Genetic diversity assessment of oat core collection in China with SSR markers

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Introduction

Oat is one of the important food and feed cereal crops in China. It is mainly cultivated in Shanxi, Inner Mongolia, Hebei, Gansu, Shaaxi, Qinghai, Xinjiang, Jilin provinces and autonomous regions. Oat is characterized by its strong tolerance to drought and adoptability to poor environments (Qu et al. 2006). Oats can reduce blood fat and cholesterol and is considered as healthy food. There are about 4000 accessions of oat germplasm collected and conserved in the National Genebank of China, and as such, a core subset of oat collection was developed through the procedures of grouping the accessions by origins, determining the number of accessions in each group by the proportion of square root method, and selecting the individual accessions for the core collection by cluster analysis (Zhang, et al. 2008).

Microsatellite or simple sequence repeat (SSR) markers are useful tool in genomic study due to their high level of polymorphism, and co-dominant expression. SSR markers were developed and used in studying the genetic diversity of oat (Leona Leišov et al. 2007). In this study, we use SSR markers to assess the genetic diversity of oat core collection developed in China and provide the molecular basis for its validation.

Material and methods

The oat core collection composed of 458 accessions was used in this study. It included 315 accessions originated from different provinces and regions in China and 143 accessions from abroad including America, Western Europe, Eastern Europe and other countries. Genomic DNA was extracted with the modified CTAB method. The polymerase chain reaction (PCR) was conducted with 20ul reaction mix containing 5.0ul DNA (10ng/ul), 3.0ul primer (25ng/ul), 2ul 10×PCR buffer, 0.25ul dNTP (10mmol/l), 0.5 ul Taq (2.5u/ul) and 9.25ul dd H_2O. Amplification profiles was consisted of one cycle at 94ºC for 3 min; thirty three-step cycles of 30 sec at 94ºC, 30 sec at 55ºC, 30 sec at 72ºC; and finally one cycle of 7 min at 72ºC. PCR products were denaturized at 95ºC for 5 min, and then electrophoresed on a 6% polyacrylamide gel for 1 hr and 20 min. Genetic diversity of oat core collection was assessed by using SSR markers. More than 300 SSR primers of oat, wheat and barley were screened and 15 SSR primers with polymorphism were selected for amplification of oat DNA (Table 1). Data were analyzed for Shannon-Weaver index, genetic similarity and principle coordinate analysis with NTSYSpc2.1 software.

Table 1. SSR primers used in this study

Primers	F/R	Sequences (5'-3')	Size
AF033096	F	TGCATGTTTTTGTTTGTTGTTG	136
	R	CACGATCCAAATACACGCAG	
AM3	F	CTGGTCATCCTCGCCGTTCA	280
	R	CATTTAGCCAGGTTGCCAGGTC	
AM7	F	GTGAGCGCCGAATACATA	156
	R	TTGGCTAGCTGCTTGAAACT	
AM87	F	GAGCAAGCTCTGGATGGAAA	150
	R	CCCGTTTATGTGGTTGTTAGC	
AM102	F	TGGTCAGCAAGCATCACAAT	213
	R	TGTGCATGCATCTGTGCTTA	
AM112	F	AGCGGTGTAGGGGAAAGAGT	234
	R	TTCTTGGTTTAGATGGGAGGA	
HVM4	F	AGAGCAACTACCAGTCCAATGGCA	198
	R	GTCGAAGGAGAAGCGGCCCTGGTA	
HVM20	F	CTCCACGAATCTCTGCACAA	151
	R	CACCGCCTCCTCTTTCAC	
HVM62	F	TCGCGACCAGACGAGAAG	251
	R	AGCTAGCCGACGACGCAC	
L39777	F	CTTCTGCCCATGAAACCCTA	202
	R	ACTCAGCACATGCACCCTC	
M83381	F	ATCTGTCAGGTGACGAGGCA	172
	R	CCTTGCATCTGAGGTTGGTT	
Xgwm88	F	CACTACAACTATGCGCTCGC	230
	R	TCCATTGGCTTCTCTCTCAA	
Xgwm99	F	AAGATGGACGTATGCATCACA	182
	R	GCCATATTTGATGACGCATA	
Xgwm471	F	CGGCCCTATCATGGCTG	220
	R	GCTTGCAAGTTCCATTTTGC	
Z48431	F	CAGCAACAACAACAACCACC	122
	R	CACTGGTAGCCGTCCTTGAC	

Results and discussion

Fifteen SSR markers presented different polymorphic levels at the oat core accessions. Table 2 shows that a total of 72 alleles were identified, with average 4.8 alleles per locus. M83381 presented 11 alleles, the highest among the all markers. PIC values for the different primers were ranged from 0.329 to 0.789, which indicated that high genetic diversity at the core collection of Chinese oats was revealed by SSR markers. Shannon-Weaver index ranged from 0.459-1.118 (Table 3), with the highest diversity showed by accessions from America and the lowest diversity presented by accessions from Xinjiang, China. For naked type of oats, the accessions from Shanxi and Inner Mongolia showed the highest diversity. The similarity coefficients between different geographical origins showed that accessions from Yunnan were more closely related to those from Shaanxi (r=0.517), while accessions from Qinghai were closer to those from Xinjiang (r=0.507). This in turn indicated that the closer the geographical areas, the closer the accessions from those areas.

Table 2. Polymorphisms revealed by SSR markers in the core collection of oat

No.	Primers	Alleles	PIC
1	AF033096	4	0.566
2	AM3	4	0.728
3	AM7	4	0.618
4	AM87	4	0.567
5	AM102	5	0.673
6	AM112	8	0.690
7	HVM4	4	0.752
8	HVM20	4	0.603
9	HVM62	2	0.329
10	L39777	6	0.621
11	M83381	11	0.789
12	Xgwm88	7	0.658
13	Xgwm99	2	0.344
14	Xgwm471	3	0.664
15	Z48431	4	0.400
Average		4.8	0.600

Fig. 1 showed the result of the principle coordinate analysis. Generally, three groups were formed, 1) including all accessions from Shanxi, Gansu and Ningxia; 2)including accessions mainly from southwest of China; and 3) accessions mainly from Western Europe. With the cluster analysis conducted with NTSYSpc2.1, 71 accessions with high genetic similarity (r=0.929) were identified, which could be eliminated for optimizing the core collection of oat.

Table 3 Shannon-weaver index of accessions with different origins

Origin	Diversity Index
America (AM)	1.118
Western Europe (WE)	1.091
Shanxi (SX)	1.085
Inner Mongolia (IM)	1.033
Other countries (OT)	0.951
Eastern Europe (EE)	0.931
Hebei (HB)	0.923
Qinghai (QH)	0.918
Gansu/Ningxia (GN)	0.833
Southwest (SW)	0.828
Northeast (NE)	0.824
Shaanxi (SH)	0.73
Xinjiang (XJ)	0.459
Total	1.107

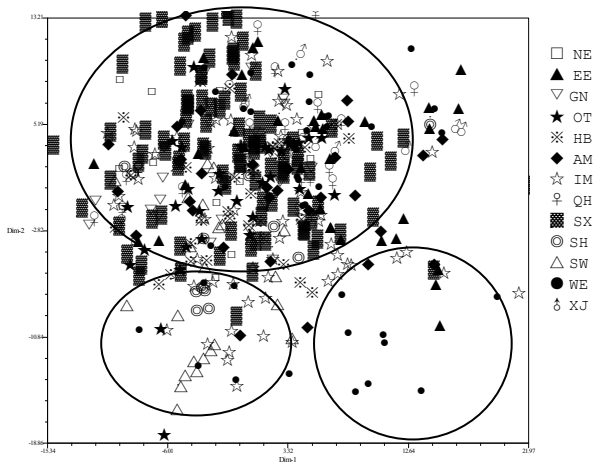

Fig 1. Two-dimension principal coordinate analysis based on SSR data

Conclusion

Some 72 alleles of 15 SSR marker loci were expressed in the oat core collection in China. Close geographic relationships of the accessions were identified. The oat core collection can be further validated and optimized by eliminating accessions with high similarity within each original group based on SSR data.

Reference

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