	2 / -0.5247**	2 / -0.0897**
me9/em13	1 / 0.2176**	2 / -0.1769**	1 / -0.0602	1 / -0.3945**	1 / -0.2778**	2 / 0.1167*
me9/em18	3 / 0.2433**	1 / -0.0383**	3 / 0.1414**	1 / -0.2816**	1 / -0.1019*	3 / 0.1798**
me10/em14	1 / 0.0766**	1 / 0.1693**	0 / 0.0828**	2 / 0.0927*	0 / 0.0062	1 / -0.0865**
me10/em15	2 / 0.1784**	3 / 0.0971**	2 / -0.0685**	2 / -0.0813	1 / -0.2469**	2 / -0.1656**
me10/em19	0 / 0.0126	0 / 0.0776**	0 / 0.0250	3 / 0.0650	1 / 0.0123	2 / -0.0526*
me11/em12	1 / 0.4742**	2 / 0.0184	1 / 0.0915**	2 / -0.4558**	0 / -0.3827**	0 / 0.0731**
me12/em12	2 / 0.3302*	3 / -0.0218	2 / 0.0401*	2 / -0.3520**	2 / -0.2901*	2 / 0.0619*
me12/em19	3 / 0.0490	3 /-0.1215**	2 / -0.0251*	1 / -0.1705**	2 / -0.0741*	5 / 0.0964**
Mean	1.8/0.1731**	2.6 / -0.0307**	2.2 / -0.0043*	1.6 / -0.2038**	1.0 / -0.1774**	2 / 0.0264**

*, ** Significant at probability levels of 0.05 and 0.01, respectively.

A total of 324 alleles were detected in the entire samples among 20 primer pairs with an average of 16.2 alleles per primer pair (Table 3). The number of alleles ranged from 5 (me9/em13) to 26 (me5/em17, me6/em18 and me10/em14) in the entire sample. The mean number of alleles in Sichuan Province of China (SC), Gansu Province of China (GS), Xizang (Tibet) Autonomous Region of China (XZ) and Qinghai Province of China (QH) was 8.0, 3.2, 6.1 and 6.6, respectively. The number of common alleles between the two arbitrary geographic populations of SC, GS, XZ and QH also varied with the different SRAP markers (Table 4). The highest value for mean number of common alleles (2.6) existed between XZ and SC, whereas the lowest (1.0) was between GS and QH.

Genetic diversity and genetic differentiation

Genetic diversity from 66 accessions (two accessions from Yunnan province of China were not included) of the subgroups SC, GS, XZ and QH was analyzed using 20 pairs of primers. According to data in Table 3, the ranges of genetic diversity from subgroup SC, GS, XZ and QH were from 0.2176 (me9/em13) to 0.8928 (me5/em17, me6/em18) with an average of 0.6773, from 0.0000 (me9/em13) to 0.7778 (me6/em13, me6/em18, me10/em14 and me10/em19) with an average of 0.5042, from 0.3945 (me9/em13) to 0.8927 (me12/em19, me9/em18) with an average of 0.7080, from 0.1975 (me5/em19) to 0.9352 (me7/em17) with an average of 0.6816, respectively, and the range of genetic diversity in entire sample was from 0.0864 (me8/em12) to 0.9412 (me5/em17) with an average of 0.6529. Generally, mean genetic diversity order of the four subgroups was XZ > QH > SC > GS.

The comparisons of genetic diversity (Table 4) indicated that the significance of genetic diversity difference between the arbitrary two of four subgroups varied along with different primer pairs. According to Z-test's results, significant differences existed between most pairs of four subgroups. On the average, the differences of genetic diversity between the two arbitrary subgroups of the four subgroups were highly significant ($P < 0.01$) except for that the difference of genetic diversity between SC and

QH was significant ($P < 0.05$).

The genetic differentiation (Table 3) among the developed qingke varieties from the four subgroups ranged from 7.60% (me12/em19) to 48.22% (me7/em17) with

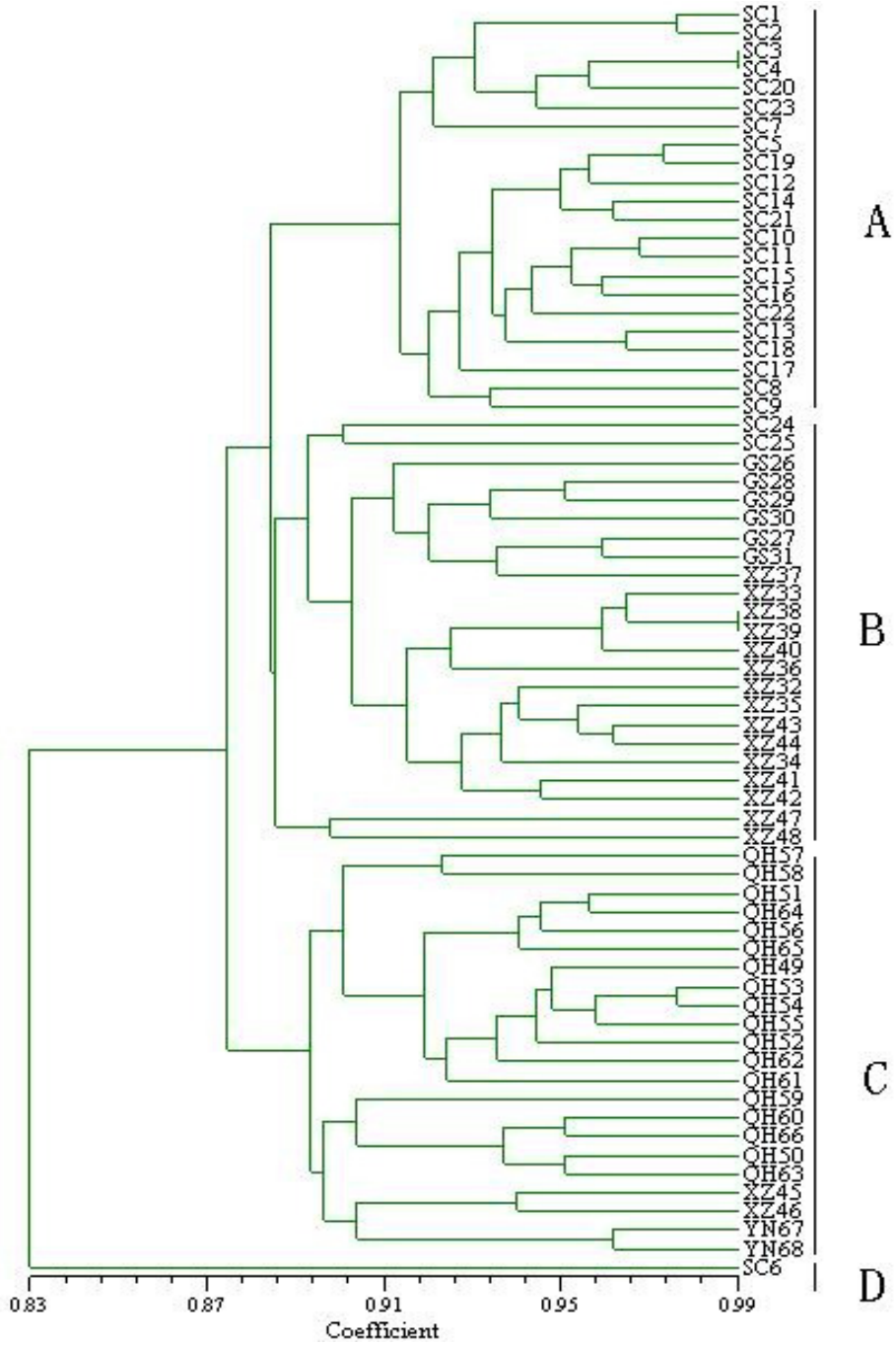

Figure 2. A dendrogram of 68 accessions of hulless barley varieties from the Qinghai-Tibet Plateau of China generated from SRAP markers.

different SRAP primer pairs, and the average value was 18.77%. This indicated that lower genetic differentiation existed among the accessions from the four regions.

Cluster analysis

The dendrogram using UPGMA analysis clustered the 68 accessions into four primary groups (Figure 2). Group A was composed of the 22 SC accessions except for SC6 which clustered alone in Group D, and SC24 and SC25 were clustered into Group B with all 6 accessions from

GS and 15 accessions from XZ. All the accessions from QH were classified into Group C with 2 XZ accessions (XZ45 and XZ46) and 2 YN entries (YN67 and YN68). The dendrogram revealed significant relationships among

Table 5. Average of genetic distance (AGD) within each subgroup.

Subgroup	AGD	SC	GS	XZ	QH
SC	0.0418	0	0.9593	0.9672	0.9521
GS	0.0657	0.0407	0	0.9705	0.9417
XZ	0.0605	0.0328	0.0295	0	0.9420
QH	0.0921	0.0479	0.0583	0.0580	0

Genetic distance (below diagonal) and genetic similarities (above diagonal) among the four subgroups.

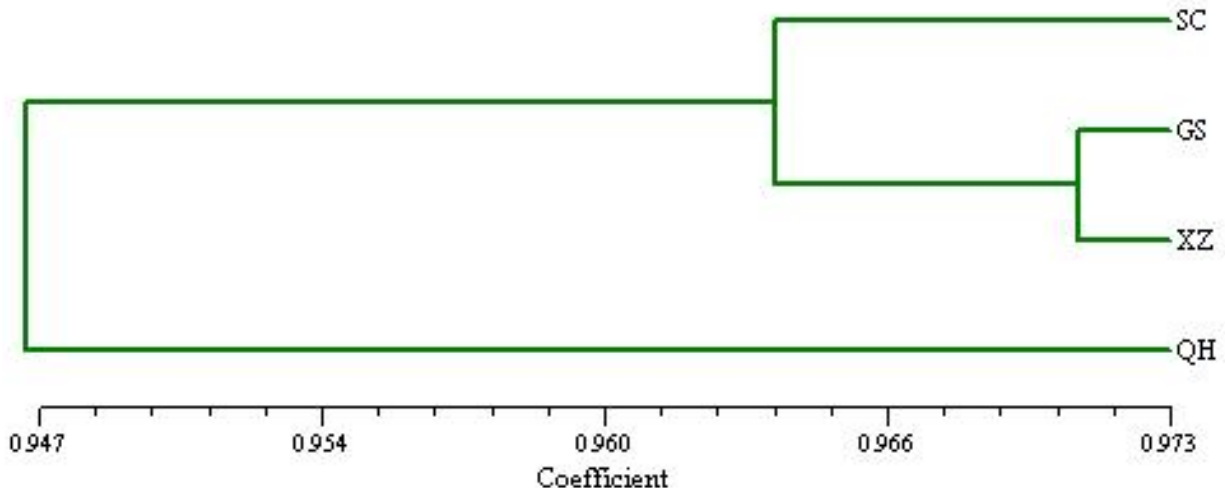

Figure 3. Dendrogram of the four subgroups based on genetic similarity.

accessions with original locations.

Genetic distance and relationship among the four subgroups

That POPGENE ver.3.11 was used to analyze the genetic distance among the four subgroups in Table 5 which shows that genetic distance (0.0583) between the QH and GS accessions is higher than (0.0580) between the QH and XZ accessions, whereas genetic distance is only 0.0479 between QH and SC accessions. Lowest (0.0295) existed between the GS and XZ accessions. Genetic relationships among the four subgroups (Figure 3) were analyzed using the NTSYS-pc (5.1) program. It indicated that genetic difference between the SC and QH accessions was higher than that between the SC accessions and either of both the GS and XZ accessions as well. The genetic difference between GS and XZ accessions was lowest. This result was also identical with that shown in Figure 2.

DISCUSSION

The SRAP marker system was primarily developed for *Brassica* species, and then was tested in other crop plants. Previous results proved that SRAP markers had many advantages that was simplicity, reliability, moderate output ratio and easy sequencing of selected bands (Li and Quiros, 2001). Ferriol et al. (2003) reported that the information given by SRAP markers in genetic diversity analysis was more consistent with the morphological variability and to the evolutionary history of the morphotypes than that of amplified fragment length polymorphism (AFLP) markers. In barley, genetic-diversity results, which were first reported for qingke varieties from Sichuan province of China using SRAP markers (Yang et al., 2008), also indicated that the SRAP markers had high polymorphism and higher correlation between the clusters and origins of the accessions as well. In this study, 20 SRAP primer combinations produced significant polymorphic bands (Table 3). Three hundred and twenty-four alleles, which were amplified by 20 primer combinations in 68 accessions, were higher in number than that given in the genetic diversity on the qingke landraces from the Qinghai-Tibet plateau of China using simple sequence repeat (SSR) markers (Feng et al., 2006b) and also in cultivated qingke varieties from China by SSR markers (Pan et al., 2008). The

dendrogram of SRAP markers given in the present study identified geographical origins of the accessions (Figure 2). Results show that the SRAP marker could be efficiently used in the study of genetic variability of barley.

The amount of genetic diversity depends on the types and frequencies of alleles. Three hundred and twenty-four alleles were detected over 20 primer pairs in the entire samples, with an average of 16.2 alleles per primer pair (Table 3). The order for the mean number of alleles based on Table 3 was as follows: SC (8.0 ± 3.5) > QH (6.6 ± 3.5) > XZ (6.1 ± 2.7) > GS (3.2 ± 1.3), while the rank for the mean genetic diversity from these regions was XZ (0.7080 ± 0.1234) > QH (0.6816 ± 0.2113) > SC (0.6773 ± 0.2129) > GS (0.5042 ± 0.2284). In general, genetic diversity values were relatively low, indicating that it is necessary to widen the genetic base of qingke barley through breeding. However, the mean number of common alleles between XZ and SC accessions was the highest (2.6), while the lowest common alleles (1.0) occurred between GS and QH accessions (Table 4). Moreover, genetic diversity was able to show significant difference between the arbitrary subgroup pairs (Table 4). Table 3 also shows that the lower genetic differentiation (18.77%) existed among the four subgroups.

Collecting genetic resources from different geographical areas can bring genetic diversity required for barley breeding. Table 5 indicates that lower genetic distance occurred not only between the accessions pairs within the four subgroups but also between the subgroup pairs among the four subgroups. This study has also shown relatively low genetic diversity among qingke varieties. The findings stated that narrow genetic basis existed within and among the four subgroups. It is proposed that collection and conservation of barley genetic resources are important for improvement of this crop. Thus, elite hulless barley germplasm could be effectively used to enhance the genetic background for breeding superior hulless barley.

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Abbreviations

GD, Genetic distance; **GS,** Gansu Province of China; **GSI,** genetic similarities; **NPB,** number of polymorphic bands; **QH,** Qinghai Province of China; **SC,** Sichuan Province of China; **SD**, standard deviation; **TNB,** total number of bands; **XZ,** Xizang (Tibet) Autonomous Region of China; **YN,** Yunnan Province of China; **A-PAGE,** acid-polyacrylamide gel electrophoresis; **SRAP,** sequence-related amplified polymorphism; **CTAB,** cetyl trimethyl ammonium bromide; **PCR,** polymerase chain reaction.

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