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Genetic diversity analysis of barley landraces and cultivars in the Shanghai region of China

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ABSTRACT. We analyzed the genetic diversity of 115 barley germplasms, including 112 landraces and three new barley cultivars grown in the Shanghai region, using a set of 11 SSR markers. Sixty-six alleles were observed at the 11 SSR loci, ranged from three to ten, with a mean of six alleles per locus. The polymorphism information content ranged from 0.568 to 0.853, with a mean of 0.732, indicating considerable genetic variation in barley in the Shanghai area. Clustering analysis indicated that these barley accessions could be divided into two categories (A and B). Ninety-seven six-rowed barley cultivars were classified in the A category; sixteen two-rowed and two six-rowed barley cultivars were classified in the B category. This demonstrated genetic differences between two-rowed and six-rowed barley varieties. In addition, we found that the three new barley cultivars are closely related.

Key words: Barley landraces; *Hordeum vulgare*; Genetic diversity; SSR markers; PIC

INTRODUCTION

Farmer preference and breeder selection create a narrow germplasm base (Duvick, 2005). Some elite germplasms are often used as parents for the next cycle of breeding (Graner et al., 1994). As a result, it leads to greater genetic uniformity and germplasm disappearance. To assure the safety of crop production, it is necessary to extend genetic background in crop breeding. It is well known that genetic diversity is the basis of biological diversity, and thus, it plays a key role in future breeding progress (Yao et al., 2007).

Barley is a cash crop, being the fourth most important cereal crop in the world, where it is used for brewing malts, animal feed and human consumption (Hayes et al. 2002). Barley is widely grown in various environments in China, including the Shanghai area. Once upon a time, there were many barley landraces in the Shanghai area. As we know, the original landraces represent a vast array of germplasms, which can be used in barley breeding for improving biotic or abiotic resistance, nutritional quality, and other traits of interest (Yao et al., 2007). Therefore, the protection and utilization of barley landraces will be more important for us.

The development of molecular markers makes it easy to assess genetic diversity in crops at the DNA level (Reif et al., 2003). Molecular markers such as RAPD (Fernández et al., 2002; Meszaros et al., 2007), AFLP (Zhang and Ding, 2007), ISSR (Fernández et al., 2002), STS (Meszaros et al., 2007), and SSR (Turuspekov et al., 2001; Matus and Hayes, 2002; Feng et al., 2006; Meszaros et al., 2007; Zhang et al., 2007) can be used to estimate genetic diversity.

There have been many studies on genetic diversity in barley (Graner et al., 1994; Turuspekov et al., 2001; Matus and Hayes, 2002; Fernández et al., 2002; Feng et al., 2006; Meszaros et al., 2007; Zhang et al., 2007; Zhang and Ding, 2007; Mikel and Kolb, 2008); however, little is known about the genetic diversity in barley landraces, especially for barley landraces from the Shanghai area. SSRs are codominant, abundant, and informative and their detection is very simple (Matus and Hayes, 2002). This makes them an excellent molecular marker system for analysis of genetic diversity. In this study, we used a set of SSRs from seven linkage groups of barley to survey 112 accessions of barley landraces and 3 new barley cultivars from the Shanghai area. The objectives of this study were i) to estimate genetic diversity of a group of barley landraces from the Shanghai area, ii) to reveal the relationship between the present major cultivars and the landraces in the Shanghai area, and to provide a basis for barley improvement in Shanghai, and iii) to protect the barley landraces of the Shanghai area by genetic classification.

MATERIAL AND METHODS

Plant materials

A total of 112 barley landraces from the Shanghai area and 3 barley cultivars, which are the major barley cultivars in the Shanghai area, were used in this study (Table 1). The barley landraces were provided by Shanghai Agrobiological Gene Center, and the 3 new barley cultivars were bred by our group.

Genomic DNA extraction

Ten seeds of each genotype were sown, and the seedlings were grown in a room at

room temperature. Two weeks later, approximately 100 mg young leaves of each genotype was used for DNA extraction. The DNA extraction was carried out according to Dellaporta et al. (1983). DNA quality and concentration were estimated using NanoDrop ND-100. DNA samples were then diluted to a concentration of 100 ng/μL using ddH_2O and stored at -20°C.

Table 1. Barley accessions used in this study.

Code	Name	Code	Name	Code	Name	Code	Name
1	Sanyuehuang1	31	Zidamai2	61	Baoshanlaolaibai	91	Congmingheiliuzhu
2	Zidamai1	32	Shanghaizidamai	62	Wusongdamai1	92	Bailiuzhuyuanmai
3	Xingshandamai	33	Shanghailiulengzidamai	63	Shanghaibendidamai	93	Shanghaibailiuzhu
4	Qianqiandamai	34	Zidamai3	64	Shanghaidongyangdamai	94	Jiadinghongjinliuzhutou
5	Zaohuangdamai	35	Xiaodamai	65	Baoshanliulengdamai	95	Jiadinghongjinyuanmai
6	Luocihongmangdamai	36	Shanghaixiaodamai	66	Qingpuhongdamai	96	Liuzhutou
7	Aizaosan	37	Laotuoxu1	67	Chundamai	97	Hongjinliuzhutou1
8	Humaiyihao	38	Laotuoxu2	68	Liuzhutouzidamai	98	Hongjinliuzhutou2
9	Baoshanerleng	39	Laotuosui	69	Liulengzidamai	99	Hongjinliuzhutou3
10	Daerdamai	40	Wanlaotuoxu	70	Liulengdamai1	100	Hongjinliuzhutou4
11	Bendamai1	41	Zaolaotuoxu	71	Wusongdamai2	101	Liuzhutouyuanmai
12	Bendamai2	42	Chuanshalaotuoxu	72	Liuzhutouhongdamai	102	Benyuanmai
13	Shuanglengbendamai	43	Songjianglaotuoxu1	73	Shanghailaotuoxu1	103	Shanghaisanyuehuang4
14	Hongdamai	44	Songjiangzaolaotuoxu	74	Baoshanlaotuoxu	104	Baisanyuehuang
15	Yangdamai	45	Songjianglaotuoxu2	75	Shanghaishuanglengbiandamai	105	Shanghaiciguqing
16	Hongjinerlengdamai	46	Wandamai1	76	Shanghaiyoumangbendamai	106	Shanghaisizhutou1
17	Qingpuerlengdamai	47	Wandamai2	77	Bendiyuanmai	107	Shanghaisizhutou2
18	Dongyangdamai	48	Chuanshawandamai1	78	Bendiguangyuanmai	108	Shanghailaotuoxu2
19	Bianjidamai	49	Chuanshawandamai2	79	Sizhutouyuanmai1	109	Wumangdamai
20	Eryuehuang	50	Jiadingwandamai	80	Sizhutouyuanmai2	110	Liulengdamai2
21	Sanyuehuang2	51	Zaodamai1	81	Fengxiansizhutou	111	Sandunhongsileng
22	Sanyuehuang3	52	Zaodamai2	82	Sizhutoubenyuanmai	112	Bailiuzhu
23	Sanyuehuang4	53	Fengxianzaodamai1	83	Cigubaiyuanmai	113	Hua30
24	Shanghaisanyuehuang1	54	Fengxianzaodamai2	84	Shanghaisanyuehuang3	114	Hua11
25	Shanghaisanyuehuang2	55	Jiadingzaodamai	85	Ciguqingyuanmai	115	Hua22
26	Baoshansanyuehuang	56	Zhongdamai	86	Shanghaidenglongtou		
27	Hongsanyuehuang	57	Damai	87	Jiadingliuzhutou		
28	Youmangdamai	58	Jinshandamai	88	Cimaomai		
29	Shanghaiyoumangdamai	59	Laolaibai1	89	Heiliuzhuyuanmai1		
30	Huoshaotoudamai	60	Laolaibai2	90	Heiliuzhuyuanmai2		

SSR analysis

A total of 28 SSR markers (Table 2) were selected for genotype identification according to Zhang et al. (2007). Three replicate DNA extractions from leaves of each genotype were used to assess the consistency of the band profiles. SSR amplifications were repeated at least three times and only the repetitive PCR products were scored.

Table 2. SSR markers and chromosome locations.

Chr.	SSR marker	Chr.	SSR marker	Chr.	SSR marker	Chr.	SSR marker
Chr. 1	Bmac213	Chr. 3	HVLTPPB	Chr. 5	scssr02306	Chr. 7	Bmag206
	Bmag0345		Bmag603		EBmac0970		GBM1464
	Bmag0105		BMAG0841		Bmac0303		Bmag0321
	Bmag0579		EBmac541		HvLOX		Bmac0156
Chr. 2	Bmac0134	Chr. 4	GBM1465	Chr. 6	Bmac0316		
	Bmag0692		EBmac0775		Bmag500		
	Bmag140		EBmac679		Bmac602		
	GBM1437		GBM1324		GBM1404		

PCR was carried out in a 25-μL volume containing 1 μL 100 ng/μL genomic DNA template, 2.5 μL 10X PCR buffer containing 15 mM Mg^{2+}, 0.5 μL 10 mM dNTP mixture, 0.75 U Taq DNA polymerase, and 0.75 μL 10 μM forward and reverse primers. Depending on the primer pair used, DNA amplification was performed in a 2720 thermocycler (ABI, USA) with a preliminary step of 5 min at 94°C, followed by 40 cycles of 30 s at 94°C, 30 s at 50-60°C and 30 s at 72°C and a final 6-min extension at 72°C. Next, 2.5 μL 98% formamide electrophoresis loading buffer containing 0.25% (w/v) bromophenol blue and 0.25% (w/v) xylene cyanole FF was added to the reactants of each reaction. After denaturation, the PCR products were separated on 8% denaturing polyacrylamide gels using 8 M urea and 1X TBE buffer at constant voltage (200 V) for about 2 h. A 700-bp DNA ladder ranging from 25 to 700 bp was used as a size standard. DNA fragments were visualized following silver staining according to the method of Sanguinetti et al. (1994).

Data analysis

Data obtained from SSR analysis were scored as presence (1) or absence (0) of fragments for each barley genotype, and polymorphism information content (PIC) was calculated from the following formula (Anderson et al., 1993).

$$PIC_i = 1 - \sum_{j=1}^{n} P^2ij,$$

where *Pij* is the frequency of the j^{th} SSR allele for the i^{th} marker, and is summed over *n* alleles. The dendrogram was constructed by UPGMA cluster analysis using the simple matching coefficient (SM) (Sneath and Sokal, 1973) and NTSYS-pc version 2.10 package (Rohlf, 2002).

RESULTS

SSR amplification and PIC statistics

In this study, 28 SSR markers were adopted for SSR analysis, and 12 of them with clear and steady bands were chosen for statistics, of which 11 were polymorphic among 115 barley germplasms. A total of 66 alleles were observed at the 11 SSR loci, ranging from 3 to 10 with an average of 6 (Table 3), and PIC values ranged from 0.568 to 0.853 with an average of 0.732.

Table 3. Polymorphic SSR markers and the polymorphism information content (PIC).

SSR marker	Chr.	Total alleles	Polymorphic alleles	PIC
Bmac213	1	7	7	0.79
Bmag0579	1	5	2	0.75
Bmac0134	2	8	8	0.82
EBmac0775	4	7	7	0.74
EBmac679	4	6	5	0.72
GBM1324	4	4	4	0.67
Bmac0303	5	5	5	0.72
HvLOX	5	6	3	0.67
Bmag500	6	10	10	0.85
Bmac602	6	5	5	0.75
GBM1404	6	3	1	0.57
Mean		6	5.18	0.73

Dendrogram obtained with SSR markers

The dendrogram (Figure 1) obtained using the SM coefficient consisted of two main categories (A and B). The A category was divided into two subgroups (A1 and A2). In addition, the A1 subgroup was further divided into two subclasses (A_{11} and A_{12}). On the dendrogram, the 3 new two-rowed barley cultivars (Hua30, Hua11 and Hua22) were all in the B category, and they were linked very close. Except for accessions 27 and 28, all accessions in the B category were two-row barley, while all accessions in the A category were six-row barley.

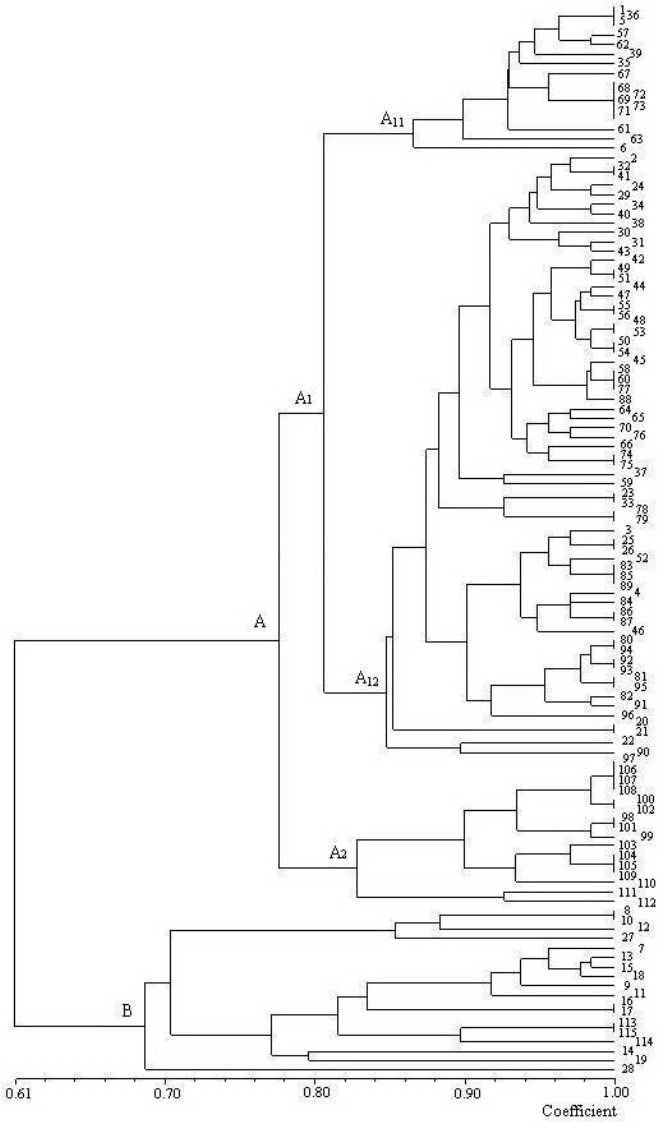

Figure 1. Dendrogram showing the genetic relationships of 115 barley germplasms based on SSR markers.

DISCUSSION

SSR markers in barley genetic diversity analysis

In this study, we just chose 11 SSR markers for barley genetic diversity analysis (Table 3), and they were from Chr. 1, Chr. 2, Chr. 4, Chr. 5, and Chr. 6. Considering the low coverage of the whole genome, it was disadvantageous to analyze barley genetic diversity. However, we still obtained many alleles, and most of them were polymorphic. Although some barley germplasms were not discriminated by the cluster analysis (Figure 1), the general classification was successful, especially for two-row and six-row barley. It indicated that the genetic structure of barley germplasms in the Shanghai area was high, and the genetic background was different between two-row and six-row barley. Therefore, we inferred that the SSR marker was a kind of high efficiency molecular marker for barley genetic analysis and could reveal the genetic differences of barley germplasms as described in previous studies (Saghai Maroof et al., 1994; Struss and Plieske, 1998; Turuspekov et al., 2001; Matus and Hayes, 2003; Feng et al., 2006; Zhang et al., 2007; Mikel and Kolb, 2008).

Genetic diversity for barley germplasm protection and barley breeding

Cluster analysis did not clearly identify all the barley germplasms (Figure 1). There maybe two reasons, one is that the SSR markers we used were not enough to discriminate all the germplasms, and the other is that some germplasms were duplicated in the collection of barley landraces of the Shanghai area and were stored for a long time and that there might have been some error. Therefore, SSR markers appear to provide an optimal system to identify duplicate materials in the barley germplasm collection (Struss and Plieske, 1998), and they are helpful in protecting and managing the barley collections.

In addition, we found that the genetic relationships of three new barley cultivars were very close, especially for the accessions 113 and 115. This may cause disadvantages for barley production in the Shanghai area. While there are many barley landraces in the Shanghai area, they have a variety of traits for adaptation in this region. Thus, they will provide many excellent characters for barley breeding in the future and will promote the development of better and more secure barley production in Shanghai and surrounding areas.

SSR markers in new variety protection

As we know, molecular fingerprinting is an effective and accurate way to identify crop varieties (Nandakumar et al., 2004). In this study, we found that the SSR marker Bmac0303 could distinguish the 3 new barley cultivars from other barley landraces, and we could then protect the breeder rights of the 3 barley cultivars by this locus. Similarly, we could identify all barley landraces by SSR fingerprinting, and we would then find out whether there were preservation repeats or mistakes for varieties. Therefore, we could protect these barley landraces better by SSR fingerprinting.

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REFERENCES

Anderson JA, Churchill GA, Autrique JE, Tanksley SD, et al. (1993). Optimizing parental selection for genetic linkage maps. *Genome* 36: 181-186.

Dellaporta SL, Wood J and Hicks JB (1983). A plant DNA minipreparation: version II. *Plant Mol. Biol. Rep.* 1: 19-21.

Duvick DN (2005). The contribution of breeding to yield advances in maize (*Zea mays* L.). *Adv. Agron.* 86: 83-145.

Feng ZY, Liu XJ, Zhang YZ and Ling HQ (2006). Genetic diversity analysis of Tibetan wild barley using SSR markers. *Yi. Chuan Xue. Bao.* 33: 917-928.

Fernández M, Figueiras A and Benito C (2002). The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barley cultivars with known origin. *Theor. Appl. Genet.* 104: 845-851.

Graner A, Ludwig WF and Melchinger AE (1994). Relationships among European barley germplasm: II. Comparison of RFLP and pedigree data. *Crop Sci.* 34: 1199-1205.

Hayes PM, Castro A, Marquez-Cedillo L, Corey A et al. (2002). Genetic Diversity for Quantitatively Inherited Agronomic and Malting Quality Traits. In: Diversity Barley (Von Bothmer R, Knupfeer H, van Hintum T and Sato K, eds.). Elsevier Science Publishers, Amsterdam.

Mikel MA and Kolb FL (2008). Genetic diversity of contemporary North American barley. *Crop Sci.* 48: 1399-1407.

Matus IA and Hayes PM (2002). Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats. *Genome* 45: 1095-1106.

Meszaros K, Karsai I, Kuti C, Banyai J, et al. (2007). Efficiency of different marker systems for genotype fingerprinting and for genetic diversity studies in barley (*Hordeum vulgare* L.). *S. Afr. J. Bot.* 73: 43-48.

Nandakumar N, Singh AK, Sharma RK, Mohapatra T, et al. (2004). Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. *Euphytica* 136: 257-264.

Prevost A and Wilkinson MJ (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theor. Appl. Genet.* 98: 107-112.

Reif JC, Melchinger AE, Xia XC, Warburton ML, et al. (2003). Genetic distance based on simple sequence repeats and heterosis in tropical maize populations. *Crop Sci.* 43: 1275-1282.

Rohlf FJ (2002). NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System, Version 2.10. Exeter Software, New York.

Saghai Maroof MA, Biyashev RM, Yang GP, Zhang Q, et al. (1994). Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, and population dynamics. *Proc. Natl. Acad. Sci. U. S. A.* 91: 5466-5470.

Sanguinetti CJ, Dias NE and Simpson AJ (1994). Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques* 17: 914-921.

Sneath PHA and Sokal RR (1973). Numerical Taxonomy. The Principles and Practice of Numerical Classification. W.H. Freeman and Co., San Francisco.

Struss D and Plieske J (1998). The use of microsatellite markers for detection of genetic diversity in barley populations. *Theor. Appl. Genet.* 97: 308-315.

Turuspekov Y, Nakamura K, Yoshikawa R and Tuberosa R (2001). Genetic diversity of Japanese barley cultivars based on SSR analysis. *Breed. Sci.* 51: 215-218.

Yao Q, Yang K, Pan G and Rong T (2007). Genetic diversity of maize (*Zea mays* L.) landraces from southwest China based on SSR data. *J. Genet. Genomics* 34: 851-859.

Zhang D and Ding Y (2007). Genetic diversity of wild close relatives of barley in Tibet of China revealed by AFLP. *Yi. Chuan* 29: 725-730.

Zhang DL, Gao HY and Li SP (2007). Analysis of genetic diversity on beer barley varieties in China by SSR. *Acta Agr. Boreali-Occidentalis Sin.* 16: 72-76.