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Exploring agro-morphological and nutritional diversity in maize (*Zea mays* L.) inbred lines under temperate conditions

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ABSTRACT

Maize (Zea mays L.) is a versatile cereal crop of global significance, widely cultivated for food, feed, and industrial purposes due to its broad adaptability and genetic diversity. Hidden hunger, caused by inadequate intake of essential nutrients, affects nearly one-third of the global population, primarily due to deficiencies in minerals such as iron (Fe) and zinc (Zn).While current varieties support food production under variable environments, future demands necessitate the development and adoption of more resilient and improved cultivars. However, simultaneous improvement of grain yield and micronutrient concentration remains challenging due to their complex inheritance and strong environmental influence. The present study aimed to evaluate 48 maize inbred lines for agro-morphological and micronutrient traits, including grain Fe and Zn content, using five standard checks (CML-451, DML-1084, IML-418-1, PV-1, and IML-187). The field experiment was conducted during Kharif 2023-24 at the Division of Genetics and Plant Breeding, Faculty of Agriculture, Wadura, SKUAST-K, following an Augmented Block Design, while laboratory analyses were carried out under a Completely Randomized Design (CRD). Significant genetic variability was observed among the inbreds for all traits. High PCV, GCV, heritability, and genetic advance as were recorded for anthesis-silking interval, plant height, cob placement, yield per plant, and kernel Fe and Zn concentrations. Correlation studies revealed positive associations of yield per plant with shelling percentage, seed weight, cob traits, and plant height. PCA grouped the variability into five principal components explaining 79.86% of total variation, while cluster analysis categorized the inbreds into two major groups. Cluster I combined early maturity with superior yield potential. Genotypes KDM-519, KDM-530, KDM-502, and KDM-529 showed high yield with medium maturity, whereas KDM-560 and KDM-504 exhibited superior Fe and Zn enrichment, making them promising candidates for biofortification in maize breeding programs.

Keywords: *ASI, Biofortification, Kernel Fe and Zn, Maize (Zea mays L.), Hidden hunger, PCA.*

Introduction

Maize (*Zea mays* L.), commonly referred to as corn, is the sole cereal crop of American origin that is extensively cultivated across tropical and subtropical regions worldwide.It is often hailed as the "queen of cereals" due to its versatility and wide cultivation across the globe.It is a monocotyledonous, annual cereal crop and the most widely cultivated species within the genus *Zea*.It belongs to the family Poaceae, with a somatic chromosome number of 2n = 20, a genome size of approximately 2.3 gigabases, and more than 32,000 genes encoded in its genome . Around 7,000 to 10,000 years ago, maize (*Zea mays* L.) evolved from teosinte (*Zea mays* ssp. *mexicana*) in the Western Hemisphere . It is cultivated across a wider range of latitudes and altitudes than any other food crop, tolerating temperatures from cool to very hot, growing in both humid and semi-arid environments, and adapting to a variety of soil types. Its adaptability to varied agroecological conditions, combined with high productivity, makes maize a vital crop for sustaining food systems and supporting agricultural resilience.

Globally, maize covers around 201.98 million hectares, with an annual production exceeding 1,247.7 million metric tonnes . In India, maize has emerged as the third most important food grain, following wheat and rice.It is cultivated across 10.04 million hectares in India, with an annual production of 422.8 lakh tonnes, resulting in an average productivity of approximately 4.21 tonnes per hectare .The major maize-growing states in the country include Uttar Pradesh, Bihar, Rajasthan, Madhya Pradesh, Punjab, Haryana, Maharashtra, Andhra Pradesh, Himachal Pradesh, West Bengal, Karnataka, and Jammu & Kashmir. Together, these states contribute over 95% of India's total maize production . In UT of Jammu and Kashmir, maize cultivation spans nearly 2.76 lakh hectares, producing around 5.47 lakh tonnes annually, with an average yield of 1,979 kg/ha . Maize is well known for its rich nutritional profile, providing a balanced mix of key macronutrients

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including carbohydrates, fats, proteins, and insoluble fibre essential for fulfilling daily dietary requirements .A maize kernel typically consists of 82% endosperm, 12% germ, and 6% pericarp, each contributing uniquely to its nutritional value. The endosperm, being the largest component, contains roughly 70% starch and about 10% protein, making it a key source of energy .As maize and its by-products make up 30% of the food supply in the Americas, 38% in Africa, and 6.5% in Asia, it serves as an essential pillar of both food security and economic advancement. Despite significant technological and economic advancements globally, malnutrition remains a major challenge, with 149.2 million children under the age of five stunted, 45 million wasted, and 38 million overweight. Malnutrition is responsible for nearly 50% of child deaths worldwide .This alarming situation calls for a collective and concerted effort to reduce human suffering and improve the quality of life.More than one in three individuals worldwide suffers from one or more micronutrient deficiencies, affecting over two billion people globally. Alarmingly, nearly half of these individuals reside in India, making it a major hotspot for micronutrient malnutrition .Micronutrient deficiencies are on the rise, largely driven by the growing global population. Deficiencies in iron and zinc can lead to severe health issues, including impaired cognitive and mental development, stunted growth, anaemia, weakened immunity, and increased mortality .Micronutrient malnutrition, primarily resulting from zinc and iron deficiencies, now affects approximately 3 billion people globally and poses significant health risks .The primary cause of iron and zinc deficiencies in developing countries is the limited dietary diversity, with populations relying heavily on one or two staple foods. Due to widespread poverty, diets are often dominated by cereals and limited animal-based products. Unfortunately, cereal crops naturally contain low levels of zinc and iron, and a significant portion of these micronutrients is lost during food processing ;.

Maize serves as a dietary staple for over 200 million people worldwide, contributing approximately 15% of global protein intake and 20% of total caloric consumption.Despite supplying many essential macro- and micronutrients, maize kernels often lack adequate levels of certain vital nutrients, posing a challenge for those who rely on maize as a major part of their diet .Phytic acid (PA), an anti-nutritional compound found in maize (*Zea mays* L.) grains, binds essential mineral elements like Fe^{3+}, Zn^{2+}, and Mg^{2+} to form insoluble salts, thereby reducing their bioavailability .While the micronutrient content in widely grown maize varieties is generally insufficient to meet nutritional needs, the significant genetic variability within maize allows for the development of enhanced cultivars with increased micronutrient concentrations through biofortification . Biofortified maize, enriched with key micronutrients, offers a practical solution to improve nutrition in underserved rural communities lacking access to balanced diets and fortified products. Over the past two decades, significant advancements have been made in maize biofortification through conventional breeding and modern molecular approaches. As an integral part of the biofortification program, this study focuses on the agro-morphological evaluation and characterization of maize inbred lines for iron (Fe) and zinc (Zn) concentrations, with the aim of identifying lines with higher micronutrient content to be utilized as potential parents in future hybridization programs. The goal is to explore the diversity among a set of maize inbred lines with respect to various morphological traits and nutritional quality.

Material and Methods

The present investigation was carried out during *Kharif* 2023-24 in the research fields of the Division of Genetics and Plant Breeding, Faculty of Agriculture, SKUAST-K, Wadura, Jammu and Kashmir. A total of 48 maize inbred lines, along with five standard checks (CML-451, DML-1084, IML-418-1, PV-1, and IML-187) presented in and were evaluated for 14 agro-morphological traits, including days to 50% tasseling, days to 50% anthesis, days to 50% silking, days to maturity, plant height, cob placement, cob length, cob diameter, number of rows per cob, number of kernels per row, 100-seed weight, shelling percentage, and yield per plant, using an Augmented Block Design (ABD).

For micronutrient traits, particular emphasis was placed on kernel iron (Fe) and zinc (Zn) contents, which were estimated following diacid digestion (nitric acid: perchloric acid in a 9:4 ratio) and analyzed using an Atomic Absorption Spectrophotometer (Model: PERKIN-ELMER 2380) as per the protocol of with minor modifications, under a Completely Randomized Design (CRD).

The collected data were analyzed using R Studio software (version 4.4.1), employing various statistical tools, including correlation analysis, principal component analysis (PCA), clustering analysis for agro-morphological traits, and variability analysis for both agro-morphological and micronutrient traits.

Result and Discussion

This chapter presents the results of the study along with their interpretations, with a comprehensive discussion of the findings in relation to previously reported literature under the following headings:

Analysis of Variance for Agro-morphological traits and Micronutrient Traits :

The analysis of variance for 14 agro-morphological traits was performed using an augmented block design (ABD) which is tabulated in the and the results revealed that the mean sum of squares exhibited significant differences across all sources of variation for maximum traits. In the first source of variation (block ignoring treatments), most traits exhibited significant block effects. However, when treatments were accounted for (block eliminating treatments), block effects turned non-significant for all traits. This indicates that the apparent block significance in the former case was primarily due to the unequal distribution of treatments across blocks rather than genuine block effects. Treatment effects were found to be significant for all traits, both with and without considering block effects. This indicates that the observed variation among treatments is primarily governed by genetic factors rather than block influences. The mean sum of squares for test entries was significant across all traits, indicating the presence of substantial variability among the evaluated lines and highlighting their distinct genetic potential for the traits under study while the mean sum of squares for checks was significant for most of the traits, indicating that the checks differed significantly for these traits. However, for anthesis–silking interval and number of rows per cob, the checks were non-significant, suggesting relative uniformity among them for these traits. The analysis for Test vs check revealed significant across most traits indicating that the newly evaluated test entries differ markedly from check varieties while non-significant relation was observed for traits like days 70 to maturity, cob placement, number of rows per cob, yield per plant and shelling percentage

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concluding that performance of the test entries was statistically similar to the standard checks, indicating limited differentiation between newly developed lines and the existing checks.

The results of analysis of variance align with the close proximity of the previous findings of [30, 20, 6, 25, 22, 35] who reported similar significance level of mean sum of square due to treatment.

The analysis of variance for micronutrient traits was performed using a Completely Randomized Design (CRD), and the results, presented in , revealed that all treatments were highly significant ($p \leq 0.01$) for both Fe and Zn concentrations, indicating substantial genetic variability among the genotypes for these traits. This variability suggests ample scope for the identification and selection of promising lines, thereby facilitating genetic enhancement and biofortification of maize with essential micronutrients.These findings are in close agreement with the results reported by [10, 1, 2] and [9] comparable significance was noticed across treatments.

Descriptive Statistics for Agro-morphological and Micronutrient Traits :

Significant difference was observed in panel of 48 inbred lines for agro-morphological as well as micronutrient traits which is tabulated in . Thus the variability ranged between the traits is summarized as follows days to 50% tasseling (75-106 days), days to 50% anthesis (77-110 days), days to 50% silking (80-113 days), anthesis-silking interval (2-7 days), days to maturity (113-146 days), plant height (61.65-177 cm), cob placement (26-102.65 cm), cob length (7.10-21 cm), cob diameter (2.50-5.10), number of rows per cob (8-16 rows), number of kernels per row (15-38 kernels), 100 seed weight (15-29 g), shelling percentage (60-83%), yield per plant (38.06 - 106 g), Fe (9.5-39.8 ppm) and Zn (4.2-33.3 ppm) the range of Fe and Zn closely align the findings of [10, 1, 2, 9, 45] The present study's outcomes are highly comparable to those reported by [31] for traits like days to 50% tasseling, anthesis, silking, cob length, cob diameter, number of kernels per rows, 100 seed weight and yield per plant [23]. shows close conformity with traits like yield per plant, number of rows per cob, number of kernels per row [38] results show a similar trend with traits like cob length, cob diameter, number of rows per cob, number of kernels per row, 100 seed weight, and yield per plant.

Estimates of Variance and Coefficient of Variance for Agro-morphology and Micronutrient Traits:

The coefficient of variation serves as a dependable statistical measure to compare the extent of variability among different traits. To assess the inherent variability within the material, both the phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were estimated. According to the classification proposed by [48], PCV and GCV values less than 10% are considered low, those between 10–20% as moderate, and values exceeding 20% as high.

The estimates of genotypic (GCV) and phenotypic coefficients of variation (PCV) were observed to be high (>20%) for agro-morphological traits such as anthesis-silking interval(ASI), plant height (PH), cob placement (CP), cob length (CL), and yield per plant (YP). Moderate variation (10–20%) was recorded for cob diameter (CD), number of rows per cob (RC), number of kernels per row (KR), and 100-seed weight (X100SW), whereas low variation (<10%) was noted for days to 50% tasseling (D50%T), days to 50% anthesis (D50%A), days to 50% silking (D50%S), and shelling percentage (S) were as it was recorded high (>20%) for micronutrient traits such as Iron (Fe) and Zinc (Zn), tabulated in Table 6.

Since phenotypic variance was consistently higher than genotypic variance, it can be inferred that environmental effects had a notable impact on trait manifestation. This indicates that relying only on phenotypic performance for selection may not be efficient, emphasizing the need for evaluation across multiple environments to capture the true genetic potential. The estimates of both phenotypic and genotypic coefficients of variation were found to be low (20%) was observed for anthesis–silking interval, plant height, cob placement, cob length, and yield per plant where whereas Iron (Fe) and Zinc (Zn) were also categorized into high for PCV and GCV. The low values of PCV and GCV indicate limited variability and, hence, lower prospects for selection, whereas moderate values suggest a reasonable amount of variation that can be exploited with careful selection. High values reflect wide genetic diversity among genotypes, providing greater opportunities for effective selection and improvement, particularly when coupled with high heritability and genetic advance. The following results are in agreement with [20, 6, 25, 17, 31] similar trend of GCV, PCV was observed across traits [32] reported high estimates of GCV and PCV for grain yield per plant.

Estimates of Heritability and Genetic Advance for Agro-morphology and Micronutrient Traits:

Heritability estimates provide the assessment of the amount of transmissible genetic variability to total variability. These values are essential in assessing genetic improvement and predicting the response to selection. As per the classification of [21] heritability estimates are categorized as low (<30%), moderate (30–60%), and high (>60%).

Genetic advance reflects the expected improvement in a trait achieved through selection. When expressed as a percentage of the mean (GAM), it serves as a more dependable indicator of selection efficiency, as it incorporates heritability, phenotypic standard deviation, and selection intensity into its estimation. According to the classification given by ,genetic advance as percent of the mean is categorized as low (<10%), moderate (10–20%), and high (>20%).

Broad-sense heritability values across different agro-morphological traits as presented in ranged between 67.14 and 95.50 percent. All the traits exhibited higher heritability (> 60%) were as the estimates were also high for micronutrient traits that is Iron (99.87) and Zinc (99.90). Among the evaluated traits, Fe (87.56), Zn (98.36), cob placement (57.43), anthesis–silking interval (52.69), cob length (49.98.06), plant height (44.28), yield per plant (42.22), number of kernels per row (27.02), number of rows per cob (26.30),100-seed weight (24.40) and cob diameter (20.08) recorded the highest genetic advance as percent of mean.

Heritability in the broad sense estimates the proportion of phenotypic variance that is genetic in origin, while genetic advance reflects the expected gain from selection. It should be emphasized that a trait with high heritability does not necessarily exhibit a high genetic advance. Although genetic advance provides the absolute expected gain under selection, it is expressed in the same unit as the trait and is not suitable for direct comparison across traits. Therefore, genetic advance as percent of mean (GAM) is preferred, as it standardizes the gain relative to the mean. Considering these two parameters together provides a clearer understanding of the inheritance pattern high heritability with high genetic advance suggests

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additive gene action and greater effectiveness of selection, whereas high heritability with low genetic advance points towards non-additive gene action. Heritability estimates were found to be high (>60%) for all the traits studied, with values ranging from 67.14% to 95.50% for agro-morphological traits, while exceptionally high estimates were recorded for micronutrients, reaching 99.87% for iron (Fe) and 99.90% for zinc (Zn). High heritability estimates suggest that the expression of the trait is largely governed by genetic factors, with minimal environmental influence. This implies that phenotypic selection would be reliable and effective for improving such traits, particularly when coupled with high genetic advance, which further confirms the predominance of additive gene action. Genetic advance as a percent of mean was observed to be high (>20%) for traits such as anthesis–silking interval, plant height, cob placement, cob length, cob diameter, number of rows per cob, number of kernels per row, and yield per plant. It was also observed exceptionally high for iron and zinc. Moderate values (10 20%) were recorded for days to 50% tasseling, days to 50% anthesis, days to 50% silking, and shelling percentage, whereas days to maturity exhibited low values(<10%).High values suggest the predominance of additive gene action, indicating that substantial genetic improvement can be achieved in the next generation through simple phenotypic selection. Moderate values reflect the involvement of both additive and non-additive gene effects, where selection remains effective but genetic progress is likely to be moderate and gradual, often requiring recurrent selection or evaluation across multiple environments. Low values imply that the trait is largely influenced by environmental factors or governed by non-additive gene action, making direct phenotypic selection less effective; hence, alternative breeding approaches such as heterosis breeding, hybrid development, or marker-assisted selection may be more appropriate.

Heritability considered in conjunction with genetic advance provides a more reliable measure of selection efficiency. Traits that recorded high heritability coupled with high genetic advance as a percent of mean—such as anthesis–silking interval, plant height, cob placement, cob length, cob diameter, number of rows per cob, number of kernels per row, 100-seed weight, yield per plant, Iron concentration and Zinc indicate the predominance of additive gene action, suggesting these traits emerge as prime targets for selection, as they combine genetic stability with considerable potential for improvement. The results are in close agreement with the findings of of [20, 6, 25, 17, 31] which showed very similar trend across traits like days to 50% tasseling, anthesis, silking, cob length, cob diameter, number of kernels per row, 100 seed weight and yield per plant [18] reported high estimates of heritability in broad sense for similar traits [32] higher estimates of heritability where recorded for yield per plant and days to 50% silking.

Pearson Correlation for Agro-morphological Traits

In the present study, the relationships among 14 agro-morphological traits were examined through correlation analysis which is presented in and the heat map revealing that days to maturity had a significant positive association with days to 50% tasseling (0.55), days to 50% anthesis (0.56), and days to 50% silking (0.55). This indicates that flowering-related traits are closely aligned with physiological maturity, suggesting that selection for early tasseling, anthesis, or silking could indirectly facilitate the development of early-maturing maize varieties.

Yield per plant exhibited a significant positive association with shelling percentage (0.75), number of rows per cob (0.37), number of kernels per row (0.37), cob diameter (0.35), 100-seed weight (0.46), plant height (0.35), and cob placement (0.35). This implies that genotypes expressing superior performance for these traits tend to achieve higher yields, highlighting their importance as key selection criteria in maize breeding programs focused on yield improvement.

The findings align with previous studies of [11, 53, 15] also reported a strong correlation among maturity traits, while yield-related traits were found to be positively associated with each other [12, 25, 31] the findings where very similar as yield per plant shows close association with yield contributing traits like 100 seed weight, number of kernels per row, cob length and cob diameter [18] studied maize inbreds and also reported positive correlation of cob length, rows per cob, kernels per row and 100 seed weight with grain yield per plant.

PCA (Principal Component Analysis) for Agro-morphological Traits

Principal Component Analysis (PCA) is a powerful multivariate approach designed to simplify complex datasets by reducing their dimensionality while preserving the majority of the information. It achieves this by converting a group of correlated variables into a new set of independent variables, known as principal components, which capture the maximum variability present in the data. The main motive of PCA is to simplify complex data, identify patterns, and highlight the most influential traits contributing to overall variation.In plant breeding studies, PCA helps in grouping genotypes, detecting trait associations, and selecting key traits that can be prioritized for efficient breeding and crop improvement.

In the present study, 48 maize inbred lines, including 5 checks, were subjected to Principal Component Analysis (PCA) based on 14 agro-phenotypic traits. The analysis initially generated a number of principal components equal to the traits evaluated. However, in accordance with Kaiser's criterion, only components with eigenvalues greater than one (>1) were considered significant contributors to total variability. Based on this criterion, five principal components (PC1, PC2, PC3, PC4, and PC5) were retained, accounting for 31.88%, 16.82%, 12.89%, 10.14%, and 8.12% of the variance, respectively, and together explaining 79.86% of the cumulative variance which is summarized in

Based on the contribution of variables to each principal component as mentioned in , Dimension 1, which explains the highest variance (31.88%), was mainly influenced by days to 50% tasseling, days to 50% anthesis, days to 50% silking, plant height, cob placement, yield per plant, shelling percentage, and cob length. Dimension 2, accounting for 16.82% of the variance, was predominantly shaped by days to 50% tasseling, days to 50% silking, days to 50% anthesis, yield per plant, and shelling percentage. The variation in Dimension 3 (12.89%) was largely explained by number of rows per cob, the number of kernels per row, and 100-seed weight. Dimension 4, contributing 10.14% of the variance, was primarily associated with anthesis–silking interval, cob diameter, number of rows per cob, and 100-seed weight. Finally, Dimension 5, which accounted for 8.12% of the variance, was mainly contributed by anthesis–silking interval, plant height, cob placement, number of kernels per row, and yield per plant. In this study the key contributing traits towards maturity and yield are predominantly represented in principal component one and two which contributes maximum variance.

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On the basis of first three dimensions the biplot was graphed as they are the highest contributors of total variance among the five retained principal components. In the first biplot (Dim1 vs. Dim2) ,the yield and yield contributing traits such as shelling percentage, cob diameter, 100 seed weight, number of kernels per row, number of rows per cob, plant height, cob placement are pointed in top right quadrant showing their interrelation while the highest contributors are showing longest arrows that is shelling percentage and yield per plant followed by plant height and cob placement while the maturity traits are pointed in top left quadrant including days to 50% tasseling, anthesis, silking and maturity with close proximity with each other showing their correlation while longest pointing showing their contribution. The maturity and yield traits are making an angle of (90^0) thus they are uncorrelated with each other. The inbred which are located in close proximity each other are more closely related to each other, while the distantly located are uncorrelated. In the second biplot (Dim1 vs. Dim2), , traits such as number of kernels per row, anthesis–silking interval, cob length, plant height, and cob placement are clustered in the top-right quadrant, indicating a strong inter-relationship among them. On the other hand, traits like shelling percentage, yield per plant, cob diameter, 100-seed weight, and number of rows per cob are grouped in the lower-right quadrant, reflecting their close association. In contrast, the maturity-related traits are positioned in the opposite direction, highlighting their negative correlation with the yield-contributing traits.

The findings are consistent with the results reported by [13, 30, 19, 29, 43, 47, 27] studied maize accessions and reported two principal components with eigen values greater than one with cumulative variance of 66.99% [34] reported seven principal components with similar traits [15] reported 3 PC's with cumulative variance of 67.7% [50] reported 3 principal components with similar traits contributing 71.76 % total variability, [40] reported 3 principal component with 81.41 % total variance while grain yield per plant, plant height and days to 50% tasseling were major contributors of variance.

Clustering Analysis for Agro-morphological Traits

Cluster analysis is a multivariate statistical technique used to classify a set of objects (genotypes, traits) into groups or clusters based on their similarity or dissimilarity. The goal is to ensure that objects within the same cluster are highly similar to each other, while those in different clusters are more dissimilar. The panel of 48 inbred lines along with 5 checks was subjected to cluster analysis, which classified the genotypes into two clusters based on agro-morphological traits, as summarized in The graphical representation of the hierarchical dendrogram and optimal number of clusters are presented in Fig. 5 and Fig. 6. A high degree of genetic variability was evident among the genotypes, as revealed by the clustering pattern. Greater variation was observed among clusters than within individual clusters. In the current study, intra-cluster distances of (38.18) and (35.25) were recorded for Cluster I and Cluster II, respectively, whereas the inter cluster distance between the two clusters was (63.18). The high inter-cluster diversity indicates that crosses between genotypes from Cluster I and Cluster II may result in enhanced heterosis (hybrid vigour) and a broader genetic base. Since Cluster II is larger, it reflects greater genetic variability and can serve as a valuable reservoir of diverse parental lines. The average intra and inter cluster distance are presented in Table 11.

The cluster means of cluster I and cluster II for 14 agro-morphological traits are presented in Table 13. Cluster mean represents the average performance of all germ plasm lines grouped within a particular cluster. The genetic differences among clusters are reflected in their mean values, which varied for one or more traits. According to the cluster means obtained through analysis cluster I recorded highest values for anthesis-silking interval (4.19), plant height (148.84 cm), cob placement (71.97 cm), cob length (15.77 cm), cob diameter (3.82 cm), number of rows per cob (12.67 rows), number of kernels per row (24.81 kernels), 100 seed weight (23.06 g), yield per plant (71.33 g) and shelling percentage (74.37 %). In contrast, it exhibited the lowest values for days to 50% tasseling (84.62 days), days to 50% anthesis (87.05 days), days to 50% silking (91.29 days) and days to maturity (130 days).This indicates that Cluster I harbours high-performing genotypes with superior yield and yield-contributing traits, making it a valuable group for selecting elite lines in maize improvement programs.

Cluster-II exhibited highest values for days to 50% tasseling (89.75 days), days to 50% anthesis (93.00 days), days to 50% silking (96.50 days) and days to maturity (136.34 days). In contrast lowest values where observed in anthesis-silking interval (3.50), plant height (104.74 cm), cob placement (45.33 cm), cob length (12.78 cm), cob diameter (3.48 cm), number of rows per cob (12.25 rows), number of kernels per row (22.02 kernels), 100 seed weight (21.62 g), yield per plant (56.43 g) and shelling percentage (67.77 %).

Considering the highest and lowest values across both clusters, it can be inferred that Cluster I contributes more to yield and yield-attributing traits, whereas Cluster II is associated with maturity-related traits but as early maturity in more desirable and favourable we must opt for lines in cluster I which shows lowest mean values for maturity and highest for yield and yield attributing traits. The results are in close conformity with the findings of [42] worked with similar traits and reported 3 clusters [13, 38, 26, 49] studied maize in breds and reported 6 clusters he also found that inter cluster distances where higher than that of intra-cluster distances [27] studied on 92 maize accessions and reported 4 clusters in which high yielding and early maturity genotypes where grouped into cluster I, [15] 260 entries were grouped into 5 clusters [40] reported 8 clusters with first cluster being largest followed by others.

Table.1 List of inbreds

S.No	INBRED	S.No	INBRED	S.No	INBRED	S.No	INBRED
1.	KDM-312	13.	KDM-445	25.	KDM-483	37.	KDM-526
2.	KDM-320	14.	KDM-446	26.	KDM-487	38.	KDM-529
3.	KDM-325	15.	KDM-449	27.	KDM-491	39.	KDM-530
4.	KDM-379	16.	KDM-450	28.	KDM-498	40.	KDM-540
5.	KDM-405	17.	KDM-456	29.	KDM-501	41.	KDM-543
6.	KDM-406	18.	KDM-457	30.	KDM-502	42.	KDM-548
7.	KDM-424	19.	KDM-458	31.	KDM-504	43.	KDM-558
8.	KDM-426	20.	KDM-459	32.	KDM-512	44.	KDM-560
9.	KDM-427	21.	KDM-460	33.	KDM-513	45.	KDM-562
10.	KDM-436	22.	KDM-461	34.	KDM-518	46.	KDM-576
11.	KDM-438	23.	KDM-466	35.	KDM-519	47.	KDM-579
12.	KDM-443	24.	KDM-469	36.	KDM-520	48.	KDM-600

Table.2 List of Checks

Sr.No	Checks
1.	CML-451
2.	DML-1084
3.	IML-418-1
4.	PV-1
5.	IML-187

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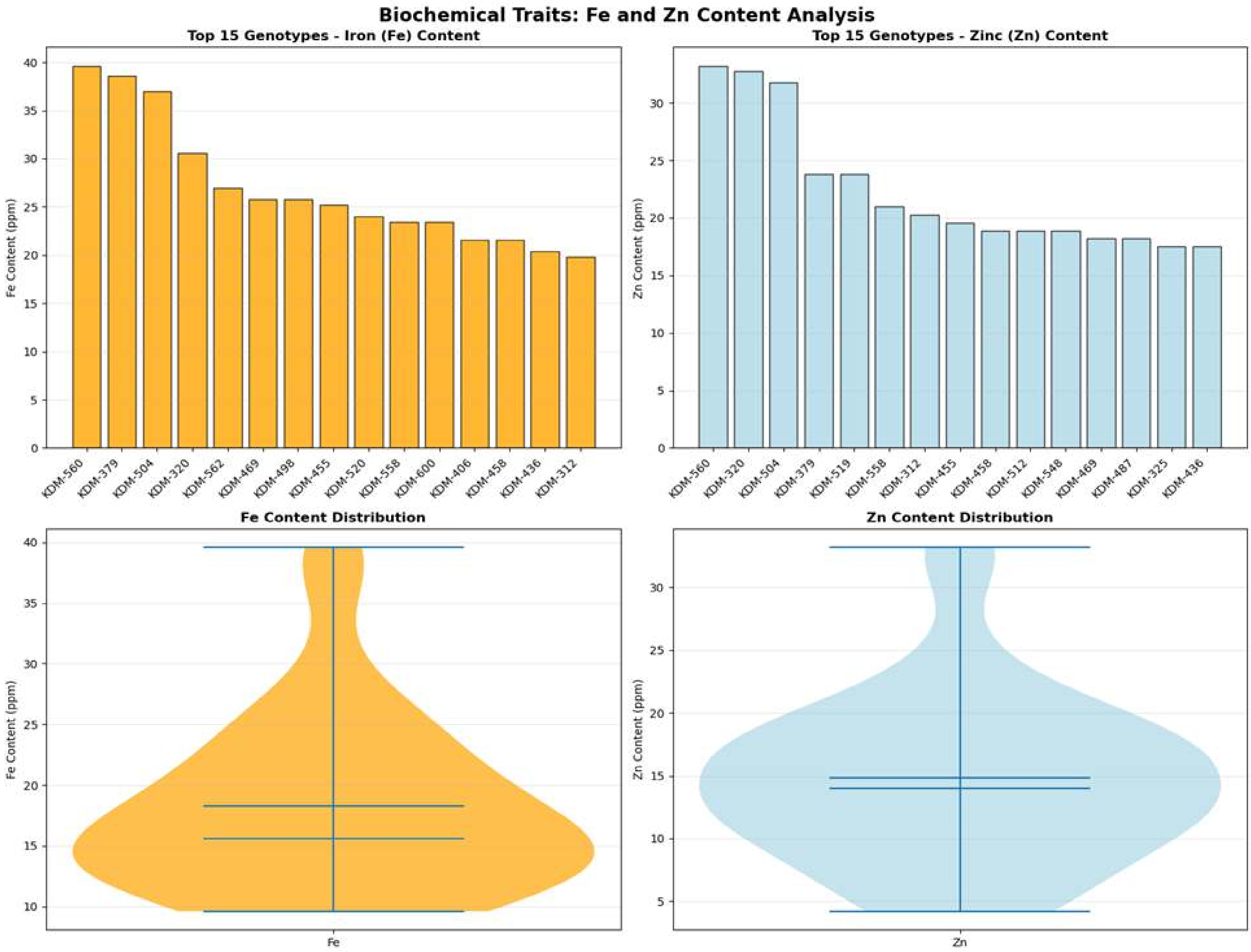

Fig.1 Bar graph and violin plot representation of top 15 genotypes for Fe and Zn

Table.3 Analysis of variance for 14 agro-morphological traits of maize (Zeamays L.) inbred lines (ABD)

Source of Variation	Df	D50T	D50A	D50S	ASI	DM	PH	CP	CL	CD	RC	KR	100SW	S	YP
Block (ignoring Treatments)	5	24.14 *	29.16 **	27.82 **	0.61 ns	13.52 ns	446.95 **	71.89 *	14.71 **	0.48 **	6.48 **	25.19 *	13.96 *	163.94 **	9.58 ns
Block (eliminating Treatment)	5	14.40 ns	10.69 ns	8.48 ns	0.13 ns	6.14 ns	28.37 ns	1.73 ns	1.78 ns	0.18 ns	1.52 ns	10.69 ns	8.89 ns	6.72 ns	2.12 ns
Treatment (ignoring Blocks)	52	63.50 **	66.19 **	66.98 **	1.38 **	61.51 **	894.01 **	370.11 **	13.31 **	0.48 **	4.50 **	22.64 **	14.92 **	242.90 **	40.75 **
Treatment (eliminating Blocks)	52	62.56 **	64.41 **	65.12 **	1.34 **	60.80 **	853.76 **	363.37 **	12.06 **	0.45 **	4.02 **	21.24 **	14.44 **	227.78 **	40.03 **
Treatment: Test	47	48.34 **	57.81 **	56.37 **	1.42 **	39.27 **	757.77 **	291.42 **	13.20 **	0.28 **	3.79 *	20.42 **	14.34 **	36.13 *	183.28 **
Checks	4	75.05 **	47.30 **	45.62 **	0.18 ns	203.07 **	941.42 **	730.70 **	8.70 **	1.69 **	3.19 ns	38.07 **	22.09 **	57.36 *	614.15 **
Test vs Checks	1	743.44 **	529.24 **	637.21 **	4.62 **	24.06 ns	5664.62 **	10.73 ns	27.06 **	2.75 **	20.68 **	27.14 *	15.43 *	30.49 ns	5.28 ns
Error	20	6.62	6.08	6.05	0.27	6.07	34.11	26.12	0.96	0.10	0.72	6.71	4.37	10.53	4.97

Table.4 Analysis of variancefor micronutrient traits (CRD)

Source of Variation	Df	Fe	Zn
Treatment	52	193.230***	162.152***
Error	106	0.085	0.055

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Table.5 Descriptive Statistics for Agro-morphological and Micronutrient Traits

Morphological Traits						
Traits	**Minimum**	**Maximum**	**Mean**	**SD**	**SE**	**CV (%)**
D50T (days)	75	106	88.14	6.95	1.00	7.88
D50A (days)	77	110	91.02	7.60	1.09	8.34
D50S (days)	80	113	94.87	7.50	1.08	7.90
ASI (days)	2	7	3.83	1.19	0.17	31.07
DM (days)	113	146	134.04	6.26	0.90	4.67
PH (cm)	61.65	177	120.81	27.52	3.97	22.77
CP (cm)	26	102.65	55.89	17.07	2.46	30.54
CL (cm)	7.10	21.00	13.79	3.63	0.52	26.32
CD (cm)	2.50	5.10	3.64	0.53	0.07	14.56
RC (rows)	8.00	16.00	12.45	1.94	0.28	15.58
KR (kernels)	15.00	38.00	23.05	4.51	0.65	19.56
100SW (g)	15.00	29.40	22.17	3.78	0.54	17.05
YP (g)	38.06	106.00	62.22	13.53	1.95	21.74
S (%)	60.00	83.00	70.00	6.01	0.86	8.58
Micronutrient Traits						
Fe (ppm)	9.5	39.8	18.86	8.03	1.1	42.57
Zn (ppm)	4.2	33.3	15.38	7.35	1.0	47.78

Table.6 Estimates of Genetic Variability for Agro-morphological and Micronutrient Traits

Morphological Traits										
Trait	**Adjusted Mean**	**Variance**		**Coefficient of Variance with category**						
		PV	**GV**	**EV**	**GCV**	**GCV Category**	**PCV**	**PCV Category**	**ECV**
D50T	87.55	48.34	41.72	6.62	7.38	**Low**	7.94	**Low**	2.94
D50A	90.52	57.81	51.73	6.08	7.95	**Low**	8.40	**Low**	2.72
D50S	94.32	56.37	50.32	6.05	7.52	**Low**	7.96	**Low**	2.61
ASI	3.79	1.42	1.15	0.27	28.35	**High**	31.46	**High**	13.64
DM	133.93	39.27	33.20	6.07	4.30	**Low**	4.68	**Low**	1.84
PH	122.47	757.77	723.66	34.11	21.97	**High**	22.48	**High**	4.77
CP	55.82	291.42	265.30	26.12	29.18	**High**	30.58	**High**	9.15
CL	13.91	13.20	12.24	0.96	25.16	**High**	26.12	**High**	7.03
CD	3.61	0.28	0.19	0.10	11.99	**Medium**	14.76	**Medium**	8.61
RC	12.36	3.79	3.07	0.72	14.17	**Medium**	15.74	**Medium**	6.87
KR	23.17	20.42	13.71	6.71	15.98	**Medium**	19.51	**Medium**	11.18
100SW	22.27	14.34	9.97	4.37	14.18	**Medium**	17.01	**Medium**	9.39
S	70.45	36.13	31.15	4.97	7.92	**Low**	8.53	**Low**	3.17
YP	62.34	183.28	172.75	10.53	21.08	**High**	21.71	**High**	5.20
Micronutrient Traits									
Fe	18.86	64.46	64.38	0.08	42.53	**High**	42.56	**High**	1.54
Zn	15.38	54.08	54.03	0.05	47.77	**High**	47.89	**High**	1.53

Table 7. Estimates of Heritability and Genetic Advance for Agro-morphological and Micronutrient Traits

Morphological Traits						
Trait	**Unit**	**h^2 (Broad Sense)**	**Category**	**GA**	**GAM**	**Category**
D50T	days	86.31	**High**	12.38	14.14	**Medium**
D50A	days	89.49	**High**	14.04	15.51	**Medium**
D50S	days	89.27	**High**	13.83	14.66	**Medium**
ASI	days	81.20	**High**	2.00	52.69	**High**
DM	days	84.54	**High**	10.93	8.16	**Low**
PH	cm	95.50	**High**	54.23	44.28	**High**
CP	cm	91.04	**High**	32.06	57.43	**High**
CL	cm	92.76	**High**	6.95	49.98	**High**
CD	cm	65.95	**High**	0.72	20.08	**High**
RC	rows	80.98	**High**	3.25	26.30	**High**
KR	kernels	67.14	**High**	6.26	27.02	**High**
100SW	g	69.55	**High**	5.43	24.40	**High**
S	%	86.23	**High**	10.69	15.18	**Medium**
YP	g	94.25	**High**	26.32	42.22	**High**
Micronutrient Traits						
Fe	ppm	99.87	**High**	16.51	87.56	**High**
Zn	ppm	99.90	**High**	15.13	98.36	**High**

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Table. 8 Pearson's Correlation coefficient for 14-Agro-morphological Traits

Trait	D50T	D50A	D50S	ASI	DM	PH	CP	CL	CD	RC	KR	100SW	YP	S
D50T	**1.000**	0.993	0.979	-0.128	0.550	-0.255	-0.283	-0.320	-0.119	-0.089	-0.003	-0.065	-0.171	-0.210
D50A	0.993	**1.000**	0.988	-0.118	0.564	-0.270	-0.320	-0.312	-0.148	-0.091	-0.001	-0.079	-0.176	-0.218
D50S	0.979	0.988	**1.000**	0.037	0.551	-0.241	-0.294	-0.290	-0.130	-0.077	0.014	-0.106	-0.175	-0.183
ASI	-0.128	-0.118	0.037	**1.000**	-0.117	0.177	0.161	0.146	0.113	0.097	0.100	-0.189	0.000	0.223
DM	0.550	0.564	0.551	-0.117	**1.000**	-0.308	-0.266	-0.043	0.053	0.006	-0.107	-0.025	-0.155	-0.198
PH	-0.255	-0.270	-0.241	0.177	-0.308	**1.000**	0.876	0.441	0.247	-0.013	0.165	0.224	0.350	0.435
CP	-0.283	-0.320	-0.294	0.161	-0.266	0.876	**1.000**	0.417	0.307	-0.058	0.160	0.286	0.352	0.455
CL	-0.320	-0.312	-0.290	0.146	-0.043	0.441	0.417	**1.000**	0.223	-0.075	0.418	-0.133	0.255	0.347
CD	-0.119	-0.148	-0.130	0.113	0.053	0.247	0.307	0.223	**1.000**	0.447	-0.050	0.085	0.352	0.284
RC	-0.089	-0.091	-0.077	0.097	0.006	-0.013	-0.058	-0.075	0.447	**1.000**	-0.312	-0.045	0.372	0.129
KR	-0.003	-0.001	0.014	0.100	-0.107	0.165	0.160	0.418	-0.050	-0.312	**1.000**	-0.373	0.371	0.286
100SW	-0.065	-0.079	-0.106	-0.189	-0.025	0.224	0.286	-0.133	0.085	-0.045	-0.373	**1.000**	0.456	0.432
YP	-0.171	-0.176	-0.175	0.000	-0.155	0.350	0.352	0.255	0.352	0.372	0.371	0.456	**1.000**	0.748
S	-0.210	-0.218	-0.183	0.223	-0.198	0.435	0.455	0.347	0.284	0.129	0.286	0.432	0.748	**1.000**

Abbreviation: Days to 50% tasseling – (D50T), Days to 50% silking – (D50S), Days to 50% anthesis – (D50A), Anthesis-Silking Interval –(ASI), Plant height (cm), – (PH), Cob Placement (cm), – (CP),Days to Maturity– (DM), Cob length (cm), –(CL), Cob diameter (cm), –(CD), Number of rows per cob –(RC), Number of kernel per row –(KR), 100 seed weight (g), –(100SW), Shelling percentage (%), –(S), Yield per plant (g), –(YP).

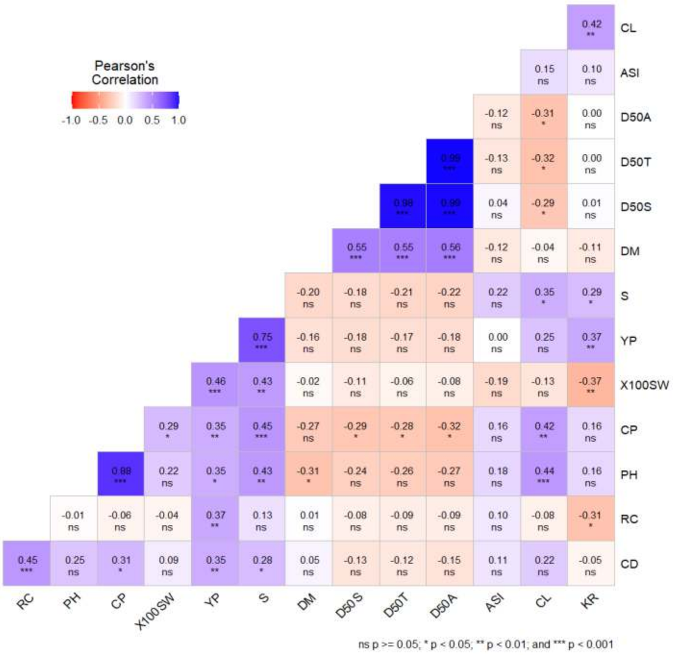

Fig.2 Pearson's Correlation HeatMap for 14-Agromorphological Traits

Abbreviation: Days to 50% tasseling – (D50T), Days to 50% silking – (D50S), Days to 50% anthesis – (D50A), Anthesis-Silking Interval –(ASI), Plant height (cm), – (PH), Cob Placement (cm), – (CP),Days to Maturity– (DM), Cob length (cm), –(CL), Cob diameter (cm), –(CD), Number of rows per cob –(RC), Number of kernel per row –(KR), 100 seed weight (g), –(100SW), Shelling percentage (%), –(S), Yield per plant (g), –(YP).

Table. 9 Latent scores (Eigen values) for Principal Component Analysis

Component	Eigenvalue	% of Variance	Cumulative % Variance
Comp 1	**4.4639**	**31.88**	**31.88**
Comp 2	**2.3544**	**16.82**	**48.70**
Comp 3	**1.8049**	**12.89**	**61.59**
Comp 4	**1.4202**	**10.14**	**71.74**
Comp 5	**1.1373**	**8.12**	**79.86**
Comp 6	0.9582	6.84	86.71
Comp 7	0.7124	5.09	91.79
Comp 8	0.4736	3.38	95.18
Comp 9	0.3390	2.42	97.60
Comp 10	0.2097	1.50	99.10
Comp 11	0.1088	0.78	99.87
Comp 12	0.0120	0.09	99.96
Comp 13	0.0055	0.04	99.99
Comp 14	0.0002	0.00	100.00

Table. 10 Contribution of variables towards each principal component %

Trait	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5
D50T	**14.11**	**13.86**	0.15	0.23	0.35
D50A	**14.64**	**13.29**	0.23	0.16	0.19
D50S	**13.63**	**14.36**	0.55	0.03	0.99
ASI	0.94	0.42	3.00	**14.22**	**11.96**
DM	**7.33**	5.90	0.28	1.26	0.17
PH	**10.05**	5.85	1.76	2.68	**18.28**
CP	**10.81**	5.55	1.11	3.67	**17.74**
CL	**6.45**	1.64	**12.42**	2.78	0.00
CD	2.97	5.13	6.71	**17.22**	2.52
RC	0.48	0.60	**21.50**	**28.25**	0.13
KR	1.04	3.30	**30.75**	0.95	**20.20**
100SW	1.59	3.79	**18.20**	**28.20**	0.04
YP	**7.24**	**13.61**	3.03	0.04	**21.21**
S	**8.73**	**12.69**	0.30	0.31	6.21

Abbreviation: Days to 50% tasseling – (D50T), Days to 50% silking – (D50S), Days to 50% anthesis – (D50A), Anthesis-Silking Interval –(ASI), Plant height (cm), – (PH), Cob Placement (cm), – (CP),Days to Maturity– (DM), Cob length (cm), –(CL), Cob diameter (cm), –(CD), Number of rows per cob –(RC), Number of kernel per row –(KR), 100 seed weight (g), –(100SW), Shelling percentage (%), –(S), Yield per plant (g), –(YP).

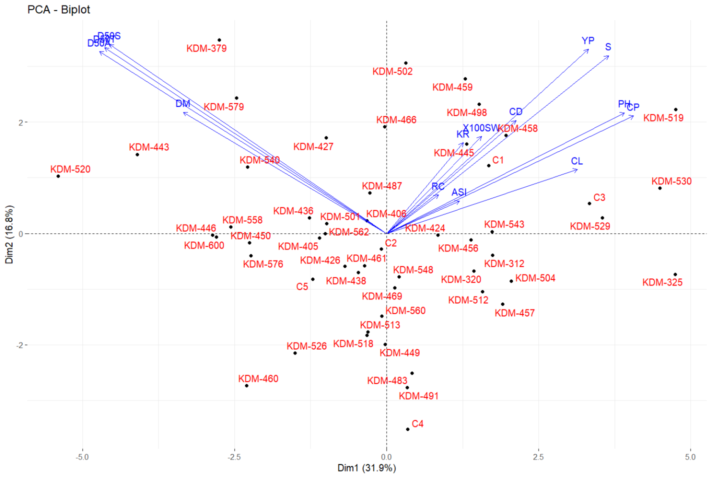

Fig.3 PCA Biplot Dimension I (PC-I) vs Dimension II (PC-II)

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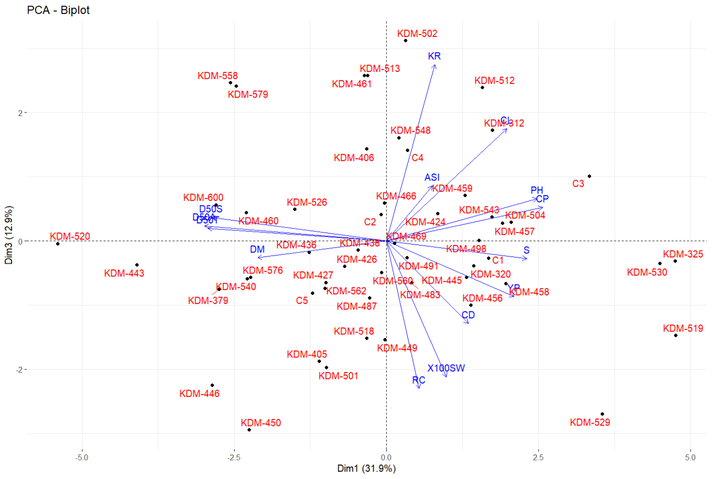

Fig.4 PCA Biplot Dimension I (PC-I) vs Dimension III (PC-III)

Table.11 Average values of intra and inter cluster distances

Clusters	Cluster -1	Cluster -2
Cluster -1	**38.18**	63.71
Cluster -2	63.71	**35.29**

Table. 12 Distribution of Inbreds across clusters

Clusters	No. of inbreds	Genotypes
Cluster- 1	**21**	KDM-529, C3, KDM-519, KDM-530, KDM-543, KDM-457, KDM-548, KDM-424, KDM-456, KDM-320, KDM-512, KDM-312, KDM-504, KDM-325, KDM-466, KDM-459, KDM-445, C1, KDM-498, KDM-458, KDM-502
Cluster- 2	**32**	KDM-562, C5,KDM-576, KDM-501, KDM-427, KDM-406,C2, KDM-461, KDM-600, KDM-558, KDM-540, KDM-579, KDM-379, KDM-518, KDM-449, KDM-491, KDM-469, KDM-560,C4, KDM-513, KDM-438, KDM-483, KDM-436, KDM-405, KDM-426, KDM-450, KDM-446, KDM-520, KDM-443, KDM-526, KDM-460, KDM-487

Table. 13 Cluster means based on 14 agro-morphological trait

Trait	Unit	Cluster 1	Cluster 2
D50T	days	84.62	**89.75**
D50A	days	87.05	**93.00**
D50S	days	91.29	**96.50**
ASI	days	**4.19**	3.50
DM	days	130.43	**136.34**
PH	cm	**148.84**	104.74
CP	cm	**71.97**	45.33
CL	cm	**15.77**	12.78
CD	cm	**3.82**	3.48
RC	rows	**12.67**	12.25
KR	kernels	**24.81**	22.02
100SW	g	**23.06**	21.62
YP	g	**71.33**	56.43
S	%	**74.37**	67.77

Abbreviation: Days to 50% tasseling – (D50T), Days to 50% silking – (D50S), Days to 50% anthesis – (D50A), Anthesis-Silking Interval –(ASI), Plant height (cm), – (PH), Cob Placement (cm), – (CP),Days to Maturity– (DM), Cob length (cm), –(CL), Cob diameter (cm), –(CD), Number of rows per cob –(RC), Number of kernel per row –(KR), 100 seed weight (g), –(100SW), Shelling percentage (%), –(S), Yield per plant (g), –(YP).

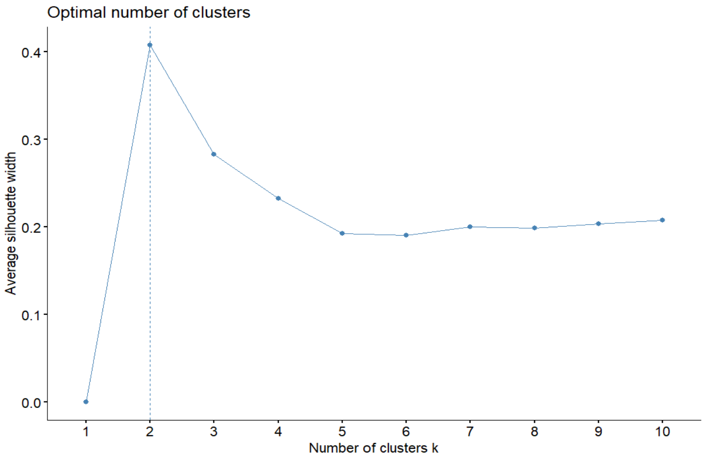

Fig.5 Optimal`number of clusters for 14- agro-morphological traits

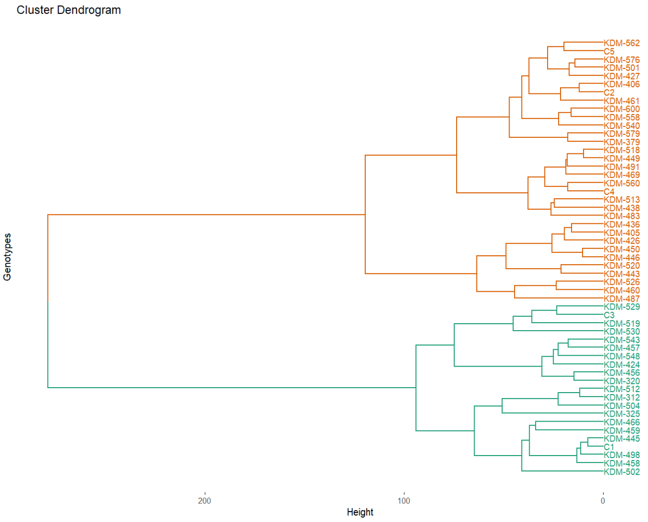

Fig.6 Hierarchical dendrogram based on 14 agro-morphological traits

Conclusion

In view of the results obtained from the present investigation, several important conclusions can be articulated regarding theextent of variability, heritability, trait associations, and genetic diversity among the studied maize inbreds

1. A significant level of variability was observed among the maize inbred lines for morphological, maturity, yield, and quality attributes, which can be harnessed in future breeding strategies.
2. Anthesis–silking interval (ASI), plant height, cob placement, yield per plant, and kernel Fe and Zn concentrations exhibited high magnitudes of PCV and GCV, in conjunction with elevated heritability and genetic advance estimates. These parameters collectively signify the presence of ample genetic variability and the predominance of additive gene action, thereby identifying these traits as key candidates for selection owing to their genetic stability and substantial potential for genetic gain.
3. Correlation analysis revealed that days to maturity was positively associated with flowering traits, while yield per plant showed significant positive correlations with shelling percentage, seed weight, cob traits, and plant height, indicating that improvement in these traits can substantially enhance grain yield potential.

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4. PCA reduced the trait variability into five major components, cumulatively explaining 79.86% of the total variation. PC1,PC2 and PC3 captured the maximum variance, largely influenced by flowering, maturity and yield and yield attributing traits.
5. Cluster analysis classified the maize inbreds into two distinct groups, indicating considerable genetic diversity. Cluster I was identified as favourable, combining early maturity with superior yield performance. The higher inter-cluster distances compared to intra-cluster distances highlight the potential of inter-cluster hybridization to enhance heterosis and expand the genetic base.
6. KDM-519, KDM-530, KDM-502, KDM-459 and KDM-529 where found high on yield with medium maturity duration.
7. KDM-504 and KDM-320 was observed as early maturing.
8. KDM-560 (39.8 ppm), KDM-379 (38.6 ppm) and KDM-504 (37.26 ppm) outperformed the checks and were identified as high in Fe content.
9. KDM-560 (33.33 ppm), KDM-320 (32.63 ppm) and KDM-504 (31.63 ppm) were identified as high Zn lines.

Future Scope of the Study

The identified maize inbred lines can be exploited in hybrid breeding programs to develop high-yielding, micronutrient-enriched cultivars. Multi-location and multi-season evaluations are required to validate yield stability and grain iron and zinc concentrations. Integration of molecular and genomic tools will further enhance selection efficiency and accelerate biofortification-oriented maize improvement.

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Conflict of interest

The authors declare that there are no conflicts of interest associated with this study.

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