Indian J. Genet., 81(1): 144-147 (2021)
DOI: 10.31742/IJGPB.81.1.18

Short Communication

Molecular characterization of oats (*Avena sativa* L.) germplasm with microsatellite markers

Gagandeep Kaur, Rahul Kapoor*, Priti Sharma[1] and Puja Srivastava

Department of Plant Breeding and Genetics, School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana

(Received: August 2020; Revised: January 2021; Accepted: January 2021)

Abstract

Assessment of genetic diversity between selected genotypes serves as an essential tool to exploit the genetic variability. In the present study, Ninety nine accessions of oat acquired from different geographical regions were used to study the molecular genetic variability by the use of 91 microsatellite primers. A total of 248 alleles from 91 primer pairs in the 99 oat genotypes were detected. The average alleles per primer pair were recorded as 2.69. The primer pairs having the highest PIC values *viz*., AM 6 (0.50), AM 55 (0.49) and M 83381 (0.49) can be further used in the association mapping studies. Clustering of the oat genotypes into two main clusters A and B was done by using the symmetric matrix of DICE coefficient based on UPGMA method in Software – DARwin 6.0. In this study, the moderate amount of dissimilarity was observed between the whole set of accessions, the values ranging from 0.33 to 0.79. The individuals belonging to the different clusters may be useful in hybridization studies of oat improvement programmes.

Key words: Genetic diversity, SSRs, polymorphic information content (PIC), cluster analysis, dissimilarity coefficient, DARwin

Oat is called a multipurpose crop as it is used for staple food directly for human consumption and indirectly for livestock feed (Varma et al. 2016). For the development of the new oat varieties, germplasm acquired from different eco-geographic areas is thought to be the finest resource which would provide the essential variation in the different traits. In any crossing programme, more diverse the individuals larger are the effects of heterosis observed and there are the increased chances of getting desirable segregants. The exotic germplasm should be properly evaluated so that it can be used in research and plant breeding (Jellen and Legget 2006). The estimation of the genetic variability of any crop is done by means of both molecular characterization and morphological method (Greene et al. 2008).

For characterization of genetic resources, genetic markers are crucial tools to analyze the variation present in the sequences of the DNA of different genotypes and, therefore evading the issues caused because of the environmental effects. Microsatellites markers are the ideal markers for molecular studies (Powell et al.1996) due of their higher reliability (reproducibility), co-dominant inheritance, simplicity, cheap usage and usually high polymorphism. These can be used in number of studies like genomic identification and protection of varieties, conservation of germplasm, genetic mapping, QTL analysis, marker-assisted selection breeding and diversity studies. Information on genetic diversity at molecular level has been reported earlier by many researchers. Genotypic diversity of 369 accessions of wild species of oat using 230 microsatellite markers has been reported by Fu et al. (2007). Similarly, a study was conducted in which 91 microsatellite primer pairs which belonged to AM, AB_AM series were used (Li et al. 2000; Dumlupinar et al. 2016). Assessment of genetic diversity at morphological or molecular level in crop plant is essential for crop improvement through hybridization

*Corresponding author's e-mail: rahulkapoor@pau.edu
Published by the Indian Society of Genetics & Plant Breeding, A-Block, F2, First Floor, NASC Complex, IARI P.O., Pusa Campus, New Delhi 110 012; Online management by www.isgpb.org; indianjournals.com

Table 1. Polymorphic information content of SSR markers

S.No.	Name	Amplified	Value	S. No.	Name	Amplified	Value	S. No.	Name	Amplified	Value
1	AM1	3	0.38	32	AM32	1	0.36	63	AM102	3	0.48
2	AM 2	3	0.33	33	AM33	3	0.42	64	AM112	2	0.24
3	AM3	3	0.47	34	AM34	2	0.32	65	HVM4	3	0.37
4	AM4	6	0.34	35	AM35	4	0.34	66	HVM20	1	0.36
5	AM5	5	0.45	36	AM36	3	0.41	67	HVM62	2	0.41
6	AM6	5	0.5	37	AM37	2	0.44	68	L39777	3	0.27
7	AM7	4	0.47	38	AM38	5	0.31	69	M83381	1	0.49
8	AM8	5	0.41	39	AM39	3	0.44	70	Xgwm88	2	0.40
9	AM9	3	0.36	40	AM40	3	0.33	71	Xgwm471	3	0.41
10	AM10	2	0.45	41	AM41	5	0.40	72	Z48431	3	0.30
11	AM11	4	0.48	42	AM42	3	0.42	73	AB_AM_059	2	0.42
12	AM12	3	0.40	43	AM43	2	0.27	74	AB_AM_065	3	0.34
13	AM13	3	0.48	44	AM44	4	0.34	75	AB_AM_066	1	0.44
14	AM14	2	0.38	45	AM45	2	0.46	76	AB_AM_068	3	0.40
15	AM15	2	0.47	46	AM46	3	0.37	77	AB_AM_070	1	0.44
16	AM16	4	0.38	47	AM47	1	0.44	78	AB_AM_073	2	0.42
17	AM17	1	0.32	48	AM48	3	0.46	79	AB_AM_074	1	0.48
18	AM18	4	0.41	49	AM49	2	0.39	80	AB_AM_076	2	0.48
19	AM19	3	0.43	50	AM50	3	0.40	81	AB_AM_077	2	0.37
20	AM20	3	0.45	51	AM51	2	0.41	82	AB_AM_079	2	0.40
21	AM21	4	0.45	52	AM52	4	0.26	83	AB_AM_081	4	0.43
22	AM22	3	0.38	53	AM53	4	0.39	84	AB_AM_083	2	0.48
23	AM23	3	0.44	54	AM54	1	0.41	85	AB_AM_087	2	0.46
24	AM24	2	0.40	55	AM55	2	0.49	86	AB_AM_093	3	0.39
25	AM25	3	0.46	56	AM56	3	0.42	87	AB_AM_095	4	0.43
26	AM26	2	0.31	57	AM57	2	0.27	88	AB_AM_108	2	0.38
27	AM27	3	0.44	58	AM58	1	0.33	89	AB_AM_111	3	0.41
28	AM28	4	0.39	59	AM59	3	0.38	90	AB_AM_123	3	0.43
29	AM29	2	0.43	60	AM60	2	0.26	91	AB_AM_124	2	0.45
30	AM30	3	0.43	61	AFO33096	3	0.46	92	AB_AM_130	3	0.37
31	AM31	2	0.35	62	AM87	2	0.41				

and selection. Therefore, an investigation was carried out to assess the genetic variation in genotypes of geographically diverse source of oat. A total of 99 accessions of oat (*Avena sativa* L.) comprising of 65 exoctic lines, 30 indigenous stocks and four cultivars belonging to different regions of the world were collected from the NBPGR, New Delhi, India for molecular diversity studies (Table 2).

The genomic DNA extraction was performed on the young seedlings of about 30 days by using the CTAB method (Murray and Thompson 1980). *In vitro* amplification of the DNA was performed in Eppendorf master cycler by using specific SSR primers by using the method described by Oliver et al (2011). Once the PCR product was amplified, 6 % PAGE gel which is polyacrylamide gel was used to resolve the products.The gel was observed under the UV light and photo gel documentation system (Alphaimager HP, Alpha Innotech) was used to take the photograph.The analysis of the genetic variability of the genotypes was done by using the DARwin 6.0 software (Perrier et al. 2006). The dendrogram was generated by UPGMA method of analysis which is unweighted pair groups methods with arithmetic mean.

Results showed that all the 91 primer pairs yielded a total of 248 alleles. The PIC values for all 91 SSR markers and no. of alleles detected per primer are given in Table 1. Figure 1 represents the gels showing the PCR amplification of different genotypes by SSR primers. The PIC values ranged from 0.24 (AM 112) to 0.5 (AM 6). The markers with higher PIC values *viz.*, AM 6 (0.50), AM 55 (0.49) and M 83381

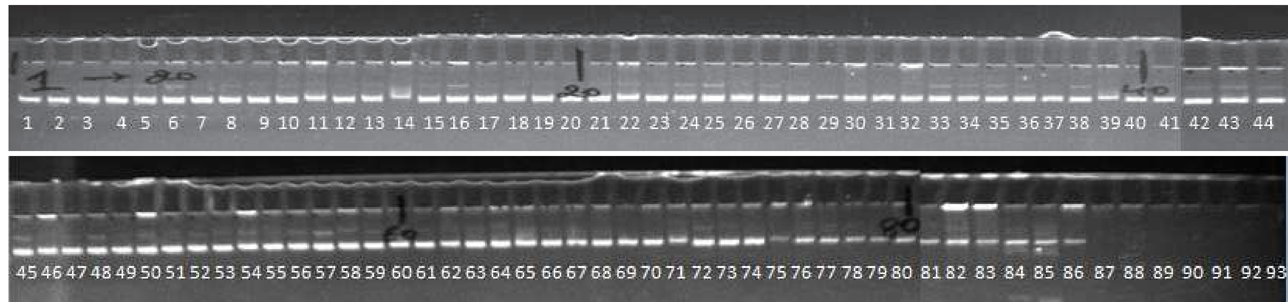

Fig. 1. PAGE Plate representing thepolymorphism generated by SSR primer AB_AM_073 on amplification of the DNA of 99 genotypes. Upper plate (1-44): Kent, OL9, OL10, IC 372496, IC 372457, IC 372452, IC 372467, IC 372563, IC 372503, EC 605839, EC 209589, IC 372523, IC 372527, IC 372437, EC 209272, EC 605833, P 7 292561, IC 108477, IC 372510, IC 372530, IC 372531, IC 372481, EC 605829, EC 537851, EC 537849, EC 537856, EC 528864, IC 372489, EC 537808, IC 372497, IC 372442, EC 533788, P 7 295932, EC 246179, IC 372458, IC 372477, EC 605835, EC 18850, EC 537878, EC 528896, EC 537869, EC 537811, IC 372478, IC 372474, IC 372493, IC 372441, EC 209570; Lower plate (45-99):EC 528871, EC 209402, IC 372424, EC 528906, EC 209307, EC 537834, EC 537825, EC 209346, EC 528919, IC 372415, EC 537819, EC 537924, EC 528907, EC 528916, EC 537836, IC 372502, IC 372466, EC 209524, EC 528888, EC 246200, EC 537815, EC 131639, EC 246158, EC 528925, EC 209576, EC 246178, EC 246112, EC 528874, EC 528902, EC 246132, IC 372529, EC 528899, EC 528923, EC 246166, IC 372462, EC 209452, EC 246176, EC 537850, EC 246181, EC 537853, EC 246131, EC 528908, EC 537885, EC 528905, EC 372463, EC 537867, IC 372482, EC 537875, EC 537855, *94-99: The bands were missing in these genotypes

Table 2. Cluster analysis of the genotypes on the basis of microsatellite markers

Cluster	Sub cluster	No. of acessions	Accession names
A	I	92	IC 372467, IC 372563, IC 372503, EC 605839, EC 209589, IC 372523, IC 372527, EC 209272, EC 605833, P 7 292561, IC 108477, IC 372510, IC 372530, IC 372531, IC 372481, EC 605829, EC 537851, EC 537849, EC 537856, EC 528864, IC 372489, EC 537808, IC 372497, IC 372442, EC 533788, P 7 295932, EC 246179, IC 372458, IC 372477, OL11, EC 605835, EC 18850, EC 537878, EC 528896, EC 537869, EC 537811, IC 372478, IC 372474, IC 372493, IC 372441, EC 209570, EC 528871, EC 209402, IC 372424, EC 528906, EC 209307, EC 537834, EC 537825, EC 209346, EC 528919, IC 372415, EC 537819, EC 537924, EC 528907, EC 528916, EC 537836, IC 372502, IC 372466, EC 209524, EC 528888, EC 246200, EC 537815, EC 131639, EC 246158, EC 528925, EC 209576, EC 246178, EC 246112, EC 528874, EC 528902, EC 246132, IC 372529, EC 528899, EC 528923, EC 246166, IC 372462, EC 209452, EC 246176, EC 537850, EC 246181, EC 537853, EC 246131, EC 528908, EC 537885, EC 528905, EC 372463, EC 537867, IC 372482, EC 537875, EC 537855, EC 246120 and EC 246147
	II	1	IC 372437
B	I	5	Kent, OL9, OL10, IC 372496 and IC 372457
	II	1	IC 372452

(0.49) can be helpful in the association mapping. In a similar study conducted by Kapoor and Chaudhary (2017), it was found that the total no. of alleles which were amplified for all the primers varied from 2-6. The PIC values ranged from 0.08 to 0.82 with an average of 0.47. The analysis of the genetic variability of the genotypes was done by using the DARwin 6.0 software (Perrier et al. 2006). The clustering of the germplasm lines was done by UPGMA method. The 99 oat genotypes were separated into two main clusters A and B (Dendrogram figure not given) (Table 2). Cluster A was the largest and was divided into two main sub-clusters I and II where sub-cluster I contained 92 individuals and sub-cluster II contained one individual. On the other hand cluster II was smaller and was divided further into two sub-clusters I and II, where

sub-cluster I had 5 individuals and sub-cluster II had only one individual. The pattern of clustering which was obtained from the study confirmed the dependability on the microsattelite markers for diversity studies. The dissimilarity values were found to be in the range of 0.33-0.79 indicating a moderate level of dissimilarity amongst the oat accessions under evaluation. The genotypes with utmost dissimilarity were EC 537875 and KENT (0.79), EC 537875 and IC 372496 (0.78), EC 246131 and IC 372457 (0.76), EC 528925 and IC 372477 (0.76). The results indicated that the genotypes can get higher dissimilarity, thus these could be further used to create mapping populations for different studies and the desirable segregants with high heterotic effects can be obtained on crossing of the germplasm lines present in distant clusters.

Authors' contribution

Conceptualization of research (GK, RK); Designing of the experiments (GK, RK, PS); Contribution of experimental materials (RK); Execution of field/lab experiments and data collection (GK, PK, PS, PS); Analysis of data and interpretation (GK, PK, PS, PS); Preparation of manuscript (GK, RK).

Declaration

The authors declare no conflict of interest.

References

Dumlupinar Z., Brown R., Campbel R., Jellen E. N., Aderson J., Bonman M., Carson M., Chao S., Obert D. and Jackson E. 2016. The art of attrition: development of robust oat microsatellites. Plant Breed., **135**: 323-334.

Fu Y. B., Chong J., Fetch T. and Wang M. L. 2007. Microsatellite variation in *Avenasterilis*o at germplasm. Theor. Appl. Genet., **114**: 1029-1038.

Greene N. V., Kenworthy K. E., Quesenberry K. H., Unruh J. B. and Sartain J. B. 2008. Diversity and relatedness of common carpetgrass germplasm. Crop Sci., **48**(6): 2298-2304.

Hoisington D., Listman G. M. and Morris M. L. 1998. Varietal development: Applied biotechnology. In: Maize seed industries in developing countries. (ed. Morris) Lynne Rienner Boulder, Co USA, Publishers, CIMMYT: 77-102.

Jellen E. and Leggett J. M. 2006.Cytogenetic manipulation in oat improvement. In: Singh, R. J., Jensen C. F. 1976. Embryo culture as an implement in hybridization, haploid production and in genetical and developmental studies.News Int. Ass. Pl. Tissue Cult., **18:** 10.

Kapoor R. and Choudhary K. 2017. Genetic diversity analysis of fodder Oats (*Avena sativa* L.) germplasm by microsatellite markers. J. Agr. Sci. Tech., **19:** 1369-1379.

Li C. D., Rossnagel B. G. and Scoles G. J. 2000. The development of oat microsatellite markers and their use in identifying relationships among *Avena* species and oat cultivars. Theor. Appl. Genet., **101**: 1259-1268.

Murray M. G. and Thompson W. F. 1980. Rapid isolation of high molecular weight DNA. NucleicAcids Res., **8**: 4321-4325.

Nikoloudakis N., Bladenopoulos K. and Katsiotis A. 2016. Structural patterns and genetic diversity among oat (*Avena sativa*) landraces assessed by microsatellite markers and morphological analysis. Genet. Resour. Crop Evol., **63**: 801-811.

Oliver R. E., Jellen E. N., Ladizinsky G., Korol A. B., Kilian A., Beard J. L., Dumlupinar Z., Wisniewski-Morehead N. H., Svedin E., Coon M., Redman R. R., Maughan P. J., Obert D. E. and Jackson E. W. 2011. New Diversity Arrays Technology (DArT) markers for tetraploid oat (*Avena magna* Murphy et Terrell) provide the first complete oat linkage map and markers linked to domestication genes from hexaploid *A. sativa* L. Theor. Appl. Genet., **123**: 1159-1171.

Perrier X. and Jacquemond-Collet J. P. 2006. DARwin software.http://darwin.cirad.fr/darwin/

Powell W., Marchray G. C. and Provan J. 1996. Polymorphism revealed by simple sequence repeats. Trends Plant Sci., **1**: 215-222.

Ramanatha R. V. and Hodgkin T. 2002.Genetic diversity and conservation and utilization of plant genetic resources.Pl. Cell Tiss. Org. Cult., **68**: 1-19.

Rana M., Gupta S., Kumar N., Ranjan R., Sah R. P., Gajghate R., Dwivedi K. K. and Ahmed S. 2019. Genetic architecture and population structure odf oat landraces (*Avena sativa* L.) using molecular and morphological descriptors. Ind. J. Trad. Knowl., **18**(3): 439- 450.

Ruwali Y., Verma J. S. and Kumar L. 2013.Comparative genetic diversity analysis of oat (*Avena sativa* L.) by microsatellite markers and morphological rainfed expressions. Afr. J. Biotech., **12**: 3414-3424.

Sharma P., Tiwari S., Tripathi N. and Mehta A. K. 2016. Polymorphism analysis in advanced mutant population of oat (*Avena sativa* L.) using ISSR markers.Physiol. Mol. Biol. Plants, **22**: 115-120.

Varma P., Bhankharia H. and Bhatia S. 2016. Oats: A multifunctional grain. J. clin. Prev. Cardiol., **5**(1): 9-17.