Molecular Biology: Open Access
ISSN: 2168-9547

Mei et al. Mol Biol 2012, 1:1
DOI: 10.4172/2168-9547.1000101

Research Article Open Access

Malt Genotypic Screening of Polymorphism Information Content (PIC) of SSR Markers Based on Physiological Traits in Barley

Lulu Mei[1], Junjiao Ping[2], Dazhong Wang[2], Zhen Zhang[2], Suyuan Luo[2], Mingzhen Yang[3] and Gang Qian[1,2]*

[1]*Department of biochemistry, Zhuhai Campus of Zunyi Medical College, Zhuhai 519041, China*
[2]*Department of Cell Biology and Genetics, Zunyi Medical College, Zunyi 563099, China*
[3]*Department of Interventional Radiology, the Affiliated Hospital of Zunyi Medical College, Zunyi 563099, China*

Abstract

Brewing genotypes were described by polymorphism information content (PIC) of 22 SSR markers in barley (*Hordeum vulgare* L.), associated with malt quality. Notably, the values of diastatic power set ranged from 5.53 to 24.13 U/Kg in these samples, suitable to assess the relative importance of biochemical components of malt variations and genetic characteristics. Cluster analysis revealed a significant correlation between eco-geographic origin and SSR marker clustering, resolving the accessions into four subgroups. Moreover, SSRs with higher PIC values and higher average number of alleles per marker in the population with higher values of diastatic power were found to be an efficient utility for distinguishing brewing genotypes in barley. These results possibly indicated that SSRs linked to brewing traits could be very useful for application in monitoring malt traits, evaluating genetic diversity, and determining the sampled eco-geographic origins in barley.

Keywords: Malt quality; SSR; Polymorphism information content (PIC); Barley (*Hordeum vulgare* L.)

Abbreviations: DP: Diastatic Power; PCR: Polymerase Chain Reaction; SSR: Simple Sequence Repeat; MAS: Marker Assisted Selection; CTAB: Cetyltrimethyl Ammonium Bromide; SPSS: Statistical Package for the Social Sciences; ANOVA: Analysis of Variance; GS: Genetic similarity; UPGMA: Unweighted pair-group method with arithmetic averages; SM: Similarity matrix; PIC: Polymorphism information content

Introduction

Characteristics of importance for malting quality include grain size, grain protein concentration, malt extract and diastatic power. Diastatic power, the total activity of starch-degrading enzymes in barley malt, is considered to be an important quality characteristic for malting and brewing [1,2]. Among the standard and nonstandard measures of malt quality, malt extract, osmolyte concentration, diastatic power, and α-amylase activity should be indicators of rapid sugar production during the malting and mashing processes [3]. However, many studies found a discrepancy in activities of amylolytic enzymes and diastatic power [4,5], suggesting that its precise functional role in the determination of grain malting quality could be indefinite.

The genetic basis of malting quality is considered complex, and a major drawback in barley breeding is the lack of molecular markers for the evaluation of quality [6]. Molecular markers are useful tools to dissect complex malting quality traits, providing breeders with the genetic information to develop strategies for the accelerate breeding of new varieties [7]. SSR markers are mostly codominant, are readily amplified by PCR and are effective at detecting genotype variation caused by a high degree of polymorphism [8]. From the barley linkage maps, SSR markers have been identified that show close linkage to many traits, such as malt quality and disease-resistant loci. Several of the barley SSR markers have now been validated in other genetic backgrounds and the results indicate that the genetic locations are correct and that they can be reliably used to monitor trait loci [9,10]. SSR markers are routinely used to construct genetic linkage maps and phylogenetic studies in barley, however, few experiments report the use of marker assisted selection (MAS) for the definite relationships between malting quality and molecular markers in the past. In contrast to the biochemical aspects of germination, little success has been reported thus far in attempts to elucidate the genetic basis of germination in barley and their potential effects on malt quality [6]. Understanding the correlation between malting quality and microsatellite markers will help us to make use of molecular markers in MAS and varietal identification in cultivars. Therefore, the general aims of this study are: 1) to examine the malt parameters such as diastatic power and malt extract in order to understand the relationships of malt quality; and 2) to use highly polymorphic molecular markers in order to obtain essential genetic information to enable application for MAS in brewing genotypes in barley.

Experimental

Plant materials

103 barley accessions (*Hordeum vulgare* L.) were collected from different eco-geographic regions of Yunnan-Guizhou Plateau in China involving Zunyi (BZ1), Bijie (BZ2), Anshun (BZ3) and Kaili (BZ4), representing different genetic backgrounds. The experimental seeds were sown on 25th October 2007 and harvested in May 2008 at the Plant Breeding Centre (lat. 27.32°S., alt. 980 m) of Zunyi Medical College, in Guizhou region of China and a basal application of 80 kg ha^{-1} double superphosphate (17% P, 4% S) was applied at sowing. The experimental plots were 5 × 1.2 m^2 with eight rows and the experiments were laid out as randomised un-complete blocks with three replicates.

Assays of diastatic power and malt extract

Harvested seed of the same maturity was passed over a series of sieves and only the size of fraction between 2.5 and 2.8 mm was taken for subsequent analyses. The malting programme used was: steeping (6 h, 16°C), air-rest (10 h, 16°C), steeping (6 h, 16°C), germination (96 h

***Corresponding author:** Gang Qian, Department of Cell Biology and Genetics, Zunyi Medical College, No. 201, Dalian Road, Zunyi city, Guizhou Provence, China. Tel: +86-852-8609692; Fax: +86-852-8609692; E-mail: qiangang69@yahoo.com.cn

Received October 28, 2011; **Accepted** November 19, 2011; **Published** November 21, 2011

Citation: Mei L, Ping J, Wang D, Zhang Z, Luo S, et al. (2012) Malt Genotypic Screening of Polymorphism Information Content (PIC) of SSR Markers Based on Physiological Traits in Barley. Mol Biol 1:101. doi:10.4172/2168-9547.1000101

with grain moisture maintained at 44%, 16°C), and kilning (from 0 to 8 h, temperature rising from 30 to 50°C; from 8 to 11 h, temperature rising from 50 to 65°C; from 11 to 13 h, temperature rising from 65 to 75°C; from 13 to 19 h, temperature rising from 75 to 80°C). The malt was then cooled to 30°C. Diastatic power was measured in finely ground malts with a small-scale method [2], with an increased extraction time (10 to 30 min). A colorimetric assay using *p*-hydroxybenzoic acid hydrazide was employed for the determination of reducing sugars. One unit of activity was defined as the amount of enzyme that produced one mmol of reducing sugars. Malt extract was determined by a modification to the method of the Institute of Brewing [11].

Analysis on SSR genetic markers

Total genomic DNA was extracted from 7-day-old fresh seedlings following a modified cetyltrimethyl ammonium bromide (CTAB) protocol [12]. Microsatellite markers were obtained from the published sequences of Karakousis [13]. Details of respective primers, the studied loci, quality traits and No. of repeats are given in Table 1. PCR reactions were performed in a volume of 20 μL containing 50 ng genomic DNA, 1 × PCR buffer, 200 μM dNTPs, 10 mM of SSR primer and 0.5 U of *Taq* polymerase (TOYOBO Co., LTD., China). Amplification of DNA was run on a C1000™ Thermal Cycler (BIO-RAD Co., LTD., USA), using the procedures described by Karakousis *et al.* [9]. The PCR products were separated on 6% polyacrylamide denaturing gels of 25 cm in length and silver stained. The band patterns were analyzed with an automated BIO-RAD Visadoc 3.0 (BIO-RAD Co., LTD., USA), and fragment sizes were determined using GENESCAN v. 2.02 and GENOTYPER v. 1.1 (Apllied Biosystems, Inc.). Allele peak profiles were identified at each locus and a genotype was assigned to each individual.

Data analysis

Measurements of malt quality were replicated three times with independent plant seed samples. Statistical analysis was done with SPSS 16.0 for Windows statistical software package. The Duncan test was employed to detect possible differences between the treatments. Two-way ANOVAs were applied in order to evaluate the effects of manganese, population and the interaction between these two factors. All analyses were done on a completely randomized design.

The presence or absence of alleles for each SSR marker was recorded for all accessions and then converted to a genetic similarity (GS) matrix using the Jaccard or Dice coefficients. The resulting genetic distance matrix was used for a cluster analysis according to the unweighted pair-group method with arithmetic averages (UPGMA) from the software program NTSYSpc version 2.0 based on the calculated similarity matrix (SM) as described by Gower [14]. The polymorphism information content (PIC) of SSR marker was determined according to the method described by Karakousis *et al.* [9]. The calculating formula of PIC value was as follows:

$$PIC = 1 - \left(\sum_{i=1}^{n} p_i^2\right) - \sum_{i=1}^{n-1}\sum_{j=i+1}^{n} 2 p_i^2 p_j^2$$

Where n is the total number of fragments (bands) for an SSR and p_i and p_j are the frequencies of the ith and jth fragment in the barley varieties investigated. A PIC value of 1 indicates that the marker can differentiate each line, and 0 indicates a monomorphic marker.

Results

Variations in diastatic power, malt extract and amylolytic enzymes

As presented in Table 2, the values of diastatic power set ranged from 5.53 to 24.13 U/Kg in the accessions, suitable to assess the relative importance of biochemical components of malt quality and genetic diversity. Malt extract showed a highly variable score among the tested accessions, within and between populations and ranged from 29.56% to 78.50%. In a simple regression analysis, diastatic power exhibited no really large correlation with malt extract in these samples (r = -0.127).

Polymorphic profiles in the contrasting subgroups

To confirm the genetic informativeness of brewing genotypes, the suitable number of subgroups was optimized to analyze the PIC in the sampled barleys. When possible, the 103 accessions were split

SSR marker	Trait	chromosome location	No. of repeats
AWBMS80	Malt extract, Diastatic power	1HS	$(CT)_{11}$
Bmac90	Malt extract, Diastatic power	1HS	$(AC)_{20}$
EBmac501	Malt extract, Diastatic power	1HS	$(AC)_{13}$
HVM2	Malt extract, Diastatic power	1HS	$(GA)_{11}$
HVLAAT	Malt extract, Diastatic power	1HS	$(CT)_{10}$
HVM20	Malt extract, Diastatic power	1HS	$(GA)_{19}$
Bmag692	Malt extract	2HL	$(CT)_{19}$
HVM36	Malt extract	2HS	$(GA)_{13}$
Bmac134	Malt extract	2HS	$(AC)_{28}$
HVM54	Malt extract	2HL	$(GA)_{14}$
EBmac415	Malt extract	2HL	$(AC)_{17}$
EBmac541	Diastatic power	3HL	$(AC)_{9}$
Bmag877	Diastatic power	3HL	$(GA)_{15}$
HVM67	Diastatic power	4HL	$(GA)_{11}$
HvBTAI3	*Alpha*-amylase inhibitor	4HL	$(GCC)_{4}$
HdAMYB	*Beta*-amylase	4HL	$(TG)_{10}(G)_{16}$
HvBAMY	5-Upsteam region of *beta*-amylase	4HL	$(A)_{17}$
HVM6	Malt extract, Diastatic power	5HL	$(GA)_{9}$
GMS1	Malt extract, Diastatic power	5HL	$(CT)_{7}TTT(CT)_{27}$
HvAMY2	Diastatic power	7HS	$(GCT)_{5}$
HVCMA	*Alpha*-amylase inhibitor	7HL	(AT)9
HVWAXYG4	Starch synthase	7HL	$(AT)_{9}$

Table 1: Characters of SSR markers linked to physiological traits.

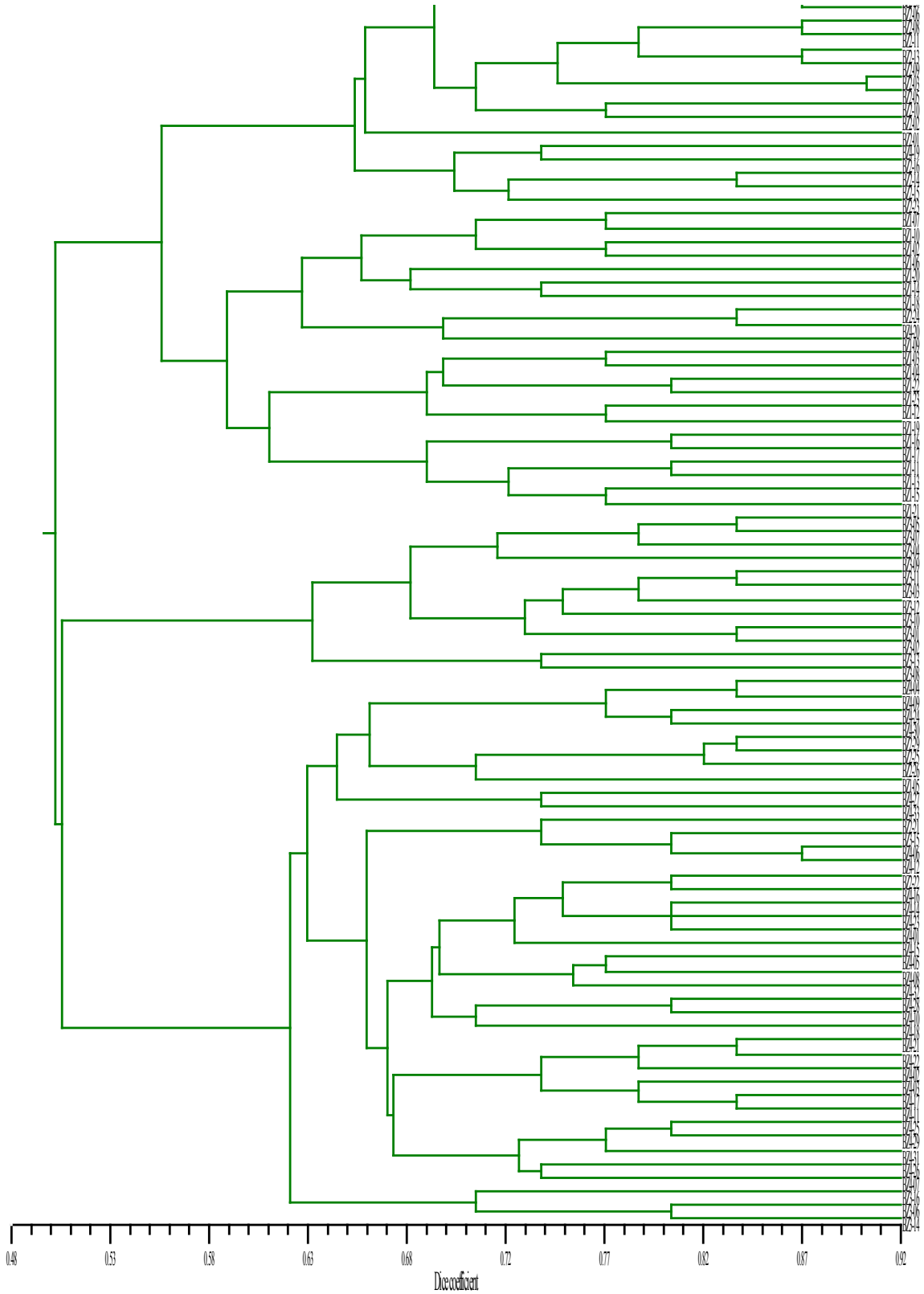

Figure 1: Dendrogram for 103 barley accessions (*Hordeum vulgare* L.) derived from a UPGMA cluster analysis using 22 SSR markers related to malting quality based on the Dice similarity coefficient.

Accession	DP (U/kg)	Malt extract (%)	Accession	DP (U/kg)	Malt extract (%)	Accession	DP (U/kg)	Malt extract (%)
BZ1-01	10.97±0.52	78.50±2.75	BZ2-13	11.52±0.64	39.05±1.37	BZ4-01	13.69±1.07	48.11±1.68
BZ1-02	11.79±0.61	49.81±1.74	BZ2-14	10.72±0.62	33.04±1.16	BZ4-02	11.14±0.94	51.54±1.80
BZ1-03	13.15±0.98	35.65±1.25	BZ2-15	10.44±0.50	44.36±1.55	BZ4-03	10.27±0.92	62.78±2.20
BZ1-04	13.53±1.06	39.20±1.37	BZ2-16	12.10±0.61	40.47±1.42	BZ4-04	9.19±0.61	44.40±1.55
BZ1-05	11.19±0.76	37.30±1.31	BZ2-17	11.34±0.59	38.59±1.35	BZ4-05	14.04±0.78	59.09±2.07
BZ1-06	8.90±0.50	53.86±1.89	BZ2-18	13.56±0.85	31.99±1.12	BZ4-06	9.64±0.57	47.03±1.65
BZ1-07	13.14±1.17	35.70±1.25	BZ2-19	11.04±0.61	36.03±1.26	BZ4-07	11.25±1.03	56.57±1.98
BZ1-08	11.5±0.68	50.24±1.76	BZ2-20	13.57±0.82	47.32±1.66	BZ4-08	10.25±0.75	43.24±1.51
BZ1-09	12.73±0.72	50.90±1.78	BZ2-21	9.20±0.17	51.38±1.80	BZ4-09	12.63±0.96	29.56±1.03
BZ1-10	13.72±0.78	42.41±1.48	BZ2-22	14.49±0.98	31.14±1.09	BZ4-10	10.11±0.81	56.76±1.99
BZ1-11	12.22±0.89	40.77±1.43	BZ2-23	13.48±0.72	33.03±1.16	BZ4-11	10.46±0.44	38.63±1.35
BZ1-12	13.54±0.97	43.24±1.51	BZ2-24	5.53±0.04	36.36±1.27	BZ4-12	9.59±0.72	37.65±1.32
BZ1-13	13.95±1.17	59.57±2.08	BZ2-25	11.64±0.51	55.36±1.94	BZ4-13	10.06±0.91	48.46±1.70
BZ1-14	11.84±1.09	55.94±1.96	BZ2-26	11.67±0.55	31.54±1.10	BZ4-14	8.20±0.35	51.36±1.80
BZ1-15	14.23±1.06	64.31±2.25	BZ2-27	11.82±0.48	41.06±1.44	BZ4-15	14.18±1.14	48.15±1.69
BZ1-16	15.27±1.33	58.27±2.04	BZ2-28	8.79±0.09	54.91±1.92	BZ4-16	10.51±0.69	44.11±1.54
BZ1-17	9.44±0.58	65.40±2.29	BZ2-29	13.7±0.67	50.49±1.77	BZ4-17	11.76±0.86	55.71±1.95
BZ1-18	15.08±1.15	40.50±1.42	BZ2-30	12.38±0.99	34.39±1.20	BZ4-18	10.87±0.93	47.65±1.67
BZ1-19	13.52±0.86	39.84±1.39	BZ2-31	14.07±1.06	56.81±1.99	BZ4-19	17.00±0.44	51.41±1.80
BZ1-20	7.64±0.49	41.33±1.45	BZ3-01	16.66±1.28	57.88±2.03	BZ4-20	13.57±0.58	46.73±1.64
BZ1-21	13.86±0.88	42.05±1.47	BZ3-02	18.05±1.34	52.48±1.84	BZ4-21	9.22±0.49	62.91±2.20
BZ1-22	14.16±0.97	44.09±1.54	BZ3-03	12.39±0.97	58.20±2.04	BZ4-22	9.31±0.56	62.51±2.19
BZ1-23	14.55±1.12	36.30±1.27	BZ3-04	10.43±0.86	50.71±1.77	BZ4-23	8.40±0.37	65.80±2.30
BZ2-01	11.00±0.83	42.30±1.48	BZ3-05	11.28±0.90	42.04±1.47	BZ4-24	10.8±0.98	42.82±1.50
BZ2-02	20.95±1.77	70.67±2.47	BZ3-06	13.40±1.01	41.16±1.44	BZ4-25	8.52±0.54	64.35±2.25
BZ2-03	24.13±2.09	50.94±1.78	BZ3-07	11.35±0.71	49.85±1.74	BZ4-26	10.64±0.91	49.46±1.73
BZ2-04	10.72±0.85	45.71±1.60	BZ3-08	12.04±0.92	44.87±1.57	BZ4-27	13.44±1.12	40.99±1.43
BZ2-05	21.26±2.06	43.08±1.51	BZ3-09	10.45±0.63	53.76±1.88	BZ4-28	10.90±0.83	55.96±1.96
BZ2-06	12.92±0.75	65.48±2.29	BZ3-10	9.45±0.66	44.97±1.57	BZ4-29	8.19±0.46	70.28±2.46
BZ2-07	10.27±0.63	46.08±1.61	BZ3-11	13.28±0.94	48.12±1.68	BZ4-30	11.38±0.92	48.72±1.71
BZ2-08	14.1±0.68	51.62±1.81	BZ3-12	13.26±1.48	50.71±1.77	BZ4-31	11.30±1.08	50.99±1.78
BZ2-09	10.14±0.74	60.69±2.12	BZ3-13	9.60±0.36	46.07±1.61	BZ4-32	8.33±0.33	62.75±2.20
BZ2-10	16.59±0.89	70.86±2.48	BZ3-14	9.51±0.82	45.97±1.61	BZ4-33	12.67±0.68	43.23±1.51
BZ2-11	15.2±0.93	65.79±2.30	BZ3-15	10.28±0.78	50.45±1.77			
BZ2-12	9.26±0.07	76.49±2.68	BZ3-16	7.98±0.51	52.88±1.85			

Note: Four populations were gathered from diverse eco-geographic regions of Yunnan-Guizhou plateau involving Zunyi (BZ1), Bijie (BZ2), Anshun (BZ3) and Kaili (BZ4), representing different genetic backgrounds. The values (means of three replicates±S.E.) in the same column followed by different letters were significantly different from each other at $P<0.05$, as revealed by the Duncan test.

Table 2: Physiological traits involving malt quality in 103 barley accessions.

into two subgroups according to their scores of diastatic power. The 31 individuals with values of diastatic power >13.00 U/kg were sorted into "putative brewing genotypes", compared with "general genotypes" involving the other accessions. Of the 22 primer pairs identifying polymorphic markers in the "putative brewing genotypes", the number of alleles detected by a single marker ranged from 2 (EBmac501) to 12 (EBmac415) with an average of 7.05 alleles. The PIC was calculated for each marker as a relative measure of informativeness and ranged from 0.21 (HVCMA) to 0.92 (GMS1) with an average PIC of 0.58. SSR markers were similarly informative in the "general genotypes", revealing 2-14 alleles with PIC values ranging from 0.21 to 0.92. Lower mean numbers of alleles per locus (6.09) and PIC (0.54) were observed in general genotypes, as compared with brewing genotypes (Table 3).

Relationships between clustering and eco-geographic origin

A high cophenetic correlation (r = 0.91) between the original similarity matrix and those given by the clustering process was observed. Genetic relationships among the sampled eco-geographic origins were observed by cluster analysis. Four major populations involving their respective origins of Zunyi (BZ1), Bijie (BZ2), Anshun (BZ3) and Kaili (BZ4) were resolved in the dendrogram and the minimal similarity was <0.50, suggesting an overall high genetic variation in these accessions. As shown in Figure 1, the clustering analysis also indicated that a highly positive relationship existed between SSR marker clustering and the eco-geographic origins, regardless of only partial correlations excluded.

Usefulness of SSR markers for discrimination of brewing genotypes

The result from comparisons of PIC values between those populations was summarized in Table 4. Many SSR markers with higher number of alleles per SSR loci in the brewing genotypes, revealing 2-12 alleles with PIC values ranging from 0.18-0.87; 59% of SSR markers generated PIC values >0.50. Higher scores involved in average of PIC values, number of alleles, SSR with PIC >0.5 and av. no. of alleles for SSR with PIC >0.5 were detected in the brewing genotypes. A few SSR markers such as HVM2, Bmag692, HvBAMY, HvAMY2, Bmag877 and GMS1 not only identified valid polymorphic bands (Table 3), but also exhibited higher values of PIC in brewing genotypes. Thus, all of the polymorphic PCR products derived from these SSR sets indicated that the potential usefulness of these markers could be sufficient for monitoring malt trait.

SSR marker	Allele length in bp	No. of alleles in total	PIC in total	Brewing genotypes*		General genotypes	
				No. of alleles	PIC	No. of alleles	PIC
AWBMS80	186-211	6	0.47	6	0.53	4	0.41
Bmac90	181-237	11	0.92	9	0.87	11	0.93
EBmac501	156-166	2	0.26	2	0.34	2	0.22
HVM2	189-211	9	0.79	9	0.82	6	0.58
HVLAAT	117-139	6	0.44	6	0.46	4	0.38
HVM20	135-158	5	0.53	5	0.58	3	0.41
Bmag692	179-266	7	0.72	7	0.46	5	0.57
HVM36	110-116	8	0.61	7	0.63	8	0.49
Bmac134	142-168	4	0.22	4	0.32	3	0.28
HVM54	138-182	5	0.78	5	0.72	5	0.81
EBmac415	225-247	12	0.74	12	0.84	11	0.62
EBmac541	110-113	6	0.46	5	0.34	6	0.49
Bmag877	213-218	7	0.28	7	0.35	4	0.23
HVM67	96-122	3	0.56	3	0.42	3	0.48
HvBTAI3	178-243	14	0.91	11	0.79	14	0.92
HdAMYB	199-221	8	0.55	8	0.61	5	0.37
HvBAMY	118-145	12	0.76	12	0.56	9	0.88
HVM6	172-213	10	0.73	10	0.74	7	0.55
GMS1	121-169	9	0.88	9	0.82	6	0.58
HvAMY2	136-240	6	0.82	6	0.86	4	0.62
HVCMA	117-132	6	0.31	4	0.18	5	0.33
HVWAXYG4	192-236	11	0.68	8	0.49	9	0.77
Average	-	7.59	0.61	7.05	0.58	6.09	0.54

*"Putative" brewing genotypes: the individuals with diastatic power >13.00 U/kg

Table 3: Allelic variation of the 22 SSR markers in the total samples, "putative" brewing genotypes and general genotypes.

	Brewing genotypes*	General genotypes	Total accessions
No. of SSR examined	155	134	167
No. of alleles	2-12	2-14	2-14
No. of alleles per SSR marker	7.05	6.09	7.59
PIC range	0.18-0.87	0.22-0.92	0.22-0.92
Av. of PIC	0.58	0.54	0.61
R^2 alleles: PIC	0.8	0.7	-
SSR with PIC >0.5	59%	46%	68%
Av. no. of alleles for SSR with PIC >0.5	8.38	7.0	8.53

*"Putative" brewing genotypes: the individuals with diastatic power >13.00 U/kg

Table 4: A summary of polymorphic information content (PIC) value revealed by SSR markers among "putative" brewing genotypes, general genotypes and total accessions.

Discussion

Traditionally, breeders, maltsters and brewers regarded measurement of total diastatic power and the individual enzyme activities as sufficient for selection of barley cultivars suitable for malting and brewing [2]. However, indefinite relationships (Table 2) between diastatic power and malt extract indicated that the production of grain high in both extract and diastatic power could be difficult in this study. Diastatic power of barley malt represented the collective activity of several starch degrading enzymes that accumulated or were activated during malting [5]. Many studies have also found a negative correlation between malt extract and grain protein, and a positive correlation between malt extract and diastatic power [3,6], suggesting that genetic and/or environmental effects on the differences in malt extract levels could play important roles [15,16].

Traditional methods of plant genotype identification are not always adequate for plant variety rights, seed certification, and seed purity control and are often difficult and unreliable to screen for due to the level of polymorphism and influence of different environmental parameters [1,7]. Knowledge of the genetic variation on the molecular level will facilitate marker assisted selection (MAS) and breeding of improved cultivars with DNA-markers [17]. Accordingly, one of the main objectives of this research was to study the association of PIC informativeness generated by microsatellites with physiological traits in barley.

So far, breeders and scientists have primarily targeted elite germplasm from different geographic regions to improve and analyze malting quality traits in barley [18]. Just as shown in Figure 1, the accessions included in this study clustered into two major groups with a UPGMA analysis and could be further divided into four respective subclusters corresponding to the sampled geographic origins. Remarkably, PCR-based markers linked to malt trait could have been used for evaluating the genetic diversity and determining the genetic relationships among these accessions [19]. Similarly, Kraic, *et al.* [17] also demonstrated that the use of 5 SSRs was sufficient to distinguish 22 of 23 European barley genotypes that could be further divided into spring and winter subgroups. Cao *et al.* [20], as parallel with our results, reported a considerable genetic differentiation in weedy rice populations from various regions. Similarly, cluster analysis on genetic diversity of rice (*Oryza sativa* L.) cultivars indicated that SSR markers used for genotyping could be applied to identify diverse geographic accessions in Argentine [21].

Interestingly, our findings indicated that the potential usefulness of highly informative SSR markers as an efficient screening for brewing genotypes in barley (Table 3 and Table 4). Genetic relationships between lotus varieties revealed by genetic similarity at SSR levels were in agreement with their roles in agricultural production and breeding [22]. As a good confirmation, Karakousis, *et al.* [9] argued the usefulness of polymorphic SSR markers for the discrimination of breeding material in Australian barley. The potential usefulness of SSR markers linked to trait loci for MAS was variable and the success of monitoring a donor trait with SSR marker in a variety of crosses was corrected with the PIC value. Highly saturated genetic maps constructed with molecular markers are basic tools for developing specific marker assisted selection in crop breeding, and for cloning of genes controlling the traits of interest through map-based strategies. Genetic segregation and association analyses were performed to define SSR linked with multiple important biological traits [23]. Overall, the authors argued that SSRs linked to brewing traits could be applied to monitor malt traits, evaluate genetic diversity, and determine the sampled eco-geographic origins in barley.

Acknowledgements

We express our deep appreciations to the financial supports of this research from Guizhou Province Natural Sciences Foundation of China (J-2007-2050), the Key Project of Zunyi City Natural Sciences Foundation of China (N-2007-05).

References

1. Henry RJ (1990) Barley quality: an Australian perspective. Aspects of Applied Biology 25: 5-14
2. Arends AM, Fox GP, Henry RJ, Marschke RJ, Symons MH (1995) Genetic and environmental variation in the diastatic power of Australian barley. J Cereal Sci 21: 63-70
3. Duke SH, Henson CA (2008) A comparison of barley malt quality measurements and malt sugar concentrations. J Am Soc Brew Chem 66: 151-161
4. Henson CA, Duke SH (2008) A comparison of standard and nonstandard measures of malt quality. J Am Soc Brew Chem 66: 11-19
5. Gibson TS, Solah V (1995) Diastatic power in malted barley: contributions of malt parameters to its development and the potential of barley grain beta-amylase to predict malt diastatic power. J Inst Brew 101: 277-280
6. Fechter I, Rath F, Voetz M (2010) A single PCR marker predicting the activity levels of various enzymes responsible for malting quality. J Am Soc Brew Chem 68: 41-47
7. Panozzo JF, Eckermann PJ, Mather DE, Moody DB, Black CK, et al. (2007) QTL analysis of malting quality traits in two barley populations. Aust J Agric Res 58: 858-866
8. Yonemaru J, Ando T, Mizubayashi T, Kasuga S, Matsumoto T, (2009) Development of genome-wide simple sequence repeat markers using whole-genome shotgun sequences of sorghum (*Sorghum bicolor* (L.) Moench). DNA Research 16: 187-193
9. Karakousis A, Barr AR, Chalmers KJ, Ablett GA, Holton TA, et al. (2003) Potential of SSR markers for plant breeding and variety identification in Australian barley germplasm. Aust J Agric Res 54: 1197-1210
10. Yamamoto T, Yonemaru J, Yano M (2009) Towards the Understanding of Complex Traits in Rice: Substantially or Superficially? DNA Research 16: 141-154
11. Institute of Brewing (1982) Recommended Methods of Analysis. (The Institute: London.)
12. Saghai-Maroof MAS, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci U S A 81: 8014-8018
13. Karakousis A (2002) The development, identification and application of SSR markers for use in Australian barley (*Hordeum vulgare* L.) breeding programs. PhD thesis, Department of Plant Science, University of Adelaide, Australia.
14. Gower JC (1966) Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 325-338
15. Duke SH, Henson CA (2009) A comparison of barley malt osmolyte concentrations and standard malt quality measurements as indicators of barley malt amylolytic enzyme activities. J Am Soc Brew Chem 67: 206-216
16. Eagles HA, Bedggood AG, Panozzo JF, Martin PJ (1995) Cultivar and environmental effects on malting quality in barley. Aust J Agric Res 46: 831-844
17. Kraic J, Zakova M, Gregova E (1998) Comparison of differentiation capability of RAPD and SSR markers in commerical barley (*Hordeum vulgare* L.) cultivars. Cereal Research Communication 26: 375-382
18. Matthies IE, Weise S, Förster J, Röder M (2009) Association mapping and marker development of the candidate genes (1 → 3), (1 → 4)-β-D-glucan-4-glucanohydrolase and (1 → 4)-β-xylan-endohydrolase 1 for malting quality in barley. Euphytica 170: 109-122
19. Von Korff M, Wang H, Léon J, Pillen K (2008) AB-QTL analysis in spring barley: III. Identification of exotic alleles for the improvement of malting quality in spring barley (*H. vulgare* ssp. *spontaneum*). Molecular Breeding 21: 81-93
20. Cao Q, Lu BR, Xia H. Rong J, Sala F, et al. (2006) Genetic diversity and origin of weedy rice (*Oryza sativa* f. *spontanea*) populations found in North-eastern China revealed by simple sequence repeat (SSR) markers. Annals of Botany 98: 1241-1252
21. Giarrocco LE, Marassi MA, Salerno GL (2007) Assessment of the genetic diversity in Argentine rice cultivars with SSR markers. Crop Science 47: 853-860
22. Pan L, Xia Q, Quan Z, Liu H, Ke W, (2010) Development of novel EST-SSR from sacred lotus (*Nelumbo nucifera* Gaertn) and their utilization for the genetic diversity analysis of *N. nucifera*. Journal of Heredity 101: 71-82
23. Jing HC, Kornyukhin D, Kanyuka K, Orford S, Zlatska A, (2007) Identification of variation in adaptively important traits and genome-wide analysis of trait-marker associations in *Triticum monococcum*. Journal of Experimental Botany 58: 3749-3764.