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Phenotypic and molecular divergence analysis of oat germplasm (*Avena sativa*) under Northern Indian conditions

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Oat (*Avena sativa* L.) is an important winter cereal forage crop and ranks sixth in world cereal production after wheat, rice, maize, barley and sorghum. It is capable of giving green fodder yield of 33.30-55.80 tonnes per ha under the single cut system, whereas it gives 40.80-41.60 tonnes per ha under multi-cut system. In Jammu and Kashmir, livestock is emerging as an important sector and plays a major role in the socio-economic development. Therefore, there is need to put concentrated efforts to evolve high yielding fodder oat varieties. Study of phenotypic traits makes it possible to estimate genetic parameters, while magnitude of estimated parameter defines the genetic properties of the population (Falconer 1989). Genetic diversity studies, assessed by various tools including DNA markers, provide important information both for genetic conservation and for use in efficiently breeding new commercial varieties. In the present study an attempt has been made to assess genetic divergence employing both phenotypic as well as molecular markers so as to identify diverse oat genotypes for their potential use in hybridization programme.

Material for the present study comprised of 28 diverse germplasm lines of oat collected from different centres of the country. These germplasm lines were evaluated in RBD design during *rabi* 2018-19 at SKUAST research farm, Jammu in three replications with a plot size of 2 m^2 for morpho-physiological yield and quality traits. Recommended agronomic practices were followed to raise the oat field experiment. The statistical analysis was carried out by using computer software INDOSTAT 9.2.The data collected on individual characters were subjected to analysis of variance to test the genotypic differences among the genotypes commonly applicable to the randomized block design as per method suggested by Panse and Sukhatme (1985). Phenotypic genetic divergence (D^2 statistics) based on morphological traits was carried out by using Tocher method (Rao 1952) for grouping the genotypes into different clusters. Molecular divergence of oat genotypes was carried out by using ten SSR markers to validate the results of phenotypic genetic divergence (Table 1). Genomic DNA was isolated, purified and amplified *in vitro* through Polymerase chain reaction (PCR) using SSR primers following standard protocols given by Oliver *et al*. (2011).

Analysis of variance for all characters indicated significant differences among the genotypes revealing presence of sufficient variability among them. The presence of considerable variability might be due to diverse genetic origin and different geographic background, which is well reflected from their originating centre and pedigree backgrounds. These results are in confirmation with the results reported by many workers (Ahmed *et al.* 2013, Khan *et al.* 2014, Jaipal and Shekhawat 2016, Bind *et al.* 2016, Kakad *et al.* 2017, Sahu and Tiwari 2020). Precise information about genetic divergence is critical for a productive breeding programme, as genetically diverse parents are known to produce high heterotic effects. The twenty-eight genotypes were grouped into 6 clusters in such a way that the genotypes within the cluster had smaller intra cluster distance than the inter cluster distance belonging to different clusters.

The cluster I was the largest cluster consisting of 19 genotypes followed by cluster III having 3 genotypes, cluster V and cluster VI having 2 genotypes each, cluster II and cluster IV had only 1 genotype. Similar results were also reported by Singh and Singh (2011), Ahmed *et al.* (2011), Jaipal and Shekhawat (2016), Kaur *et al.* (2018) in their respective studies. The pattern of group constellations proved that geographical diversity was not an essential factor to group the genotypes from a particular source or origin into one particular cluster. Cluster means for different morphological, maturity and forage related traits revealed that substantial variability existed for all the characters. The highest cluster mean for green fodder yield (354 q/ha)

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Table 1 List of SSR primers, sequence information and their attributes used in the study

Marker	Repeat Motif	Forward primer (5' to 3')	Reverse primer (5' to 3')	Tm (°C)	Size range (bp)	No. of alleles scored per locus	Polymorphism percentage (%)	PIC value
AM03	(AG) 39	GGTTGCATCCTGAACCTATGGC	GTACGTGTATCCCCGTGCTCTA	55	138-190	4	75	0.48
AM04	(AG) 37	GGCGATAACTTCCTACCCACTC	GGATAGGAGGCCATCGGTTTCT	57	129-170	4	25	0.59
AM7	AG	GTGAGCGCCGAATACATA	TTGGCTAGCTGCTTGAAAACT	48	140-180	3	33.3	0.46
AM8	AG	CAAGGCATGGAAAGAAGTAAGAT	TCGAAGCAACAAATGGTCACAC	47	170-190	3	50	0.35
AM9	AG	CAAAGCATTGGGCCCCTTGT	GGCTTTGGGACCTCCTTTCC	48	125-150	3	33.3	0.19
AM10	AG	AAAATCGGGGAAAGGAAAACC	GAAGGCAAAAATACATGGAGTCAC	46	255-270	4	25	0.36
AM11	(AG)(AAAG)	TCGTGGCAGAGAATCAAAGACAC	TGGGTGGAGGCAAAAAACAAAAC	49	150-160	2	50	0.068
AM12	AG	TGCTGAAGTGAACAATCGC	CCTTCTCCAACAACTCTAC	44	210-235	4	66.3	0.25
CWM27	A	AGCATCCTCGCATTTCTTGTA	GCAGCCGCTTTTGATTCTA	55	150-160	3	33.3	0.48
CWM29	AAC	CGCATGCAAGCTCACAAGT	GCTGAGGCTGCTGGTAGGAGAC	55	140-150	5	40	0.36
Average						3.5	43.12	0.356

was exhibited by cluster II (OS 6).The estimates of intra and inter cluster distance for six clusters. The highest intra cluster distance was found in cluster III (113.1), followed by cluster VI (99.9), cluster V (77.1) and cluster I (63.4). The inter cluster distance observed 110.2 (between cluster II and cluster IV) to maximum of 15853530.0 (between cluster III and cluster VI) followed by cluster III. So crossing between lines belonging to these clusters may result into high heterosis, which could be exploited in oat improvement, as the crossing among the pure lines leads to formation of different transgressive segregants which are utilized to study heterotic effects.

The genotypes OS 6 in cluster II exhibited desirable plant height (115.63 cm), number of tillers per plant (13.07), dry matter yield (1.41 kg/plot), leaf width (1.77 cm), number of leaves per plant (83.17), leaf area index (16.13), days to 50% flowering (96), days to maturity (137) and green fodder yield (7.08 kg/plot) based on *per se* performance of these characters. OS 403, belonging to cluster I was found to have highest leaf length (48.01 cm) based on *per se* performance. SKO 98 was found to be having highest value for crude protein (18.49 %) and leaf stem ratio (0.55) based on *per se* performance. JHO 88-2 was found to be having highest stem girth (2.37 cm). The choice of parents for further breeding programme can be made by selecting individuals from the maximum divergent cluster such as cluster III and cluster VI, clusters have single genotypes II and IV and genotypes having better *per se* performance in the present study, which would exert high heterosis and wide variability in genetic architecture in subsequent generations. The JHO 88-2, SKO-90, SKO 98, Local-1 and OS 6 belonging to the distant clusters and having high mean performance could be used in hybridization program for obtaining a wide spectrum of variation among the segregants.

All 28 oat genotypes representing different geographical origin and morphological diversity as detected from divergence analysis were further evaluated for genetic diversity at molecular level using polymorphism generated by SSR primers. A set of 10 SSR primer pairs belonging to AM and CWM series were selected from the published source to assess the genetic diversity among the 28 oat genotypes. The AM primer sequences and CWM series markers were graciously provided by the published article of Wu *et al*. (2012), Zhang *et al.* (2012), Li *et al.* (2000), Kapoor and Choudhary (2017) respectively. DARwin 5.0 software was used to generate the dendrogram. All the genotypes were grouped into five clusters of different sizes. The genotypes of clusters may be used for the development of suitable mapping population for the identification and mapping of quantitative trait loci (QTL). A total of 35 alleles were detected by 10 primers in the 28 oat genotypes with an average of 3.5 alleles per primer. Total number of alleles amplified for each primer ranged from 2-5. The maximum number of alleles (5) was amplified by CWM29. The amplicon size ranged from 125 to 270 bp. The lower average number of alleles amplified per SSR marker in the present study is due to use of local *Avena sativa* genotypes, where no ploidy changes were there.

The PIC values provide an estimate of the prejudiced power of a locus by taking into account the number of alleles that are expressed as well as the relative frequencies of the alleles in question. PIC values ranged from 0.068 to 0.59 with an average of 0.35. The highest PIC value was recorded by AM04 (0.59), followed by AM03 (0.48) and CWM27 (0.48). Polymorphism percentage ranged from 25-75% with an average of 43.12%. The highest polymorphism percentage was recorded for AM03 (75%), followed by AM12 (66.3%).Similarly, the average PIC value of 0.35 and 0.45 was obtained by Fu *et al.* (2007) and Kapoor and Choudhary (2017) respectively, for evaluation of genetic diversity in oat genotypes. The most informative primer was AM04 since it gave a PIC value greater than 0.5. Effectiveness of SSR markers in genetic diversity analysis of *Avena* genotypes has also been examined by several authors (Li *et al*. 2000, 2007, Nersting *et al.* 2006, Fu *et al.* 2007, Sood *et al.* 2014, Rana *et al.* 2019).

From the dissimilarity and similarity coefficient it can be concluded that the genotype JHO 88-2, SKO-90, SKO 98, JHO-99-2 and SKO-188 were genetically very dissimilar to each other since the genomic dissimilarity value for them was high based on molecular marker (SSR) data analysis. This marks a possibility that the SSR markers used in the study may be unlinked to the genomic region in these genotypes. Association between morphological and molecular diversity analysis revealing similarities between clustering patterns of both studies. Based on association between morphological and molecular diversity analysis genotypes JHO 88-2, SKO-90 and SKO 98 are more diverse genetically.

SUMMARY

The present study was undertaken to assess the genetic diversity among 28 oat (*Avena sativa* L.) germplasm lines through both phenotypic and molecular approach during *rabi* 2018-19 in RBD design. D^2 analysis grouped all 28 genotypes into six clusters. The mean for green fodder yield, dry matter yield and number of leaves per plant was highest in cluster number II. The inter-cluster distance was highest between VI and III cluster pair suggesting significant high genetic diversity among genotypes of these clusters. On the basis of mean performance and genetic distance of different characters, genotypes JHO 88-2 – OS 6 for green fodder yield and OS 403 – OS 346 for dry matter yield were found to be diverse. Crude protein, leaf stem ratio and days to maturity contributed maximum towards variability which served as source characters for genetic divergence. The molecular diversity analysis using ten SSR markers clustered genotypes into five clusters and significant level of dissimilarity was depicted among them. PIC values ranged from as low as 0.19 to as high as 0.59 (AM04). Genetic divergence revealed JHO 88-2, SKO 90 and SKO 98 to be most diverse at both morphological and molecular level. These genotypes can further be utilized for oat hybridization programme.

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