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Assessment of Nutritional and Agro morphological Traits in Zea mays Inbred Lines for Aflatoxin Resistance Using the Side Needle Inoculation Technique

Imran Manzoor [1,4*], Kotb A. Attia[2], Muhammad Arshad [1] , Rao Muhammad Samran Gul[3], Tahir Naqqash [6], Itoh Kimiko[5], Muhammad Tahir [8], Momina Hussain [7]

[1]Department of Biotechnology, University of Okara, Pakistan.
[2]Center of Excellence in Biotechnology Research, King Saud University, Saudi Arabia.
[3]Chinese Academy of Agricultural Sciences, Beijing, China.
[4]Laboratory, Maize and Millet Research Institute (MMRI), Yousaf Wala, Sahiwal, Pakistan.
[5]Institute of Science and Technology, Niigata University, Japan.
[6]Department of Molecular Biology and Biotechnology, BZU Multan, Pakistan.
[7]Chinese academy of Tropical Agriculture and Sciences. Sanya, China.
[8]Department of Microbiology, Molecular biology and Biotechnology CUI, Vehari, Pakistan.

Correspondence
Imran Manzoor
Signmein32@gmail.com

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Abstract

Maize is an important staple food, and there is a need to increase its productivity. However, its production is limited by the occurrence of aflatoxin-producing fungi *Aspergillus flavus* and *Aspergillus parasiticus*. This research pursued to estimate the variation in agro-morphological and biochemical characteristics in relation to fungal resistance through principal component analysis and clustering. There was great diversity identified for resistance against *Aspergillus flavus* infections. 49 inbred lines were highly resistant, 23 were resistant, 22 maize inbred lines were fairly resistant, thirty exhibited moderate susceptibility, twenty two were vulnerable, and four displayed high susceptibility to fungal infection. Out of 49 elite maize inbred lines identified as highly resistant, UO45, UO43, UO44, UO86, UO68, and UO91 exhibited excellent yield performance and could be openly utilized or integrated into breeding the maize to improve high-yielding genotypes susceptible to *Aspergillus flavus.* Principal component analysis indicated that the first four components, with eigenvalues exceeding 1, accounted for 70.2% of the total variability in agro-morphological and biochemical traits. Through clustering investigation, 150 maize inbred lines were characterized into five different groups, with clusters 1 to 5 consisting of 37, 29, 26, 46, and 12 lines, correspondingly. Cluster 5 enclosed high yielding lines distinguished by promising traits. Pearson correlation analysis revealed noteworthy relations across all analyzed characteristics. Based on a comprehensive evaluation, UO45, UO43, UO44, UO68, UO92, UO112, and UO86 appeared as favorable high-yielding and *Aspergillus flavus* resistant lines, endorsed to their greater plant height, longer ears, higher 100 kernel weight, and better grain yield per plot.

KEYWORDS
Aspergillus flavus; *Zea mays L.*; Biotic stress; Inbred lines; Association; Yield.

1.0 INTRODUCTION

Maize is a miracle food crop in cultivation [1]. Maize is the most important cereal crop and considered as an essential staple food in the world [2]. The importance of maize around the world cannot be denied because it reduces hunger in economically developing and poor countries. In the industrial sector of the world, maize is used as the most important fodder and industrial resource. Pakistan has the 16th rank in the world in terms of maize productivity [3]. Understanding the genetic variation is important for assessing the extent of genetic variation within the foundational material [4]. Humans began to use genetic variation of species about 10000 years ago, and agriculture started to meet their needs. Breeders inserted the desired gene and deleted different processes, including selection, migration, population size, variation, and genetic drift [6]. The main cause of genetic variation is gene flow between the individual species in plants. By the estimation of intraspecific variation, knowledge about the development of diversity and successful breeding programs in the genetic structure of plants can be obtained [7]. The assessment of genotypic and phenotypic traits can play an important role in improving the yield, but these approaches should be reliable and reproducible [8].

Globally common pathogens of maize are the species of the genus *Aspergillus* and *Fusarium*. The popular species are *F. graminearum*, *F. verticillioides*, and *A. flavus* among them [9]. *Aspergillus flavus* generates aflatoxins, a group of mycotoxins frequently found contaminating food and animal feed[10]. These toxins pose significant health risks to both humans and animals [11]. Among the twenty identified aflatoxins, the most critical are B1, B2, G1, and G2 [12]. The prevalence of aflatoxins, particularly B1, B2, G1, G2, and M1, in food products is notably higher compared to other variants. [13]. Aflatoxin B1 can attach to DNA and cause transversions of G to T. The main target of aflatoxin B1 is human tumor gene p53 [14], which is why IARC classifies AFs B1 as human cancer-causing. The limit of aflatoxin varies greatly between countries; in the United States, the acceptable threshold for aflatoxin contamination is set at 20 ppb (μg/kg), whereas in Europe, the controlling limit is stricter at 4 ppb.

For aflatoxin control in food, strict rules are not the best policy [15]. In Pakistan, the health of local customers is harmfully impacted as high quality food is often exported, leaving behind aflatoxin-contaminated products for domestic consumption. [16]. In the local market, contaminated products are sold at low prices, so aflatoxin exposure in low-income people may increase [17]. Due to low-quality imported food products in Pakistan, the local population is at higher risk of aflatoxin exposure [18]. There should be regulation for the application of aflatoxin standards for exported products. In these inbred lines, the genetic diversity is still basically unidentified to make the germplasm resistant to aflatoxin. It needs to be categorized and made more effective in the present landraces and varieties and also to expand the base material to get elite lines for developing new maize varieties.

The present proper selection and genetic variation against yield-related characters develop superior crop varieties [19]. The effectiveness of breeding programs depends on the extent of genetic diversity, variability, and the inheritance of essential traits [20]. Multivariate statistical methods play a crucial role in assessing genetic variation and classifying germplasm [21]. Principal component analysis (PCA), a widely applied technique, simplifies complex datasets by grouping variables into key components [22]. PCA also allowing for better interpretation of genetic relationships and trait contributions [23]. Additionally, hierarchical clustering provides a systematic approach to structuring and categorizing germplasm based on genetic similarities [24]. Evaluating genetic diversity is essential for selecting superior parental lines for hybridization [25]. Principal component analysis is a statistical technique used to reduce the complexity of large datasets, enhance interpretability, and minimize data loss [26]. Economic yield, a highly complex trait, is influenced by genetic factors, environmental conditions, and various biotic and abiotic stressors [27]. Introducing new germplasm is vital for expanding genetic variation and strengthening breeding programs. Bridging the existing yield gap and developing high-yielding maize varieties require advancements in crop productivity and stress tolerance [28]. Understanding genetic variability is fundamental for breeding strategies aimed at producing superior progeny [29]. Researchers have emphasized the importance of selecting diverse parents to develop improved inbred lines during segregating generations [30,31]. Yield-related traits have frequently been analyzed using multivariate approaches to assess genetic diversity and categorize crop genotypes effectively [32]. Given these attentions, this study directed to assess the performance of numerous maize inbred lines, examine genetic variance in key agro-morphological and biochemical traits, and classify high-yielding genotypes with resistance to *Aspergillus flavus*. Additionally, the research sought to pinpoint required traits that could enlarge the maize gene pool, contributing to breeding programs

Table-1. A brief outline of the research approach for this study.

concentrated on developing resilient and high-performing maize varieties.

2. Materials and Methods

There were three main experiments in the present research work. These were performed under laboratory conditions and in open field conditions. The outline of work plan is presented in (Table 1).

Level of experiment	Explanation	No. of inbred lines	Trial conditions
Agro morphological-I	Study of qualitative and quantitative traits in autumn 2023	150 inbred lines from MMRI, Sahiwal	Research field at MMRI Sahiwal, Pakistan
Agro morphological-II	Study of qualitative and quantitative traits in spring 2024	150 inbred lines from MMRI, Sahiwal	Research field at MMRI Sahiwal, Pakistan
Agro morphological-III	Study of quantitative and qualitative traits in autumn 2024	150 inbred lines from MMRI, Sahiwal	Research field at MMRI Sahiwal, Pakistan

2.1 Plant material and location of experiment

A total of 150 elite inbred lines of maize were selected for this experiment. These inbreed lines were locally derivated from local germplasm. These inbred lines were sourced from the Maize and Millet Research Institute (MMRI) located in Yousaf Wala, District Sahiwal, Pakistan. For the genetic diversity estimation based on morphological traits, the first field experiment was accompanied during autumn-2023, second in spring-2024 and third in autumn-2024 in open research fields at MMRI, Sahiwal, Pakistan. Situated at latitude of 31°41′N and a longitude of 73°12′E, the location stands at an elevation of 175 meters above sea level. In 2024, the recorded annual rainfall in this region was approximately 200 mm.

2.2 Field management and experimental layout

The experiment was planted using a randomized block design (RBD) with three replications depending upon the objectives of the research work. In the east-west direction it comprised 3 beds, and in each row 20 plants. Each plot maintained an inter-row spacing of 45 cm and intra-row spacing of 10 cm. To grow healthy crop from sowing to the harvesting all suggested traditional practices were accomplished. Chemical fertilizer, accurate irrigation and pesticides were used when required. From central part of each row indiscriminately five plants were carefully chosen and for recording the agro-morphological data the plants were tagged.

2.3 *Aspergillus flavus* inoculum application

A total of 150 maize inbred lines were designated to evaluate genetic diversity in reaction to aflatoxin contamination. *Aspergillus flavus* strain 464, sourced from MMRI, was utilized in field trials due to its well-documented ability to induce high aflatoxin levels in maize grain. The fungal inoculum was cultured on PDA media to boost its growth [33]. Spore concentrations were measured using a hemocytometer and diluted with sterile distilled water to the required level [34]. To facilitate infection, maize kernels in each plot were inoculated with an *A. flavus* conidial suspension using the side-needle technique, two weeks after mid silking to ensure consistent exposure across all test entries (when at least 50% of plants in the plot had visible silk emergence). Each ear was injected with 3.4 mL of suspension containing 3 × 10^8 conidia per mL through the husk (Figure 1).

Fig-1. Inoculum application by side needle technique.

Maize ears were harvested at physiological maturity, approximately 60 days post-mid-silk. The disease responses of various elite inbred lines to *A. flavus* infection were carefully documented. After harvesting 5 plants were selected at random from each line and scored for morphological data and fungal attack. The development of the disease was started by artificial infection, and the disease infection was created by side needle technique.

2.4 Evaluation of agronomic traits and biochemical properties

The biochemical and agro morphological traits of total 150 inbred lines of maize were valued. According to the standard descriptors for maize these traits were note down. Five randomly selected plants from each entry in every replication were evaluated, and their recorded observations were averaged by dividing the total value by five. The data collected included plant height (PH) in centimeters, ear height (EH) in centimeters, ear length (EL) in centimeters, number of kernel rows (NKR), number of kernels per row (NK/R), 100-kernel weight (HKW) in grams, kernel length (KL) in millimeters, grain yield per plant (GY/Plant), and grain yield per plot (GY/Plot) in grams. Additionally, biochemical traits such as protein content (PC), crude oil (CO), and starch content (SC) were evaluated. Grain yield and yield-related characteristics were measured on a separate plant basis. Ear length (EL), number of kernel rows (NKR), number of kernels per row (NK/R), 100-kernel weight (HKW), kernel length (KL), grain yield per plant (GY/Plant), and grain yield per plot (GY/Plot) were determined after harvesting five randomly selected plants from each plot. Plant height (PH) was measured from the base of the stem to the plant's apex, while ear height (EH) was recorded as the distance from the soil surface to the node bearing the uppermost ear, measured after the milk stage. To determine the 100-kernel weight (HKW), a sample of 100 dried and cleaned seeds was counted and weighed. Yield per plant was assessed by harvesting five randomly chosen plants, separating the kernels, drying, and cleaning them before recording the final yield.

2.5 Biochemical Trait Analysis

The biochemical composition of maize kernels, including protein, starch, neutral detergent fiber (NDF), ash, and crude oil content, was evaluated using the AgriNIR Analyzer W. This portable near-infrared (NIR) spectrometer enables rapid and efficient assessment of grain quality parameters. Maize samples were placed in the sample compartment of the AgriNIR Analyzer W, where they were scanned using near-infrared light. This light interacts with molecular bonds within the grain components, allowing for analysis through near-infrared reflectance (NIR) spectroscopy. The instrument, equipped with pre-calibrated mathematical models, interprets spectral data to determine the concentration of various biochemical constituents. The analyzer provides quick, high-precision, and reproducible measurements, displaying results within seconds. This non-destructive, reagent-free technique is ideal for analyzing large sets of maize inbred lines, ensuring an efficient and reliable approach to characterizing maize germplasm.

2.6 Statistical Analysis

Both agronomic and biochemical characteristics were assessed to evaluate the maize inbred lines. A total of nine agronomic traits and three biochemical parameters were analyzed. Observations were recorded from five plants per inbred line, and the trait values were averaged to determine the mean for each replication. Statistical and biometric analyses were employed to interpret the aggregated data. Analysis of variance (ANOVA), descriptive statistics, and least significant difference (LSD) were computed using Statistix 8. The disease severity was evaluated using a standardized scoring scale ranging from 1 to 7.

The Least Significant Difference (LSD) test at a 5% significance level was used to differentiate the means. Data collected from three consecutive seasons autumn 2023, spring 2024, and autumn 2024 were combined for analysis. Principal Component Analysis (PCA) was used to study trait associations, while cluster analysis grouped the maize inbred lines based on their agro-morphological and biochemical properties. The descriptive statistics analysis provided mean values and standard deviations for each trait. PCA calculations included eigenvalues, percentage variance, cumulative variance, and principal component loadings. Scatter plots for the first four principal components were generated to illustrate the genetic diversity patterns among the maize inbred lines. All statistical analyses were conducted using Statistix 8.1 and SPSS 16.0 software.

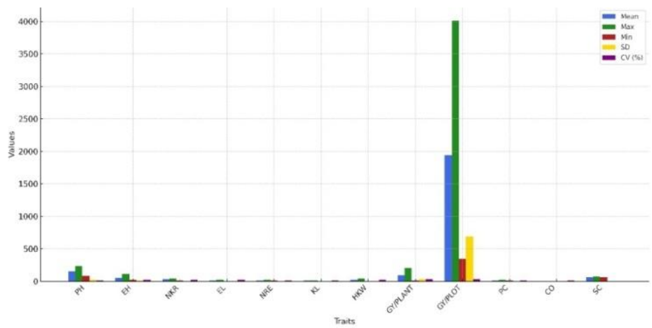
3. Results

Our results showed significant variation against the fungal attack and the traits studied in the elite maize inbred lines. There was a highly resistant response shown in 49 maize inbred lines (32.67% of the total), and the resistant response was found in 45 maize inbred lines (30%). It was investigated that 22 maize inbred lines (14.67%) were moderately resistant to *A. flavus* attack. Moderately susceptible responses were found in 30 maize inbred lines, which were 20% of the total maize inbred lines. It was found that 22 maize inbred lines (14.6%) were susceptible, and 4 inbred lines showed a highly susceptible response against fungal attack, respectively.

3.1 Assessment of variation among maize inbreed lines by descriptive statistical analysis

The variation among maize inbreed lines based on the quantitative traits listed in figure 2 was evaluated through descriptive statistical measures (Mean, maximum, minimum, coefficient of variance, and standard deviation). The coefficients of variance for 12 quantitative traits, varied from 3.4 to 35.6%. The GY/Plant of maize inbred lines had the maximum CV (35.6%), followed by GY/Plot (35.5), EH (27), and HKW (26.6).

Fig-2: The descriptive statistics of agro-morphological and biochemical traits (Combined data of Aut-2023, Spr-2024, and Aut-2024).



EH=Ear height, PH=Plant height, EL=Ear length, NKR=No. of kernel per row, NR/E=No. of rows per ear, HKW=hundred kernel weight, GY/PLANT=Grain Yield per plant, GY/PLOT=Grain yield per plot, PC=Protein content, CO=Crude oil, SC=Starch content.

In figure 2 blue (Mean) represents the average value of each trait across all accessions. Green (Max) indicates the highest recorded value for each trait. Red (Min) shows the lowest observed value for each trait. Gold (SD - Standard Deviation) measures the variation or dispersion of the trait values. Purple (CV% - Coefficient of Variation) expresses variability as a percentage of the mean, indicating relative dispersion

Fig-3: Frequency distributions of the 150 maize inbred lines for twelve agronomic traits.

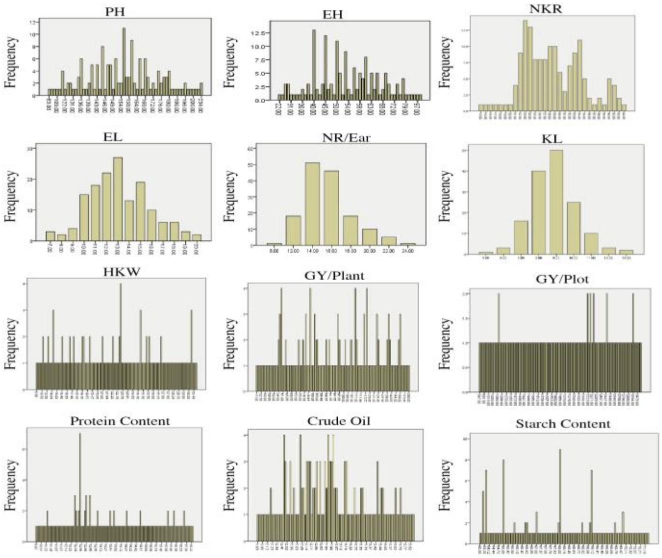


Figure 3 illustrates the frequency distributions of elite maize inbred lines based on quantitative traits. The histogram reveals significant variation among the evaluated maize inbred lines across the assessed biochemical and agro-morphological traits.

3.2 Genotypic variations in agronomic traits

The analysis of variance through seasons exposed no significant differences among the assessed maize inbred lines. Subsequently, data from three seasons Aut-2023, Spr-2024, and Aut-2024 were pooled for diversity assessment and multivariate analysis. The calculation of mean performance across numerous parameters revealed significant variation in all studied traits, with prominent differences observed in yield, plant height, ear height, and disease response.

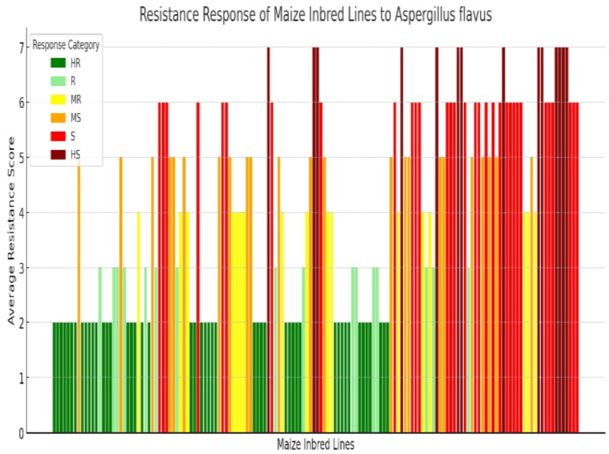
3.3 Response of Maize Inbred Lines to *Aspergillus flavus* Infection

The performance of elite maize inbred lines was assessed under field conditions through three growing seasons. To assess their response to fungal infection, the inbred lines were deliberately inoculated with a virulent strain (464) of *Aspergillus flavus*, known for its high aflatoxin-producing potential. The results exposed considerable variation in disease susceptibility between the tested lines, indicating differences in their resistance mechanisms against fungal colonization and toxin accumulation. Among the 150 elite maize inbred lines evaluated, 49 lines (UO01, UO02, UO03, UO04, UO05, UO06, UO07, UO09, UO10, UO11, UO12, UO13, UO15, UO16, UO17, UO22, UO23, UO24, UO26, UO28, UO40, UO41, UO43, UO44, UO45, UO46, UO47, UO59, UO60, UO61, UO62, UO68, UO71, UO72, UO73, UO74, UO75, UO84, UO85, UO86, UO88, UO89, UO90, UO91, UO93, UO94, UO95, UO97, and UO98) demonstrated a high level of resistance to *Aspergillus flavus* infection. This accounted for 32.66% of the total inbred lines, with a Disease Severity Scale rating of 2.

Out of the 150 maize inbred lines evaluated, 23 lines (UO14, UO18, UO19, UO21, UO27, UO30, UO36, UO65, UO76, UO92, UO96, UO101, UO109, UO110, UO112, UO118, UO119, UO124, UO132, UO134, UO142, UO148, and UO149), representing 15.3% of the total, exhibited resistance to *Aspergillus flavus* infection with a Disease Severity Scale rating of 3. Additionally, 22 lines (UO25, UO37, UO39, UO52, UO53, UO54, UO55, UO58, UO67, UO69, UO77, UO78, UO83, UO103, UO111, UO115, UO120, UO121, UO135, UO136, and UO150), accounting for 14.66%, displayed moderate resistance with a Disease Severity Scale rating of 4. Furthermore, 30 lines (UO08, UO20, UO29, UO34, UO35, UO38, UO48, UO51, UO56, UO57, UO66, UO70, UO81, UO87, UO99, UO100, UO104, UO105, UO113, UO114, UO116, UO117, UO125, UO126, UO127, UO128, UO133, UO141, UO145, and UO146), making up 20% of the total, were categorized as moderately susceptible with a Disease Severity Scale rating of 5. Twenty two (UO31, UO32, UO33, UO42, UO49, UO50, UO63, UO64, UO79, UO80, UO102, UO106, UO107, UO108, UO122, UO123, UO129, UO131, UO137, UO138, UO140 and UO143) i.e. 14.66% were susceptible and four (UO130, UO139, UO144 and UO147) i.e. 2.66% were highly susceptible.

Fig-4: *A. flavus* response variations in the elite maize inbred lines UO45 and UO123.

Fig-5: Fungal disease response of 150 maize inbred lines in Aut-23, Spr-24, and Aut-24 screened against *Aspergillus flavus.*

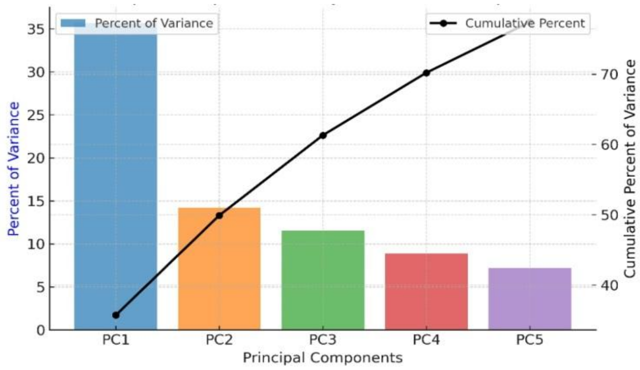


The bar chart in figure 5 representing of resistance response of maize inbred lines to *Aspergillus flavus*. The colors indicate different resistance categories: Green (Highly Resistant - HR), Light Green (Resistant - R), Yellow (Moderately Resistant - MR), Orange (Moderately Susceptible - MS), Red (Susceptible - S) and Dark Red (Highly Susceptible - HS).

3.4 Principal component analysis

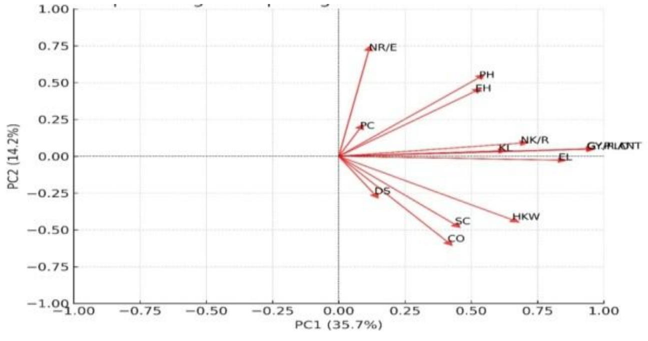
Principal component analysis revealed that only the first four principal components had eigenvalues exceeding 1.00, accounting for approximately 70.2% of the total variation in yield-related traits among elite maize inbred lines (fig. 6). In maize yield improvement programs, these four PCs may be prioritized when selecting for key traits. A total of nine agro-morphological and three biochemical traits were analyzed. The first three principal components (PC1, PC2, and PC3) collectively explained 61.3% of the total variance, contributing 35.7%, 14.2%, and 11.5%, respectively (fig.6).

Fig-6: Principal component analysis and variance explained.



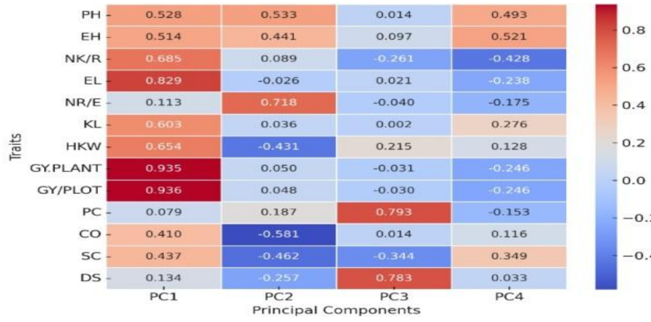
Colored bars represent the percent of variance explained by each principal component (PC1 to PC5). Black line with markers represents the cumulative percent of variance, showing the accumulated contribution of each PC. Blue Y-axis label indicated the percent of variance explained by each principal component. Black Y-axis label indicated the cumulative percent of variance across components.

Fig-7: Contribution of variables to PC-1 and PC-2.



Red arrows represent the contribution of each agro-morphological and biochemical trait to PC1 (35.7%) and PC2 (14.2%). Arrow Direction indicated the relationship of traits with the principal components. Traits pointing in the same direction are positively correlated, while those pointing in opposite directions are negatively correlated. Arrow length represented the strength of the trait's influence on the respective principal components. Longer arrows indicate a stronger contribution. Dashed Axes (X = 0, Y = 0) mark the origin, separating positive and negative loadings. Each trait is labeled at the end of its corresponding arrow.

Fig-8: First four principal components of agro morphological and biochemical traits.



PH=Plant height, EH=Ear height, NKR=No. of kernel per row, EL=Ear length, NR/E=No. of rows per ear, HKW=hundred kernel weight, GY/PLANT=Grain Yield per plant, GY/PLOT=Grain yield per plot, PC=Protein content, CO=Crude oil, SC=Starch content, DS=Disease scale

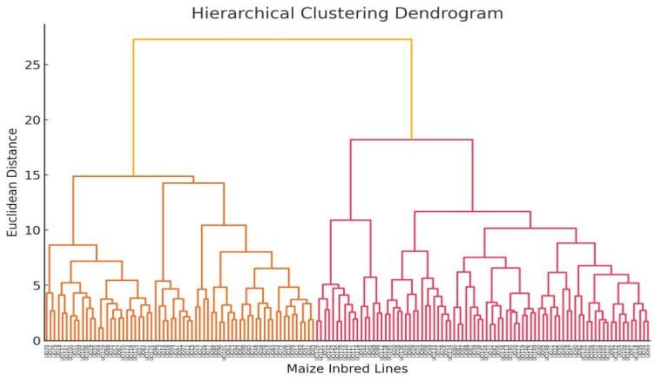
Color Gradient represents the magnitude and direction of trait loadings. Red shades represent positive loadings, indicating a strong positive contribution of the trait to the principal component. Blue shades represent negative loadings, indicating a strong negative contribution of the trait to the principal component. Lighter shades (White to Light Blue/Red) indicate weaker contributions. Numerical values showed the exact loading value of each trait for the corresponding principal component (PC1 to PC4). X-axis (Principal Components) displayed the first four principal components (PC1, PC2, PC3, PC4). Y-axis (Traits) listed the 13 agro-morphological and biochemical traits analyzed.

The findings align with previous research [22], further validating the current analysis. The eigenvalues guided the selection of the most influential variables, with PC1 accounting for 35.7% of the variance. Notably, all agro-morphological and biochemical traits made positive contributions to PC1, highlighting their collective influence on the observed variation. The PC2 contributed for 14.2% of the complete variance and showed negative contribution by PH, EH, NK/R, NR/E, KL, Y/Plant, GY/Plot and Protein Content and show positive contribution by ear length, hundred kernel weight, crude oil, starch content and disease response.

3.5 Cluster analysis

Cluster analysis categorized the 150 maize inbred lines into five distinctive groups. The number of elite inbred lines per cluster varied between 12 and 46, with Cluster 4 holding the highest number, adding 46 inbred lines. Cluster 5 comprised the fewest number of inbred lines twelve. Inbred lines in clusters 1, 2, and 3 were 37, 29, and 26, respectively (Figure 9).

Fig-9: Dendrogram using the Ward Method.



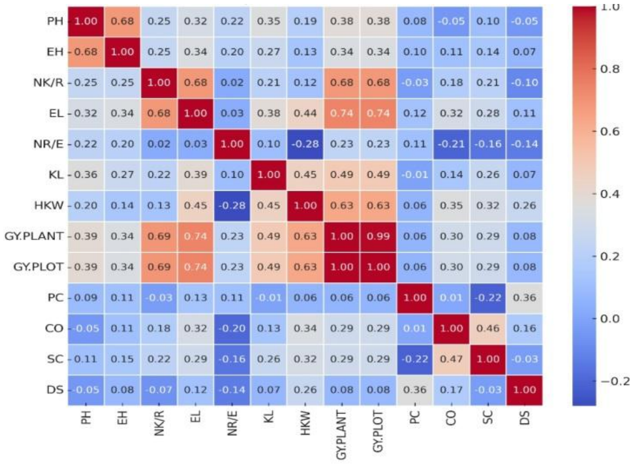
The dendrogram represent a gradient from yellow (top) to red (bottom) to indicate hierarchical clustering levels. Yellow represent higher Euclidean distances, marking distinct clusters. Orange to red denotes progressively smaller clusters, grouping maize inbred lines based on their similarity. X-axis maize inbred lines examined in the study. Inbred lines positioned closely within the dendrogram share more similar characteristics, particularly in terms of nutrient composition and aflatoxin resistance. Y-axis the Euclidean distance measures the dissimilarity between maize inbred lines. Higher distances indicate greater differences, while shorter distances suggest closely related inbred lines

Taskin et al., stated that great variety was present in the material under evaluation, as evidenced by the discrimination of inbred lines into so many distinct clusters [24]. Therefore, it is suggested that to enhance seed production and yield-related traits through non-allelic interactions, breeding programs should incorporate inbred lines from these distinct groups along with other required characteristics. Among the five clusters, Cluster 3 and Cluster 4 displayed the lowest genetic diversity, sharing alike genetic structure, as specified by the smallest inter-cluster distance (2.06). This was shadowed by the genetic proximity between Cluster 4 and Cluster 5 (3.40). To break negative correlations between yield and its associated traits, inbred lines from the most genetically diverse and closely related groups could be strategically used to develop bi-parental crosses, enhancing genetic gains in maize breeding programs.

3.6 Correlation analysis

A strong interrelationship was perceived among the estimated traits (Figure 10). The correlation coefficient calculates the strength and direction of the linear association between two variables.

Fig-10: Heat map of Correlation matrix of agro morphological and biochemical traits.



PH=Plant height, EH=Ear height, NKR=No. of kernel per row, EL=Ear length, NR/E=No. of rows per ear, HKW=hundred kernel weight, GY/PLANT=Grain Yield per plant, GY/PLOT=Grain yield per plot, PC=Protein content, CO=Crude oil, SC=Starch content and disease scale.

Heat map represents the correlation values, ranging from -1 to 1. Red (1.0 to ~0.5) described strong positive correlation. Light colors (~0.5 to 0.0) showed weak to no correlation and blue (-1.0 to ~-0.5) represent strong negative correlation among different traits.

The correlation analysis of 13 characteristics under field conditions is summarized in (Figure 10). Plant height (PH) exhibited a significant positive correlation with ear height (EH) (r = 0.68) and the number of kernels per row (NKR) (r = 0.25). However, PH showed a negative correlation with crude oil content. Correspondingly, EH demonstrated a significant positive correlation with ear length (EL) (r = 0.34) and grain yield per plot (GY/Plot) (r = 0.34). Additionally, the hundred-kernel weight (HKW) displayed a negative correlation with the number of rows per ear (NRE) but showed a highly significant positive correlation with grain yield per plant (GY/Plant) (r = 0.63). These findings highlight the complicated associations between morphological and yield-related traits, which are critical for choosing maize inbred lines with required characteristics.

4. Discussion

For an effective breeding program study on genetic diversity and multivariate analysis is important. These inbred lines uncover an extraordinary amount of agro morphological difference. To construct superior cultivars of essential crops a prominent amount of diversity is used. All examined maize inbred lines revealed a divergence through numerous characters which describe an extensive range of variability between the traits. By a number of researchers, the same result of variability for the characters in maize was documented. For upcoming improvement breeders might decide on improved lines by means of morphological classification in various extents. Evaluating agro morphological behaviors is a collective technique for shaping genetic diversity for numerous crop species, together with maize. It is efficaciously used on the 150 elite maize inbred lines. By the previous research the answers are confirmed and expression that traits like PH, HKW, EL, EH and GY.PLANT, presented a precise variation in mean performance. In this experiment by the mean performance the high amount of diversity in yield related characters was highlighted (Figure 3), proposing that upcoming breeding programs will have additional prospects to use of these characters. In yield associated characters here was a share of phenotypic diversity. A number of investigators revealed significant diversity in maize yield and its related traits. Due to the variance in genetic structure of investigated inbred lines there was significant variance for calculated characters among the experimented maize inbred lines. For maize crop production the results of investigated inbred lines validated with the outcomes of [28] in maize. Significant variations across inbred lines were also described by that relate with the present results. There was an enormous yield loss in crops that were infected by the *Aspergillus flavus* disease. For resistance to aflatoxin producing fungus maize breeding needs tangible disease screening procedures. When the maize crop grows for seed yield, *Aspergillus flavus* infection is larger as the crop matures the disease attack is more severe. With varying disease responses in maize inbred lines natural bases of resistance to *Aspergillus flavus* have been found.

Significant losses of yield determine in crops that are stressed from *Aspergillus flavus* disease. The usage of the accurate disease screening methods is very important although breeding for maize to resist against aflatoxin producing fungus. The genetic built resistance to injurious diseases is the chief alternative for maize breeding. In order to choose fungus-resistant lines for documentation and identification of elite lines for general crop growing and the *Aspergillus flavus* disease resistance the selected maize inbred lines were evaluated. For the identification of fungal disease resistance maize inbred lines, the investigators have applied a mixture of methodologies, but the consistent and actual technique is artificial inoculation. In this study, 150 elite maize inbred lines were inoculated in the research field for the identification of the resistance to aflatoxin producing fungus. Out of 150 maize inbred lines there were not a single inbred line selected as immune. On the other hand, 49 inbred lines were highly resistant and 23 inbred lines were detected resistant. Twenty-two inbred lines verified moderately resistant, and the left behind thirty inbred lines displayed moderate susceptibility. The twenty-two inbred lines showed susceptible to aflatoxin producing fungus disease. There were only four inbred lines were highly susceptible to the fungal disease. More evidence must be collected to know the response of genetic diversity and agronomic traits against aflatoxin producing disease. Elite maize inbred lines, that have great response of resistance and agronomic dominance may lessen the time of plant breeders that is required to remove the undesired genes by repetitive backcrossing method. Many researchers investigate that first four components are the utmost significant to show the arrays of variation amongst the inbred lines, and the characters linked to genotypic diversity.

The principal component analysis and cluster analysis divided 150 maize inbred lines into five groups. [29] Showed a related clustering design utilizing principal component analysis and hierarchical cluster analysis. According to Euclidean distance, the source of genotypes or topographical places mostly forms related clusters. With the help of multivariate statistical analysis, such as principal component analysis we can easily recognize the association among variables. It could help to simplify the data and understand the nature of the traits. In this experiment the first four principal components of the thirteen agro morphological and biochemical traits explained 70.2% variance, demonstrating the important linkage among the traits in this experiment. The principal component 1 was the very important, and it contributes for 35.7% of the variance. Because of their importance, the PC-1 differentiating the maize inbred lines for the GY/P, HKW, PH, EL, KL and NKR were important. Similar results were set up in the maize crop by [22,23], Consequently, principal component analysis showed different characteristic diversity in addition to important variation through the 5 groups of the 150 maize inbred lines (Figure 6). The traits PH, EL, HKW, and GY/Plant considerably explained the variations of the UO40, UO44, UO45, UO60, and UO86 inbred lines, and it is suggested that through selection by these characters the genetic diversity of the maize inbred lines could be improved. The selection of an appropriate cluster for further genetic improvement and hybridization should prioritize traits that exhibit the greatest genetic divergence [24]. The dendrogram analysis identified five distinct clusters, with cluster five displaying the largest intra-cluster distance, followed by clusters two, one, and four. The most significant inter-cluster distance was observed between clusters two and three, followed by clusters one and three.

Cluster four, which contained 46 inbred lines, exhibited notable advancements in key agronomic traits, including the number of kernels per row (NKR), ear length (EL), hundred-kernel weight (HKW), ear height (EH), and grain yield per plant (GY/Plant). The results of this study highlight that yield-related traits with positive and significant correlations have strong potential to enhance seed production. Given their substantial association with grain yield, these traits were prioritized in the selection process. Consequently, these maize inbred lines may serve as valuable parental sources in breeding programs. The multivariate statistical analysis conducted across different growing seasons (autumn 2023, spring 2024, and autumn 2024) revealed significant genetic diversity among and within the evaluated maize inbred lines for both quantitative and qualitative traits. This study provides strong evidence of sufficient genetic variation, suggesting promising opportunities for genetic enhancement in maize yield and associated agronomic traits. Furthermore, the findings demonstrated significant variability in seed yield, yield-associated traits, and resistance to *Aspergillus flavus*, indicating the feasibility of selecting superior gene pools. These gene pools could either serve as direct sources of resistance or be utilized in hybridization to improve high-yielding but disease-prone genotypes. Notably, the inbred lines UO43, UO44, UO45, UO86, and UO68 exhibited both high yield potential and strong resistance to *A. flavus,* making them suitable candidates for advanced yield trials or hybridization programs. Additionally, UO142, UO92, UO112, and UO149 were identified as high-yielding and resistant to *A. flavus*, further expanding the pool of promising lines for breeding.

For breeding applications, elite lines can be selected from both high-yielding, resistant gene pools (UO43, UO44, UO45, UO86, and UO68) and low-yielding, resistant gene pools (UO27, UO19, UO96, UO124, and UO101). Principal component analysis (PCA) indicated that four principal components (PCs) had eigenvalues greater than one, collectively explaining 70.2% of the total genetic variability. PC1 accounted for 35.7% of the variation, followed by PC2 and PC3, which contributed 14.2% and 11.5%, respectively. Traits such as EL, NKR, HKW, and GY/Plant were strongly associated with PC1, while PC2 was predominantly influenced by plant height (PH), EH, and the number of rows per ear (NRE). Overall, the germplasm evaluated in this study demonstrated a high degree of resistance to aflatoxin-producing fungi, along with substantial genetic diversity and strong agronomic performance. These findings suggest that the identified inbred lines hold significant potential for breeding high-yielding, *A. flavus* resistant maize varieties. This research can serve as a foundation for phenotypic selection and agronomic performance assessments in maize breeding programs, leading to the development of superior maize lines. In the present study, seven inbred lines UO43, UO44, UO45, UO68, UO86, UO92, and UO112 were identified as both highly resistant to *A. flavus* and high-yielding. These lines could be instrumental in developing molecular markers, mapping populations for molecular breeding, identifying quantitative trait loci (QTL) for *A. flavus* resistance, and breeding resistant maize varieties.

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Conflicts of Interest

The authors declare no conflicts of interest.

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