GENETIC POLYMORPHISM IN ENDOGENOUS LANDRACES OF WILD OAT (*AVENA FATUA* L.) COLLECTED FROM AN UNEXPLORED AREA

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Abstract. The current study was conducted based on the morphological, biochemical, and SSR characterization of wild Oat (*Avena fatua* L.) genotypes collected from three different Districts of Malakand Division, Pakistan. A significant variance was observed across all twenty morphological parameters, indicating a high likelihood that breeding programs would introduce fresh variety into adapted oat cultivars. A substantial variation was also found for leaf length (32.55%) and stem diameter (28.33%), as compared to the number of spikelet (15.66%). The harvest index and plant height had a high and positive correlation (0.79**), while a negative correlation (-0.76**) was observed for plant height and plant biomass. All genotypes were arranged into three groups based on the cluster analysis, each having a Euclidian distance of 87%. A total of 15 bands were visible for the total seed storage proteins, out of which 10 were polymorphic and 5 were monomorphic. The entire dataset of 54 oat genotypes was split into 2 lineages (L-1 and L-2) based on two-way cluster analysis, with a genetic distance of 36.5% between them and further subdivided into three subgroups at 60% genetic distance. The SSR markers used in this study successfully amplified genomic regions from oat genotypes. Out of 5 SSRs, HVM62 showed the prominent polymorphism, and among eight alleles detected, two were monomorphic and six were polymorphic. Z48431 had the highest PIC value (0.93), followed by HVM62 (0.89). Band-14 had the highest PIC value at 0.90%, followed by bands 9 and 10 (0.80% and 0.50%, respectively). Bands 8, 11, 12, and 13 had the lowest PIC value, correspondingly. The overall findings showed a significant degree of variety in the oat genotypes growing in District Swat and Dir, which offers the potential for the introduction of distinctive diversity in well-adapted oat cultivars.
Keywords: *wild oat, genetic diversity, morphology, SDS-PAGE, SSR markers*

Introduction

Oat is an annual grass of Asiatic origin and is used for food as well as forage across the world (Liu et al., 2016). As a cereal plant, the oat is rated sixth in the globe, followed by wheat, rice, maize, barley, and sorghum (Ihsan et al., 2021). Traditionally, there are two primary types of oat crops: wild and farmed. The most severe weed of temperate cereals, the wild oats are present as weeds in more than 20 crops across different countries (Farooq et al., 2011). According to Abberton et al. (2011), germplasm usually includes primitive landraces and wild species related to particular

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crops, and developed varieties and breeders' lines such as fiber, turf, forages, ornamentals, industrial, and medicinal purposes. All over the world, oats are utilized for human and animal feed (Dapic et al., 2019). Oat is a nutritionally intriguing crop with a range of positive aspects, among them an interesting content of soluble fibers, high protein content, and well-composed lipid fraction. Traditionally rolled oats and various breakfast cereals have been in the main interest, but later a range of liquid products have been developed. As compared with other cereals, oat is said to be more suitably produced in marginal conditions, such as chilly, damp weather and low fertility soils (Duda et al., 2021; Xue et al., 2023). In other production areas, however, oat yield is reduced relative to wheat and barley grain. According to FAO STAT (2008) and FAO (2012), the majority of the production areas require higher grain yields. Some of the most familiar weeds, both wild oats and winter wild oats are tall, stout annual grasses similar to the cultivated or 'tame' oats. Very similar species, both have large loose drooping seed heads or panicles. Grain yield is the consequence of a series of complex morphological and physiological processes that interact and occur at various stages of growth (Dumlupinar et al., 2012). Traits such as antioxidant potency, high soluble fiber, and nutritional benefits of oats, render them more suitable for increased intake (Rasane et al., 2015). Oats are characterized as high in antioxidants such as avenanthramides, alpha-tocopherol, and alpha-tocotrienol, as well as total dietary fiber, which includes beta-glucans (Oliver et al., 2010). Recent studies have assessed the impact of oat consumption on human health, and the advantages are not only confined to the lowering of cardiovascular risk factors but also include lowering the onset of diabetes, blood pressure, blood cholesterol levels, maintaining body weight, and improving gastro-intestinal health (Clemens, 2014).

It is well-accepted that genetic diversity must be valued for crop development and is considered effective in the selection of superior varieties (Nisar et al., 2016). In several crop species, genetic characterization has traditionally been based on physical features. However, environmental influences may also have an impact on the physical features (Boffetta et al., 2014; Kapoor et al., 2016). Recently, the characterization of the plant genome and its morphological features has been executed with the help of biochemical and molecular techniques, with the objective of amplifying the degree of genetic variety for crop development (Nisar and Ghafoor, 2011). On the other hand, molecular characterization (based on the makers of proteins and DNA) is however trustworthy and unaffected by the environment (Ahmad et al., 2014; Khalid et al., 2020). It is also recognized that differences in protein bands highlight the links between different collections from several geographical areas (Botstein et al., 1980). Among the molecular markers, the microsatellites or simple sequence repeats (SSR) are more potent methods for analyzing the diversity and thus attracting the attention of scientists (Buerstmayr et al., 2007). These multiallelic markers (co-dominant) which can be quite polymorphic are extensively dispersed across genomes. SSRs have been used to analyze genetics, pedigree, phylogeny, and/or identify different characteristics and/or germplasm accessions with success; they have been particularly crucial in evaluating genetic diversity and genetic maps (Burnette et al., 1992; Knörzer et al., 2009). These factors have led to the widespread use of SSR markers for multiple purposes such as gene tagging, genome mapping, marker-assisted selection (MAS) breeding, genetic diversity detection, and differentiate of the varieties based on their phenotypic traits at the gene level (Ordon et al., 1995; Butt et al., 2008; Huang et al., 2022). Among the different molecular PCR-based active markers like SSRs are standardly used, as they do

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not need any previous sequence of data. SSRs are commonly, informative genetic markers used for genetic diversity analysis because of their easiness, high levels of polymorphism, high development, and co-dominant inheritance patterns (Idrees and Irshad, 2014). Along with morphological traits, these markers are also utilized as an additional marker system (Ajmal et al., 2016). Throughout the growing regions of the world, the microsatellite markers have successfully been employed to examine genetic differences in different cultivars and landraces (Jenkins et al., 2002; Capps Jr et al., 2017).

The present study was conducted to appraise genetic diversity using different morphological traits. The total seed storage proteins and genetic distance in selected lines will also be estimated using SDS-PAGE analysis and SSR markers, respectively.

Material and methods

Morphological evaluation

The present research work was conducted at the Department of Botany, University of Malakand. A total of 54 Oat genotypes were collected from different areas of Malakand Division (*Figure S1, Table S1*), and were analyzed for different morphological traits using the IBPGR descriptor of Oat (Rome, 1985). Different exploratory trips were arranged to different areas, plant materials were identified, and data were scored. Five plants from each area/site were randomly selected and the mean value was used for data analysis. The growth behavior, plant height, stem thickness, hairiness of nodes, stiffness of leaves, panicle form, panicle erectness, spikelet erectness, seed colour, and kernel coating were all taken as qualitative traits. Plant height, number of grains, seed length, seed diameter, number of spikelets, plant weight, seed weight, leaf length, leaf breadth, and stem diameter were among the ten quantitative qualities, although these traits were not quantifiable. The traits such as seed and leaf length and breath, respectively were measured by a vernier caliper and a standard measuring scale.

SDS-PAGE analysis

All the genotypes used in morphological traits were subjected to SDS-PAGE analysis to find out the total seed storage protein. Seeds from each genotype were finely grounded with the help of mortar and pestle and about 0.02 gm was added to an Eppendorf tube containing 400 µL protein extraction buffers and were vortexed for 1 min. After vertexing, the tubes were centrifuged at 13000 rpm for 15 minutes. About 14% polyacrylamide gel was used for the estimation of genetic diversity for total seed storage proteins (Nisar et al., 2009).

Analysis of microsatellite markers (SSR)

Based on the contrasting morphological traits, the sixteen potential genotypes were subjected to SSR markers analysis. For the PCR reaction, the genomic DNA was extracted from the fresh leaves of five selected candidate lines grown in the pots in the lab by CTAB method described by Doyle and Doyle (1990). After extraction of DNA, it was diluted in deionized water up to the 50 µL volume. The DNA from the genotypes examined was used in the amplification procedures in semi-automated multi-locus genotyping systems using 10 pairs of primers that flanked microsatellite regions Each SSR primer PCR reaction took place in a 20-µL reaction volume (*Table 1*).

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Table 1. *SSR primers used in this investigation*

Sr. No	Markers	Sequence Forward/Reverse	Reference
1	AF033096	TGCATGTTTTTGTTTGTTGTTG	Barzin et al., 2016
		CACGATCCAAATACACGCAG	
2	HVM62	TCGCGACCAGACGAGAAG	Barzin et al., 2016
		AGCTAGCCGACGACGCAC	
3	Z48431	CAGCAACAACAACAACCACC	Barzin et al., 2016
		CACTGGTAGCCGTCCTTGAC	
4	M83381	ATCTGTCAGGTGACGAGGCA	Barzin et al., 2016
		CCTTGCATCTGAGGTTGGTT	
5	L39777	CTTCTGCCCATGAAACCCTA	Barzin et al., 2016
		ACTCAGCACATGCACCCTC	

Data analysis

Both the qualitative and quantitative data were subjected to different statistical tools. For qualitative traits, the frequency distribution was used. For quantitative data, five plants were randomly selected, and their mean value was subjected to different statistical packages like descriptive statistics (Mean, Maximum, minimum, standard deviation, standard error), using Microsoft Office Excel 2013, Cluster analysis using PC ORD version 6, and correlation analysis by using the SPSS version 22 software. For total seed storage protein, the data matrices were subjected to two-way cluster analysis, where 0, 1 was used for data scoring, 0 was used for the absence of a band, and 1 was used for the presence of the band. Similarly, for molecular markers analysis, the 0, 1 data was also used and was subjected to two-way cluster analysis using PC-ORD version 6 software and genetic linkages and genetic similarity, and PIC using power marker software version 5.

Results

Morphological traits

Diversity in qualitative traits

The present study revealed the significant diversity of growth habits. 48.14% of genotypes were semi prostrate while 24.07% were prostrate and 27.77% were found to be erect. In terms of plant height, tall or medium-sized plants predominated in the samples (40.74%), with short-length plants having a minimal frequency of 18.51%. Thin, Intermediate, and Thick stem data for thickness were calculated for each selected category, with the largest % frequency recorded for thick stem plants (42.59%) and the lowest % frequency noted for thin stem plants (18.51%). There was a significant difference for the provided parameter between the highest frequency for node hairs that were mildly pubescent (42.59%) and the minimum frequency for extremely pubescent (3.70%). The largest frequency for leaf stiffness was obtained for slightly bent leaves (53.70%), while the lowest frequency was for bent leaves (20.37%). The two types of panicles, unilateral and equilateral, were both recorded, with the greatest frequency for unilateral panicles being 53.70% and for equilateral ones being 46.29%. Three distinct categories of Panicle erectness were observed: drooping, semi-erect, and erect. In which the greatest frequency for semi-erect is 61.11%, and the minimum frequency is 22.22 for both erect and drooping, respectively.

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Spikelets' erectness was also seen in two forms, including semi-erect and drooping. Spikelets were recorded with a maximum of 55.55% for semi-erect and a minimum of 22.22% for erect and drooping. Out of 54 Oat genotypes, four distinct seed colors white, yellow, grey, and red were found. In which the greatest frequency of 62.96% for seeds of the hue grey, 18.51% white, and 9.25% yellow and red. There were two different kinds of seeds identified: covered seeds and bare seeds. In the 54 Oat accessions, 83.33% of covered seeds and 16.66% of bare seeds were recorded.

Quantitative traits

The current study collected data on 10 quantitative features in total, including plant height, number of grains, seed length, diameter, and number of spikelets, as well as plant weight, weight of 100 seeds, leaf length, leaf breadth, and stem diameter. *Table 2* summarizes the quantitative trait values. Leaf length was found to vary by 32.55%, with a range of 10.9–33.00, a standard deviation of 6.49, a standard error of 0.70, and a mean value of 20.99. The percentage of spikelets revealed a CV of 15.66%, a standard deviation of 2.58, a range of 12.00–25.00, a standard error of 0.29, and a mean value of 17.25, respectively. Indicative of the promise of the oats germplasm growing in the research region, all characteristics have shown beneficial variability.

Table 2. *Descriptive statistics for quantitative traits*

Traits	Mean	Standard Error	Standard Deviation	Range	Minimum	Maximum	Coefficient of Variation%
Plant height	89.54	2.99	21.96	67	63	129	25.36
Number of grains	22.32	0.76	2.99	16	13	35	15.76
Seed length	0.5	0.01	0.14	0.4	0.29	0.79	23.45
Seed diameter	4.99	0.16	1.5	5	3	7	28.33
Number of spikelets	17.25	0.29	2.58	9	12	25	15.66
Plant weight	3.45	0.15	1.26	3.99	1.9	6.8	28.76
100 seed weight	2.65	0.04	0.43	1.87	1.27	3.1	19.22
Leaf length	20.99	0.7	6.49	21.90	10.9	33	32.55
Leaf width	1.09	0.02	0.25	0.99	0.4	1.6	21.22
Stem diameter	1.02	0.03	0.24	1	0.4	1.4	19.99

Correlation analysis

The correlation analysis for different morphological traits is presented in *Table 3*. High significance correlations were found between plant height and harvest index (0.79**), between plant height and seed diameter (0.39**), between plant height and leaf length (0.39**), and between plant height and plant biomass negative correlation was found (-0.76**). Additionally, it was shown that seed length was found negatively correlated with seed diameter (-0.78**) and positively correlated with plant biomass

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(0.62*), stem diameter (0.38*), and leaf width (0.33*), respectively. Additionally, there was a positive correlation between seed diameter and harvest index (0.48*), and a positive correlation with leaf length (0.77*). Additionally, it was found that the plant biomass had a +ve correlation with stem diameter at (0.37*) and a -ve correlation with harvest index and leaf length at (-0.83) and (-0.50), respectively (*Table 3*).

Table 3. *Correlation analysis for different quantitative traits used in the present study*

Traits	PH	NG	SL	SD	NSP	PBM	SW	SL	LW	SD
PH	1									
NG	-0.15	1								
SL	-0.42	0.14	1							
SD	0.39*	-0.02	-0.8	1						
NSP	-0.09	0.12	-0.03	0.15	1					
PBM	-0.76	0.2	0.62*	-0.58	0.12	1				
SW	0.26	-0.04	0.05	0.03	-0.02	-0.05	1			
LL	0.39	0.05	-0.77	0.77**	0.1	-0.50	0.06	1		
LW	-0.06	0.16	0.33	-0.25	0.05	0.03	-0.07	-0.26	1	
SD	-0.57	0.11	0.38*	-0.36	-0.01	0.37*	-0.14	-0.33	0.24	1
HI%	0.79**	-0.24	-0.47	0.48	-0.1	-0.83	0.47*	0.40*	-0.07	-0.39

Note: PH-Plant height, NG-Number of grains, SL-Seed length, SD-Seed diameter, NSP-No. Spikelets, PBM-Plant biomass, SW-seed weight, LL-Leaf length, LW-leaf width, SD-Stem diameter, HI-Harvest index%

Cluster analysis

The entire data set of 54 genotypes was split into three clusters, C-I, C-II, and C-III, based on the cluster dendrogram for morphological traits computed using PC-ORD software (*Fig. 1*). These clusters are 87% genetically distant from one another. The C-1 included 18 genotypes, the C-II included 24 genotypes, and the C-III included 12 genotypes, respectively. The groups created from the data set were based on the quantitative traits that were linked together based on specified attributes. i.e., the genotypes that grouped in the C-I in the current study exhibited resemblance and were further split into two groups. Twelve genotypes made up Group-1, and four made up Group-2. Similar to C-II, Group-1 and Group-2 were further separated into two groups. Group-2 had two subgroups: Grou-1, which was made up of 16 genotypes, and Group-2, which was made up of 6 genotypes. However, Group-2 only included 2 genotypes, which were grouped together based on certain qualities. Twelve genotypes with a comparable genetic relationship between the quantitative characteristics made up the third cluster C-III.

SDS-PAGE analysis of the total seed proteins

For biochemical characterization of the collected landraces, total crude proteins were characterized in the current investigation using SDS-PAGE. Electrophorogram displayed a total of 15 bands, 10 of which were polymorphic and 5 of which were monomorphic, demonstrating the high level of protein diversity (*Fig. 2*). The seed total proteins are sufficiently variable, according to gel pictures. In the current investigation, out of 15 protein bands, B-2 was shown to have the most genetic variety at 0.12%, followed by B-3 at 0.11% and B-14 at 0.9%, whereas B- 8, B-11, B-12, and B-13,

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respectively, had the lowest genetic diversity. According to table, the remaining 8 bands similarly displayed a large difference in the genetic diversity of proteins.

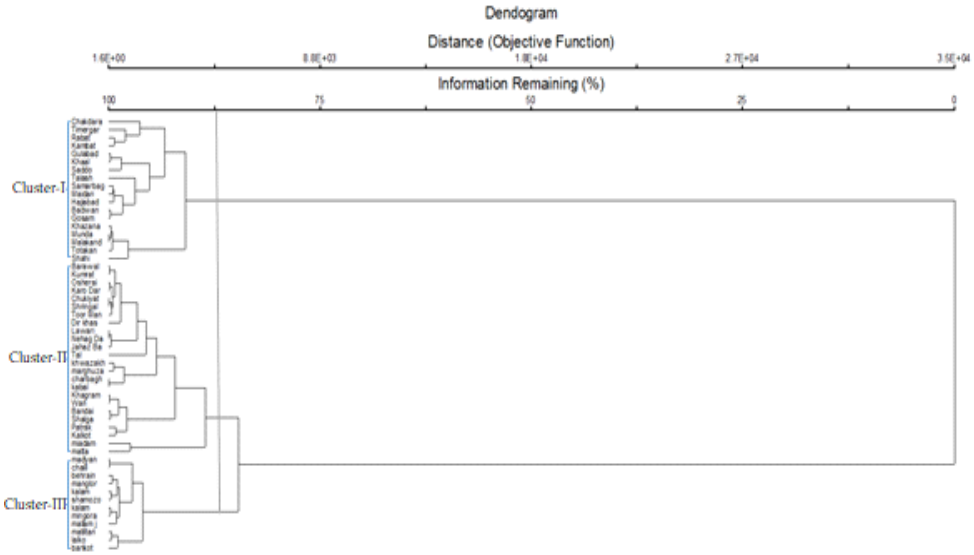

Figure 1. *Cluster dendrogram of the oats genotypes based on quantitative traits*

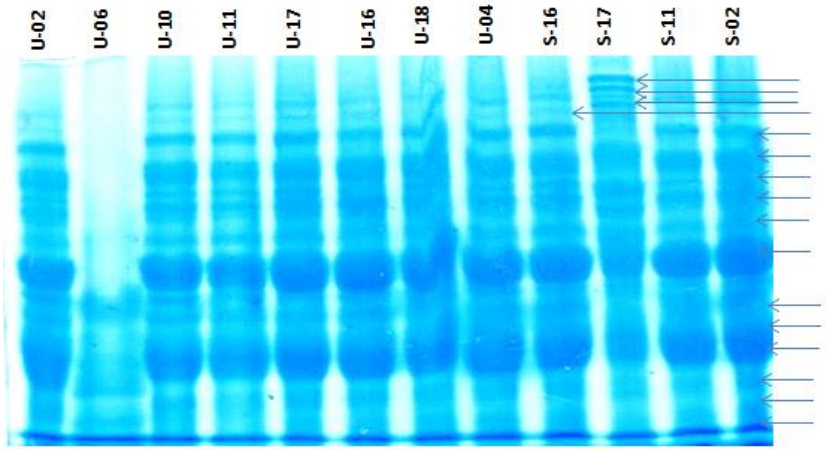

Figure 2. *Representative gel image of the total seed protein showing variation in banding pattern*

Two-way cluster dendrogram based on seed protein profiling

The entire data set of 54 oat genotypes was separated into 2 lineages (L-1 and L-2) at a genetic distance of 36.5% using a two-way cluster dendrogram using the PC-ORD program (*Figure 3*). Three groups were discernible based on band acquired and grouping at 60% genetic distance: Cluster-1 (C-I), Cluster-II (C-II), and Cluster-III (C-III). Only 2 bands made up C-1, 5 bands made up C-II, and 8 bands made up C-III, each of which demonstrated similarities within the cluster and distinctions across clusters.

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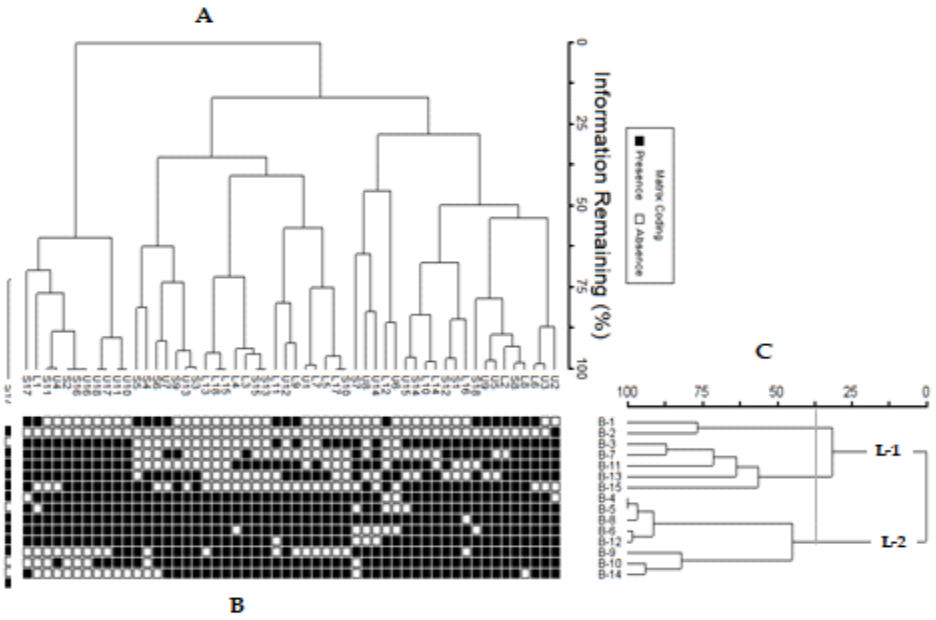

Figure 3. *Two-way cluster dendogram for proteomic data by using PC-ORD software*

Molecular characterization (Simple Sequence Repeats (SSRs) markers)

In the current study, the genetic diversity and allelic variation among the selected oat genotypes were estimated using a total of five SSR-markers. The fact that these primers had a 100% success rate suggests that the oat genome contains many SSRs. *Figure 4* shows that the bands observed by markers HVM62, Z48431, L39777, AF033096, and M83381 ranged from 210 to 320, 100 to 350, 192 to 210, 190 to 260, and 230 to 380, respectively.

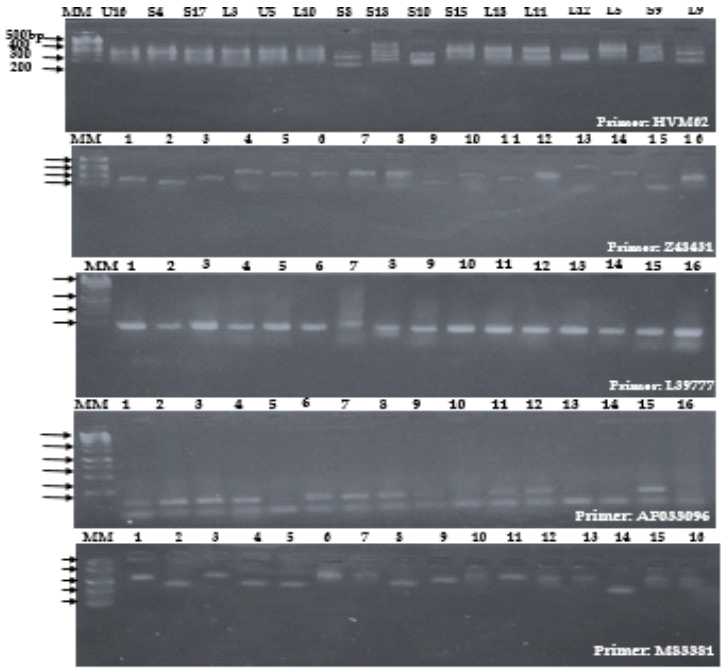

Figure 4. *Gel amplification picture of SSR-Marker used in Oat genotypes*

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In this instance, binary scoring was used, and one allele (as described in the literature) was scored for each primer, yielding a total of five alleles from the five SSR-markers. All had a high amount of genetic variety, but primer Z48431 recorded the most genetic diversity. The genotypes were classified into four groups (G-I, G-II, G-3, and G-4) at 50% genetic distances using the two-way cluster dendrogram produced from 0, 1 data using the PC-ORD software (*Fig. 5*). 3 genotypes made up G-I, 4 landraces made up G-2, 3 landraces made up G-3, and 4 landraces made up G-4, respectively. The primer L39777 had a low level of genetic diversity and a polymorphic information content (PIC) value of 0.74, while primer AF033096 had a PIC value of 0.88. These primers were followed by HVM62 and M83381 with PIC values of 0.90 and 0.89, respectively (*Table 4*).

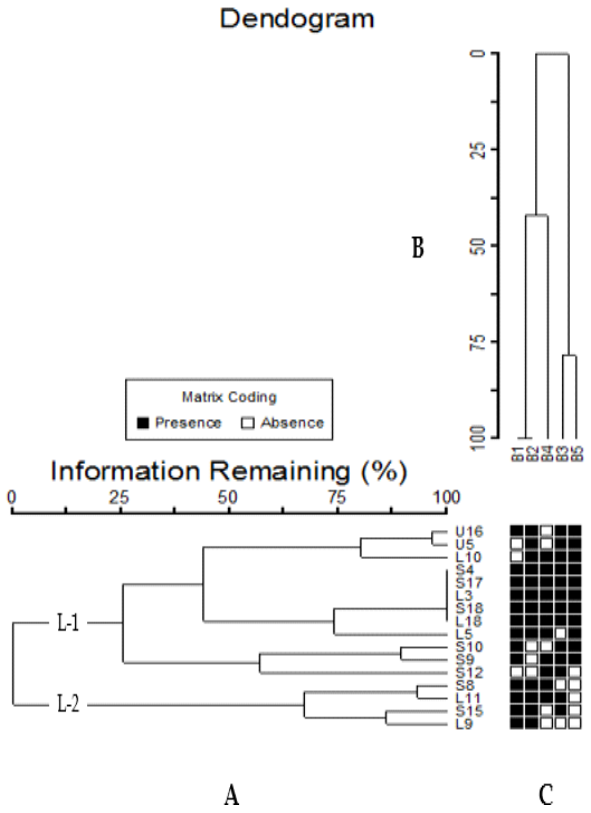

Figure 5. *Two-way cluster dendrogram of SSR-Markers*

Table 4. *Statistical analysis of SSR markers*

Marker	NOB	RS (bp)	AN	AS (bp)	MAF	SS	AVL	PIC
HVM62	15	249	11	209-319	0.18	15	1	0.89
Z48431	15	119	14	99-349	0.12	15	1	0.92
L39777	15	199	7	189-209	0.43	15	1	0.75
AF033096	15	129	10	189-259	0.18	15	1	0.87
M83381	15	168	11	229-379	0.18	15	1	0.88
Mean	15	-	12	-	0.17	15	1	0.88

Note: NOB=Number of observations, RS= Reported size, AN= Allele Number, AS= Allele Size, MAF= Major Allele Frequency, SS= Sample Size, AVL= Availability, PIC= Polymorphic Information Content

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Discussion

Oats are not an exception to the rule that genetic variety is essential in the cultivar and breeding development programs related to any crop (Lithourgidis et al., 2011; Kuzay et al., 2020). To quantify the genetic variety among various oat genotypes, the current effort has been executed to combine the morphological variation with biochemical as well as SSR-based diversity (Dapic et al., 2019). Since many years ago, morphological analysis and agronomic features have been employed to characterize smaller groups of oat species, cultivars, and landraces or massive data sets of *Avena taxa* in gene banks. Because of this, the morphological description has developed into an important source of data for breeding and agronomic research programs (Doehlert et al., 2010; Munir et al., 2016). Although, morphological features are frequently used to estimate the variation in genetics; however, diversity estimates based on morphological traits have drawbacks of their own. The environment has a significant impact on characteristics, and there is a chance that features of agronomical value may be unintentionally selected (Kaur et al., 2018).

54 Oat genotypes were evaluated in the current study based on morphological characteristics, and considerable variance was discovered across genotypes for almost all key parameters that were taken into consideration (Dvořáček et al., 2003). Although expensive technology is not necessary for morphological characterization, considerable areas of land are frequently needed for these investigations. Due to the fact that these variables are frequently subject to phenotypic plasticity, it is possible to quantify variety even in the presence of environmental variation (Nisar et al., 2020).

Here, the variations for both qualitative and quantitative attributes were also evaluated. Ten qualitative characteristics were taken into account, and each displayed some difference in look (Gemma et al., 2007; Glenn et al., 2017). These variations may be attributed to genetic influences, but environmental variables like altitude, climate, soil type, etc. may also contribute to variability through epigenetic modification of the chromatin (Kim and Xing, 2009; Ahmad et al., 2014). In the study area of PGRI, NARC, Islamabad, 124 oat accessions, three checks, and diverse germplasm were assessed for several agro-morphological parameters in the 2013–14 academic years. Regarding the parameters selected in our study, Tanoli et al. (2016) found that there were significant variations in these parameters. The present study was also compared to the study conducted by Ahmad et al. (2014), in which the researchers discovered a large amount of variability among several morphological features. Similar findings have been published by Beyene et al. (2015), who performed a field experiment to assess the performance of seven fodder oat types at the Debre Berhan University, Agricultural Experiment Station in the 2014–2015 academic years. Plant height, leaf number tiller-1, plant number tiller-1, tillers per m^2, and green fodder output were all noted by the authors. The present study was also compared to that by Amare et al. (2016), in which the researchers discovered a large amount of variability among several morphological features (Weih et al., 2008).

A wide range of DNA molecular markers are included in molecular studies and can be used to examine variance. According to Grover (2016) and Gupta et al. (2000), different markers have various potencies as well as restrictions (they may be dominant/co-dominant, amplifying uncharacterized or characterized loci, including expressed or non-expressed sequences, etc.) (Nei et al., 1979; Rauf et al., 2016). Linkage mapping uses SSR markers more frequently than other types of markers because of their co-dominance, excellent repeatability, and polymorphism (Liebhard et

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al., 2002). These are 1 to 6 nucleotide units long repeating sequences. SSR markers support the direct assessment between the alleles of various samples, and the findings are generalizable to other investigated samples (Wendt et al., 2008). Five SSR markers were employed in this study to examine the genetic diversity among selected Oat genotypes from three districts in the Malakand Division. According to Arora et al. (2014) and Zhang et al. (2016), SSRs are transferable markers that are successfully amplified across generic borders. All of the 100% markers generated amplicons from the genomic DNAs, and the primers Z48431 and L39777, respectively, showed the largest genetic diversity (0.93) and the lowest genetic variation (0.74). Genetic diversity of 0.89 was on average. Using 24 SSR primers, Arshad et al. (2003) investigated the genotypes of white and black oats, reporting variance and varying primer performance. The authors concluded that the mean genetic variation was 0.15 and that the genetic variety ranged from 0.66 (for primer GMS001) to 0.99 (for primer HVHVA1) (Hameed et al., 2009; Raza et al., 2019). The results presented here are entirely consistent with those of the authors; among the five primers, amplification effectiveness varied, and these SSRs amplified several bands. Because of their impact on chromatin organization, gene activity, DNA replication, and mismatch repair, SSR distribution is non-random (Au et al., 2006). According to Gioia et al. (2019) and Guzmán et al. (2017), SSRs could offer an evolutionary benefit of quick adaptability to novel settings. When compared to the reference sizes, the size variation of the amplicon was also discovered in the current investigations (Loskutov et al., 2008; Pattananyak et al., 2019). This would point to the SSR region's rapid evolution, and these shifts might not be the result of homologous recombination between various chromosomal regions. Instead, a wide range in size might be linked to unauthorized recombination that is mediated by transposable elements (Boczkowska et al., 2017). Primer HVM62 identified the most alleles, five, while primer AF033096 discovered four alleles, and the remaining primers, Z48431, L39777, and M83381, each detected two alleles. 209 alleles in all, with an average of 14.65 alleles per primer, were produced by the SSR primers (Kianian et al., 1999; Kristensen et al., 2019). Only five of the alleles were shared by all genotypes, meaning that the bulk of them were polymorphic. Additionally, 105 alleles had a frequency lower than 0.05, and 49 alleles were unique. Based on molecular linkage, cluster analysis separated all genotypes into 2-Lineages and 3-Clusters, where C-1 included 9 landraces, C-2 contained 3 landraces, and C-3 contained 4 landraces. It was interesting to see that the impacts of geography were evident in certain genotypes but not in others. This is likely due to the regular exchange of genes and seed materials within the region. Similar research (Nikoloudakis et al., 2016; Osawaru et al., 2015) discovered that geographical locations (such as Greece and Cyprus) had a significant impact on the grouping of various genotypes based on SSR profiling.

Conclusions

A total of 20 morphological features were evaluated in the current study, and there was enough variation in each trait to suggest that there is a very high likelihood of introducing fresh variation into breeding programs for adapted Oat cultivars. The current investigation discovered a strong and favorable association between many qualities. These qualities can be considered while breeding to introduce beneficial features into genotypes of adapted Oats. These 54 genotypes were grouped into three primary clusters using cluster analysis based on the morphological characteristics. In the

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present study total of 15 bands were recorded using SDS-PAGE profiling, with band sizes ranging from 4 to 45 KDa. Band-14 had the highest genetic diversity, which was 0.90%, followed by bands 9 and 10 (0.80% and 0.50%, respectively). The overall findings showed a significant degree of variety in the oat genotypes growing in District Swat and Dir, which offers the potential for the introduction of distinctive diversity in well-adapted Oat cultivars. Future research should strongly consider the testing of nutritional or quality features, which were not addressed in this study.

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APPENDIX

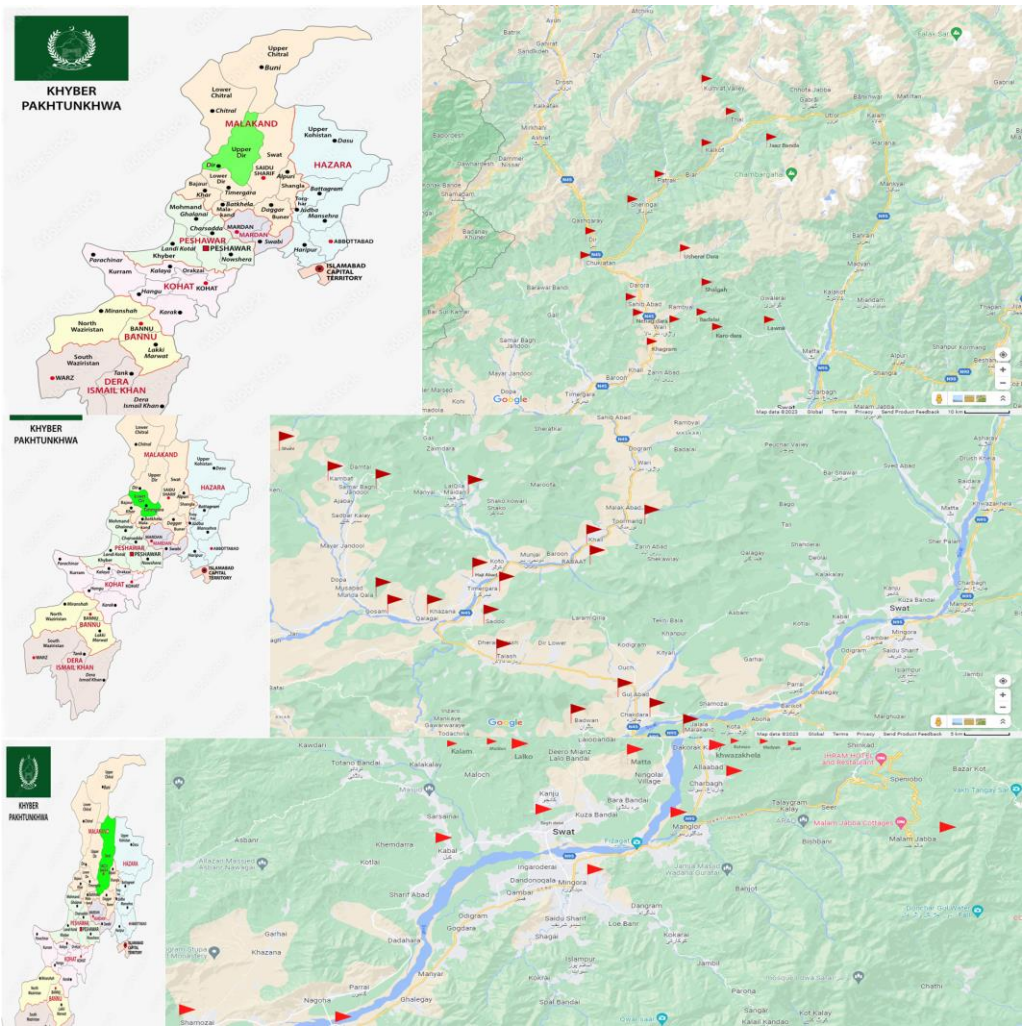

Figure S1. *Oat genotypes collected from different ecological zones of Dir Upper, Dir Lower, and Swat KhyberPakhtun Khwa, Pakistan*

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Table S1. *Passport information of Oat genotypes collected from different ecological zones of Dir Upper, Dir Lower, and Swat KhyberPakhtun Khwa, Pakistan*

S. No	Species name	Area of collection	District	GPS coordination
1	*Avena Fatua*	Chakdara	Dir (L)	34.6666° N, 72.0290° E
2	*-do-*	Gulabad	Dir (L)	34.6960^0 N, 72.0308^0 E
3	*-do-*	Talash	Dir (L)	34.7415^0 N, 71.8720^0 E
4	*-do-*	Khazana	Dir (L)	34.8029^0 N, 71.7934^0 E
5	*-do-*	Saddo	Dir (L)	34.7900^0 N, 71.8579^0 E
6	*-do-*	Timergara	Dir (L)	34.8278^0 N, 71.8423^0 E
7	*-do-*	Munda	Dir (L)	34.8237^0 N, 71.6866^0 E
8	*-do-*	Samarbagh	Dir (L)	34.9117^0 N, 71.6436^0 E
9	*-do-*	Badwan	Dir (L)	34.6576^0 N, 71.9698^0 E
10	*-do-*	Toormang	Dir (L)	34.9137^0 N, 72.0201^0 E
11	*-do-*	Rabat	Dir (L)	34.8646^0 N, 71.9537^0 E
12	*-do-*	Khaal	Dir (L)	34.8945^0 N, 71.9816^0 E
13	*-do-*	Maidan	Dir (L)	34.9519^0 N, 71.8082^0 E
14	*-do-*	Haji Abad	Dir (L)	34.8554^0 N, 71.8482^0 E
15	*-do-*	Malakand	Dir (L)	34.8302^0 N, 71.8295^0 E
16	*-do-*	Kambat	Dir (L)	34.9729^0 N, 71.6688^0 E
17	*-do-*	Gosam	Dir (L)	34.8068^0 N, 71.7022 E
18	*-do-*	Shahi	Dir (L)	35.9440^0 N, 71.4132^0 E
19	*-do-*	Barawal	Dir (U)	35.0901795° N, 71.7634998° E
20	*-do-*	Osherai Dara	Dir (U)	35.1040^0 N, 72.0098^0 E
21	*-do-*	Chukyathan	Dir (U)	35.1224^0 N, 71.5236^0 E
22	*-do-*	Khagram	Dir (U)	34.9263^0 N, 72.0434^0 E
23	*-do-*	Wari	Dir (U)	34.99798^0 N, 72.07295^0 E
24	*-do-*	Sheringal	Dir (U)	35.2781^0 N, 72.0029^0 E
25	*-do-*	Karo Dara	Dir (U)	35.1035^0 N, 71.5071^0 E
26	*-do-*	Dir khas	Dir (U)	35.1655239^0 N, 72.0468164^0 E
27	*-do-*	Lawari	Dir (U)	35.3497^0 N, 71.8023^0 E
28	*-do-*	Nehag Dara	Dir (U)	35.1667^0 N, 71.8333^0 E
29	*-do-*	Patrak	Dir (U)	$35.20\ 47^0$ N, $72.\ 338^0$ E
30	*-do-*	Kalkot	Dir (U)	35.1035^0 N, 71.507183^0 E
31	*-do-*	Thal	Dir (U)	35.32165^0 N, 72.13872^0 E
32	*-do-*	Jaaz Banda	Dir (U)	35.3688^0 N, 72.3444^0 E
33	*-do-*	Kumrat	Dir (U)	35.3222^0 N, 71.1495^0 E
34	*-do-*	Bandai	Dir (U)	35.1667^0 N, 71.8333^0 E
35	*-do-*	Shalga	Dir (U)	35.0867^0 N, 72.1233^0 E
36	*-do-*	Sahib Abad	Dir (U)	35.0493^0 N, 72.0036^0 E
37	*-do-*	Shamozo	Swat	34.684807° N, 72.127991° E
38	*-do-*	Barikot	Swat	34.677778^0 N, 72.221944^0 E
39	*-do-*	Kabal	Swat	34.7923^0 N, 72.2825^0 E
40	*-do-*	Mingora	Swat	$34.\ 462512^0$ N, 72.2135^0 E
41	*-do-*	Matta	Swat	35.093611^0 N, 72.313056^0 E
42	*-do-*	Lalko	Swat	34.706837^0 N, 72.452850^0 E
43	*-do-*	Miadam	Swat	35.0519997^0 N, 7.725564^0 E
44	*-do-*	Marghuzar	Swat	34.6794^0 N, 72.3389^0 E

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S. No	Species name	Area of collection	District	GPS coordination
45	-*do*-	Malam jabba	Swat	34.793163^0 N, 72.569664^0 E
46	-*do*-	Charbagh	Swat	34.8358^0 N, 72.4436^0 E
47	-*do*-	Kalam	Swat	35.4902^0 N, 72.5796^0 E
48	-*do*-	Mangl0r	Swat	34.8076^0 N, 72.4312^0 E
49	-*do*-	Khwaza khela	Swat	34.9371^0 N, 72.4687^0 E
50	-*do*-	Matiltan	Swat	34.8554^0 N, 71.8482^0 E
51	-*do*-	Chail	Swat	35.0902^0 N, 72.3643^0 E
52	-*do*-	Madyan	Swat	35.1404^0 N, 72.5353^0 E
53	-*do*-	Bahrain	Swat	35.2072^0 N, 72.5456^0 E
54	-*do*-	Bagh Derai	Swat	34.706837^0 N, 72.452850^0 E

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