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Original scientific article

MOLECULAR CHARACTERIZATION OF OATS (*AVENA SATIVA* L.) DIVERSITY: IMPLICATIONS FOR DUAL-PURPOSE BREEDING

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Oat (*Avena sativa* L.) is one of the world's healthiest and gluten-free grains that are packed with essential vitamins, minerals, fiber, and antioxidants. It is also one of the most important cereal fodder crops. The present study was conducted from 2016 to 2018 and morphological and molecular diversity was analyzed for dual-purpose oat based on ten fodder and eight grain traits among 96 oats accessions including four wild accessions (*A. vavilioviana, Guiena oats, A. maroccana and A. sterilis)* collected from various eco-geographical regions of India. Thirty-one out of one hundred seventy (18%) simple sequence repeats (SSR) markers detected polymorphism among the 96 oat accessions with average polymorphic information content (PIC) of 0.47. A total of 100 alleles were detected with an average of 3.2 alleles per primer. The molecular diversity analysis grouped all the 96 germplasm lines into two major clusters, 'A' and 'B'. The similarity coefficients ranged from 0.37 to 1. The genotypic pairs *viz;* UPO 276: SKO 315 (46%); SKO 314: OL-125 (46%); SKO 314: OS 363 (49%); SKO 314: UPO 032 (49%) exhibited least genetic similarity and these pairs can be potentially used as parents to conduct various mapping studies and further contributing to the oat breeding community. Moreover, 6 accessions (JHO-2001-1, JHO-99-2, OL 1635, SKO 27, UPO 093 and OS329) had been identified which were superior to OL-10 (best check in the northwest India) for fodder as well as grain yield. This study showed the opportunity of utilizing SSR markers with morphological characteristics to breed for dual purpose oats.

Keywords: Breeding, cluster analysis, dual-purpose, genetic diversity, and simple sequence repeats

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INTRODUCTION

Oat is an important cereal grown in different regions of the world. The common oat (*A. sativa* L.) is allohexaploid (2n = 6x = 42, AACCDD sub-genomes) and belongs to the *Poaceae* family. It ranks sixth in cereal production worldwide after wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), and sorghum (*Sorghum bicolor* L.) (AMANDEEP and KAPOOR, 2022; GUDI *et al*., 2022; SINGH *et al*., 2024). Oats are grown on 10.18 million hectares around the world, with a production of 25.56 million metric tons per year (USDA, 2021). Oat is the most important multipurpose winter season crop grown for grain, pasture, and forage under irrigated conditions. Since long, oats were mainly utilized as a livestock feed because of its outstanding growth, regeneration potential after first cut, and better palatability and high concentrations of proteins, and minerals (HILLI *et al*., 2023; SINGH *et al*., 2021). It produces a high fodder production per unit area and time with minimal irrigation, and its multi-cut nature assures a consistent supply of fodder over a long period of time (HEDAYETULLAH and ZAMAN, 2018; SINGH *et al.*, 2022).

Oats have gained a lot of attention as a "Super-grain" because of its excellent grain and nutritional quality along with high dietary fiber (POONIA *et al*., 2022). Oats are used as an affluent source of trace nutrients which are required by humans and have been reported to be an effective food of metabolic regulation (KAPOOR *et al*., 2022). It contains high quantity and quality of proteins (17%) compared to other widely consumed cereal grains (KAPOOR *et al*., 2022) and have positive effects on human health due to β-glucans These polysaccharides (β-glucans) have a beneficial effect on human health, as they can lower food glycemic index (LAFIANDRA *et al*., 2014), reduce blood cholesterol (KARMALLY *et al*., 2005), including low-density cholesterol (DAVY *et al*., 2002), improve liver function, and prevent excess body weight (EL KHOURY *et al*., 2012). For oats to be considered as a dual-purpose, it should have higher green fodder yield than best check variety and its grain yield should not be less than 10% of check variety (FCU, 2020). The first cut is taken for fodder at 70 days after sowing and afterwards, the crop is harvested at the time of grain maturity (AMANDEEP *et al*., 2021; KAUR and KAPOOR, 2017).

Molecular markers are valuable genomic tools that are used in characterization of genetic diversity, and it overcomes the limitations of morphological and agronomical descriptors, including low polymorphism and heritability, late expression, and vulnerability to environmental influences (SINGH *et al*., 2022). In recent past, simple sequence repeats (SSR) are preferred in crop genetics, breeding, and genomics, including genetic diversity, since they are PCR-based, hypervariable, co-dominant, robust, chromosome specific, and multi-allelic in nature (KUMAR *et al*., 2008; GUDI *et al*., 2020; 2024). SSR have emerged as marker of choice for various genetic diversity studies in oats (KAPOOR and CHOUDHARY, 2017; KAUR *et al*., 2021; NIKOLOUDAKIS *et al*., 2016; RANA *et al*., 2019; SOOD *et al.*, 2016).

Due to the rapidly growing human and livestock populations, as well as the limited availability of land resources for fodder, the forage breeders must develop multipurpose varieties that are acceptable in different cropping systems. To develop dual-purpose oats, high fodder and grain yields are required. The present study was conducted to estimate the genetic diversity in oats germplasm for dual purpose oats based on morpho-agronomic traits and SSR markers for cataloguing the germplasm lines into different groups to identify desirable dual-purpose oats

accessions, which could further assist the overall dual-purpose oats cultivar development in India.

MATERIAL AND METHODS

Plant material and experimental design

The experimental material consists of 96 oats accessions including four wild accessions (*A. vavilioviana, Guiena oats, A. maroccana and A. sterilis)* belonging to diverse eco-geographic regions of the India. The oat germplasm used in the present investigation along with its geographic region is given in Table S1. The germplasm was procured from State Agricultural Universities all over the India. The exotic germplasm lines or Exotic collection (EC) were procured from The National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India. The germplasm was evaluated in augmented block design for dual purpose. The material was planted along with three checks *i.e*., Kent (national check), OL-10 (state check), and OL-125 (zonal check) in single row of 2m with row-row spacing 30cm at the experimental area of Forage Research Farm, Punjab Agricultural University, Ludhiana, Punjab, India.

Morphological assessment

Ten fodder traits and eight grain traits were considered to evaluate diversity for morphological characters in 96 oat germplasm lines for dual purpose. Five random plants from each entry were used to record observations for 10 fodder traits *viz*: Plant height (PH-cm), Leaf length (LL-cm), Leaf width (LW-cm), number of tillers per plant (NOT/P), Green fodder yield (GFY-q/ha), Dry matter yield (DMY-q/ha), Acid detergent fibre (ADF%), Neutral detergent fibre (NDF%), In-vitro dry matter digestibility percentage (IVDMD%), Crude protein (CP%) and eight grain yield and quality related traits viz: Panicle length (PL-cm), Grain length (GL-mm), Grain width (GW-mm), Thousand grain weight (1000 GW-gm), Spikelet no. per panicle (SNPP), Grain no. per panicle (GNPP), beta-glucan (β-G%), Grain yield (GY-q/ha). IVDMD was analyzed according to the method of HOLDEN (1999). ADF and NDF were analyzed by method given by GEORING and VAN SOET (1970). CP was estimated by microkjeldhal method (AOAC, 1990) and β-G was analyzed by method given by WOOD *et al*. (1977).

Genomic DNA extraction and PCR amplification

Total genomic DNA of 96 germplasm lines was extracted using Cetyl Trimethyl Ammonium Bromide (MURRAY and THOMPSON, 1980). The DNA quality and quantity was accessed by 0.8% agarose gel electrophoresis. A total of 170 SSR markers belonged to AM (*Avena sativa* Microsatellite), cAM series were selected from the published data (JANNINK and GARDNER, 2005; OLIVER *et al*., 2010) and were tested on 96 oat germplasm lines. AM and cAM series were provided by LI *et al*. (2000). The stock and final concentration of different components used in PCR are given in Table S2. PCR amplification was performed using temperature profile mentioned in Table S3.

For preparing the PAGE gel solution, 22.5 ml of 40% acrylamide: bisacrylamide solution (19:1) and 7.5 ml of TBE (10X) were mixed. Then, 120 µL TEMED and 0.5 % ammonium persulfate (APS) were added to 100 ml distilled water and were mixed with acrylamide: bisacrylamide solution. The solution was gently poured in the gap between the plates and the gel was left for about one hour for proper solidification. The PAGE apparatus consists of two chambers for electrophoresis buffer:

upper chamber and lower chamber. Both the chambers of PAGE apparatus were filled with about one litre 0.5X TBE buffer and 60-80 µl of ethidium bromide was added. After solidification the PAGE gel was given a pre-run (without loading the samples) for 2-3 hours so that the ethidium bromide is uniformly imbibed by the gel to get visible bands of DNA. About 10µL of sample was loaded in 6% polyacrylamide gel and was resolved by running the gel at 250V for 5-6 hours. The gels were visualized under UV light and photographed using photo gel documentation system (ALPHAIMAGER HP, ALPHA INNOTECH).

Statistical analysis

Statistical analysis of morphological traits including analysis of variance (ANOVA), correlation analysis, and principal component analysis (PCA) was done by using the R software version 4.1.2 (Team, 2013) with agricolae packages (MENDIBURU, 2020). PCA analysis was done by using 'PCA tools' package (BLIGHE and LUN, 2020). Box plot analysis was done by SPSSv20.0 software (SPSS IIBM, 2011). ANOVA for an augmented block design used in the present study was done by R package 'augmented RCBD' (ARAVIND *et al*., 2021) and is given in Table S4. Correlation analysis was done by using the 'correlation package' (MAKOWSKI *et al*., 2020). Pearson's correlation coefficient formula used is as follows,

$$r = n(\Sigma xy) - (\Sigma x)(\Sigma y)/\sqrt{[n\Sigma x^2 - (\Sigma x)^2]\,[n\Sigma y^2 - (\Sigma y)^2]}$$

where, r= Pearson coefficient
n=number of pairs of variables
Σx=Sum of the 1st variable value
Σy=Sum of the 2nd variable value
Σxy=Sum of the product of 1st & 2nd variable
Σx^2=Sum of the squares of the 1st variable
Σy^2=Sum of the squares of the 2nd variable

Genetic clustering

The genetic diversity among the oat germplasm lines was calculated using NTSYS-PC, version 2.1 (ROHLF, 1998). Similarity matrices of 96 oats accessions were generated by using SIMQUAL format of NTSys software. The dendrogram was made based on genetic distance matrix by using the UPGMA (unweighted pair group method with arithmetic mean) to show phenetic representation of the genetic relationship among 96 oat germplasm lines. PIC was estimated using the following equation of BOTSTEIN *et al*., (1980).

$$PIC = 1- \sum_{i=1}^{n}(P_{ij})^2 - \{\sum_{i=1}^{n}(P_{ij})^2\}^2 + \sum_{i=1}^{n}\{(P_{ij})^2\}^2$$

Where P_{ij} is the frequency of j^{th} allele in i^{th} primer and summation extends over 'n' patterns.
We also calculated effective number of alleles (Ne) using following formula:

$$Ne = \frac{1}{\sum_{i=1}^{n}(1-PIC_i)}$$

Where, Where, Ne is the effective number of alleles.

- n is the total number of markers.
- PICi is the PIC value of the i^{th} marker.

RESULTS AND DISCUSSION

Morphological assessment

The experimental material containing 96 genotypes comprising three checks was sown in augmented block design for dual purpose and data recorded from morpho-agronomic traits was used for the analyses of variances (Table 1). The mean sum of square among test genotypes was found significant for NOT, GFY, DMY, CP, IVDMD, GW, 1000GW, GY, and SNPP, indicating that the significant differences were present between the genotypes for these traits. To examine the variation, present among the checks (controls), and test genotypes *vs* checks, the experimental material was analyzed by contrast analysis. Significant differences were present between the three checks for LW, NOT, ADF, NDF, CP, β-G, PL, and GY. Significant differences were also present among checks vs test genotypes for NOT, ADF and NDF. Frequency distribution using box plots for the 96 genotypes for different morphological traits are shown in Figure 1.

Table 1. ANOVA for fodder and grain traits in 96 oat germplasm lines.

Source	Among test genotypes	Among control	Test-vs control	CV
PH (cm)	54.02	133.48	1622.51	1.8
LL(cm)	63.47	20.21	244.36	2.3
LW(cm)	0.43	0.11*	1.53	8.5
NOT	17.24**	37.12**	41.68**	13.6
ADF%	19.16	6.64**	6.73**	2.2
NDF%	22.66	3.17*	3.10*	1.9
GFY(kg/plot)	11961*	20732	242201	2.8
DMY(kg/plot)	447.5*	1418.1	6822.7	3.2
CP%	2.22*	2.31**	0.027	4.9
IVDMD%	1.86**	8.49	41.63	1.2
β-G%	0.28	0.20*	1.8	6.1
PL (cm)	19.59	8.44**	1481.16	3.5
GL (mm)	2.96	0.79	11.2	5.9
GW (mm)	0.59*	0.01	1.71	12.9
1000 GW (gm)	49.38*	406.24	15.18	2.4
GY (g/plot)	18.79*	4.02*	96.74	6.3
SNPP	22653.0**	15157	6595.5	18
GNPP	1243.59	2996.7	3121.37	3.1

PH=plant height, LL=leaf length, LW=leaf width, NOT=number of tillers per plant, GFY=green fodder yield, DMY=dry matter yield, β-G%=beta glucan, PL=panicle length, GL=grain length, GW=grain weight, 1000GW=1000 grain weight, GY=grain yield, SNPP=spikelet number per panicle, GNPP=grain number per panicle, CV=coefficient of variation *=significant at 5% level of significance, **=significant at 1% level of significance

The major cause for variation within the different germplasm accessions for different traits is that all the accessions were of diverse origin and they performed differently when subjected to the same environment. Plant breeding programs based on improvement of the traits related to forage and grain yield requires correlation studies to understand relationship among these traits (AHMAD *et al*., 2013). Analysis of variance revealed that enough variability was present among the genotypes for characters studied.

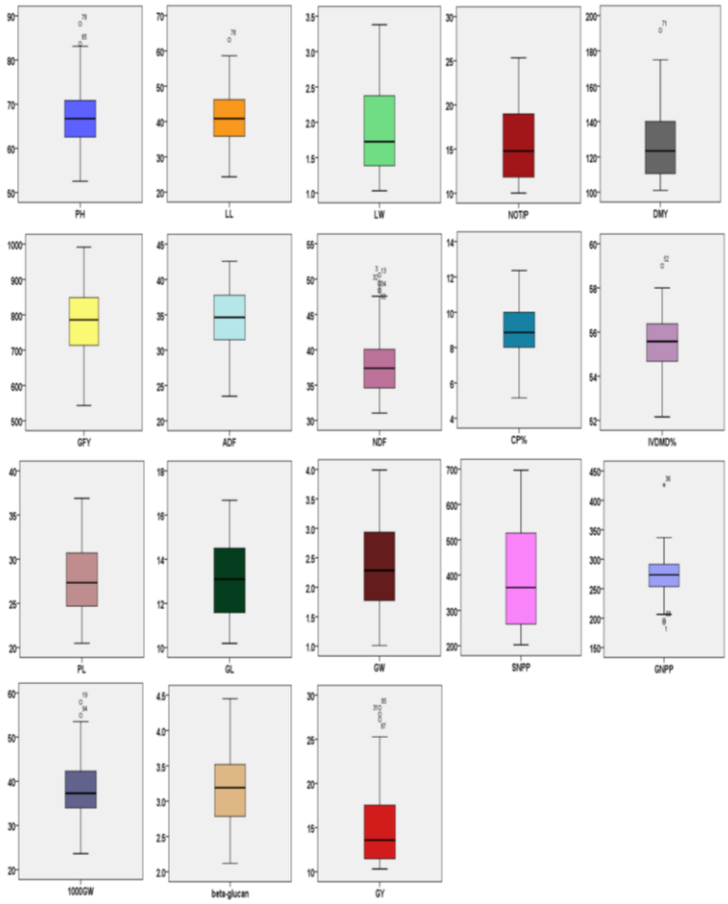

Fig 1. Box plots results for 10 fodder and 8 grain related traits in 96 germplasm accessions of oats.
(Left to right-Plant height (PH-cm), Leaf length (LL-cm), Leaf width (LW-cm), number of tillers per plant (NOT/P), Dry matter yield (DMY-q/ha), Green fodder yield (GFY-q/ha), Acid detergent fibre (ADF % , Neutral detergent fibre (NDF%), In-vitro dry matter digestibility percentage (IVDMD%), Crude protein (CP%), Panicle length(PL-cm), Grain length (GL-mm), Grain width (GW-mm), Thousand grain weight (1000 GW-gm), Spikelet no. per panicle (SNPP), Grain no. per panicle (GNPP), beta-glucan (β-G%), Grain yield (GY-q/ha).

Correlation analysis

Correlations between 18 traits are shown in Figure 2. Positive correlations are shown in blue circles, whereas negative correlations are shown in red circles. The size and color of the circle are proportionate to the correlation coefficients. The legend color on the right side of the correlogram displays the correlation coefficients and their corresponding colors. A significant and positive correlation was observed between GFY and DMY with r=0.900; followed by r=0.868, between PH and LL; and, r=0.566, between PL and SNPP.

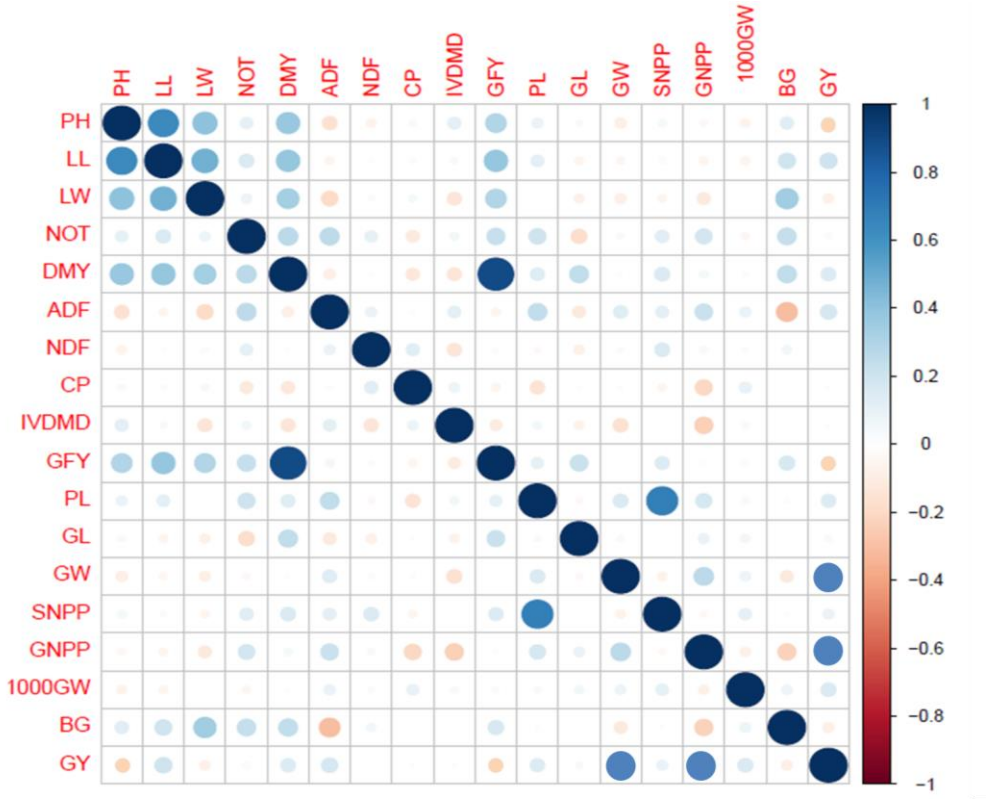

Fig 2. Correlation analysis among all eighteen traits.

Plant height (PH-cm), Leaf length (LL-cm), Leaf width (LW-cm), number of tillers per plant (NOT), Dry matter yield (DMY-q/ha), Green fodder yield (GFY-q/ha), Acid detergent fibre (ADF %) , Neutral detergent fibre (NDF%), In-vitro dry matter digestibility percentage (IVDMD%), Crude protein (CP%), Panicle length(PL-cm), Grain length (GL-mm), Grain width (GW-mm), Thousand grain weight (1000 GW-gm), Spikelet no. per panicle (SNPP), Grain no. per panicle (GNPP), beta-glucan (BG%), Grain yield (GY-q/ha).

Results indicated that GFY had positive and highly significant correlation with many yield contributing traits viz., PH (0.298), LL (0.383), LW (0.297), NOT/P (0.228) and DMY (0.900). DMY also had a significant positive correlation with PH (0.377), LL (0.383), LW

(0.330), NOT/P (0.263). Similarly, grain yield was significantly and positively correlated with GW (0.217) and GNPP (0.266). PL was significantly correlated to SNPP (0.566). The correlation coefficients result also showed that GFY had a significant negative correlation with GY (-0.113). The most important grain quality trait, beta-glucan manifested positive and significant correlation with PH, LL, LW, NOT, DMY and GFY. But it had a highly significantly negative correlation with ADF (-0.301), GNPP (-0.204) and GW (-0.141). Another important fodder quality parameters viz; ADF and were found to be significantly and positively correlated to GNPP, NOT, PL and GY. IVDMD was negatively correlated with GNPP (-230). CP had a negative and significant correlation with PL, GNPP, NOT and DMY. The results are in accordance with earlier findings of AHMAD *et al*. (2013), KAPOOR (2018) and POONIA *et al*. (2017). The selection-based PH, LW, NOT/P and DMY will result in improving the fodder yield in oat. Similarly, GY was significantly and positively correlated with PL (0.143), GW (0.217) and GNPP (0.266). Also, PL was highly significantly correlated to SNPP (0.566) So, the bigger the panicle length and greater the grain number per panicle, spikelet number per panicle and also the grain width will consequently lead to the higher grain yield. Our data is consistent with the findings of other authors (KAZIU *et al*., 2019; SURJE and DE, 2014). Consequently, selection of traits that are significantly and positively associated with grain yield will be effective in improving oat grain yield. GY was significantly and negatively correlated with PH (-0.115). In previous works, BUERSTMAYR *et al*. (2007) and DUMLUPINAR (2012) also determined significant and negative correlations between PH and GY in oat and reported that PH and lodging were positively correlated. So, lodging due to higher PH causes reduction in GY. The results also showed that GFY had a significant negative correlation with GY (-0.113). SAIT and RAMZAN (2020) found significant positive correlation between GFY and GY (-0.506). So, a direct selection for traits like PH, LL, LW, DMY, NOT/P and GFY will help in improvement of fodder yield, whereas for grain yield traits like PL, SNPP, GNPP and GW will prove effective while going for breeding oats for yield components.

Principal component analysis

In the PCA for fodder traits, a total of ten principal components (PCs) were extracted (Fig. 3a) and it revealed that the first three principal components have eigen values more than one i.e. 2.26, 1.84 and 1.31 and the amount of variation explained by these PCs was 22.61%, 18.67% and 13.11% respectively which constitutes about 54.39% of the total variation among all the 96 germplasm lines (Table S5). As per the PCA-biplot (Fig. 3c) and factor loading of different characters (Table S5), the first principal factor was loaded on plant height, leaf length and dry matter yield which indicate that this component is the weighted average of the characters, which determines the green fodder yield. These traits had the major role in divergence and responsible for major portion of its variability. Second principal factor has positive loading for acid detergent fiber, dry matter yield and number of tillers. The third principal factor was characterized by the higher loading values of leaf width, green fodder yield and neutral detergent fiber. Among these principal factors, PF-1 regarded as fodder yield factors. The value was observed for plant height (0.52), leaf length (0.52) and dry matter yield (0.43) were highest suggesting that PF-1 can be regarded as fodder yield factor as all the fodder yield contributing factors are present.

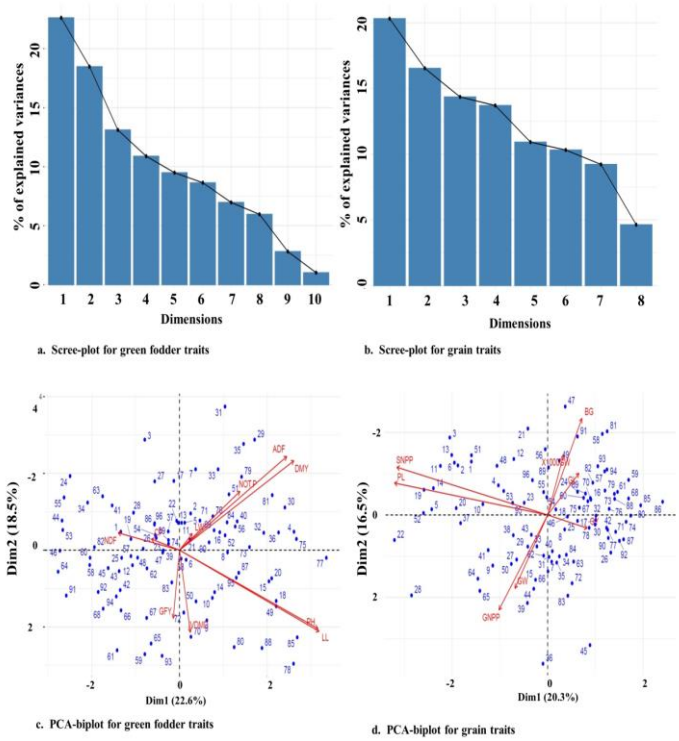

Fig 3. PCA-Scree plot and biplot of mean values for fodder (a) and grain traits (b) in 96 germplasm accessions of oats.

PCA analysis for seed traits revealed total eight principal components (Fig. 3b). The first three principal components have eigen values 1.63, 1.32 and 1.15 and the amount of variation explained by these PCs was 20.32%, 16.54% and 14.36% which constitutes about 51.21% of total variations among all the lines (Table S6). According to the PCA-biplot (Fig. 3d) and factor loading of different seed yield characters (Table S6), the first principal factor was loaded for grain yield, beta-glucan content and grain length. Second principal factor was loaded for beta-glucan content, 1000 grain weight and grain length. The higher values of grain length, grain width and 1000 grain weight were observed for the third principal factor. Among these principal factors, PF-3 can be regarded as seed yield factor as it has higher values for grain length (0.67), grain width (0.47) and 1000 grain weight (0.36) as these traits are seed yield contributing traits. The principal component analysis identifies the major traits that are contributing to most of the observed variation in a set of genotypes. This technique has a practical application as it allows the direct selection of best genotypes that are clustered around

vector for particular trait. In the current study, genetic variability was mainly explained by first three PCs which accounted for 54.39% of the total variance for fodder traits. For grain related traits, total variance of 51.21% was explained by first three PCs. GUNGOR *et al*. (2021), KUMARI and JINDAL (2019) and POONIA *et al*. (2021) reported a similar amount of cumulative variance.

The three principal factors (PFs) were loaded for different traits that contributed towards the fodder yield and seed yield. Among these principal factors, PF-1 and PF-3 regarded as fodder yield as well as seed yield factors because the value of major characters such plant height (0.52), leaf length (0.52) and dry matter yield (0.43) that contributes towards higher green fodder yield have been recorded higher in PF-1 and for seed yield higher values for grain length (0.67), grain width (0.47) and 1000 grain weight (0.36) have been recorded for PF-3. By utilizing these traits in breeding programmes, green fodder yield as well as seed yield of oat can be increased further. AMANDEEP *et al.* (2023), KRISHNA *et al*. (2014), and POONIA *et al*. (2021) also studied PCA in oat and recommended to allocate correlated variables into a few PCs explaining considerable variability of the material analyzed.

SSR polymorphism

Thirty-one polymorphic SSR markers detected 100 alleles in the 96 oats genotypes. Number of alleles amplified by primers ranged from 2 to 8, with an average of 3.2 alleles per primer; the highest allele number was achieved by the cAM19 marker. The average polymorphic information content (PIC) value obtained was 0.47 with a range of 0.08 (cAM 38) to 0.82 (1950-2). The number of alleles detected per primer pair and the PIC values of the 31 SSR primers are given in Table 2.

Table 2. Alleles amplified and PIC values of primers.

S.no.	Primer	PIC value	Alleles amplified	S.no.	Primer	PIC value	Alleles amplified
1	cAM 03	0.43	3	17	cAM 37	0.81	2
2	cAM 04	0.71	6	18	cAM 38	0.08	2
3	cAM 05	0.67	4	19	cAM 39	0.20	2
4	cAM 06	0.19	2	20	cAM 41	0.27	2
5	cAM 09	0.41	2	21	AM 87	0.50	2
6	cAM 10	0.71	6	22	AM 88	0.25	2
7	cAM 12	0.61	3	23	AM92	0.69	5
8	cAM 16	0.57	4	24	AM93	0.45	3
9	cAM 19	0.81	8	25	AM105	0.49	2
10	cAM 25	0.81	6	26	AM106	0.49	2
11	cAM 26	0.52	3	27	AM107	0.49	2
12	cAM 28	0.31	2	28	AM113	0.48	2
13	cAM 29	0.37	3	29	AM115	0.57	5
14	cAM 31	0.22	2	30	AM120	0.29	2
15	cAM 34	0.22	2	31	1950-2	0.82	6
16	cAM 35	0.17	3				

PAGE results obtained by two primers, cAM03 and cAM10 are presented in Figure S1. Genetic diversity plays an important role in crop improvement (GUPTA *et al*., 2009; TANIN *et al*., 2022; SINGH *et al*., 2022). In present study, SSR markers detected a huge amount of polymorphism among oat germplasm lines. In present study, the average numbers of alleles per each SSR locus was comparable to 3.22 alleles per primer reported by KAPOOR and CHOUDHARY (2017). Alleles observed were relatively more than 2, 2.58, and 2.69 reported by RANA *et al*. (2019), SOOD *et al*. (2016) and KAUR *et al*. (2021) and less than 3.49, 3.63, and 4.8 alleles which were reported by YARVAAN *et al*. (2020), ARORA *et al*. (2021), and FU *et al*. (1970) respectively.

PIC value is supposed to be a reliable index during selection of markers for molecular characterization of germplasm. PIC values of each primer pair in the present study ranged from 0.08 to 0.82 with an average of 0.47, which is more than 0.24, 0.28, 0.45, 0.37, 0.42, reported by BOCZKOWSKA and TARCZYK (2013), FU *et al*. (2007), KAPOOR and CHOUDHARY (2017), ISABEL *et al*. (2019); RANA *et al*. (2019) and less than 0.80, 0.50, 0.60 reported by MONTILLA-BASCÓN *et al*. (2013), ARORA *et al*. (2021), YARVAAN *et al*. (2020). The differences in the number of alleles detected in all these studies are mainly due to the use of high-resolution power of 6% polyacrylamide gel, the geographical distribution of the genotypes and SSR markers used.

Genetic similarity coefficient

The similarity coefficients ranged from 0.37 to 1 among cultivated oats germplasm lines with minimum genetic similarity observed between genotypic pairs**:** UPO 276: SKO 315 (46%); SKO 314: OL-125 (46%); SKO 314: OS 363 (49%); SKO 314: UPO 032 (49%). Similarly, among wild and cultivated oats accessions, minimum genetic similarity was observed between following pairs: *A. maroccana***:** EC 605829, EC 209750, EC 209472, EC 209616, EC 209408, EC 209402 (37%); *A. sterilis*: OL 1710 (38%); *A. sterilis*: OL 1714 (38%); *A. sterilis*: OL 1722 (39%). Genotypic pairs, OL 1635, OL 1636**:** OL 1542, OL 1611, OL 1612, OL 10, OL 1615, OL 1624, OL 1625, KENT, OL 1680 had the maximum genetic similarity of 100%. Genetic diversity between different genotypes was analyzed by calculating similarity coefficients which ranged from 0.37 to 1. Consequently, these results showed the genotypic pairs which showed minimum similarity can be used as parents in developing mapping populations for various mapping studies. These results were in agreement with those of KAPOOR and CHOUDHARY (2017), SOOD *et al*. (2016) and YARVAAN *et al*. (2020).

Cluster analysis

The cluster analysis approach revealed a relative pattern of differentiation among 96 oats genotypes. To understand the genetic relationships among 96 oats accessions, a dendrogram was generated with unweighted pair group method with arithmetic averages (UPGMA) (NEI and LIE 1979) using sequential agglomerative hierarchical and non-overlapping (SAHN) (SNEATH and SOKAL, 1979) clustering method based on the genetic distance matrix (Fig. 4) from the pooled data of 96 *Avena sativa* entries which revealed two distinct clusters *i.e.* cluster ‘A’ and cluster ‘B’ (Table S7). Cluster ‘A’ held all four wild oats accessions. Cluster ‘B’ was the largest cluster and further sub-divided into five sub-clusters *i.e.* sub-cluster ‘B1’, ‘B2’, ‘B3’,’B4’ and ‘B5’. Sub-cluster ‘B1’, ‘B2’, ‘B3’,’B4’ and ‘B5’ held 9, 12, 19, 7 and 45 genotypes, respectively. Cluster analysis showed that the genotypes within different clusters were distantly related than the

individuals in the same clusters. All the four wild oat accessions were grouped into same cluster i.e., major cluster 'A', implying that they are highly diverse from all other accessions analyzed in the present study. Major cluster 'B' held all the cultivated oats accessions and it was sub-divided into five sub-clusters *i.e.* sub-cluster '1B', '2B', '3B', '4B' and '5B'. Sub-cluster '5B' held largest number of genotypes (45).

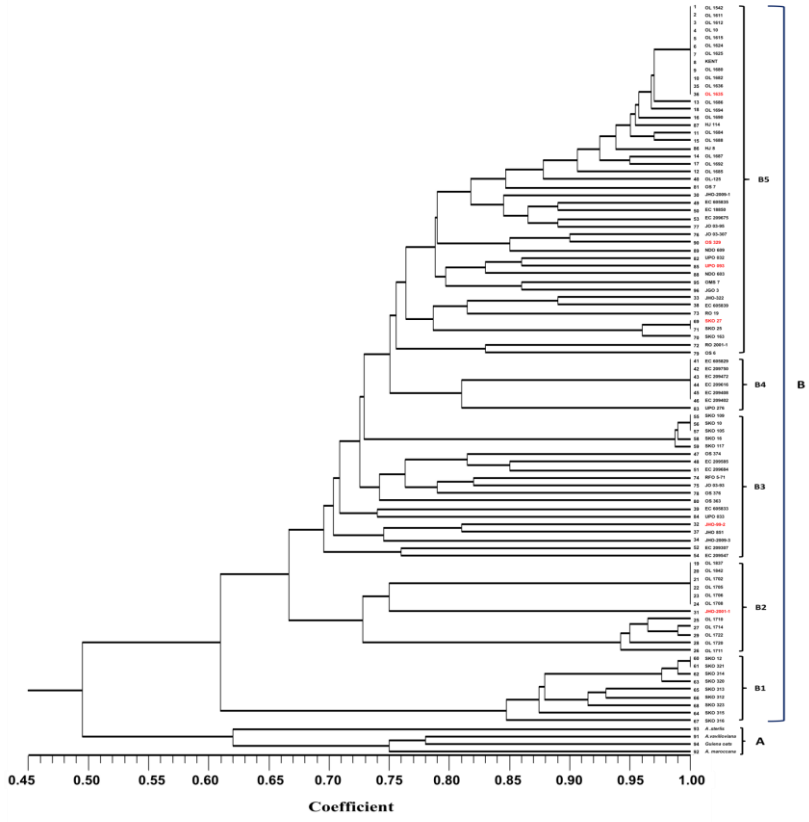

Fig 4. Dendrogram based on genetic distance matrix obtained by SSR marker analysis.
Text in red indicates superior genotypes with high fodder as well as grain yield

The genotypes which lied in same cluster were more closely related and had low genetic divergence among them. Hybridization programs using genotypes with low genetic divergence will have no practical significance in oat improvement program. The clustering pattern obtained in present study clearly showed that all the genotypes collected from same place may not group together in same cluster. It may be due to that the microsatellite classification is only based on a small sequence of whole genomic DNA. Therefore, such a grouping pattern does not seem to be correlated with the geographical distribution of these genotypes. HUANG *et al.* (2002) also showed that it is not mandatory that all genotypes collected from the same geographic location

must be assembled in the same cluster. As per the clustering pattern obtained in the present study, it is observed that all the commercial cultivars (OL-10, OL-125, Kent, JHO-99-2, RO 19, HJ 8, HJ 114, OS 6, OS 7) were grouped in the same sub-cluster i.e. cluster '5B' implying that these have narrow genetic base. These findings are supported by ACHLEITNER *et al*. (2008), TINKER *et al*. (2009) and SOOD *et al*. (2016). So, wild relatives or landraces of oats are one of the most important resources that can be utilized for broadening the genetic base of commercial oats cultivars.

Best dual purpose accessions were identified on basis of their high green fodder as well as grain yield. Only those accessions were shortlisted as best dual purpose oats which had higher green fodder and grain yield not less than 10% as compare to the best check variety. For both traits, OL-10 was superior among both commercial checks (Kent and OL 125). Only six accessions viz; JHO-2001-1, JHO-99-2, OL 1635, SKO 27, UPO 093 and OS329 had been identified as superior to OL-10 for fodder as well as grain yield. Results from the previous studies on fodder oats revealed that high fodder yielding genotypes were not high seed yielding types. Therefore, the above-mentioned genotypes and OL-10, a popular local check variety in Punjab can be exploited as dual-purpose oats and can be further used in dual-purpose oats breeding programs. Based on SSRs classification, JHO-2001-1 lied in sub-cluster 'B2' and JHO-99-2 lied in sub-cluster 'B3'. OL 1635, SKO 27, UPO 093 and OS329 lied in same cluster i.e. sub-cluster 'B5' (highlighted in red text, fig 5). So, in present study, six diverse and superior genotypes had been identified which had high fodder yield and grain yield.

CONCLUSION

Conclusively breeding programs employing hybridization between diverse oats genotypes would be beneficial for overall dual-purpose oats cultivar development programs. In the present study, genotypic pairs:- UPO 276:SKO 315 (46%); SKO 314:OL-125 (46%); SKO 314:OS 363 (49%); SKO 314:UPO 032 (49%); *A. maroccana***:** EC 605829,EC 209750,EC 209472,EC 209616,EC 209408,EC 209402 (37%); *A. sterilis*: OL 1710 (38%); *A. sterilis*: OL 1714 (38%); *A. sterilis*: OL 1722 (39%) are implicated to get high dissimilarity, thus they can be used as parents to develop mapping populations. The genetic variability detected in present study would be of great importance in oat breeding programs. It is vital to enriching the oat gene pool with diverse alleles. So, introgression of beneficial alleles from wild relatives into present commercial oat cultivars could be a possible and excellent source of new allelic variation. JHO-2001-1, JHO-99-2, OL 1635, SKO 27, UPO 093, OS 329 and OL-10 were identified as diverse and superior genotypes which had high fodder yield and grain yield and these could be used in dual-purpose oats breeding program. Based on the results of this study, breeding programs could be planned by using superior genotypes to generate dual purpose oat cultivars for food security and livestock production.

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MOLEKULARNA KARAKTERIZACIJA DIVERZITETA OVSA (*Avena sativa* L.): IMPLIKACIJE NA OPLEMENJIVANJE ZA DVOSTRUKU NAMENU

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Izvod

Ovas (*Avena sativa* L.) je jedna od najzdravijih žitarica na svetu, bez glutena, prepuna esencijalnih vitamina, minerala, vlakana i antioksidansa. Takođe je jedna od najvažnijih krmnih žitarica. U ovoj studiji analiziran je morfološki i molekularni diverzitet za ovas dvostruke namene na osnovu deset krmnih i osam osobina zrna među 96 uzoraka ovsa, uključujući četiri divlja uzorka (*A. vavilioviana*, *Guiena* ovas, *A. maroccana* i *A. sterilis*) sakupljene iz različitih eko-geografske regija Indije. Generalno, morfološka i molekularna raznolikost je pokazala velike varijacije. Trideset jedan od 170 (18%) SSR markera detektovao je polimorfizam među 96 uzoraka ovsa sa prosečnim sadržajem polimorfnih informacija (PIC) od 0,47. Detektovano je ukupno 100 alela sa prosečno 3,2 alela po prajmeru. Analiza molekularne raznovrsnosti grupisala je svih 96 linija u dva glavna klastera, „A“ i „B“. Koeficijenti sličnosti su se kretali od 0,37 do 1. Genotipski parovi, tj. UPO 276: SKO 315 (46%); SKO 314: OL-125 (46%); SKO 314: OS 363 (49%); SKO 314: UPO 032 (49%) su pokazali najmanju genetsku sličnost i oni se potencijalno mogu koristiti kao roditelji za sprovođenje različitih studija mapiranja i daljeg doprinosa zajednici oplemenjivača ovsa. Štaviše, identifikovano je 6 uzoraka (JhO-2001-1, JhO-99-2, OL 1635, SKO 27, UPO 093 i OS329) koji su bili superiorniji od OL-10 (najbolji standard u severozapadnoj Indiji) za stočnu hranu i prinos zrna. Ova studija je pokazala mogućnost korišćenja SSR markera u oplemenjivanju ovsa za dvostruke namene.

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