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# 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)

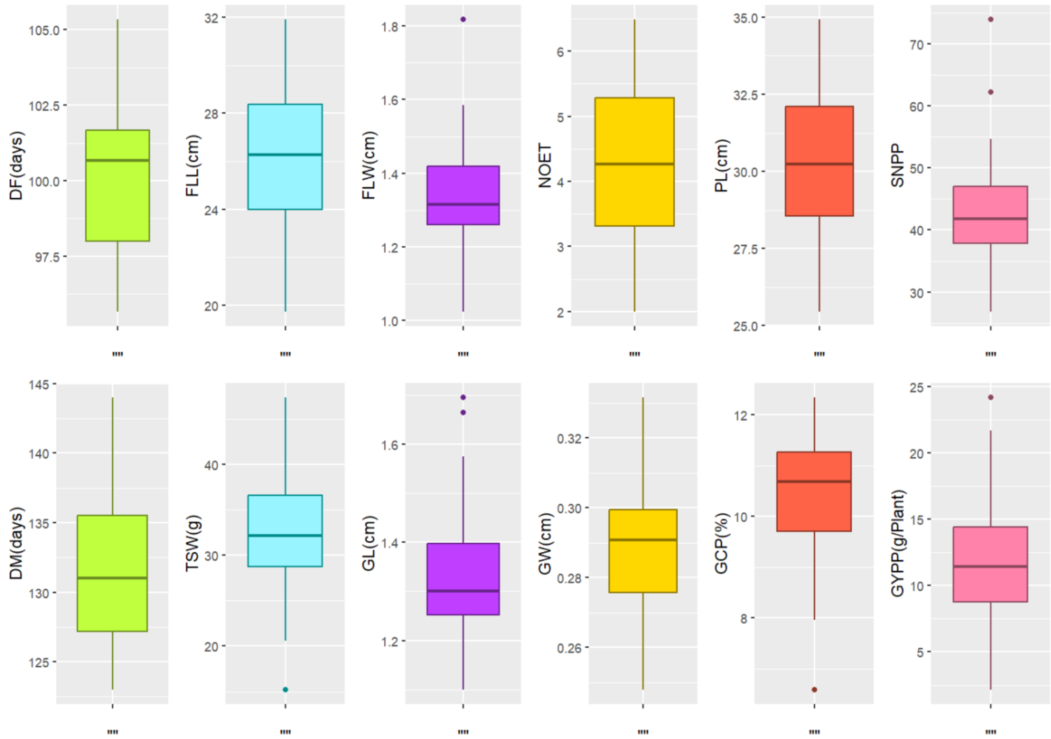


**Fig. 1. Boxplots representing the distribution phenotypes for grain yield and related traits**

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)

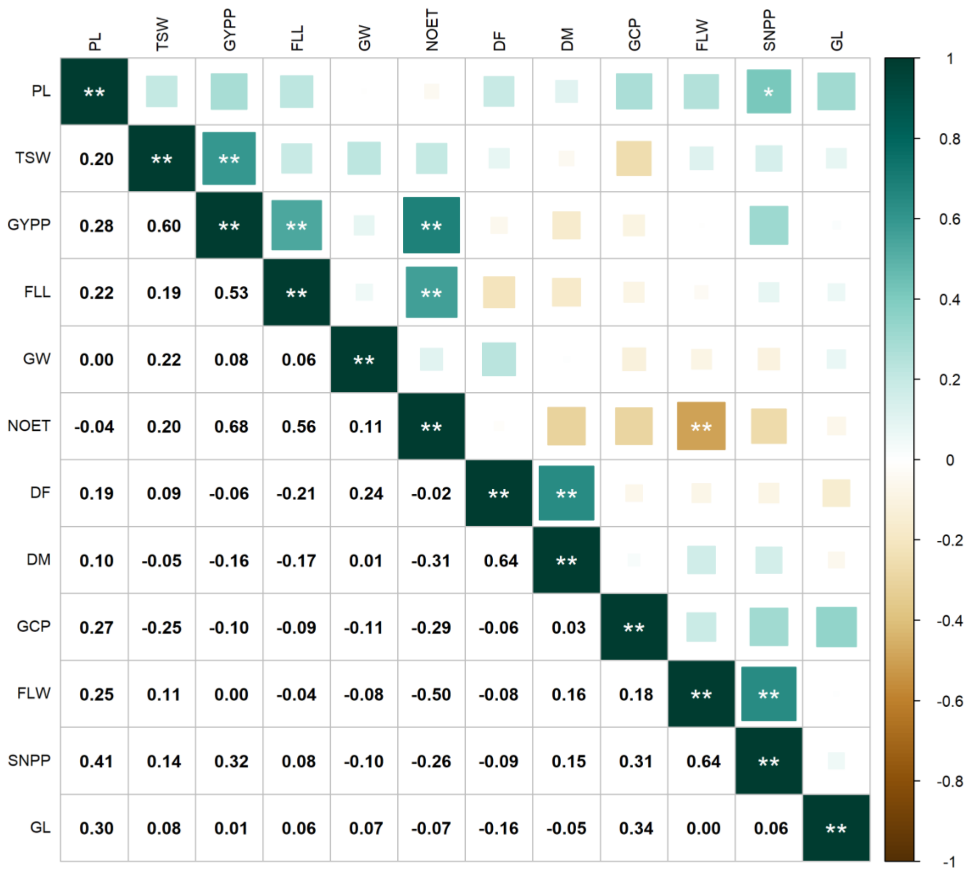


**Fig. 2. Correlogram representing the correlation coefficient among various twelve grain traits**

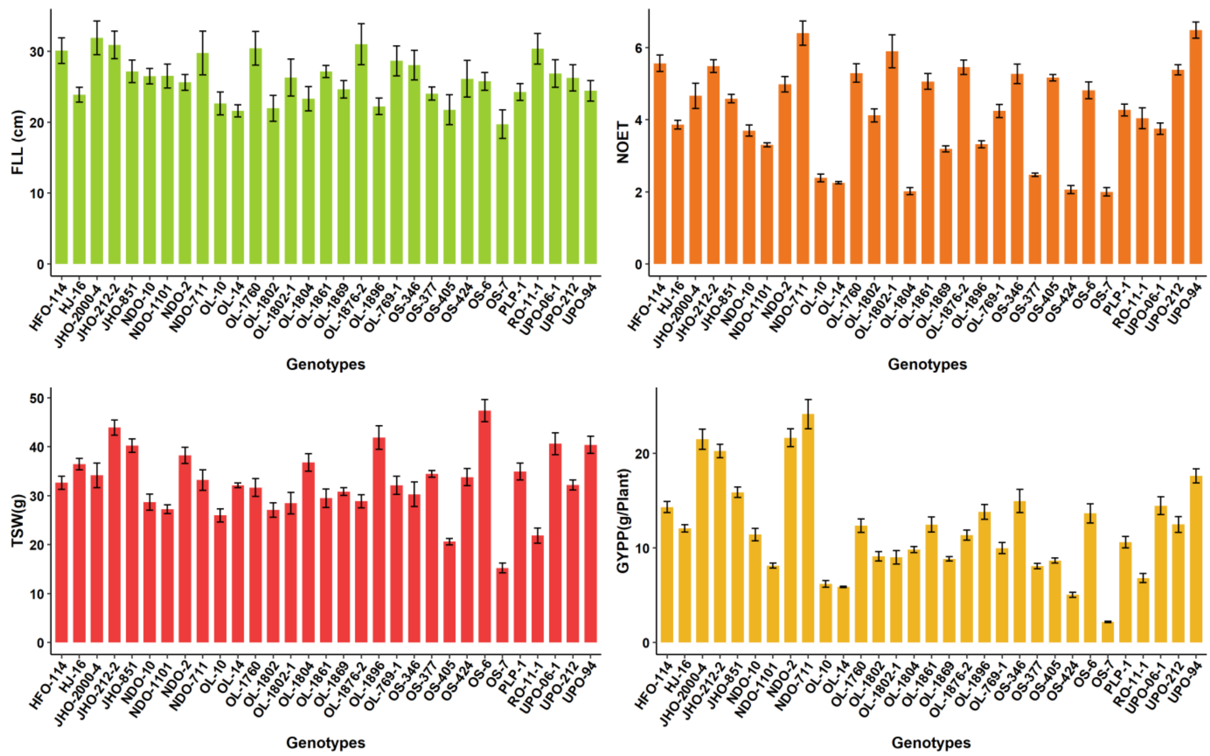


**Fig. 3. Mean performance of the traits that are significantly correlated with grain yield per plant**

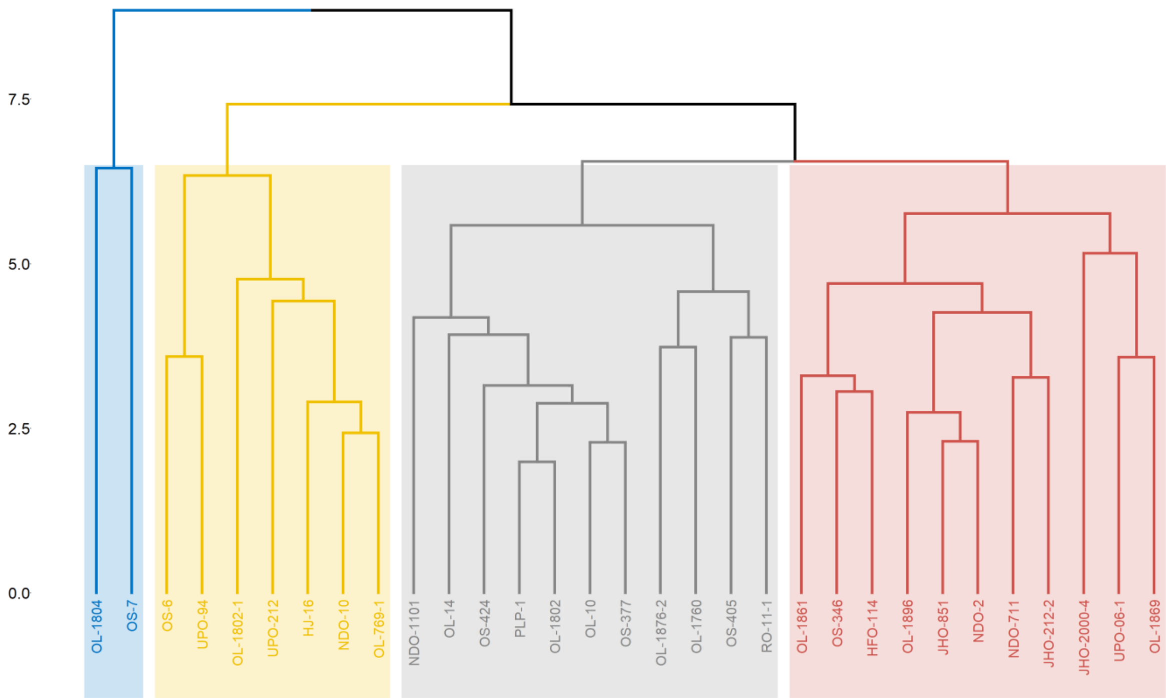


**Fig. 4. Dendrogram representing the hierarchical clustering of genotypes based on grain-related phenotypes**

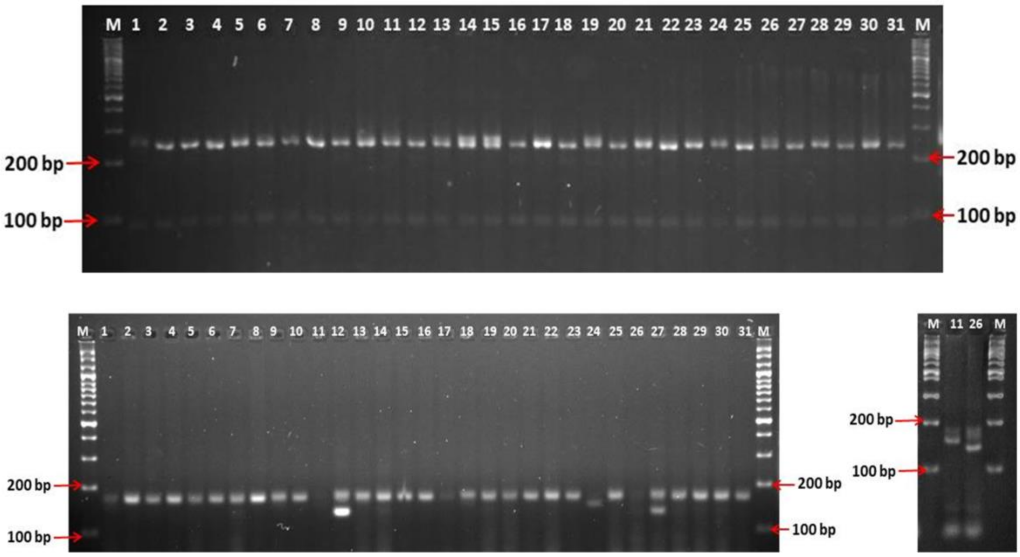


**Fig. 5. SSR amplification profile of AM87 and AAM06 in *A. sativa* L. genotypes**

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** |

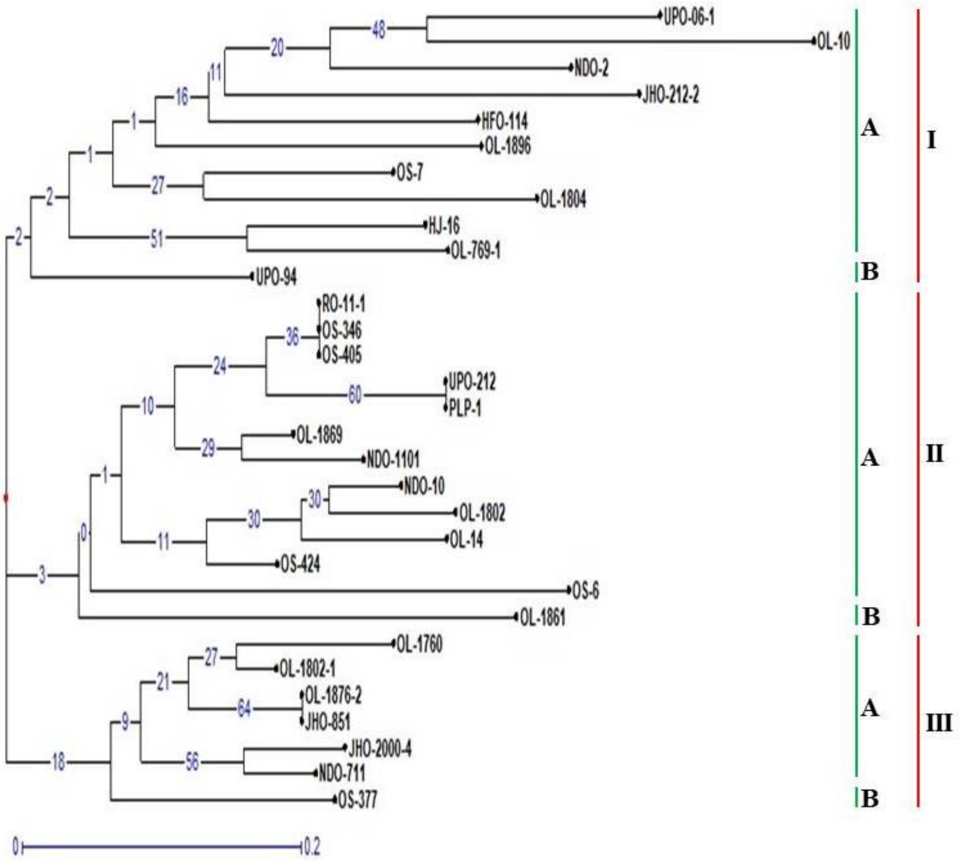


**Fig. 6. Dendrogram showing the relationship among 31 oats genotypes using Unweighted Neighbour Joining method** (The numbers mentioned on the branches were bootstrap values. The distance scale is given below the dendrogram to understand how two genotypes related to each other)

**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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