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**Original Research Article**

# Role of Genetic Variability Present in Germplasm for The Development of High-Yielding Maize Hybrids

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**Abstract:** *The exploitation of genetic variability in maize germplasm is essential for the development of high-yielding, stress-resilient hybrids that meet the demands of diverse agroecological conditions. In Pakistan's semi-arid regions, where environmental stresses challenge crop productivity, systematic evaluation of indigenous and multinational maize hybrids is crucial for sustainable agricultural advancement.* ***Objective:*** *To assess the genetic diversity and agronomic performance of indigenous and multinational maize hybrids through morphological, yield-related, and biochemical traits for hybrid development in semi-arid conditions.* ***Methods:*** *This experimental study was conducted at the Maize and Millets Research Institute, Yusafwala-Sahiwal, during the spring of 2024. A total of 70 maize hybrids (58 indigenous and 12 multinational) were evaluated in a randomized complete block design (RCBD) with three replications. Data were collected for days to tasseling and silking, plant height, ear height, cob length, thousand grain weight, protein content, fresh cob weight, and grain yield. Statistical analyses included analysis of variance (ANOVA) for trait differentiation, Pearson's correlation analysis to determine inter-trait associations, and principal component analysis (PCA) to assess genetic diversity and trait contribution to yield variability.* ***Results:*** *ANOVA revealed significant genotypic differences ($P < 0.05$) for most traits. Grain yield showed a perfect positive correlation with fresh cob weight ($r = 1.00$), and moderate correlations with ear height ($r = 0.70$) and plant height ($r = 0.36$). PCA indicated that the first two principal components accounted for 53.06% of total variance, identifying fresh cob weight, grain yield, and plant structural characteristics as primary contributors to genetic diversity. Multinational hybrids achieved higher yields (>7,000 kg/ha), while select indigenous hybrids exhibited comparable performance, indicating their breeding potential.* ***Conclusion:*** *The study demonstrates the substantial genetic variability within maize germplasm and emphasizes the utility of multivariate statistical tools for hybrid selection. The competitive performance of certain indigenous hybrids highlights their strategic role in future breeding efforts aimed at enhancing maize productivity and resilience under semi-arid climates.*

***Keywords:*** Agronomic traits, Genetic diversity, Maize, Multivariate analysis, Principal component analysis, Semi-arid, Yield traits

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## Introduction

Maize (*Zea mays L.*), a versatile cereal crop, is a cornerstone of global agriculture, serving as a staple food, animal feed, and industrial raw material. Originating in Mesoamerica over 10,000 years ago, maize has evolved into a globally cultivated crop due to its adaptability and high yield potential (1). It ranks third among cereals in terms of production, with global output exceeding 1.2 billion metric tons annually (2). In Pakistan, maize is the third most important cereal after wheat and rice, contributing significantly to food security and economic stability. Its uses range from direct human consumption (e.g., flour, cornmeal) to livestock feed and industrial products like ethanol and corn syrup (9-11). However, achieving optimal maize yields in Pakistan is challenged by biotic stresses (e.g., pests, diseases), abiotic stresses (e.g., drought, heat), and limited access to high-quality germplasm, necessitating innovative breeding strategies to harness genetic variability (5-6).

In Pakistan, maize production faces significant constraints, including suboptimal soil fertility, water scarcity, and variable climatic conditions, particularly in rain-fed regions (7, 8). Low adoption of improved hybrids, coupled with reliance on traditional varieties, limits yield potential, with average yields (4.5–5.5 t/ha) lagging behind global benchmarks (8–10 t/ha) (Saeed et al., 2021; Khalid et al., 2020). Genetic variability in germplasm is critical for overcoming these constraints, as it provides a reservoir of traits for breeding resilient, high-yielding hybrids. Indigenous and multinational maize hybrids offer diverse genetic backgrounds, enabling the selection of traits such as drought tolerance, disease resistance, and enhanced grain yield (5, 6). By exploiting genetic diversity, breeders can develop hybrids tailored to Pakistan's agroecological zones, addressing region-specific challenges and boosting productivity.

The role of genetic variability in key morphological and agronomic traits has been extensively studied to enhance maize yield (6). Traits such as days to tasseling and silking influence reproductive success and adaptability to varying growing seasons, with early-maturing hybrids being critical for short-season environments. Plant height and ear height affect lodging resistance and harvest efficiency, while thousand grain weight and grain yield per hectare directly correlate with productivity (3). Cob length contributes to grain number, a key yield component, and protein content percentage is vital for nutritional quality, particularly in regions with dietary protein deficiencies (18). Studies, such as those by Yousaf et al., 2018; Yousaf et al., 2020, have shown significant variability in these traits among maize hybrids, with correlation analyses revealing strong associations between grain yield and traits like thousand grain weight and cob length. Principal component analysis (PCA) has further highlighted the contribution of these traits to overall genetic diversity, guiding hybrid selection (11-14).

Despite advances in maize breeding, gaps remain in understanding how genetic variability in indigenous and multinational hybrids can be optimized for Pakistan's diverse environments. Previous research has often focused on individual traits, with limited integration of multivariate analyses like PCA to assess trait interactions (15). Moreover, the potential of indigenous germplasm, which may harbor unique alleles for stress tolerance and yield stability, remains underexplored compared to multinational hybrids (10). This study addresses these gaps by evaluating 70 maize hybrids (indigenous and multinational) for key morphological and agronomic traits, using ANOVA to assess genotypic differences, correlation analysis to identify trait associations, and PCA to elucidate trait contributions to yield variability. Such an approach will provide insights into the genetic potential of diverse germplasm for developing high-yielding hybrids.

The objective of this study is to evaluate the genetic variability among 70 indigenous and multinational maize hybrids for key agronomic traits (days to tasselling, days to silking, plant height, ear height, thousand grain weight, grain yield per hectare, cob length, and protein content percentage) and to determine their contributions to yield potential using ANOVA, correlation coefficient, and principal component analysis. The findings aim to identify superior hybrids and inform breeding strategies for enhancing maize productivity in Pakistan's agroecological conditions.

## Methodology

### Experimental Site and Season

The study was conducted at the Maize and Millets Research Institute (MMRI), Yusafwala-Sahiwal, Pakistan, during the spring season of 2024. The experimental site is located in a semi-arid region characterized by hot summers and moderate winters, with an average annual rainfall of 300–400 mm. The soil type was sandy loam, with a pH of 7.8 and organic matter content of 0.85%. A total of 70 maize (*Zea mays* L.) hybrids were evaluated, comprising 58 indigenous hybrids from MMRI, 8 hybrids from Kissan Seed Corporation (KSC), Sahiwal, and 4 multinational hybrids from Corteva, Bayer, and Syngenta (Table 1).

**Table 1: Indigenous and Multinational maize hybrids used in the study**

| Sr # | Genotypes | Sr # | Genotypes | Sr # | Genotypes | Sr # | Genotypes |
|---|---|---|---|---|---|---|---|
| **1** | MMRI-1101 | **19** | MRI-1119 | **37** | MMRI-1137 | **55** | MMRI-1155 |
| **2** | MMRI-1102 | **20** | MRI-1120 | **38** | MMRI-1138 | **56** | MMRI-1156 |
| **3** | MMRI-1103 | **21** | MRI-1121 | **39** | MMRI-1139 | **57** | MMRI-1157 |
| **4** | MMRI-1104 | **22** | MRI-1122 | **40** | MMRI-1140 | **58** | MMRI-1158 |
| **5** | MMRI-1105 | **23** | MRI-1123 | **41** | MMRI-1141 | **59** | KSC-1 |
| **6** | MMRI-1106 | **24** | MRI-1124 | **42** | MMRI-1142 | **60** | KSC-2 |
| **7** | MMRI-1107 | **25** | MRI-1125 | **43** | MMRI-1143 | **61** | KSC-3 |
| **8** | MMRI-1108 | **26** | MRI-1126 | **44** | MMRI-1144 | **62** | KSC-4 |
| **9** | MMRI-1109 | **27** | MRI-1127 | **45** | MMRI-1145 | **63** | KSC-5 |
| **10** | MMRI-1110 | **28** | MRI-1128 | **46** | MMRI-1146 | **64** | KSC-6 |
| **11** | MMRI-1111 | **29** | MRI-1129 | **47** | MMRI-1147 | **65** | KSC-7 |
| **12** | MMRI-1112 | **30** | MRI-1130 | **48** | MMRI-1148 | **66** | KSC-8 |
| **13** | MMRI-1113 | **31** | MRI-1131 | **49** | MMRI-1149 | **67** | DK-6317 |
| **14** | MMRI-1114 | **32** | MRI-1132 | **50** | MMRI-1150 | **68** | DK-6321 |
| **15** | MMRI-1115 | **33** | MRI-1133 | **51** | MMRI-1151 | **69** | P-1543 |
| **16** | MMRI-1116 | **34** | MRI-1134 | **52** | MMRI-1152 | **70** | Bambus |
| **17** | MMRI-1117 | **35** | MRI-1135 | **53** | MMRI-1153 | | |
| **18** | MMRI-1118 | **36** | MRI-1136 | **54** | MMRI-1154 | | |

These genotypes were selected to represent a diverse genetic pool, encompassing both local and global germplasm, to assess variability in agronomic and yield-related traits. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Each hybrid was sown in plots consisting of four rows, with a row-to-row distance of 75 cm and a plant-to-plant distance of 20 cm. Each plot measured 5 m in length, ensuring uniform plant density across replications. The sowing was carried out on February 26, 2024, and harvesting was completed on June 21, 2024.

Standard agronomic practices were followed to ensure optimal crop growth. The field was prepared with two plowing followed by planking to achieve a fine seedbed. A basal dose of fertilizer (NPK: 120-80-60 kg $ha^{-1}$) was applied at sowing, with an additional 60 kg $ha^{-1}$ nitrogen top-dressed at the V6 stage. Irrigation was applied at critical growth stages (V4, V8, tasseling, and grain filling) to avoid water stress. Weeds were controlled through manual weeding and the application of pre-emergence herbicides (atrazine at 1.5 kg $ha^{-1}$). Pest and disease management followed integrated pest management protocols, with no significant pest or disease incidence recorded during the season.

### Data Collection

Data were recorded for key morphological and agronomic traits, including days to tasseling, days to silking, plant height (cm), ear height (cm), fresh cob weight (kg $plot^{-1}$) thousand grain weight (g), grain yield per hectare (kg $ha^{-1}$), cob length (cm), and protein content percentage. Days to tasselling and silking were recorded when 50% of plants in a plot reached the respective stages. Plant height and ear height were measured at physiological maturity on ten randomly selected plants per plot. Thousand grain weight was determined by weighing 1000 randomly selected kernels from each plot. Grain yield per hectare was calculated after harvesting and adjusting grain moisture to 14%. Cob length was measured on ten randomly selected cobs per plot. Protein content was analyzed using the Spectrophotometer equipment Partin NIR.

### Statistical Analysis

Data were subjected to analysis of variance (ANOVA) to assess genotypic differences for each trait, with significance tested at $P < 0.05$ (Steel et al., 1997). Correlation coefficients were calculated to determine relationships among traits, particularly their associations with grain yield. Principal component analysis (PCA) was performed to elucidate the contribution of each trait to overall genetic variability and to identify key traits influencing yield potential. Statistical analyses were conducted using R software (version 4.3.2).

### Analysis of Variance (ANOVA)

The analysis of variance (ANOVA) conducted on the 70 maize hybrids revealed significant genotypic variability for most evaluated traits (Table 2). Mean squares for genotypes were highly significant ($P < 0.05$) for plant height ($254.1^{**}$), ear height ($167.5^{**}$), cobs harvested ($12.17^{**}$), cob length ($12.32^{**}$), thousand grain weight ($2777.9^{**}$), and grain yield ($2,676,314^{**}$). Replication mean squares were significant for plant height (31,548.9), ear height (19,326.7), and cobs harvested (395.67), indicating consistent environmental effects across blocks. However, the variations among maize hybrids for days to 50% tasseling and days to 50% silking were non-significant ($4.70^{NS}$; $4.58^{NS}$), respectively.

**Table 2. Analysis of Variance (ANOVA) for agronomic and yield-related traits of 70 maize hybrids evaluated at MMRI, Yusafwala-Sahiwal, Spring 2024.**

| | **Mean Squares** | | |
|---|---|---|---|
| Parameters/ Sources of Variation | Replications | Genotypes | Error |
| Df | 2 | 69 | 138 |
| Days to 50% Tasselling | 5.1619 | $4.7014^{NS}$ | 4.7126 |
| Days to 50% Silking | 1.9857 | $4.5814^{NS}$ | 5.1161 |
| Plant Height (PH) | 31548.9 | $254.1^{**}$ | 78.7 |
| Ear Height (EH) | 19326.7 | $167.5^{**}$ | 68.3 |
| Cobs harvested (CH) | 395.67 | $12.17^{**}$ | 4.4 |
| Cob Length (CL) | 1.4949 | $12.3235^{**}$ | 0.0005 |
| Fresh Cob Weight (FCW) | 34.285 | $0.355^{**}$ | 0.158 |
| Moisture Percentage (Moisture %) | 3.5523 | $4.3962^{**}$ | 1.4624 |
| Thousand Grain Weight (1000GW) | 1154 | $2777.9^{**}$ | 0.0006 |
| Protein Contents Percentage (Protein %) | 3.8021 | $2.6435^{**}$ | 0.0008 |
| Yield | 2.5E+08 | $2676314^{**}$ | 1283437 |

## Correlation Analysis

Correlation analysis revealed significant relationships among agronomic traits and grain yield for the 70 maize hybrids (Table 3). Grain yield showed strong positive correlations with fresh cob weight ($r = 1.00^{***}$, $P < 0.01$), cob height ($r = 0.70^{***}$, $P < 0.01$) and plant height ($r = 0.36^{**}$, $P < 0.05$), indicating their substantial contribution to yield potential. However, grain yield also has significantly negative correlation protein content percentage ($r = -0.62^{***}$, $P < 0.01$), thousand grain weight ($r = -0.33^{**}$, $P < 0.05$), days to 50% tasseling ($r = -0.28^{*}$), days to 50% silking ($r = -0.24^{*}$) and moisture contents percentage ($r = -0.24^{*}$). Similarly, days to 50% tasseling and days to 50% silking had a strong positive correlation ($r = -0.86^{***}$) between them. Moreover, ear height and plant height ($r = 0.58^{***}$) also had strong positive correlation followed by cob length and thousand grain weight ($r = 0.69^{***}$).

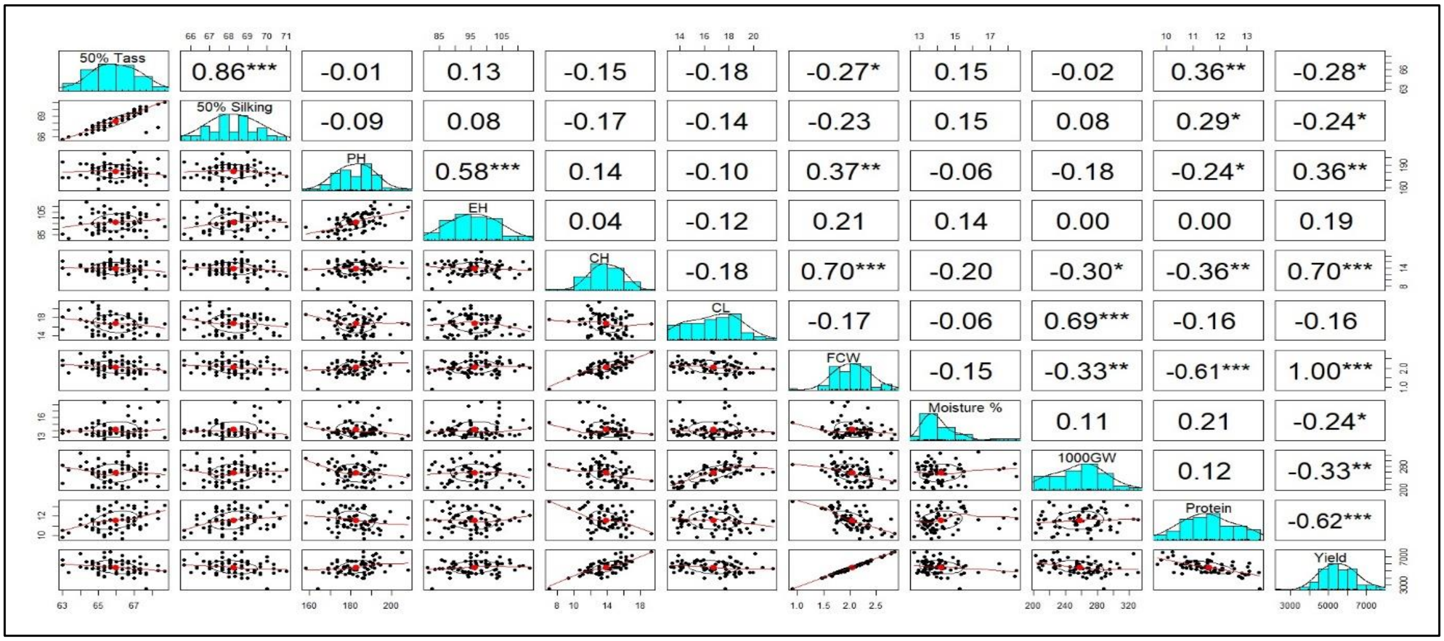

**Figure 1. Correlation coefficients among agronomic traits and grain yield for 70 maize hybrids.**

## Principal Component Analysis (PCA)

Principal component analysis (PCA) elucidated the contribution of traits to genetic variability among the 70 maize hybrids, with the scree plot indicating the percentage of variance explained by each principal component (Figure 1). The first two principal components (PC1 and PC2) accounted for a cumulative 53.2% of the total variance, with PC1 explaining 33.9% and PC2 contributing 19.2%. Subsequent components showed a steep decline, with PC3 (13.5%), PC4 (10.7%), and PC5 (8.2%) adding progressively less variance (5.5%, 3.4%, 2.6%, 2.1%, and 1.1% for PC6 to PC10, respectively). This suggests that the first two components capture the majority of the variability, with diminishing returns from higher components.

## Principal Component Analysis (PCA) Results Description

The principal component analysis (PCA) results for the 70 maize hybrids, as presented in the table, indicate that the first two principal components (PC1 and PC2) collectively explain 53.06% of the total variance, with PC1 accounting for 33.90% (eigen value 1.9310) and PC2 contributing 19.15% (eigen value 1.4515). This suggests that these components capture the majority of the variability in the dataset, primarily driven by key agronomic traits such as grain yield and thousand grain weight. The subsequent components (PC3 to PC5) add incremental variance, with PC3 (13.45%, eigen value 1.2164), PC4 (10.70%, eigen value 1.0850), and PC5 (8.18%, eigen value 0.9486) together increasing the cumulative proportion to 85.39%, highlighting their importance in explaining additional trait variability.

Beyond the fifth component, the explained variance diminishes significantly, with PC6 to PC11 contributing only 14.61% collectively, ranging from 5.51% (PC6, eigen value 0.7785) to a mere 0.09% (PC11, eigen value 0.0119). This steep decline, as confirmed by the scree plot, indicates that higher components contribute minimally to the overall variability, suggesting that focusing on the first five principal components is sufficient for a comprehensive analysis of the genetic diversity among the hybrids. The cumulative proportion reaches 100% by PC10 (eigen value 0.3451), underscoring that the dataset's multidimensional structure is adequately represented by the initial components, with PC1 and PC2 providing a robust foundation for interpreting trait contributions to yield potential. The first 4 PCs, having eigen value higher than 1, are of great importance and will be considered to study the nature, magnitude and direction of the variability.

The table presents the loadings of various agronomic traits on the first eleven principal components (PC1 to PC11) derived from the PCA of 70 maize hybrids, indicating the contribution of each trait to the variance explained by each component. PC1, explaining 33.9% of the variance, shows notable positive loadings for plant height (PH, 0.235) and ear height (EH, 0.106), suggesting these structural traits are significant contributors to the first axis of variability. Conversely, days to tasseling (-0.235) and days to silking (-0.233) exhibit moderate negative loadings, indicating an inverse relationship with PC1, while yield (0.485) and protein content (0.360) have strong positive loadings, highlighting their importance in driving the primary source of genetic variation. Traits such as thousand grain weight (1000GW, -0.226) and moisture percentage (-0.158) show negative loadings, suggesting they contribute oppositely to the traits driving PC1.

PC2, accounting for 19.2% of the variance, is characterized by strong positive loadings for cobs harvested (CH, 0.378) and cob length (CL, 0.480), indicating these reproductive traits are key contributors to the second axis of variability. Fresh cob weight (FCW, 0.480) and moisture percentage (0.139) also show positive loadings, reinforcing their association with PC2, while plant height (-0.226) and ear height (-0.306) have negative loadings, suggesting a divergence from structural traits in this component. The negative loadings for days to tasseling (-0.496) and days to silking (-0.447) on PC2 further emphasize the importance of early maturity in differentiating hybrids along this axis.

Higher components (PC3 to PC11) exhibit diminishing contributions to variance, with PC3 (13.5%) showing significant loadings for plant height (0.482) and ear height (0.580), suggesting these traits gain prominence in explaining additional variability. PC4 (10.7%) highlights positive loadings for cobs harvested (0.272) and cob length (0.335), while PC5 (8.2%) is influenced by thousand grain weight (0.297) and protein content (0.513). The loadings taper off in PC6 to PC11, with values approaching zero (e.g., PC11 loadings range from -0.003 to 0.010), indicating that these components capture residual variance with minimal trait-specific influence. Overall, the first five components, particularly PC1 and PC2, effectively encapsulate the major sources of variability, with yield, structural traits (PH, EH), and reproductive traits (CH, CL) playing dominant roles in differentiating the maize hybrids.

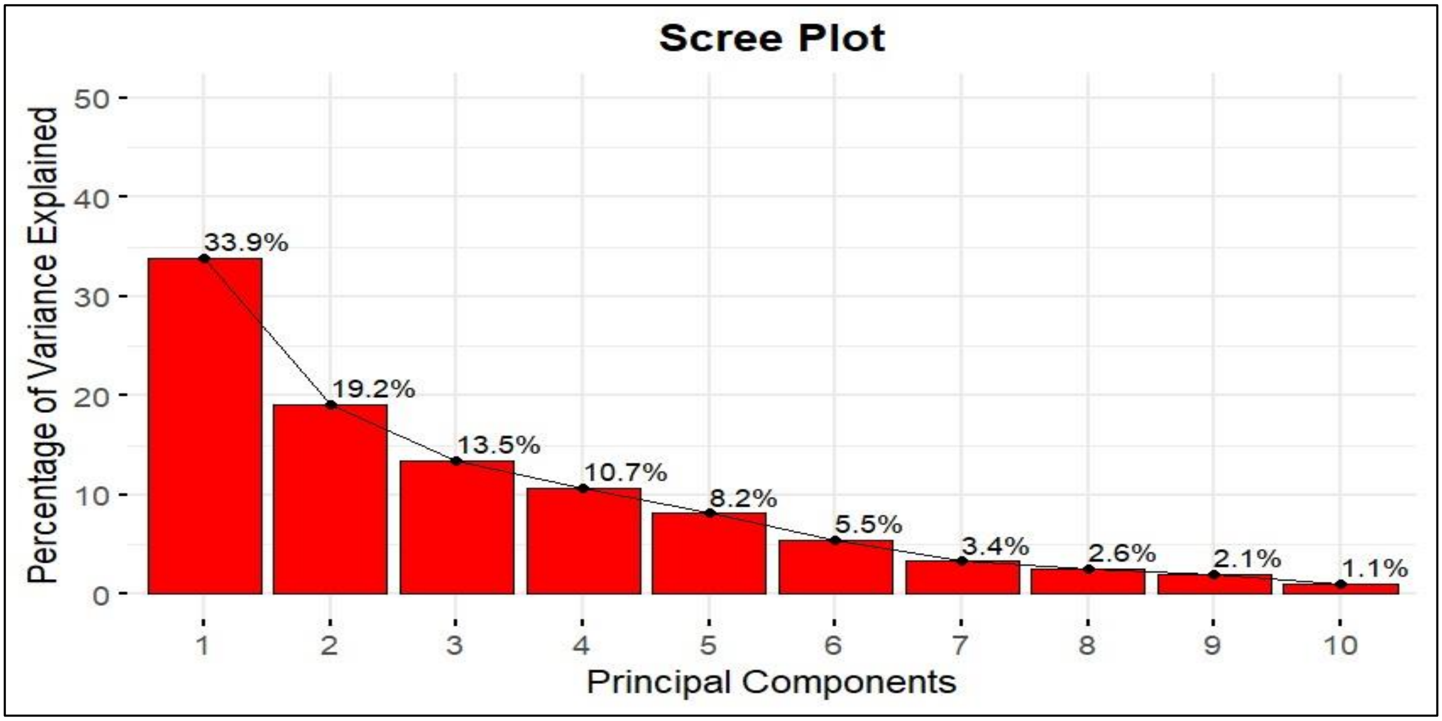

**Figure 2: Scree plot showing the percentage of variance explained by the first ten principal components for 70 maize hybrids.**

**Table 3. Eigenvalues, Proportion of Variance, and Cumulative Proportion of Variance Explained by Principal Components for 70 Maize Hybrids.**

| | Eigen Value | Proportion of Variance | Cumulative Proportion |
|---|---|---|---|
| **PC1** | 1.9310 | 0.3390 | 0.3390 |
| **PC2** | 1.4515 | 0.1915 | 0.5306 |
| **PC3** | 1.2164 | 0.1345 | 0.6651 |
| **PC4** | 1.0850 | 0.1070 | 0.7721 |
| **PC5** | 0.9486 | 0.0818 | 0.8539 |
| **PC6** | 0.7785 | 0.0551 | 0.9090 |
| **PC7** | 0.6072 | 0.0335 | 0.9425 |

| | | | |
|---|---|---|---|
| **PC8** | 0.5357 | 0.0261 | 0.9686 |
| **PC9** | 0.4755 | 0.0206 | 0.9892 |
| **PC10** | 0.3451 | 0.0108 | 1.0000 |
| **PC11** | 0.0119 | 0.0000 | 1.0000 |

**Table 4: Loadings of Agronomic Traits on Principal Components (PC1 to PC11) for 70 Maize Hybrids.**

| | PC1 | PC2 | PC3 | PC4 | PC5 | PC6 | PC7 | PC8 | PC9 | PC10 | PC11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Tasselling** | -0.235 | -0.496 | -0.057 | -0.402 | 0.056 | -0.080 | -0.141 | -0.123 | -0.160 | -0.680 | 0.001 |
| **Silking** | -0.233 | -0.447 | -0.057 | -0.503 | -0.004 | -0.141 | 0.088 | 0.125 | 0.101 | 0.660 | 0.000 |
| **PH** | 0.235 | -0.226 | -0.482 | 0.256 | 0.305 | -0.168 | -0.618 | 0.224 | 0.197 | 0.059 | 0.002 |
| **EH** | 0.106 | -0.306 | -0.580 | 0.255 | 0.079 | 0.260 | 0.509 | -0.380 | -0.116 | 0.052 | 0.000 |
| **CH** | 0.378 | -0.079 | 0.148 | -0.272 | -0.178 | 0.543 | -0.367 | -0.463 | 0.264 | 0.096 | 0.006 |
| **CL** | -0.102 | 0.474 | -0.397 | -0.335 | 0.016 | -0.015 | -0.277 | -0.204 | -0.593 | 0.158 | -0.003 |
| **FCW** | 0.480 | -0.078 | -0.049 | -0.173 | -0.194 | 0.038 | 0.125 | 0.364 | -0.218 | -0.065 | -0.702 |
| **Moisture** | -0.158 | -0.139 | -0.178 | 0.249 | -0.894 | -0.159 | -0.181 | -0.019 | -0.029 | 0.016 | 0.062 |
| **1000GW** | -0.226 | 0.341 | -0.445 | -0.297 | -0.115 | 0.287 | 0.144 | 0.316 | 0.534 | -0.213 | 0.003 |
| **Protein** | -0.360 | -0.182 | 0.107 | 0.228 | 0.051 | 0.687 | -0.177 | 0.391 | -0.330 | 0.080 | 0.000 |
| **Yield** | 0.485 | -0.062 | -0.031 | -0.191 | -0.113 | 0.046 | 0.143 | 0.363 | -0.220 | -0.064 | 0.710 |

The bar graph comparing grain yield (kg/ha) across 70 maize hybrids reveals a wide range of performance, with distinct patterns among hybrids developed by different institutions. The majority of hybrids, represented in green, are from the Maize and Millets Research Institute (MMRI), totalling 58 hybrids, and exhibit yields predominantly ranging between 4,000 and 7,000 kg/ha, with a few peaks exceeding 7,000 kg/ha (e.g., MMRI-132 and MMRI-136). This indicates a generally consistent yield potential among MMRI hybrids, with some standout performers, suggesting effective breeding for local adaptability in Pakistan's semi-arid conditions. In contrast, the eight black bars, representing hybrids from Kissan Seed Corporation, Sahiwal, show yields clustering around 5,000 to 6,000 kg/ha, with a notable dip below 4,000 kg/ha for some hybrids, indicating variable performance that may reflect less optimization for the specific trial environment.

The four red bars, denoting multinational hybrids (e.g., Corteva, Bayer, Syngenta), stand out with the highest yields, consistently exceeding 7,000 kg/ha and reaching up to approximately 8,000 kg/ha, particularly for hybrids labelled as "Pioneer" and "DKC." This suggests that multinational hybrids leverage advanced genetic resources and breeding technologies, potentially offering superior yield potential under the trial conditions at MMRI, Yusafwala-Sahiwal during Spring 2024. However, the limited number of multinational hybrids compared to the larger MMRI and Kissan cohorts highlights the need for further evaluation to assess their stability across diverse agroecological zones in Pakistan, while the MMRI hybrids' broad yield range underscores their role as a valuable genetic base for future improvement.

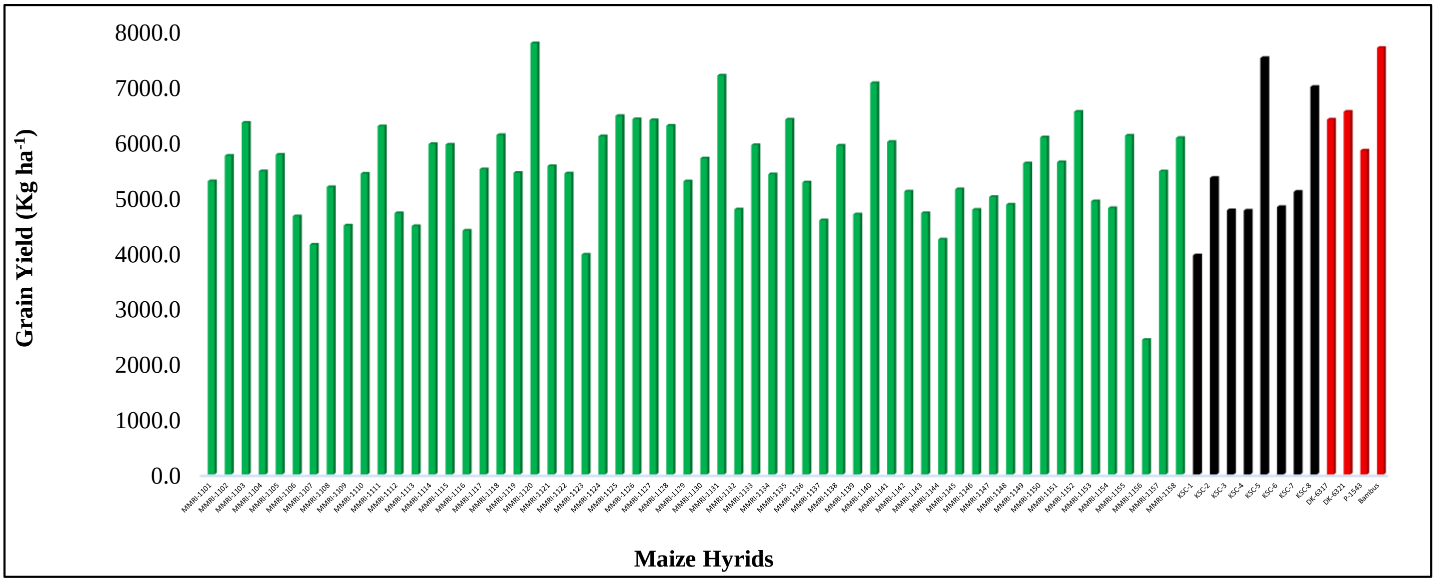

**Figure 3: Grain Yield of 70 Maize Hybrids at MMRI, Yusafwala-Sahiwal, Spring 2024.**

The Individual Variables Plot illustrates the relationships between agronomic traits and the first two principal components (PC1 and PC2), which together explain 53.06% of the total variance in the 70 maize hybrids. PC1, on the x-axis, and PC2, on the y-axis, are represented as orthogonal axes, with trait vectors (arrows) indicating their contributions and correlations (Figure 3a). Traits such as yield, fresh cob weight (FCW), plant height (PH), and ear height (EH) are positioned with strong positive loadings on PC1 (extending toward the right), suggesting they are major drivers of the first axis of variability. In contrast, days to 50% tasseling (50% Tass) and days to 50% silking (50% Silking) show negative loadings on PC1, indicating an inverse relationship, while traits like thousand grain weight (1000GW), cob length (CL), cobs harvested (CH), protein content (Protein), and moisture percentage (Moisture %) are distributed across both axes, with varying degrees of influence on PC2, highlighting their role in the secondary axis of variation.

The Variable Contributions to PC1 and PC2 bar chart quantifies the percentage contribution of each trait to the variance explained by PC1 and PC2, with a threshold of 7% (dashed line) indicating significant contributors (Figure 3b). Yield and FCW exhibit the highest contributions (approximately 15% each), underscoring their dominant role in PC1 and PC2, respectively, aligning with their strong loadings in the biplot. Traits like 50% Tasseling, 50% Silking, and Protein also contribute notably (around 10–12%), reflecting their influence on the primary components, while CH, CL, 1000GW, PH, EH, and Moisture % contribute less (5–8%), with Moisture % showing the lowest impact (below 5%). This distribution confirms that yield-related traits and reproductive timing are critical in differentiating the maize hybrids, while structural and nutritional traits play a secondary but supportive role in the PCA structure.

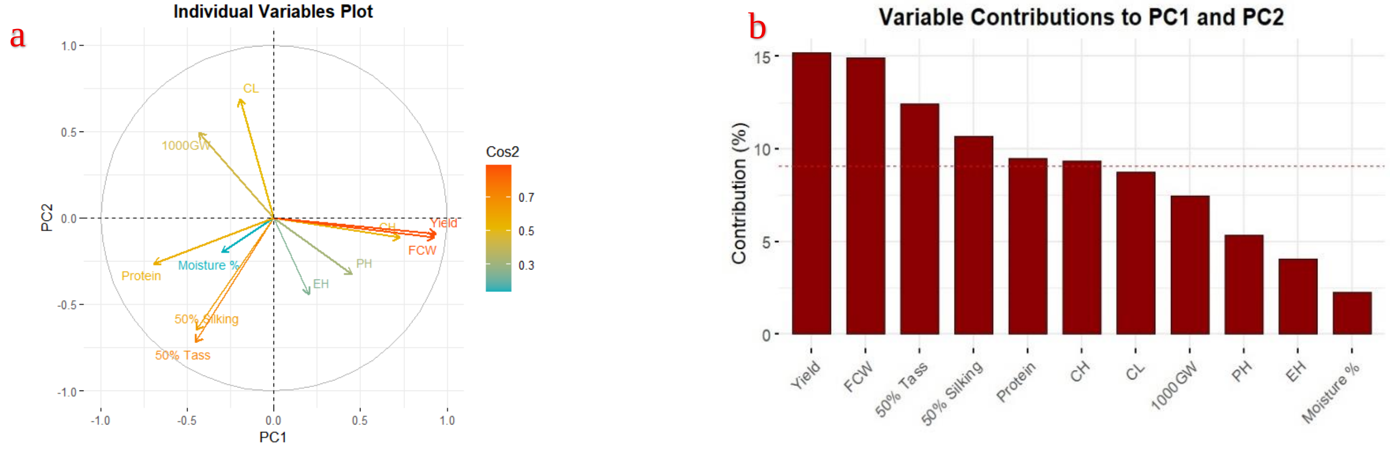

**Figure 4: (a-b): Pricipal Component Analysis (a) Individual Variables Plot (PCA Biplot) (b) Variable Contributions to PC1 and PC2**

## Discussion

The significant genotypic variability observed in the analysis of variance (ANOVA) for traits such as days to 50% tasseling, plant height, thousand grain weight, and grain yield among the 70 maize hybrids underscores the critical role of genetic diversity in germplasm for developing high-yielding maize hybrids, aligning with the study's focus. The high mean squares for yield and thousand grain weight indicate substantial genetic potential within the indigenous (MMRI) and multinational hybrids, which is essential for overcoming yield constraints in Pakistan, such as drought and suboptimal soil fertility (12-14). The elevated error mean square for yield (suggests genotype-by-environment interactions, a common challenge in Pakistan's variable agroecological zones, necessitating further multi-location trials to ensure stability (8).

The correlation analysis further supports the importance of genetic variability, with strong positive correlations of grain yield with cob height, plant height and fresh cob weight, consistent with findings by Yousaf et al. (2022) that highlight these traits as key yield components. The negative correlations of days to tasseling and silking with yield suggest that early-maturing hybrids could enhance productivity in Pakistan's short spring season, addressing climatic constraints like heat stress during late growth stages as suggested by Saeed et al. (2021). The negative correlation of protein content with yield indicates that nutritional quality can be maintained alongside yield improvements, a vital consideration for food security in protein-deficient regions (9).

The principal component analysis (PCA) reinforces the role of genetic variability, with PC1 and PC2 explaining 53.06% of the total variance, driven by yield, fresh cob weight, and structural traits like plant height and ear height. The biplot and variable contribution chart highlight yield and fresh cob weight as the most influential traits, aligning with their high loadings and contributions (approximately 15% each), which supports their use as selection criteria in breeding programs (21). The negative loadings of days to tasseling and silking on PC1 suggest that early maturity is a valuable genetic attribute for adapting maize to Pakistan's growing conditions, while the distribution of indigenous and multinational hybrids in the yield comparison graph indicates diverse genetic bases that can be exploited.

The yield comparison bar graph reveals that multinational hybrids (e.g., Pioneer, DKC) outperform MMRI and Kissan Seed Corporation hybrids, achieving yields up to 8,000 kg/ha, compared to the 4,000–7,000 kg/ha range for indigenous varieties. This superiority likely stems from advanced breeding technologies and diverse germplasm in multinational lines, offering a benchmark for local programs (19). However, the consistent performance of MMRI hybrids across a broad yield range suggests that indigenous germplasm harbors unique alleles for resilience, which, when combined with multinational traits through hybridization, could lead to superior hybrids tailored to Pakistan's needs.

In the context of the study's objective to develop high-yielding maize hybrids, these results emphasize the strategic use of genetic variability from both indigenous and multinational germplasm. The integration of traits like early maturity, high thousand grain weight, and yield stability, as identified through ANOVA, correlation, and PCA, provides a foundation for targeted breeding. Future research should incorporate genomic selection and multi-environment testing to refine these hybrids, ensuring they address Pakistan's yield constraints and enhance productivity.

## Declarations

### Data Availability statement

All data generated or analysed during the study are included in the manuscript.

### Ethics approval and consent to participate

Approved by the department concerned.

### Consent for publication

Approved

### Funding

Not applicable

### Conflict of interest

The authors declared the absence of a conflict of interest.

### Author Contribution

*AG, MS, ARM Conceptualization, Validation, Supervision, Planning, Execution of Experiment; AS, UF, GM Writing – original draft; II, AR Writing – review & editing; MUS, ZUR Formal analysis, Resources, Statistical Analysis, Graphic improvement*

*All authors reviewed the results and approved the final version of the manuscript. They are also accountable for the integrity of the study.*

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