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RESEARCH ARTICLE

# Multivariate analysis for grain yield and yield attributing traits in maize (*Zea mays* L.) inbred lines under acidic and neutral soil conditions

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## Abstract

Evaluating maize genotypes under varying environmental conditions is essential for identifying stable genotypes with high yield potential. This study investigated the multivariate relationships of grain yield and key agronomic traits in 110 maize inbred lines grown in field conditions under both neutral (pH 6.7) and acidic (pH 4.8) soils. Analysis of variance revealed significant variation among the genotypes for all traits except anthesis-silking interval. The correlation analysis indicated that under neutral soil, yield per plant showed a positive but weak association with traits such as days to 50% silk emergence, anthesis-silking interval, days to 75% maturity, plant height and ear height. Under acidic soil conditions, however, yield exhibited negligible positive correlations with all traits. Principal component analysis revealed that the first three components explained 81.6% of the total variation. Hierarchical clustering classified the genotypes into four distinct clusters, with genotypes P53, P66, P37, P100, P60, P90, P59 and P36 showing superior performance under acidic conditions. These genotypes demonstrated higher grain yield, plant height, ear height and a shorter anthesis-silking interval, and all are promising parents for breeding programs targeting improved performance under acidic soil conditions. The findings provide valuable insights for maize breeding strategies aimed at improving performance across different soil pH environments.

**Keywords:** Maize; Multivariate Analysis; Principal Component Analysis; Soil pH; Genetic Variability; Acid Tolerance.

## Introduction

Maize (*Zea mays* L.) is the most commercially cultivated cereal crop globally, valued for its versatility as a source of food, feed, fodder, ethanol, oil, and industrial raw materials. It is grown on approximately 197 million hectares worldwide and serves as a critical pillar of food security in many regions, particularly in Asia, Africa, and Latin America (FAOSTAT 2021). The increasing global demand for maize is driven not only by its nutritional and industrial utility but also by its growing role in sustainable bioenergy production, such as bio-ethanol—a clean and renewable energy source.

As a day-neutral C4 plant, maize exhibits broad ecological adaptability and can thrive across a wide range of climates, from humid tropical zones to cool temperate regions. However, the challenge of enhancing maize productivity is intensified by shrinking arable land, dwindling water resources, and increasing biotic and abiotic stresses. Among the most critical abiotic constraints is soil acidity, which affects nearly 40–50% of the world's potentially arable land. Acidic soils (pH < 5.5) currently occupy about 3,950 million hectares of the Earth's ice-free land. In India alone, approximately 19.75% (31 million hectares) of the 157 million hectares of cultivable land are classified as acidic (Maji et al. 2012). These soils are especially prevalent in the north-eastern, eastern regions, such as Jharkhand, West Bengal, Odisha, Andhra Pradesh, Telangana, Tamil Nadu, Kerala, and

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Maharashtra—where maize is commonly grown as a rainfed upland crop during the Kharif season (Panda 1979).

Low soil pH adversely affects maize growth through a combination of proton ($H^+$), aluminium ($Al^{3+}$), and manganese ($Mn^{2+}$) toxicities, coupled with reduced availability of essential nutrients such as phosphorus (P), calcium (Ca), magnesium (Mg), potassium (K), and molybdenum (Mo). Under acidic conditions, aluminium, typically present in inert aluminosilicate forms, becomes solubilized into its phytotoxic form, $Al(H_2O)_6^{3+}$, due to silicon leaching. This form severely inhibits root elongation and nutrient absorption (Gupta et al. 2013; Dewi-Hayati et al. 2014; Tandzi et al. 2015). Furthermore, since maize roots often penetrate into deeper soil layers in search of nutrients and water, surface applications of lime are insufficient to ameliorate subsoil acidity (Bhattacharyya et al. 2006; Pal et al. 2014).

Soil acidity has been shown to reduce maize grain yield by as much as 71% under extreme conditions. The extent of yield loss depends on several interacting factors: the severity of soil acidity, agro-climatic conditions, and the genetic resilience of maize genotypes to acid stress. Consequently, the development and deployment of acid soil-tolerant maize hybrids is an eco-sustainable, cost-effective strategy to boost productivity on marginal lands.

Genetic diversity forms the foundation of any robust and sustainable crop improvement program. It provides the raw material for selection and facilitates the development of genotypes with enhanced performance under diverse environmental conditions, including stress-prone environments such as acidic soils. A wide range of biometrical and multivariate statistical techniques is available to assess genetic diversity and to elucidate the complex relationships between yield and its contributing traits. Among these, correlation analysis, path coefficient analysis, and multivariate regression are particularly useful for identifying key traits that directly or indirectly influence yield performance (El-Badawy and Mehasen 2011).

Multivariate analytical approaches offer a more holistic and reliable assessment of trait interrelationships, genotype grouping, and trait-based prediction. Principal component analysis (PCA), in particular, has been extensively employed to quantify genetic variability and to dissect complex trait architecture in maize germplasm (Mounika et al. 2018; Al-Naggar et al. 2020; Yadesa et al. 2022). These methods allow breeders to identify promising genotypes and trait combinations that can be targeted in breeding programs for stress tolerance and yield enhancement.

In the present study, the effects of low soil pH and associated edaphic stress factors on grain yield and related agronomic traits in a diverse set of maize inbred lines were investigated. A comprehensive multivariate analysis was conducted on 110 maize inbred lines evaluated under varying soil pH conditions. Six key agronomic parameters, along with grain yield, were measured to determine the impact of acid soil stress and to identify genotypes with superior performance and acid-soil tolerance. This approach enabled a detailed understanding of trait associations under stress conditions and facilitated the selection of potential parental lines for developing acid-tolerant, high-yielding maize hybrids.

## Materials and methods

A field experiment was conducted during the Rabi season of 2023–2024 at ICAR-Indian Agricultural Research Institute (IARI), Jharkhand (24.285° N, 85.360° E), to assess the response of 110 maize (*Zea mays* L.) inbred lines under contrasting soil pH conditions. These genotypes, sourced from the Winter Nursery Centre (WNC) of ICAR-IIMR and CIMMYT-India, Hyderabad, were evaluated under acidic soil (pH 4.8) and neutral soil (pH 6.7) environments. The experimental layout followed an alpha lattice design with three replications, which accounted for spatial heterogeneity and minimized error variance across plots.

Data were collected on key agronomic traits, namely: days to 50% pollen shed (DT), days to 50% silk emergence (DS), anthesis-silking interval (ASI), days to 75% maturity (DM), plant height (PH), ear height (EH), and grain yield (GY). Standard protocols were followed for recording each parameter. Statistical analysis was performed using R software (version 4.4.0), incorporating multiple packages to support variance estimation, multivariate decomposition, trait association, and clustering.

Analysis of Variance (ANOVA) was conducted using the Agricolae package based on the linear model. Principal Component Analysis (PCA), analyzed via the FactoExtra package.

Hierarchical clustering was performed using the Dendextend and Complex Heatmap packages:

Pearson correlation coefficients were calculated via the Corrplot package using the formula:

$$r_{xy} = \sum_{i=1}^{n} (xi - \bar{x})(yi - \bar{y}) / \sqrt{[\sum_{i=1}^{n} (xi - \bar{x})^2 * \sum_{i=1}^{n} (yi - \bar{y})^2]}$$

Where x and y are variables of interest, and $\bar{x}$ and $\bar{y}$ are their respective means. The statistical significance of each correlation coefficient was tested using the t-distribution with n–2 degrees of freedom:

$$t = r * \sqrt{[(n - 2) / (1 - r^2)]}$$

## Results

### *Phenotypic Identification and Variation*

Analysis of variance (ANOVA) conducted under an alpha lattice design revealed statistically significant genetic variation ($p < 0.001$) among maize inbred lines for all measured traits under both soil pH regimes, with the sole exception of the anthesis-silking interval (ASI). This underscores the inherent genetic heterogeneity present in the panel of genotypes and highlights the potential for selection under contrasting soil environments (Table 1).

The estimates of phenotypic and genotypic coefficients of variation (PCV and GCV, respectively) indicated differential sensitivity of the traits to soil pH conditions. Under neutral soil conditions (pH 6.7), ear height (EH) exhibited the highest PCV and GCV, suggesting substantial variability that could be effectively harnessed through selection. Plant height (PH) and grain yield (GY) displayed moderate PCV and GCV, indicative of a balanced genetic and environmental influence. In contrast, ASI exhibited moderate PCV but low GCV, signifying a greater environmental component relative to genetic control. Traits such as days to 50% pollen shed (DT), days to 50% silk emergence (DS), and days to 75% maturity (DM) exhibited low PCV and GCV, implying limited variation in phenological development among genotypes under optimal conditions.

Under acidic soil conditions (pH 4.8), the extent of variability shifted notably. Traits such as ASI, EH, and GY registered both high PCV and GCV, reflecting a robust genotype × environment interaction and underscoring the importance of genotype-specific responses to acid stress. PH maintained a moderate level of variation, while DT, DS, and DM again exhibited low PCV and GCV, affirming the relative stability of phenological traits across environments (Table 2).

Estimates of broad-sense heritability ($h^2$) were high for all traits except ASI in both pH conditions, suggesting that the majority of the observed variation is attributable to genetic rather than environmental factors. This pattern is especially advantageous for breeders targeting stable genetic gains in yield and architectural traits under diverse soil conditions. Assessments of genetic advance complemented the heritability estimates as percent of the mean (GAM), which provides insight into the potential efficiency of selection.

According to the classification of Johnson *et al*. (1955), GAM values were categorized into low (<10%), moderate (10–20%), and high (>20%). In neutral soil, ASI and DS exhibited low GAM, indicating limited scope for improvement through direct selection. DT, DS, and DM demonstrated moderate GAM, while PH, EH, and GY showed high GAM, suggesting strong additive genetic variance and suitability for yield-enhancing selection strategies. A similar trend was observed under acidic conditions, with PH, EH, and GY again showing high GAM. Notably, ASI under low pH exhibited moderate GAM despite low heritability, potentially reflecting the influence of non-additive genetic effects or genotype × environment interaction.

These findings collectively point toward the genetic responsiveness of structural and productivity traits under pH stress, highlighting the feasibility of selecting acid-soil-tolerant maize genotypes. The greater variability and heritability under acidic conditions for traits such as GY and EH suggest that selection pressure in such environments may reveal superior genotypes with resilience to acid soil stress conditions, which often include aluminium toxicity and nutrient imbalances. Furthermore, the differential expression of GAM between environments indicates the need for environment-specific breeding strategies or the development of broadly adapted cultivars through multi-environment selection protocols.

### *Correlation analysis*

The Pearson correlation analysis among key agronomic traits and grain yield across the 110 maize inbred lines revealed distinct patterns of trait associations under neutral and acidic soil conditions, reflecting the influence of environmental context on phenotypic interrelationships (Figs 1 and 2).

Under neutral soil conditions (pH 6.7), grain yield (GY) exhibited weak and statistically non-significant correlations with all other measured traits, suggesting an absence of strong linear dependencies in the absence of abiotic stress. Specifically, GY was marginally and positively associated with days to 50% silk emergence (DS; $r = 0.01$), anthesis-silking interval (ASI; $r = 0.07$), days to 75% maturity (DM; $r = 0.17$), plant height (PH; $r = 0.05$), and ear height (EH; $r = 0.09$). Notably, no correlation was observed between GY and days to 50% pollen shed (DT), indicating that reproductive

**Table 1.** ANOVA of traits under acidic soil and neutral soil

| **Acidic soil** | | | | | | | |
|---|---|---|---|---|---|---|---|
| Trait | DT | DS | ASI | DM | PH | EH | GY |
| Df | 109 | 109 | 109 | 109 | 109 | 109 | 109 |
| MSS | $23.135^{***}$ | $23.305^{***}$ | $0.904^{***}$ | $48.753^{***}$ | $625.73^{***}$ | $153.827^{***}$ | $3.933^{***}$ |
| **Neutral soil** | | | | | | | |
| Trait | DT | DS | ASI | DM | PH | EH | GY |
| Df | 109 | 109 | 109 | 109 | 109 | 109 | 109 |
| MSS | $22.357^{***}$ | $22.375^{***}$ | $0.250^{***}$ | $46.913^{***}$ | $625.66^{***}$ | $165.400^{***}$ | $4.064^{***}$ |

**Table 2.** Estimates of genetic variability parameters of various traits in maize inbred lines

| | Mean | PCV (%) | GCV (%) | $h^2$ | GAM (5%) |
|---|---|---|---|---|---|
| **Neutral soil condition** | | | | | |
| DT | 53.1909 | 6.5110 | 6.0523 | 0.8641 | 11.5892 |
| DS | 47.0000 | 6.2409 | 5.7737 | 0.8559 | 11.0036 |
| ASI | 2.4455 | 19.92 | 4.7688 | 0.0573 | 2.3513 |
| DM | 84.9318 | 5.8380 | 5.5635 | 0.9082 | 10.9219 |
| PH | 111.3333 | 16.5090 | 15.2394 | 0.8521 | 28.9787 |
| EH | 30.8485 | 30.3374 | 28.7355 | 0.8972 | 56.0692 |
| GY | 9.2129 | 15.5456 | 15.3994 | 0.9813 | 31.4244 |
| **Acidic soil condition** | | | | | |
| DT | 55.2500 | 6.4278 | 5.8714 | 0.8344 | 11.0483 |
| DS | 59.7045 | 6.0851 | 5.3246 | 0.7657 | 9.5979 |
| ASI | 4.5409 | 20.3951 | 10.2538 | 0.2528 | 10.6190 |
| DM | 86.9364 | 5.8488 | 5.5044 | 0.8857 | 10.6714 |
| PH | 101.5164 | 18.1275 | 16.6903 | 0.8477 | 31.6564 |
| EH | 27.0207 | 33.5098 | 31.3684 | 0.8763 | 60.4896 |
| GY | 6.8270 | 20.9938 | 20.0775 | 0.9146 | 39.5547 |

phenology alone may not be a reliable proxy for yield performance in optimal conditions.

Strong and statistically significant correlations were observed among several developmental and structural traits, suggesting potential pleiotropic or tightly linked genetic control. The highest positive correlation was recorded between DT and DS (r = 0.99), underscoring the synchrony in male and female flowering across genotypes. Likewise, PH and EH were moderately correlated (r = 0.61), reflecting coordinated growth dynamics of the vegetative and reproductive axes. Maturity traits also clustered tightly: DT correlated with DM (r = 0.58), and DS with DM (r = 0.58), reinforcing the physiological coherence of phenological events. Interestingly, EH exhibited negative correlations with DT (r = -0.28) and DS (r = -0.28), suggesting that earlier flowering genotypes may exhibit increased ear placement, which could have agronomic implications for plant stability and harvesting efficiency.

Under acidic soil conditions (pH 4.8), the correlations between grain yield and the remaining traits remained uniformly weak and non-significant, with r-values ranging from 0.02 to 0.15. Specifically, GY was only marginally correlated with DT (r = 0.02), DS (r = 0.02), ASI (r = 0.03), DM (r = 0.15), PH (r = 0.03), and EH (r = 0.05). These low correlations may be attributed to the confounding effects of acid stress, which can disrupt the physiological integrity of yield-related pathways, thereby decoupling them from otherwise predictable phenotypic patterns.

Despite the stress conditions, certain trait inter-

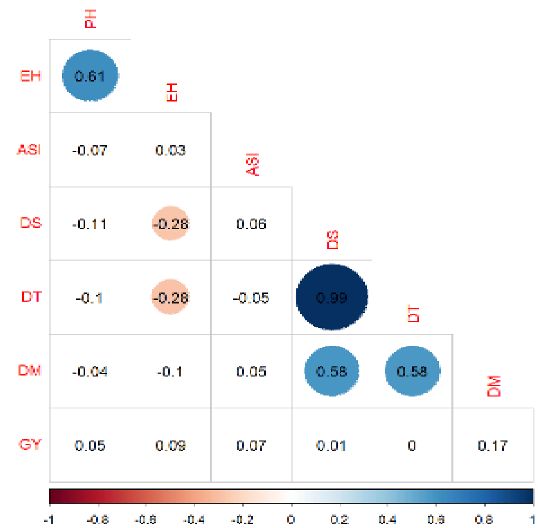


**Fig. 1.** Correlations among seven agronomic traits in 110 maize lines under neutral soil

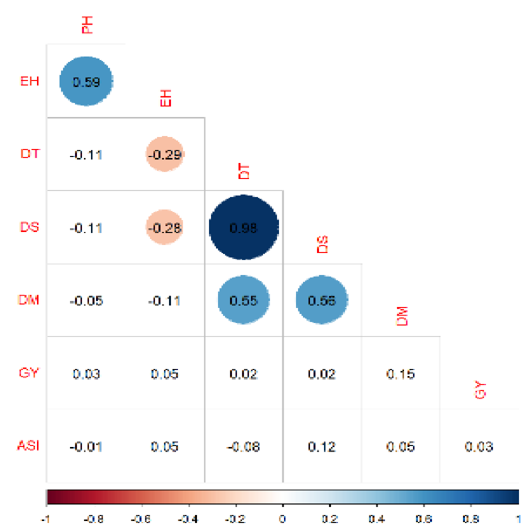


**Fig. 2.** Correlations among seven agronomic traits in 110 maize lines under acidic soil

relationships remained consistent with those observed under neutral pH. The correlation between DT and DS remained high (r = 0.98), reinforcing the tight temporal regulation of flowering events even under adverse conditions. PH and EH maintained a moderate correlation (r = 0.59), indicating the continued coordination of vegetative and reproductive development. Similarly, strong positive correlations were observed between DM and DS (r = 0.56), and between DM and DT (r = 0.55), suggesting that flowering time remains a principal determinant of maturity duration across soil types. Notably, as under neutral conditions, EH exhibited negative correlations with DS (r = -0.28) and DT (r

= -0.29), implying a recurring inverse relationship between flowering time and ear placement across environments.

Collectively, the correlation structure highlights the complex and environment-dependent nature of trait associations in maize. While phenological traits exhibit strong intrinsic coherence, their direct relationship with grain yield is weak or non-existent under both pH regimes, emphasizing the multifactorial nature of yield expression. These findings underscore the necessity for integrative multivariate selection strategies rather than reliance on single-trait indicators when breeding for yield under acid soil stress.

### *Principal Component Analysis (PCA)*

Principal component analysis was employed to elucidate the underlying structure of multivariate trait variation across 110 maize inbred lines evaluated under both acidic and neutral soil conditions. The analysis revealed that the first three principal components (PCs) cumulatively explained 81.6% of the total phenotypic variation, indicating a high degree of dimensional reduction with minimal information loss. Notably, each of these components had eigenvalues exceeding the threshold of 1.0, justifying their retention based on the Kaiser criterion.

The scree plot (Fig. 3) illustrates the progressive decline in eigenvalues across successive components, with a pronounced inflection observed after PC3, supporting the significance of the first three PCs in capturing the dominant axes of variation. Detailed contributions of each PC are provided in Table 3. Specifically, PC1 accounted for 44.0% of the total variance and primarily reflected variation in flowering phenology and vegetative architecture. The trait contributing most strongly to PC1 was days to 50% silk emergence (27.64%), while grain yield exhibited the lowest relative influence (7.50%).

PC2 contributed an additional 20.5%, bringing the cumulative variance explained to 64.5%. The largest trait loading in PC2 was observed for plant height (25.24%), suggesting that this component captured variation related to structural vigor and plant stature. In contrast, days to 50% silk emergence contributed least (5.93%) to this axis, highlighting a decoupling between reproductive timing and vertical growth in this dimension.

PC3 contributed a further 17.1%, raising the total explained variance to 81.6%. This component was dominated by grain yield (28.74%), underscoring a unique axis of variation driven predominantly by yield performance, largely independent of phenological or structural traits. Conversely, days to 50% silk emergence contributed minimally (0.42%), reinforcing the orthogonality of this yield-centric component from temporal traits. Trait-wise contributions to each principal component are systematically tabulated in Table 4.

The biplot derived from the first two principal components (PC1 vs. PC2; Fig. 4) provided a two-dimensional visualisation of genotype dispersion and trait interrelationships. Under acidic soil conditions, genotypes P99, P37A, P105, and P88 were positioned at extreme distances from the origin across different quadrants, indicating substantial divergence in their multivariate trait expression and potential utility as contrasting parental lines for hybrid development. Similarly, under neutral pH conditions, genotypes P99, P37, P36, and P26 were distantly located from the centroid, suggesting

**Table 3.** Eigenvalue, percentage variability and cumulative variability of different principal components

| Principal component | Eigenvalue | Variance percent | Cumulative variance percent |
|---|---|---|---|
| PC 1 | 3.1 | 44.0 | 44.0 |
| PC 2 | 1.4 | 20.5 | 64.5 |
| PC 3 | 1.2 | 17.1 | 81.6 |
| PC 4 | 0.5 | 7.8 | 89.4 |
| PC 5 | 0.4 | 5.8 | 95.2 |
| PC 6 | 0.3 | 4.8 | 100.0 |
| PC 7 | 0.0 | 0.0 | 100.0 |

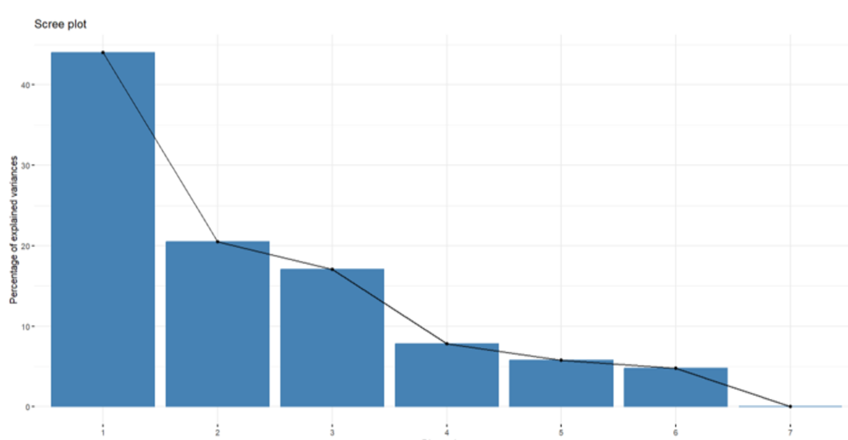


**Fig. 3.** Scree plot depicting the contribution of various PCs toward total variation

broad phenotypic variability and potential as candidates for broad adaptation.

The spatial separation of genotypes across quadrants of the biplot signifies trait diversity and multivariate uniqueness, with implications for ideotype breeding under both optimal and stress-prone environments. Overall, the PCA results highlight key trait dimensions governing phenotypic diversity and enable the identification of genotypes with unique trait combinations suitable for targeted breeding interventions.

### *Hierarchical cluster analysis*

To elucidate the genetic divergence and phenotypic structuring among the 110 maize inbred lines, hierarchical cluster analysis was performed using Ward's minimum variance method, which minimizes the total within-cluster sum of squares. This approach effectively grouped genotypes based on multivariate trait similarity under both neutral (pH 6.7) and acidic (pH 4.8) soil conditions.

The resulting dendrograms (Figs 5 and 6) revealed four distinct clusters under each soil environment, indicating substantial phenotypic heterogeneity among the inbred lines. Under neutral soil conditions, Cluster II was the largest, encompassing 43 genotypes, while Cluster III, with only 14 genotypes, represented the smallest grouping. Conversely, under acidic soil conditions, Cluster III emerged as the most populous with 43 genotypes, whereas Cluster I was the smallest cluster, consisting of 12 genotypes.

This shift in cluster composition between soil conditions underscores the influence of edaphic stress on trait expression and genotype-environment interaction. The differential distribution of genotypes across clusters suggests the presence of genotypic plasticity, with certain lines exhibiting stable phenotypes across environments, while others display soil condition-specific divergence.

The hierarchical clustering outputs, validated by the cophenetic correlation coefficient (not shown), provide a robust framework for identifying genotypes with contrasting phenotypic profiles, facilitating their deployment in heterotic group formation and stress-resilient hybrid development. Furthermore, the clustering results complement the PCA-based multivariate structure, jointly offering a comprehensive understanding of phenotypic diversity under variable pH regimes.

To support and validate the principal component findings under both neutral and acidic soil conditions, heatmaps were generated to visualize the patterns of trait associations among the 110 maize inbred lines, as shown in Figs 7 and 8, respectively. Under neutral pH conditions, three distinct clusters of traits emerged. The first cluster comprised anthesis-silking interval and grain yield, indicating a shared expression pattern potentially driven by physiological interactions or co-regulation. The second cluster grouped together days to 50% silk emergence, days

**Table 4:** Per cent contributions of seven characters towards the first three principal components

| *Traits* | *PC 1* | *PC 2* | *PC 3* |
|---|---|---|---|
| Days to 50% pollen shed | 22.781647 | 12.741702 | 0.8460261 |
| Days to 50% silk emergence | 27.638090 | 5.934516 | 0.4279602 |
| Anthesis silking interval | 11.784812 | 6.752905 | 25.9853619 |
| Days to 75% maturity | 12.255277 | 20.155125 | 0.9235995 |
| Plant height | 7.680001 | 25.241405 | 17.9922701 |
| Ear height | 10.356046 | 14.064178 | 25.0797331 |
| Grain yield | 7.504127 | 15.110169 | 28.7450491 |

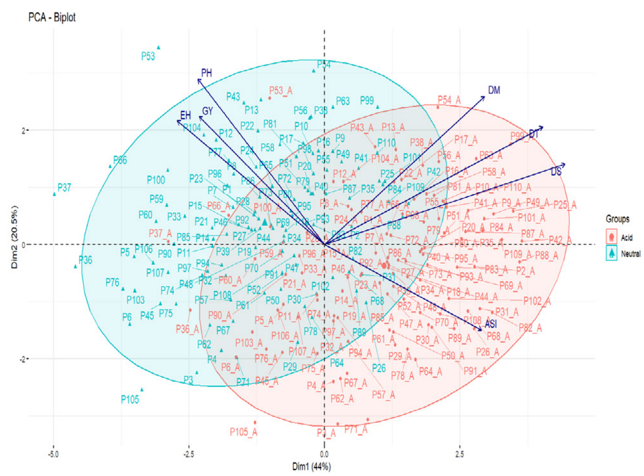


**Fig. 4.** A principal component analysis biplot illustrating the variations between 110 maize inbred lines

to 50% pollen shed, and days to 75% maturity, suggesting these phenological traits are developmentally synchronized. The third cluster included plant height and ear height, reflecting their structural or architectural relatedness.

Notably, under neutral conditions, genotypes such as P63, P104, and P17 were found to cluster in regions characterized by high grain yield and low anthesis-silking interval, highlighting them as promising candidates for productivity and early reproductive synchrony. Conversely, genotypes like P61, P27, and P66 were associated with lower yield potential and prolonged anthesis-silking intervals, rendering them less favorable for selection under these soil conditions.

In the acidic soil environment, the heatmap revealed a shift in the genotypic response profile. A contiguous group of genotypes, particularly those spanning from P63 to P107, demonstrated consistently higher grain yield and reduced anthesis-silking interval, indicating superior adaptation to low pH stress. These genotypes may therefore serve as valuable sources of acid-soil tolerance

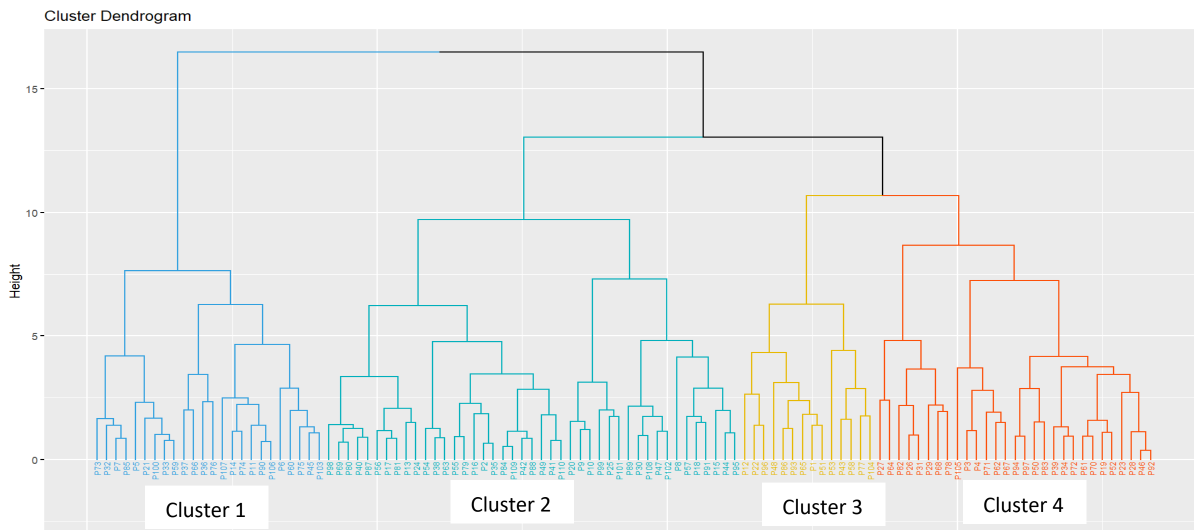


**Fig. 5.** Dendrogram based on Ward's method using Euclidean distance under neutral soil

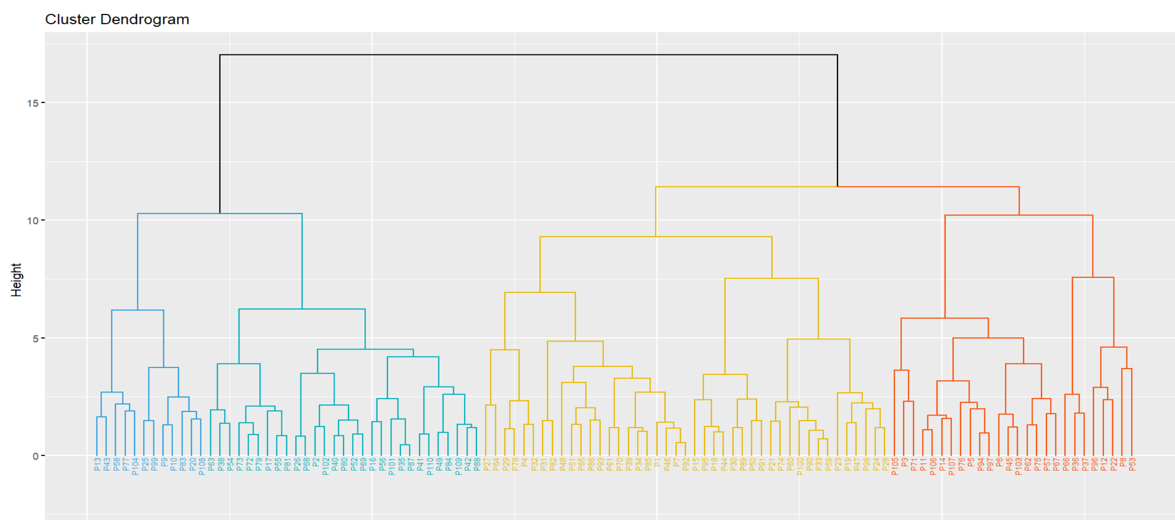


**Fig. 6.** Dendrogram based on Ward's method using Euclidean distance underacidic soil

in breeding pipelines. In contrast, genotypes positioned in the middle to lower regions of the heatmap, notably P66 to P68 and P71 to P11, exhibited suboptimal trait profiles characterized by reduced yield and extended reproductive intervals, suggesting their limited suitability under acidic conditions.

The heatmap analysis not only corroborates the results of the PCA and clustering but also provides a visual confirmation of trait co-expression and genotypic performance, thereby enhancing the robustness of genotype selection under differential soil pH environments.

## Discussion

Maize yield is a complex quantitative trait, modulated by the cumulative effects of multiple phenological and morphological parameters, each subject to both genetic and environmental influences. In this study, multivariate analyses were conducted to dissect the relationships among six key agronomic traits—days to 50% pollen shed (DT), days to 50% silk emergence (DS), anthesis-silking interval (ASI), days to 75% maturity (DM), plant height (PH), and ear height (EH)—and grain yield across 110 maize inbred lines grown under contrasting soil pH regimes. These analyses aimed to identify trait combinations and genotypes that confer resilience and superior yield under acidic stress, which remains a critical abiotic constraint in many maize-producing regions.

The results from the analysis of variance (ANOVA) revealed significant genotypic differences for all studied traits, reaffirming the presence of substantial genetic variation in the genotypes. This observed variability is essential for the implementation of selection-based

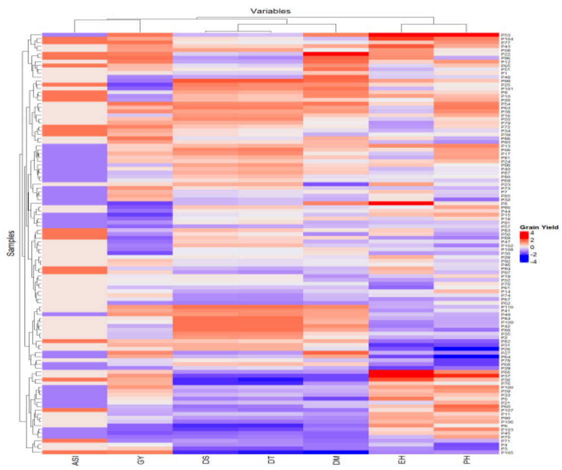


**Fig. 7.** Heat map based on hierarchical clustering among 110 maize inbred lines and 7 traits under neutral soil conditions

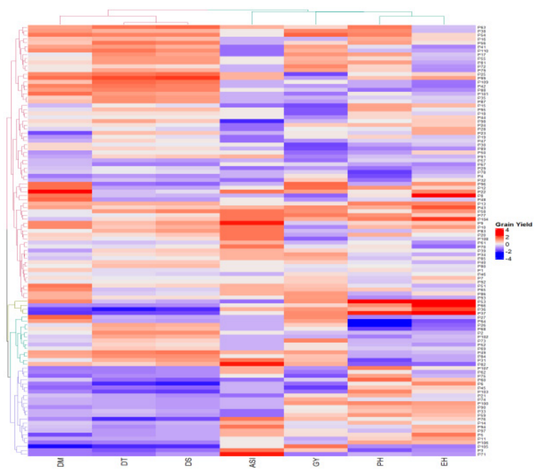


**Fig. 8.** Heat map based on hierarchical clustering among 110 maize inbred lines and 7 traits under acidic soil conditions

breeding strategies, particularly those targeting the development of acid-soil tolerant germplasm. Similar patterns of significant phenotypic diversity under varying environmental conditions have been reported by Kumar *et al*. (2024) and Singh *et al*. (2024), further substantiating the robustness of the current findings.

In both soil environments, phenotypic coefficients of variation (PCV) exceeded their corresponding genotypic coefficients of variation (GCV) for all measured traits. However, the relatively narrow magnitude of the PCV-GCV differential suggests that environmental influence, while present, is not the predominant factor driving phenotypic expression. This indicates the potential for effective selection, particularly for traits such as plant height, ear height, and grain yield, where the genotypic component accounts for a major portion of the observed variation. These findings are in concordance with those reported by Magar *et al*. (2021), Sahu *et al*. (2022), and Antony *et al*. (2024), who observed similar trait dynamics under multi-environment trials.

Importantly, traits such as plant height, ear height, and grain yield exhibited both high heritability and high genetic advance as a percentage of mean (GAM) under both neutral and acidic conditions. This combination is indicative of additive gene action and suggests that direct selection for these traits would be highly effective in advancing stress-resilient maize ideotypes. The classical quantitative genetic theory supports this interpretation, as traits governed predominantly by additive gene effects tend to respond predictably and favorably to selection pressure, in contrast to traits controlled by dominant or epistatic interactions, which may show high heritability but low GAM (Krishna *et al*. 2009).

Taken together, the multivariate analyses underscore the effectiveness of integrating genetic variability assessments with robust statistical modeling to guide genotype selection under abiotic stress conditions. The identification of heritable, yield-associated traits across diverse soil pH environments offers a valuable roadmap for the genetic improvement of maize lines targeted for deployment in marginal acid-affected agroecosystems.

Under acidic soil conditions, a subset of maize inbred lines—specifically genotypes P53, P66, P37, P100, P60, P90, P59, and P36—exhibited superior agronomic performance. These lines were characterized by consistently higher grain yields, increased plant stature, and reduced anthesis-silking intervals, highlighting their potential as elite donors for breeding programs targeting soil acidity tolerance. In contrast, genotypes such as P68, P26, P89, P31, and P64 displayed suboptimal performance, suggesting heightened susceptibility to acidic stress and limited utility in low-pH environments.

Correlation analysis revealed only weak positive associations between grain yield and traits such as days to 50% silk emergence, anthesis-silking interval, days to 75% maturity, plant height, and ear height under neutral soil conditions. Similarly, in acidic environments, all evaluated traits—including phenological (days to 50% pollen shed, silk emergence, and maturity) and morphological (plant and ear height) parameters—showed negligible correlations with grain yield. These results align with the complexity of trait interactions influencing yield under stress, as documented in prior studies. For instance, Mishra *et al*. (2023) reported negative correlations between grain yield and traits such as leaf length, leaf width, days to 50% silking, and maturity, while Yadesa et al. (2022) observed positive associations between yield and attributes like plant height, ear height, ear diameter, ear number per plant, and thousand kernel weight. Such contrasting findings emphasize the polygenic and context-dependent nature of yield determination, especially under suboptimal edaphic conditions.

Principal component analysis (PCA) proved instrumental in dissecting the multivariate trait architecture across the genotypes. In this study, seven principal components were extracted, with the first three cumulatively accounting for 81.6% of the total phenotypic variance, underscoring their sufficiency in capturing the key patterns of genotypic divergence. Trait loading patterns on the PCA axes revealed distinct clustering, with days to 50% pollen shed, silk emergence, and days to 75% maturity primarily contributing to the first quadrant, while plant height, ear height, and grain yield were more strongly associated with the second quadrant. The anthesis-silking interval was uniquely positioned in the fourth quadrant, indicating a divergent contribution to the total variation. The spatial distribution of genotypes across these quadrants further enabled the identification of contrasting ideotypes. Genotypes situated farther from the origin exhibited greater trait divergence, whereas those positioned near the origin contributed minimally to variation, consistent with earlier interpretations by Molosiwa et al. (2016) and Sinana et al. (2023).

Notably, promising acid-soil-adapted inbred lines were predominantly located within the second and third quadrants of the biplot, suggesting that these zones encapsulate genotypic profiles with favorable trait combinations for acidic environments. As shown in prior studies (Anand et al. 2023; Visakh et al. 2023), PCA serves as a powerful tool for narrowing down breeding populations by identifying key traits and reducing the evaluation burden, thereby accelerating the development of stress-resilient maize cultivars.

Hierarchical cluster analysis has long been recognized as a robust multivariate technique for classifying germplasm collections based on genetic similarity and dissimilarity (Van Hintum, 1995). In the present study, Ward's minimum variance method effectively grouped the 110 maize inbred lines into four distinct clusters under both neutral and acidic soil conditions. This classification reflects underlying genetic divergence and trait associations, which are critical for selecting genetically diverse parental lines to enhance heterosis in hybrid breeding programs (Suryanarayana et al. 2017).

The clustering pattern revealed clear genotype differentiation according to soil pH tolerance. In particular, inbred lines exhibiting superior agronomic performance under acidic soil conditions—including genotypes P53, P66, P37, P100, P60, P90, P59, and P36—were consistently grouped within clusters III and IV. These genotypes demonstrated enhanced grain yield potential, greater plant stature, and shorter anthesis-silking intervals under stress conditions, suggesting strong adaptability to acidic environments. Their placement in the same clusters highlights shared genetic and phenotypic profiles, reinforcing their suitability as candidate parental lines for breeding programs aimed at improving acid soil tolerance in maize.

The ability to distinguish such high-performing genotypes through hierarchical clustering offers a valuable decision-support tool for breeders. By integrating this approach with PCA and correlation analyses, it is possible to design strategic crosses that maximize genetic gain while addressing the challenges posed by variable edaphic conditions.

## Authors' contribution

Conceptualization of research (SK); Designing of the experiments (SK, PS); Contribution of experimental materials (BK); Execution of field/lab experiments and data collection (SK, PS, AMC); Analysis of data and interpretation (AK, NRP); Preparation of the manuscript (BK, PRK, SKM).

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