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Genetic architecture and population structure of Oat Landraces (*Avena sativa* L.) using molecular and morphological descriptors

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Oat is grown as winter forage in India. It is a self-pollinated crop with less variability. However, the variation for different morphological traits in oat germplasm may be available at genotypic level. The present study was conducted to find out the genetic diversity among 24 oat landraces using 9 morphological traits and 24 SSR primers. Morphological data observed across the 24 landraces showed wide variation and grouped various landraces into two clusters. GFY and DMY were positively and significantly correlated with most of the traits studied. The molecular analysis using 24 SSR primers resulted amplification of 62 polymorphic alleles with an average of 2.58 alleles per primer. Size of amplified alleles ranged from 70 to 480 bp. Mean polymorphic information content was 0.42 showing moderate level of SSR polymorphism. Cluster analysis based on SSR data differentiated 24 oat landraces into three major clusters. Bayesian model-based STRUCTURE analysis assigned landraces into two clusters and showed the extent of admixture within individuals. Clustering pattern of oat landraces based on SSR marker profiles were different from that of morphometric traits. So, based on the pooled analysis at morphological and molecular level, the landraces IG-02-121, IG-02-129 and IG-02-113 were found superior for morphological traits as well as most distant among all the landraces under study. Hence, these landraces could be used in for future breeding programmes for genetic improvement in oats.

Keywords: Genetic diversity, Landraces, Oat, Population structure, SSR markers
IPC Code: Int. Cl.[19]: C12N 1/11, A61K 47/48, A23L 5/40, G16B 20/40, H04Q 3/48

Oat (*Avena sativa* L.) is a cool season, annual, temperate crop having both food and feed value. Its cultivation for grain and fodder in Asia Minor is available since Christian era (Vavilov 1926). However Mediterranean region is considered as centre of origin especially for the cultivated red oat (*A. byzantina*) (Zade 1918; Trabut 1914; Malzew; 1930). There are about 50 known species of oat (Hackel 1890) which have been summarized in three groups, having n=7, n=14 and n=21 respectively (Stanton 1936). Common oat (*A. sativa* L.) belonging to hexaploid group is more likely evolved from *A. sterilis* (Trabut 1914) instead of *A. fatua* (Zade 1918). *A. sativa* is an allo-hexaploid having three distinct genomes, designated as A, B and C (Nishiyama 1929; Rajhathy 1960). Progenitor of A genome is one of the *strigosa* group (Nishiyama 1929). *A. ventricosa* is donor of C genome (Thomas 1970). B genome is possibly a modified form of A genome (Sadasivaiah and Rajhathy 1968). Being important feed and fodder crop, it is now gaining importance due to its unique and important quality characteristics, particularly the lipid and protein in grains. Green fodder contains about 10 to 13% protein and 30 to 35% dry matter. Oat grain are known for rich nutritional profile and well known cereal food for humans and feed for livestock. It is also reported that oat leafs has various medicinal properties, traditionally it is known to cure the menstrual imbalance ailment and treatment of osteoporosis and urinary tract infections (Duke, 2002). Oat grain makes a good balanced concentrate in the rations for poultry, cattle, sheep and other animals. The competition for utilization of land for food grains and fodder necessitates intensified efforts toward more efficient forage production which involves development of high yielding, fast growing, multicut with good regeneration capacity, dual purpose, nutritious and disease resistant varieties of fodder crops through genetic improvement.

In recent years, oat has gained considerable attention with respect to collection and conservation,

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due to its valuable nutrients and dual purpose utilization as livestock feed and human consumption. Oats are rich in lipid and protein content and its beta-glucans have role in lowering cholesterol and risk of heart disease (Whitehead et al., 2014). More than 80,000 landraces of wild and cultivated *Avena* spp. are stored in seed gene banks worldwide (Diederichsen, 2008). Landraces in particular, made a significant contribution to the oat germplasm, since several modern varieties are derived from them. Different definitions for landraces have been given through time, the most comprehensive being ''landrace is a highly diverse population or mixture of landraces naturally developed in a certain region under the influence of the regionally prevailing conditions of climate, soil and management, without or with only little mass selection'' (Banga, 1944). Transfer of beneficial traits from landraces is relatively easy as compared to their wild relatives. Thus, several studies suggest that landraces may be a good source of new allelic diversity for breeding programmes (Bascon et al., 2013). However, better characterization of germplasm is needed to facilitate its use in plant breeding and in research (Hawkes, 1990; Jellen and Leggett, 2006), to the introgression of germplasm into a plant breeding program.

Earlier for germplasm characterization, morphological traits were used by the plant breeders to assess the diversity, but they have a number of limitations, including low polymorphism, low heritability, late expression, and vulnerability to environmental influences (Smith and Smith, 1992). But the knowledge of the phenotype given by the morphological and agronomical descriptors is still important. In contrast, molecular markers are not influenced by environmental conditions and have been successfully used to assess the genetic diversity among various crops. Among various molecular markers available for germplasm characterization, the microsatellites or simple sequence repeat (SSR) markers are widely preferred for diversity analysis. The SSR loci are well-suited for the study of population structure due to their high allelic diversity and co-dominant nature (Garris et al., 2005). Since simple sequence repeat (SSR) markers were firstly used in human genetics, they have now become one of the most widely used markers in the related researches in crops, especially in the molecular characterization of genetic resources, e.g., rice (Nachimuthu et al., 2015) and wheat (Arora et al., 2014). Effectiveness of SSR markers in the genetic diversity analysis of oat germplasm has been well documented by different researchers (Achleitner et al., 2008, Bascon et al., 2013, Sood et al., 2014, Nikoloudakis et al., 2015). Keeping this in view, the study of genetic architecture and population structure in *Avena sativa* landraces using morphological and microsatellite based molecular markers was undertaken.

Materials and method

Plant material

The plant material used in the present study comprised of 24 indigenous collections of hexaploid (6x) Oat (*Avena sativa* L.) collected from different regions of the country and were assigned IC number. These landraces were stored at Mid Term Storage module (MTS) of ICAR-Indian Grassland and Fodder Research Institute (ICAR-IGFRI), Jhansi, Uttar Pradesh India (Table 1).

Field experiment and Morphological evaluation

A Field study was conducted at the experimental farm of ICAR-IGFRI, Jhansi. Geographically, in a Central Plain Zone with latitude 25.5114° N, 78.5337° E longitude. The experimental material was evaluated in the Rabi 2015-16. The experiments were laid out in a randomised complete block design with two replications. The observations were recorded for yield and its component characters viz., plant height (PHT), number of leaves (NL), flag leaf length (FLL), flag leaf width (FLW), number of tillers (NT), stem diameter (SD), number of florets (NF), green fodder yield (GFY) and dry matter yield (DMY). The morphological data were analysed statistically for mean, variances, correlations and genetic diversity to deduce the genetic similarity/dissimilarity using SYST AT-12 and Indostat 7.5 softwares. The estimates of variability parameters were analysed as per method suggested by Lush (1940). PCV and GCV were calculated based on the method advocated by Burton (1952) using Indostat 7.5 software. Heritability (h^2) in broad sense was estimated as per Allard (1960) and expressed in percentage using Indostat 7.5 software. Genetic advance was estimated by the method suggested by Johnson et al., (1955). Correlation between nine observed traits and PCA were estimated using StatistiXL version 1.10.

DNA Extraction and Purification

Isolation of genomic DNA was carried out from the leaves of *Avena sativa* landraces by using CTAB method (Doyle and Doyle, 1990). Quality and

Table 1 — Details of the plant material used along with source

S. No.	Institute ID	Landraces	Species	Source
1	IG-02-75	IC372428	*Avena sativa*	IGFRI, Jhansi
2	IG-02-65	IC372418	*Avena sativa*	IGFRI, Jhansi
3	IG-02-63	IC372416	*Avena sativa*	IGFRI, Jhansi
4	IG-02-74	IC372427	*Avena sativa*	IGFRI, Jhansi
5	IG-02-72	IC372425	*Avena sativa*	IGFRI, Jhansi
6	IG-02-62	IC372415	*Avena sativa*	IGFRI, Jhansi
7	IG-02-68	IC372421	*Avena sativa*	IGFRI, Jhansi
8	IG-02-71	IC372424	*Avena sativa*	IGFRI, Jhansi
9	IG-02-66	IC372419	*Avena sativa*	IGFRI, Jhansi
10	IG-02-64	IC372417	*Avena sativa*	IGFRI, Jhansi
11	IG-02–59	IC372412	*Avena sativa*	IGFRI, Jhansi
12	IG-02-112	IC372465	*Avena sativa*	IGFRI, Jhansi
13	IG-02-131	IC372484	*Avena sativa*	IGFRI, Jhansi
14	IG-02-122	IC372475	*Avena sativa*	IGFRI, Jhansi
15	IG-02-130	IC372483	*Avena sativa*	IGFRI, Jhansi
16	IG-02-123	IC372476	*Avena sativa*	IGFRI, Jhansi
17	IG-02-125	IC372478	*Avena sativa*	IGFRI, Jhansi
18	IG-02-126	IC372479	*Avena sativa*	IGFRI, Jhansi
19	IG-02-127	IC372480	*Avena sativa*	IGFRI, Jhansi
20	IG-02-132	IC372485	*Avena sativa*	IGFRI, Jhansi
21	IG-02-128	IC372481	*Avena sativa*	IGFRI, Jhansi
22	IG-02-121	IC372474	*Avena sativa*	IGFRI, Jhansi
23	IG-02-129	IC372482	*Avena sativa*	IGFRI, Jhansi
24	IG-02-113	IC372466	*Avena sativa*	IGFRI, Jhansi

quantity of DNA preparations was checked by standard spectrophotometry (Ausubel et al., 1995) and the samples were diluted to 13 ng DNA/µL concentration.

PCR amplification

Selection of Primers

Primers were initially screened using DNA from 10 random samples of the subjected landraces. Primers that produced maximum number of reproducible and scorable polymorphic bands were selected for SSR analysis (Table 2).

Optimization of Conditions for PCR Amplification

SSR primers were amplified using Polymerase Chain Reaction using the protocol of Williams et al. (1990). DNA amplification was performed in thermocycler (BIOER XP Cycler, Hangzhou, China). The PCR was performed in a 10 µL volume consisting of 2.0 µL DNA (13 ng/µL), 1.0 µL 10 X Taq Buffer, 0.50 µL dNTP (10 mM), 0.50 µL $MgCl_2$ (25 mM), 0.50 µL each forward and reverse primer (10 mM), 0.20 µL of Taq polymerase (5U/uL) and the final volume is made up to 10 µL with autoclaved distilled water for every reaction. This mixture was equally distributed to each 0.2 mL thin walled PCR reaction tube (Axygen Scientific Pvt. Ltd, New Delhi, India) and then 2.0 µL of DNA was added separately to each tube. The tubes were placed in a thermo cycler (BIOER XP Cycler, Hangzhou, China) for cyclic amplification with a thermal cycling programme having initial denaturation for 4 min at 94°C, followed by 35 cycles of 1 min at 94°C for denaturation, 1 min for 50-55°C for annealing and extension at 72°C for 2 min and finally, 8 min at 72°C with final extension.

Gel Electrophoresis

The amplified DNA was mixed thoroughly with 6X loading dye and then electrophoresed on 3% agarose (Genei, Bangalore, India) gel in 1X TBE buffer for SSR analysis. The gel was run at constant voltage at the rate of 5 V/cm under submerged conditions for about 3 h. The size of the amplified product was determined by co-electrophoresis of 100 bp standard molecular weight marker (Genei, Bangalore, India). DNA profiles were visualized on a UV transilluminator and photographed by using Gel Documentation System (INGENIUS-3, Syngene, USA).

Table 2 — List of SSR primers used in molecular characterization of *Avena sativa* landraces

S. No.	Primer Name	Forward primer Sequence	Reverse Primer Sequence
1	Asa-2014-162	5' ACAATGAAGGCACGGAGTCT 3'	5' TGTGGTGGCCTTCTACTTCC 3'
2	Asa-2014-170	5' GCAAACTAGGCTCCACTTGC 3'	5' CCAAATTAATAGTTGCCTTGTGTG 3'
3	Asa-2014-177	5' AACCAAAAGCTCATCGGAGA 3'	5' ATCATACTTCCTCCGTTCGC 3'
4	Asa-2014-178	5' GGGTTAGCCAACGAAGACAA 3'	5' GAGCTGGGAAGAACAGATGC 3'
5	Asa-2014-179	5' CCACCGACGAATCATCTTTT 3'	5' TCCAATCCATTTCCTTTCCC 3'
6	Asa-2014-180	5' TTTCTTTCTCGGTGGGTCTC 3'	5' ACATGCAATTCGTCACCGTA 3'
7	Asa-2014-184	5' CTACGACGGGGACATCAAGT 3'	5' CTCTGTTTCGTGCTCCCTTC 3'
8	Asa-2014-190	5' TCCTCCTCCAGGATGTGACT 3'	5' TTCTATCTGAAACCGGCCAC 3'
9	Asa-2014-196	5' GGCCAGGTCGACAAGTAGAT 3'	5' GAGAGGAAGATGGATGCTCG 3'
10	Asa-2014-206	5' CCATTTGTACAACAAGAACAACC 3'	5' GCAGAAGTGGCTGGAGAAAC 3'
11	Asa-2014-207	5' GTTTAGTTTTGCCGGTGGTC 3'	5' CGACACGGACAGAGAGATGA 3'
12	Asa-2014-215	5' AGATCTCCAAATCGACACGG 3'	5' GTAGTGGACTCCGCCTCGTA 3'
13	Asa-2014-216	5' CACTGACCGAACGATCTCAA 3'	5' TGCATAGTTAGCATGCGTCC 3'
14	Asa-2014-232	5' GCACTCACCCTCTCCTCAAC 3'	5' CAGCGGAGGAGTAAAGCAAC 3'
15	Asa-2014-239	5' AAGAGAAGAGATTGCGCTCG 3'	5' TGGTTTGGTTGCTTCTAGGG 3'
16	Asa-2014-245	5' CTCATGGGCTTCAACAAGGT 3'	5' GCACATTCTGAGCTTCCTCC 3'
17	Asa-2014-254	5' CAGGACTTGGGCTCCAATAA 3'	5' ATGCATGCAGTGTGGACAAG 3'
18	Asa-2014-257	5' ACCATCGCTAAGCCTCGTAA 3'	5' AACAGCGAGAGAGCTTGAGG 3'
19	Asa-2014-262	5' TCACACCTACCGACGAATGA 3'	5' CCATGAACTGGATCAACGG 3'
20	Asa-2014-263	5' AGGTTGCAAGGAACCCACTA 3'	5' TCCCTCTGCCTAAGCTGCTA 3'
21	Asa-2014-264	5' CAGTGTCCAGTCATTGGTGG 3'	5' AGTTCGCCTCCGAATCCT 3'
22	Asa-2014-276	5' ATATTAGCGACGAACCGACG 3'	5' CCATTGTCGTGTTTCCACTG 3'
23	Asa-2014-278	5' GGCTGTTCCTGGTAACGTGT 3'	5' GTACTGCTGGGTCTGTCCGT 3'
24	Asa-2014-292	5' CCACCAGCAGAGTCATGTGT 3'	5' GAAGGGCGCCTCTAGTCTCT 3'

Marker Scoring and Data analysis

All fragments were scored manually and converted into binary data, i.e., 1 for presence of band and 0 for absence of band. The polymorphism information content (PIC) of each primer pair was calculated according to the following formula given by Botstein et al. (1980):

$$PICi = 1 - \sum_{j=i}^{n} P2ij$$

Where Pij is the frequency of the jth pattern for marker i and summation extends over n patterns. Distance-based cluster analysis was performed and dendrogram based on the unweighted pair group method of arithmetic mean (UPGMA) was constructed using Jaccard's similarity coefficient with the help of NTSYS pc2.0 (Rohlf, 1998). Neighbor-joining (N-J) tree and PCoA was constructed with the help of DARwin software (Perrier et al. 2003). Bootstrapping with 1000 replicates was also performed with DARwin software. Bayesian model based clustering method implemented in STRUCTURE software version 2.3.3 (Pritchard et al. 2000; Falush et al. 2007) was utilized to assess the genetic structure at population level as well as to detect genetic stocks contributing to this germplasm collection. Evanno's method (Evanno et al. 2005) based program, STRUCTURE HARVESTER, (Earl and Vonholdt, 2011) was used to determine the value of estimated Ln probability of data-LnP (K) and to get the best fit value of K for the data.

Results and Discussion

Genotypic mean and variation

The average mean, range and coefficients of variation (CV) of morphological traits of Oat is

shown in Table 3. The mean performance of the landraces was in high range for PHT (65.87-37.67 cm), FLL (10.53-38.50), NT (2-10 numbers), NF (21.33-108.67 numbers), GFY (4.99-23.73 t/ha) and DMY (1.43-4.50 t/ha). Whereas, a moderate range of variation was observed for NL (5.00-7.33 numbers) and SD (4.33-7.33). The average green fodder yield of 24 landraces was 11.29±1.25 t/ha and average dry matter yield was 2.56±0.19 t/ha. This yield was contributed by morphological or yield attributing traits such average as plant height (93.56±4.36), average number of leaves (5.99±0.11), average flag leaf length (21.60±1.50), average flag leaf width (1.63±0.08), average number of tillers (6.83±0.41), average stem diameter (5.72±0.16) and average number of florets (45.40±4.31). The mean and standard error showed that landraces were diverse based on traits chosen for study. The variation in oat lines with respect to range, mean morphological and yield traits was higher in different landraces. This variation was an ideal to initiate the breeding programme. The genotypes expressing larger proportion of genetic variability (genotypic variance) for particular character or group of characters may be more amenable to selection. But, the presence of genetic variability alone does not imply which traits to be selected, so heritability was estimated to separate the proportion of heritable variation from total phenotypic variation which is transmissible to progeny.

Genetic variables assessment

Variability parameters were estimated and are presented in Table 3. High GCV and PCV was observed for NL (69.36% and 77.46%), FLW (33.18% and 34.21%), GFY (54.60% and 68.11%) and DMY (76.60% and 94.30%). Relatively moderate GCV and PCV were recorded for FLL (33.18% and 34.20%) and NF (45.52% and 46.54%). Low GCV and PCV was observed for PHT (22.87% and 23.10%), NT (29.52% and 29.73%) and SD (23.59 and 28.22). PCV was slightly invariably higher than GCV for all traits indicating a higher contribution of genetic constitution of plants to phenotypic expression and very little effect due to environmental variations. This can be further justified by the high values of h^2 for all the traits, which was higher than $h^2 = 0.70\%$ (heritability is high at >0.60). Hence, the genetic materials possess sufficient variability and high heritability for traits. We have observed trait specific diversity in oat landraces from the above parameter which will be useful for improving the selection efficacy and identification of desirable variants for breeding programme. Similarly, GA as a percent of mean provide the quantification of trait specific selection and improvement in landrace possible. Higher GA was observed for PHT (46.65%), FLL (70.37%), NT (60.37%), NF (95.78%) and GFY (90.17%).

Based on the mean performance of landraces, IG-02-127, IG-02-128, IG-02-121, IG-02-129, IG-02-113 were found superior for green fodder yield whereas, the landraces IG-02-122, IG-02-127, IG-02-121, IG-02-129, IG-02-125 and IG-02-128 produced high dry matter yield. However, IG-02-212, IG-02-122, IG-02-126, IG-02-121, IG-02-129 were found superior for higher number of florets and IG-02-126, IG-02-128, IG-02-121, IG-02-129 and IG-02-113 showed highest plant height.

Correlation studies

Pearson's correlation coefficients among the morphological parameters were calculated and presented in Table 4. Among morphological parameters, GFY and DMY were positively and significantly ($p \leq 0.05$) correlated with most of the traits except NL for GFY; NT for GFY and DMY both. Which implies that the traits in this study have direct relevance with yield traits. However, NT had very poor association with other traits. Besides, other morphological traits such as, PHT had significant correlation with FLL, FLW, SD, NF, GFY and DMY.

Table 3 — Range, mean, variance and coefficient of variation among quantitative traits measured in 24 Oat landraces

Variability parameter	PHT	NL	FLL	FLW	NT	SD	NF	GFY	DMY
Range	65.87 – 137.67	5.00 – 7.33	10.53 – 38.50	1.10 – 2.27	2.00 -10.00	4.33 – 7.33	21.33 – 108.67	4.99 – 23.73	1.43 – 4.50
Mean ± SE	93.56 ± 4.36	5.99 ± 0.11	21.60 ± 1.50	1.63 ± 0.08	6.83 ± 0.41	5.72 ± 0.16	45.40 ± 4.31	11.29 ± 1.25	2.56 ± 0.19
CV %	12.43	5.27	8.37	2.52	4.8	1.74	11.32	15.48	10.49
GCV	22.87	69.36	33.18	124.74	29.52	23.59	45.52	54.60	76.60
PCV	23.10	77.46	34.21	168.10	29.73	28.22	46.54	68.11	94.30
h^2	0.99	0.90	0.97	0.74	0.99	0.84	0.98	0.80	0.81
GA at 5%	46.65	2.33	70.376	7.501	60.366	13.487	95.788	90.173	29.262

Table 4 — Correlation coefficients among quantitatively measured traits in 24 Oat landraces

Traits	PHT	NL	FLL	FLW	NT	SD	NF	GFY	DMY
PHT	1.00	0.20	0.85**	0.71**	0.27	0.49*	0.60**	0.89**	0.66**
NL		1.00	-0.02	0.27	-0.36	0.08	0.37	0.28	0.45*
FLL			1.00	0.83**	0.36	0.49*	0.62**	0.88**	0.65**
FLW				1.00	0.10	0.50	0.65**	0.78**	0.58**
NT					1.00	0.26**	0.10	0.26	0.33
SD						1.00	0.58**	0.53*	0.42*
NF							1.00	0.66**	0.58**
GFY								1.00	0.86**
DMY									1.00

*Correlation is significant at the 0.05 level (1-tailed); **Correlation is significant at the 0.01 level (1-tailed)

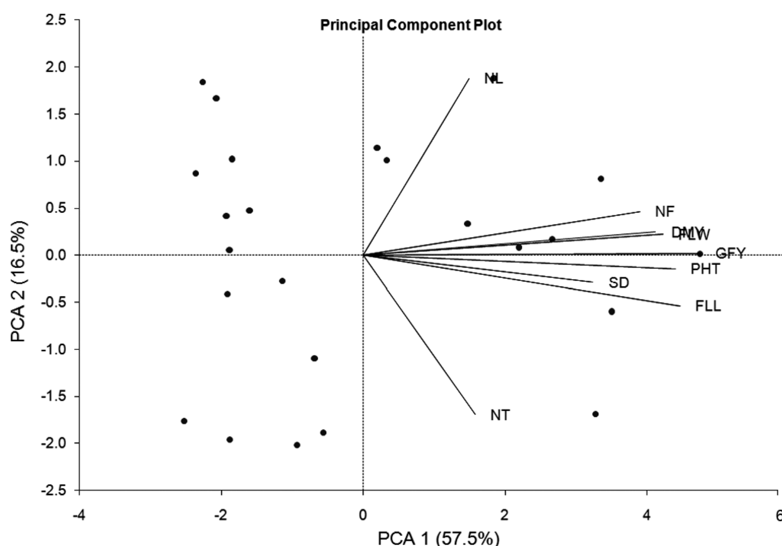

Fig. 1 — Biplot of different variables loaded on PC1 and PC2

The association with yield traits was perfect to estimate diversity among the landraces using the above traits.

PCA and cluster analysis

Principal component analysis (PCA) helps in identifying the most relevant characters that can be used as descriptors by explaining as much of total variation in the original set of variables. According to the Principal component analysis (PCA; Fig. 1), the first principal component (PC1), explained 57.5% of the total variance, contributed mainly by GFY, FLL, PHT, FLW and DMY. PC2 accounted for 16.5% variation through NL, NF and FLW, together PC1 and PC2 contributed 74% of the total variation. In all the principal components, NL resulted in highest positive values followed by FLW, NF, GFY and DMY, so these traits should be the maximum contributors towards genetic divergence. Hence, selection on the basis of these characters would be effective for yield improvement in oats. Dendrogram constructed on the basis of morphological traits using squared Euclidean distance and group average clustering method showed two clear groups (Fig. 2). Cluster-I comprises of 15 landraces while Cluster-II comprised of 9 landraces. Moreover, landraces of Cluster-II are more divergent as compared to landraces of Cluster-I. Thus, selection on the basis of landraces of different clusters would be effective for yield improvement in oats.

Molecular diversity in *Avena sativa*

One hundred and thirty-six SSR primer pairs with length of 18 to 25 bp were used in the SSR-PCR reaction. Out of these, 24 SSR primer pairs were polymorphic. Sixty-nine amplicons with size ranged from 70-480 bp were generated with an average of 2.87 amplicons per primer pair (Table 5). A high degree of molecular polymorphism was exhibited by

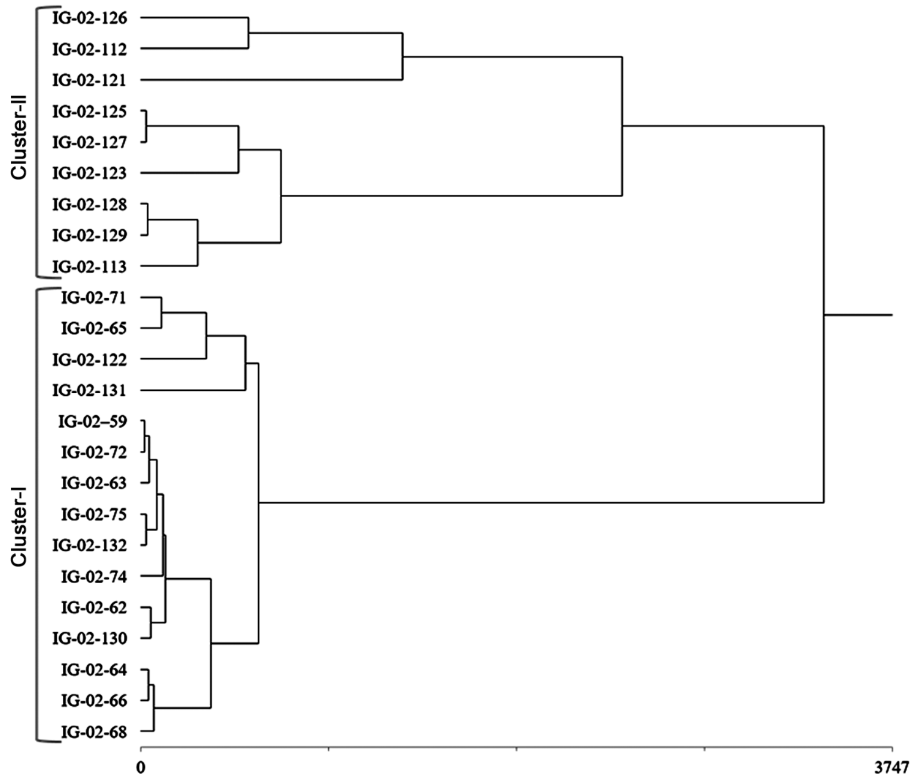

Fig. 2 — Dendrogram based on morphological traits constructed using squared Euclidean distance and group average clustering method

Table 5 — Amplified primers, number of PCR amplified bands, their product range, amplicons and PIC value obtained in SSR analysis of *Avena sativa* landraces

S. No.	Primer	Total bands	Polymorphic bands	Monomorphic bands	Polymorphism %	Amplified Product Range (bp)	Number of amplified fragments	PIC Value
1	SSR-162	2	2	0	100.00	220-240	27	0.49
2	SSR-170	3	3	0	100.00	120-200	44	0.48
3	SSR-177	2	2	0	100.00	180-200	22	0.50
4	SSR-178	5	5	0	100.00	160-230	48	0.48
5	SSR-179	2	2	0	100.00	180-200	21	0.49
6	SSR-180	3	3	0	100.00	160-200	46	0.46
7	SSR-184	2	2	0	100.00	200-220	25	0.50
8	SSR-190	2	2	0	100.00	280-300	24	0.50
9	SSR-196	2	2	0	100.00	110-180	36	0.38
10	SSR-206	6	6	0	100.00	70-190	56	0.48
11	SSR-207	4	4	0	100.00	120-190	25	0.39
12	SSR-215	3	3	0	100.00	230-480	50	0.42
13	SSR-216	3	2	1	66.67	210-270	48	0.44
14	SSR-232	2	1	1	50.00	400-480	45	0.12
15	SSR-239	2	1	1	50.00	220-240	40	0.28
16	SSR-245	3	2	1	66.67	190-330	67	0.13
17	SSR-254	3	1	2	33.33	260-300	71	0.03
18	SSR-257	4	3	1	75.00	200-300	54	0.49
19	SSR-262	3	3	0	100.00	250-290	33	0.50
20	SSR-263	2	2	0	100.00	250-270	24	0.50
21	SSR-264	4	4	0	100.00	230-450	46	0.50
22	SSR-276	3	3	0	100.00	250-340	46	0.46
23	SSR-278	2	2	0	100.00	210-250	28	0.49
24	SSR-292	2	2	0	100.00	120-150	24	0.50
Total (Average)		**69 (2.87)**	**62 (2.58)**	**7 (0.29)**			**950**	**0.42**

all the markers studied. The details of the SSR amplified products obtained from the 24 landraces of *Avena* is summarised in Table 6. Usefulness of markers is described mainly through the percentage of polymorphic fragments. A varying level of genetic polymorphism was revealed in the banding pattern across 24 *Avena sativa* landraces (Fig. 3). The pattern of polymorphism revealed by SSR marker showed high level of polymorphism, thus greater potential of this marker for analysis and discrimination of oat landraces (Sood et al. 2014). High levels of polymorphism clearly depict that the different landraces under study were genetically diverse.

Table 6 — Summary of SSR amplified products obtained from 24 landraces of *Avena sativa* examined in the study

Description	SSR
Total Number of primers used	136
Number of informative primers	115
Number of polymorphic primers	24
Total bands scored	69
Number of monomorphic bands	07
Number of polymorphic bands	62
Number of unique bands	02
Percentage of polymorphism	89.85
Average number of bands per primer	2.87
Maximum Polymorphism Information Content	0.50
Minimum Polymorphism Information Content	0.03
Maximum Similarity Coefficient	1.00
Minimum Similarity Coefficient	0.35
Total number of amplified fragments	950

The PIC value provides an estimate of the discriminatory power of a locus or loci, by taking into account not only the number of alleles that are expressed, but also relative frequencies of these alleles. Referring to PIC value recorded for all the informative SSR primers, the PIC varies from a minimum of 0.03 for SSR-254 and maximum of 0.50 for SSR-177, SSR-184, SSR-190, SSR-262, SSR-263, SSR-264 and SSR-292 with an average of 0.42. Similarly, the average PIC value of 0.52 was obtained by Sood et al. (2014) for evaluation of genetic diversity in oat landraces.

Comparative analysis of the SSR-PCR of 24 *Avena sativa* landraces showed the amplification of two specific bands which could be used as molecular markers to label and identify different oat landraces. Only SSR primer 206 and 207 was able to fingerprint landrace number IG-02-127 and IG-02-68 by giving 1 unique bands of sizes 120 and 190 bp respectively. Further, the similarity coefficient was as low as 0.35 to as high as 1.00 indicated substantial diversity present in the germplasm. The average genetic similarity coefficient was found to be 0.70. Maximum

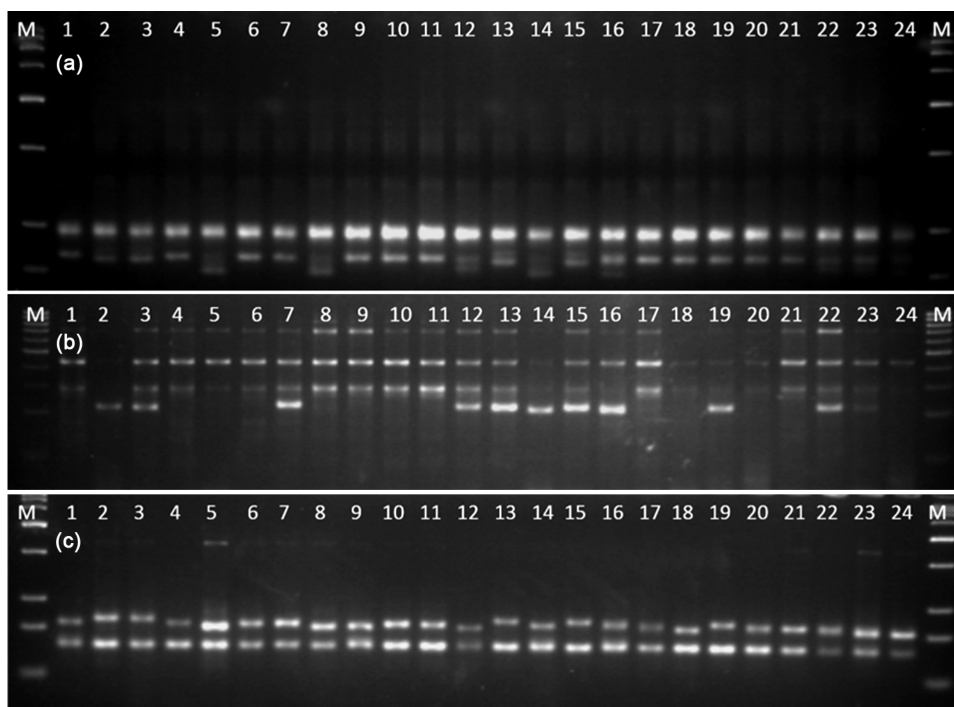

Fig. 3 — Gel showing DNA banding profiles using SSR marker SSR-257 (Fig. 3a), SSR-264 (Fig. 3b), and SSR-178 (Fig. 3c), respectively, where; M: 100 bp ladder, 1-24: *Avena sativa* landraces

similarity coefficient of 1.00 was observed between (IG-02-71) & (IG-02-72) and minimum of 0.35 was observed between IG-02-62 & IG-02-126.

Cluster analysis

The dendrogram obtained showed that the *Avena sativa* landraces were broadly divided into three major clusters comprising of 16 (Cluster I), 6 (Cluster II) and 2 (Cluster III) landraces respectively, truncated at similarity value of 0.48 (Fig. 4). Thus revealed 48% similarity with each other and 52% genetic diversity as a whole. First major cluster consisted of 2 subclusters Ia and Ib. Subcluster Ia and Ib comprised of 8 landraces each. Subcluster Ia comprised of all diverse landraces as compared to subcluster Ib which contains 100% (IG-02-71 and IG-02-72) as well as 96% (IG-02-130 and IG-02-131) similar landraces also. Second major cluster contained only 6 landraces. Out of these, 2 landraces (IG-02-59 and IG-02-64) were almost 90% similar, and rest were diverse from each other. Third major cluster contains only two landraces and these were more divergent then others.

Comparative results were also obtained by different workers as Grezeda (2004) and Drossou et al. (2004) observed lower RAPD polymorphism (30%) than amplified fragment length polymorphism (AFLP) (42.9%) in different oat landraces. Fu et al. [2004; 2007] in the analysis of genetic diversity of Canadian oat cultivars obtained 43% of polymorphism for AFLP and SSR marker systems. Boczkowska and Tarczyk (2013) in the studies of various Polish landraces of common oat (*Avena sativa* L.) obtained 59.3% of polymorphism for inter simple sequence repeat (ISSR) markers. However, higher level of polymorphism (50-100%) was reported by Ruwali et al. (2013) in selected fodder and dual purpose oat (*Avena sativa* L.) landraces. Higher level of polymorphism obtained in the present investigation is thus comparable with the genetic diversity studies of oat landraces.

Structure analysis

ΔK, which is used to determine the best fit value of K, was computed by STRUCTURE harvester for the given range, i.e., 1-10 and highest value was shown at K=2 (Fig. 5). Therefore, STRUCTURE analysis was

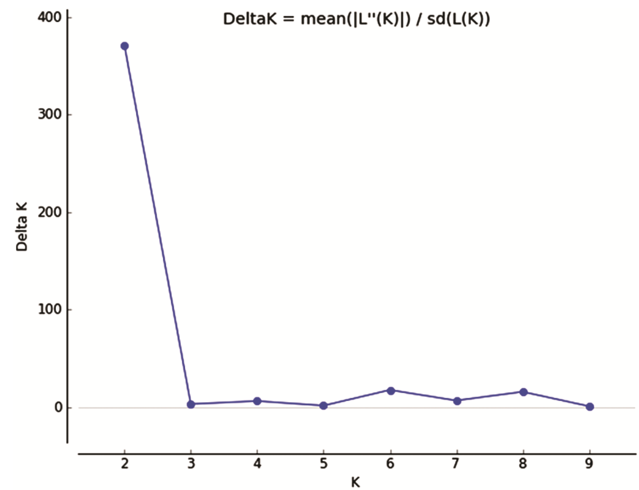

Fig. 5 — STRUCTURE inferences of *Avena sativa* populations based on SSR genotyping. Changes in the log likelihood, Δ(K), for different number of groups

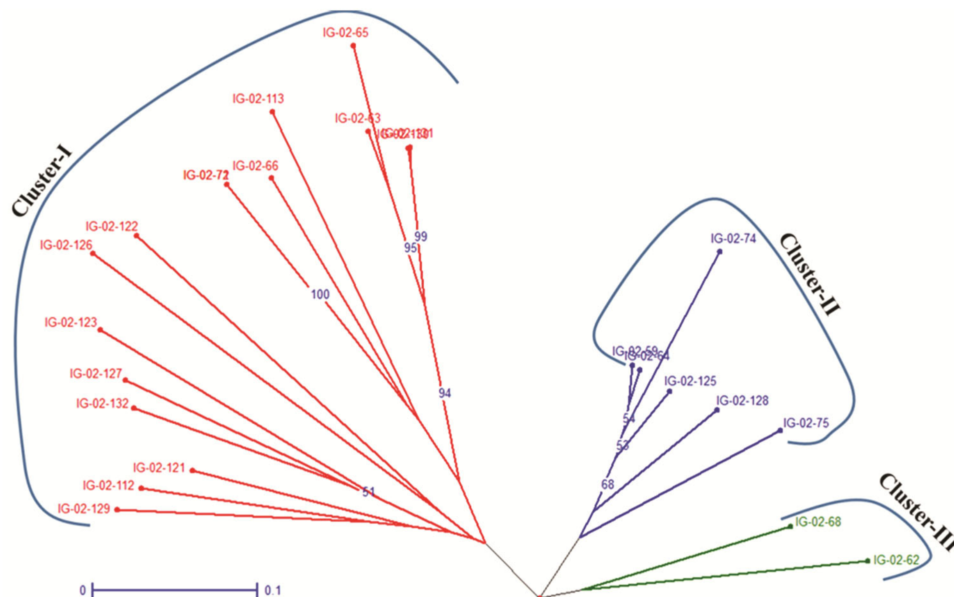

Fig. 4 — Radial neighbor-joining tree based on 69 alleles from 24 SSR loci among 24 oat landraces

conducted for K=2 (Fig. 6). Bayesian genetic structure and admixture analysis performed using software STRUCTURE assigned landraces into two genetic clusters, thus depicting the presence of 2 gene pools. In addition to assigning individuals to different clusters on the basis of allele frequencies, it also detected the extent of admixture within landraces. Further, the concept of two gene pools obtained from the present study is supported by Nikoloudakis et al., 2015 who also detected two gene pools for oat landraces. This means that oat landraces were belonged to two gene pools.

PCoA analysis

Principal coordinate analysis is used to explain genetic variation, show the variation pattern in a multi-dimensional pattern and do a better interpretation of the relationship between individuals. Clustering patterns in PCoA was in correspondence with clustering of both UPGMA tree and the STRUCTURE (Fig. 7). Principal

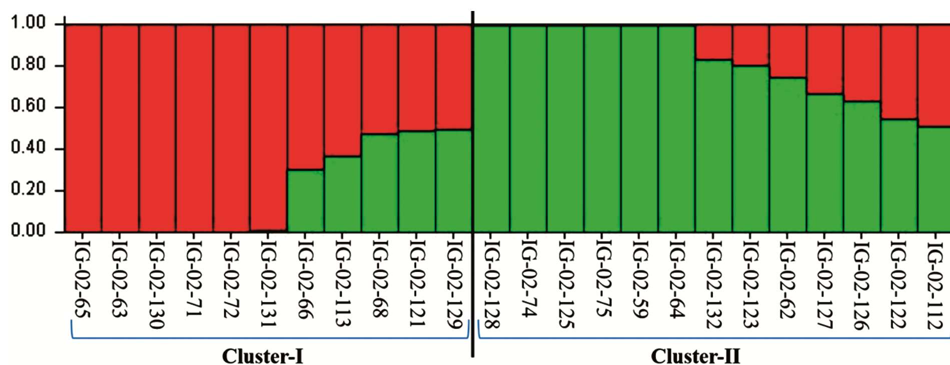

Fig. 6 — Genetic structure of 24 oat landraces as inferred by STRUCTURE v2.3.3 with 24 SSR markers data set

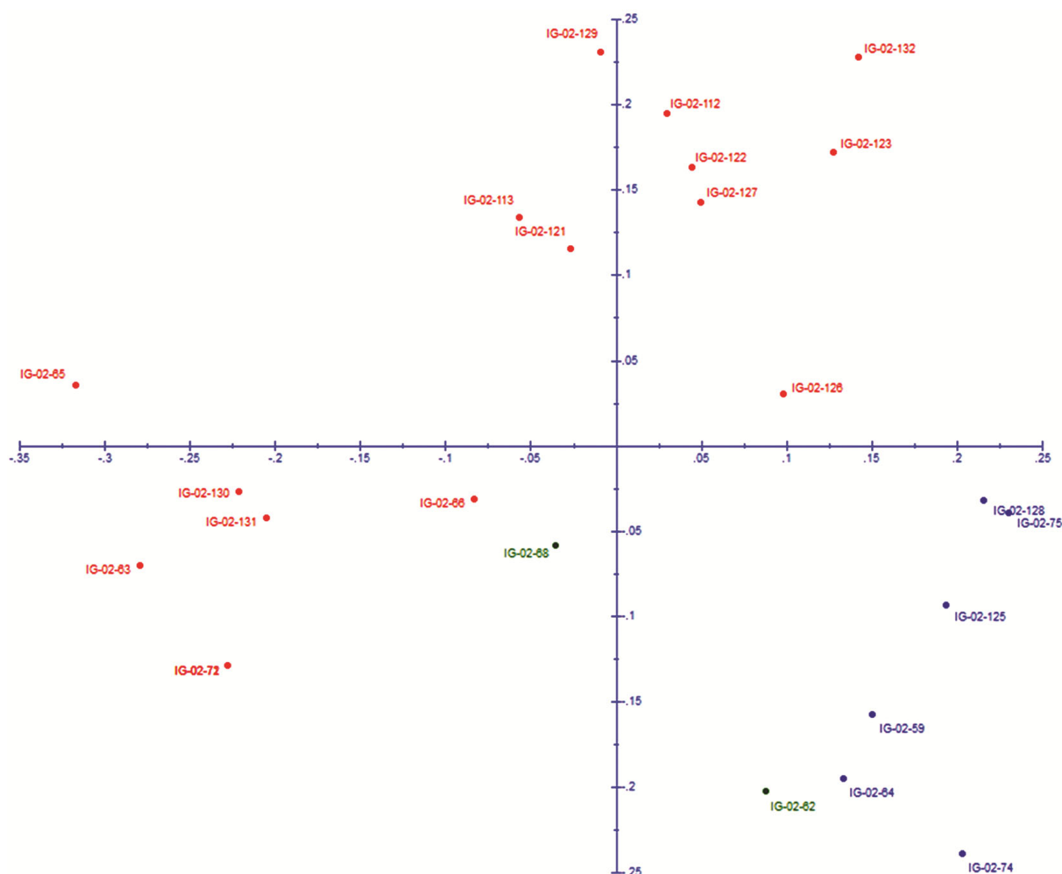

Fig. 7 — Principal coordinate analysis of the oat landraces using SSR marker data set

coordinate analysis as well as population structure have been shown to be good predictors of grouping patterns and they can be used to complement the clustering method analysis, since different combinations of genetic distance matrices and clustering algorithms can give rise to somewhat different groups (Reif et al., 2006). Clustering pattern based on SSR markers grouped the landraces into three main clusters. Clustering of population into three distinct groups represents the diversity between populations and indicates a significant influence of environment on genetic diversity. Clustering pattern of oat landraces based on SSR marker profiles were different from that of morphometric traits. So, based on the pooled analysis at morphological and molecular level, the landraces IG-02-121, IG-02-129 and IG-02-113 were found superior for morphological traits as well as most distant among all the landraces under study.

Based on these observations, it is obvious that SSR marker is more efficient and reliable marker system for genetic diversity analysis in oats. Effectiveness of SSR markers in genetic diversity analysis of *Avena* landraces has also been examined by several authors (Fu et al. 2007; Li et al. 2000, 2007; Nersting et al. 2006; Sood et al. 2014).

Utilization of genetics resources is possible if quantum of diversity and the genetic relationships of the collection are known, narrow genetic base in genetically interrelated (Fu et al., 2003). Thus, the need to investigate the genetic diversity among the germplasm collections is obvious. Investigations into nature and structure of genetic diversity gives a fair idea of relatedness as well as ability to identify germplasm sources having valuable genes for yield, quality and other important traits.

In conclusion, it was revealed from this comprehensive study that huge level of genetic diversity was present in *Avena* landraces. Further, the SSRs used in the present study can be recommended for further germplasm characterization projects. The results of this investigation showed that oat landraces have been essentially comprised of 2 gene pools. This study also gave us insight into the landraces which have been derived from pure gene pools. In brief, the results from the present study will be extremely beneficial in designing studies relating to oat phylogeny as well as formulating future breeding programs.

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