

Morphophysiological evaluation of hybrid maize lines from peak and test crosses for high-yield development

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Abstract. Nasaruddin, Farid M, Anshori MF, Nur A, Iriany N, Fadhilah AN. 2025. *Morphophysiological evaluation of hybrid maize lines from peak and test crosses for high-yield development. Biodiversitas* 26: 5204-5210. The increasing demand for maize in Indonesia amid stagnant productivity underscores the need for innovative breeding approaches to enhance yield potential and resilience. This study integrates morphophysiological trait analysis with hybrid maize lines derived from transgressive segregants and elite line, aiming to identify promising genotypes and key traits associated with high productivity under tropical conditions. A total of 96 genotypes, consisting of 60 hybrids, 20 parental lines and 4 commercial controls, were evaluated using an augmented design. Observations were made on agronomic and physiological traits. This is the first report combining peak and test cross schemes with morphophysiological evaluation in tropical maize, which broadens the genetic base and addresses the limitation of narrow parental pools. Several hybrid maize lines, particularly G29 (8.7 t.ha⁻¹), G28, and G39, outperformed their parents in yield performance. Traits such as cob weight, number of seeds per row, and plant height exhibited high heritability and strong direct effects on yield, highlighting their suitability as selection criteria in breeding programs. Overall, integrating source traits (e.g., plant height, chlorophyll index) and sink traits (e.g., cob characteristics) provides an effective strategy for developing high-yielding and resilient maize hybrids suitable for smallholder-based production systems in tropical environments.

Keywords: Hybrid maize, morphophysiology, peak crosses, transgressive segregation, yield expanding

INTRODUCTION

Maize (*Zea mays* L.) plays a central role in Indonesia as a staple food, animal feed, and industrial raw material. Demand continues to rise sharply, driven by the feed and processing sectors, which together account for over 80% of national maize use (USDA 2024). In response, Indonesian government has targeted maize self-sufficiency through increased productivity and reduced imports, particularly in feed-grade maize. However, actual productivity gains remain limited, especially in stress-prone and marginal production zones such as South Sulawesi, East Nusa Tenggara, and parts of Kalimantan (Syahrudin et al. 2020). These areas experience a combination of low soil fertility, erratic rainfall, and high temperatures, which reduce hybrid performance and yield stability.

Despite the adoption of commercial hybrids bred for broad adaptability, their agronomic performance often falls short under such conditions. Many existing hybrids lack resilience to abiotic stresses and are derived from a narrow genetic base, limiting the potential for further genetic improvement (Engida 2022; Choudhary et al. 2023). In addition, the high cost of hybrid seeds discourages widespread adoption among smallholder farmers, who dominate maize production in Indonesia. Thus, there is an

urgent need for breeding strategies that not only emphasize high yield but also local adaptation, stress tolerance, and affordability, key pillars of sustainable maize production in tropical regions.

Recent advances in breeding technologies, such as Marker-Assisted Selection (MAS), Genomic Selection (GS), and High-Throughput Phenotyping (HTP), have accelerated hybrid development in many tropical maize programs (Ashan et al. 2020; Makore et al. 2021; Prasanna et al. 2021). However, these methods are not always accessible or fully optimized in resource-constrained environments. Many breeding efforts in Southeast Asia still rely heavily on conventional field-based selection, which, although effective, must be enhanced to increase efficiency, especially under diverse agroecological constraints.

A promising solution involves the use of transgressive segregants, progeny lines that exhibit superior performance relative to both parents. These lines can capture novel allelic interactions and are highly valuable for creating high-heterosis hybrids (Bahtiar et al. 2020). Peak crosses, defined as deliberate crosses among top-performing recombinant lines selected from early segregating generations, are designed to capture optimal trait combinations and maximize heterosis (Anshori et al. 2021).

To further refine selection accuracy, the inclusion of morphophysiological traits, such as chlorophyll content, canopy temperature depression, NDVI, and biomass accumulation, has gained traction as a complementary approach. These traits tend to exhibit higher heritability and environmental stability compared to yield alone, enabling more reliable early-generation selection under variable conditions (Saritha et al. 2020; Anshori et al. 2022; Utari et al. 2023). This is especially relevant in tropical systems, where stressors are both biophysical and unpredictable. However, few studies have systematically combined transgressive segregants, targeted crossing strategies, and morphophysiological trait selection under resource-limited conditions. The lack of integrative approaches in tropical breeding pipelines limits the development of hybrids with both high yield potential and stress adaptability.

The novelty of this study lies in its integrative breeding approach, combining transgressive segregant selection, peak and test crossing schemes, and morphophysiological trait-based evaluation within an augmented experimental design. The augmented design enables the evaluation of a large number of genotypes with minimal replication, making it suitable for preliminary selection stages in resource-limited settings. By focusing on traits with high heritability and direct yield impact, the study seeks to identify hybrid lines with improved physiological efficiency, heterosis expression, and yield resilience.

The overarching objective is to develop high-yielding, climate-resilient maize hybrids tailored to Indonesia's diverse agroecological conditions. In doing so, this research addresses critical gaps in hybrid maize breeding. It offers a scalable, locally relevant model for accelerating genetic gain, ensuring food security, and enhancing sustainability in tropical maize systems.

MATERIALS AND METHODS

Research location

This research was conducted at the Congko Village Experimental Garden, Bone District, South Sulawesi, Indonesia, from March to June 2024. The location has an altitude of ± 80 m above sea level with ultisol soil type with

sandy clay texture, soil pH around 5.4, and low organic matter content. Rainfall during the research period ranged from 150-200 mm/month with an average daily temperature of 29-33°C.

Materials and research design

The genetic material consisted of 60 hybrid maize lines from peak cross crosses, 20 transgressive segregant lines, and 4 control varieties. The experiment was conducted in a single growing season at one location and followed an augmented design consisting of four blocks. The study used an Augmented Design with 4 blocks as treatment units. The augmented design was chosen due to its efficiency in handling a large number of unreplicated test genotypes alongside replicated control varieties. This approach allows preliminary evaluation of a wide genetic base under field conditions with minimal resource input, which is especially practical for early-generation hybrid testing (Kumar et al. 2025). Each block consisted of 24 genotypes, resulting in a total of 96 experimental units. Four control varieties were repeated in each block, while the other lines were not repeated (without replication). Each genotype was planted in one plot measuring 2.5 m $\times$ 3 m (7.5 m²) with a planting distance of 75 cm $\times$ 25 cm (approximately 160 plants/plot). Basic fertilization and maintenance were carried out according to Indonesian Cereal Research Institute (ICERI) recommendations.

Observed characters and measurement time

The morphological and physiological characteristics of maize plants observed in this study included plant height, cob height, stem diameter, number of leaves, leaf chlorophyll index, length of filled cobs, cob diameter, number of seed rows per cob, number of seeds per row, cob weight, seed weight, and yield (Table 1). Plant height was measured during the flowering stage, from the ground surface to the highest point of the plant using a measuring stick. Cob height was measured from the ground to the position of the primary ear. Stem diameter was recorded at the first internode above the ground using a vernier caliper at 45 Days After Planting (DAP). The number of leaves was manually counted when the plant reached full flowering.

Table 1. Summary of observed traits, measurement methods, and observation timing

Trait	Measurement method	Time of measurement
Plant height (PH)	Measuring stick (ground to plant tip)	Flowering stage
Stem diameter (SD)	Vernier caliper (first internode)	45 DAP
Number of leaves (NL)	Manual count	Full flowering
Number of seeds per row (NSR)	Manual count (5 cobs)	Harvest
Length of seeded cob (LSC)	Ruler (10 cobs per plot)	Harvest
Cob diameter (CD)	Caliper/ruler (10 cobs per plot)	Harvest
Leaf chlorophyll index (LCI)	SPAD meter (5th leaf from top)	50 DAP
Ear height	Measuring stick (ground to ear position)	Flowering stage
Number of seeds rows per cob (NSC)	Manual count (5 cobs)	Harvest
Cob weight	Weighing (under dry conditions)	Harvest
Seed weight	Weighing (under dry conditions)	Harvest
Yield	Conversion of dry seed weight to t/ha	Harvest
NDVI	Calculated from spectral reflectance (NIR and Red bands)	70 DAP

Note: DAP: Days After Planting

The leaf chlorophyll index was assessed using a SPAD meter on the fifth leaf from the top at 50 DAP. Measurements of filled cob length and cob diameter were taken at harvest from ten sample cobs per plot. The number of seed rows per cob and seeds per row was manually counted from five randomly selected cobs. Cob weight and seed weight were recorded under dry storage conditions to ensure accuracy and consistency. Yield was calculated by converting the dry seed weight per plot to tons per hectare. All observations were conducted systematically and consistently to ensure data reliability as a foundation for selecting superior genotypes.

Data analysis

The data obtained from observations were analyzed using Analysis of Variance (ANOVA) based on the augmented design to determine the significance of differences among genotypes and control varieties. The ANOVA results were also used as a basis for estimating the heritability of each trait. Broad-sense Heritability (H^2) was calculated as: $H^2 = \sigma^2_g / \sigma^2_p$, where σ^2_g is the genotypic variance and σ^2_p is the phenotypic variance. Genetic variance was estimated from the difference between the mean square of genotypes and the error mean square, adjusted by the number of replications in the control genotypes (Redhu et al. 2025).

In addition to ANOVA, Pearson correlation analysis was conducted to examine the strength and direction of relationships between traits. The correlation coefficient (r_{xy}) between traits X and Y was calculated as: $r_{xy} = \text{Cov}(X,Y) / (\sigma_x \times \sigma_y)$ (Luthfiyah et al. 2023; Manalu and Sudrajat 2025). Traits that were significantly correlated with yield were further analyzed using path analysis to determine their direct and indirect contributions to yield. The total correlation (r_{iy}) between an independent variable i and yield (y) was partitioned as: $r_{iy} = p_{iy} + \sum (r_{ij} \times p_{jy})$, where p_{iy} is the direct effect of trait i on yield, and $\sum (r_{ij} \times p_{jy})$ represents the indirect effects via other traits j . Principal Component Analysis (PCA) was not applied in this study, as the primary objective was to identify traits with high heritability and strong direct effects for inclusion in selection indices. Future studies are planned to incorporate multivariate approaches such as PCA to enable dimensionality reduction and trait clustering. All statistical analyses were performed using Microsoft Excel and STAR.

RESULTS AND DISCUSSION

Analysis of variance and heritability

ANOVA revealed significant genotypic differences for key morphological traits such as Plant Height (PH), Cob Weight (CW), Seed Weight (SW), Yield (Y), Length of Seeded Cob (LSC), and Cob Diameter (CD) (Table 2). Most traits exhibited low coefficients of variation (<10%) and moderate to high heritability (>50%), indicating reliable data and strong potential for selection. Number of Leaves (NL) showed the highest heritability (92%), while Number of Seed rows per Cob (NSC) had the lowest (49%) (Table 5). Physiological traits such as LCI and NDVI also displayed considerable variability, though more influenced by environmental factors. Overall, traits with significant differences, low CV, and high H^2 (e.g., PH, NSR, CW, CD) can serve as effective selection criteria in hybrid maize breeding.

Correlation and path analysis

Correlation analysis indicated that several traits were positively associated with yield, with the strongest relationships observed for length of seeded cob, cob weight, and seed weight (Table 3). These findings are further illustrated in the heatmap of Pearson correlation (Figure 1), which visually represents the strength of associations among traits. In the heatmap, darker blue shades indicate stronger positive correlations, particularly between yield and traits, highlighting their strong contribution to yield potential. Lighter shades reflect weaker, yet still positive, associations. The diagonal values (1.00) represent perfect self-correlation. Overall, the heatmap confirms the quantitative results and visually supports the identification of key traits associated with yield improvement. Path analysis further highlighted cob weight as the most influential trait with the highest direct effect on yield, followed by number of seeds per row and plant height (Table 4). In contrast, traits such as seed weight and cob diameter contributed mainly through indirect pathways, suggesting their effects were mediated by cob weight. This combination of correlation and path analysis underscores cob weight and related kernel traits as the most reliable selection criteria for improving yield potential in hybrid maize.

Table 2. Mean squares, coefficients of variation, and heritability estimates for morphophysiological traits in maize

Character	Mean square (Control)	Mean square (Genotype)	Mean square (Interaction)	CV (%)	H^2 (%)
PH	555.45*	369.34*	2294.14**	5.59	65.89
SD	7.45ns	5.34ns	16.70*	7.30	56.35
NL	0.05ns	0.87**	3.04**	2.31	92.36
NSR	5.98ns	37.79**	152.83**	7.09	85.38
LSC	5.05*	5.13**	5.42*	3.20	79.99
CD	55.16**	11.81**	117.29**	6.17	81.88
LCI	4325.58**	1492.15ns	8224.56**	5.03	62.99

Note: Trait abbreviations: PH: Plant Height, SD: Stem Diameter, NL: Number of Leaves, NSR: Number of Seeds per Row, LSC: Length of Seeded Cob, CD: Cob Diameter, LCI: Leaf Chlorophyll Index. **: Significant at the 1% level ($p < 0.01$), *: Significant at the 5% level, ns: Not significant. CV: Coefficient of Variation, H^2 : Broad-sense Heritability. Different superscript letters within a row indicate significant differences among treatments at $p < 0.05$ (LSD test). The check mark (✓) indicates characters with statistically significant variation, supported by a low Coefficient of Variation (CV) and high Heritability (H^2), indicating strong potential for genetic selection

Table 3. Pearson correlation for selected characters of yield

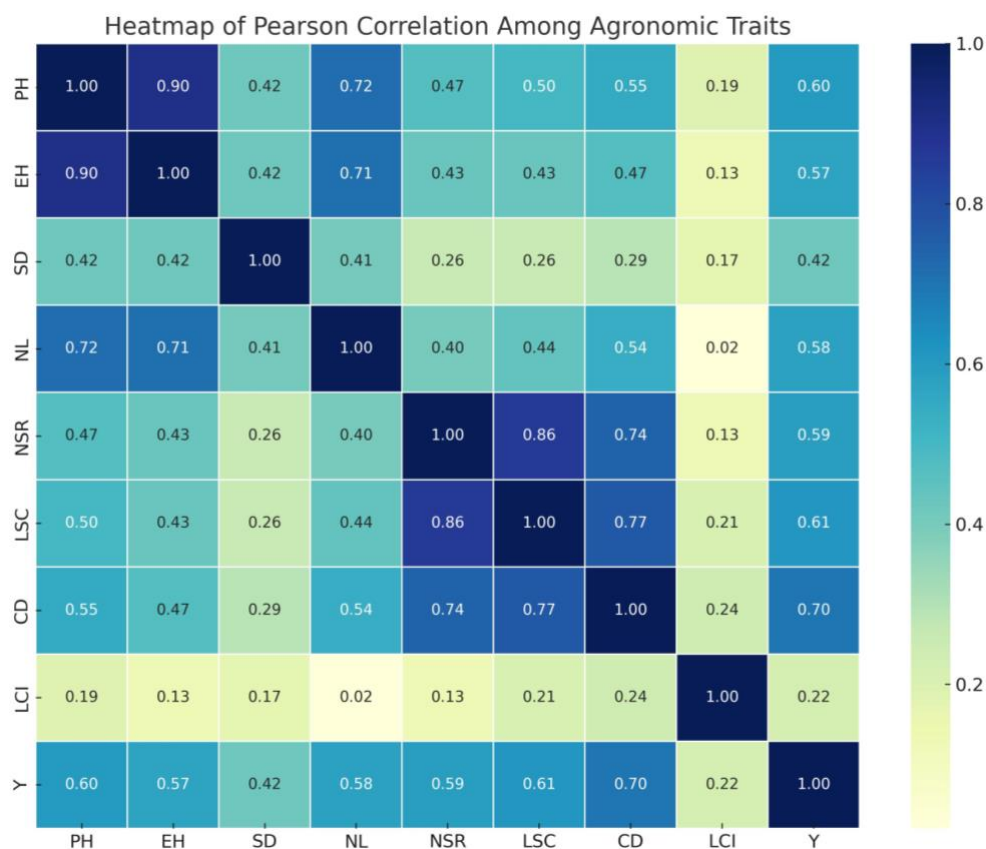
	PH	EH	SD	NL	NSR	LSC	CD	LCI	Y
PH	1.00								
EH	0.90**	1.00							
SD	0.42**	0.42**	1.00						
NL	0.72**	0.71**	0.41**	1.00					
NSR	0.47**	0.43**	0.26*	0.40**	1.00				
LSC	0.50**	0.43**	0.26*	0.44**	0.86**	1.00			
CD	0.55**	0.47**	0.29*	0.54**	0.74**	0.77**	1.00		
LCI	0.19tn	0.13tn	0.17tn	0.02tn	0.13tn	0.21tn	0.24*	1.00	
Y	0.60**	0.57**	0.42**	0.58**	0.59**	0.61**	0.70**	0.22tn	1.00

Notes: **: Significant at the 1% level ($p < 0.01$), *: Significant at the 5% level, ns: Not significant. Trait abbreviations: PH: Plant Height, EH: Ear Height, SD: Stem Diameter, NL: Number of Leaves, NSC: Number of Seeds rows per Cob, NSR: Number of Seeds per Row, CW: Cob Weight, SW: Seed Weight, Y: Yield, LSC: Length of Seeded Cob, CD: Cob Diameter, LCI: Leaf Chlorophyll Index, NDVI: Normalized Difference Vegetation Index

Table 4. Path analysis for maize yield based on the characters with the highest correlation with yield

Character	Direct effect	Indirect effect						Total effect
		PH	SD	NL	NSR	CD	LCI	
PH	0.15		0.06	0.12	0.06	0.21	0.01	0.60
SD	0.13	0.06		0.07	0.03	0.11	0.01	0.41
NL	0.16	0.11	0.05		0.05	0.21	0.00	0.58
NSR	0.13	0.07	0.03	0.06		0.28	0.01	0.59
CD	0.38	0.08	0.04	0.09	0.09		0.01	0.69
LCI	0.05	0.03	0.02	0.00	0.02	0.09		0.21

Note: Numbers in bold indicate the greatest direct effect. Trait abbreviations: PH: Plant Height, SD: Stem Diameter, NL: Number of Leaves, NSR: Number of Seeds per Row, CD: Cob Diameter, LCI: Leaf Chlorophyll Index

**Figure 1.** Heatmap of Pearson Correlation among agronomic traits in maize. Darker blue colors represent stronger positive correlations (closer to +1), while lighter shades indicate weaker associations. The diagonal values (1.00) show perfect self-correlations

Selection of comparative elders

Eight hybrid maize lines (G19, G23, G28, G29, G31, G39, G46, and G47) demonstrated superior productivity compared to their respective parents (Figure 2). Among these, G29 achieved the highest yield, with G28 and G39 also performing consistently above parental and control varieties. This highlights the effectiveness of peak and test crosses in capturing heterosis. In addition, parent T19 (CB1.5.2) showed strong potential as a valuable source for generating productive populations in future breeding programs.

Discussion

The success of tropical maize breeding strongly relies on identifying traits that are not only heritable but also physiologically meaningful for yield formation. In this study, key morphophysiological traits such as plant height, cob weight, number of seeds per row, cob diameter, and seed weight exhibited moderate to high heritability along with low coefficients of variation, confirming their stability across environments and a strong genetic basis. This makes them highly suitable as selection criteria for early breeding decisions, consistent with recent studies showing that high-heritability traits accelerate breeding efficiency by enabling indirect selection (Rana et al. 2024; Singh et al. 2024). Such evidence aligns with the growing emphasis on predictive trait-based breeding, where heritability and stability are used to prioritize resource allocation in selection pipelines (Ahmad et al. 2024).

Trait-yield relationships in this study further emphasize the importance of both sink and source attributes. Seed weight, cob weight, and number of seeds per row were the strongest contributors to yield, representing reproductive sink capacity. Plant height and stem diameter, although less strongly correlated, contributed to vegetative vigor and canopy development, reinforcing source strength for photosynthate supply. Path coefficient analysis identified cob weight as the most influential trait with the largest direct effect, whereas seed weight and cob diameter acted indirectly through their influence on cob weight. This pattern highlights the central role of cob-related traits in maize yield formation, consistent with reports by Prasanna et al. (2021) and Ojeda-

Rivera et al. (2025). It is further supported by a recent multi-environment analysis showing that cob traits explained the largest portion of yield variation under tropical conditions (Singamsetti et al. 2023).

Physiological efficiency also played a supporting role in hybrid performance. Although Leaf Chlorophyll Index (LCI) and NDVI were less directly correlated with yield, their association with photosynthetic activity, nitrogen assimilation, and stress resilience makes them valuable secondary traits. Previous studies have shown that high LCI values contribute to improved grain filling under nitrogen-limited environments (Sandhu et al. 2021; Noor et al. 2023). Similarly, UAV-based NDVI assessments have demonstrated strong correlations with yield ($r = 0.80-0.84$), confirming its predictive power for hybrid performance (Khoddamzadeh et al. 2023). The incorporation of such physiological markers into breeding pipelines complements conventional morphological traits, especially when targeting resilience to abiotic stresses like drought and heat.

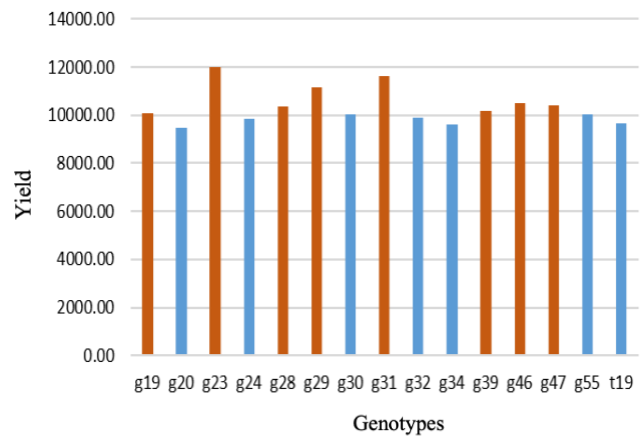


Figure 2. Bar chart of the top 15 maize genotypes based on yield, highlighting genotypes that surpassed their parent yield performance

Table 5. Mean squares, coefficients of variation, and heritability estimates for morphophysiological traits without significant variation in maize

Character	Mean square (Control)	Mean square (Genotype)	Mean square (Interaction)	CV (%)	H ² (%)
EH	184.67ns	251.57*	1353.60**	7.57	75.55
NSC	13.71**	1.44ns	26.30**	7.07	49.03
CW	4248.25*	2717.30*	6873.60*	11.91	75.63
SW	3090.92**	1878.00*	6077.75**	12.78	76.14
Y	4.69*	4.42*	21.70**	13.16	75.10
NDVI	0.003ns	0.009*	0.042**	13.61	73.80

Note: Trait abbreviations: EH: Ear Height, NSC: Number of Seed rows per Cob, CW: Cob Weight, SW: Seed Weight, Y: Yield, NDVI: Normalized Difference Vegetation Index. **: Significant at the 1% level ($p < 0.01$), *: Significant at the 5% level, ns: Not significant. CV: Coefficient of Variation, H²: Broad-sense Heritability. Different superscript letters within a row indicate significant differences among treatments at $p < 0.05$ (LSD test). The check mark (✓) indicates characters with statistically significant variation, supported by a low Coefficient of Variation (CV) and high Heritability (H²), indicating strong potential for genetic selection

The evaluation of hybrid performance in this study reinforces the functional significance of these traits. Eight hybrids consistently outperformed both parental lines and controls, with G29, G28, and G39 showing the strongest yield advantages. These results highlight the successful capture of heterosis through peak and test cross designs. At the same time, the identification of T19 as a promising parental line reflects strong combining ability and genetic potential for future use. Comparable outcomes were reported by Bahtiar et al. (2023), where careful parental choice significantly enhanced hybrid performance across multiple environments. More recently, multi-trait index selection under variable moisture regimes also confirmed that hybrids with superior source-sink integration outperform checks under both drought and waterlogging (Singamsetti et al. 2023), echoing the strategy demonstrated here.

The genetic implications of these findings extend to the broader design of breeding pipelines. High heritability traits provide opportunities for effective early-generation selection, minimizing the need for extensive multilocation testing and reducing costs (Singamsetti et al. 2023). Conversely, traits with lower heritability, such as the number of seed rows per cob, are more environmentally influenced and require multilocation trials to accurately capture Genotype $\times$ Environment ($G \times E$) interactions (Wondaferew et al. 2024). Expanding the genetic base by including transgressive segregants, as applied in this study, is particularly important in tropical maize, where reliance on narrow elite pools has limited adaptive capacity (Prasanna et al. 2021). The incorporation of transgressive segregants introduces novel allelic combinations that extend beyond parental variation, thereby reducing genetic vulnerability and providing a deeper genetic foundation for future hybrid development (Mackay et al. 2021). While advanced genomic tools were not applied here, the combination of ANOVA, heritability estimation, correlation, and path analysis represents comprehensive yet operationally feasible analytical framework that strengthens the reliability of trait-based selection in resource-limited breeding programs. This strategy introduces novel allelic combinations, enhances heterosis, and improves resilience against climatic variability (Al Ashkar et al. 2023; Zeinalzadeh-Tabrizi 2023).

Beyond empirical trait analysis, these results resonate with global breeding priorities. The International Maize and Wheat Improvement Center (CIMMYT, Mexico) and the International Rice Research Institute (IRRI, Philippines) are leading international agricultural research centers under the CGIAR consortium. Both institutions have developed global breeding programs focusing on genetic improvement of cereals, particularly emphasizing stress resilience, yield potential, and climate-smart technologies. Programs at CIMMYT and IRRI increasingly emphasize trait-based and genomic-assisted selection, integrating high-throughput phenotyping tools such as canopy spectral indices, chlorophyll meters, and UAV imaging (Crossa et al. 2025). Recent advances include Multispectral Canopy Vegetation Indices (MSCVI), which achieved yield prediction accuracies above 90% under irrigated maize (Guo et al. 2024), and genomic prediction models that improve selection accuracy for yield-related traits at early generations (Wu et al. 2024).

Incorporating such tools with trait-based selection strategies can accelerate breeding cycles while maintaining precision in hybrid identification.

Overall, the integration of morphophysiological evaluation with peak and test crossing provides a robust framework for tropical maize improvement. By strategically combining source traits (plant height, LCI) with sink traits (cob weight, seed number), breeders can optimize both physiological efficiency and genetic gain. The superior performance of hybrids such as G29, G28, and G39 illustrates the successful capture of heterosis and functional trait complementarity. Future work should validate these hybrids across diverse agroecological zones through multilocation trials to ensure yield stability and adaptability. This approach not only accelerates genetic gain but also contributes to climate-resilient maize breeding and supports food security for smallholder farmers in tropical regions.

In conclusion, morphophysiological traits such as cob weight, number of seeds per row, and plant height are reliable indicators of maize yield due to their strong genetic control and significant contribution to source-sink balance. Integrating these traits with physiological efficiency offers a robust approach to developing high-yielding and stress-resilient hybrids for tropical environments. Beyond the experimental context, the findings support breeding programs in prioritizing trait-based selection strategies, in line with global initiatives such as those promoted by CIMMYT and IRRI. We recommend that maize breeding programs adopt a combined approach of phenotypic trait evaluation and genetic diversity enhancement to accelerate genetic gain. At the policy level, investment in multilocation testing, high-throughput phenotyping, and genomic-assisted selection should be strengthened to ensure that improved hybrids are both climate-resilient and suitable for smallholder farmers in tropical regions.

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