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Genetic diversity of oat accessions revealed by SSR markers

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Abstract
Oat is an important cereal crop for the food and feed industries. Genetic resources of oat are the basic materials for sustainable breeding programs. Microsatellites (SSR) are a useful tool for understanding the genetic background of oat germplasm resources. We used 83 SSR primer pairs to assess the genetic diversity of 286 oat accessions from five countries. The results indicated the presence of considerable variation among accessions. The number of alleles per SSR locus ranged from 2 to 8, with an average of 3.49; allelic frequencies ranged from 0.002 to 0.709; Nei's genediversity was 0.67, ranging from 0.44 to 0.85; and polymorphism information content (PIC) values ranged from 0.37 to 0.83. Genetic distance estimated by the Jaccard model was compared between accession groups as well as among all accessions using all marker alleles. The principle coordinates analysis based on genetic dissimilarity also revealed distinct groups of accessions. We concluded that SSR markers are effective for identification of oat germplasm resources and use of germplasm from North America and Asia should lead to significant progress in Mongolian oat breeding programs.

Keywords: Oat, germplasm, population, simple sequence repeat markers (SSR)

Introduction
Oat (*Avena* spp.) is a cereal crop that is mainly grown in North America, northern Europe, Mongolia and China and Australia. It is usually managed as a low input crop adapted to marginal land while playing an important role in supporting livelihoods (Hoffmann 1995; Marshall *et al.* 2013) [6]. Oat grain contains high levels of protein, oil and microelements, and has high nutritional value as both human food and animal feed (Tian *et al.* 2003) [3]. The world oat cultivation area is around 7 million ha with Mongolia as the biggest producing country followed by Canada, Australia, USA and Poland (FAO 2014) [4]. Oat has a high potential for research and development. Oat genetic resources are essential for oat breeding and improvement. Over 200,000 accessions of oat and its wild relatives are stored in gene banks worldwide (Diederichsen 2008) [2]. Exchange of germplasm among breeders across continents could enrich the diversity and availability of oat materials in breeding programs. Phenotypic evaluation is necessary to provide genetic information on oat accessions for breeding use. However, genotypic variation is important to understand the genetic relationships among oat accessions and to identify useful markers for trait selection in variety improvement. Simple sequence repeats (SSR) are small segments of DNA, usually 2 to 5 base pairs in length that repeat themselves a number of times, and are evenly distributed throughout the nuclear, chloroplast, and mitochondrial genomes of eukaryotes. The genesis of SSR is an evolutionarily dynamic process and has proven to be exceedingly complex (Ellegren 2004; Pearson *et al.* 2005). SSR markers have been used in genetic analysis of oat and have provided a backbone of genetic information due to their high levels of polymorphism and ease of use. Li *et al.* [7]. Developed SSR markers for identifying relationships among *Avena* species and oat cultivars and found that polymorphisms among cultivars were relatively low. Fu *et al.* [5] used 26 SSR primers to assess genetic variation in 369 *Avena sterilis* accessions from 26 countries. The SST patterns were useful for understanding the progenitor species of cultivated oat. Li *et al.* [7] compared the diversity of wild populations of *A. fatua* from China and America and concluded that the wild population in China had a similar level of diversity to that in the USA. Montilla-Bascón *et al.* [10] assessed genetic diversity and population structure of oat cultivars and landraces from Spain and showed that SSR markers had high potential for use in identifying commercial cultivars. Nersting *et al.* [12] evaluated oat varieties from Nordic breeding programs by SSR markers

showed that there was loss of genetic diversity at both the agronomic and molecular levels. However, there is no report on analysis of genetic diversity and identification of relationships among accessions from Asian and American countries with SSR markers. Breeders often exchange oat germplasm. Sometimes it may be difficult to identify suitable parental materials for breeding due to unclear genetic backgrounds. Although phenotypic methods are useful to determine the genetic similarity or dissimilarity of various oat accessions, molecular markers help in understanding the genetic relationships of materials and allele frequencies among different populations. The current study assessed genetic diversity, population structure, and genetic relationships among 286 oat accessions from Brazil, Canada, China, Mongolia and USA, using SSR markers.

Materials and Methods

The 286 oat lines used in the study included 19 accessions from Brazil, 30 from Canada, 69 from China, 50 from Mongolia, and 118 from the USA

DNA Isolation and PCR amplification

DNA extracted from leaf tissue by grinding in liquid nitrogen using a modified CTAB method (Dellaporta *et al*. 1983) [1] was diluted to a final concentration of 30 ng µl−1 for polymerase chain reactions (PCR). Microsatellite primers were developed by the oat research group at the CAAS Institute of Crop Science (Wu *et al*. 2012) [13]. Eighty-three SSR primer pairs that produced polymorphic amplifications were chosen to genotype the entire germplasm set. The reaction solutions for PCR amplification contained 50 ng of DNA, 1.5 mmol L–1 MgCl2, 0.2 mmol L–1 dNTP, 1.0 U Taq D NA p olymerase, and 0.1 µmol L –1 primer. The PCR program included denaturization at 94 °C for 5 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 60 s, extension at 72°C for 10 min, and then cooling at 4 °C. Amplified products were detected on 8% non-degenerating polyacrylamide gels (PAGE) and electrophoresis for 2.5 h at 200 V with an electric current of 500 mA. Gels were stained with silver nitrate.

Allele scoring

Most SSR markers were visualized as clusters of two to eight bands. Stained gels were dried and placed on a light box. Allele scores were based on the presence of a particular size allele in each accession. The presence of an allele was denoted as 1 and its absence as 0.

Data analysis

A 1/0 matrix was constructed based on the presence or absence of alleles for the set of 83 SSR markers. The SSR genotype data was analyzed for genetic diversity and population structure. Descriptive genetic diversity statistics were calculated using Power Marker software version 3.25 (Liu and Muse 2005) [9]. The average number of alleles per locus was computed as the arithmetic mean of the total number of alleles observed at each SSR locus. Genetic diversity was computed for each marker locus and all loci. Unbiased heterozygosity values (Nei 1978) [11] were calculated. Neighbor joining (NJ) trees were computed on the basis of the symmetrical matrix of pair-wise genetic dissimilarity estimates

Results

SSR marker polymorphism

A total of 450 SSR markers were screened and 83 SSR with polymorphisms were selected to obtain PCR arrays on the 286 oat accessions. A total of 290 alleles were identified at 83 SSR marker loci. The mean number of alleles per SSR locus was 3.49, ranging from of 2 to 8 (AM2185). Sixteen, 29, 25, 19, 1, 1, and 1 SSR markers identified 2, 3, 4, 5, 6, 7 and 8 alleles, respectively. Allele frequencies ranged from 0.002 to 0.709, averaging 0.262. Nine of the 290 alleles had frequencies less than 0.03; these could be identifying unique accessions. A total of 625 genotypes were generated by these alleles across all accessions. A minimum of 2 genotypes was generated by marker AM579 and a maximum of 28 genotypes was generated by AM2185; and the average genotype number was 7.53 per locus. The mean gene diversity of all SSR markers computed over all accessions was 0.67, and ranged from of 0.44 for AM939 to 0.85 for AM2673. The heterozygosity of all SSR markers across accessions was 0.49, ranging from 0.00 for AM623, AM1046 and AM1057 to 0.98 for AM2438. Polymorphic information content (PIC) varied from 0.37 for AM939 to 0.83 for AM2673, indicating that SSR analysis revealed a relatively high number of informative markers among the oat accessions. Markers AM2185 (with 8 alleles), AM2673 (with 7 alleles) and AM2438 (with 4 alleles) showed the highest levels of polymorphism in genotype number, genetic diversity and heterozygosity.

Genetic variation among oat accessions

The genetic variation of oat accessions from different countries was calculated using the SSR data. Genetic variation was compared among groups of oat accessions from five countries by calculating the number of alleles, number of genotypes, major allele frequency, Nei's genetic diversity and heterozygosity (Table 1). Allele numbers within groups ranged from 222 (Brazil) to 279 (USA) and increased with increased number of accessions within groups. Major allele frequencies within groups ranged from 0.35 (Brazil and Canada) to 0.37 (USA).

Nei's genetic diversity was not significantly different among groups from different countries, indicating a relatively uniform genetic background across all accessions. Heterozygosity ranged from 0.46 (US accessions) to 0.52 (Chinese accessions), indicating a more complicated genetic background among Chinese accessions.

Table 1: Genetic parameters of oat groups from different countries

Group	No. Of accessions	No. of alleles	Major allele frequency	Nei's genetic diversity	Heterozygosity
Brazil	19	222	0.35	0.74	0.48
Canada	30	255	0.35	0.74	0.48
China	69	261	0.36	0.74	0.52
Mongolia	50	253	0.36	0.73	0.50
USA	118	279	0.37	0.74	0.46
Total	286	290	0.36	0.74	0.49

Genetic relationships among oat accessions

Genetic distance estimated by the Jaccard model was compared between accession groups as well as among all accessions using all marker alleles. The principle coordinates analysis based on genetic dissimilarity also revealed distinct groups of accessions (Fig. 2). Generally, two groups comprised only US accessions, one group was a mixture of accessions from Brazil, Canada and USA, one group was composed of accessions from China and Mongolia, and one group comprised accessions from Canada and China. However, Mongolian accessions that clearly formed a unique group in cluster analysis grouped with Chinese accessions [14]. All accessions from Brazil and the USA were positively associated with the first coordinate, whereas accessions from China and Mongolia were negatively associated with the first coordinate. Accessions from Canada were both positively and negatively associated with the first coordinate.

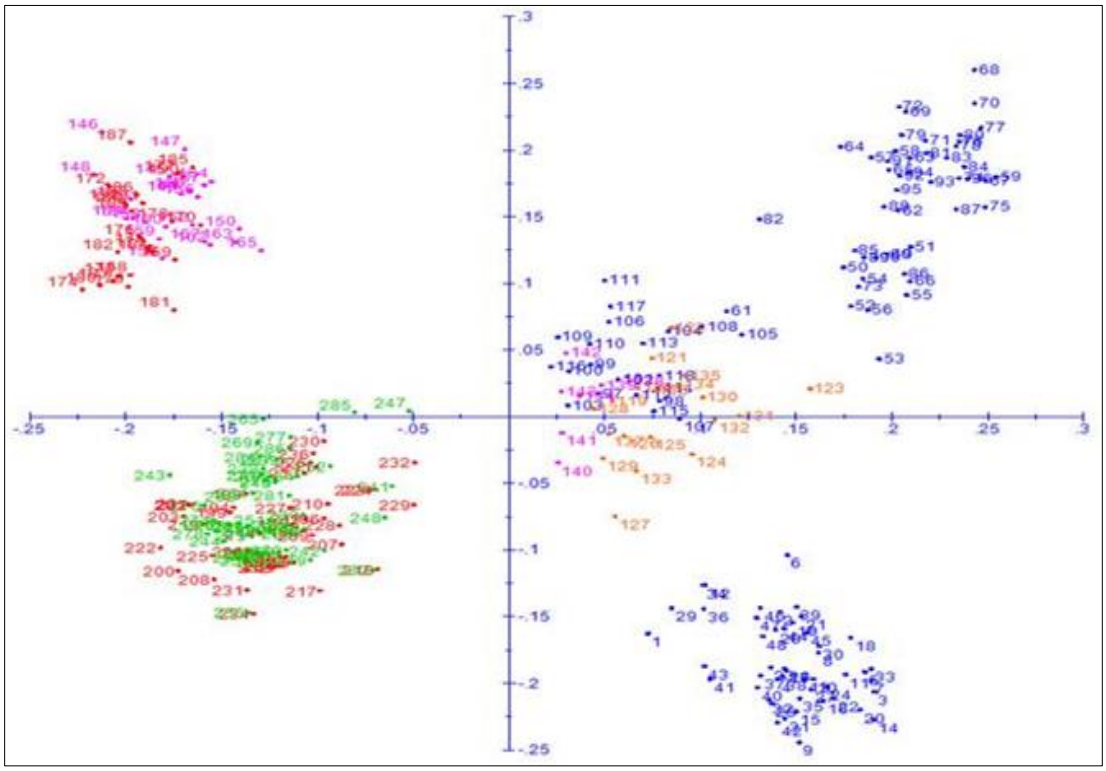

Fig 2: Principle coordinates analysis of 286 oat accessions based on genetic dissimilarity calculated with allele frequencies of SSR markers. Yellow for Brazilian accessions; pink for Canadian accessions; red for Chinese accessions; green for Mongolian accessions; blue for USA accessions.

Discussions

SSR Polymorphism and allelic frequencies

Microsatellite markers have been used by researchers to identify relationships among Avena species and oat cultivars (Li *et al*. 2000) [7] variation in *Avena sterilis* germplasm (Fu *et al*. 2007) [5] and genetic diversity of oat cultivars and landraces (Montilla-Bascón *et al*. 2013) [10]. We screened a total of 526 primer pairs and identified 83 (15.8%) showing polymorphism, considerably less than the 36% reported earlier (Li *et al*. 2000) [7]. Nersting *et al*. (2006) identified 66 alleles from seven SSR loci in 84 oat landraces and cultivars, averaging 9.43 alleles per locus. The average number of alleles of 3.49 in the current study was much lower, but similar to that reported by Li *et al*. [7] where two to eight alleles were found at 16 polymorphic SSR sites. PIC values in different studies were all relatively high, i.e. 0.37 - 0.83 in the current study, 0.49 - 0.88 reported by Nersting *et al*. [12] and 0.46 - 0.96 reported by Montilla-Bascón *et al*. [10] Fu *et al*. [5] used 26 pairs of SSR primers to evaluate 369 oat accessions and found allele frequencies ranging from 0.01 to 0.99 and averaging 0.28. In the current study, frequencies ranged from 0.002 to 0.709, averaging 0.262. There was a similar averaged allele frequency among accessions in the current study, but the frequencies of some alleles, e.g. AM072-4, AM074-6, AM033-8 and AM022-2 at <0.01 were much lower than those reported in the other studies. Such alleles could have specific uses; for example, AM072-4 was found only in accession S007, whereas AM074-6 was found only in S028. Another two alleles, namely AM033-8 and AM022-2, occurred in three accessions (S018, S029 and S036) and 5 accessions (S008, S014, S170, S182 and S188), respectively. Ten accessions with rare alleles included eight from the USA and two from China.

Genetic variation and relationships of oat accessions from different countries

Genetic variation is the basis for genetic selection and improvement in oat breeding programs. Nei's genetic diversity ranged from 0.73 to 0.74 in oat groups from different countries [11]. The genetic diversity of oat accessions in this study was higher than reported in other populations, but was not significantly different between countries. This result is quite different from the significant variation found among accessions from various countries using AFLP markers (Fu *et al*. 2005) [5]. Generally, cluster analysis in this study classified oat genotypes into six clusters that were consistent with their geographic origins. For example, Clusters I, II and III were mainly from American countries, and V and VI were mainly from Asia. However, Cluster IV comprised genotypes from China and

Canada, indicating some exchange of germplasm between the two countries. Cluster III was a mixture of accessions from Brazil, Canada and USA, indicating a high level of interchange of oat germplasm among those three countries.

Conclusion

In this study, 290 alleles at 83 SSR loci were identified in 286 oat accessions from five countries. Nine alleles had frequencies less than 0.03 and were therefore identified as rare. The distribution of genetic diversity was clearly associated with the geographical origin of accessions and reflected the trends of interchange of germplasm between American countries and between Asian and American countries.

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References

1. Dellaporta SL, Wood J, Hicks JB. A plant DNA minipreparation: Version II. Plant Molecular Biology Reporter. 1983;1:19-21.
2. Diederichsen A. Assessments of genetic diversity within a world collection of cultivated hexaploidoat (*Avena sativa* L.) based on qualitative morphological characters. Genetic Res and Crop Evol. 2008;55:419-440.
3. Tian C, Hao R, Zhao S, Chen S. Nutrition and medical value and health-care food of oats. Journal of Zhangjiakou Teachers College. 2003;19:12-15.
4. FAO. FAOSTATS, 2014. http://faostat.fao.org/.
5. Fu YB, Chong J, Fetch T, Wang ML. Microsatellite variation in *Avenasterilis*oat germplasm. Theor Appl Genet. 2007;114:1029-1038.
6. Hoffmann LA. World production and use of oats. In: Welch RW (ed) The oat crop-production and utilization. Chapman and Hall, London, 1995.
7. Li CD, Rossnagel BG, Scoles GJ. The development of oat microsatellite markers and their use in identifying relationships among Avena species and oat cultivars. Theor Appl Genet. 2000;101:1259-1268.
8. Li R, Wang S, Duan L, Li Z, Christoffers MJ, Mengistu LW. Genetic diversity of wild oat (*Avenafatua*) populations from China and the United States. Weed Sci. 2007;55:95-101.
9. Liu K, Muse SV. PowerMarker: Integrated analysis environment for genetic marker data. Bioinformatics. 2005;21:2128-2129.
10. Montilla-Bascón G, Sánchez-Martín J, Rispail N, Rubiales D, Mur L, Langdon T, *et al*. Genetic diversity and population structure among oat cultivars and landraces. Plant Mol Biol Reptr. 2013;31:1305-1314.
11. Nei M. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics. 1978;76:379-390.
12. Nersting LG, Andersen SB, Bothmer R, Gullord M, Jørgensen RB. Morphological and molecular diversity of Nordic oat through one hundred years of breeding. Euphytica. 2006;150:327-337.
13. Wu B, Lu P, Zhang Z. Recombinant microsatellite amplification: a rapid method for developing simple sequence repeat markers. Mol Breeding. 2012;29:53-59.
14. Munkhtuya Y, Zhang Z. Analyses on Diversity of Germplasm Resources and QTLs for Important Agronomic Traits in Oat. Ph.D. dissertation GSCAAS. China. Beijing, 2017.