

Genetic Diversity Analysis of Fodder Oats (*Avena sativa* L.) Germplasm by Microsatellite Markers

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ABSTRACT

The present study was undertaken to assess the genetic diversity in the ninety six oat (*Avena sativa* L.) elite cultivars representing the collection from various eco-geographical regions of India. The molecular diversity analysis using 40 SSR markers clustered all the 96 cultivar into ten clusters and significant level of distinction (dissimilarity coefficient ranged from 0.12 to 0.96) was depicted among the lines indicating a high degree of divergence among these lines. Genotypic pairs having utmost genetic dissimilarity (0.96) were OL1634 and OL1688, OL1702 and OL1688, OL1705 and OL1634, UPO03-3 and OL1688, and UPO03-3 and OL1705 that can be used as parents in purposeful hybridization programs. Polymorphic Information Content (PIC) values ranged from as low as 0.06 to as high as 0.75 (AM 7). Owing to their highest PIC values, primer pairs AM7 (0.75), AM2 (0.69) and AM10 (0.69) can be further used in association mapping studies in oat. The Un-weighted Pair Group Method with Arithmetic Mean (UPGMA) based dendrogram revealed the cluster V (19 genotypes) as the largest and cluster X (3 genotypes) as the smallest one. Thus, genotypes within clusters can be predicted as similarity pool in further oat improvement programs. The selected panel of SSR markers performed well in detection of genetic diversity patterns and can be recommended for future germplasm characterization studies in oats.

Keywords: Dissimilarity coefficient, Molecular diversity analysis, Polymorphic information Content, SSRs.

INTRODUCTION

The genus *Avena* belongs to the grass family Poaceae. Oat (*A. sativa* L.) is an economically important crop and ranks sixth in world cereal production after wheat, rice, maize barley and sorghum (FAO, 2012). It is an important winter forage crop in many parts of the world and is also grown as multipurpose crop for grain, pasture, and forage. Differing from other cereal grains such as wheat and barley, it is rich in the antioxidants α -tocotrienol, α -tocopherol, and avenanthramides, as well as total dietary fibre including the soluble fibre β -glucan (Oliver *et al.*, 2010). In recent years, with the advent of exaggerated dairy industry in our country, oat has fascinated the attention

of breeders for its improvement due to its nutritious quality fodder for livestock and its grains as animal feed with high net energy gains (Ruwali *et al.*, 2013). Hence, the first and foremost need is the identification or cataloguing of oats genotypes along with the assessment of genetic diversity prevalent in different geographical regions in the world. Moreover, there are different genetic approaches for assessment of genetic diversity in

germplasm accessions, breeding lines, and segregating populations based on morpho-agronomic traits and DNA markers. Among them, the use of DNA marker technology in varietal improvement has progressed rapidly during the last decade. The discovery and use

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of molecular markers based on DNA differentiation offers great opportunity to understand and identify the diverse genetic material in crop species. Molecular markers are indispensable tools for characterizing genetic resources by detecting variation of the DNA sequences among cultivars and, therefore, directly bypass the problems related to environmental effects and thus have many applications of value to crop improvement. In oats, extensive studies have been done for genetic diversity analysis using molecular markers such as Random Amplified Polymorphic DNA (RAPD) (Loskutov, 2007; Loskutov and Perchuk, 2000; Hanif et al., 2008; Abbas et al., 2008; Boczkowska et al., 2014), restriction fragment length polymorphism (RFLP) (Pal, 2002), Sequence-Characterized Amplified Region (SCAR) and Cleaved Amplified Polymorphic Sequence (CAPS) (Orr and Stephen, 2008). But, at certain point, these studies were to be less edifying due to highly monomorphic nature as well as in bi-parental mapping population. Therefore, SSRs are the logical choice to characterize genetic variability for understanding phylogenetic and evolutionary patterns owing to their high reliability, effectiveness in assessment of crop germplasm at genetic level (O'Neill et al., 2003) and being used extensively in studies of variability and genotype characterization. These markers are PCR based (Weber and May, 1989), co-dominant (Litt and Luty, 1989) and consist of short tandem repeats of between 1 and 6 base pairs (Queller et al., 1993). As compared to RFLP and AFLP analysis, assays involving SSRs are rapid, cost effective (Jannink and Gardner, 2005), work well with minute or even degraded samples of DNA, and display consistency in scoring of alleles with clear comparisons across various gels (Queller et al., 1993). These characteristics, along with an evenly dispersed genomic distribution, are properties which make SSRs ideal genetic markers (Morgante and Olivieri, 1993; Powell et al., 1996). To date, 174 oat genomic microsatellites have been identified from SSR-enriched libraries, related species, and by

mining sequence databases (Li et al., 2000, Holland et al., 2001, Pal et al., 2002, Jannink and Gardner, 2005, Oliver et al., 2010, Wight et al., 2010). In addition, 216 microsatellites with good PIC (0.42) have been mined from available Expressed Sequence Tag (EST) databases in oat (Becher, 2007); however, the availability of this EST-based resource is restricted. Montilla-Bason et al., (2013) utilized 31 of these existing SSR markers having very high PIC values (mean 0.80) and successfully identified four clusters among 177 oat accessions consisting of cultivars, two groups of four white oat landraces, and red oat accessions. More recently, Boczkowska et al. (2014) employed eight ISSR primers, with mean PIC value of 0.24, as part of a larger study to measure genetic variation in pre-1939 Polish oat varieties. Thus, the purpose of this study was to assess the genetic diversity among oat germplasms based on microsatellite markers (SSRs).

MATERIALS AND METHODS

The experimental material comprised of 96 elite cultivars from diverse eco-geographic regions of the country (Figure 1) maintained at the experimental area of Forage Research Farm, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The names and origin of the plant material used in the present investigation is given in Table 1. Dendrogram was constructed using symmetric matrix of euclidean distance coefficients based on UPGMA using DARwin 5.0 (Perrier *et al.*, 2006)

Isolation of Genomic DNA

Total genomic DNA of 96 *Avena sativa* accessions was isolated from young leaves from four-week old plants following the procedure of Poulsen *et al.* (1993). DNA samples were evaluated

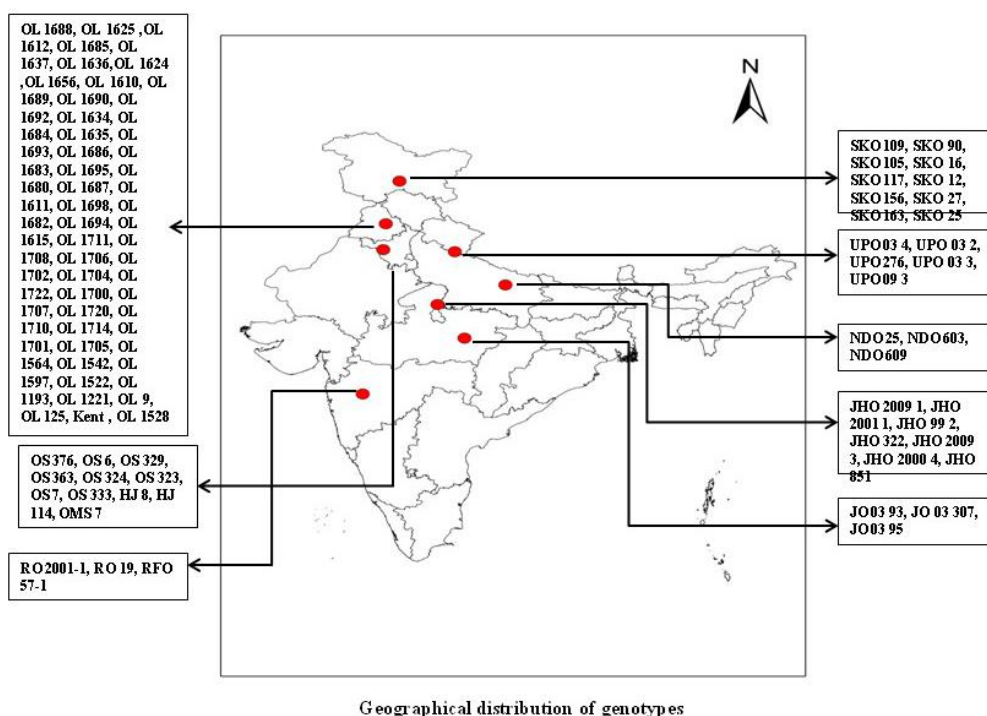


Figure 1. Geographic distribution of 96 germplasm accessions on Indian map.

both quantitatively and qualitatively using spectrophotometer and λ (lambda) DNA (concentration marker), respectively.

Selection of Primers

For the present study, 40 SSR primer pairs (Table 2) belonging to AM, CWM, WISC series were selected from the published source. AM series were provided by Li *et al.* (2000), the WISC primers were developed by Zhu and Kaeppler (2003), and CWM series was provided by Gao *et al.* (2003).

SSR Analysis

PCR amplification of SSR markers was carried out using reaction mixture containing 50 ng of template DNA, 1X PCR buffer, 1.5 mM MgCl₂, 200 μ M of dNTPs, 0.4 μ M each of forward and reverse primers and 1 unit of Taq DNA polymerase. Amplification was performed using the following conditions:

denaturation at 94 °C for 4 minutes; 40 cycles of 1 minute denaturation at 94 °C, 1 minute annealing at temperatures adjusted depending upon the SSR primer sequence, 1 min extension at 72°C and a final extension at 72°C for 10 minutes. The SSR amplification products were separated in a vertical denaturing 6% polyacrylamide. DNA fragments were revealed using the ethidium bromide staining procedure. The gels were stained for 30-35 minutes and visualized under UV light and photographed using photo gel documentation system (Alphaimager HP, Alpha Innotech).

RESULTS AND DISCUSSION

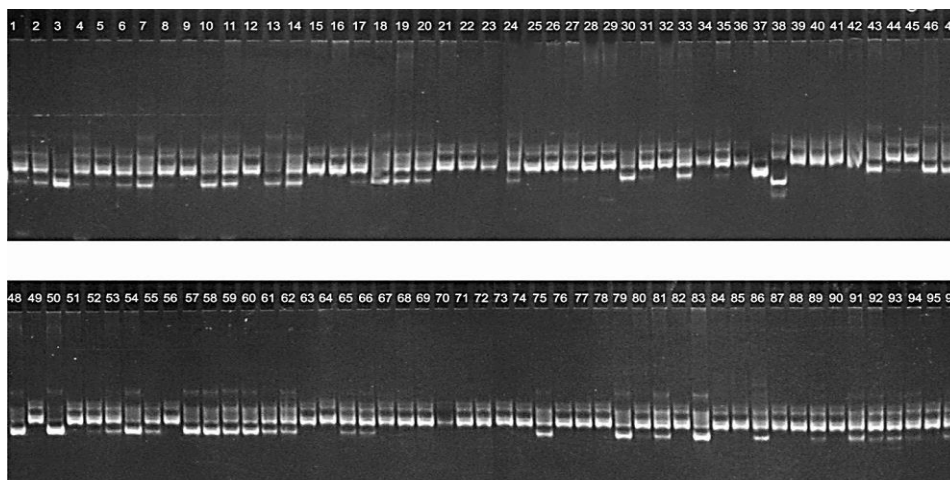
Summarized data for number of alleles detected per primer pair and the Polymorphic Information Content (PIC) values for each of the 40 SSR primers are presented in Table 3. PCR amplification results by one primer are presented in Figure 2. A total of 129 alleles were detected by 40

**Table 1.** The list of all the germplasm accessions evaluated for diversity analysis.

S No	Genotype	Origin	S No	Genotype	Origin	S No	Genotype	Origin	S No	Genotype	Origin
1	OL 1688	Ludhiana	25	OL 1694	Ludhiana	49	UPO 03 2	Pantnagar	73	SKO 90	J & K
2	OL 1625	Ludhiana	26	OL 1615	Ludhiana	50	UPO 276	Pantnagar	74	SKO 105	J & K
3	OL 1612	Ludhiana	27	OL 1711	Ludhiana	51	UPO 03 3	Pantnagar	75	SKO 16	J & K
4	OL 1685	Ludhiana	28	OL 1708	Ludhiana	52	UPO 09 3	Pantnagar	76	SKO 117	J & K
5	OL 1637	Ludhiana	29	OL 1706	Ludhiana	53	HJ 8	Hisar	77	SKO 12	J & K
6	OL 1636	Ludhiana	30	OL 1702	Ludhiana	54	HJ 114	Hisar	78	SKO 156	J & K
7	OL 1624	Ludhiana	31	OL 1704	Ludhiana	55	NDO 25	Faizabad	79	SKO 27	J & K
8	OL 1656	Ludhiana	32	OL 1722	Ludhiana	56	NDO 603	Faizabad	80	SKO 163	J & K
9	OL 1610	Ludhiana	33	OL 1700	Ludhiana	57	NDO 609	Faizabad	81	SKO 25	J & K
10	OL 1689	Ludhiana	34	OL 1707	Ludhiana	58	JHO 2009 1	Jhansi	82	OL 1564	Ludhiana
11	OL 1690	Ludhiana	35	OL 1720	Ludhiana	59	JHO 2001 1	Jhansi	83	OL 1542	Ludhiana
12	OL 1692	Ludhiana	36	OL 1710	Ludhiana	60	JHO 99 2	Jhansi	84	OL 1597	Ludhiana
13	OL 1634	Ludhiana	37	OL 1714	Ludhiana	61	JHO 322	Jhansi	85	OL 1522	Ludhiana
14	OL 1684	Ludhiana	38	OL 1701	Ludhiana	62	JHO 2009 3	Jhansi	86	OL 1193	Ludhiana
15	OL 1635	Ludhiana	39	OL 1705	Ludhiana	63	JHO 2000 4	Jhansi	87	OL 1221	Ludhiana
16	OL 1693	Ludhiana	40	OS 376	Hisar	64	JHO 851	Jhansi	88	OL 9	Ludhiana
17	OL 1686	Ludhiana	41	OS 6	Hisar	65	RO 2001 1	Rahori	89	OL 125	Ludhiana
18	OL 1683	Ludhiana	42	OS 329	Hisar	66	RO 19	Rahori	90	Kent	Ludhiana
19	OL 1695	Ludhiana	43	OS 363	Hisar	67	RFO 57 1	Rahori	91	EC 605830	Exotic collection
20	OL 1680	Ludhiana	44	OS 324	Hisar	68	JO 03 93	Jabalpur	92	EC 605839	Exotic collection
21	OL 1687	Ludhiana	45	OS 323	Hisar	69	JO 03 307	Jabalpur	93	EC 605833	Exotic collection
22	OL 1611	Ludhiana	46	OS 7	Hisar	70	JO 03 95	Jabalpur	94	EC 605829	Exotic collection
23	OL 1698	Ludhiana	47	OS 333	Hisar	71	OMS 7	Hisar	95	EC 605832	Exotic collection
24	OL 1682	Ludhiana	48	UPO 03 4	Pantnagar	72	SKO 109	J&K	96	OL 1528	Ludhiana

Table 2. The list of SSR primers and their sequence information used in present study.

S No	Primer	Repeat motif	Left primer 5' to 3'	Right primer 5' to 3'	Tm (°C)
1	AM1	(AG)(CAGAG)	GGATCTCCACGGTGTGA	CTCATCCGATGGGGTTTA	46
2	AM2	AG	TGAATTCGTGGCATTAGTCAAGA	AAGGAGGGCATAGGAGGTAATT	49
3	AM3	AG	CTGGTCTCTCCGGCTTCA	CATTTAGCCAGGTGGCCAGGTC	51
4	AM4	AG	GGTAAGGTTTCGAAGAGCAAAG	GGGCTATATCCATCCCTCAC	48
5	AM5	AG	TTGTCAGCGAAATAAGCAGAGA	GAATTCGTGACCAGCAACAG	46
6	AM6	AG	AATGAAGAACCAGGGTGGGAAGTG	CCAGCCCACTAGTTCAGCCCATCT	52
7	AM7	AG	GTGAGCCCGAATACATA	TTGGCTAGCTGCTTGAACCT	48
8	AM8	AG	CAAGGCATGAAAAGAGTAAGAT	TCGAAAGCAAAATGGTCCACAC	47
9	AM9	AG	CAAAAGCATTGGGCCCTTGT	GGCTTTGGACCCTCCTTCC	48
10	AM10	AG	AAAATCGGGGAAGGAACC	GAAGGCAAAATACATGGAGTCCAC	46
11	AM11	(AG)(AAAG)	TCGTGGCAGAGAATCAAAGCAC	TGGGTGGAGGCAAAAACAAAAC	49
12	AM12	AG	TGCTGAAGTGAACATCTCC	CCTTCTCCAACAATCTTAC	44
13	AM13	AG	CGCGGTGATTTGGGAAGAAG	CTAGTAACGGCCCGCAGTGTGCTG	54
14	wise48	AG	CAATGGGCATTGAGAGAITAAG	TATGGCTGGTGGAGTGTGTTTG	52
15	CWM26	A	GGCAGCAGCAAAGCAGGTC	ACTCACATAGGGGCAAAATG	55
16	CWM27	A	AGCATCTCGCATTTCTTGA	GCAGCCGCTTTTGGATTCTA	55
17	CWM28	A	CGCATGGAAGCTCACAGTTT	TTGCTGAGATGGCTGGAAGGAG	55
18	CWM29	AAC	CGCATGCAAGCTCACAGT	GCTGAGGCTGCTGGTAGGAGAC	55
19	CWM30	AAC	GCAGTCCCAAGCCATCC	ACATTTGACAGTACGGTCTCT	55
20	CWM31	AAC	ATGCAGGATGTTGTATTG	CTGTGGGTTTTGGCTGAGAT	55
21	CWM32	AAC	GCGGTGCCAAGCCATCCA	CACATTCAGGTAGCGGTCTCT	55
22	CWM33	AAC	CAGCCCATGGAAGATCACAA	GTT GGAGCCCTGGCCATATGGA	55
23	CWM34	AAC	GCAGCATCCATAGCGTCCG	TG AAATGGTCCCTGATGATGGAG	55
24	CWM35	AAC	ATGCAGGATGTTGTATTG	GAGGCTGTGGAAGGAGAC	55
25	CWM36	AAC	CATGCAGGATGTTGCTTG	ATATGCTGCTGAGGCTGTTGG	55
26	CWM37	A	AGCATCTCGCATTTCTTGA	GCAGCCGCTTTTGGATTCTA	55
27	CWM38	A	TTGGTCTGTAGGGGTGAAA	AATGTAAAACGACGGCCAGTAAT	55
28	CWM39	A	TCCTGGCCCTCTGATGTAAT	TAATAAATGGCCCTCCAGAG	50
29	CWM40	A	TCGCTCCCTACCGCCGAGAA	ATGAAAAGGCCCGCCAGTGAAC	55
30	CWM41	A	TTACCCGTTCCCGATTACAG	AGGGATGGCCCGGGATGGTGGAG	50
31	CWM42	A	TGTCCCGTTGCCAGTTTGT	TAG AITACCCCGGGCCAGTGAAC	50
32	CWM43	A	AGCGTGGAGGAAAGTGGAG GG	GGGTGGTGGGGTAG 55	55
33	CWM44	A	AATGGAAAGTGGGGTTGA	GGGGCCCGGTCTTTTT 5	55
34	CWM45	AAC	GCCGATGGAAGCTCACAG	TTTTGAAGAAGCCCTGGCTGATGGATA	55
35	CWM46	ATG	GGTTCCTCTGGTGTGAG	ATAACAAGGATGCTGGGAG	55
36	CWM47	ATCCCT	ATCGCCCTCCAGCA	ACCAGACGACCGCAGAGGACGAG	55
37	CWM48	ATG	GATCGGCACTTCTCCCT	ACCCGCTCTTCCCAATAAT	55
38	CWM49	A	TCGCTCCCTACGGCCGAGAA	TGAAAAGGCCCGCAGTGAAC	55
39	CWM50	AAC	AGCGCATGGAAGATCAAA	GGAGCCCTGCTAATGGA	55
40	CWM51	A	GCGTATGCGTGCACAAA CG	ATAATCAAAAAGGGTCACTAC	55



Genotypes are marked by order (1-96) as per sequence given in table 1

Figure 2. PAGE Plate: Polymorphism pattern from amplification of genomic DNA of 96 test genotypes with SSR primer CWM 51.

Table 3. Alleles amplified and *PIC* values of primers.

S No	Primer	Number of alleles amplified	<i>PIC</i> value	S No	Primer	Number of alleles amplified	<i>PIC</i> value
1	AM1	2	0.33	21	CWM33	2	0.34
2	AM2	4	0.69	22	CWM34	3	0.55
3	AM3	2	0.37	23	CWM35	4	0.57
4	AM4	2	0.36	24	CWM36	4	0.58
5	AM5	3	0.56	25	CWM37	2	0.16
6	AM6	3	0.57	26	CWM38	2	0.37
7	AM7	5	0.75	27	CWM39	3	0.49
8	AM8	4	0.48	28	CWM40	4	0.23
9	AM9	6	0.63	29	CWM41	4	0.37
10	AM10	5	0.69	30	CWM42	4	0.40
11	AM11	4	0.54	31	CWM43	3	0.41
12	AM12	4	0.53	32	CWM44	3	0.30
13	AM13	3	0.43	33	CWM45	3	0.59
14	CWM26	3	0.31	34	CWM46	2	0.53
15	CWM27	4	0.60	35	CWM47	2	0.37
16	CWM28	2	0.28	36	CWM48	3	0.50
17	CWM29	4	0.48	37	CWM49	2	0.38
18	CWM30	3	0.40	38	CWM50	2	0.06
19	CWM31	3	0.45	39	CWM51	3	0.48
20	CWM32	2	0.36	40	WISC 48	4	0.63

primers in the 96 genotypes with an average of 3.22 alleles per primer. To mention but a few, the main causes of this divergence may be the deletions, insertions, and chromosomal inversion at the DNA level which generate polymorphism or allelic diversity. However, Fu *et al.* (2007) found higher level of diversity by observing the number of alleles per primer pair ranged from 1 (CWM204) to 24 (AM3) and averaged 4.8 alleles. These differences in our study pertaining to the number of alleles detected are primarily due to the use of high resolution 6% polyacrylamide gel, lines from different geographic regions and SSR markers used. Total number of allele amplified for each primer ranged from 2 to 6. The maximum number of alleles (6) was amplified by AM9. The lower average number of alleles amplified per SSR marker and that by the individual marker in the present study is due to the use of local *Avena sativa* accessions, which had no ploidy changes and had not been used by any of the studies enlisted above. So, these genotype specific alleles couldn't add on to the alleles amplified per primer and the average alleles per primer.

Polymorphic Information Content (PIC) Values

PIC values ranged from 0.06 to 0.75 with an average of 0.45. The highest *PIC* value was recorded by AM7 (0.75), followed by AM2 (0.69), AM10 (0.69), WISC 48 (0.63) and AM9 (0.63). *PIC* values of each primer pair ranged from 0.02 to 0.95 with an average of 0.35 reported by Fu *et al.* (2007) in *Avena sterilis*. These polymorphic primers can be used in further molecular studies like association mapping, tagging of gene of interest, and the so called approach Marker Assisted Selection (MAS).

Cluster Analysis in Germplasm Collection Based on SSR Data

Ten clusters were obtained upon clustering the 96 genotypes under study

(Figure 3) with the symmetric matrix of DICE coefficients based on UPGMA using DARwin 5.0 (Perrier *et al.*, 2006). The perusal of the clustering analysis revealed that the individuals within any one cluster were more closely related than the individuals in different clusters (Table 4). The cluster I consisting of 19 genotypes was the largest amongst all and was closely followed by cluster V having 14 genotypes. Among all, cluster X was the smallest one consisting of three genotypes. The check cultivars OL 9 and KENT used in the present study fell in clusters II and IX, respectively. The clustering pattern thus obtained in the study confirmed the discriminating power and reliability of the SSR markers for genetic diversity studies.

The dissimilarity coefficients ranged from 0.12 to 0.96 signifying a high degree of dissimilarity among the *A. sativa* accessions evaluated in the present investigation. Genotypic pairs having utmost genetic dissimilarity of 96% were OL1634 and OL1688, OL1702 and OL1688, OL1705 and OL1634, UPO03-3 and OL1688, UPO03-3 and OL1705. These results showed that genotypes OL1634, OL1688, OL1705, OL1702 and UPO03-3 were implicated to get high dissimilarity, consequently, they can be used to facilitate as mapping population in various mapping studies as well as establishing the utility of microsatellite markers in identifying diverse pairs. In contrast, the minimum genetic dissimilarity of about 12% was observed between the lines SKO12 and OMS7, SKO12 and SKO16, SKO12 and HJ114, SKO16 and OL1695. This marked a possibility that the SSR markers used in the study may be linked to the genomic region in these genotypes. OL1684 was grouped into the separate cluster from OL1615, so, they being diverse as reported by the SSR study could be used to develop mapping populations for number of tillers/plant, green fodder yield, and dry fodder yield because they have extreme value for these traits and also are present

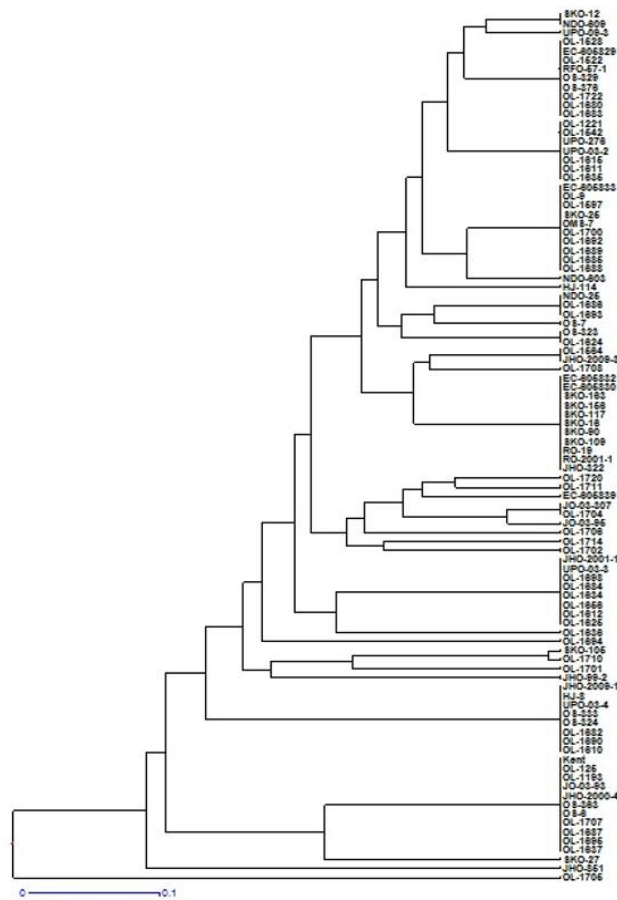


Figure 3. Dendrogram obtained by SSR marker analysis using UPGMA method.

Table 4. Clustering pattern obtained by SSR analysis.

Cluster	Number of genotypes	Genotypes
I	19	SKO12, NDO609,UPO00-3, OL1528, EC605829, OL1622, RFO57-1, OS329, OS376, OL1722, OL1680, OL1683, OL1221, OL1542, UPO276, UPO03-2, OL1615, OL1611, OL1635
II	11	EC 165833,OL9, OL1597, SKO25, OMS7, OL1700, OL1692, OL1689, OL1688, NDO603
III	7	HJ114, NDO25, OL1693, OS7, OS323, OL1624,OL1625
IV	14	OL1684, JHO2009-3, OL1708, EC605832, EC605830, SKO163, SKO109, SKO117, SKO18, SKO90, SKO169, RO19, RO2001-1, JHO322
V	9	OL1720, OL1711, EC605839, JO03-307, OL1704, JO 03-95, OL1708, OL1714, OL1702
VI	9	JHO2001-1, UPO03-3, OL1693, OL1684, OL1634, OL1658, OL1612, OL1625, OL1638
VII	5	OL1694, SKO105, OL1710, OL1701, JHO99-2
VIII	8	JHO 2009-1, HJ 8, UPO 03-4, OS 333, OS 324, OL 1682, OL 1690, OL 1710
IX	11	KENT, OL125, OL1193, JO03-93, JHO2000-4, OS 363, OS8, OL1707, OL1687, OL1696, OL1637
X	3	SKO27, JHO851, OL1705

in separate clusters. While excellent mapping population for number of leaves/plant could be developed between UPO03-4 and UPO276, as they have extreme values for the target character, as well as they are placed in the extreme groups by the SSR dendrogram. Similarly, genes for plant height could be mapped from a mapping population using any genotypes among OL1625, OL1686, UPO03-4, JHO99-2 and JO03-93 because all have extreme trait values, as well as are grouped in distinct groups by microsatellite analysis. Greater number of genotypes in a single cluster manifests that these genotypes were more closely related and had less genetic variation among them. It further implies that hybridization program employing these genotypes inhabiting a common cluster will be of little use in oat improvement program. In studies of SSR diversity in accessions of *Triticum aestivum* L., Huang *et al.* (2002) observed that not all accessions originating from a geographic region clustered in the same group. The authors concluded that either similar genetic variation occurred independently among accessions in the different geographical regions or that the artificial transfer of accessions between regions resulted in the incorrect/false determination of geographical origin. SSRs with similar attributes could be exploited for genome/ species differentiation, because repetitive sequences associated with SSRs are relatively stable and genome-specific (Li *et al.*, 2000). Additionally, these results probably linked with the morphological attributes of the same cultivars and need to be validated through precise investigation for association either through bulk segregation analysis or near isogenic lines (Tanhuanpaa *et al.*, 2007).

CONCLUSIONS

Conclusively, induction of diverse oat genotypes followed by their utilization in the well defined scientific oat hybridization program could be a useful tool to cater the

overall fodder oats cultivar development program. Further, among marker discriminating indices, PIC (Polymorphic Information Contents) are believed to be more reliable indices for selecting a marker or combination of markers for characterization of germplasm. Also, between marker systems, microsatellites (SSRs) could be suitable for studying genetic diversity among oats genotypes because of their ability to produce more number of bands per reaction, while their co-dominant nature with high value of expected heterozygosity will make them more suitable for genome mapping.

REFERENCES

1. Abbas, S. J., Shah, S. R. U., Rasool, G. and Munir, I. 2008. Analysis of Genetic Diversity in Genus *Avena*. *Pak. J. Weed Sci. Res.*, **14**: 33-41.
2. Becher, R. 2007. EST-Derived Microsatellites as a Rich Source of Molecular Markers for Oats. *Plant Breed.*, **126**: 274–278.
3. Boczkowska, M., Nowosielski, J., Nowosielska, D. and Podyma, W. 2014. Assessing Genetic Diversity in 23 Early Polish Oat Cultivars Based on Molecular and Morphological Studies. *Gene. Resour. Crop Evol.*, **61**(5): 927–941.
4. FAO. 2012. *Production Statistics*. Food and Agriculture Organisation, Rome.
5. Fu, Y. B., Cho ng, J., Fetch, T. and Wang, M. L. 2007. Microsatellite Variation in *Avena sterilis* Oat Germplasm. *Theor. Appl. Genet.*, **114**: 1029–1038.
6. Gao, L. F., Tang, J. F., Li, H. W. and Jia, J. Z. 2003. Analysis of Microsatellites in Major Crops Assessed by Computational and Experimental Approaches. *Mol. Breed.*, **12**: 245-261.
7. Hanif, Z., Swati, Z.A., Khan, I., Hassan, G., Marwat, K.B., Ali, S. and Khan, M. I. 2008. RAPD and SSR Analysis of Wild Oats (*Avena* Species) from North West Frontier Province of Pakistan. *Afr. J. Plant Sci.*, **11**: 133–139.
8. Holland, J. B., Helland, S. J., Sharopova, N. and Rhyne, D. C. 2001. Polymorphism of PCR-Based Markers Targeting Exons,



- Introns, Promoter Regions, and SSRs in Maize and Introns and Repeat Sequences in Oat. *Genome*, **44**: 1065–1076.
9. Huang, X. Q., Borner, A., Roder, M. S. and Ganal M. W. 2002. Assessing Genetic Diversity of Wheat (*Triticum aestivum* L.) Germplasm Using Microsatellite Markers. *Theor. Appl. Genet.*, **105**: 699–707.
 10. Jannink, J. L. and Gardner, S. W. 2005. Expanding the Pool of PCR Based Markers for Oat. *Crop Sci.*, **45**: 2383–2387.
 11. Li, C. D., Rosnagel, 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.
 12. Litt, M. and Luty, J. A. 1989. A Hyper Variable Microsatellite Revealed by *In Vitro* Amplification of a Dinucleotide Repeat within the Cardiac Muscle Actin Gene. *Am. J. Hum. Genet.*, **44**: 397–401.
 13. Loskutov, I. G. 2007. *Oat (Avena sativa) Distribution, Taxonomy, Evolution and Breeding Value*. VIR, St. Peterberg, Russia.
 14. Loskutov, I. G. and Perchuk, I. N. 2000. Evaluation of Interspecific Diversity in *Avena* Genus by RAPD Analysis. *Oat Newslett.*, 46 PP.
 15. Montilla-Bason, G., Sanchez-Martin, J., Rispail, N., Rubiales, D., Mur, L., Langdon, T., Griffiths, I., Howarth, and Prats, E. 2013. Genetic Diversity and Population Structure among Oat Cultivars and Landraces. *Plant Mol. Biol. Res.*, **31**: 1305–1314.
 16. Morgante, M. and Olivieri, A. M. 1993. PCR-Amplified Microsatellites as Markers in Plant Genetics. *Plant J.*, **3**: 175–182.
 17. O'Neill, R., Snowdon, R. J. and Kohler, W. 2003. Population Genetics Aspects of Biodiversity. *Progress Bot.*, **64**: 115-137.
 18. Oliver, R. E., Obert, D. E., Hu, G., Bonman, J. M. and Jackson, E. W. 2010. Development of Oat based Markers from Barley and Wheat Microsatellites. *Genome*, **6**:458-71.
 19. Orr, W. and Stephen, J. M. 2008. Development of PCR-based SCAR and CAPS Markers Linked to β -Glucan and Protein Content QTL Regions in Oat. *Genome*, **51**: 421-425.
 20. Pal, S. 2002. Oat-Derived SSRs and RFLPs. *Crop Sci.*, **42**: 912-918. Perrier, X. and Jacquemond-Collet, J. P. 2006. *DARwin Software*. <http://darwin.cirad.fr/darwin/>.
 21. Poulsen, G. B., Kahl, G. and Weising, K. 1993. Abundance and Polymorphism of Simple Repetitive DNA Sequences in *Brassica napus* L. *Theor. Appl. Genet.*, **85**: 994-1000.
 22. Powell, W., Marchray, G. C. and Provan, J. 1996. Polymorphism Revealed by Simple Sequence Repeats. *Trend. Plant Sci.*, **1**: 215–222.
 23. Queller, D. C., Strassmann J. E. and Hughes, C. R. 1993. Microsatellites and Kinship. *Trend. Ecol. Evol.*, **8**: 285–328.
 24. Ruwali, Y., Singh, K., Kumar, S. and Kumar, L. 2013. Molecular Diversity Analysis in Selected Fodder and Dual Purpose Oat (*Avena sativa* L.) Genotypes by Using Random Amplified Polymorphic DNA (RAPD). *Afr. J. Biotech.*, **12(22)**: 3425-29.
 25. Tanhuanpää, P., Kalendar, R., Schulman, A. H. and Kiviharju, E. 2007. A Major Gene for Grain Cadmium Accumulation in Oat (*Avena sativa* L.). *Genome*, **50(6)**: 588-594.
 26. Weber, J. L. and May, P. E. 1989. Abundant Classes of Human DNA Polymorphisms which Can Be Typed Using the Polymerase Chain Reaction, *Am. J. Hum. Genet.*, **44**: 388–396.
 27. Wight, C. P., Yan, W., Fetch, J. M., Deyl, J. and Tinker, N. A. 2010. A Set of New Simple Sequence Repeat and Avenin DNA Markers Suitable for Mapping and Fingerprinting Studies in Oat (*Avena* Spp.). *Crop Sci.*, **50**:1207–1218.
 28. Zhu, S. and Kaeppler, H. F. 2003. Identification of Quantitative Trait Loci for Resistance of New Sources to Crown Rust in Oat Line MAM17- 5. *Crop Sci.*, **43**: 358-366.

تحلیل تنوع ژنتیکی ژرم پلاسماهای جو دو سر (*Avena sativa* L.) با نشانگرهای ریزماهوره ای

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چکیده

هدف از این پژوهش ارزیابی تنوع ژنتیکی در ۹۶ کولتیوار الیت جودوسر (*Avena sativa* L.) بود که نمونه های جمع آوری شده از مناطق مختلف بوم-جغرافیایی هندوستان بودند. با تجزیه و تحلیل تنوع ملکولی با استفاده از ۴۰ SSR، همه این ۹۶ کولتیوارها در ده خوشه متمایز در سطح معنا دار دسته بندی شد (ضریب عدم تشابه بین ۰/۱۲۰ تا ۰/۹۶) که نشاندهنده درجه واگرایی بالایی بین این رگه ها بود. جفت های ژنوتیپیک با بیشترین عدم تشابه (۰/۹۶) عبارت بودند از OL 1634 و OL1688، OL1702 و OL1688، OL 1705 و OL 1634، UPO 03-3 و UPO 03-3، UPO 03-3 و OL1705 که می توان آنها را به عنوان والد ها در برنامه های دورگ گیری هدفمند به کار گرفت. مقدار محتوای اطلاعاتی چند شکلی (PIC) از مقدار کم ۰/۰۶ تا مقدار زیاد ۰/۷۵ (AM7) تغییر میکرد. به علت داشتن بالاترین مقدار PIC، جفت های آغاز گر AM 7 (۰/۷۵) و AM2 (۰/۶۹) و AM10 (۰/۶۹) را میتوان در مطالعات association mapping در جو دو سر استفاده کرد. روش Un-weighted Pair Group با دندروگرام مبتنی بر میانگین حساسی آشکار ساخت که خوشه V (شامل ۱۹ ژنوتیپ) بزرگترین خوشه و خوشه X (شامل ۳ ژنوتیپ) کوچکترین خوشه بود. به این قرار، در برنامه های بهبود و اصلاح ژنتیکی جو دو سر می توان ژنوتیپ های درون خوشه ها را به عنوان مجموعه ای مشابه قلمداد کرد. در این پژوهش، مجموعه انتخاب شده SSRها در شناسایی الگوهای تنوع ژنتیکی به خوبی عمل کرد و می توان آن ها را در مطالعات بیشتر برای تشخیص ژرم پلاسماها در جو دو سر توصیه کرد.