Unveiling Genetic Potential: Phenotypic and Genetic Analysis of BC_2F_1 Maize Lines from *Zea nicaraguensis* Crosses

Abstract

Maize (*Zea mays* L.) is a crucial cereal crop with significant applications in food, feed, and industry. This investigation aimed to enhance maize yield and quality by evaluating kernel traits in *Zea nicaraguensis* introgressed individuals having LM13 and LM14 maize inbred lines as recipient. Kernel type, shape, colour, dimensions, and weight were evaluated in 35 BC_2F_1 plants using standardized visual methods. Analysis of Genotypic, Phenotypic Coefficients of Variation, heritability, and genetic advance showed significant genetic diversity and influence of environmental factors. Essential sources of variability highlighted through Principal Component Analysis were kernel colour, endosperm colour, and 100 seed weight. High heritability and genetic advance for kernel colour and length-to-breadth ratio show strong potential for selection. The re-introduction of the diversity that is inherent in *Zea nicaraguensis* improved both agronomic performance and quality. Future research should aim to identify genetic markers and explore 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 and Kernel traits

Comment [EC1]: Include another keyword

Introduction

Maize (*Zea mays* L.) is one of the most vital cereal crops globally, with extensive applications in food, feed, and industry purposes **(Murdia *et al.*, 2016).** As a staple food for millions of people, particularly in developing countries, maize plays a crucial role in food security and nutrition. It is also a significant component of animal feed and serves as a raw material for various industrial products, including biofuels, starch, and sweeteners **(Klopfenstein *et al.*, 2013).** Provided its economic and nutritional importance, continuous improvement of maize yield and quality traits is imperative to meet the demands of a growing global population **(Shiferaw *et al.*, 2011).**

Kernel traits, such as kernel type, shape, colour, dimension, and weight, are critical determinants of maize's commercial value. These traits influence not only the appearance and marketability of maize but also its nutritional quality and suitability for various end uses **(Xu *et***

***al.*, 2009).** For instance, kernel size and weight are directly linked to grain yield, while kernel colour can indicate the presence of specific nutrients or anti-nutritional factors **(Hassan *et al.*, 2021).** The genetic basis of these traits is complex, involving multiple genes and environmental interactions. Therefore, understanding the genetic variability underlying these traits is essential for developing effective breeding programs **(Liu *et al.*, 2016)**.

The introduction of genetic diversity into breeding populations is a cornerstone of crop improvement. In maize, the use of wild relatives has proven to be an effective strategy for broadening the genetic base and introducing novel traits that are not present 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 study focuses on the BC$_2$F$_1$ population derived from crosses between LM13 and LM14 inbred maize lines and *Zea nicaraguensis.* In this population, we observed a substantial amount of kernel variability, encompassing traits such as kernel type, shape of the upper surface of the kernel, kernel colour, endosperm colour, kernel width, kernel length, kernel length to breadth (kl/b) ratio, and 100 seed weight. These traits were chosen for their importance in determining the yield, quality, and marketability of maize **(Jaradat *et al.*, 2013).**

Comment [EC2]: Delete space here

To analyse the genetic variability of kernel traits, we used various genetic and statistical methods. Descriptive analysis offered an overview of observed traits, while variability analysis, including Genotypic Coefficient of Variation (GCV), Phenotypic Coefficient of Variation (PCV), Heritability (H^2), and Genetic Advance (GA), provided insights into genetic improvement potential. High heritability and genetic advance suggest effective selection for these traits **(Jilo *et al.*, 2018).** Principal component analysis (PCA) identified key traits contributing to overall variability, reducing data dimensionality and highlighting significant traits **(Okporie, 2008).** This analysis aids in understanding trait relationships and identifying primary factors for selection, facilitating the identification of superior genotypes **(Prasanna, 2012).** The study's results enhance knowledge on maize genetic variability, offering a foundation for breeding programs to improve kernel traits, agronomic performance, nutritional quality, and commercial value **(Araus *et al.*, 2008).**

Comment [EC3]: Take this paragraph to material and methods specifically under analysis subsection

Comment [EC4]: Take this sentence to recommendation

Materials and Methods

Experimental Design and Plant Material

This experiment was conducted at the N.E.B. Crop Research Centre, GBPUA&T Pantnagar. 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_1F_1 and subsequently BC_2F_1 generation. A total of 35 BC_2F_1 plants from each biparental cross combination (LM13 x *Z. nicaraguensis* and LM14 x *Z.nicaraguensis*) were analysed for kernel traits including kernel type, shape of the upper surface, kernel colour, endosperm colour, kernel width, kernel length, K L/B ratio, and 100 seed weight. These traits were measured using standardized visual assessments.

Comment [EC5]: Provide coordinates for the study area

Comment [EC6]: Do you mean standardised visual assessment tools????

Statistical Analysis

Genetic variability was evaluated through genotypic and phenotypic coefficients of variation (GCV and PCV), broad-sense heritability (H^2), and genetic advance (GA) using R software version 4.1.2 and the *'Agricolae'* package. Principal Component Analysis (PCA) was conducted to identify key traits contributing to variability, involving data standardization, covariance matrix calculation, extraction of eigenvalues and eigenvectors, and biplot visualization with the *'Factoextra'* package. Descriptive statistics of range were computed to provide an overview of variability within the BC_2F_1 population. This approach aimed to clarified the genetic basis of kernel traits and identify promising candidates for breeding programs, leveraging the genetic diversity introduced by *Zea nicaraguensis*.

Result and Discussion

Comment [EC7]: Separate Results 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

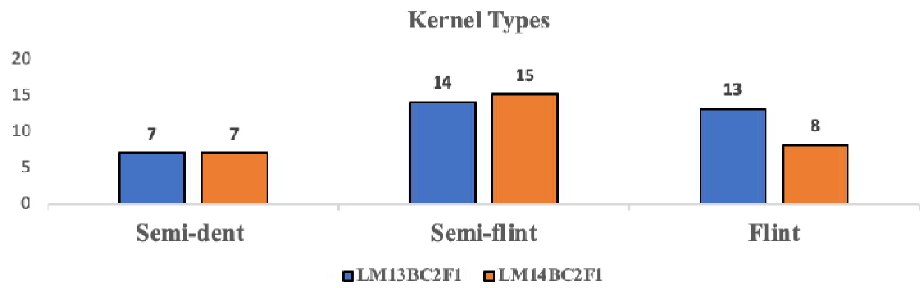
LM13BC$_2$F$_1$ having 7 , suggests possible genetic divergence outcomes favouring different kernel shapes in these populations **(Prasanna, 2012).**

Endosperm colour analysis indicated a strong preference for yellow in both populations, with LM13BC$_2$F$_1$ having 15 yellow kernels and LM14BC$_2$F$_1$ 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$_2$F$_1$ showed a higher frequency of orange endosperm (5 kernels) compared to LM13 BC$_2$F$_1$ (1 kernel), indicating genetic diversity .The lower occurrence of white endosperm (4 in LM13 BC$_2$F$_1$ and 2 in LM14 BC$_2$F$_1$) and cream endosperm (5 in LM13 BC$_2$F$_1$ and 1 in LM14 BC$_2$F$_1$) across both populations suggested that these traits were less desirable.

Kernel colour (Colour of top of the grain) data reinforced these findings, with both populations predominantly showing yellow kernels (23 in LM13 BC$_2$F$_1$, 21 in LM14 BC$_2$F$_1$). The slight variation in white kernel frequencies between the populations (5 in LM13 BC$_2$F$_1$, 7 in LM14 BC$_2$F$_1$) suggested residual genetic variation . **(Jaradat *et al.*, 2013).**

Comment [EC8]: on not of

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 dissect the genetic mechanisms and environmental factors contributing to trait variation, providing 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. 1



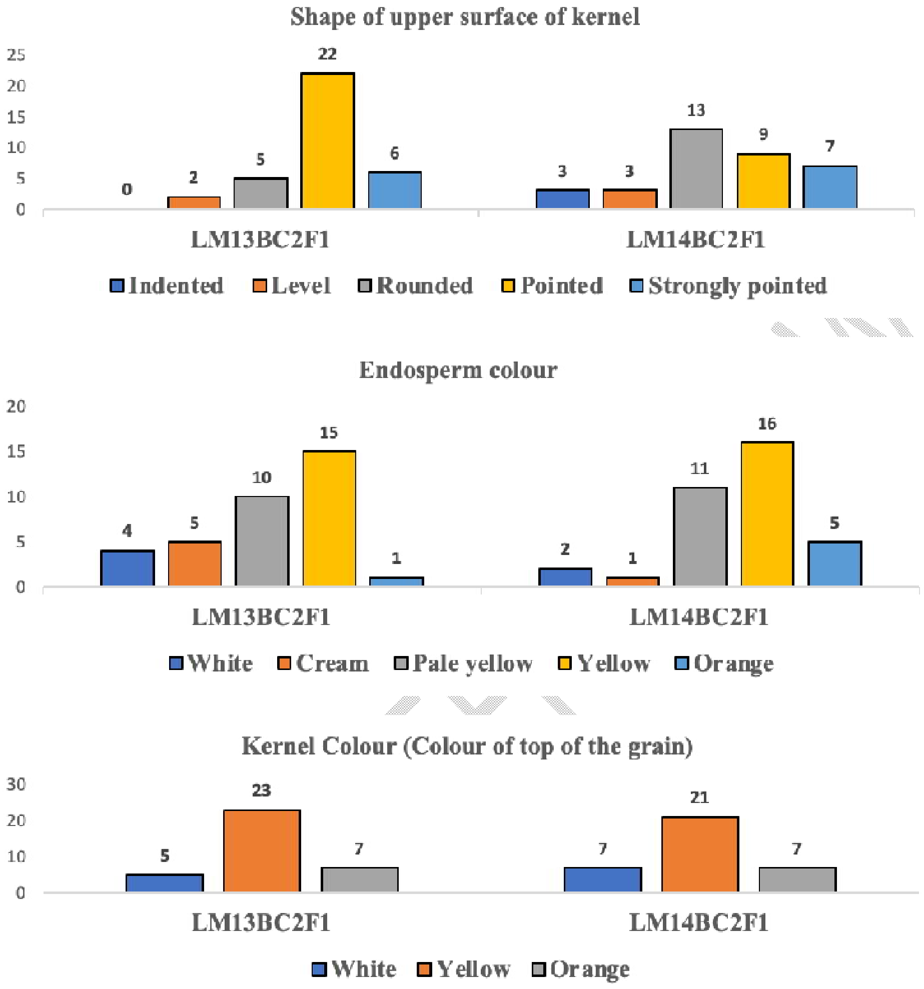
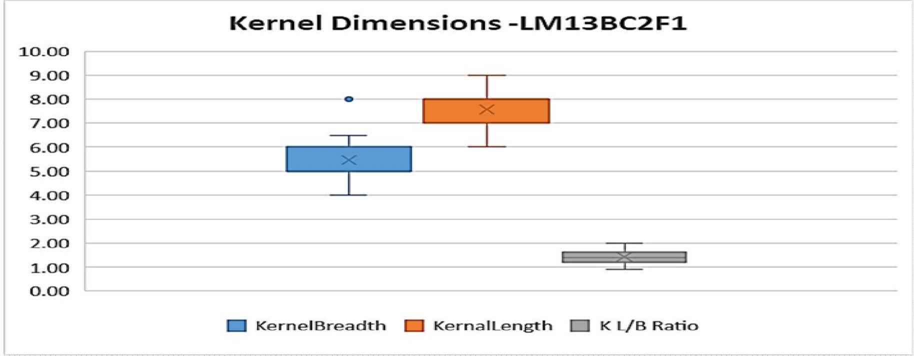


Figure 1. Kernel Trait Distribution in LM13BC$_2$F$_1$ and LM14BC$_2$F$_1$

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 highlight 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 4, 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 2.



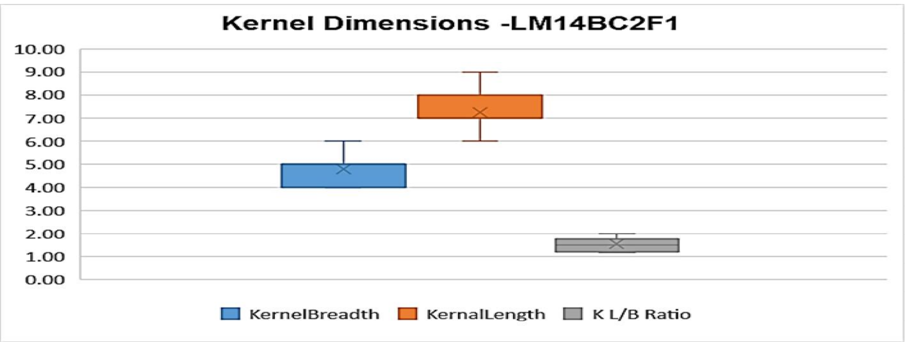


Figure 2. Kernel dimensions and 100 Seed Weight (g) Distribution in LM13BC$_2$F$_1$ and LM14BC$_2$F$_1$

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.

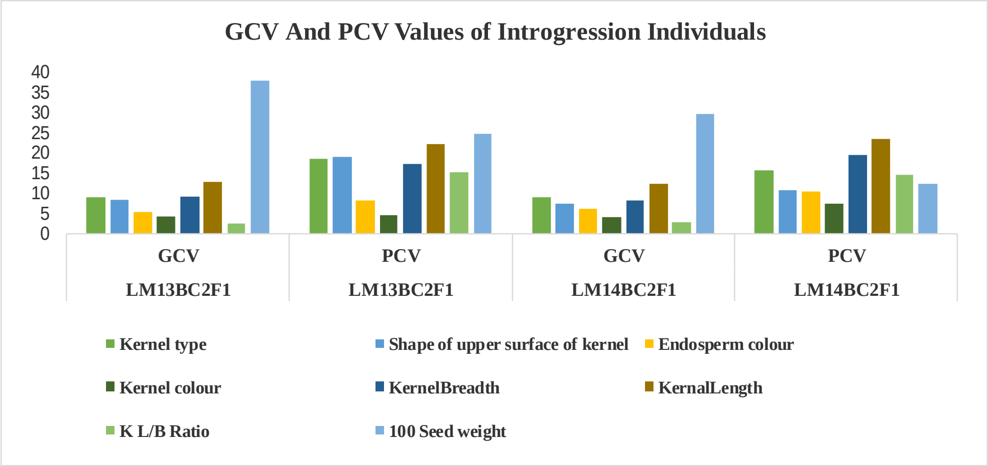


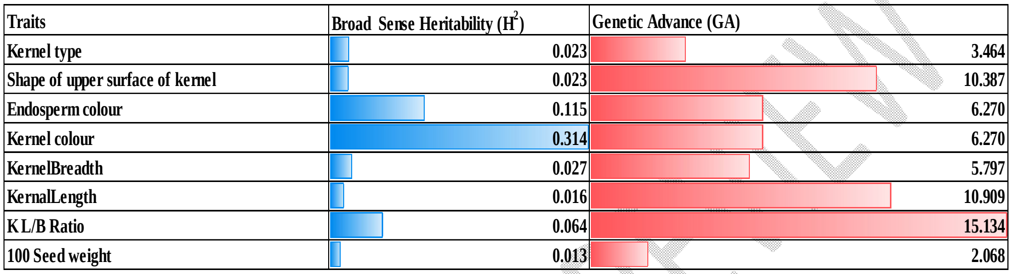
Figure 3. GCV and PCV Values of Introgression Individuals

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 1. 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)**.

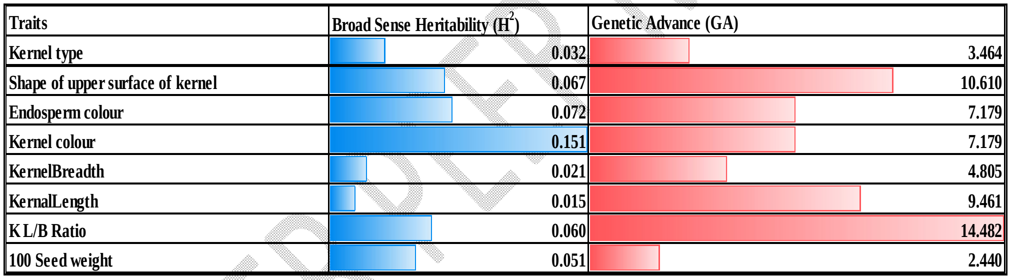
Table 1. Heritability and Genetic Advance Values of Introgression Individuals

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 for LM13BC$_1$F$_2$ individuals provided a comprehensive view of the variation in kernel traits, with the first two principal components explaining a combined 47.1% of the total variance (28.9% for PC1 and 18.2% for PC2). Key traits such as kernel colour, endosperm colour, and 100 seed weight (HSW) had significant contributions to PC1, indicating that these traits were major determinants of variability within the population. The length-to-breadth ratio also aligned closely with PC1, highlighting its role in differentiating kernel shapes. On the other hand, traits like kernel breadth and the shape of the upper surface of the kernel had substantial loadings on PC2, suggesting that these traits contributed to the secondary axis of variation. The biplot revealed that individuals 28 and 32 were outliers, characterized by notably high HSW and distinct endosperm colours, indicating potential candidates for selection based on these desirable traits.

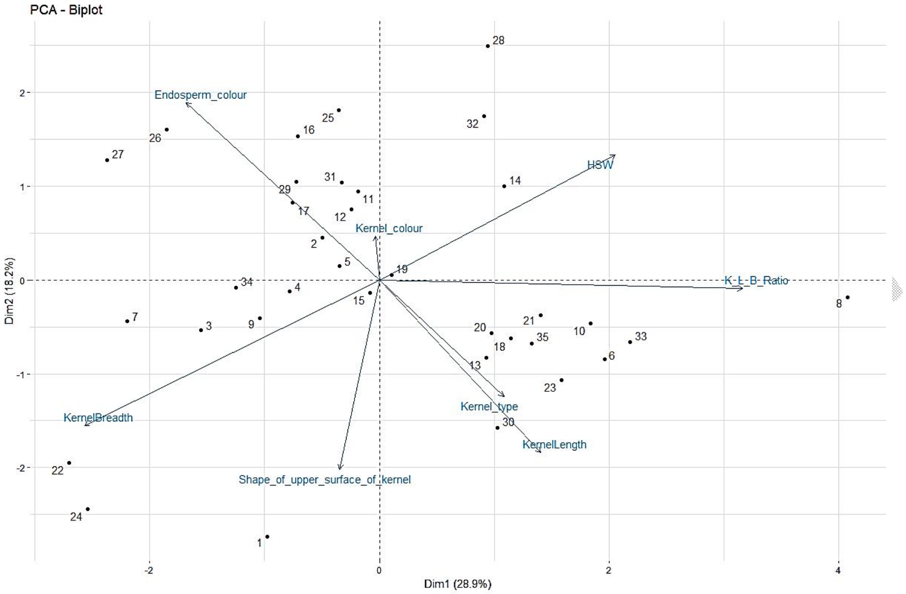


Figure 4. PCA Biplot for LM13BC$_1$F$_2$ Individuals

PCA of LM14BC$_1$F$_2$ Population

The PCA biplot for LM14 BC$_1$F$_2$ individuals shows the first two principal components explaining a combined 60.8% of the total variance (31.5% for PC1 and 29.3% for PC2). Key traits such as kernel length, kernel type, and endosperm colour strongly contribute to PC1, while kernel breadth and 100 seed weight (HSW) significantly influence PC2. Notably, individuals 14 and 17 are distinct, likely due to higher kernel length and endosperm colour variations. The separation of individuals along PC1 and PC2 underscores substantial genetic diversity within the population. These findings highlight the critical traits contributing to variability and suggest valuable targets for selective breeding, particularly focusing on kernel length and weight for improved yield and adaptability. The positioning of individuals along these principal components underscores the genetic diversity within the population, particularly in traits associated with kernel morphology and weight. The clear separation of certain individuals suggests opportunities for targeted breeding programs aimed at enhancing specific traits. Overall, the PCA highlights the critical traits contributing to genetic variability and offers a strategic direction for future breeding efforts to improve yield and adaptability in *Zea nicaraguensis* introgression lines **(Okporie, 2008)**.

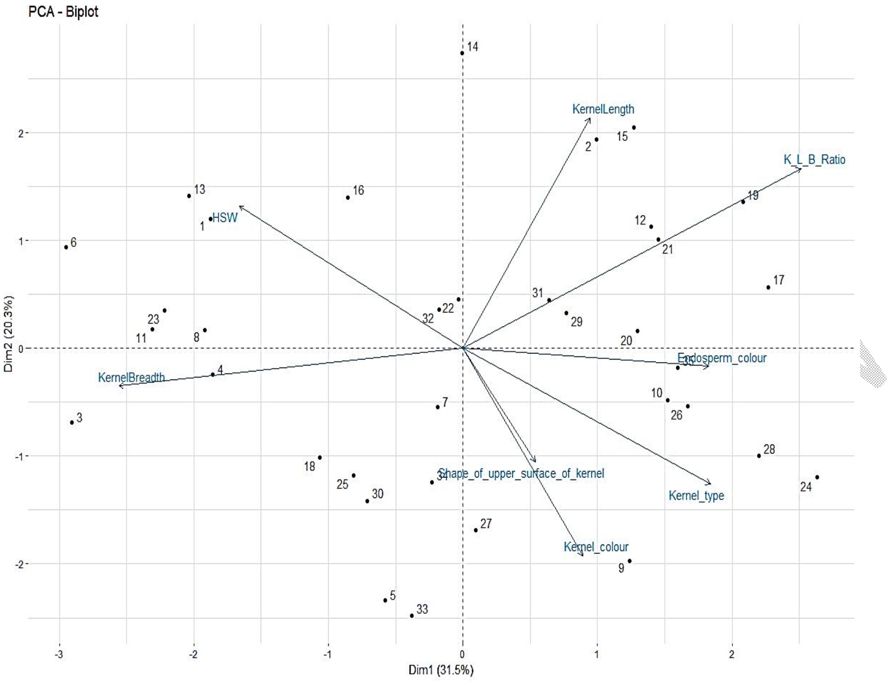


Figure 5. PCA Biplot for LM14BC$_1$F$_2$ Individuals

Conclusion and Future Prospects

This study comprehensively analysed kernel traits of BC$_2$F$_1$ maize lines created 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 are 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$_2$F$_1$ 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 marker-assisted and genomic selection methods. 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. 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 for posterity.

References

Anjorin, F. B., & Ogunniyan, D. J. (2014). Comparison of growth and yield components of five quality protein maize varieties. *International Journal of Agriculture and Forestry*, *4*(1), 1-5.

Araus, J. L., Slafer, G. A., Royo, C., & Serret, M. D. (2008). Breeding for yield potential and stress adaptation in cereals. *Critical Reviews in Plant Science*, *27*(6), 377-412.

Hassan, Z. M., Sebola, N. A., & Mabelebele, M. (2021). The nutritional use of millet grain for food and feed: a review. *Agriculture & food security*, *10*, 1-14.

Hossain, F., Muthusamy, V., Bhat, J. S., Zunjare, R. U., Kumar, S., Prakash, N. R., & Mehta, B. K. (2022). Maize breeding. In *Fundamentals of Field Crop Breeding* (pp. 221-258). Singapore: Springer Nature Singapore.

Jaradat, A. A., & Goldstein, W. (2013). Diversity of maize kernels from a breeding program for protein quality: I. Physical, biochemical, nutrient, and color traits. *Crop science*, *53*(3), 956-976.

Jilo, T., Tulu, L., Birhan, T., & Beksisa, L. (2018). Genetic variability, heritability and genetic advance of maize (*Zea mays* L.) inbred lines for yield and yield related traits in southwestern Ethiopia. *Journal of plant breeding and crop science*, *10*(10), 281-289.

Kajla, P., Chaudhary, V., Dewan, A., & Goyal, N. (2023). Colored cereals: Botanical aspects. In *Functionality and Application of Colored Cereals* (pp. 1-25). Academic Press.

Klopfenstein, T. J., Erickson, G. E., & Berger, L. L. (2013). Maize is a critically important source of food, feed, energy and forage in the USA. *Field Crops Research*, *153*, 5-11.

Liu, Z., Garcia, A., McMullen, M. D., & Flint-Garcia, S. A. (2016). Genetic analysis of kernel traits in maize-teosinte introgression populations. *G3: Genes, Genomes, Genetics*, *6*(8), 2523-2530.

Lorant, A. (2018). *Plasticity and genetic adaptation as contributors to the evolutionary history of cultivated maize and its wild relatives* (Doctoral dissertation, Université Paris Saclay (COmUE)).

Mano, Y., & Omori, F. (2013). Flooding tolerance in interspecific introgression lines containing chromosome segments from teosinte (*Zea nicaraguensis*) in maize (Zea mays subsp. mays). *Annals of Botany*, *112*(6), 1125-1139.

Murdia, L. K., Wadhwani, R., Wadhawan, N., Bajpai, P., & Shekhawat, S. (2016). Maize utilization in India: an overview. *American Journal of Food and Nutrition*, *4*(6), 169-176.

Nawaz, R., RIAZ, U., Gouhar, H., Mukhtar, M., Arshad, A., HUSSAIN, A., ... & AMJAD, I. (2023). Harnessing genetic diversity for sustainable maize production. *Journal of Physical, Biomedical and Biological Sciences*, *2023*(1), 15-15.

Okporie, E. O. (2008). Characterization of maize (*Zea mays* l.) germplasm with principal component analysis. *Agro-Science*, *7*(1), 66-71.

Prasanna, B. M. (2012). Diversity in global maize germplasm: characterization and utilization. *Journal of biosciences*, *37*(5), 843-855.

Sahoo, S., Adhikari, S., Joshi, A., & Singh, N. K. (2021). Use of wild progenitor teosinte in maize (*Zea mays* subsp. *mays*) improvement: present status and future prospects. *Tropical Plant Biology*, *14*(2), 156-179.

Shiferaw, B., Prasanna, B. M., Hellin, J., & Bänziger, M. (2011). Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food security*, *3*, 307-327.

Shrestha, J. (2013). Agro-morphological characterization of maize inbred lines. *Wudpecker Journal of Agricultural Research*, *2*(7), 209-211.

Smale, M., Bellon, M. R., & Aguirre Gomez, J. A. (2001). Maize diversity, variety attributes, and farmers' choices in Southeastern Guanajuato, Mexico. *Economic development and cultural change*, *50*(1), 201-225.

Varalakshmi, S., Sahoo, S., Singh, N. K., Pareek, N., Garkoti, P., Senthilkumar, V., ... & Nankar, A. N. (2023). Marker–Trait Association for Protein Content among Maize Wild Accessions and Coix Using SSR Markers. *Agronomy*, *13*(8), 2138.

Xu, Y., Skinner, D. J., Wu, H., Palacios-Rojas, N., Araus, J. L., Yan, J., ... & Crouch, J. H. (2009). Advances in maize genomics and their value for enhancing genetic gains from breeding. *International journal of plant genomics*, *2009*(1), 957602.

Yadav, O. P., Hossain, F., Karjagi, C. G., Kumar, B., Zaidi, P. H., Jat, S. L., ... & Dhillon, B. S. (2015). Genetic improvement of maize in India: retrospect and prospects. *Agricultural research*, *4*, 325-338.