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Research Article

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Trait-Based Clustering and PCA Reveal Genetic Resources for Fodder Maize Improvement

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ABSTRACT

Maize is a versatile crop used for food, animal feed, fodder, and industrial purposes. Known for its high nutritional value and food safety for livestock. The genetic diversity of 50 maize genotypes was assessed based on eight morphological traits using an RCBD design at NARC Islamabad during the spring season of 2024. The analysis of variance revealed significant variations for all recorded traits except the number of cobs observed, with non-significant variations(0.41^{ns}). Hierarchical cluster analysis with the complete linkage method grouped all genotypes into four clusters, indicating a broad genetic base. Cluster I comprise of maximum number of genotypes (22) indicating the genetic similarity among them, while Cluster IV has with minimum number of genotypes (4). In principal component analysis, the first four components had eigenvalues greater than 1, explaining 77.82% of the total variance. The identification of substantial genetic diversity in this study supports maize germplasm characterization, conservation, and future breeding improvements.

Keywords: Maize germplasm, fodder, cluster analysis, morphological traits and PCA.

INTRODUCTION

Maize (*Zea mays* L.) is widely consumed staple cereal after wheat and rice. It is also used as raw material for both human food and animal feed products (Alam *et al*. 2022). Maize is utilized as fundamental raw material in a wide range of industrial products, pharmaceuticals, textiles, paper industries, alcoholic beverages, packaging industries, protein, films, food sweeteners, gums, cosmetics, starch and oil. Maize grains at physiological maturity contains 60-72% starch, 8-11% protein, and 4-6% oil (Wang *et al*. 2023).

Corn holds significant global importance as a fodder crop due to its dual-purpose utility, serving both as a substantial source of energy for livestock and as a staple food for humans. Its contribution to livestock nutrition is particularly noteworthy, as maize fodder is characterized by its high digestibility and palatability (Wolińska *et al*., 2023). Studies highlight that maize is commonly utilized as silage in ruminant diets, where its biomass and energy content are pivotal for livestock productivity (Horst *et al*., 2020; Zafar *et al*., 2020). This versatility allows for optimal growth and reproduction in livestock, significantly boosting agricultural productivity and enhancing food security (Aguk *et al*., 2021).

In Pakistan maize is one of the dominant cereals and fodder crop and it covers the area of about 1.32 million hectares with an average yield of 4,787 kg/ha in Province of Punjab (Naderi *et al*. 2024). In Pakistan maize crop gain a lot of importance by supporting livestock sector as well as communities in rural areas. It is also cultivated as a winter crop across the country.

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High-quality fodder is crucial for enhancing animals' nutrition requirements, lactation, and enhancing livestock general health and breeding performance (Rangasami *et al*., 2024). Dairy nutrition highly depends on green fodder which effects the reproduction, health development, reproduction and general productivity of livestock. To tackle the issue of food security caused by the growing populations, it is crucial to boost the maize production.

Maize productivity in Pakistan has indeed shown a positive trend over the past five years. It is classified as the fourth most important crop, following wheat, rice, and cotton. During the 2019-2020 period, maize recorded a yield increase of approximately 6%, contributing significantly to Pakistan's agricultural output (Shahid *et al*., 2024). Although, still have great deal of room for the improvement for the yield and productivity of maize crop. Unfortunately, there is substantial scarcity of green fodder which hardly meets the one third the livestock requirement and this deficiency has a detrimental impact on the livestock performance (Ahmad *et al*. 2024). Fodder yield in maize crops is a complex trait and influenced by morphological traits. To enhance yield potential, selection for desirable genotypes with desirable traits should be focused.

Genetic variability is critical for the advancement of maize as it serves as the foundation for breeding programs aimed at improving crop traits. Studies highlight that genetic diversity not only affects resilience and adaptability but also contributes to improved nutritional quality of maize, crucial for food security (Iqbal *et al*., 2024). Our country possesses an enormous source of maize traditional races and local germplasm. To establish a diverse genetic foundation for improvement of overall productivity of maize crop through breeding programs it is important to evaluate the both indigenous exotic collections of germplasm. The main objective of this study was to evaluate the genetic diversity of maize germplasm collected from different climatic regions of the Pakistan using morphological traits, in order to identify genetically diverse accessions suitable for future breeding programs for green fodder yield.

MATERIALS AND METHODS

Total fifty accessions of maize were obtained from PGRI, NARC Islamabad, Pakistan. The accessions were evaluated by using RCBD design with two replications in February 2023. The distance between plants and rows maintained at 2.5 feet and 6 inches apart. The weather conditions were presented in Figure 1. All suggested cultural practices were implemented with the passage of time to protect the crop from abiotic and biotic factors.

Morphological traits and biometrical analysis

The data for eight morphological traits were recorded. These traits include plant height (PH), ear height (EH), number of leaves (NL), leave width (LW), leave length (LL), number of cobs (NC), number of nodes (NN) and internodal length (IL). The average of the recorded data of the morphological traits analyzed statistically using Statistix 8.1. The analysis included calculations of the mean, minimum, maximum, standard deviation, and coefficient of variation to evaluate the genetic diversity among the studied germplasms. The correlation among morphological traits was calculated using mean values through Pearson's coefficient correlation method in R software (version 2025.05.1-513) with the help of 'psych' package. Analyzing the data, numerical taxonomic methods such as cluster analysis and principal component analysis were performed by using approach given by Sheraz *et al*. (2025) and Iqbal *et al*. (2015).

RESULTS

Analysis of Variance

The study aimed to variations among morphological traits of fifty maize genotypes. Data for eight morphological traits were collected and analyzed to explore possible variations. Table 1 presents the ANOVA results for the eight morphological traits. Table 1 shows statistically significant variations ($p \leq 0.05$) among genotypes in three replications for the recorded traits. All the recorded traits presented significant differences except the number of cobs showed non-significant differences.

Table 1. Analysis of Variance of morphological traits

SOV	DF	PH	EH	NL	LW	LL	NC	NN	IL
Replication	2	1633.29	38.73	2.06	3.55	8.41	0.08	0.86	5.36
Entries	49	2288.85**	706.39**	8.42**	4.79**	253.39**	0.41^{ns}	4.48**	12.93**
Error	98	812.97	214.26	2.46	1.6	67.26	0.28	1.97	4.64
Total	149								
CV		15.05	20.59	14.8	13.89	11.87	24.23	15.76	12.61

Correlation

The correlation among eight recorded morphological traits presented in Figure 1. Plant height showed positive and significant association with ear height, number of leaves, internodal length, leaf width and leaf length while negative association recorded with no of cobs. Ear height showed negative correlation with number of cobs and number of nodes while observed in positive and significant correlation with PH, NL, LW and LL. No of leaves showed positive significant association with PH, EH, LL and LW. At the same time no of leaves showed a negative correlation no of cobs and internodal length. Leaf width showed positive significant effect with plant height, ear height, no of leaves, and leaf length while negative association was observed with no of cobs and no of nodes. Positive significant association of leaf length was observed with plant height, ear height, no of leaves and leaf width. No of cobs was observed having positive association with leaf length, no of nodes and internodal length while negative correlation with remaining traits. A positive association of no of nodes was observed with internodal length, no of cobs, LL, NL and plant height while negative association with leaf width and EH. Internodal length was observed to have a negative association with no of leaves while positive association with all remaining morphological traits.

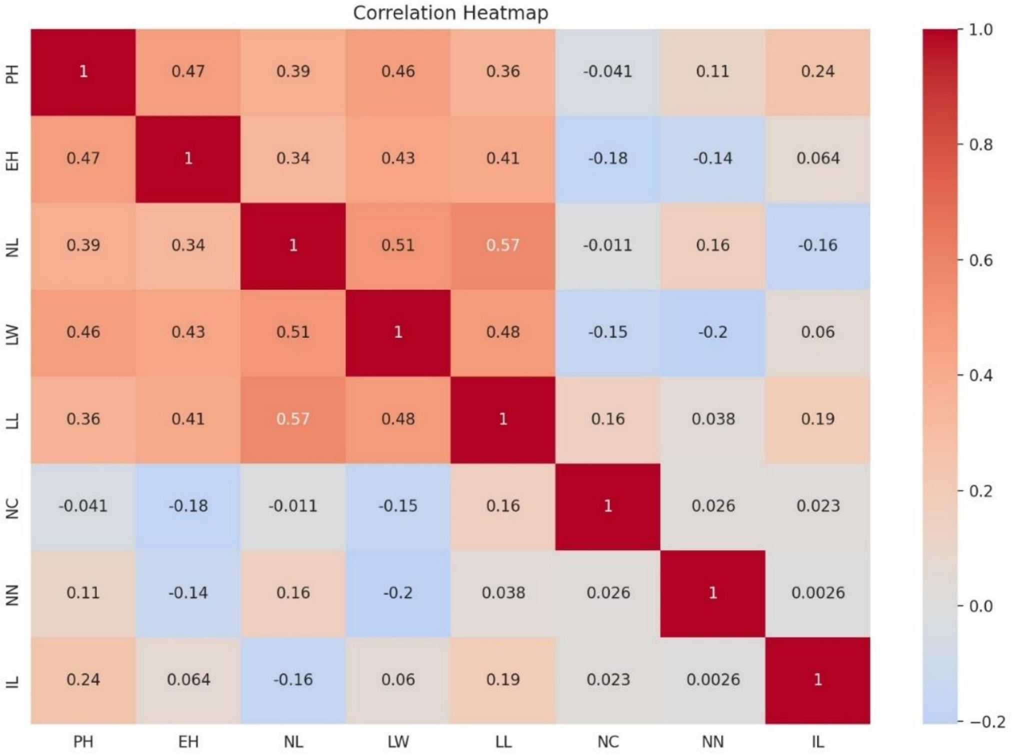

Figure 1. Correlation analysis among morphological traits

Cluster Analysis

Hierarchical cluster analysis was performed by using eight agronomic traits and the dendrogram presented in Figure 2. In current study the segregation among 50 genotypes was calculated and divided into four clusters (Table. 2). Cluster I comprises of maximum number of twenty-two genotypes (26, 7, 20, 48, 28, 21, 12, 50, 44, 49, 22, 19, 48, 42, 31, 25, 29, 14, 11, 15, 16 and 3). Cluster II and cluster III comprise of equal number of genotypes 12, cluster II had (6, 2, 4, 18, 30, 23, 17, 47, 45, 37, 40 and 35) while cluster III had (36, 24, 5, 43, 29, 48, 41, 1, 10, 9, 8 and 13). Cluster IV was observed with minimum number of genotypes (34, 32, 33 and 27).

Mean values of cluster for eight morphological characters were presented in Table 3. The highest mean value for plant height was recorded in cluster II (226.93), followed by Cluster III (198.04). The highest mean value for ear height was recorded in cluster III (87.56) followed by cluster II (74.93). Maximum value for number of leaves was observed in cluster III (11.33) followed by cluster II (11.23). Highest value for leaf width was observed in cluster III (9.45) followed by cluster II (9.3). The maximum value for leaf length was observed in cluster II (74.33) followed by cluster III (71.46). Cluster II showed maximum value for number of cobs (2.27) followed by the cluster I (2.23). Maximum value of number of nodes recorded in the cluster II (9.53) followed by the cluster IV (8.98). Maximum value of internodal length was observed in cluster II (18.13) following the cluster I (17.17).

Table 2. Distribution of 50 genotypes of maize in four clusters

	No of genotypes	**Genotypes in clusters**
Cluster I	22	26, 7, 20, 48, 28, 21, 12, 50, 44, 49, 22, 19, 48, 42, 31, 25, 29, 14, 11, 15, 16, 3
Cluster II	12	6, 2, 4, 18, 30, 23, 17, 47, 45, 37, 40, 35
Cluster III	12	36, 24, 5, 43, 29, 48, 41, 1, 10, 9, 8, 13
Cluster IV	4	34, 32, 33, 27

Table 3. Cluster means for eight different characters of 50 genotypes

Traits	**Cluster I**	**Cluster II**	**Cluster III**	**Cluster IV**
PH	186.1	226.93	198.04	155.05
EH	60	74.93	87.56	60.17
NL	10.27	11.23	11.33	9.55
LW	8.7	9.3	9.45	8.05
LL	67.67	74.33	71.46	63.67
NC	2.23	2.27	2.12	2.21
NN	8.67	9.53	8.64	8.98
IL	17.17	18.13	16.62	16.79

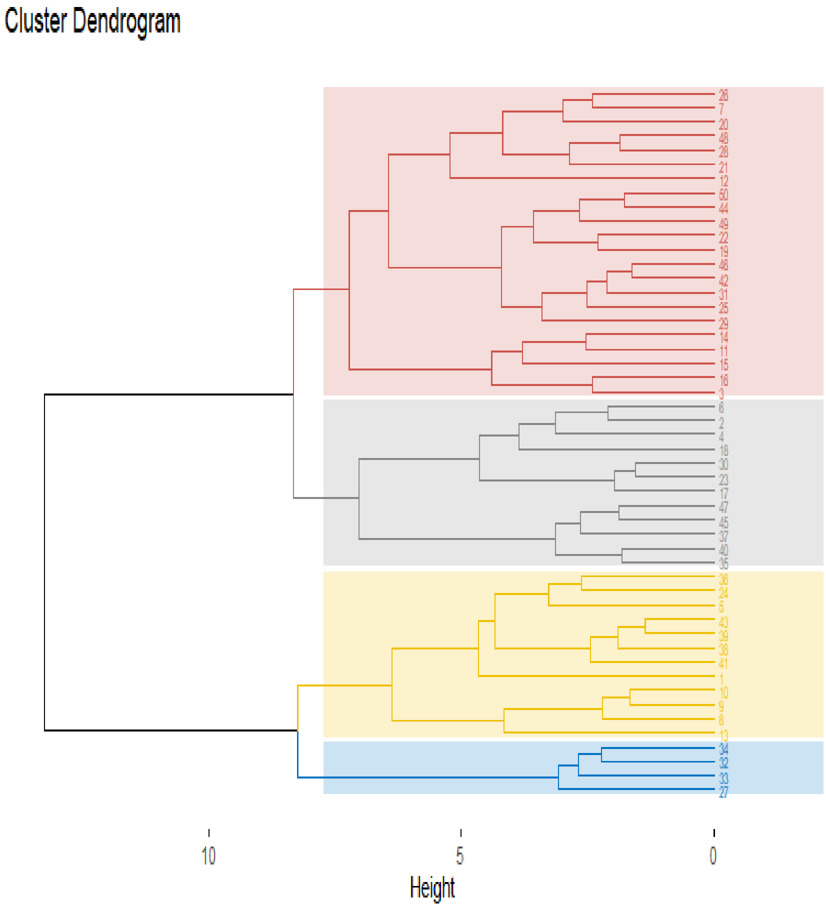

Figure 2. Dendrogram of hierarchical cluster analysis based on eight agronomic traits

PCA Analysis Based on Morphological Traits

To explore the genitive variability of maize genotypes, a principal component analysis was applied on the mean data of the studied traits to check the relationship between these traits and genotypes. The eigenvalues and proportion of variance accounted by each principal component, based on morphological parameters, are presented in Table 4, while the scree plot is shown in Figure 3. The 1st four PCs (PC1 to PC4) showed eigen values greater than 1 showing significant contribution of these components in variation. These 4 components contributed 77.82% of the total variation. The 1st principal component, which is the most important component, accounted for 1.67 eigen value and contributed 34.99% in total variation. The major contributing traits were number of cobs (0.0542) and number of nodes (0.01). The 2nd principal component explained 15.51% of the total variation and accounted for the eigen value of 1.11. The major contributing traits were number of nodes, number of cobs, leaf length and number of leaves. The 3rd principal component showed eigen value 1.07 and contributed 14.45% in variation. The major contributing traits were internodal length, plant height and number of cobs. The 4th principal component explained 12.87% in total variation with eigen value 1.01. The major contributing traits were number of nodes, plant height and internodal length.

The factor loading results based on recorded traits are presented in Table 4. Factor loading indicates the correlation of each variable with the respective principal component. It is observed that the first principal component (PC1) shows a negative association with two variables number of cobs and number of nodes. All other recorded traits number of leaves, leaf length, plant height, leaf width and ear height have positive correlation in 1st principal component. In the 2nd principal component, ear height and leaf width showed negative correlations while positive correlation was observed for other traits such as number of cobs, number of nodes, leaf length and plant height. The 3rd principal component is positively correlated with PH, EH, LL, NC and IL. Positive correlation for PH, EH, NN and IL was observed in 4th principal component.

Table 4. Factor Loadings of the traits

	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
PH	-0.43272	0.063345	0.223898	0.259229	0.450441	-0.53566	0.274374	0.355399
EH	-0.42236	-0.27369	0.028464	0.060911	0.572094	0.544306	-0.17949	-0.29396
NL	-0.44121	0.241594	-0.40389	-0.02082	-0.23363	-0.03637	0.539726	-0.486
LW	-0.46929	-0.22414	-0.06993	-0.13178	-0.30811	-0.42654	-0.63851	-0.15069
LL	-0.45529	0.265405	0.063027	-0.24156	-0.30523	0.448016	-0.01035	0.604945
NC	0.054212	0.577507	0.176808	-0.64832	0.35174	-0.13938	-0.15476	-0.21182
NN	0.010056	0.638011	-0.11925	0.640266	0.008801	0.085716	-0.39541	-0.07006
IL	-0.09564	0.038301	0.855328	0.150593	-0.32145	0.077808	0.114436	-0.33562

Table 5. Eigen values and variance percentage

	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
Standard deviation	1.6731	1.1139	1.0751	1.0148	0.79632	0.73851	0.56917	0.52037
Proportion of Variance	0.3499	0.1551	0.1445	0.1287	0.07927	0.06817	0.04049	0.03385
Cumulative Proportion	0.3499	0.505	0.6495	0.7782	0.85748	0.92566	0.96615	1.00000
Eigen Values	2.7991935	1.2408031	1.1559143	1.0298196	0.6341312	0.5453955	0.3239592	0.2707836

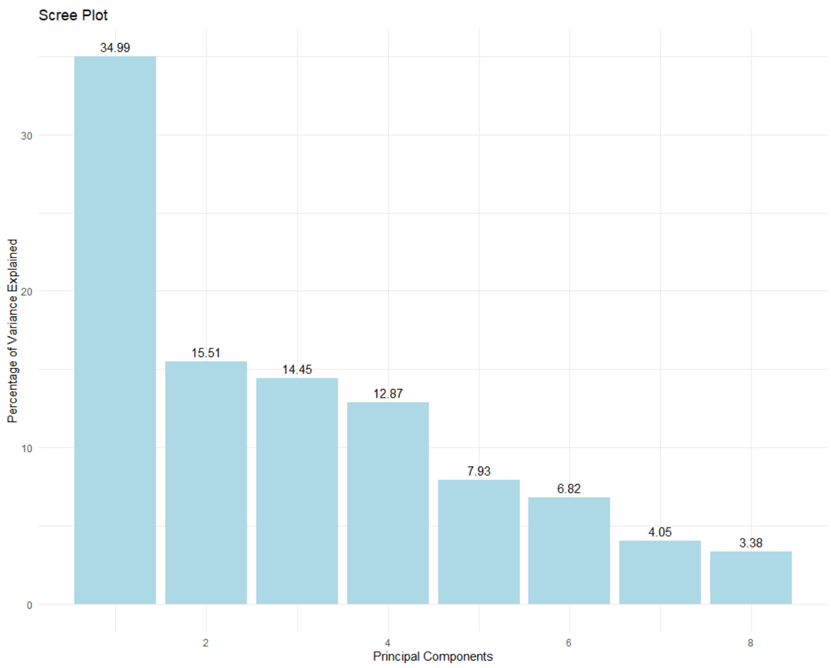

Figure 3. Scree plot of principal components

Genotype × Environment Interaction (Biplot Analysis)

The biplot technique is often used to assess the relationships among different variables (Yan & Rajcan, 2002). The biplot analysis (genotypes × traits association) based on PC1 and PC2 presented in **Figure 4.** The two PCs explained 50.5% of the total variation. Genotype numbers are displayed in blue numbers, while recorded traits are represented as red vectors. The vectors for LL, PH, EH, NL, and LW align with the PC1 (Dim 1) axis, indicating a higher contribution of these traits to PC1 (Dim 1). Similarly, the NC and NN vectors are aligned with the PC2 (Dim 2) axis, reflecting the contribution of these traits to PC2 (Dim 2).

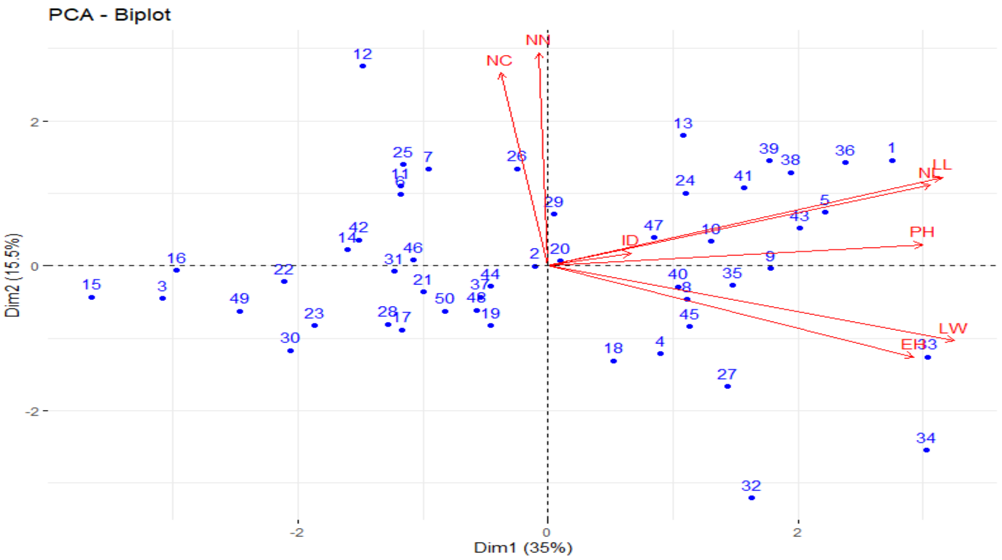

Figure 4. PCA biplot of morphological traits

DISCUSSION

Analysis of variance is a statistical approach used to determine the significant difference among the means of various traits. In the current study, ANOVA showed substantial variations for all recorded traits except the number of cobs. It showed non-significant differences (0.41^{ns}) among the recorded traits. The finding that seven out of eight morphological traits show statistically significant variation indicates a strong genetic basis for these characteristics within the fifty genotypes studied. The significant genetic diversity observed for the majority of morphological traits among the fifty maize genotypes highlights their strong potential for use in breeding programs. Similar findings for number of cobs were reported by Tanveer *et al*. (2014), indicating that the number of cobs might not be a highly heritable trait under certain conditions. In contrast, other studied traits exhibited significant variation, reflecting the influence of genetic diversity and the potential for selection in breeding programs. These findings align with those of Khan *et al*. (2018) and Rocandio-Rodríguez *et al*. (2020) further confirming the role of genotype in controlling morphological traits and highlighting the traits with the greatest breeding value for crop improvement.

Correlation analysis plays a vital role for breeders to help in the identification of suitable characteristics for breeding purposes. Correlation is the result of the presence of linked genes of various traits. Genotype and environmental interaction also influence the association of traits. Sometimes, the environment directly influences both traits in a similar and synchronized manner, while in other cases, it affects them.

The recorded morphological traits (PH, EH, LL, and LW) exhibited a highly significant positive correlation with one another, indicating their collective contribution to enhancing green fodder yield in maize. This interdependence suggests that selection for taller plants with broader and longer leaves may result in a higher photosynthetic surface area, improved light interception, and overall vegetative biomass, which are critical factors for green fodder production. On the other hand, the number of leaves and internodal length showed a significant negative correlation, implying that an excessive number of leaves or longer internodes may not necessarily translate into increased biomass, possibly due to resource partitioning inefficiencies or structural limitations in biomass accumulation. These findings align with the earlier studies of Iqbal *et al*. (2015) and Khan *et al*. (2018), reinforcing the idea that a balance of plant architecture traits is crucial for optimizing fodder yield. Additionally, Barua *et al*. (2017) reported a significant positive association between plant height and ear height, which further supports our observation and highlights the potential use of ear height as a secondary trait for indirect selection of high-yielding green fodder genotypes.

Cluster analysis is a perfect biometrical technique used to group the set of elements into various clusters based on similarities. In this clustering a specific technique called Agglomerative Hierarchical Clustering was applied. In the current study, all genotypes were classified into four distinct clusters, with Cluster I comprised the highest number of genotypes, followed by Clusters II, III, and IV. Cluster IV contained the lowest number of genotypes, indicating its members may possess unique or rare traits compared to those in other clusters. The formation of four distinct clusters is direct statistical evidence of significant genetic diversity within the studied germplasm, confirming it is a valuable resource for breeding. Breeders can strategically select parents from different clusters to create structured breeding populations with maximum genetic variability. The large size of Cluster I suggest contains genotypes with common, widely adapted alleles. This group could be a source of well-adapted, stable parents for breeding programs. This suggests that genetic diversity is not solely governed by geographical origin but may be influenced by factors such as mutation, gene flow, and artificial selection pressures in local breeding practices. The dendrogram (Figure 2) reflects the genetic divergence among the clusters and within genotypes, providing insights into the potential use of diverse parental lines in hybridization programs. Genotypes from distant clusters can be crossed to maximize heterosis, while genotypes within clusters with superior values for key traits can be used for line improvement. Notably, some accessions from the same or nearby geographical locations were placed in different clusters. Similar clustering patterns were reported by Alam *et al*. (2022) and Ganesan *et al*. (2010) in maize, supporting the consistency of genetic variability within diverse germplasm. Matin *et al*. (2017) also observed comparable results, reinforcing the reliability of clustering methods in identifying genetic relationships.

Principal Component Analysis (PCA) is a powerful multivariate tool that not only quantifies trait contributions to overall variability but also helps dissect the underlying genetic diversity among genotypes. In this study, PCA effectively distinguished maize accessions based on their morphological traits, underscoring its utility in genetic evaluation and germplasm classification. The traits showed high contributions to the major PCs are number of cobs, leaf length, leaf size, no of nodes and plant height which points to the complex genetic architecture of maize and its responsiveness to environmental pressures. These results suggest that these traits having higher contributions should be considered as key selection criteria in future breeding programs for fodder maize improvement. Pakistan's heterogeneous climatic zones from arid to temperate regions create diverse selection pressures that likely contribute to the maintenance of high genetic variability in local maize germplasm.

The observed clustering patterns in PCA imply that certain genotypes share adaptive trait combinations, possibly due to similar evolutionary or selection histories. These clusters may represent distinct heterotic groups that can be exploited in hybrid development. The presence of such diversity is crucial for breeding programs aiming to exploit heterosis and develop improved varieties with broad genetic backgrounds. This study effectively pre-screens the germplasm collection, allowing breeders to prioritize a subset of genetically distinct and promising genotypes for more intensive (e.g., molecular) evaluation, saving time and resources.

Our findings align with those of Annicchiarico (2006), who reported that the first principal component accounted for 35% of total variability in lucerne, suggesting a common pattern of major traits dominating early PCs in forage crops. Similarly, studies by Okporie (2018) and Shrestha (2016) corroborate our results, reinforcing the robustness of PCA in identifying trait combinations useful for selection. Moreover, the findings of Mengistu *et al*. (2021) validate that components with eigenvalues greater than one are informative in explaining genetic divergence, further confirming the biological relevance of our results.

CONCLUSION

This study demonstrated extensive phenotypic variability across the most examined traits in fifty maize accessions. The genetic analysis revealed highly significant differences among them. Cluster analysis grouped the germplasm into four main clusters, while PCA distinguished most genotypes within the first four principal components with eigenvalues exceeding one. Overall, the findings suggest that selecting these genotypes could be beneficial for developing improved genotypes for fodder purpose.

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