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Research Article

Assessment of genetic variability in oat (*Avena sativa* L.) germplasm using agro-morphological traits and microsatellite markers

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Abstract
This study assessed genetic variability among oat genotypes for twelve traits, including yield. Significant differences were found across all traits, with high heritability and genetic advance for the number of effective tillers, 1000-seed weight, and grain yield. Hierarchical clustering using morphometric traits grouped the genotypes into four distinct clusters. We identified 665 potential microsatellites using 1000 contigs from NCBI and designed possible primer pairs to develop PCR-based markers in orphan crop like oats. Validation with a panel of 31 diverse genotypes revealed that seven of ten newly developed markers detected expected alleles, with four being polymorphic. Additionally, eight reported SSRs were used to assess genotypic differences. The markers showed a mean allele richness of 2.86 (range: 2-4) and a mean polymorphism information content (PIC) of 0.37 (range: 0.15-0.96). Cluster analysis indicated three distinct clusters with a mean dissimilarity of 0.54, demonstrating the markers' effectiveness for genetic diversity assessment and breeding.

Keywords: Oats, heritability, GCV, PCV, microsatellites, molecular diversity.

INTRODUCTION

Cultivated Oats (*Avena sativa* L.) is a natural allohexaploid (2n=6x=42, AACCDD) which have been domesticated more than three thousand years ago. Oats have become important among popularly cultivated cereal crops due to their unique quality traits such as rich protein, lipids and beta-glucan. It demands comparatively fewer nutrients (i.e., NPK) than wheat and maize. Furthermore, oats are dual-purpose crop that can satisfy the diet demands of livestock as well as the human population. Oats supply highly nutritious and highly palatable, succulent green herbage as fodder and provide grains for human utilization. In the livestock and poultry sector, oat grain constitutes a well-balanced concentrate feed (Chawla *et al.,* 2024). To address the rising competition for arable land, crop breeders must prioritize developing dual-purpose oat varieties that maximize fodder production, possess strong regeneration capabilities, resist biotic stresses, and yield high-quality grain.

Progress in crop breeding relies on the availability of genetic variation in germplasms. Analyzing genetic variability in targeted traits, particularly grain yield and related characteristics, is crucial. But grain yield is attributed to various other yield related traits that should also be focussed while searching for variation among germplasms. Molecular markers effectively reveal diversity among genotypes, facilitating the identification of valuable traits for improvement in breeding programs.

Microsatellites (SSR markers) are effective for assessing molecular polymorphism in diverse crop accessions due to their codominant inheritance and high reproducibility (Arulselvi, 2022; Kanchana and Kalra, 2023). However, the limited number of SSR markers in oats compared to wheat and barley hinders breeding progress. Bioinformatics approaches can expedite the development of additional SSR markers from genomic sequences. Therefore, this study investigates genetic variability in oat genotypes for agro morphological traits, designs microsatellite markers using in-silico approaches, and assesses SSR-based molecular diversity for crop improvement.

MATERIALS AND METHODS

Genetic variability analysis: The study included 31 oat genotypes obtained from the Golden Jubilee Forage Farm of Assam Agricultural University, Jorhat. The trial was laid out in a randomized block design with three replications, each containing two rows, spaced 30 x 10 cm apart, during the *Rabi* season of 2021-22. Measurements on flag leaf length (FLL), flag leaf width (FLW), number of effective tillers (NOET), panicle length (PL), spikelets per panicle (SNPP), 1000-seed weight (TSW), grain length (GL), grain width (GW), and grain yield per plant (GYPP) were recorded from five plants per genotype. Additional observations included days to 50% flowering (DF), days to maturity (DM), and grain crude protein percentage (GCP) were recorded on row basis. Since oats can be used as a dual-purpose crop (for both food and fodder), all the agro-morphological observations mentioned above were taken after the first cut for fodder to investigate the grain yield potential following the fodder cut. GCP determined using the micro Kjeldahl method with KELPLUS KES 8L and CLASSIC - DX VATS equipment. Data were analyzed for variation using ANOVA with a randomized block design, and genetic parameters such as phenotypic and genotypic coefficients of variation, broad-sense heritability, and genetic advance were calculated (Allard, 1960).. ANOVA as well as genetic parameters were analyzed using the package "*variability*" developed by Popat *et al.* 2020 in R Studio (version 4.3.2). Visualizations such as boxplots, bar graphs, correlograms, and cluster dendrograms were generated using R Studio (version 4.3.2).

Primer evaluation**:** Fresh young and healthy leaves were collected from all the genotypes on 30 DAS for extraction of genomic DNA. Total genomic DNA was extracted using CTAB method. For the exploration of repeat motifs, a FASTA file containing 16,620 oat genomic sequence contigs was utilized. The first 1,000 contigs were analyzed using the Simple Sequence Repeats Identification Tool (SSRIT) from the Gramene database and MISA-web.

Table 1. Primer pairs designed *in silico* and evaluated for PCR amplification

Sequence IDs	Name	Primer Pairs (5'-3')	GC (%)	T_m (°C)	Repeat Motif	Product size (bp)
PKQH01000100	AAM01	F: CTCCAATTCGTCTGTTCCGC	55	54	$(CCT)_8$	228
		R: AGCAAGACAGGACACAGACA	50	52		
PKQH01000124	AAM02	F: AGACCTCAAGCTGCGATTC	52	51	$(TCC)_8$	248
		R: CAGCTTTCATCTCTAGGACCA	48	52		
PKQH01000181	AAM03	F: GCTGGTGTTGCTAAGACGTT	50	52	$(AGA)_{14}$	165
		R: TCCATGGACTCACTTGACCT	50	52		
PKQH01000196	AAM04	F: TCTTGTAAACCTGCCACTCC	50	52	$(TCA)_{19}$	241
		R: CAAGTTGATGGTGATGGTCA	45	50		
PKQH01000342	AAM05	F: GATCAGCTGGTGAAAGTGCT	50	52	$(GCA)_8$	232
		R: CGACGATGGAGATAACCTTG	50	52		
PKQH01000011	AAM06	F: ATCATCCGGCATGCTAAAG	47	50	$(TTTC)_7$	237
		R: AAGGCTCATCTTGCTTCTCA	45	50		
PKQH01000029	AAM07	F: GCATGGTCACACGGATAGAT	50	52	$(CACG)_6$	222
		R: CATGGATGAGCAGGCTAAGT	50	52		
PKQH01000294	AAM08	F: TATCGTGGGCGAAATGTAGT	45	50	$(GCAG)_6$	243
		R: ATTTGCAGGTAGTCCAGCAG	50	52		
PKQH01000294	AAM09	F: CGATCTCCATGGTACACACA	50	52	$(AAGC)_5$	198
		R: ATGCATGGTCGGTCATATTC	45	50		
PKQH01000076	AAM10	F: TGCTTCAGGTGTCCTCTTTC	50	52	$(AACA)_5$	221
		R: AACCCGTGTTACAGAACAGC	50	52		

Primer pairs for amplifying identified SSR regions were designed using Primer3Plus, aiming for a length of 18 to 27 bp, a T_m of 50°C to 65°C, and a GC content of 45% to 80%. Ten random markers (**Table 1**) from the designed set were tested for SSR amplification from oat genomic DNA. The amplification conditions included an initial denaturation at 94°C for 4 minutes, followed by 35 cycles of denaturation at 94°C, annealing from 58°C to 48°C, extension at 72°C for 1 minute each and final extension at 72°C for 4 minute. PCR products were resolved on 3.0% agarose gel, with bands visualized using the Gel Doc™ XR+ Imaging System and Image Lab™ Software.

SSR-based genotypic difference: In addition to the *in silico* designed microsatellite markers, eight previously published oat SSR markers (**Table 2**) were utilized to assess molecular diversity. PCR conditions were consistent with prior methods. Amplicons from each informative primer were scored based on band presence (1) or absence (0), recorded in a binary matrix. The size of the amplicons was determined using a 100 bp DNA ladder. The total number of alleles for each SSR marker was counted sequentially. For estimating genetic dissimilarity among genotypes, the binary data was analyzed using DARwin v6.0.21 software, calculating genetic distance (GD) as one minus Jaccard's similarity index. A dendrogram was constructed for the 31 genotypes based on the Unweighted Neighbour Joining (UNJ) method. Additionally, the Polymorphism Information Content (PIC) was calculated for each primer pair to evaluate the discriminatory power of the SSR loci. The PIC was estimated using the formula

$$PIC = 1 - \Sigma P_i^2$$

where,

P_i is the frequency of the i^{th} allele in the set of genotypes investigated.

RESULTS AND DISCUSSION

Genetic variability for agro morphological traits: Analysis of variance revealed significant variation among oat genotypes for recorded agro morphological traits (**Table 3**). Except for panicle length and grain width, all other traits showed highly significant variation (**Fig. 1**). The phenotypic coefficient of variation (PCV) exceeded the genotypic coefficient of variation (GCV) for all traits, indicating substantial environmental influence (**Table 4**). Pearson correlation analysis demonstrated a highly significant positive correlation between grain yield per plant and traits such as 1000-seed weight, flag leaf length, and number of effective tillers (**Fig. 2**), supporting the findings of Nagesh *et al.* (2023). This suggests that selection for higher values of these traits could enhance grain yield. Mean performances of genotypes for traits significantly correlated with grain yield are shown in bar plots (**Fig. 3**). Hierarchical clustering identified hidden similarities among genotypes, grouping them into four distinct clusters, as illustrated in the dendrogram (**Fig. 4**). Cluster I contained two genotypes, while Cluster II included seven genotypes. Clusters III and IV each comprised 11 genotypes, highlighting the genetic diversity present among the studied oat accessions.

Table 2: List of reported SSR markers used for diversity analysis

Name	Forward & Reverse Primer (5'-3')	% GC	T_m (°C)	Repeat Motif	Product size (bp)	Reference
AM30	F: TGAAGATAGCCATGAGGAAC	45	50	$(GAA)_{14}$	178-231	Li *et al.* (2000)
	R: GTGCAAATTGAGTTTCACG	42	47			
AM31	F: GCAAAGGCCATATGGTGAGAA	47	52	$(GAA)_{23}$	132-198	
	R: CATAGGTTTGCCATTCGTGGT	47	52			
AM32	F: AGTGAAGGCGATGGCGAA	55	50	$(GAA)_{19}$	295	
	R: GGATAATGCACCCGAGTTGC	55	54			
AM42	F: GCTTCCCGCAAATCATCAT	47.	49	$(GAA)_{16}$	143-205	
	R: GAGTAAGCAAAGGCCAAAAAGT	40.	51			
AM87	F: GAGCAAGCTCTGGATGGAAA	50	52	$(AC)_{13}$	92–171	Pal *et al.* (2002)
	R: CCCGTTTATGTGGTTGTTAGC	47	52			
AM104	F: AACAATGATGGGGATGGTGT	45	50	$(AG)_{36}$	186	
	R: GTCGTGAGCAAGTTGAACCA	50	52			
AM115	F: CGCAACTCTTCCTACTTTTTGTT	39	52	$(AC)_9$	214	Grain genes database
	R: TGGCAAACTCCCTCGATTTA	45	50			
MAMA_4	F: GGAGTGGGCGTTTGACATTA	50	52	$(TCTA)_n$	352-420	Wight *et al.* (2003)
	R: CAGCTACCGGTTTTCATTCC	50	52			

Table 3. Analysis of variance (ANOVA) for grain yield and attributing traits

Source of variation	df	Mean Squares											
		DF	FLL	FLW	NOET	PL	SNPP	DM	TSW	GL	GW (x10^{-2})	GCP	GYPP
Genotype	30	19.02**	31.02**	0.07**	5.22**	17.02*	261.47**	81.86**	142.32**	0.07**	0.12*	4.79**	79.62**
Replication	2	1.17	14.11	0.02	0.04	5.89	6.78	45.46	3.08	0.01	0.05	0.43	0.74
Error	60	2.89	10.25	0.01	0.13	9.92	16.62	22.65	8.51	0.02	0.07	0.64	1.50

*, ** - Significance at 5% and 1% probability levels respectively
DF – Days to 50% Flowering, FLL – Flag Leaf Length (cm), FLW – Flag Leaf Width (cm), NOET – Number of Effective Tillers per plant, PL – Panicle Length (cm), SNPP – Number of Spikelet per Panicle, DM – Days to Maturity, TSW – 1000 Seed Weight (g), GL – Grain length (cm), GW – Grain Width (cm), GCP – Grain Crude Protein (%), GYPP – Grain Yield per Plant (g/plant)

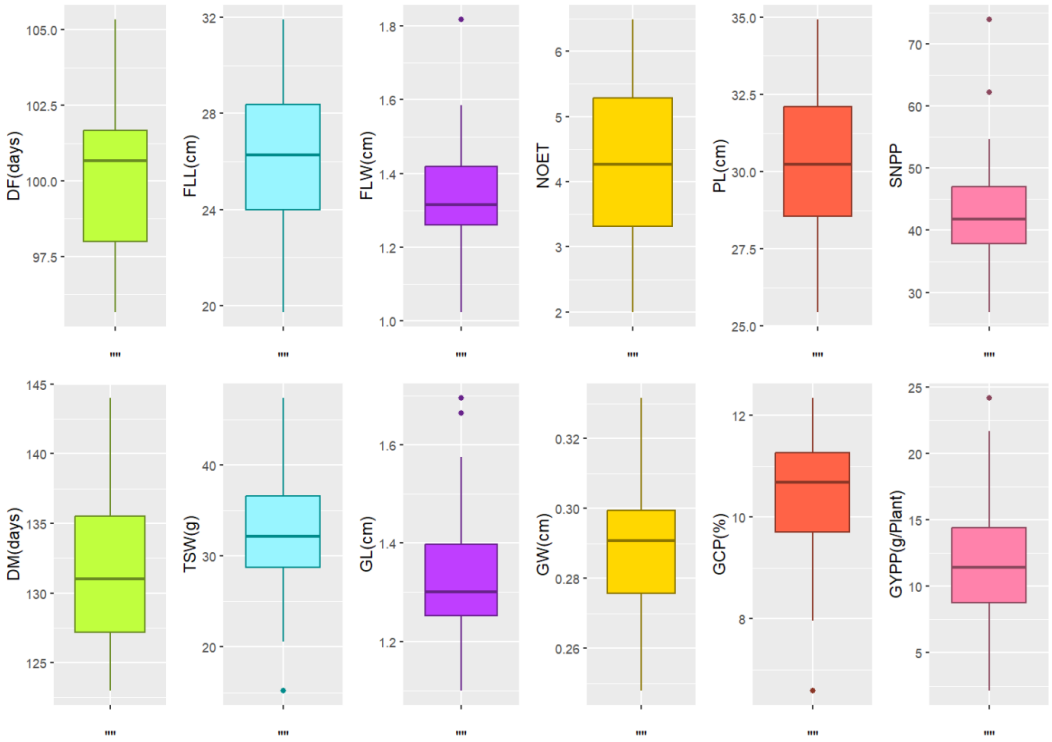

Fig. 1. Boxplots representing the distribution phenotypes for grain yield and related traits

Detection and characterization of microsatellites: A total of 665 perfect SSR sequences were identified from 1000 contigs. Among these, 514 were dinucleotide, 127 trinucleotide, 20 tetranucleotide, and 4 pentanucleotide repeats. Notably, dinucleotide repeats showed considerable variation in repeat counts (5 to 41), while trinucleotide repeats displayed moderate variation (5 to 30). Tetranucleotide and pentanucleotide repeats showed limited variation (5 to 9 and 5 to 6, respectively). The mean numbers of repeats for di-, tri-, tetra-, and penta-nucleotide classes were 5.25, 5.65, 6.94, and 6.49, respectively. In contrast to previous studies that identified AT as the predominant dimeric repeat (Morgante and Olivieri, 1993; Powell *et al.,* 1996), our analysis found AG/TC (37.74%, 194 out of 514) to be the most common, followed by AT/TA (35.41%, 182 out of 514) and AC/TG (24.71%, 127 out of 514). Trinucleotide repeats also favoured CTG/GAC (23.62%, 30 out of 127). These findings align with earlier reports highlighting the prevalence of AG/TC and AC/TG repeats in cereals (Varshney *et al.,* 2002; Gao *et al.,* 2003; Pal *et al.,* 2002).

Validation of the selected set of microsatellites: Out of the 665 SSRs identified using SSRIT, thirteen located at the beginning or end of contigs were omitted due to the absence of suitable flanking sequences. Primer pairs for the remaining 652 SSRs were designed in silico. To validate these markers, ten random markers—five with

Table 4. Estimates of genetic parameters for different traits related to grain yield

Traits	Heritability (%)	GCV (%)	PCV (%)	GA	GAM (%)
DF	64.99	2.32	2.87	3.85	3.85
FLL	40.33	10.07	15.85	3.44	13.17
FLW	64.86	10.65	13.23	0.24	17.68
NOET	93.17	30.73	31.83	2.59	61.10
PL	19.27	5.07	11.56	1.39	4.59
SNPP	83.08	20.79	22.81	16.96	39.04
DM	46.56	3.38	4.96	6.25	4.75
TSW	83.98	20.47	22.34	12.60	38.65
GL	53.40	9.90	13.55	0.20	14.91
GW	22.22	4.89	10.36	0.02	4.73
GCP	68.35	11.37	13.75	2.00	19.36
GYPP	94.57	42.44	43.64	10.22	85.02

DF – Days to 50% Flowering, FLL – Flag Leaf Length (cm), FLW – Flag Leaf Width (cm), NOET – Number of Effective Tillers per plant, PL – Panicle Length (cm), SNPP – Number of Spikelet per Panicle, DM – Days to Maturity, TSW – 1000 Seed Weight (g), GL – Grain length (cm), GW – Grain Width (cm), GCP – Grain Crude Protein (%), GYPP – Grain Yield per Plant (g/plant)

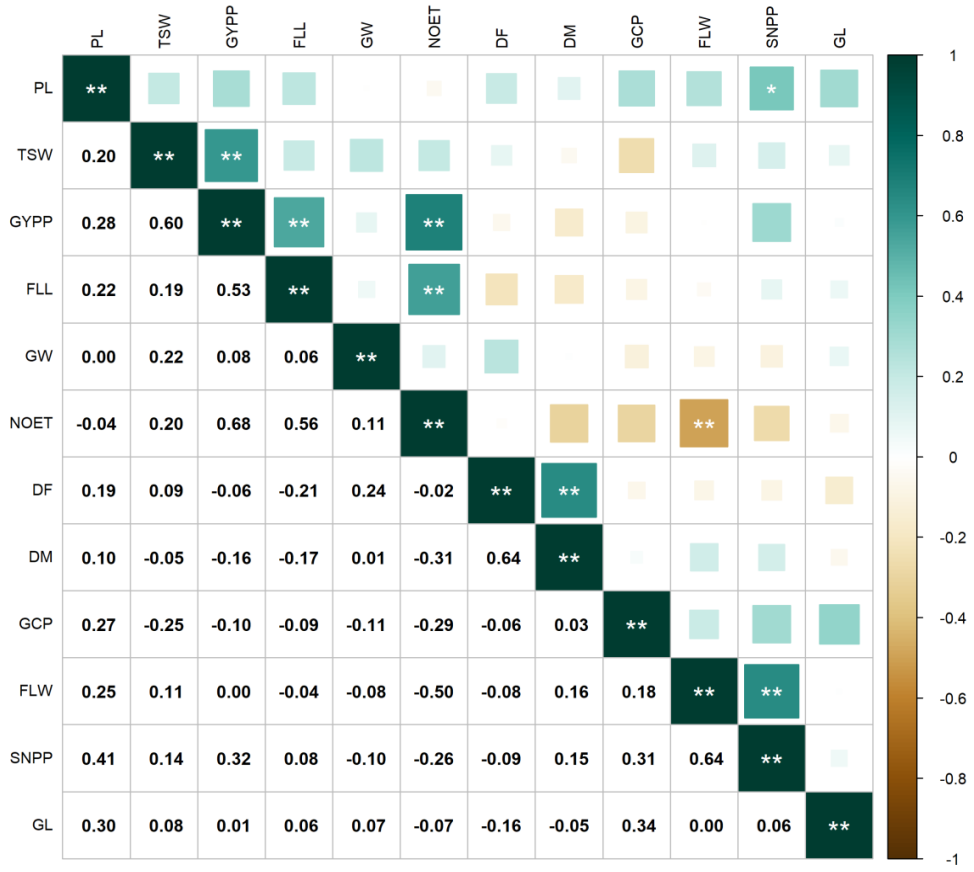

Fig. 2. Correlogram representing the correlation coefficient among various twelve grain traits

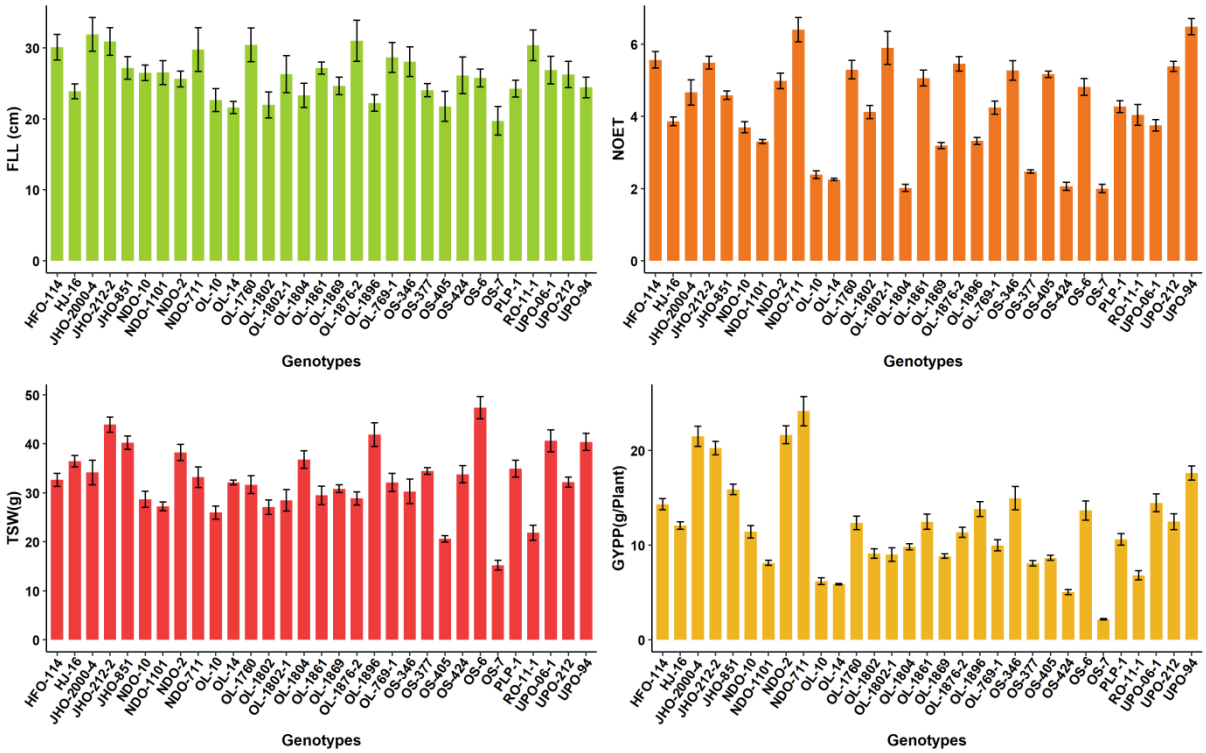

Fig. 3. Mean performance of the traits that are significantly correlated with grain yield per plant

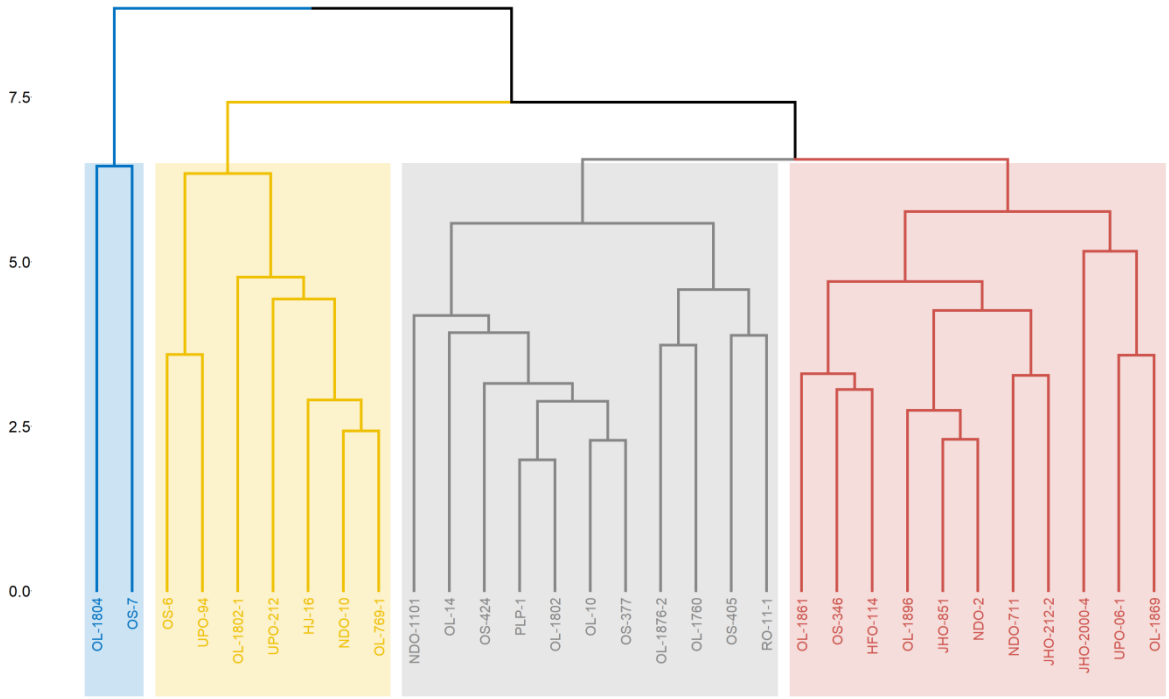

Fig. 4. Dendrogram representing the hierarchical clustering of genotypes based on grain-related phenotypes

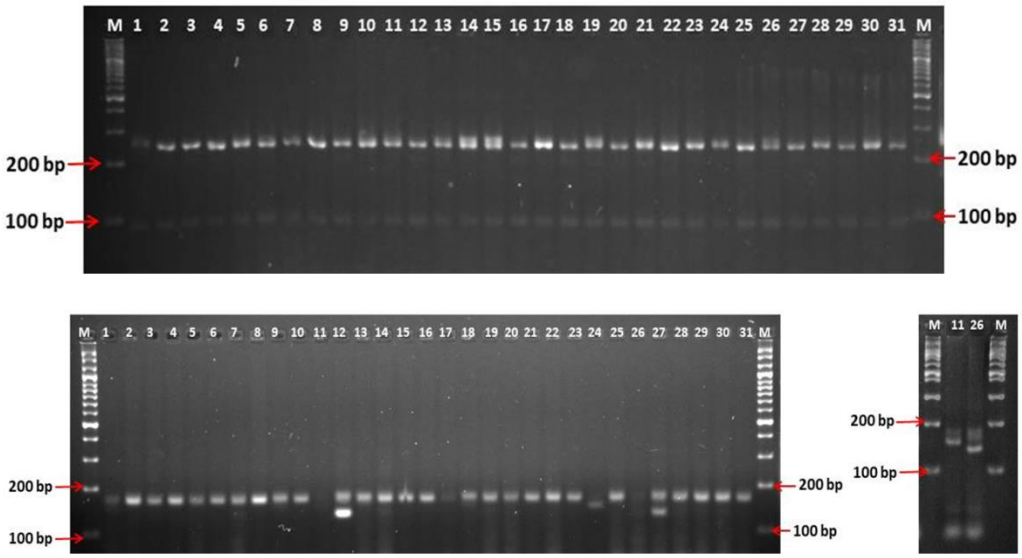

Fig. 5. SSR amplification profile of AM87 and AAM06 in *A. sativa* L. genotypes

trinucleotide and five with tetranucleotide repeats—were selected from the 652 and tested in a panel of 31 oat genotypes. The markers (M) were designated with acronyms (AAM01–AAM10) to indicate their origin from Assam Agricultural University (A) and *Avena sativa* (A). Among the ten markers, four revealed polymorphic products, three produced monomorphic products, and three failed to amplify despite multiple attempts (**Fig. 5**). This resulted in 70% amplification efficiency for the designed microsatellite markers. Product sizes ranged from 165 bp to 248 bp, with most exceeding 200 bp, and the amplified markers averaged two alleles, ranging from 2 to 4. The successful amplification of seven markers and polymorphism in four indicates the efficacy of the design conditions, suggesting that the remaining markers will also be effective in population studies. Additionally, the identified microsatellites are likely functional markers, as they originate from genomic regions encoding NB-LRR proteins associated with pathogen resistance (Dubey and Singh, 2018; Bezerra -Neto *et al.,* 2020).

SSR marker-based genotypic difference: Seven out of fifteen markers demonstrated polymorphism, enabling the distinction of oat genotypes (**Table 5**). The mean number of alleles per polymorphic SSR locus was 2.86, with a range of two to four. The marker AAM03 generated the highest number of alleles (4), while AAM01 and AAM06 produced two alleles each, with Polymorphism Information Content (PIC) values ranging from 0.15 to 0.96. A significant correlation (r = 0.69) was observed between allele richness and PIC, indicating that an increased number of alleles contributes to a higher PIC value (Rakshit *et al.,* 2012). Mwangi *et al.* (2021) noted that high allele frequencies can lead to lower PIC values, suggesting that markers with PIC values below 0.25 may arise from closely related varieties. The percentage of polymorphic fragments reflects the effectiveness of microsatellite markers in diversity studies; markers with PIC values between 0.25 and 0.50 are moderately informative, while those above 0.50 are highly informative. For genetic dissimilarity analysis, Jaccard's index was applied to assess pairwise genetic relationships among the 31 oat genotypes. Dissimilarity values ranged from 0 to 0.98, with a mean value of 0.54 (**Table 6**), consistent with findings in sorghum accessions (Rakshit *et al.,* 2012). The highest dissimilarity was noted between genotypes OS-6 and OL-10 (0.98) and OL-10 and OL-1861 (0.95), reflecting substantial diversity among the studied genotypes. Cluster analysis, using the Unweighted Neighbor Joining (UNJ) method, revealed three major clusters: I, II, and III, further divided into two sub-clusters each (**Fig. 6**). Within cluster IIA, three accessions (RO-11-1, OS-346, and OS-405) were inseparable, as were UPO-212 and PLP-1. Similar results were observed in cluster IIIA with OL-1876-2 and JHO-851, suggesting a need for additional markers to enhance discrimination. As the genomic regions representing SSRs were not associated with the traits under study and the coverage of polymorphic markers used in this study across the *A. sativa* genome was extremely low, to extend and establish the true/exact relationship between the classifications formed by morphometric traits and SSR data is too optimistic.

Overall, this study elucidates the genetic variability in agro morphological traits that informs the scope for improving grain yield post first cut in oats crop and enriches the oat microsatellite marker pool. We identified 665 microsatellites and validated seven novel SSR markers. Since the contigs from which these SSRs are

Table 5. Detection of polymorphism by the microsatellite markers

S.No.	Marker	Allele richness	No. of polymorphic allele	Polymorphic amplicons (%)	PIC
1.	AM30	1	0	0.00	0.00
2.	AM31	1	0	0.00	0.00
3.	AM32	1	0	0.00	0.00
4.	AM42	1	0	0.00	0.00
5.	AM87	3	2	66.67	0.96
6.	AM104	2	0	0.00	0.00
7.	AM115	3	3	100.00	0.16
8.	MAMA_4	3	3	100.00	0.28
9.	AAM01	2	1	50.00	0.35
10.	AAM02	1	0	0.00	0.00
11.	AAM03	4	3	75.00	0.35
12.	AAM05	1	0	0.00	0.00
13.	AAM06	2	2	100.00	0.15
14.	AAM08	1	0	0.00	0.00
15.	AAM10	3	3	100.00	0.32
	Mean	**2.86**			**0.37**

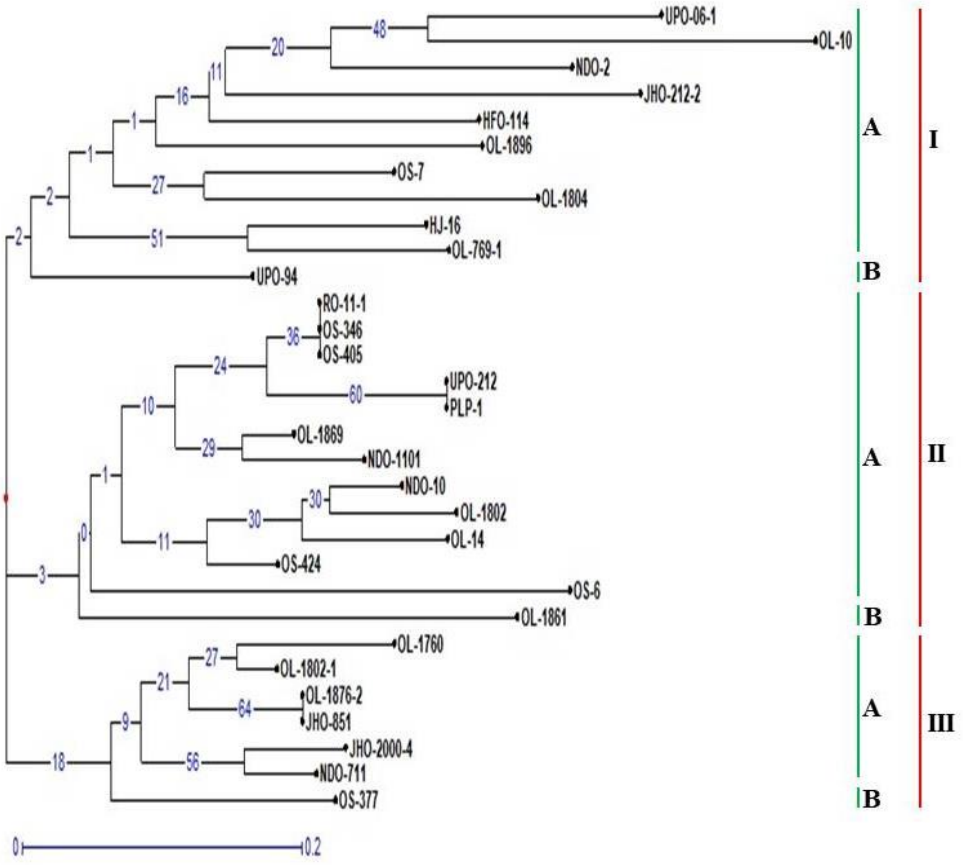

Fig. 6. Dendrogram showing the relationship among 31 oats genotypes using Unweighted Neighbour Joining method (The numbers mentioned on the branches were bootstrap values. The distance scale is given below the dendrogram to understand how two genotypes related to each other)

Table 6. Pair-wise Jaccard's dissimilarity coefficient among 31 oat genotypes based on SSR markers

i	j	d(i,j)	i	j	d(i,j)	i	j	d(i,j)	i	j	d(i,j)	i	j	d(i,j)
2	1	0.894	10	4	0.406	14	1	0.901	16	13	0.482	19	4	0.528
3	1	0.803	10	5	0.663	14	2	0.472	16	14	0.663	19	5	0.785
3	2	0.167	10	6	0.388	14	3	0.382	16	15	0.624	19	6	0.510
4	1	0.791	10	7	0.254	14	4	0.534	17	1	0.628	19	7	0.375
4	2	0.527	10	8	0.406	14	5	0.791	17	2	0.653	19	8	0.528
4	3	0.436	10	9	0.416	14	6	0.516	17	3	0.562	19	9	0.538
5	1	0.444	11	1	0.944	14	7	0.382	17	4	0.550	19	10	0.223
5	2	0.783	11	2	0.577	14	8	0.534	17	5	0.518	19	11	0.578
5	3	0.693	11	3	0.486	14	9	0.544	17	6	0.532	19	12	0.599
5	4	0.680	11	4	0.577	14	10	0.229	17	7	0.562	19	13	0.492
6	1	0.773	11	5	0.834	14	11	0.584	17	8	0.550	19	14	0.216
6	2	0.509	11	6	0.559	14	12	0.605	17	9	0.560	19	15	0.177
6	3	0.418	11	7	0.486	14	13	0.499	17	10	0.533	19	16	0.657
6	4	0.146	11	8	0.577	15	1	0.862	17	11	0.704	19	17	0.654
6	5	0.662	11	9	0.588	15	2	0.433	17	12	0.741	19	18	0.559
7	1	0.803	11	10	0.456	15	3	0.343	17	13	0.479	20	1	0.815
7	2	0.167	12	1	0.982	15	4	0.495	17	14	0.660	20	2	0.550
7	3	0.000	12	2	0.598	15	5	0.752	17	15	0.621	20	3	0.460
7	4	0.436	12	3	0.507	15	6	0.477	17	16	0.466	20	4	0.298
7	5	0.693	12	4	0.615	15	7	0.343	18	1	0.822	20	5	0.704
7	6	0.418	12	5	0.872	15	8	0.495	18	2	0.558	20	6	0.280
8	1	0.791	12	6	0.597	15	9	0.505	18	3	0.467	20	7	0.460
8	2	0.527	12	7	0.507	15	10	0.190	18	4	0.262	20	8	0.298
8	3	0.436	12	8	0.615	15	11	0.545	18	5	0.712	20	9	0.308
8	4	0.000	12	9	0.625	15	12	0.566	18	6	0.244	20	10	0.430
8	5	0.680	12	10	0.477	15	13	0.460	18	7	0.467	20	11	0.601
8	6	0.146	12	11	0.665	15	14	0.143	18	8	0.262	20	12	0.639
8	7	0.436	13	1	0.720	16	1	0.707	18	9	0.125	20	13	0.412
9	1	0.801	13	2	0.491	16	2	0.655	18	10	0.437	20	14	0.558
9	2	0.537	13	3	0.400	16	3	0.565	18	11	0.609	20	15	0.519
9	3	0.446	13	4	0.388	16	4	0.552	18	12	0.646	20	16	0.576
9	4	0.241	13	5	0.610	16	5	0.596	18	13	0.419	20	17	0.574
9	5	0.691	13	6	0.370	16	6	0.534	18	14	0.565	20	18	0.329
9	6	0.223	13	7	0.400	16	7	0.565	18	15	0.526	20	19	0.552
9	7	0.446	13	8	0.388	16	8	0.552	18	16	0.584	21	1	0.719
9	8	0.241	13	9	0.398	16	9	0.563	18	17	0.581	21	2	0.768
10	1	0.773	13	10	0.371	16	10	0.535	19	1	0.895	21	3	0.678
10	2	0.345	13	11	0.542	16	11	0.706	19	2	0.466	21	4	0.665
10	3	0.254	13	12	0.580	16	12	0.744	19	3	0.375	21	5	0.609
21	6	0.647	23	4	0.418	24	21	0.681	26	13	0.458	28	1	0.520
21	7	0.678	23	5	0.674	24	22	0.636	26	14	0.639	28	2	0.720
21	8	0.665	23	6	0.400	24	23	0.586	26	15	0.600	28	3	0.629
21	9	0.676	23	7	0.189	25	1	0.803	26	16	0.568	28	4	0.617
21	10	0.648	23	8	0.418	25	2	0.167	26	17	0.566	28	5	0.410
21	11	0.819	23	9	0.428	25	3	0.000	26	18	0.560	28	6	0.599
21	12	0.857	23	10	0.236	25	4	0.436	26	19	0.633	28	7	0.629

Table 6. Continued..

i	j	d(i,j)	i	j	d(i,j)	i	j	d(i,j)	i	j	d(i,j)	i	j	d(i,j)
21	13	0.595	23	11	0.468	25	5	0.693	26	20	0.552	28	8	0.617
21	14	0.776	23	12	0.489	25	6	0.418	26	21	0.681	28	9	0.627
21	15	0.737	23	13	0.382	25	7	0.000	26	22	0.573	28	10	0.599
21	16	0.581	23	14	0.363	25	8	0.436	26	23	0.523	28	11	0.770
21	17	0.503	23	15	0.325	25	9	0.446	26	24	0.608	28	12	0.808
21	18	0.696	23	16	0.547	25	10	0.254	26	25	0.541	28	13	0.546
21	19	0.769	23	17	0.544	25	11	0.486	27	1	0.790	28	14	0.727
21	20	0.689	23	18	0.449	25	12	0.507	27	2	0.615	28	15	0.688
22	1	0.835	23	19	0.357	25	13	0.400	27	3	0.524	28	16	0.533
22	2	0.330	23	20	0.442	25	14	0.382	27	4	0.512	28	17	0.454
22	3	0.239	23	21	0.659	25	15	0.343	27	5	0.680	28	18	0.648
22	4	0.468	23	22	0.125	25	16	0.565	27	6	0.494	28	19	0.721
22	5	0.725	24	1	0.806	25	17	0.562	27	7	0.524	28	20	0.641
22	6	0.450	24	2	0.695	25	18	0.467	27	8	0.512	28	21	0.545
22	7	0.239	24	3	0.604	25	19	0.375	27	9	0.522	28	22	0.661
22	8	0.468	24	4	0.592	25	20	0.460	27	10	0.495	28	23	0.611
22	9	0.478	24	5	0.696	25	21	0.678	27	11	0.666	28	24	0.632
22	10	0.286	24	6	0.574	25	22	0.239	27	12	0.704	28	25	0.629
22	11	0.518	24	7	0.604	25	23	0.189	27	13	0.442	28	26	0.632
22	12	0.539	24	8	0.592	25	24	0.604	27	14	0.623	28	27	0.616
22	13	0.433	24	9	0.602	26	1	0.806	27	15	0.584	29	1	0.857
22	14	0.414	24	10	0.575	26	2	0.631	27	16	0.552	29	2	0.593
22	15	0.375	24	11	0.746	26	3	0.541	27	17	0.549	29	3	0.502
22	16	0.597	24	12	0.783	26	4	0.529	27	18	0.543	29	4	0.229
22	17	0.594	24	13	0.521	26	5	0.696	27	19	0.616	29	5	0.746
22	18	0.499	24	14	0.702	26	6	0.511	27	20	0.536	29	6	0.143
22	19	0.407	24	15	0.663	26	7	0.541	27	21	0.665	29	7	0.502
22	20	0.492	24	16	0.568	26	8	0.529	27	22	0.557	29	8	0.229
22	21	0.710	24	17	0.566	26	9	0.539	27	23	0.506	29	9	0.307
23	1	0.785	24	18	0.623	26	10	0.511	27	24	0.591	29	10	0.472
23	2	0.279	24	19	0.696	26	11	0.682	27	25	0.524	29	11	0.643
23	3	0.189	24	20	0.616	26	12	0.720	27	26	0.273	29	12	0.681
29	13	0.454	29	28	0.683	30	15	0.561	31	1	0.894	31	16	0.655
29	14	0.600	30	1	0.703	30	16	0.465	31	2	0.000	31	17	0.653
29	15	0.561	30	2	0.592	30	17	0.463	31	3	0.167	31	18	0.558
29	16	0.618	30	3	0.501	30	18	0.520	31	4	0.527	31	19	0.466
29	17	0.616	30	4	0.489	30	19	0.593	31	5	0.783	31	20	0.550
29	18	0.328	30	5	0.593	30	20	0.513	31	6	0.509	31	21	0.768
29	19	0.594	30	6	0.471	30	21	0.578	31	7	0.167	31	22	0.330
29	20	0.364	30	7	0.501	30	22	0.534	31	8	0.527	31	23	0.279
29	21	0.731	30	8	0.489	30	23	0.483	31	9	0.537	31	24	0.695
29	22	0.534	30	9	0.499	30	24	0.375	31	10	0.345	31	25	0.167
29	23	0.484	30	10	0.472	30	25	0.501	31	11	0.577	31	26	0.631
29	24	0.658	30	11	0.643	30	26	0.505	31	12	0.598	31	27	0.615
29	25	0.502	30	12	0.681	30	27	0.489	31	13	0.491	31	28	0.720
29	26	0.594	30	13	0.419	30	28	0.529	31	14	0.472	31	29	0.593
29	27	0.578	30	14	0.600	30	29	0.555	31	15	0.433	31	30	0.592

Where,
d(i, j) – Dissimilarity coefficient between i^{th} and j^{th} genotype

1 : OL-10	9 : NDO-711	17 : HFO-114	25 : RO-11-1
2 : PLP-1	10 : OS-424	18 : JHO-2000-4	26 : OL-769-1
3 : OS-346	11 : OL-1861	19 : OL-14	27 : HJ-16
4 : JHO-851	12 : OS-6	20 : OS-377	28 : NDO-2
5 : UPO-06-1	13 : UPO-94	21 : JHO-212-2	29 : OL-1760
6 : OL-1802-1	14 : OL-1802	22 : NDO-1101	30 : OS-7
7 : OS-405	15 : NDO-10	23 : OL-1869	31 : UPO-212
8 : OL-1876-2	16 : OL-1896	24 : OL-1804	

sourced are specifically enriched for NB-LRR sequences associated with pathogen resistance, these markers hold considerable potential for future oat molecular breeding. Among these, four markers were found to be polymorphic when tested across a panel of thirty-one genotypes, demonstrating their utility in uncovering diversity among oat germplasms. The findings from this research underscore the effectiveness and value of mining microsatellite markers from public databases, particularly in terms of marker validation. The results highlight the potential of these markers for revealing genetic diversity and improving breeding strategies. The inclusion of additional polymorphic microsatellite markers could further uncover hidden genetic relationships within the oat accessions studied, providing a more reliable basis for genetic assessment and breeding improvements.

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