MULTIVARIATE ANALYSIS FOR EVALUATING DIVERSITY IN *AVENA SATIVA* GERMPLASM

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SUMMARY

Avena sativa L. is a quick-growing, highly nutritious fodder of cool climates, grown for various purposes, and can meet feed demands in scarce periods. The study aimed to identify potential genotypes that offer higher fodder yields. Over three years (2018–2020), 225 genotypes collected from the USDA and FRI were analyzed for genetic diversity based on morphological and yield-related characteristics. The experiments followed an alpha lattice design, with data recorded before panicle emergence. The assessment revealed significant genetic variability among the accessions for the studied traits. The principal component analysis demonstrated that three primary components explained the bulk of the total variability each year. Genotypes with high green fodder yield, tillers per plant, leaves per plant, and plant height acquired positions in the right quadrants of the biplots for 2018 and 2020. Positive correlations observed between tillers per plant and leaves per plant and among plant height, tillers per plant, leaves per plant, and green fodder yield. Exotic and local genotypes were widely distributed across all four quadrants, indicating substantial genetic diversity. The cluster analysis classified 225 oat genotypes into 10 groups based on phenotypic characteristics. Clusters II, VIII, IX, and X displayed higher mean values for most studied traits. Clusters with maximum inter-cluster distances, such as Clusters II and X in 2018, I and X in 2019, and III and VIII in 2020, could be useful in future hybridization programs. Genotypes 198 (Mustang) and 219 (Boppy) provided superior fodder yield than standard checks. These genotypes need further evaluation in different locations for sustainable performance and recommendations for general cultivation.

Keywords: *Avena sativa*, morphology, diversity, PCA, cluster analysis

Key findings: Oat genotypes have rich genetic diversity, evident from the dispersal of exotic and local genotypes in biplot quadrants. PCA revealed that tillers per plant and leaves per plant were the major source of variation, followed by plant height. Based on the higher mean values for traits, genotypes from Clusters II, VIII, IX, and X can benefit future hybridization programs.

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INTRODUCTION

Avena sativa L., commonly known as oat, is a member of the Poaceae family, widely cultivated for human consumption and animal feed due to its high nutritional value. *Avena*, an immense and diverse genus that encompasses diploid, tetraploid, and hexaploid species, contains oat, an allohexaploid crop that self-pollinates (2n = 6× = 42) and has the genomic composition AACCDD (Poonia and Phogat, 2017). Oats are the sixth most important cereal crop worldwide, after wheat, rice, maize, barley, and sorghum, ranking higher in significance. Oats are more suitable for cultivation in marginal environments, such as, regions with cool and wet climates and low-fertility soils, versus other cereal crops (Alshadiwi and Alrubaiee, 2022; Zhang *et al.*, 2023). This crop is grown for various purposes, including bedding, hay, haylage, silage, chaff for feed, pasture, and forage straw. It is a succulent and palatable plant that grows fast and recovers quickly from cuts (Dhakal *et al.*, 2023). The harvest stage affects the nutritional composition of green fodder. The plant yields a substantial quantity of forage within a relatively short period of 60–70 days, possessing appropriate nutrients that are abundant in energy, protein, vitamin B, phosphorus, and iron. The protein found in oats is distinct for its higher lysine content, and the amino acid composition of oats is comparatively more balanced than other grains (Bibi *et al.*, 2012). Oat forage exhibits a high nutrition value and boasts a dry matter digestibility exceeding 75% when utilized as feed for dairy cattle.

Recently, the dairy industry in Pakistan has experienced significant growth, leading to an increased interest in oat breeding among researchers. It is because of the plant's potential as a highly nutritious fodder for livestock and its grains offering high net energy gains when used as animal feed (Niazi, 2021). Farmers in central and northern regions of Pakistan faces scarcity of green fodder from mid-November to mid-January. Forage oats cultivation occurs during the winter across diverse soil and climatic conditions in Pakistan to meet feed demand during the lean period (Anwar *et al.*, 2010). Oat farmers in Khyber Pakhtunkhwa acknowledge it as a crucial winter crop that satisfies their fodder requirements, with primary use for animal consumption in its green state. Oats yield abundantly high-quality fodder during periods when other succulent forages are scarce and unavailable for grazing or harvesting as green feed, hay, or silage (Helsel and Skrdla, 1983; Ahmed *et al.*, 2011; Al-Yasari, 2022). Combined with other legumes commonly grown during the cold season, such as, berseem, lucerne, senji, shaftal, pea, and vetch, it produces a highly effective combination (Hussain *et al.*, 2010).

Evaluating *Avena sativa* germplasm for higher fodder yield is crucial in developing improved varieties. By conducting systematic evaluations, researchers can identify genetic diversity within the species and select genotypes that exhibit superior yield ability (Arora *et al.*, 2021). It allows us to identify unique genotypes that possess traits absent in existing varieties. Hence, by incorporating these traits into already established cultivars, researchers can develop new genetic material and broaden the genetic base of *Avena sativa* (Sahu and Tiwari, 2020). It will help to select and develop improved varieties tailored specifically for multi-cut fodder production, with increased productivity and enhanced feed availability for livestock and other agricultural purposes (Tester and Langridge, 2010). Researchers have only conducted a few studies to determine the high-yielding oat strains. Accordingly, a pressing need for additional feedlots and identifying oat cultivars with enhanced nutritional value is necessary, thereby augmenting the demand for this crop (Ihsan *et al.*, 2021). The initial stage of a breeding program involves assessing the extent of variability in traits of agricultural significance across a vast pool of genetic resources to identify the most promising populations for subsequent evaluation (Liaqat *et al.*, 2023; Zaman *et al.,* 2023a). Genetic diversity evaluation can succeed through morphological measurements and phenotypic characterization (Leišová-Svobodová *et al.*, 2019). The monitored agronomic traits include various aspects, such as, fodder quality traits,

flowering, maturity, plant height, tillers per plant, leaves per plant, leaf area, resistance to pests and diseases, drought, and cold tolerances, among others (Annicchiarico *et al*., 2015). Employing appropriate statistical methods to evaluate bio-agronomic and quality characteristics is a valuable strategy for the preliminary description and classification of oat assortments (Zaman *et al.,* 2023b). This approach facilitates the identification and selection of advantageous genetic resources by plant breeders, which can be readily available for farmers, integrated into breeding initiatives, or utilized for efficient germplasm preservation and exploitation (Iannucci *et al*., 2011).

Applying multivariate analysis techniques, such as principal component analysis (PCA) and cluster analysis, can aid in determining interrelationships among various accessions. Its base is on the genetic variation in the germplasm for agronomic traits (Tang *et al*., 2014). According to Kumari and Jindal (2019), several researchers have highlighted the importance of parental diversity in achieving optimal levels for producing superior genotypes in segregating generations. Cluster analysis, to calculate the group distance based on various characteristics, quantified the degree of variation. Assessing genetic divergence through evaluating multiple traits has emerged as an essential technique

(Adélaïde *et al.,* 2023). Understanding diversity patterns enables breeders to gain improved insights into the evolutionary relationships among genotypes, thereby facilitating the development of desired varieties that can produce maximum yield (Bohra *et al*., 2022). The relevant study sought to assess the genetic diversity and similarity among 225 oat accessions and characterize oat germplasm through multivariate analysis. This study contributed insights into the genetic diversity and performance of *Avena sativa* L. genotypes. Using principal component analysis, biplots, and clustering analysis enhanced further understanding of oat germplasm, providing valuable information for future breeding programs, ultimately aiming to improve fodder yield and crop productivity.

MATERIALS AND METHODS

Experimentation

The study was conducted in three consecutive rabi seasons (November – May) of 2018, 2019, and 2020 in the research fields of the Plant Breeding and Genetics department, University of Agriculture Faisalabad, Pakistan. The area has an altitude of 184.4 m between 31° – 26° N latitude and 73° – 60° E longitude, with metrological conditions shown in Figure 1. Two hundred and fifteen genotypes from the USDA
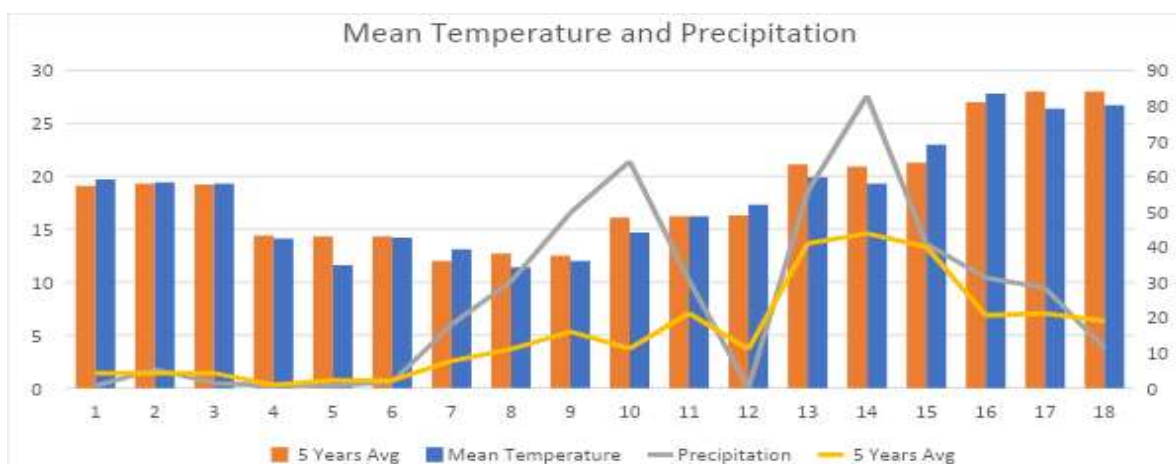
Figure 1. Meteorological conditions at the experimental site during the rabi seasons (Nov-May) of 2018, 2019, and 2020.

gene bank and 10 varieties (standards or checks) from the Fodder Research Institute Sargodha underwent evaluation and comparison of fodder yield traits in three consecutive years.

Field preparations

Sowing of 225 genotypes was in two repeats, applying alpha lattice design in a 2.5-m length row by maintaining a row-to-row distance of 45.72 cm and a plant-to-plant distance of 15.24 cm. The study was conducted on loamy soil of 3.7% clay, 53% silt, and 43.3% sand. The ground contained 0.04% nitrogen, 39.5 mg/kg of phosphorus, 128 mg/kg of potassium, and a pH of 8. The recommended methodologies included soil preparation, plowing, leveling, irrigation (48 h post-sowing), and weed management were followed. Prior to sowing, applying herbicides (Dual-gold 960 EC, Syngenta, Basel, Switzerland) helped prevent the growth of undesired plants.

Morphological evaluation

Five plants from each genotype were randomly selected before panicle emergence and data were recorded for five quantitative morphological traits, following the guidelines of IPBGR-1985 devised for oat (*Avena sativa* L.). These traits included plant height (cm), number of tillers per plant, leaves per plant, leaf area (cm^2), and green fodder yield (t/ha). The study involved quantifying tillers and leaves per plant, as well as measuring plant height from the base to the top using a meter rod upon reaching maturity. For weighing and recording the resultant biomass of selected plants, a digital balance was used. Leaf area calculation was followed by measuring leaf length and width with a meter rod, then multiplying leaf length, width, and correction factor 0.75.

Statistical analysis

The data collected for all the characters mentioned earlier got analysis for each separate year, i.e., 2018, 2019, and 2020. Analysis of variance (ANOVA) performed on the

recorded data was according to the alpha-lattice design with two replications (Patterson and Hunter, 1983). Analysis of the relationships/association between fodder yield characteristics resulted from principal component analysis (PCA), conducted separately for each growing season over three consecutive years using R software. PCA-based biplots served as multivariate analysis to visualize genotypic diversity in 225 genotypes grown under three different rabi seasons (2018, 2019, and 2020) based on collectively recorded data of various fodder yield traits (Abdi and Williams, 2010). Cluster analysis facilitated grouping sets of accessions into homogeneous classes by Pearson distance measurement with the complete linkage method using Minitab software. Cluster analysis helped evaluate the diversity of germplasm (Anderberg, 2014).

RESULTS

Combined analysis of variance

The results of the combined ANOVA indicated a statistically significant variation in all traits for genotypes and environments (years) ($P <$ 0.001). Considerable variation was present within the oat germplasm concerning yield and agronomic characteristics (Table 1).

Principal component analysis

A principal component analysis of the five traits ascertained the variation patterns and identified the relationship structures between the observed traits. The PCA showed that the studied quantitative features could subdivide into five PCs. During the study, PC1, PC2, and PC3 explained 89%, 88%, and 85% of the variation in the dataset with eigenvalues greater than one for 2018, 2019, and 2020, respectively. The highest eigenvalue (2.31) emerged in PC1 in 2018.

In 2018 and 2019, the first principal component accounted for 46% of the total variation, while in 2020, PC1 explained 42% of the total variation. The full disparity was positively associated with tillers per plant,

Table 1. Combined analysis of variance among 225 oat genotypes for fodder yield and related traits over three years.

Source	DF	PH	TPP	LPP	LA	GFY
Gen	224	1262***	42.44***	867.2***	1512.9***	739.4***
Env	2	30^{ns}	16.53***	312.7***	36.3*	2384.5***
Env: Rep	3	38097***	350.82***	8280.8***	25355.8***	16582***
Gen: Env	448	89***	8.23***	195***	124.1***	11.1^{ns}
Env: Rep: Blk	84	11^{ns}	0.35^{ns}	7.5^{ns}	8.4^{ns}	16.9^{ns}
Residuals	588	12	0.28	7.3	9.6	16.9

***= Significant at 0.001 significance level, *= Significant at 0.05 significance level, ns= Non-significant, DF= Degree of freedom, Gen= Genotype, Env= Environment, Rep= Replications, Blk= Block, PH= Plant height, TPP= Tillers per plant, LPP= Leaves per plant, LA= Leaf area, GFY= Green fodder yield.

Table 2. Principal components of traits examined for the years 2018, 2019, and 2020.

| Traits	PC1			PC2			PC3			PC4			PC5		
	2018	2019	2020	2018	2019	2020	2018	2019	2020	2018	2019	2020	2018	2019	2020
PH	0.27	-0.27	0.27	0.76	-0.69	-0.73	0.27	-0.47	0.22	-0.51	0.46	-0.58	0.11	0.13	0.12
TPP	0.58	-0.59	0.60	-0.28	0.23	0.30	-0.26	0.28	-0.20	-0.09	0.09	-0.03	0.71	0.72	0.71
LPP	0.59	-0.59	0.60	-0.24	0.23	0.29	-0.19	0.16	-0.14	-0.30	0.35	-0.27	-0.68	-0.67	-0.68
LA	0.01	0.09	-0.07	-0.49	0.64	0.47	0.83	-0.71	0.82	-0.28	0.26	-0.30	0.07	0.11	0.08
GFY	0.49	-0.47	0.45	0.21	-0.07	-0.27	0.38	-0.42	0.46	0.75	-0.76	0.71	-0.09	-0.11	-0.10
EV	2.31	2.30	2.08	1.10	1.04	1.15	1.03	1.03	1.01	0.43	0.51	0.56	0.11	0.12	0.21
Var %	46.3	46.02	41.5	22.0	20.9	22.9	20.7	20.6	20.2	8.66	10.1	11.2	2.29	2.34	4.18
Cum v.	46.3	46.02	41.5	68.3	66.9	64.4	89.1	87.5	84.6	97.7	97.6	95.8	100	100	100

PH= Plant height, TPP= Tillers per plant, LPP= Leaves per plant, LA= Leaf area, GFY= Green fodder yield, EV= Eigenvalue, Var= Variance %, Cum V.= Cumulative Variance %.

leaves per plant, and green fodder yield in the first PC in 2018 and 2020. In 2019, variation correlated negatively with tillers per plant, leaves per plant, and green fodder yield in the first PC. In PC1, tillers per plant had the most positive (2020) or negative (2019) contributions to the variance. The second PC showed variation contributions of 22%, 21%, and 23% in 2018, 2019, and 2020, respectively. For the second PC, plant height and green fodder yield in 2018 contributed positively to the variation, while in 2019 and 2020, leaf area contributed positively. A negative association with variation in the second PC came from leaf area in 2018 and plant height in 2019 and 2020. In the second main factor, plant height had the most positive and negative contributions to variance in 2018 and 2020, respectively. The third PC accounted for 21% of the overall variations in 2018 and 2019 and 20% in 2020. Tillers per plant in 2018 and 2020, plant height and leaf area in 2019 also showed negative differences in the third PC. Positive effects on variation by leaf

area appeared in 2018 and 2020, from tillers per plant in 2019 in the third PC. Maximum positive and negative associations with variation were notable for leaf area in 2018 and 2019, respectively (Table 2).

Biplot analysis helped determine how the traits relate to each other. The two PCs, PC1 and PC2, formed a biplot that showed total variations of 68%, 67%, and 64% for 2018, 2019, and 2020, respectively. The biplots, placed next to each other, found out how the genotypes differ genetically and how they associate geographically. The separation based on PC1 and PC2 revealed the distribution of the genotypes in all four quadrants. It signified that the genotypes studied had a high level of genetic diversity. Local and exotic genotypes were spread distinctively across the biplot, showing that the studied germplasm was highly diverse (Figures 2, 3, and 4).

The best performing genotypes for plant height were 198 (Mustang), 177 (Beltsville 62-85), 59 (Fulghum), 68 (Hull-less Type HA14), and 203 (Canada cluster) in
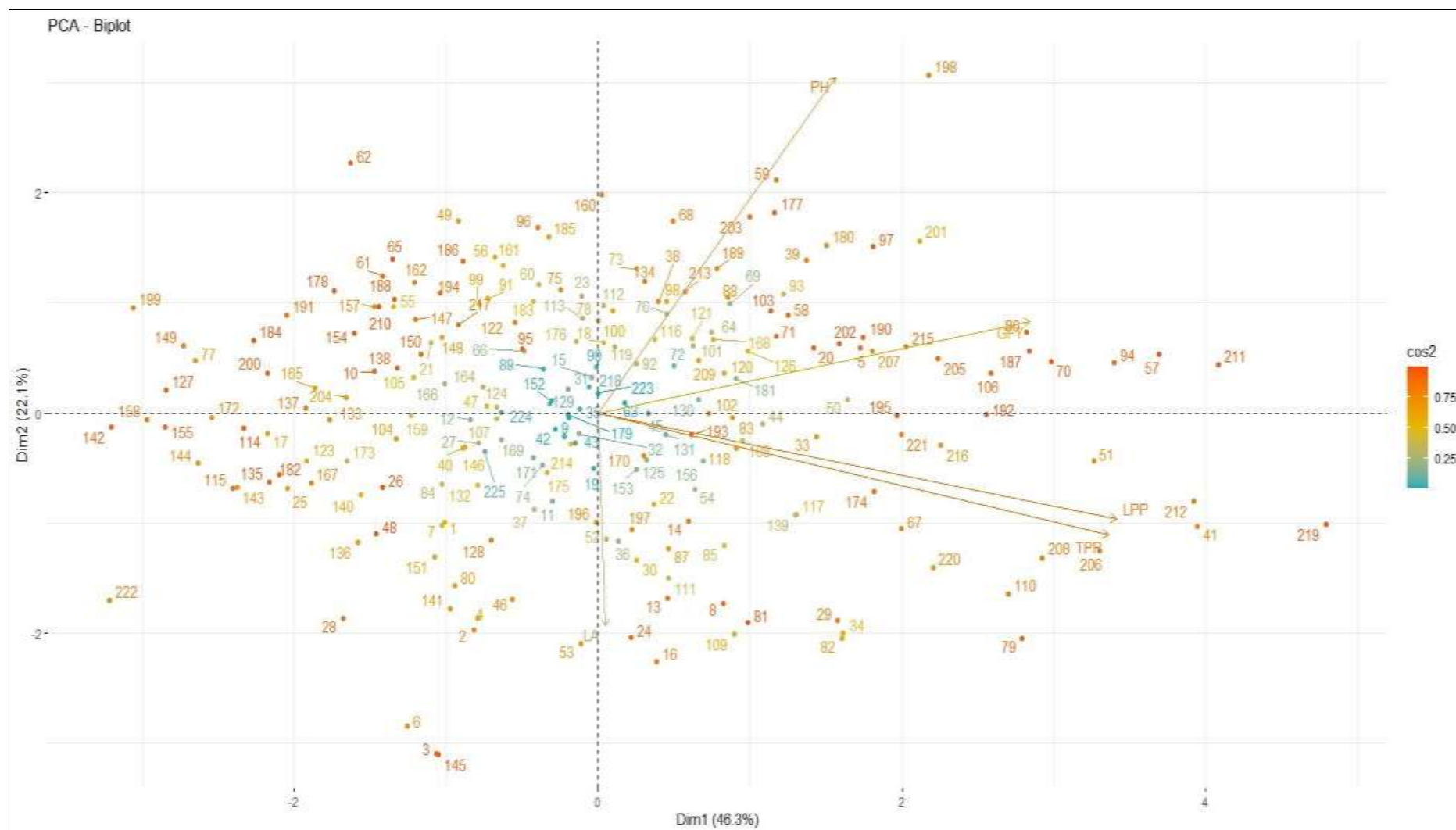
Figure 2. The PCA-Biplot of all traits evaluated in 2018. PH: Plant height, TPP: Tillers per plant, LPP: Leaves per plant, LA: Leaf area, GFY: Green fodder yield.
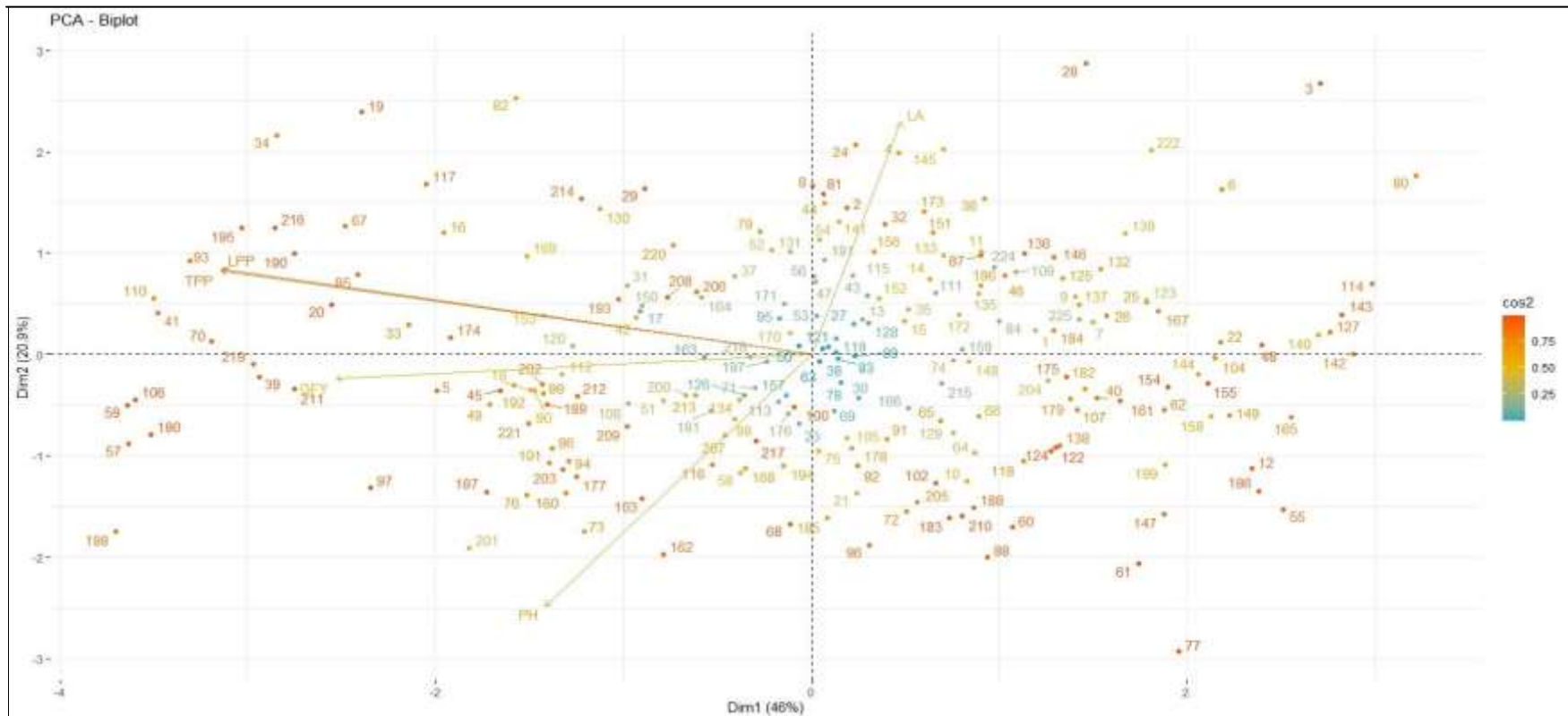
Figure 3. The PCA-Biplot of all traits evaluated in 2019. PH: Plant height, TPP: Tillers per plant, LPP: Leaves per plant, LA: Leaf area, GFY: Green fodder yield.
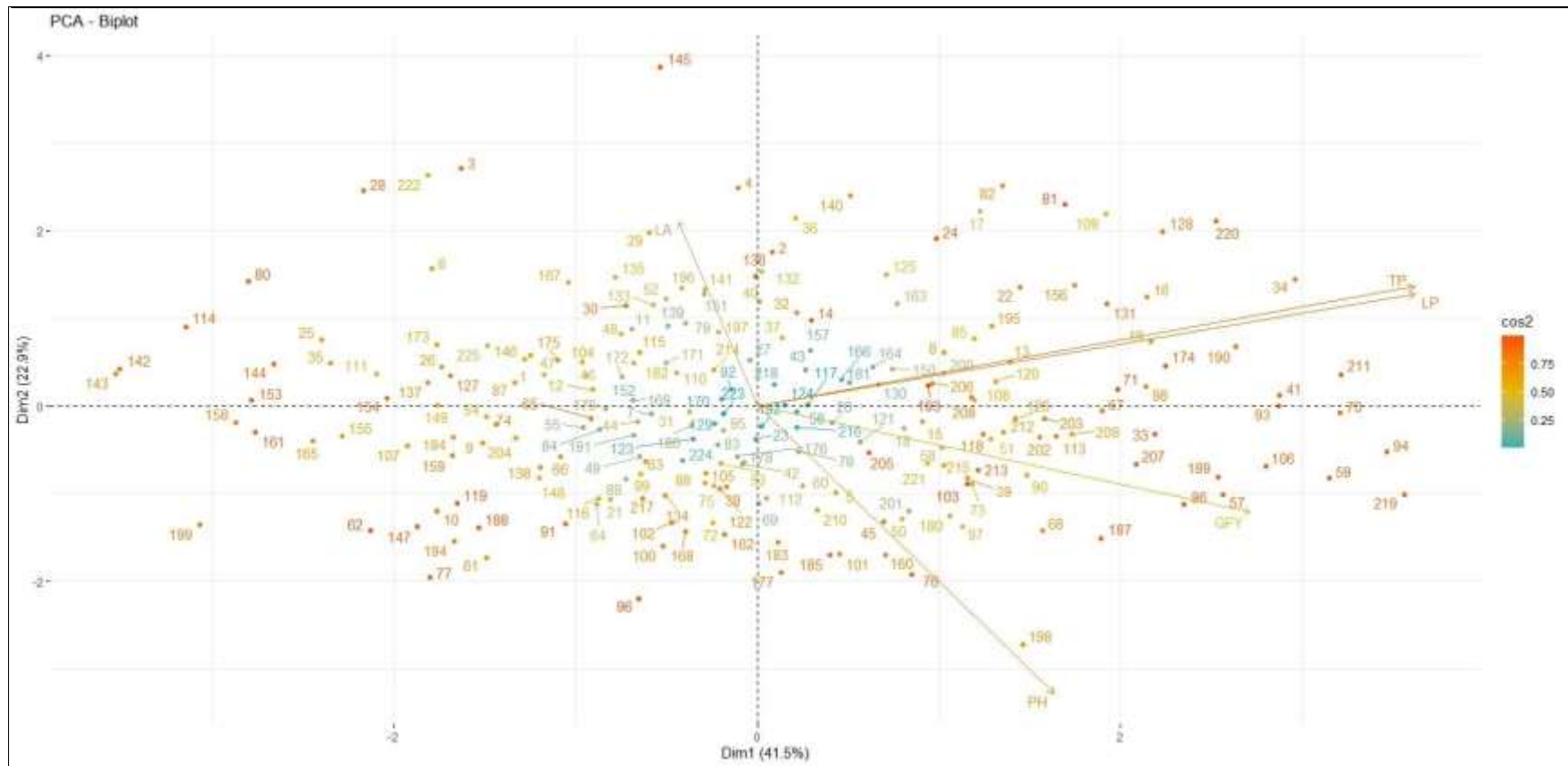
Figure 4. The PCA-Biplot of all traits evaluated in 2020. PH: Plant height, TPP: Tillers per plant, LPP: Leaves per plant, LA: Leaf area, GFY: Green fodder yield.

2018; 198 (Mustang), 201 (CW 553), 97 (CI 7396), 73 (Black Algerian), and 162 (58D-1220) in 2019; and 198 (Mustang), 97 (CI 7396), 76 (Klein Mar), 185 (Massachusetts C-2-2-58), and 177 (Beltsville 62-85) in 2020, according to the biplot. Genotypes having the best green fodder yield potential included 198 (Mustang), 201 (CW 553), 86 (CI 5509), and 211 (Guyra) in 2018; genotypes 198 (Mustang), 180 (47Ab2685), 16 (Cliff), 219 (Boppy), and 57 (Silvermine) in 2019; and genotypes 198 (Mustang), 57 (Silvermine), 219 (Boppy), 59 (Fulghum), and 106 (Missouri 04773) in 2020. Oat genotypes associated with higher leaves per plant and tillers per plant included 212 (Gray Winter), 219 (Boppy), 41 (Taxas Red), 206 (Mariner), and 208 (X424III) in 2018; 195 (Mustang Selection), 110 (Missouri 04780), 41 (Taxas Red), 70 (S.E.S. No. 42), and 190 (Kherson 34) in 2019; and genotypes 34 (Welcome), 16 (Cliff), 13 (O.A.C.No. 72), 190 (Kherson 34), and 211 (Guyra) in 2020. Better-performing genotypes for leaf area included 6 (Record), 11 (Aurora), 19 (Red Rustproof), and 36 (President) in 2018; genotypes 24 (Fulghum), 28 (College Wonder), 2 (Thousand Dollar), and 32 (Bonanza) in 2019; and genotypes 3 (White Russian), 4 (Yellow), 29 (California Red), and 145 (Florida 59-RR558) in 2020. Oat genotypes with higher green fodder yield, tillers per plant, leaves per plant, leaf area, and plant height were in the positive coordinates of the biplot for 2018 and 2020. Tillers per plant and leaves per plant indicated a significant positive correlation, and plant height, tillers per plant, and leaves per plant had a strong positive correlation with green fodder yield.

Cluster analysis of studied traits for the years 2018, 2019, and 2020

Adopting a complete linkage method with Pearson distance measurement helped perform the cluster analysis. Based on cluster analysis, all genotypes indicated separating into three primary lineages (lineage I, II, and III) at a 25% distance in 2018 and 2019 and at a 15% distance in 2020, further subdivided into 10 subclusters at about 50% distance. The cluster

analysis dendrogram classified the 225 oat genotypes into 10 groups according to the following characteristics: plant height, number of tillers per plant, number of leaves per plant, leaf area, and green fodder yield. Lineages I, II, and III in 2018 were subdivided into five, one, and four clusters, respectively. Lineages I, II, and III in 2019 were segmented into two, five, and three aggregates, respectively. In 2020, lineages I, II, and III further broke into five, two, and three clusters, respectively (Figures 5, 6, and 7).

Intra-and inter–cluster divergence

Genotypes with taller plant heights can be found in Cluster III in 2018, VIII 2020 and Cluster X in 2019. Cluster II had the highest mean values for leaf area in 2018 and 2020, and Cluster VIII in 2019. Tillers per plant were higher in genotypes from Cluster VII in 2018, VIII in 2019 and IX in 2020. For 2018, genotypes with higher LPP belonged to Cluster VII, Cluster VIII in 2019 and Cluster IX in 2020. The highest green fodder yield accessions resulted in Cluster IX in 2018, Cluster X in 2019, while in 2020, they were higher in Cluster IV. Genotypes from clusters with higher mean values can benefit future breeding programs to improve green fodder yield. Cluster VIII in 2018, IX in 2019, and X in 2020 had the lowest mean values for green fodder yield. The difference in the five investigated attributes had maximum influences from plant height, followed by leaves per plant and leaf area. (Table 3)

In 2018, 2019, and 2020, the distances within a cluster were shorter than the distances between clusters. It shows that they are similar and have less genetic diversity within them. The maximum distance between clusters with a value of 64.39 appeared between Clusters II and X in 2018, Cluster I and Cluster X with 76.47 in 2019, and Cluster III and Cluster VIII with 68.07 in 2020. Thus, parents from these distinct clusters may be useful in a hybridization program to create superior segregates. A minimum genetic diversity of 19.82 occurred between Clusters I and IV in 2018, Clusters IV and VI, with a value of 19.99 in 2019, and Clusters IV and
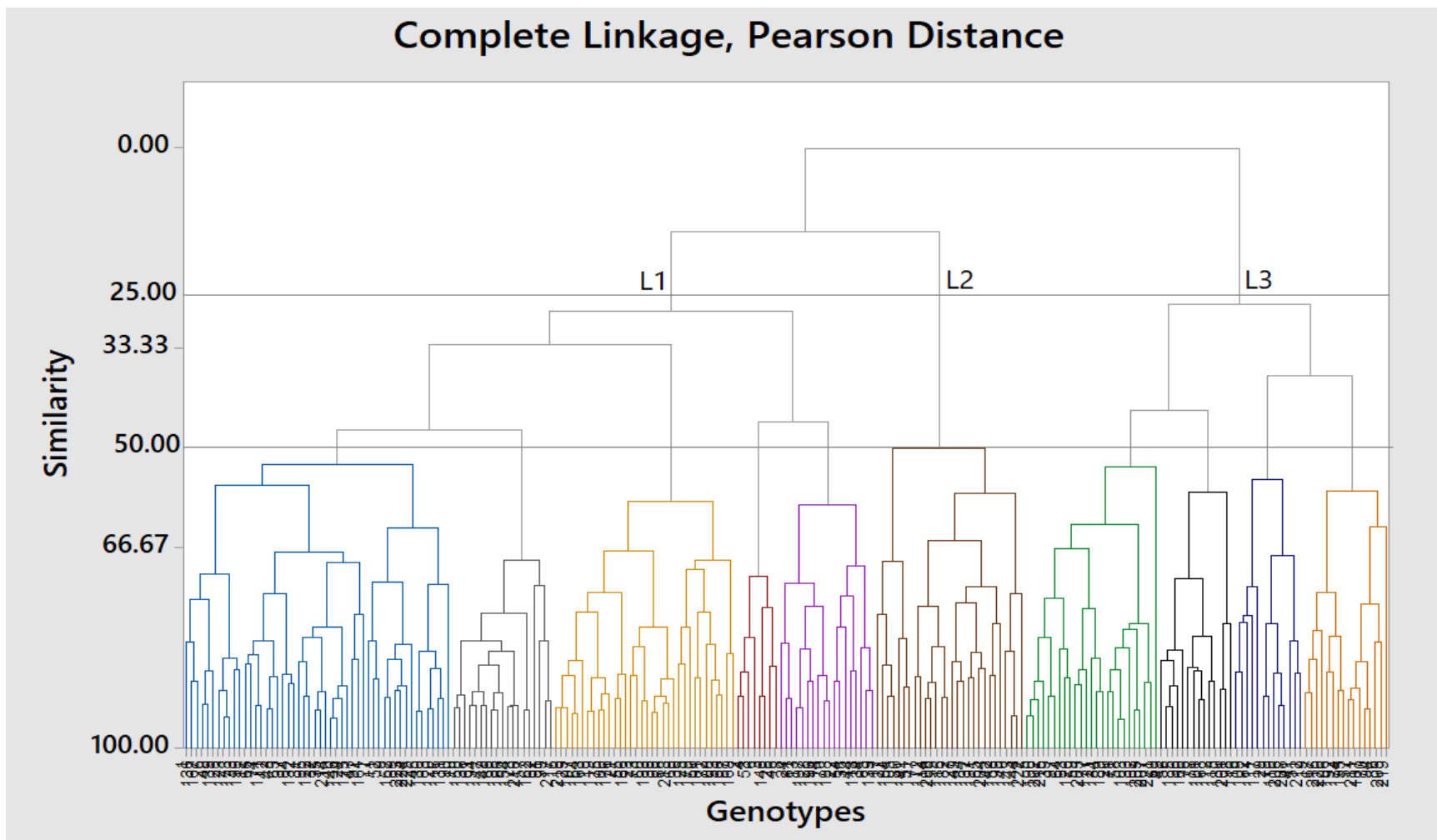
Figure 5. Cluster analysis dendrogram for the year 2018.
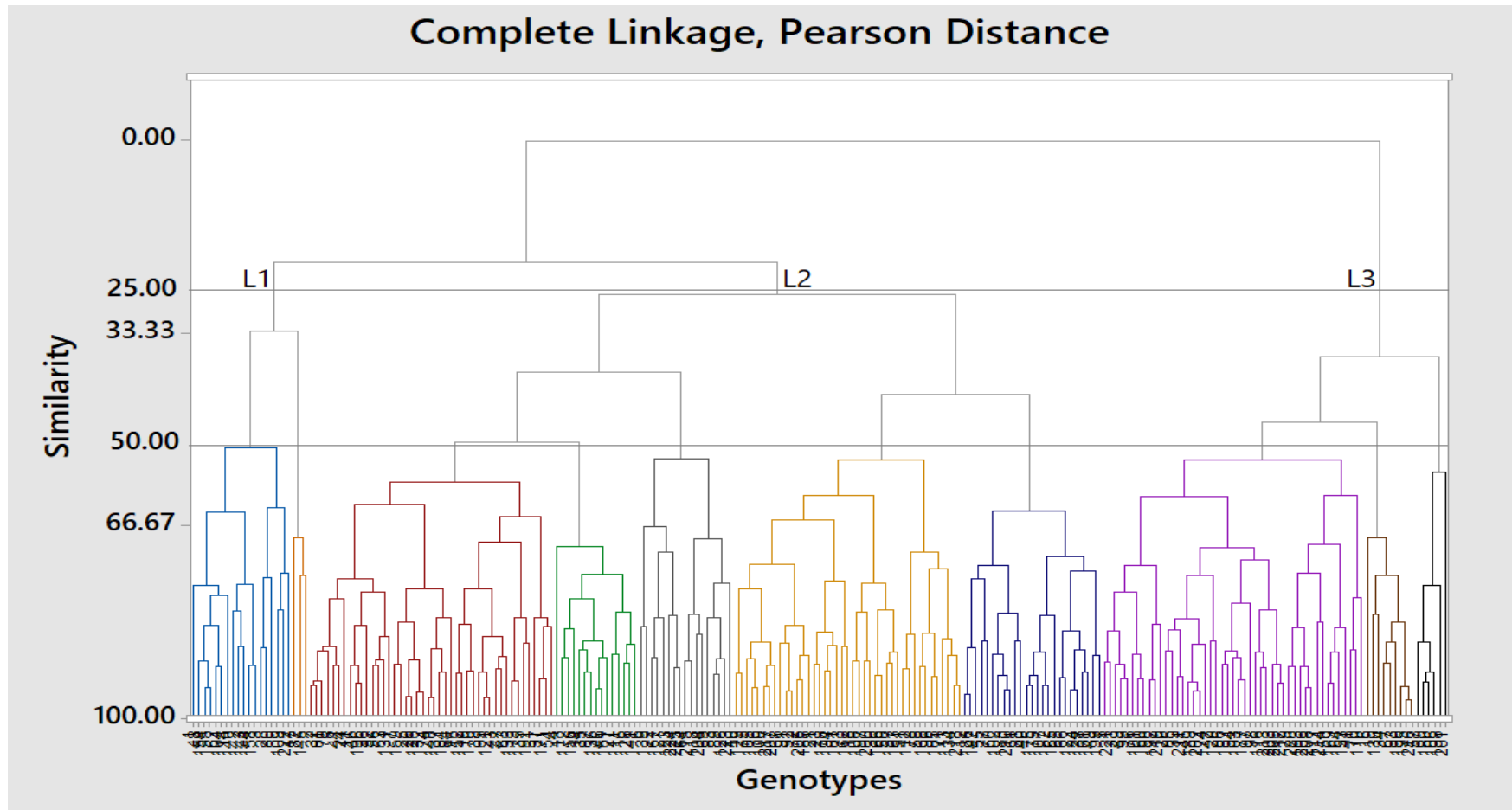
Figure 6. Cluster analysis dendrogram for the year 2019.
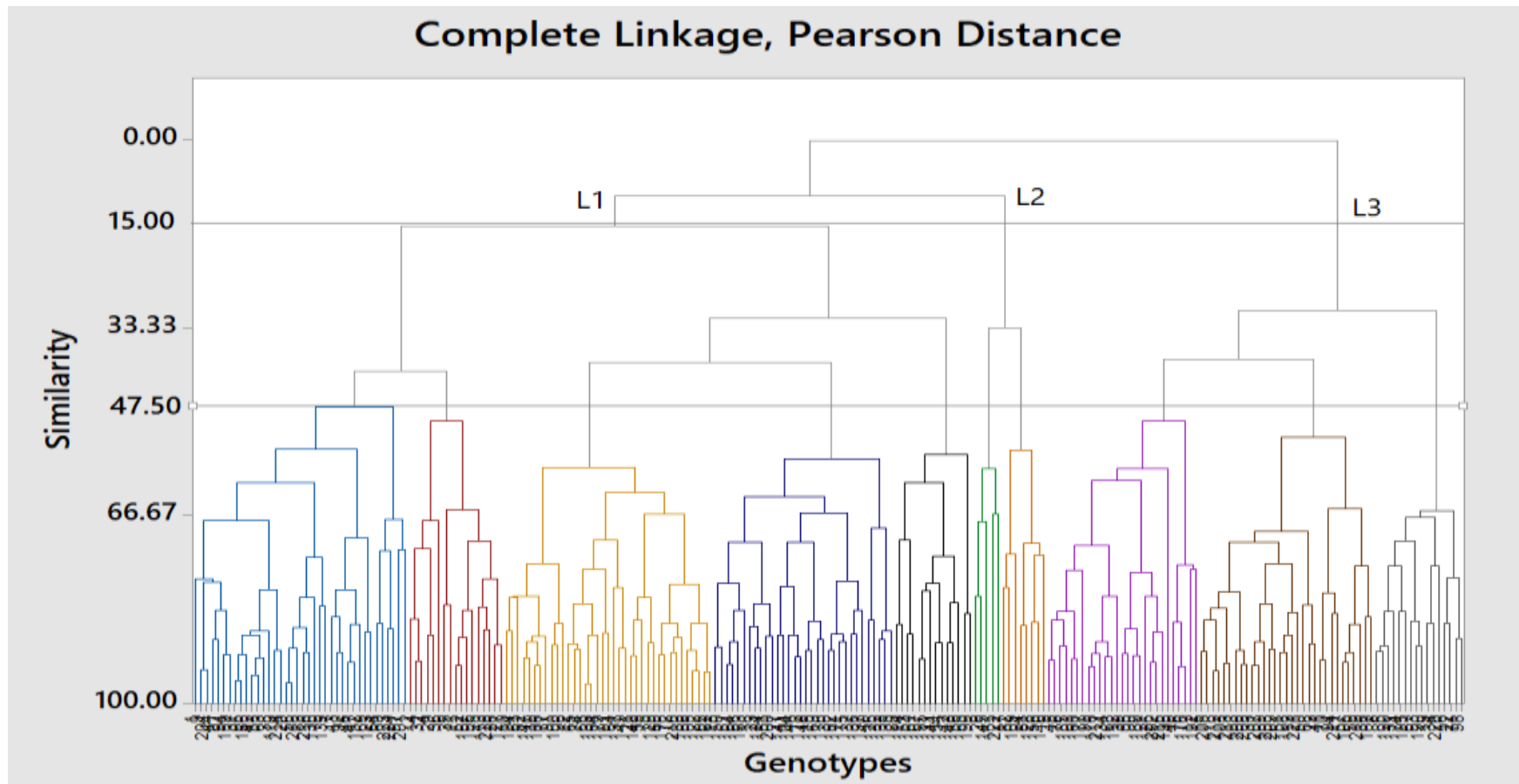
Figure 7. Cluster analysis dendrogram for the year 2020.

Table 3. Cluster centroids of examined traits for the years 2018, 2019, and 2020.

Variables	Cluster I			Cluster II			Cluster III			Cluster IV			Cluster V		
	2018	2019	2020	2018	2019	2020	2018	2019	2020	2018	2019	2020	2018	2019	2020
PH	103.7	73.8	106.5	68.4	100.4	87.4	118.4	81.4	56.1	96.5	109.0	109.0	110.3	107.2	107.5
TPP	8.3	7.2	8.2	8.4	9.1	8.2	10.8	9.9	10.6	10.9	13.0	9.2	6.7	6.8	14.6
LPP	54.5	38.9	53.5	64.9	63.4	55.5	69.9	62.7	54.3	72.2	75.9	56.9	44.2	43.9	79.3
LA	68.2	46.1	67.0	76.0	69.2	78.4	47.5	48.6	61.8	71.5	44.8	42.4	43.4	73.1	68.5
GFY	50.8	38.2	55.3	49.2	43.9	49.2	63.4	43.4	42.5	54.1	56.6	64.1	46.9	52.0	60.1
Variables	Cluster VI			Cluster VII			Cluster VIII			Cluster IX			Cluster X		
	2018	2019	2020	2018	2019	2020	2018	2019	2020	2018	2019	2020	2018	2019	2020
PH	106.7	108.7	111.0	87.7	108.4	95.3	86.0	106.8	113.5	114.4	67.8	79.8	111.5	125.3	83.8
TPP	10.3	10.2	7.8	16.5	6.5	10.7	6.8	15.9	13.6	14.6	14.7	16.5	8.8	15.5	5.6
LPP	67.6	61.7	50.2	78.4	45.1	66.8	40.5	77.3	75.1	79.2	79.7	81.5	53.8	79.4	34.0
LA	34.4	35.5	40.4	42.2	40.8	48.1	40.0	74.5	37.2	57.7	37.3	49.3	33.6	35.9	41.1
GFY	45.4	46.4	42.4	56.4	37.4	41.3	38.6	61.2	59.8	70.0	35.9	46.0	68.3	75.4	35.1

PH: Plant height, TPP: Tillers per plant, LPP: Leaves per plant, LA: Leaf area, GFY: Green fodder yield.

Table 4. Intra- and inter-cluster distances between cluster centroids of traits examined for 2018, 2019, and 2020.

Clusters	Cluster I			Cluster II			Cluster III			Cluster IV			Cluster V			Cluster VI			Cluster VII			Cluster VIII			Cluster IX			Cluster X		
	18	19	20	18	19	20	18	19	20	18	19	20	18	19	20	18	19	20	18	19	20	18	19	20	18	19	20	18	19	20
Cluster I	21	23	18	38	43	23	32	26	52	20	55	26	28	45	27	37	44	30	40	36	29	38	63	38	35	43	44	40	77	45
Cluster II				13	16	20	60	28	36	30	32	45	57	23	35	57	35	46	43	35	34	48	25	54	56	49	41	64	55	46
Cluster III							16	13	21	34	34	60	32	41	60	25	31	59	34	34	43	51	43	68	16	26	39	23	59	41
Cluster IV										18	18	19	43	43	35	40	20	23	32	37	29	49	30	20	29	47	43	47	27	45
Cluster V													12	20	15	26	42	45	43	36	33	26	36	32	45	67	37	26	60	64
Cluster VI																17	17	17	26	20	24	35	45	31	37	46	46	27	38	33
Cluster VII																			19	16	18	43	53	30	34	54	23	38	55	36
Cluster VIII																						19	12	16	61	60	39	42	45	57
Cluster IX																									19	20	20	36	70	51
Cluster X																												16	13	19

VIII in 2020, with a value of 20.40, suggesting that their members are genetically extremely close (Table 4).

DISCUSSION

The presence of diverse genetic materials provides opportunities for genetic enhancement efforts. Many methods for quantifying genetic diversity exist, but using multivariate analysis is, by far, the most common (Montilla-Bascon *et al*., 2013). Studying morphological characteristics also makes it possible to select and keep genotypes for future use. However, climate greatly influences morphological parameters, which may have a positive or negative effect on the genetic potential of plants; therefore, data from multiple years in each locality is necessary for the effective reproduction of a particular plant (Jan *et al*., 2019). Additionally, there is no restriction on the movement of seed stock between regions for direct introduction or breeding. Character patterns normally in association with a certain geographical area become generic.

Multivariate analysis is the most common method for measuring the genetic heterogeneity required for the genetic improvement of crops (Kumar *et al*., 2021). The wide use by different scientists helps assess genetic diversity in plant breeding programs (Lei *et al*., 2020; Shi *et al*., 2019)**.** Through this analysis, vast collections of germplasm may have simultaneous evaluation for several morphological traits, which are otherwise difficult to manage. Thus, selecting parents according to their level of genetic divergence has proven effective in various plant species (Priyanka *et al*., 2021). It is general for researchers to recognize that higher fodder yields can succeed through parents' selection with a broader diversity. Thus, plant breeders may find it easier to choose desirable parents if they have clear selection criteria for agronomic traits contributing directly to yield (Menon *et al*., 2016).

The principal component analysis is a unique statistical method that can turn a huge amount of linked data into a much smaller set of new variables by linearly combining the variables that explain the most variation in the original variables (Jolliffe and Cadima, 2016). It explains why the original data set was so different by breaking down the huge number of variables into smaller parts. PCA tries to group germplasms based on their PC scores and find the fewest factors that can explain the most variation (Wang *et al*., 2019).

Genotypes under this study have significant differences for studied traits, such as, plant height, tillers per plant, leaves per plant, leaf area, and green fodder yield. During the study of all variables, it was notable that the first principal component was accountable for the highest proportion of the total variation, with the subsequent elements exhibiting a decreasing trend in accounting for the discrepancy. The presented data indicated that every trait significantly contributed to the variation observed in at least one principal component. However, tillers per plant and leaves per plant described a fair amount of variation in PC1; plant height explained most of the variation in PC2, and leaf area was a major source of variation in PC3. When looking at the PC1 and PC2 biplot analyses, the main sources showed diversity in yield characteristics and the environment. Dumlupinar *et al*. (2012) said that biplot diagrams could help find genotypes with good combinations of traits that could benefit a breeding project. For example, if the objective was to increase green fodder yield, genotypes falling in the right quadrant are generally above average for this trait.

Different genotypes are considerably potential sources for improving traits in future breeding programs, as they may cause required changes in the genetic makeup of the desired plant (Kujur et al., 2017). The data obtained from the materials under investigation have uncovered distinct correlations among the traits examined. It suggested the selection process may yield genotypes possessing favorable features, such as high yield and desirable characteristics, which can serve in subsequent selection programs. Information obtained from the principal components would greatly assist the selection of potentially significant breeding

lines for future oat development programs. These findings corroborate Tanoli *et al*. (2016) and Ihsan *et al*. (2021), who found similar results for leaves and tillers per plant. They suggested further study of the germplasm's genetic diversity by converting many correlated variables into a few independent principal components. The number of tillers per plant is one of the most important factors for higher green fodder yield (Bibi *et al*., 2012). Genotypes with a higher number of tillers and leaves per plant came out with the support of biplot analysis over a three-year study, and using these potential genotypes in future breeding programs can help increase green fodder yield and meet the increasing feed demand for animals in the winter. Similar findings resulted from other investigators (Gupta and Mehta, 2020; Chawla *et al*., 2022). They found that different oat accessions had varying plant heights, tiller counts, leaf areas, and fodder yields. Differences in the count of tillers/plants among the studied genetic material may refer to variations in the genetic potential of the accessions collected from different origins (Kumar *et al*., 2022).

Breeders can also use cluster analysis to find desirable genotypes that create variability, improve required traits, and sort out potential genes in large populations (Iannucci *et al*., 2011). Hence, with the various implications of cluster analysis, 225 oat genotypes were subdivided into 10 groups based on four agronomic attributes directly linked with enhanced green forage yield. Ahmad *et al*. (2011) employed cluster analysis and inferred that days to 50% blooming, grain production, leaf-stem ratio, and green fodder yield contributed more to genetic divergence, implying that direct selection for these traits might be beneficial.

This three-year study suggested a high level of genetic diversity in the germplasm. Plants of native accessions showed distribution in more than one cluster for fodder yield-associated variables, with the genotypes of exotic germplasm spread in separate clusters. Genotypes from clusters with the highest inter-cluster distance can be useful in crossing programs to produce better genotypes. According to the results of the divergence study, the main cluster has genotypes' composition with a wide variety of indigenous origins. The pattern of groups can validate that genetic diversity and geographic variation are different. Clustering of genotypes showed no link between where accessions originated globally and genetic diversity (Klos *et al*., 2016). Most of these results align with the findings by Lei *et al*. (2020) and Shi *et al*. (2019).

The diverse genetic materials evaluated through this study offer promising prospects for genetic enhancement in oat breeding programs. It could lead to an increase in green fodder yield, thus catering to the escalating global demand for animal feed. Leveraging distinct genetic materials, employing multivariate and cluster analyses, understanding climate and geography, and selecting genotypes with desirable traits will all contribute to developing plants with higher fodder yields. Breeders can further optimize crop productivity by utilizing observed variation in the germplasm. It can happen by developing oat genotypes possessing higher tiller counts and leaves per plant, which are crucial factors in augmenting green fodder yield.

CONCLUSIONS

The significant diversity in oat germplasm is of considerable interest from a breeding perspective, as it provides valuable materials for oat improvement programs in the studied environment. Applying a multivariate approach in oat germplasm evaluation proved an effective method. This approach enables a comprehensive characterization of populations regarding their productivity and adaptation, with greater discriminatory power when compared with analyzing individual traits separately. PCA and cluster analysis assisted in identifying that genotypes 198 (Mustang) and 219 (Boppy) produced better fodder yield than standard checks and have the potential recommendation for general cultivation and use in future breeding programs.

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