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Genetic Diversity among Barley Populations from West China Based on RAMP and RAPD Markers

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Abstract: Using RAMP and RAPD for detecting the genetic diversity of 46 barley accessions, collected from west China. Seventeen primer combinations produced 104 discernible RAMP fragments of which 96 (91%) were polymorphic. The number of fragments per primer combination varied from 2 to 11, with the mean of 6.18. The mean of Polymorphism Information Content (PIC) for the RAMPs was 0.752. On the basis of 96 polymorphic fragments, each genotype had a unique banding profile and the Genetic Similarity (GS) coefficient varied between 0.450 and 0.960, with the mean of 0.803. In RAPD analysis, 28 out of 43 bands (65%) were polymorphic. The number of alleles ranged from 1 to 8, with an average of 2.53 per primer. The mean of Polymorphism Information Content (PIC) for the RAPDs was 0.282. The Genetic Similarity (GS) coefficient varied between 0.679 and 1.000, with the mean of 0.898. The mean values of GS within the HS, HA and HV groups were 0.909, 0.893 and 0.913, respectively. RAMP-based genetic similarity matrices were compared with the corresponding RAPD-based matrices by the Mantel test. A poor of correlation was found between both sets of data, indicating little relationship between these two estimators of genetic similarity. The cluster results based on RAMP more faithfully distinguished the experimental accessions than did the RAPD results. Both dendrograms generated by the RAPD matrix and the RAMP matrix all agree better with the groups of the genotypes, but the dendrogram generated by the RAPD matrix agrees better with the geographic origins of the genotypes than the dendrogram generated by the RAMP results.

Key words: Barley, RAMP marker, RAPD marker, genetic diversity, West China

INTRODUCTION

Barley, *Hordeum vulgare* L., is one of the principal cereal crops in the world crop and is cultivated in all temperate areas (von Bothmer *et al*., 1995). Wild barleys, *H. vulgare* ssp. *spontaneum* and *H. vulgare* ssp. *agriocrithon*, are the primary gene pool of cultivated barley (*H. vulgare* ssp. *vulgare*). China is known to be the genetic diversity center of barley and rich in both landrace and wild relatives of barley (Yong-Cui *et al*., 2005). The wild relatives of barley constitute a large reservoir of genetic diversity. It is known to possess high genetic variation in several useful traits (Nevo, 1992), so the wild relatives of barley have been used as a source of important genes for barley breeders for cultivar development via., interspecific crosses (Dávila *et al*., 1999a). The new varieties are more genetically homogeneous (Yong-Cui *et al*., 2005). This has promoted the search for new sources of variation that might be of use in plant breeding programs (Brown *et al*., 1990). Knowledge regarding the amount of genetic variation in germplasm and genetic relationships between genotypes are important considerations for efficient conservation and utilization of germpalsm resources (Kresovich *et al*., 1995; Russell *et al*., 1997; Dávila *et al*., 1998). In the context of plant improvement, this information provides a basis for making decisions regarding selection of parental combinations that will maximize gain from selection and maintain genetic diversity (Matus and Hayes, 2002). More knowledge regarding the genetic structure of breeding materials could help to maintain genetic diversity (Troyer *et al*., 1998; Liu *et al*., 2000).

The degree of diversity present in a sample of germplasm can be measured in terms of morphology, pedigree, allelic diversity at marker loci and allelic diversity at genes determining target phenotypes. Accordingly, diversity at markers loci is currently the most feasible strategy for characterizing diversity in wild and cultivated germpalsm (Matus and Hayes, 2002). Many types of molecular markers have been used to characterize and evaluate the genetic diversity within and between species and populations. It has been shown that different markers might uncover different classes of variation (Powell *et al*., 1996; Russell *et al*., 1997; Dávila *et al*., 1999b).

In barley, highly polymorphic microsatellites had been developed by Saghai-Maroof *et al*. (1994), Becker and Heun (1995a) and Liu *et al*. (1996) and had been

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shown to discriminate between even closely related cultivars (Russell *et al*., 1997). These studies have shown the reproducibility of the patterns generated, the Mendelian inheritance of the polymorphic amplified bands and their usefulness in the investigation of the genetic relationships between accessions or cultivars of different plant species.

In the present study, 5'-anchored oligonucleotides complementary to microsatellites in combination with oligonucleotides of arbitrary sequence were used to amplify barley DNA and generate Random Amplified Microsatellite Polymorphisms (RAMP) (Wu *et al*., 1994). Meanwhile, the arbitrary oligonucleotides were used as single primers in a parallel RAPD analysis to detect the level polymorphism of the same samples. The objectives of this study are to (1) reveal the RAMP-based genetic diversity in a barley germplasm from western China, (2) compare RAMP and RAPD diversity in the studied materials and (3) assess the genetic diversity within the selected accessions of the barley landraces as compared to that in its wild relatives by using RAPD and RAMP molecular markers.

MATERIALS AND METHODS

Plant material and DNA extraction: The material were planted at Yaan, Sichuan in 2006. A total of 46 barley accessions from 44 locations of west China were used in this study (Table 1). There were 27 landraces of *H. vulgare* ssp. *vulgare* (HV), 6 wild relative forms of *H. vulgare* ssp. *spontaneum* (HS) and 13 wild relative 0 forms of *H. vulgare* ssp. *agriocrithon* (HA). Genomic DNA was extracted from a bulk sampling of a minimum of ten individuals for each accession according to Sharp *et al*. (1988).

RAMP and RAPD analysis: Two 5' anchored oligonucleotides, GC(CA)4 and GT(CA)4, were used in combination with oligonucleotides of arbitrary sequence from Operon (kits A, B, H and R) to amplify DNA of the 46 accessions. Seventeen primer combinations were selected (Table 2). The PCR reaction mixture consisted of 20~50 ng of genomic DNA, 1×PCR buffer (10 mmol L^{-1} Tris-HCl pH 8.3, 50 mmol L^{-1} KCl, 0.001% gelatin), 2.0 mmol L^{-1} $MgCl_2$, 100 μmol L^{-1} of each dNTP, 0.1 μmol L^{-1} of each primer and 1U of *Taq* polymerase in a 25 μL volume. The amplification protocol was 94°C for 4 min to pre-denature, followed by 45 cycles of 94°C for 1 min, 42°C or 45°C for 1 min and 72°C for 1 min, with a final extension at 72°C for 10 min. Amplification products were fractionated on 2% agarose gel. Amplification reactions using the Operon oligonucleotides as single primers to produce RAPDs were carried out following the same experimental procedure.

Data analysis: RAMP and RAPD data were scored for presence (1), absence (0) or as a missing observation (9), and each band was regarded as a locus. Two matrices, one for each data, were generated. The Genetic Similarities (GS) were calculated according to Nei and Li (1979):

Table 1: The origin of 46 landraces and wild barley accessions used in this study

Acc. No.	Group	Rowed type	Hulled or Naked	Origin	Acc. No.	Group	Rowed type	Hulled or Naked	Origin
ZYM0022	HS	Two	Hulled	Gongbujiangda, Xizang	B20	HV	Six	Naked	Mianning, Sichuan
ZYM0059	HS	Two	Hulled	Longzi, Xizang	B21	HV	Six	Naked	Ma'erkang, Sichuan
ZYM0083	HS	Two	Hulled	Cuola, Xizang	B22	HV	Six	Naked	Dangxiong, Xizang
ZYM0182	HS	Two	Hulled	Qusong, Xizang	B23	HV	Six	Naked	Minshan, Gansu
ZYM0195	HS	Two	Hulled	Qiongjie, Xizang	B33	HV	Six	Naked	Xiahe, Gansu
ZYM0212	HS	Two	Hulled	Dingri, Xizang	B40	HV	Six	Naked	Li, Sichuan
ZYM0281	HA	Six	Hulled	Luolong, Xizang	B47	HV	Six	Naked	Ganzi, Sichuan
ZYM0310	HA	Six	Hulled	Nimu, Xizang	B53	HV	Six	Naked	Jinchuan, Sichuan
ZYM0315	HA	Six	Hulled	Mozhugongka, Xizang	B65	HV	Six	Naked	Lasa, Xizang
ZYM0407	HA	Six	Hulled	Luozha, Xizang	B76	HV	Six	Hulled	Lu, Sichuan
ZYM0444	HA	Six	Hulled	Naidong, Xizang	B77	HV	Six	Hulled	Peng'an, Sichuan
ZYM0469	HA	Six	Hulled	Rikaze, Xizang	B83	HV	Four	Naked	Wenchuan, Sichuan
ZYM0494	HA	Six	Hulled	Zhada, Xizang	B87	HV	Six	Naked	Xiaojin, Sichuan
ZYM0531	HA	Six	Hulled	Luolong, Xizang	B95	HV	Six	Hulled	Miyi, Sichuan
ZYM0545	HA	Six	Hulled	Chaya, Xizang	B96	HV	Four	Hulled	Santai, Sichuan
ZYM0606	HA	Six	Hulled	Milin, Xizang	B97	HV	Six	Naked	Ruo'ergai, Sichuan
ZYM0642	HA	Six	Hulled	Linzhou, Xizang	B98	HV	Four	Hulled	Nanchong, Sichuan
ZYM0830	HA	Six	Hulled	Jiangzi, Xizang	B108	HV	Six	Naked	Heishui, Sichuan
ZYM0834	HA	Six	Hulled	Renbu, Xizang	B115	HV	Six	Naked	Nanping, Sichuan
B4	HV	Six	Naked	Ruo'ergai, Sichuan	B118	HV	Six	Naked	Gongka, Xizang
B10	HV	Six	Naked	Rangtang, Sichuan	B120	HV	Six	Naked	Mozhugongka, Xizang
B12	HV	Six	Naked	Songpan, Sichuan	B124	HV	Six	Naked	Qingdao
B18	HV	Six	Naked	Hongyuan, Sichuan	B125	HV	Six	Hulled	Xichang, Sichuan

$$GS = 2Nij/(Ni+Nj)$$

where, Nij is the number of bands present in both genotypes i and j, Ni is the number of bands present in genotypes i and Nj is the number of bands present in genotype j. Based on the similarity matrix, a dendrogram showing the genetic relationships between genotypes was constructed using the unweighted pairgroup method with rithmetic average (UPGMA) (Sneath and Sokal, 1973) though the software NTSYS-pc version1.80 (Rohlf, 1993). Polymorphic Information Content (PIC) values were calculated for each RAPD primer and RAMP primer combination according to the formula:

$$PIC = 1-\Sigma(Pij)^2$$

where, Pij is the frequency of the ith pattern revealed by the jth primer summed across all patterns revealed by the primers (Botstein *et al*., 1980). Similarity matrices based on different marker system (RAMP and RAPD) were compared using the standardized Mantel coefficient (Mantel, 1967). The significance level for the correlation coefficient was calculated by Sokal and Rohlf (1995).

RESULTS

Polymorphism within barley: Seventeen oligonucleotides of arbitrary sequence were used as single primers to amplify DNA of the 46 accessions to produce RAPDs (Table 2). The Total Number of Bands (TNB), Number of Polymorphic Bands (NPB), percentage of polymorphic bands (P%) and Polymorphic Information Content (PIC) obtained per each primer were also shown in the Table 2. A total of amplified products were 43, with the average of 2.53 bands, ranged from 1 to 8 bands per primer. Twenty-eight out of 43 bands (65%) were polymorphic, among which 0 to 8 polymorphic bands were detected by each primer. The Polymorphic Information Content (PIC) of the seventeen RAPDs primer ranged from 0 for eight primer (OPA-3, OPA-6, OPA-14, OPA-15, OPA-19, OPA-20 OPP-15 and OPB-17) to 0.907 for primer OPB-20. The average PIC value was 0.282.

The same seventeen oligonucleotides of arbitrary sequence were used in combination with two 5'anchored oligonucleotides, $GT(CA)_4$ and $GC(CA)_4$ to amplify DNA of the 46 accessions to obtain RAMPs. The TNB, NPB, P% and PIC obtained per each primer combinations were also shown in Table 2. A total of 105 fragments resulting in a mean of 6.18 fragments and ranging from 2 to 11 bands per pair of primers. Ninety-six bands (91%) were polymorphic with the mean of 5.65 per primer. The average Polymorphic Information Content (PIC) was 0.752, ranging from 0.334 to 0.948. The lowest and the highest PIC values were recorded for primer combination OPB-20+ $GT(CA)_4$ (0.334) and OPH-19+ $GC(CA)_4$ (0.948), respectively. percentage of polymorphic bands; PIC, polymorphic information content.

Table 2: RAPD primers and the results of amplification

Primer	TNB	NPB	P(%)	PIC
RAPD				
OPA-3	1	0	0	0.000
OPA-6	1	0	0	0.000
OPA-14	1	0	0	0.000
OPA-15	1	0	0	0.000
OPA-18	6	6	100	0.833
OPA-19	1	0	0	0.000
OPA-20	1	0	0	0.000
OPP-15	1	0	0	0.000
OPB-17	1	0	0	0.000
OPB-18	4	1	25	0.371
OPB-20	8	8	100	0.907
OPH-4	2	1	50	0.294
OPH-13	4	4	100	0.363
OPH-19	3	2	67	0.623
OPP-2	3	3	100	0.371
OPP-4	3	2	67	0.570
OPP-8	2	1	50	0.466
Total	43	28	65	
Mean	2.53	1.65	65	0.282
RAMP				
OPA-3+ $GT(CA)_4$	8	8	100	0.808
OPA-6+ $GT(CA)_4$	2	2	100	0.589
OPA-14+ $GC(CA)_4$	4	4	100	0.779
OPA-15+ $GC(CA)_4$	5	5	100	0.726
OPA-18+ $GT(CA)_4$	7	6	86	0.905
OPA-19+ $GT(CA)_4$	7	6	86	0.869
OPA-20+ $GT(CA)_4$	4	3	75	0.534
OPP-15+ $GC(CA)_4$	7	5	71	0.647
OPB-17+ $GC(CA)_4$	5	5	100	0.798
OPB-18+ $GC(CA)_4$	11	11	100	0.940
OPB-20+ $GT(CA)_4$	5	3	60	0.334
OPH-4+ $GC(CA)_4$	5	5	100	0.760
OPH-13+ $GT(CA)_4$	7	7	100	0.789
OPH-19+ $GC(CA)_4$	9	8	89	0.948
OPP-2+ $GC(CA)_4$	6	5	83	0.683
OPP-4+ $GT(CA)_4$	6	6	100	0.800
OPP-8+ $GC(CA)_4$	7	7	100	0.873
Total	105	96	91	
Mean	6.18	5.65	91	0.752

TNB: The total No. of bands; NPB: Number of polymorphic bands; P%: Percentage of polymorphic bands

Comparing the results of the two set of experiments, it showed that RAMP marker could be revealed more polymorphism than did the RAPD results. The average PIC value produced by RAMPs (0.752) was significant higher than that revealed by RAPDs (0.282) and all of the PIC values produced by RAMP primer combinations were higher than those revealed by RAPD primer, excepted the primer combination OPB-20+ $GT(CA)_4$.

Genetic similarity estimates: Genetic similarity between 46 barley accessions was estimated from the RAPD and RAMP data using the Dice coefficient. The similarity values between each pair of accessions obtained from the

RAMP data were lower that the corresponding values from RAPD data. The average genetic similarity value using RAMP was 0.803 and it was 0.898 using RAPD data. The correlation coefficient calculated for the elements of the two similarity matrices using the Mantel test. A poor correlation (r = 0.307) was found. It suggested that both sets of markers provided unrelated estimates of genetic relationships.

All the 43 bands, generated from 17 RAPD primers, were subjected to calculate the genetic similarity index among the 46 genotypes. The RAPD-GS value ranged from 0.679 to 1.000. The lowest genetic similarity was observed between HV accession B20 (from Sichuan) and HA accession ZYM0281 (from Xizang). Four HV accessions, such as B21 (from Sichuan), B23 (from Gansu), B65 (from Lasa) and B83 (from Sichuan), had the highest genetic similarity values 1.000. It indicated that the RAPDs failed to clearly distinguish them. It was found that the mean genetic similarity among the all accessions (0.898) exhibited equivalent to that within the three groups, such as HS accessions (0.909), HA accessions (0.893) and HV accessions (0.913), respectively (Table 3).

All the 105 bands, generated from 17 RAMP primer combinations, were subjected to calculate the genetic similarity index among the 46 genotypes. The RAMP-GS value ranged from 0.450 to 0.960. The lowest genetic similarity was observed between B18 and B125, which both were HV accessions and originated from Sichuan Province. The highest genetic similarity was found between HV accession B21 (from Sichuan) and HV accession B33 (from Gansu). The average genetic similarity among the all accessions (0.803) exhibited equivalent to within HV accessions (0.809) and HA accessions (0.797). However, the average genetic similarity within HS accessions (0.845) was higher than that within HV accessions (0.809) and HA accessions (0.797), as well as among the all accessions (0.803) (Table 3). It indicated that the HS group was more diverse than the HA and HV groups. The average genetic similarity values based on RAMP markers were lower than that of RAPD markers both within landraces and wild barley forms, as well as among the all accessions. These

Table 3: RAMP and RAPD based genetic similarity within groups

Group	HV	HS	HA	All
RAMP				
Mean	0.809	0.845	0.797	0.803
Variation	0.451-0.960	0.766-0.905	0.610-0.919	0.450-0.960
RAPD				
Mean	0.913	0.909	0.893	0.898
Variation	0.800-1.000	0.853-0.963	0.733-0.984	0.679-1.000

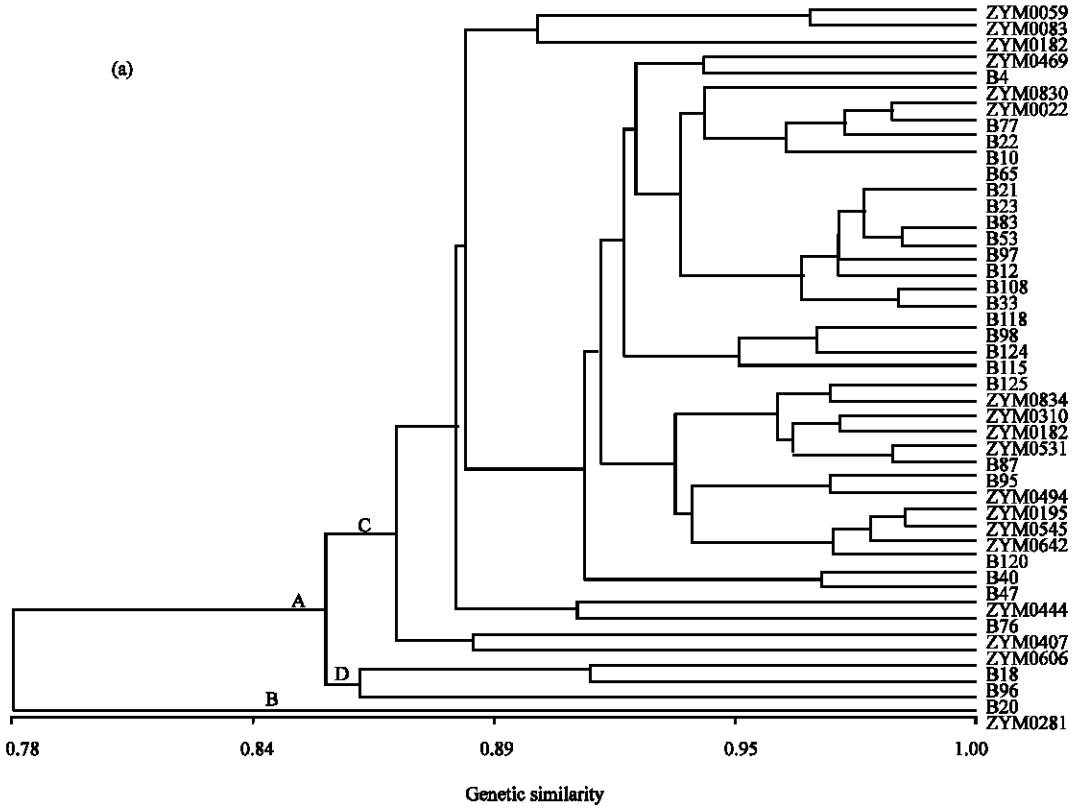

Fig. 1: Continued

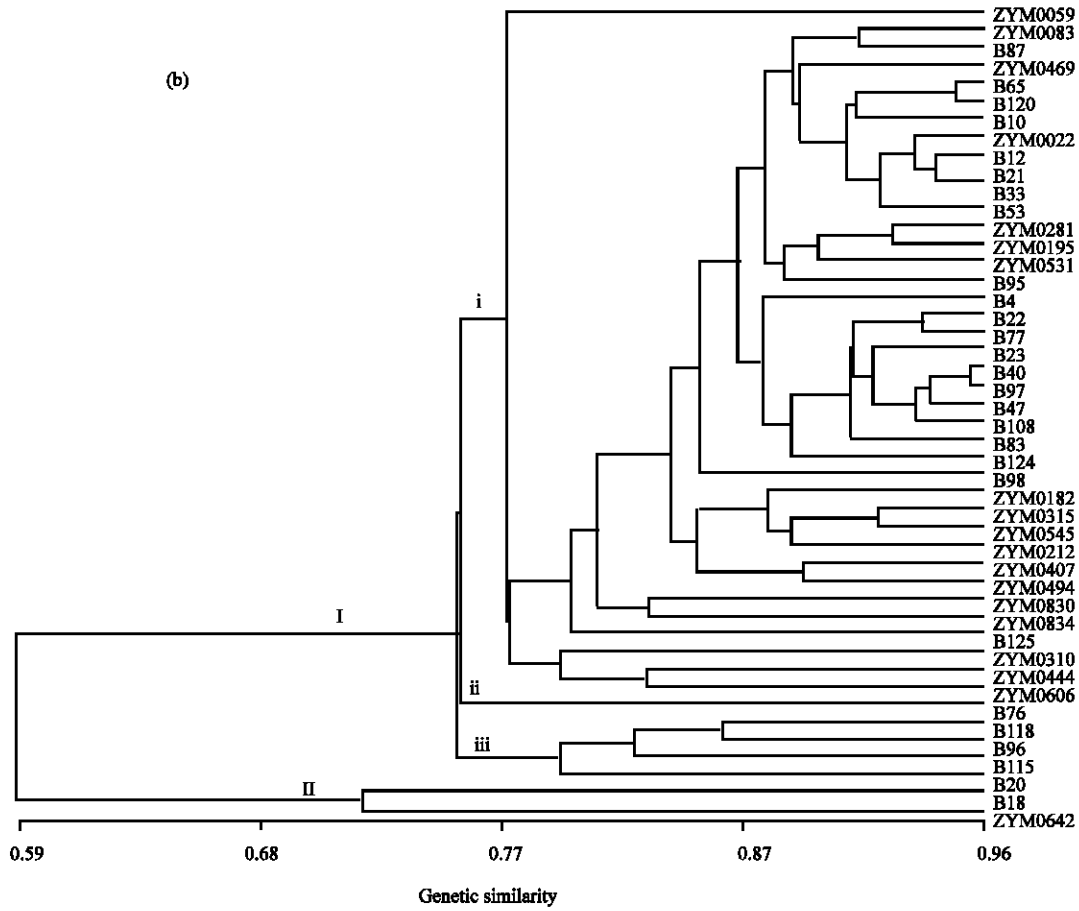

Fig. 1: Dendrogram of 46 barley accessions constructed from matrices of similarity based on RAPD data (a) and RAMP data (b)

results suggested that higher genetic diversity could be detected by RAMP markers than that of RAPD markers among the 46 barley accessions from west China.

Dendrogram: The resultant dendragrams based on RAPD and RAMP similarities were shown in Fig. 1. The shape of the two dendragrams was very similar. But the genetic relationships among the accessions as revealed by RAPD analysis were different from the relationships revealed by analysis of RAMP data. The RAPD-derived dendragram (Fig.1a) has two main clusters (A and B), the first includes all the accessions except a HA accession ZYM0281, coming from Xizang, which constitutes the second cluster (B). On the other hand, the first cluster is divided in two subclusters (C and D). In one of them (D) contains three naked HV accessions, which originated from Sichuan Province. The other subcluster (C) contains the remained 42 studied accessions. It showed that in the subcluster C there were four HV accessions did not distinguish clearly by the dendragram and most of the wild relative forms (HA and HS groups) could be cluster into a group with each other faster than with the landraces (HV group).

The RAMP-derived dendragram (Fig. 1b) also has two main clusters. The first cluster included all the accessions except a HA accession ZYM0281 and a HV accession B18. They originated from Xizang and Sichuan respectively and constituted the second cluster. The cluster is divided into three subclusters. In one of them contained a six-rowed and hulled HV accession B76, which originated from Sichuan Province. The subcluster contained four HV accessions. Three of them (B20, B115 and B118) were six-rowed and naked and the other one (B96) was four-rowed and hulled. And three of them (B20, B96 and B115) were collected from Sichuan and the other one (B118) collected from Xizang. The subcluster contained the remained 39 studied accessions. It showed that all 46 accessions were could be clearly distinguished by the dendragram and most of the wild relative forms (HA and HS groups) could be cluster into a group with each other faster than with the landraces (HV group).

DISCUSSION

In this study, genomic DNA was extracted from a bulk sampling for each accession. The advantages and inconvenient of the bulk analysis have been discussed by Michelmore *et al.* (1991) and Loarce *et al.* (1996). Bulk analysis are economic and rapid and it is possible to estimate the genetic variability between accessions, whereas it is not possible to obtain information about the genetic variability within the accessions (Fernández *et al.*, 2002). The number of individual plants bulked for the accessions is an important experimental factor whether the bulked analysis revealed the genetic relationship between the accessions. Yang and Quiros (1993) found that the bulked samples with 10, 20, 30, 40 and 50 individuals had the same banding pattern. Bustos *et al.* (1998) also found that bulks of 10 to 20 individuals resulted in the same RAPD profiles. In this study, we used a minimum of ten individuals for representing the each barley accession. Two of the Polymerase Chain Reaction (PCR)-based systems, RAMP and RAPD, were used to detect the genetic diversity among the *H. vulgare* ssp. *vulgare*, *H. vulgare* ssp. *spontaneum* and *H. vulgare* ssp. *agriocrithon* populations from west China. The barley accessions are a small component of the total gene pool of this species from China. However, both the molecular marker systems have been able to show high levels of polymorphism among these accessions.

Given the proliferation of genetic markers, comparisons between techniques are inevitable. However, there is a need for such comparison in order to decide on which technique is best studied to the issue being examined. The results using the primer combinations containing 10 mer oligonucleotides of arbitrary sequence and either $GT(CA)_4$ or $GC(CA)_4$ that produced RAMPs was in sharp contrast with the positive results obtained using the same 10 mer oligonucleotides of arbitrary sequence as single primers that produced RAPDs. To diminish the influence of errors in the analysis produced by amplifications that might have generated faint fragments, only those fragments present in at least three different accessions were considered. Every assay was repeated at least twice. When amplified products were individually considered in each assay, RAMP bands showed the higher level of polymorphism (91%) whereas only 65% of the RAPD bands were polymorphic. This in turn was reflected in the overall Polymorphism Information Content (PIC) values calculated. The mean PIC value (0.752) in RAMP analysis also was significant higher than that in RAPD analysis (0.282). Considering the entire germplasm array, the genetic similarity values ranged from 0.450 to 0.960 for RAMP analysis contrasting with the results ranged from 0.679 to 1.000 for RAPD analysis. Moreover, the average GS values for the 17 RAMPs surveyed among the *H. vulgare* ssp. *vulgare*, *H. vulgare* ssp. *spontaneum* and *H. vulgare* ssp. *agriocrithon* were lower (0.809, 0.845 and 0.797, respectively) than that obtained from the 17 RAPDs analysis among the three populations (0.913, 0.909 and 0.893, respectively). Furthermore, there were four landraces (i.e., B21, B23, B65 and B83) could not be distinguished by the 17 RAPDs analysis, although they originated from different locations. The results suggested that the RAMP markers were superior to RAPD markers in the capacity of revealing more informative bands in a single amplification and detecting the genetic diversity among the studied accessions. As previously demonstrated by Sánchez de la Hoz *et al.* (1996), genetic relationship between barley cultivars were better reflected by RAMP-based analysis than by RAPD-based analysis. Moreover, RAMP should be considered a good multilocus marker system, easy to perform and able to detect high levels of polymorphism, comparing with the coefficient parentage (COPs) (Dávila *et al.*, 1998) and SSR marker (Dávila *et al.*, 1999a).

Substantial disagreement was found when genetic similarity based on RAMP analysis was compared with RAPD eatimates. A poor correlation was observed between these two set of data. It suggested that both set of markers explore genetic variation differently. It is likely that RAMP and RAPD markers target different regions of the genome, which are subjected to different mechanisms generating genetic variation. It has been previously shown that primers used to produce RAMP markers mainly annealed to sequences containing short arrays of microsatellite motif (4 to 10) (Dávila *et al.*, 1999b). These elements are less prone to variation by strand slippage during replication than the larger ones (Weber and May, 1989). However, the RAPD technique provides a powerful tool for obtaining a large number of anonymous loci of both coding and non-coding sequences, although it is commonly considered that most RAPD loci consist of non-coding sequences (Williams *et al.*, 1990).

The poor correlation between these two set of genetic similarity data resulted in that the dendrogram based on RAPD markers was not in accord with the dendrogram based on RAMP markers (Fig. 1). However, when the studied barley genotypes divided into two groups, landraces and wild relative forms, the both dendrogram generated by the RAPD matrix and the RAMP matrix all agree better with the groups of the genotypes. Most of the genotypes within the wild barley forms and the landraces were closely related and were

classified into same branch. However, the dendrogram generated by the RAPD matrix agrees better with the geographic origins of the genotypes than the dendrogram generated by the RAMP results. For example, there were four unique branches containing two landraces (i.e., B53 and B97, B40 and B47, B18 and B96, B21 and B83), which they all originated from Sichuan Province. Several studies have previously demonstrated that the dendragrams based on RAPD or RAMP were not in accord with that based on other molecular markers (Fernández *et al*., 2002; Wu *et al*., 2005; Dávila *et al*., 1999a). It could be partially explained by the different number of informative PCR products (28 for RAPDs and 96 for RAMPs). They reinforced again the importance of the number of loci and their coverage of the overall genome and obtained reliable estimates of genetic relationship among the studied materials (Fernández *et al*., 2002). On the other hand, the relationship observed using molecular markers may provide information on the history and biology of cultivars, but it does not necessarily reflect what may be observed with respect to agronomic traits (Métais *et al*., 2000). The selection process leads to an accumulation of best alleles for the traits under selection. RAPDs and RAMPs are dispersed throughout the genome and their association with agronomic traits is influenced by the breeder only in the region under selection pressure. The other loci are subjected to random genetic drift (Fernández *et al*., 2002).

Due to its worldwide distribution, the valuation of the genetic diversity among barley germplasm from different country had been performed (Bahattin, 2003; Liu *et al*., 2002; Fernández *et al*., 2002; Matus *et al*., 2002; Dávila *et al*., 1999a, b; Dávila *et al*., 1998; Konishi, 2001; Bustos *et al*., 1998; Bjornstad *et al*., 1997). Knowledge of genetic variation and the genetic relationship between genotypes is an important consideration for efficient rationalization and utilization of germplasm resources. Better knowledge and measures of genetic similarity of accessions could help to maintain genetic diversity (Graner *et al*., 1994). Bernard *et al*. (1997) analyzed the genetic diversity in 88 genotypes from 20 populations of wild barley from Israel, Turkey and Iran by RAPD marker. When the total genetic diversity was estimated, 75% of the variation detected was partitioned within the 88 genotypes and 25% among the populations. When variation between countries was assessed, no substantial differences were found, because most of the variation detected (97%) was partitioned within the 20 populations and the remainder among the countries. Genetic similarity value based on RAPD for a sample of eighteen accessions from Netherlands, France, Great Britain, Germany and Italy, was 0.521 (Russell *et al*., 1997), which contrasts with the value obtained here, which was 0.898. Bahattin (2003) assayed 15 wild barley populations from west Turkey by using RAPD and ISSR markers. The results revealed that the average genetic similarity was 0.27 and the genetic variation was significant higher than that found in this study. Two surveys of 70 barley lines including cultivars and landraces and of 27 accessions of the wild barley *Hordeum vulgare* ssp. *spontaneum* by RAMP, gave mean genetic similarity indexes of 0.627 (Dávila *et al*., 1998) and 0.533 (Dávila *et al*., 1999a), respectively, contrasting with the value of 0.803 obtained in the present study. Overall these results reflected that the genetic variation of barley from China was relatively lower than that from other country and would justify initiatives for a wild conservation of barley germplasm.

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