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Genetic diversity in barley from west China based on RAPD and ISSR analysis

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Abstract

Two types of molecular markers, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR), were assayed to determine the genetic diversity of 46 barley accessions, including 27 landraces of *H. vulgare* ssp *vulgare* (HV), 6 accessions of *H. vulgare* ssp. *spontaneum* (HS) and 13 accessions of *H. vulgare* ssp. *agriocrithon* (HA), from west China. A high level of polymorphism was found with both RAPD and ISSR markers, and the mean polymorphism information content (PIC) values were 0.574 and 0.631 for RAPD and ISSR markers, respectively. In RAPD analyses, 84 out of 109 bands (77.06%) were polymorphic. The number of alleles ranged from 2 to 8 per primer, with an average of 4.19 per primer. The RAPD-based genetic similarity (RAPD-GS) ranged from 0.753 to 0.980, with the mean of 0.864. In ISSR analyses, a total of 107 alleles were detected, among which 105 alleles (98.13%) were polymorphic. The number of alleles per primer ranged from 2 to 10 with an average of 5.94 alleles per ISSR primer. The ISSR-derived genetic similarity (ISSR-GS) ranged from 0.212 to 0.931, with the mean of 0.674. ISSR was better than RAPD to detect genetic diversity among the barley accessions. A poor correlation (r = 0.113) was found between both sets of genetic similarity data, suggesting that both sets of markers revealed unrelated estimates of genetic relationships. Cluster analyses indicated that all 46 barley accessions could be distinguished by both RAPD and ISSR markers.

Key words: barley, RAPD markers, ISSR markers, genetic diversity, west China

Introduction

Barley, *Hordeum vulgare* L., is one of the principal cereal crops in the world 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*). The total number of barley accessions in the Genbanks, including redundant materials, is estimated to be about 280,000 (Plucknett et al. 1987). China is known to be a genetic diversity center of barley and rich in both landrace and wild relatives of barley (Yang et al., 1987). The wild relatives of barley have been considered to be a large reservoir of genetic diversity. It is known that wild barley populations possess high genetic variation in several useful characters, including earliness, biomass and yield, protein content and a high proportion of resistant genotypes against powdery mildew and leaf rust (Nevo 1992). Due to its compatibility and full interfertility with cultivated barley, these wild species have been used

as a source of important genes for cultivar development via interspecific crosses (Dávila et al. 1999a). Modern plant breeding and agriculture system have narrowed the genetic base of cultivated barley (Nevo et al. 1986). Therefore, the modern varieties are more genetically homogeneous and more exposed to pathogens and adverse environmental conditions (Asins and Carbonell 1989). This has promoted the search for new sources of variation that might be of use in plant breeding programs, and many national and international organizations have stressed the need for the collection, conservation and use of wild-species relatives of cultivated species and the endemic varieties (Brown et al. 1990). Assessment of the extent of genetic variability within barley, including the wild relatives, is fundamental for barley breeding and the conservation of genetic resources, and is particularly useful as a general guide in the choice of parents for breeding hybrids.

Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. It has been shown that different markers might reveal different classes of variation (Powell et al. 1996; Russell et al. 1997). It is correlated with the genome fraction surveyed by each kind of marker, their distribution throughout the genome and the extent of the DNA target which is analyzed by each specific assay (Dávila et al. 1999b). The advent of the polymerase chain reaction (PCR) favored the development of different molecular techniques such as random amplified of polymorphic DNA (RAPD), simple sequence repeats (SSR or microsatellite), sequence tagged sites (STS), random amplified microsatellite polymorphism (RAMP) and inter-simple sequence repeat polymorphic DNA (ISSR), and so on (Saiki et al. 1988; Welsh and McCleland 1990; Williams et al. 1990; Akkaya et al. 1992; Tragoonrung et al. 1992; Zietkiewicz et al. 1994; Wu et al. 1994; Nagaoka and Ogihara 1997). These molecular markers had been used in barley for detecting genetic diversity, genotype identification, genetic mapping (Sánchez de la Hoz et al. 1996; Matus and Hayes 2002; Dávila et al. 1999a, 1999b; Dávila et al. 1998; Tragoonrung et al. 1992; Tanyolac 2003; Fernández et al. 2002; Struss and Plieske 1998). Of these techniques, RAPD has several advantages, such as simplicity of use, low cost, and the use of small amount of plant material, etc. RAPDs were proved to be useful as genetic markers in the case of self-pollinating species with a relatively low level of intraspecific polymorphism, such as hexaploid wheat (Devos and Gale 1992; Joshi and Nguyen 1993) and cultivated barley (Barua et al. 1993; Chalmers et al. 1993; Tinker et al. 1993). ISSR markers, which involve PCR amplifications of DNA using a primer composed of a microsatellite sequence anchored at 3' or 5' end by 2-4 arbitrary, could be used to assess genetic diversity (Qian et al. 2001). ISSRs have been used for cultivar identification in maize (Kantety et al. 1995; Pejic et al 1998), potatoes (Prevost and Wilkinson 1999), trifoliate orange (Fang et al. 1997), wheat (Nagaoka and Ogihara 1997), bean (Métais et al. 2000), *Diplotaxis* (Martín and Sánchez-Yélamo 2000) and barley (Fernández et al. 2002; Tanyolac 2003).

To date, knowledge regarding the amount of genetic variation and genetic relationship at molecular marker in Chinese barley germplasm is available using RAPD (Shi et al. 2004; Chen et al. 2000a, 2000b; Hong et al. 2001) and SSR (Feng et al. 2003) markers. The objectives of this study are to (1) reveal the ISSR-based genetic diversity in a barley germplasm from western China, (2) compare RAPD and ISSR 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 ISSR molecular markers.

Materials and methods

Plant material and DNA extraction. 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 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).

RAPD and ISSR analysis. A total of twenty-six 10-mer oligonucleotides with arbitrary sequence from Operon (kits A, B, H and R) were used in RAPD analysis (Table 2) and 18 primers based on dinucleotide, tetranucleotide or pentanucleotide repeats were used in ISSR analysis (Table 3). The PCR reaction mixture consisted of 20-50ng genomic DNA, 1×PCR buffer, 2.0 mmol/L $MgCl_2$, 100 μmol/L of each dNTP, 0.1 μmol/L primer and 1U *Taq* polymerase in a 25μL volume. The amplification protocol was 94 ºC for 4 min to pre-denature, followed by 45 cycles of 94 ºC for 1 min, 36 ºC (for RAPD analysis) or 50 ºC (for ISSR analysis) for 1 min and 72 ºC for 1 min, with a final extension at 72 ºC for 10 min. Amplification products were fractionated on 1% (for RAPD analysis) or 2% (for ISSR analysis) agarose gel.

Data analysis. RAPD and ISSR 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 marker, were generated. The genetic similarities (GS) were calculated according to Nei and Li (1979): $GS = 2N_{ij}/(N_i+N_j)$, where N_{ij} is the number of bands present in both genotypes i and j, N_i is the number of bands present in genotypes i, and N_j 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 arithmetic 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 and ISSR primer according to the formula: $PIC = 1-\Sigma(P_{ij})^2$, where P_{ij} is the frequency of the ith pattern revealed by the jth primer summed across all patterns revealed by the primers (Botstein et al. 1980). The similarity between matrices based on different marker system (RAPD and ISSR) were calculated using the standardized Mantel coefficient (Mantel 1967). The significance level for the correlation coefficient was calculated following Sokal and Rohlf (1995).

Results

In RAPD analysis, a total of 109 bands were detected, among which 84 bands (77.06%) were polymorphic with the mean of 3.23 per primer (Table 2). For each primer, the number of bands ranged from 2 to 8, with an average of 4.19. The average polymorphic information content (PIC) was 0.574, ranging from 0.156 to 0.894. The lowest and the highest PIC values were recorded for primer OPH-13 and OPH-12, respectively.

In ISSR analysis, a total of 107 bands were observed, with 5.94 bands per primer (Table 3). One hundred five out of 107 bands (98.13%) were polymorphic, among which 2 to 10 polymorphic bands were detected by each primer. The average PIC was 0.636, and the lowest and highest PIC values were 0.351 (ISSR16) and 0.874 (ISSR18), respectively. Four ISSR primers (i.e. ISSR18, ISSR7, ISSR6 and ISSR8) had the higher PIC values.

All the 109 bands, generated from 26 RAPD primers, were subjected to calculate the genetic similarity index (RAPD-GS) among the 46 accessions (Table 4.). The RAPD-GS value ranged from 0.735 to 0.980, with the mean of 0.864. The highest genetic similarity was found between HV accession B65 (from Xizang) and HV accession B21 (from Sichuan), while the lowest genetic similarity was observed between HV accession B76 (from Sichuan) and HA accession ZYM0531 (from Xizang). It was found that the genetic similarities within the HV and HA groups were equal to that within HS group.

The ISSR-derived data were subjected to calculate the genetic similarity (ISSR-GS) (Table 4). The genetic similarity coefficient varied between 0.212 and 0.931, with the average of 0.674. The minimum GS value derived between HV accession B22 (from Xizang) and HA accession ZYM0834 (from Xizang), while the maximum GS value derived between HV accession B22 (from Xizang) and HV accession B40 (from Sichuan). The average genetic similarity within HS accessions (0.645), HA accessions (0.623) and all accessions (0.674) exhibited equivalent to each other, and the average genetic similarity within HV accessions (0.716) was higher than that of all accessions. In contrast to RAPD-GS, the ISSR-GS within HS accessions (0.645) and HA accessions (0.623) were significant lower than that within HV (0.716). It indicated that the wild barley groups were more diverse than the landraces group. The average genetic similarity values based on ISSR markers were lower than that of RAPD markers both within landraces and wild barley accessions, as well as among the all accessions. These results suggested that higher genetic diversity could be detected by ISSR markers than that of RAPD markers among the 46 barley accessions from west China.

The relationships within and between groups were estimated by a UPGMA cluster analysis of GS matrices (Fig 1.). It indicated that all 46 barley accessions could be distinguished by both RAPD and ISSR markers. Fig. 1a was the dendrogram based on RAPD data. One HV accession (B18) with six rowed and hulled, which collected from Hongyuan, Sichuan, was less related with other genotypes, and divergent from the others. Two subgroups were evident for the remaining 45 genotypes. The first subgroup contained 17 accessions, including 2 HS accessions (ZYM0059 and ZYM0083) and 15 HV accessions. The second subgroup consisted of 28 accessions, including 4 HS accession, 13 HA accessions and 11 HV accessions. The genotypes were more closely related with each other within the second subgroup, whereas more diverse within the first subgroup.

Fig. 1b was the dendrogram generated from ISSR data. One HA accession (ZYM0834) and One HV accession (B118), which were collected from Xizang, were less related with other genotypes, and divergent from the others. Two subgroups were evident for the remaining

44 accessions, with the first subgroup only including one HS accession (ZYM0059) and two HA accessions (ZYM0830 and ZYM0469). The second subgroup including 41 genotypes consisted of 5 HS accessions, 10 HA accessions and 26 HV accessions. The genotypes were more closely related with each other within the second subgroup, while more diverse within the first subgroup.

The correlation coefficient for the elements of the RAPD-GS and ISSR-GS matrices was calculated using the Mantel test (Mantel 1967). There was no significant correlation (r = 0.113) between the RAPD-GS and ISSR-GS matrices, indicating that both sets of markers revealed the unrelated estimates of genetic relationships.

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 analyses 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. The results indicated that the percentage of ISSR polymorphic bands (98.13%) was higher than that of RAPD (77.06%). The mean number of amplification RAPD bands (4.19) was more than that of ISSR (5.94). Moreover, the total number of polymorphic bands (105) detected by 18 ISSR primers was much higher than that of the 26 RAPD primers (84). And the mean polymorphism information content (PIC) (0.631) in ISSR analysis also was higher than that in RAPD analysis (0.574). The bulk analysis of RAPD and ISSR markers could successfully be used to investigate the genetic diversity of the barley landraces and the wild forms from west China. However, the results in this study suggested that the ISSR markers were superior to RAPD markers in the capacity of revealing more informative bands in a single amplification. The similar results were observed by Fernández et al. (2002) and Tanyolac (2003).

Due to its worldwide distribution, the valuation of the genetic diversity among barley germplasm from different countries has been performed (Tanyolac 2003; Liu et al. 2002; Fernández et al. 2002; Matus et al. 2002; Dávila et al. 1999a, 1999b; Dávila et al. 1998; Konishi 2001; Bustos et al. 1998; Bjornstad et al. 1997; Chen et al. 2000b; Feng et al. 2003). Bernard et al. (1997) analyzed the genetic diversity in 88 genotypes from 20 populations of wild barley from Israel, Turkey and Iran by RAPD markers. 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. Russell et al. (1997) found that the

average genetic diversity based on RAPD analyses of eighteen accessions from Netherlands, France, Great Britain, Germany and Italy was 0.521. 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 higher than that found by Nevo et al. (1979) and Nevo et al. (1986) both using isozyme markers. In the present study, the average genetic similarities of barley accessions from western China based on RAPD and ISSR markers were 0.864 and 0.674, respectively. The similarities detected with RAPDs are greater than the similarities observed with ISSRs. Fernández et al. (2002) also found similar results. The genetic variation found in this study was equivalent with that found by Chen et al. (2000a) (0.81), Shi et al. (2004) (0.631) and Chen et al. (2000b) (0.746) both using RAPD markers to analyze the genetic variation of different barley populations from China. But the variation was relatively lower than that from other country (Russell et al. 1997; Bahattin 2003).

In this study, it was obvious that the dendrogram based on RAPD markers was not in accord with the dendrogram based on ISSR markers. The dendrogram generated by the RAPD matrix agrees better with the groups of the genotypes than the dendrogram generated by the ISSR results (Fig. 1a). Most of the genotypes within the wild barley and the landrace were closely related. However, the dendrogram generated by the ISSR matrix agrees better with the geographic origins of the genotypes than the dendrogram generated by the RAPD results (Fig. 1b). For example, three landraces (i.e. B65, B118 and B120) originating from Xizang, were closely clustered with the wild forms originating from Xizang. Fernández et al. (2002) found the dendrogram generated by the ISSR matrix agrees better with the genealogy and the known pedigree of the barley cultivars than the dendrogram generated by the RAPD results. Wu et al. (2004) found that the data based on RAPD-GS were more correlated with the geographic distribution of the genus *Houttuynia Thunb*, while the data based on ISSRs were closely related with their number of chromosomes. It could be partially explained by the different number of informative PCR products (84 for RAPDs and 105 for ISSRs). 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). Similar results had been observed by Loarce et al. (1996). 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 ISSRs 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). Another explanation could be the putatively similar bands originating for RAPDs in different bulked samples were not necessarily homologous although they shared the same size in base pairs (Karp et al. 1997). This situation might lead to wrong results when calculating genetic relationships (Fernández et al. 2002).

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Table 1. Origin, group and rowed type of 46 landraces and wild barley accessions form west China

Acc.no.	Group	Row type	Hulled or Naked	Origin	Acc. no.	Group	Row 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

Table 2. Primer sequences, amplified bands, polymorphic bands and PIC values in RAPD analysis

Primer	Sequence (5'-3')	Amplified bands	Polymorphic bands	PIC
OPA-1	CAGGCCCTTC	4	3	0.677
OPA-4	AATCGGGCTG	2	2	0.639
OPA-11	CAATCGCCGT	5	4	0.647
OPA-13	CAGCACCCAC	4	2	0.442
OPA-18	AGGTGACCGT	6	6	0.316
OPB-1	GTTTCGCTCC	3	2	0.453
OPB-2	TGATCCCTGG	5	4	0.412
OPB-3	CATCCCCCTG	7	6	0.603
OPB-10	CTGCTGGGAC	7	3	0.762
OPB-12	CCTTGACGCA	7	6	0.293
OPB-18	CCACAGCAGT	4	1	0.360
OPB-20	GGACCCTTAC	8	8	0.550
OPH-7	CTGCATCGTG	4	4	0.619
OPH-12	ACGCGCATGT	5	5	0.894
OPH-13	GACGCCACAC	4	4	0.156
OPH-18	GAATCGGCCA	5	3	0.744
OPH-19	CTGACCAGCC	3	2	0.407
OPP-3	CTGATACGCC	4	2	0.486
OPP-5	CCCCGGTAAC	2	1	0.741
OPP-8	ACATCGCCCA	3	2	0.816
OPP-9	GTGGTCCGCA	2	1	0.753
OPP-11	AACGCGTCGG	2	2	0.724
OPP-12	AAGGGCGAGT	2	2	0.708
OPP-14	CCAGCCGAAC	3	3	0.659
OPP-16	CCAAGCTGCC	4	3	0.439
OPP-17	TGACCCGCCT	4	3	0.623
Total		109	84	
Mean		4.19	3.23	0.574

Table 3. Primer sequences, amplified bands, polymorphic bands and PIC values in ISSR analysis

Primer	Sequence (5'-3')*	Amplified bands	Polymorphic bands	PIC
ISSR-1	HVH$(CA)_7$T	6	6	0.516
ISSR-2	$(AG)_8$C	4	4	0.618
ISSR-3	$(GA)_8$T	5	5	0.763
ISSR-4	$(CT)_8$G	7	7	0.693
ISSR-5	$(CA)_6$ACAG	8	8	0.645
ISSR-6	BDB$(TCC)_5$	2	2	0.816
ISSR-7	HVH$(TCC)_5$	2	2	0.835
ISSR-8	DBDA$(CA)_7$	6	6	0.808
ISSR-9	$(CA)_8$RG	5	5	0.533
ISSR-10	$(GA)_8$YG	6	6	0.396
ISSR-11	$(AC)_8$YG	10	10	0.509
ISSR-12	$(AG)_8$YT	3	3	0.723
ISSR-13	$(AC)_8$C	7	7	0.590
ISSR-14	$(GA)_8$YC	3	3	0.760
ISSR-15	$(GA)_8$C	8	7	0.455
ISSR-16	$(AC)_8$YA	7	7	0.351
ISSR-17	$(TC)_8$C	8	8	0.478
ISSR-18	$(TG)_8$G	10	9	0.874
Total		107	105	
Mean		5.94	5.83	0.636

* R=A/T, Y=G/C, B=T/G/C; D=A/T/G, H=A/T/C, V=A/G/C

Table 4. RAPD-and ISSR-based genetic similarity within groups.

Group		HV	HS	HA	All
RAPD	Mean	0.867	0.895	0.877	0.864
	Variation	0.763-0.980	0.849-0.954	0.800-0.954	0.735-0.980
ISSR	Mean	0.716	0.645	0.623	0.674
	Variation	0.350-0.931	0.342-0.832	0.250-0.911	0.212-0.931

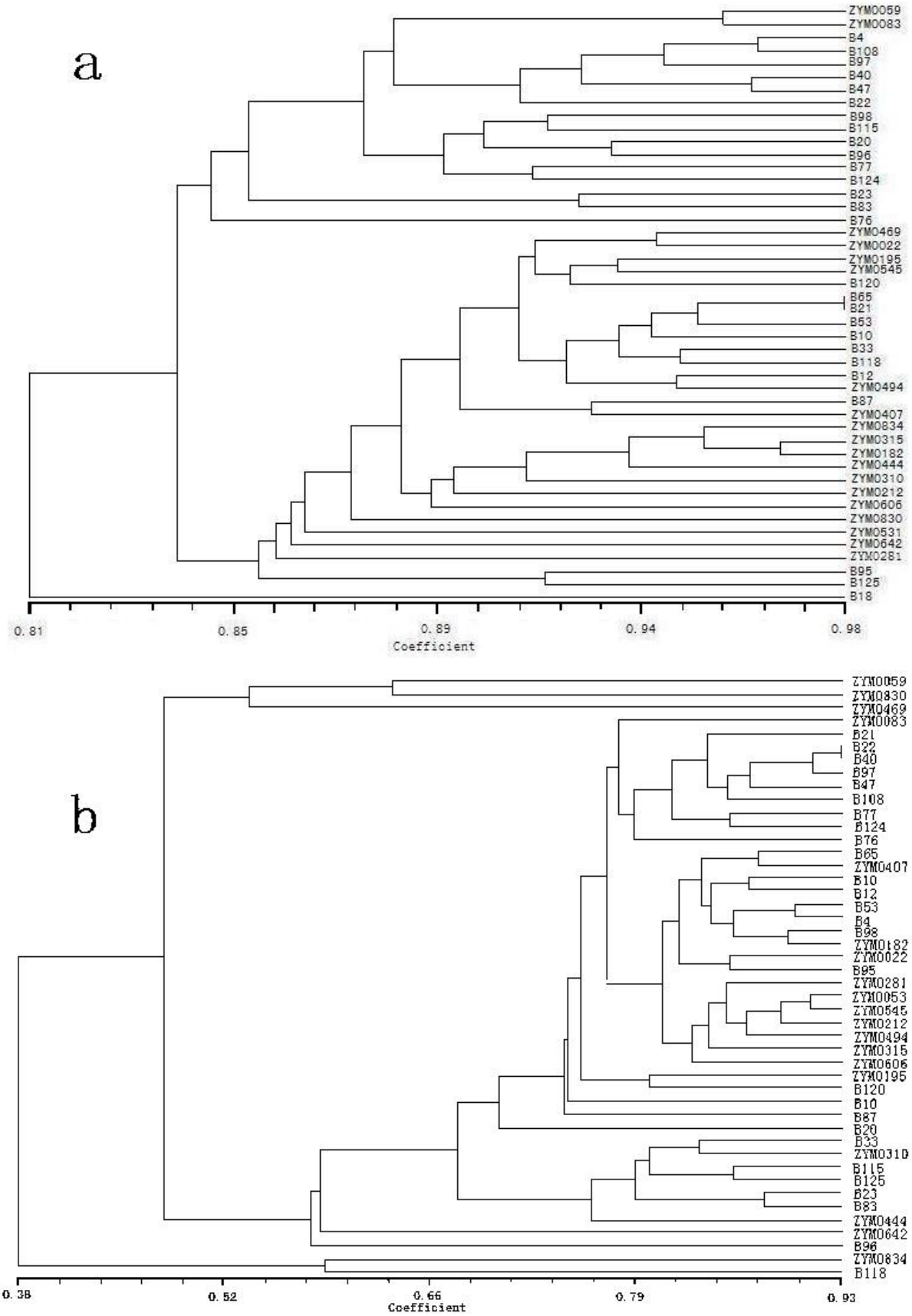

Fig. 1 Dendrograms of 46 barley accessions constructed from RAPD-GS (a) and ISSR- GS (b) matrices