Kernel Trait and Genetic Variability Analysis in BC_2F_1 Maize Lines Derived from *Zea nicaraguensis* Crosses

Abstract

Maize (*Zea mays* L.) is an important cereal crop with significant applications in food, feed and industrial applications. This study aimed to increase maize yield and quality by evaluating core traits in introgressed *Zea nicaraguensis* individual's recipients of LM13 and LM14 maize inbred lines. Kernel type, shape, color, dimensions and weight were assessed in 35 BC2F1 plants using standardized descriptor guidelines. Analysis of genotypic and phenotypic coefficients of variation, heritability and genetic progress showed significant genetic diversity and the influence of environmental factors. Significant sources of variability highlighted by principal component analysis were kernel color, endosperm color, and 100-seed weight. High heritability and genetic progress in kernel color and length-to-width ratio indicate great selection potential. Reintroducing the diversity lost in cultivated maize inherent in Zea nicaraguensis has the potential to improve both its agronomic performance and quality. Future research should aim to identify genetic markers and examine the interaction between genetic and environmental influences to optimize maize varieties for specific environments and consumer preferences, ultimately contributing to global food security and agricultural sustainability.

Keywords: Maize, *Zea nicaraguensis* , Introgression breeding , Kernel traits and Genetic Diversity

Introduction

Maize (*Zea mays* L.) is the most important cereal crop in the world, holding wide applications in food, feed, and industrial uses **(Murdia *et al.*, 2016).** As a staple food for millions of people, especially in developing countries, maize plays a critical role in food security and nutrition. It is also an important component of animal feed and serves as a raw material for various industrial products, including biofuels, starches and sweeteners **(Klopfenstein *et al.*, 2013).** It provides economic and nutritional importance, continuous improvement of maize yield and quality traits is imperative to meet the needs of a growing global population **(Shiferaw *et al.*, 2011).** Kernel characteristics such as type, shape, colour, dimension, and weight are the main determinants of maize commercial value [25-27]. These are important not only for the appearance and marketability of maize but relate to its nutritional quality and hence suitability for different uses. **(Xu *et al.*, 2009).** For instance, kernel size and weight are directly linked to grain yield, while kernel colour can indicate the existence of specific nutrients or anti-nutritional factors **(Hassan *et al.*, 2021).**

The genetic basis of kernel traits is complex, involving multiple genes and environmental interactions. Therefore, understanding the genetic variability underlying these traits is critical to developing effective breeding programs **(Liu *et al.*, 2016)**. Introducing genetic diversity into breeding populations is a cornerstone of crop improvement. In maize, the use of wild relatives has proven to be an effective approach to broadening the genetic base and introducing new traits not found in cultivated varieties **(Nawaz *et al.*, 2023)**. Wild relatives of maize, such as *Zea nicaraguensis,* possess unique traits that can enhance water-logging tolerance and nutritional quality **(Mano *et al.*,2013 and Varalakshmi *et al.*, 2022) .** Incorporating these traits into cultivated maize can lead to the development of superior genotypes with improved agronomic performance **(Sahoo *et al.*, 2021)**.

This investigation focuses on the BC$_2$F$_1$ population derived from crosses between LM13 and LM14 inbred maize lines and *Zea nicaraguensis*. To analyze the diversity of kernel traits in the descriptive analysis, genetic variability parameter analysis and principal component analysis were used **(Okporie, 2008 and Jilo *et al.*, 2018**). In this population, we found a significant level of kernel variability, which included characteristics such as kernel type, shape, color, dimensions, and weight. These characteristics were selected because of their importance in determining yield, quality, and marketability of maize **(Jaradat *et al.*, 2013).** This analysis aids in understanding trait relationships and identifying primary factors for selection of kernel traits **(Prasanna, 2012).** These study reports advance knowledge of maize genetic variability and provide a foundation for wild introgression breeding programs to improve kernel traits, agronomic performance, nutritional quality, and commercial value **(Araus *et al.*, 2008).**

Materials and Methods

Experimental Design and Plant Material

This experiment was conducted at the N.E.B. Crop Research Centre, GBPUA&T Pantnagar (Coordinates: 28.97°N, 79.41°E, Average Rainfall: 1443.9 mm). LM13 and LM14 maize inbred lines were crossed with the wild relative *Zea nicaraguensis* to develop F$_1$ hybrids. These were then backcrossed with the recurrent parents to produce BC$_1$F$_1$ and subsequently BC$_2$F$_1$ generation.

Table 1. Kernel Characteristics of Parent Materials: LM13, LM14, and *Zea nicaraguensis*

Parent Materials	**LM13**	**LM14**	***Zea nicaraguensis***
Kernel Type	Flint	Flint	Wild
Shape of Upper Surface of Kernel	Round	Round	Level
Kernel Colour(Top of grain)	Yellow	Yellow	Brown
Endosperm Colour	Pale Yellow	Pale Yellow	White
Kernel Length (mm)	6.00	6.00	6.00
Kernel Breadth(mm)	4.00	5.00	3.00
K L/B ratio	1.50	1.20	2.00
100 Seed Weight (g)	22.00	20.00	8.25

A total of 35 BC_2F_1 plants from each biparental cross combination were analysed for kernel traits including kernel type, shape of upper surface of kernel, kernel colour, endosperm colour, kernel width, kernel length, K L/B ratio, and 100 seed weight. All traits of data collection were observed based on CIMMYT Maize descriptor guidelines . This is schematically breeding process shown in Figure. 1.

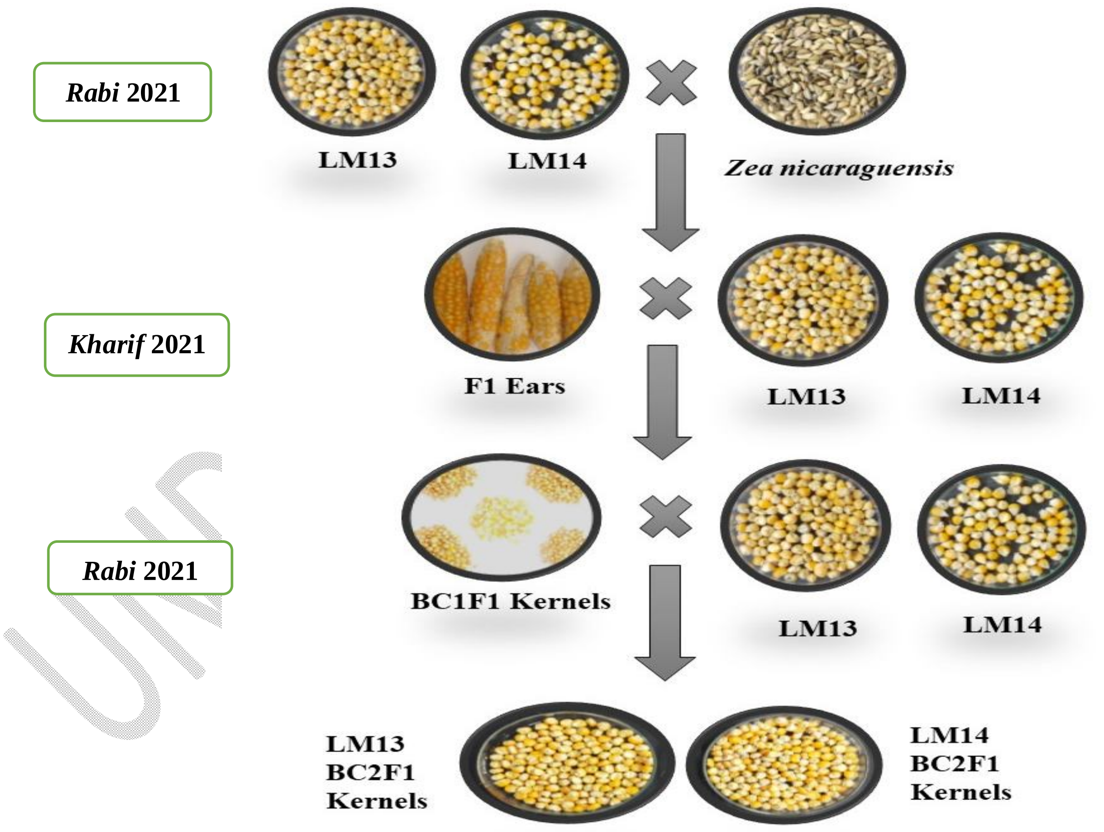


Figure 1. Breeding Scheme for BC_2F_1 Generation from Crosses of LM13 and LM14 with *Zea nicaraguensis*

Statistical Analysis

To analysed the genetic and phenotypic variability of kernel traits, various statistical methods were employed. Descriptive statistics, including the computation of the range of kernel traits, offered a basic understanding of the degree of variation found in the BC_2F_1 population. Variability analysis, including the Genotypic Coefficient of Variation (GCV), Phenotypic Coefficient of Variation (PCV), Heritability (H^2), and Genetic Advance (GA), was conducted using the ***'Agricolae'*** package in R software version 4.1.2. PCA was performed to identify key traits contributing to variability. This involved data standardization, extraction of eigenvalues and percentage of variance, and biplot visualization with the **'*Factoextra*'** package. These combined approaches aimed to clarify the genetic basis of kernel traits and identify promising genotypes for breeding programs, leveraging the genetic diversity introduced by *Zea nicaraguensis* **(Okporie, 2008 and Jilo *et al.*, 2018).**

Result and Discussion

1. Descriptive Statistics Analysis

Qualitative Trait Analysis

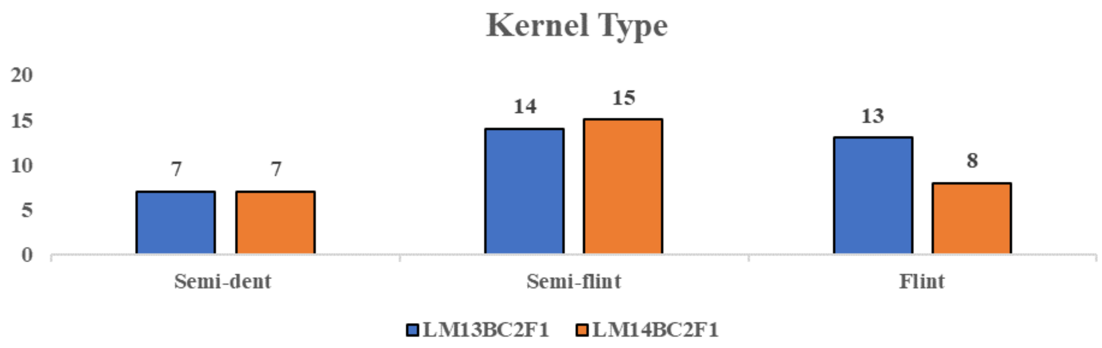
The data collected were differentiated between two populations: LM13 BC_2F_1 and LM14 BC_2F_1. The results revealed notable variations in kernel types, with LM13BC_2F_1 exhibiting a balanced distribution of semi-dent (7), semi-flint (13), and flint kernels (15), while LM14BC_2F_1 showed a significant preference for semi-dent kernels (14), with fewer semi-flint (7) and flint kernels (8). This divergence suggested genetic or environmental factors influencing the dominance of certain kernel types **(Prasanna, 2012).**

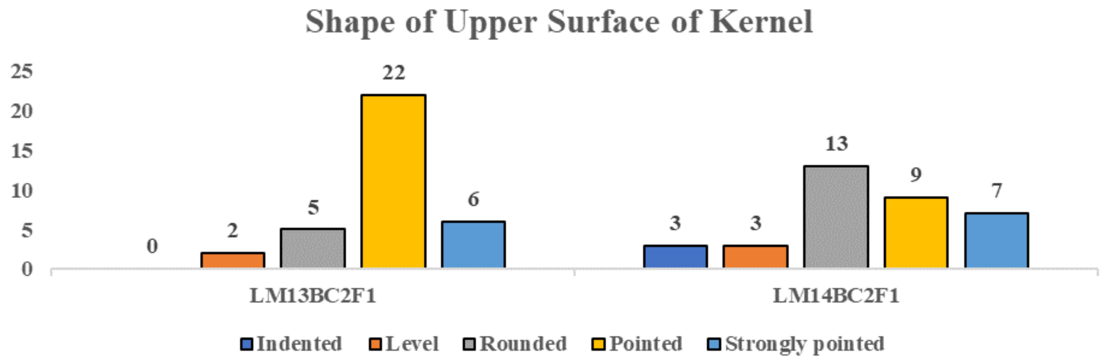
The shape of the kernel surface varied significantly between populations. LM14BC_2F_1 was dominated by indented kernels (3), whereas LM13BC_2F_1 had none, highlighting the impact of specific genetic or environmental influences. Both populations predominantly had rounded kernels, with LM13BC_2F_1 having 13 and LM14BC_2F_1 having 22, indicating a common genetic basis .The absence of strongly pointed kernels in LM14BC_2F_1, compared to their presence in LM13BC_2F_1 having 7 , suggests possible genetic divergence outcomes favouring different kernel shapes in these populations **(Prasanna, 2012).**

Endosperm colour analysis indicated a strong preference for yellow in both populations, with LM13BC_2F_1 having 15 yellow kernels and LM14BC_2F_1 having 16 yellow kernels. This suggested that yellow endosperm may have been selected for its nutritional and aesthetic benefits **(Kajla *et al.*, 2023).** In contrast, LM14 BC_2F_1 showed a higher frequency of orange endosperm (5 kernels) compared to LM13 BC_2F_1 (1 kernel), indicating genetic diversity .The lower occurrence of white endosperm (4 in LM13 BC_2F_1 and 2 in LM14 BC_2F_1) and cream endosperm (5 in LM13 BC_2F_1 and 1 in LM14 BC_2F_1) across both populations suggested that these traits were less desirable.

Kernel colour (Top of grain) data reinforced these findings, with both populations predominantly showing yellow kernels (23 in LM13 BC_2F_1, 21 in LM14 BC_2F_1). The slight variation in white kernel frequencies between the populations (5 in LM13 BC_2F_1, 7 in LM14 BC_2F_1) suggested residual genetic variation . **(Jaradat *et al.*, 2013).**

These findings emphasized the importance of understanding the genetic basis and environmental interactions influencing these traits, which could guide future breeding and selection strategies for improved *Zea nicaraguensis* derived varieties **(Lorant, 2018).** Further research was needed to analyze the genetic mechanisms and environmental factors that contribute to trait variation and provide valuable insights for optimizing breeding programs to develop varieties tailored to specific environments and consumer preferences **(Smale *et al.*, 2001).** All of these results are shown schematically in Fig. 2





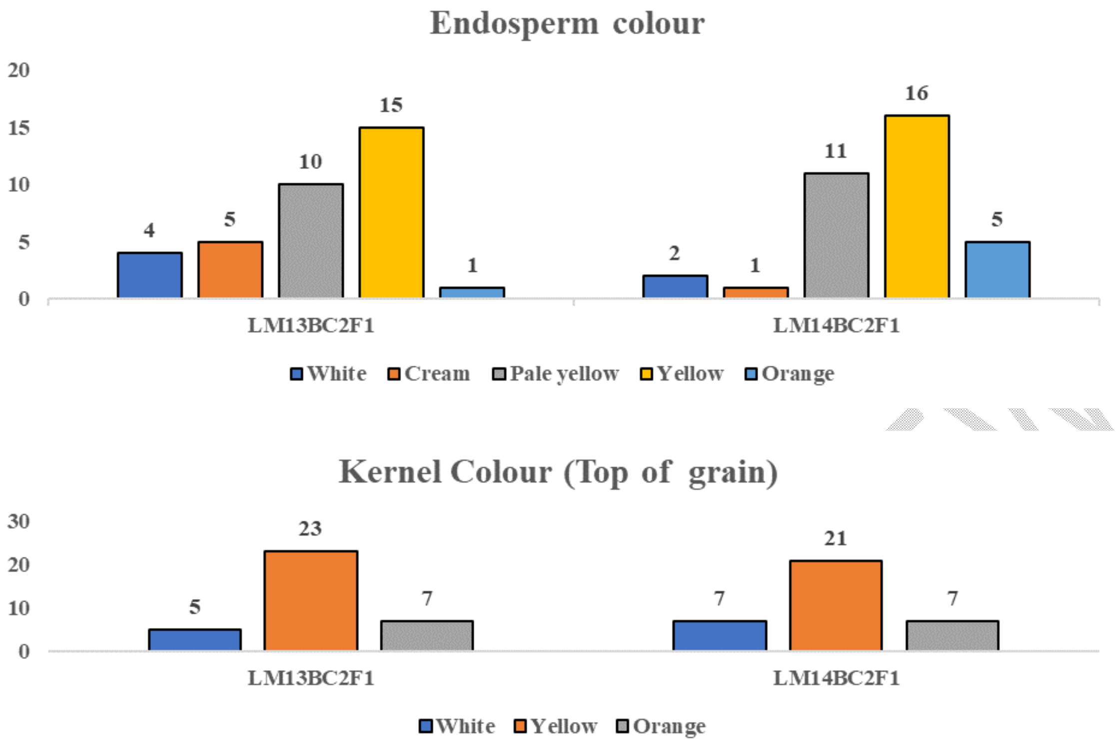
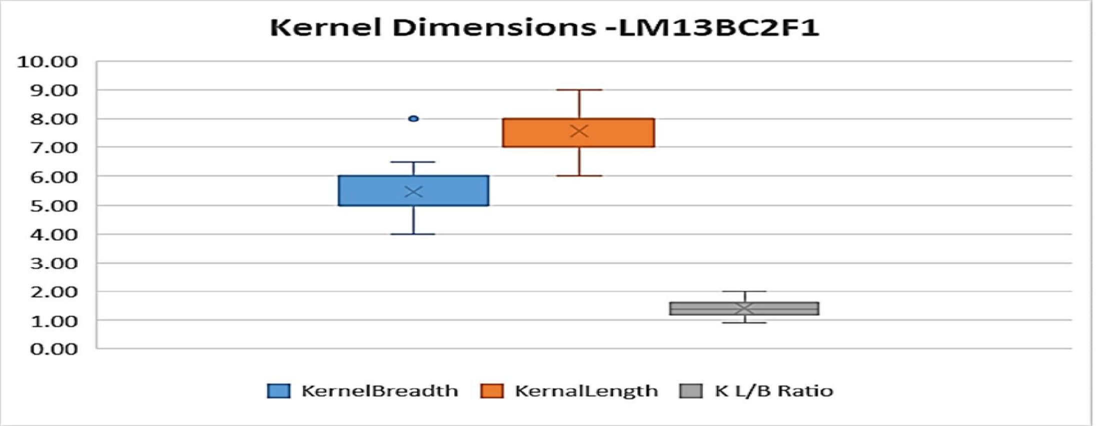


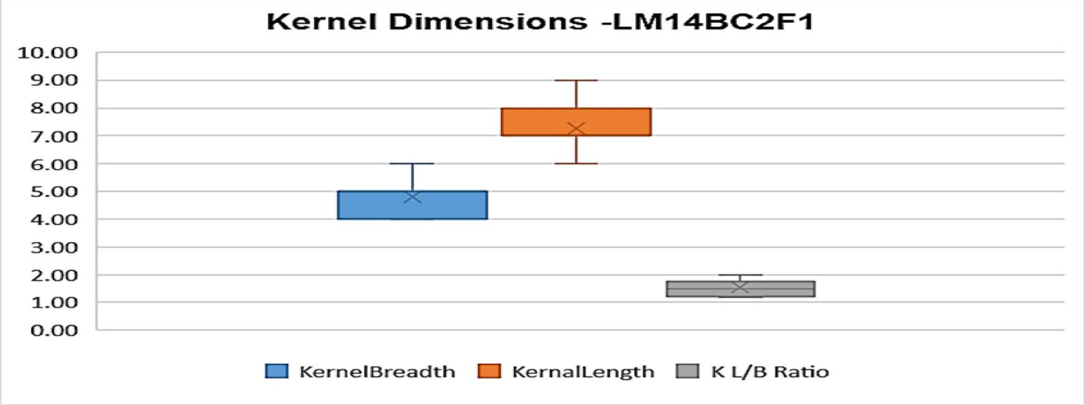
Figure 2. Kernel Trait Distribution in LM13BC$_2$F$_1$ and LM14BC$_2$F$_1$

Quantitative Trait Analysis

The quantitative analysis of kernel dimensions in *Zea nicaraguensis* introgression individuals elucidated key differences and similarities between two populations, LM13BC$_2$F$_1$ and LM14BC$_2$F$_1$. The LM13BC$_2$F$_1$ population exhibited an average kernel breadth of around 5.0 units, slightly higher than LM14BC$_2$F$_1$ having 4.5 units. Both populations have similar kernel lengths, averaging 7.5 mm units, suggesting stable genetic factors for this trait. The kernel length to breadth (K L/B) ratio varies from 1.0 to 1.8 in LM13BC$_2$F$_1$ and from 1.0 to 2.0 in LM14BC$_2$F$_1$, indicating that kernels in LM14BC$_2$F$_1$ are slightly more elongated. These findings highlighted significant genetic diversity and suggest that both genetic and environmental factors influence kernel dimensions **(Prasanna, 2012)**. Understanding these traits can inform breeding programs aimed at optimizing kernel size and shape for improved yield and adaptability in *Zea nicaraguensis* varieties **(Jaradat *et al.*, 2013).**

Focusing on the 100 seed weight of two populations: LM13BC$_2$F$_1$ and LM14BC$_2$F$_1$. The data is presented in Figure 3, showing the variation in 100 seed weight across 35 samples for each population. The 100 seed weight for LM13BC$_2$F$_1$ and LM14BC$_2$F$_1$ populations shows significant variability. The weight ranges from approximately 13.00 grams to nearly 30.00 grams across the samples. The variation in 100 seed weight between the two populations suggests a considerable genetic diversity introduced through the introgression process. The broad range of seed weights indicates that both populations possess a wide array of genetic backgrounds, which is beneficial for breeding programs aiming to enhance specific traits. In both populations, there are instances of higher and lower seed weights, reflecting the influence of both genetic and environmental factors. The presence of heavier seeds in both populations can be advantageous for traits associated with yield and vigour, whereas the lighter seeds might indicate less favourable conditions or genetic combinations that need to be considered in breeding strategies **(Hossain *et al.*, 2022)**. The quantitative analysis of the 100 seed weight in *Zea nicaraguensis* introgression individuals reveals significant genetic diversity and variability within the LM13BC$_2$F$_1$ and LM14BC$_2$F$_1$ populations. This diversity is crucial for breeding programs as it provides a broad genetic base to select for desirable traits such as seed weight, which is directly related to crop yield and quality **(Anjorin *et al.*, 2014).** The results are shown graphically in Figure 3.





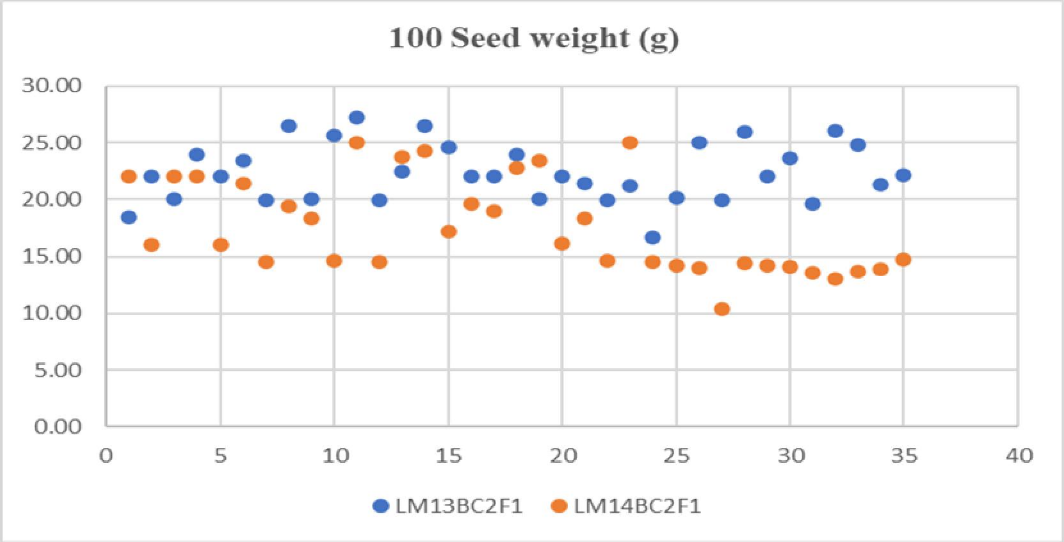


Figure 3. Kernel dimensions and 100 Seed Weight (g) Distribution in LM13BC$_2$F$_1$ and LM14BC$_2$F$_1$

2.Genetic Variability Analysis

The analysis of genetic variability parameters for kernel traits in *Zea nicaraguensis* introgression individuals revealed significant genetic variability between the LM13BC$_2$F$_1$ and LM14 BC$_2$F$_1$ populations. For 100 seed weight, LM13BC$_2$F$_1$ shows a remarkably high genotypic coefficient of variation (GCV) of 37.80, indicating substantial genetic influence, whereas LM14BC$_2$F$_1$ has a GCV of 29.69. The phenotypic coefficient of variation (PCV) is higher in LM13BC$_2$F$_1$ (24.82) than in LM14 BC$_2$F$_1$ (12.23), suggesting greater environmental impact in the former. Kernel length exhibits notable GCV values of 12.80 (LM13 BC$_2$F$_1$) and 12.27 (LM14 BC$_2$F$_1$), with corresponding PCV values of 22.10 and 23.40, highlighting considerable genetic diversity. Additionally, kernel breadth shows high PCV values in both populations, emphasizing environmental influence. These findings indicate a broad genetic base and significant environmental interaction, essential for breeding programs targeting enhanced yield and adaptability **(Yadav *et al.*, 2015)**. The considerable genetic variability, particularly in 100 seed weight and kernel dimensions, underscores the potential for selecting superior genotypes, thereby aiding in the development of improved *Zea nicaraguensis* varieties.

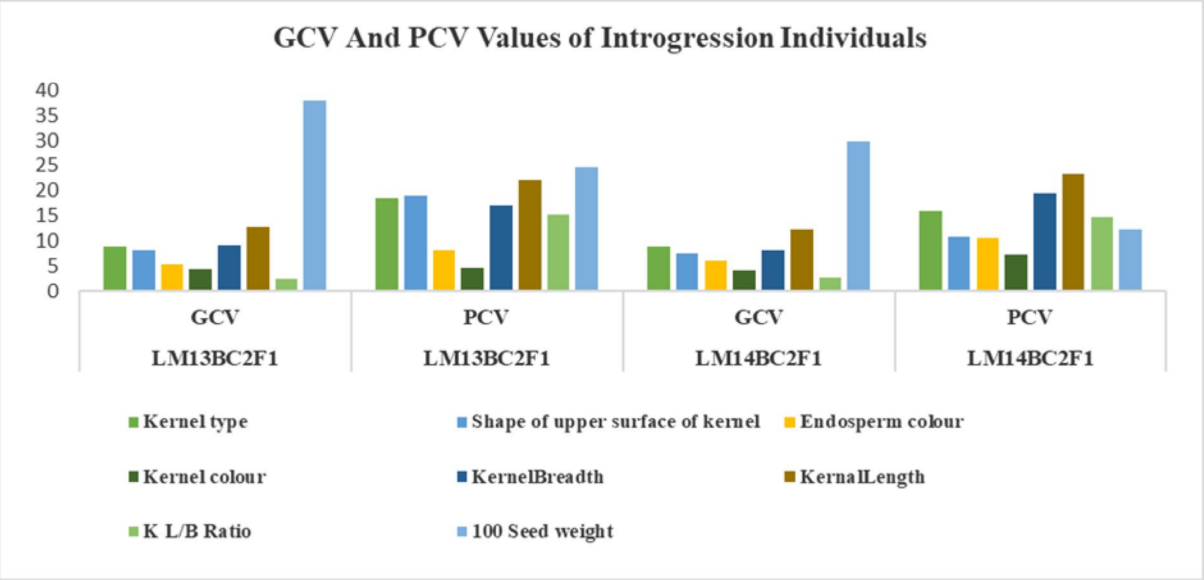


Figure 4. GCV and PCV Values of Introgression Individuals

Heritability and Genetic Advance

The analysis of broad-sense heritability (H^2) and genetic advance (GA) for kernel traits in *Zea nicaraguensis* introgression individuals revealed notable findings between the LM13BC_2F_1 and LM14BC_2F_1 populations, results are depicted in the Table 2. Kernel type exhibited low heritability (0.023 for LM13 BC_2F_1 and 0.032 for LM14 BC_2F_1) with moderate GA, indicating limited genetic control. The shape of the upper surface of the kernel showed higher heritability in LM14 BC_2F_1 (0.067) compared to LM13 BC_2F_1 (0.023), suggesting better genetic predictability in LM14 $BC_2F_1$1. Endosperm colour had moderate heritability (0.115 for LM13 BC_2F_1 and 0.072 for LM14 BC_2F_1) with substantial GA, indicating moderate genetic influence and significant improvement potential through selection. Kernel colour displayed the highest heritability (0.314 for LM13BC_2F_1 and 0.151 for LM14BC_2F_1) with considerable GA, suggesting strong genetic control and substantial selection potential. Kernel breadth and length showed low heritability and moderate GA, highlighting significant environmental influence. The K L/B ratio had moderate heritability (0.064 for LM13BC_2F_1 and 0.060 for LM14BC_2F_1) with high GA, indicating considerable genetic variability and improvement potential. The 100 seed weight exhibited low heritability (0.013 for LM13BC_2F_1 and 0.051 for LM14BC_2F_1) and low GA, suggesting limited genetic control and higher environmental impact. Overall, kernel colour and K L/B ratio emerged as significant traits with strong genetic control and high improvement potential. These findings emphasized the importance of both genetic and environmental factors in trait expression, guiding breeding programs to focus on traits with high heritability and genetic advance for developing superior *Zea nicaraguensis* varieties **(Prasanna, 2012)**.

Table 2. Heritability and Genetic Advance Values of Introgression Individuals

LM13BC$_2$F$_1$

Traits	Broad Sense Heritability (H^2)	Genetic Advance (GA)
Kernel type	0.023	3.464
Shape of upper surface of kernel	0.023	10.387
Endosperm colour	0.115	6.270
Kernel colour	0.314	6.270
KernelBreadth	0.027	5.797
KernalLength	0.016	10.909
K L/B Ratio	0.064	15.134
100 Seed weight	0.013	2.068

LM14BC$_2$F$_1$

Traits	Broad Sense Heritability (H^2)	Genetic Advance (GA)
Kernel type	0.032	3.464
Shape of upper surface of kernel	0.067	10.610
Endosperm colour	0.072	7.179
Kernel colour	0.151	7.179
KernelBreadth	0.021	4.805
KernalLength	0.015	9.461
K L/B Ratio	0.060	14.482
100 Seed weight	0.051	2.440

3.Principal Component Analysis Results

PCA of LM13BC$_1$F$_2$ Population

The PCA biplot represents the relationships between various maize kernel traits and the individual samples. Dim1 explains 28.9% of the variance and Dim2 explains 18.1%, together capturing 47% of the total variance.

Table 3: Principal Component Analysis Results of LM13 BC$_2$F$_1$

Component	Eigenvalue	Percentage of Variance
Comp 1	2.308	28.854
Comp 2	1.447	18.086
Comp 3	1.338	16.719
Comp 4	1.085	13.561
Comp 5	0.658	8.231
Comp 6	0.630	7.878
Comp 7	0.515	6.440
Comp 8	0.018	0.230

Traits such as Kernel Length, Kernel Type, HSW (Hundred Seed Weight), and K L/B ratio are positively correlated with Dim1, while Kernel Breadth shows a negative correlation. Endosperm colour is negatively correlated with Dim2, whereas the Shape of upper surface of kernel is positively correlated. Samples on the right side of the biplot (e.g., samples 8, 6, 10, 21, 23, 30) have higher values for traits like Kernel Length, Kernel Type, HSW, and K L/B ratio. Samples on the left (e.g., samples 22, 27) have higher Kernel Breadth, and those at the bottom (e.g., samples 25, 28, 26) show intense Endosperm colour. Samples near the center exhibit average trait values. This analysis visualizes the trait distribution and patterns among the maize samples, aiding in the identification of trait relationships and sample groupings.

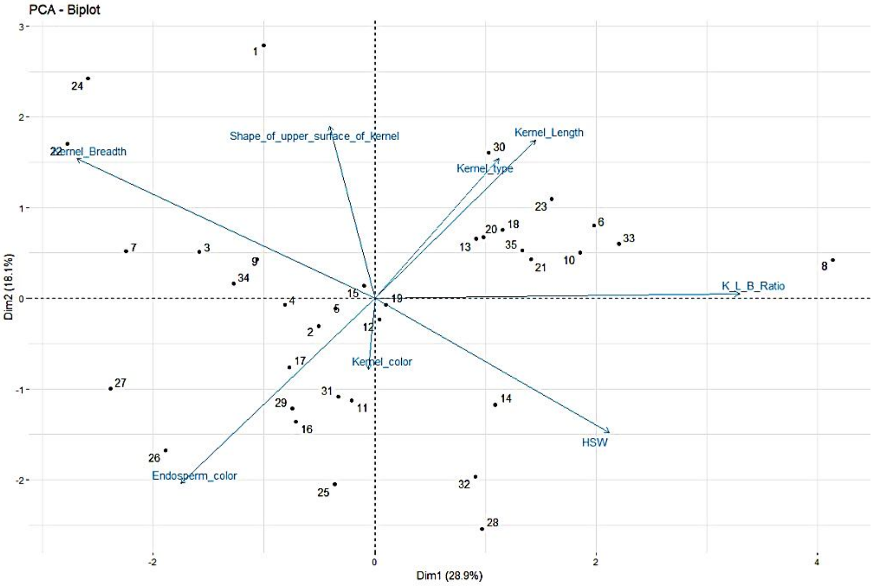


Figure 5. PCA Biplot for LM13BC$_1$F$_2$ Individuals

PCA of LM14 BC$_1$F$_2$ Population

The PCA biplot you've provided illustrates the relationships between various maize kernel traits and individual samples, with Dim1 explaining 31.5% of the variance and Dim2 explaining 20.3%, together accounting for 51.8% of the total variance.

Table 4: Principal Component Analysis Results of LM13 BC$_2$F$_1$

Component	Eigenvalue	Percentage of Variance
Comp 1	2.516	31.452
Comp 2	1.621	20.268
Comp 3	1.225	15.317
Comp 4	0.951	11.89
Comp 5	0.627	7.843
Comp 6	0.548	6.855
Comp 7	0.506	6.325
Comp 8	0.003	0.045

Traits of kernel length, kernel type, HSW (hundred seed weight), and K L/B ratio have positive loadings on Dim1, indicating that samples positioned to the right of the biplot (e.g., samples 1, 15, 19, 21, 31) are associated with higher values for these traits. kernel breadth is negatively correlated with Dim1, suggesting that samples (e.g., samples 3, 4, 11, 13) exhibited higher kernel breadth. Dim2 is positively correlated with traits like kernel length and K L/B ratio, while endosperm colour had been negative loading, indicating that samples located at the bottom (e.g., samples 5, 18, 25, 27, 30) tend to have intense endosperm colour and specific kernel colour characteristics. the central grouping of samples suggested average values for most traits. this biplot effectively visualizes the distribution and patterns of traits among the maize samples, aiding in understanding their relationships and identifying trait groupings.**(Okporie,2008)**.

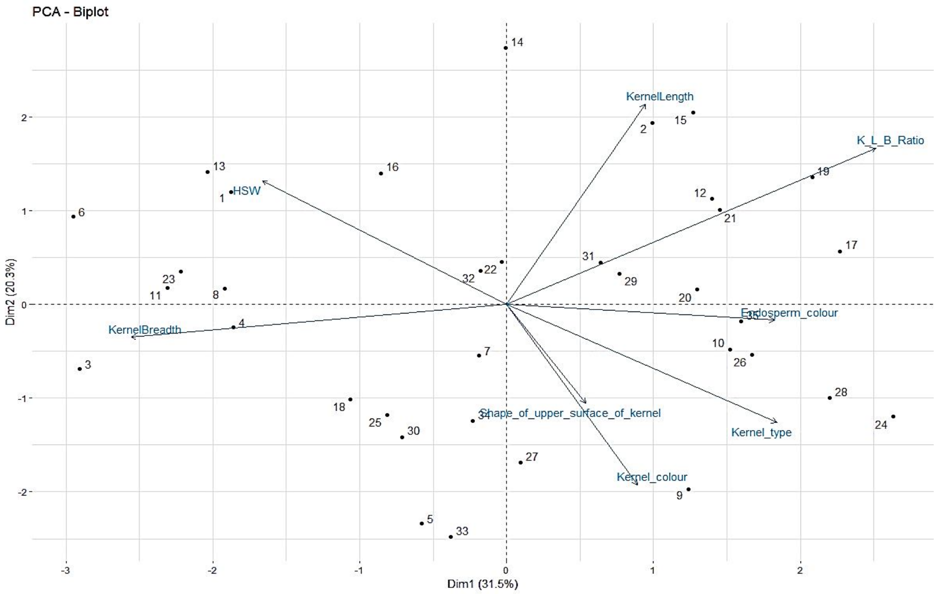


Figure 6. PCA Biplot for LM14BC_1F_2 Individuals

Conclusion and Future Prospects

This study comprehensively analysed kernel traits of BC_2F_1 maize lines obtained from crosses between the inbred lines LM13 and LM14 with *Zea nicaraguensis*. It is an inference that genetic variability is relatively high within the traits concerning the type, shape, colour, dimensions, and weight of kernels. Genetic diversity, the main effect, and interaction of environmental factors were found to be responsible for shaping all of these traits, as described both descriptively and statistically. The traits concerned with kernel colour and kernel length-to-breadth ratio showed high heritability, along with high genetic advance in most cases, indicating high potential under effective selection programs.

These critical characteristics causing the most variability within the BC_2F_1 populations were further emphasized by the results from the PCA. With this knowledge of critical traits, selection efforts can be put forth by breeders with the most influence on yield, quality, and marketability. The estimated heritability and genetic variability values obtained in the present study indicate much potential for improvement in maize with well-informed breeding strategies. Indeed, introgressions of gene material related to wild species, such as *Zea nicaraguensis*, proved beneficial to maize by contributing novel traits that could positively influence improvement for new agronomic performance and adaptability **(Prasanna, 2012)**.

Future work should focus on revealing the genetic mechanisms underlying these kernel characteristics to facilitate the estimation of gene action. Investigations on the environmental factors responsible for the expression of these traits will further guide the breeding programs in such a way that suitable genetic materials are developed for various agro-ecological zones. This can be aided with the molecular methods like MAS and genomic selection. The genes underlying the expression of traits can be effectively mapped using QTL analysis. Also, broadening the study to incorporate more advanced backcross generations and multi-location trials helps in affirming stability and adaptability in the chosen traits. This is ultimate research that would pave the way for developing superior maize varieties to meet the demands in achieving food security, nutrition, and industrial applications to ensure sustainable maize production.

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