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Morphological characterization of *Zea nicaraguensis*-derived introgression lines of maize for enhanced fodder traits

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Abstract
The study evaluated 166 BC_1F_3 maize lines derived from a biparental cross between maize inbred LM13 and *Zea nicaraguensis* to assess their potential for maize fodder improvement. The advanced backcross lines were grown under optimum field conditions, and six key traits i.e., plant height, tassel length, leaf length, leaf breadth, number of leaves per plant, and number of ears per plant were analyzed. Descriptive statistics revealed significant variability, with promising genotypes showing enhanced traits critical for fodder and biomass yield. Plant height exhibited substantial variation (100–270 cm), correlating positively with tassel length and the number of leaves, thus influencing vegetative biomass. Leaf traits such as length and breadth demonstrated strong positive correlations, emphasizing their combined importance in photosynthetic efficiency and fodder production. The introgression from *Zea nicaraguensis* contributed to genetic and phenotypic diversity, offering opportunities for targeted breeding strategies. Correlation analysis highlighted interconnected traits for optimizing yield while identifying independently regulated traits for selective improvement. Superior genotypes, including MN15, MN135, and MN136, were identified for their potential to enhance multiple traits simultaneously, paving the way for developing high-performing fodder maize varieties. These findings provide valuable insights into leveraging wild germplasm introgression to improve maize productivity.

Keywords: Wild relatives, fodder, maize, *Zea nicaraguensis*

Introduction
India, grappling with the world's highest livestock population faces chronic shortages in fodder resources. With the limitation of increasing the agricultural area, the focus on increasing the productivity of fodder crops is the greatest challenge (Gupta *et al.*, 2020) [6]. The gap between fodder supply and demand is widening, with only 4.4% of cultivated land under fodder crops. This creates challenges in sustaining India's large livestock population (Hilli *et al.*, 2023) [8]. Maize (*Zea mays* L.) is a globally important cereal crop cultivated for food, fodder, and industrial applications. However, its utility as a forage crop depends heavily on its vegetative traits, such as plant height, leaf size, and number of leaves (Dogalli *et al.*, 2024). Enhancing these traits can significantly improve maize's fodder potential.
The wild relatives of maize are traditionally cherished as cattle fodder in their native habitats. *Zea mays* ssp. *parviglumis*, is utilized as a fodder crop worldwide. It demonstrates strong tillering and ratooning abilities, contributing to its potential as a high-yield fodder crop. It has been explored for its ability to improve fodder maize breeding programs by incorporating its traits, such as multi-tillering and re-growth potential (Choudhary *et al.* 2019). The robust ratooning and adaptability of teosinte have been identified as advantageous traits for forage production, offering opportunities for multiple harvests within a single growing season. *Zea mays* ssp. *parviglumis* is valued for its contribution to biomass production, as it has been utilized in breeding programs to enhance the productivity of maize varieties through its prolific tillering and growth characteristics (Lakshmi *et al.*, 2019) [12]. A standout performer, DFH-2, an F_1 hybrid from maize inbred CAL 159 x teosinte cross, was evaluated in multi-location trials during the *kharif* season of the year 2019, 2021, and 2022. DFH-2's success also encouraged intensive efforts for development and evaluation of several backcross inbred

lines from maize x Nicaraguan teosinte crosses since 2019 at Pantnagar (Garkoti *et al.*, 2023) [5].

Other wild relatives of maize, such as *Zea nicaraguensis*, *Zea mexicana*, *Zea perennis*, and *Zea diploperennis*, are also valuable genetic resources for improving maize as a fodder crop (Singh and Dutta, 2021) [17]. These species possess desirable traits like robust vegetative growth, higher biomass production, and enhanced tolerance to abiotic stresses, such as waterlogging and drought. Their prolific leaf production and plant architecture make them suitable for fodder purposes. By introgression of these traits into cultivated maize through breeding, they can help improve key traits like leaf area, plant height, and leaf number, significantly enhancing fodder quality and yield. Their genetic diversity contributes to sustainable fodder production in diverse agro-ecological conditions.

The effectiveness of the breeding program would largely rely on the level of genetic variability available for important economic traits (Pragati *et al.*, 2024) [14]. Correlation analysis provides information on the strength and direction of relationships between variables, allowing researchers to assess how different traits are related (Kovačević *et al*., 2024) [11]. In the context of a breeding program, it helps identify which economic traits are positively or negatively correlated with each other. This information can guide selection decisions, as traits with strong positive correlations may be selected together to enhance desired characteristics, while traits with negative correlations might require balancing strategies to avoid unintended trade-offs. Understanding these relationships is crucial for optimizing genetic improvement and ensuring the success of the breeding program.

Zea nicaraguensis, a wild species endemic to Nicaragua, is well known for its ability to thrive in extreme environments, including waterlogging and reducing conditions (Watanabe *et al.*, 2017) [20]. It exhibits desirable vegetative traits, such as robust plant structure and prolific leaf production, which are critical for fodder quality. Introgression of these traits into maize through interspecific hybridization provides an opportunity to enhance maize's utility as a fodder crop. This study aims to evaluate the potential of *Zea nicaraguensis* for improving fodder-related traits in maize. Using 166 BC_1F_3 lines derived from a biparental cross between maize and *Zea nicaraguensis*, we assess key morphological traits important for fodder purposes. The findings are expected to contribute to breeding programs targeting enhanced fodder traits in maize.

Materials and Methods

Experimental Material

The study utilized 166 BC_1F_3 lines derived from a biparental cross between maize (*Zea mays* L.) and its wild relative *Zea nicaraguensis* (Accession no. E938022). The parent lines were selected based on their contrasting vegetative and fodder-related traits. To create nicaraguensis introgressed lines; LM13 (maize inbred) x *Zea nicaraguensis* cross was generated in *Kharif* 2020 using LM13 as seed parent and *Zea nicaraguensis*) as pollen parent and backcrossed with recurrent parent (LM13) in *Kharif* 2021. BC_1F_1s were obtained and eventually two generations of selfing were carried out in *Rabi* 2021-22 and *Kharif* 2022 to produce BC_1F_3 at Norman E. Borlaug Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (figure 1). These advanced backcross lines were grown and evaluated under field conditions to assess their fodder potential.

Experimental Design

The field experiment was conducted during the cropping season under controlled irrigation and fertilization conditions. The lines were sown in 3m rows with 60×20 cm planting distance at Norman E. Borlaug Crop research centre of G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand for recording observations on morphological traits.

Data Collection

Observations were recorded on six traits: plant height, tassel length, number of ears, leaf length, leaf width and number of leaves per plant, and leaf area. Plant height (cm) was measured from the base of the plant to the tip of the tassel. Tassel length (cm) was recorded from the base to the tip of the tassel. Leaf length (cm) was measured on the third fully expanded leaf from the base, and leaf width (cm) was measured at the widest point of the same leaf. The number of leaves per plant was counted from the base to the top.

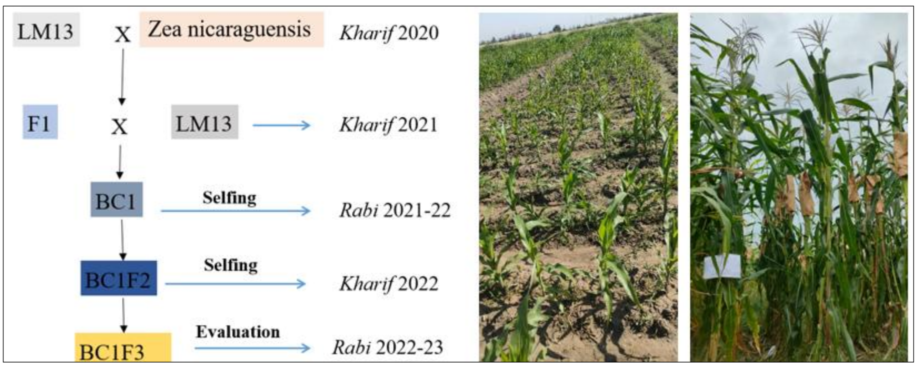

Fig 1: Development and evaluation of BC1F3 lines at N.E.B. Crop Research Centre, Pantnagar

Statistical Analysis

Descriptive statistics were performed using Microsoft Excel to calculate the mean, range, and categorize the number of genotypes into various groups, aiming to understand the distribution and variability within the dataset. Promising lines were identified based on these analyses to select candidates with desirable traits. Additionally, correlation analysis was conducted to examine relationships among vegetative traits, which is critical for identifying traits that could influence each other and guide selection strategies using R studio version 4.3.1.0.

Environmental Conditions

The present investigation was carried out at Govind Ballabh Pant University of Agriculture and Technology, Pantnagar U.S. Nagar, Uttarakhand. Pantnagar is geographically located at 29° N latitude, 79.30° E longitude and an altitude of 243.84 meters above mean sea level. It falls under humid sub-tropical zone at the foot hills of Shivalik range of the Himalayas in a narrow belt, locally known as Tarai. The average annual rainfall is about 1700 mm. The soil texture is miscellaneous type and it is generally 1.0 to 1.5 m deep. The characteristic features of the soil are high water table, shallow deep and calcareous nature.

Results and Discussion

Descriptive statistics

The descriptive statistics for the six traits studied has been given in table 1. Plant height (PH) exhibited a substantial mean of 178.78 cm, with a range of 170 cm (100–270 cm). This significant variability highlights the influence of *Zea nicaraguensis* introgression on inbred maize, suggesting its potential to enhance plant height and biomass. Such increase in plant height is particularly valuable for improving the overall forage yield, as taller plants generally produce more vegetative matter. The observed variability indicates the possibility of selecting superior lines with enhanced biomass potential. Adhikari *et al.* (2021) [1] and Varalakshmi *et al.* (2022) [19] also observed an increase in plant height across the introgression lines developed from *Zea mays* subsp. *parviglumis* as a donor. Research consistently highlights the positive correlation between plant height and forage yield across various crops. Studies on silage maize reveal that taller plants, under optimal growth conditions, produce higher forage yields due to increased vegetative matter. Similarly, findings on annual summer forage crops, including corn and Sudan grass, demonstrate that plant height is a reliable predictor of forage yield when specific growth densities are maintained (Ikeda and Takahashi, 1965). Furthermore, early plant height in silage maize has been shown to significantly influence final yields, linking rapid height growth to improved nutrient uptake and biomass accumulation (Pedersen *et al.*, 2021). Tassel length (TL) demonstrated notable variability within the population, averaging 31.94 cm with a range of 10–60 cm, reflecting genetic and phenotypic diversity. This trait indirectly contributes to silage production, as tassel biomass adds to the total forage yield. Research highlights the importance of tassel size and associated biomass in improving overall plant productivity and forage value. For example, studies have found that variations in tassel characteristics influence biomass accumulation and nutrient content, impacting the quality and yield of silage (Zhao *et al.*, 2015) [22] and (Jin-baob, 2007). Such variability provides opportunities for breeding programs aimed at optimizing tassel traits to enhance forage yields and silage quality. The introgression from *Zea nicaraguensis* may also influence tassel development, contributing to increased genetic diversity in the population.

Leaf length recorded a mean of 28.52 cm, with a range of 26 cm (14–40.5 cm), while leaf breadth showed a mean of 4 cm with a narrow range (2–6 cm). These two traits collectively contribute to leaf area (LA), a key determinant of photosynthetic capacity and biomass production. The variability observed in leaf length suggests the potential for improving photosynthetic efficiency, which directly impacts fodder yield and quality. Larger leaf area is particularly advantageous in forage crops, as it supports greater biomass accumulation. Singh *et al.* (2017) [18] developed BC1F3 lines derived from teosinte by crossing three inbred maize lines and evaluated them for various morphological traits. Notably, significant variation was observed in flag leaf length and width, demonstrating the potential of teosinte for diversifying and improving maize germplasm. Similarly, Adhikari *et al.* (2020) [1] documented modifications in flag leaf length and width within the BC1F5 population. The introgression of wild species genomes typically induces significant changes in gene expression, which may result from cis-regulation, trans-regulation, or a combination of both mechanisms in wild and cultivated species. These regulatory dynamics contribute to a wide range of phenotypic variation (Alonge *et al.*, 2020; Haas *et al.*, 2020) [2, 7].

The number of leaves per plant (NOL) showed a mean of 16.21, with a range of 13 (8–21). This indicates that *Zea nicaraguensis* introgression may influence leaf production, a critical trait for improving vegetative biomass. Similarly, the number of ears per plant (NOE) had a mean of 2.89, with a range of 4 (1–5 cobs). The variability in ear production suggests opportunities for selecting genotypes with higher cob biomass, which is essential for improving the nutritional quality of silage due to its contribution to starch content.

The plant height is divided into three categories: Low (100–155 cm) with 38 genotypes, Medium (156–199 cm) with 84 genotypes, and High (200–270 cm) with 44 genotypes. The tassel length is categorized into Low (10–25 cm) with 46 genotypes, Medium (26–35 cm) with 77 genotypes, and High (36–60 cm) with 43 genotypes. For leaf length, the categories are Low (14–22 cm) with 36 genotypes, Medium (23–30 cm) with 77 genotypes, and High (31–40 cm) with 63 genotypes. The number of leaves is grouped into Low (5–10) with 1 genotype, Medium (10–15) with 62 genotypes, and High (16–21) with 103 genotypes. The number of ears is divided into Low (1–2) with 57 genotypes, Medium (2.1–4) with 102 genotypes, and High (4.1–5) with 7 genotypes. Lastly, the leaf breadth is categorized into Low (2.0–3.0 cm) with 38 genotypes, Medium (3.1–4.1 cm) with 83 genotypes, and High (4.1–5.0 cm) with 45 genotypes.

Table 1: Descriptive statistics for the traits studied in BC1F3 lines

Parameter	Plant height (cm.)	Tassel length (cm.)	Leaf length (cm.)	Leaf Breadth (cm.)	No. of leaves	No. of ears
Mean	178.78	31.94	28.52	3.69	16.21	2.89
Median	175.00	30.00	29.00	3.75	16.00	3.00
Mode	170.00	30.00	24.00	4.00	17.00	3.00
Range	170.00	50.00	36.00	4.00	13.00	4.00
Minimum	100.00	10.00	14.00	2.00	8.00	1.00
Maximum	270.00	60.00	40.50	6.00	21.00	5.00

Table 2: Number of genotypes belonging to different categories

Trait	Category	Range	No. of genotypes
Plant Height	Low	100-155	38
	Medium	156-199	84
	High	200-270	44
Tassel Length	Low	10-25	46
	Medium	26-35	77
	High	36-60	43
Leaf Length	Low	14-22	36
	Medium	23-30	77
	High	31-40	63
No. of Leaves	Low	05-10	1
	Medium	10-15	62
	High	16-21	103
No. of Ears	Low	1-2	57
	Medium	2.1-4	102
	High	4.1-5	7
Leaf Breadth	Low	2.0-3.0	38
	Medium	3.1-4.1	83
	High	4.1-5.0	45

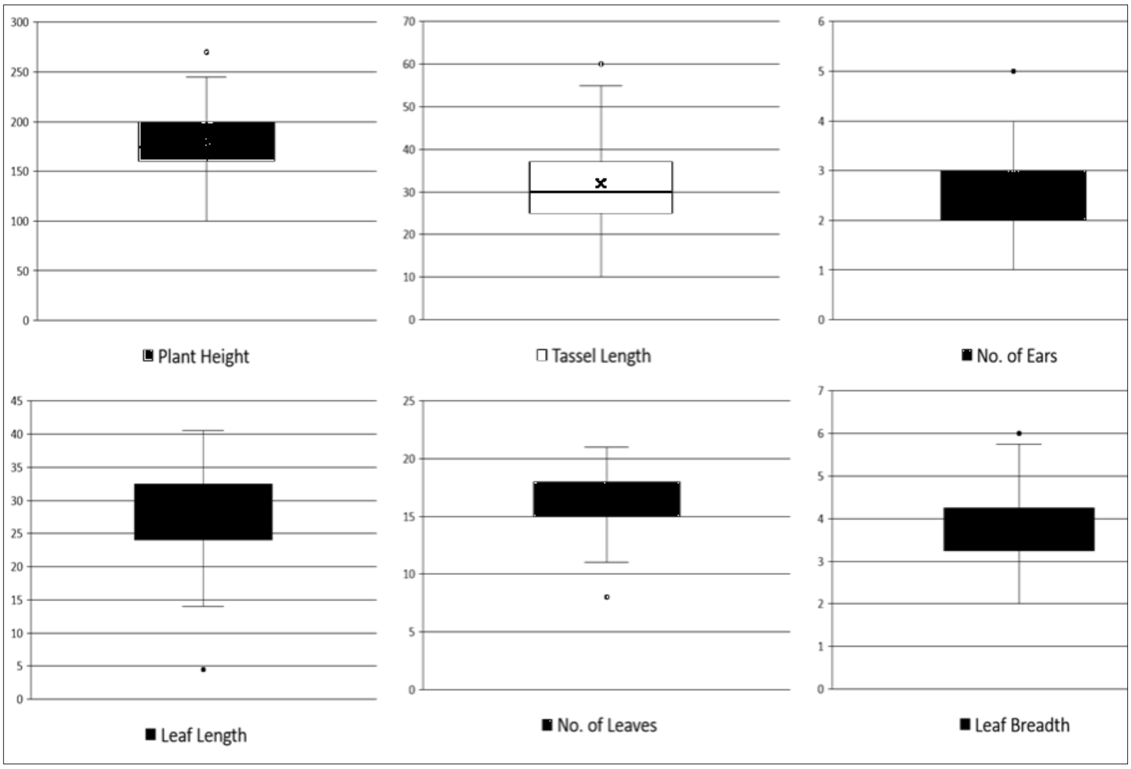

Fig 1: Boxplot of various characters observed in BC1F3 lines

Correlation studies

The correlogram (Fig 3) illustrates the pairwise correlations between six key traits: plant height (PH), tassel length (TL), number of cobs (NOC), leaf breadth (FLB), leaf length (FLL), and number of leaves. The relationships are visualized with color-coded intensity, where green indicates positive correlations, red denotes negative correlations, and the magnitude reflects the strength of the relationship. These correlations provide valuable insights into the interconnectedness of these traits and their potential impact on biomass improvement.

Plant height shows a positive correlation with tassel length, suggesting that taller plants are likely to have longer tassels, and with the number of leaves, implying that taller plants often produce more leaves, contributing to increased vegetative biomass. Similarly, leaf length and leaf breadth exhibit strong positive correlations, emphasizing their combined importance in determining leaf area, a critical factor for photosynthetic efficiency and biomass production. Negative correlations, depicted by blue circles, reveal potential trade-offs between certain traits. A weak negative correlation is observed between leaf breadth and the number of leaves. Traits such as tassel length and the number of ears exhibit limited correlations with other traits, as indicated by small or negligible circles. This suggests that these traits may be governed by independent genetic factors, allowing for selective breeding without significantly affecting other key morphological attributes. The correlogram underscores the importance of interconnected traits like plant height, leaf length, and leaf breadth in enhancing fodder yield while identifying opportunities to independently improve traits such as tassel length and cob production. These relationships provide a roadmap for targeted breeding strategies aimed at optimizing maize for fodder purposes.

Research supports the observation that plant height is positively correlated with tassel length and other yield-related traits. Studies have shown that plant height often correlates with tassel length and biomass, which are crucial factors for optimizing yield in maize. For example, research revealed that plant height had significant positive correlations with both tassel length and grain yield components, indicating that taller plants generally contribute to greater biomass and yield (Rai *et al.*, 2021)). Additionally, traits like leaf length and breadth showed strong positive correlations, emphasizing their combined importance in determining leaf area, a critical factor for photosynthetic efficiency and overall biomass production (Kanagarasu *et al.*, 2012). Negative correlations, such as a weak negative relationship between leaf breadth and the number of leaves, highlight potential trade-offs that may occur when optimizing specific traits (Ma *et al.*, 2014). Finally, the independence of traits like tassel length and the number of ears from other morphological traits, as observed, aligns with findings that such traits are often controlled by independent genetic factors, allowing selective breeding without significantly altering other key attributes (Rathod *et al.*, 2021) [16]. These insights collectively provide a robust foundation for targeted breeding strategies aimed at optimizing maize for fodder and grain production.

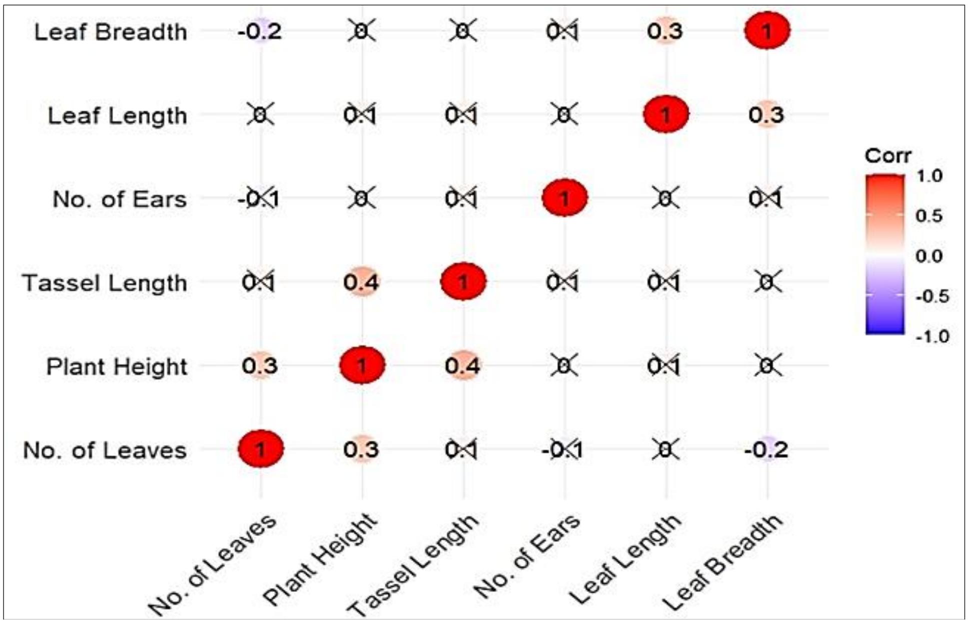

Fig 3: Correlogram showing interrelationships between various traits studied

Identification of lines having desirable characters

The identification of genotypes with desirable traits is a critical step in crop improvement programs. It allows breeders to select lines with superior performance in specific traits, enabling targeted enhancements in productivity, adaptability, and quality. In the present study, several genotypes were evaluated for their potential to contribute to key agronomic and fodder-related characteristics in maize, paving the way for the development of high-yielding and resilient varieties.

Table 3: Top ten genotypes for various traits studied

Trait	Superior genotypes
Plant height	MN159, MN49, MN15, MN93, MN114, MN135, MN136, MN157, MN165, MN72
Tasssel length	MN136, MN166, MN57, MN54, MN17, MN24, MN39, MN43, MN75, MN80
Leaf length	MN115, MN149, MN82, MN2, MN4, MN40, MN24, MN48, MN50, MN83
Leaf breadth	MN20, MN29, MN15, MN104, MN119, MN5, MN16, MN25, MN26, MN106
Number of leaves	MN91, MN124, MN157, MN161, MN48, MN123, MN125, MN126, MN135, MN153
Number of ears per plant	MN12, MN20, MN23, MN38, MN41, MN83, MN115, MN4, MN14, MN19

The analysis identified several promising genotypes with potential for improving multiple traits critical to maize productivity. MN15 was superior in both plant height and leaf breadth, making it a strong candidate for enhancing traits related to overall plant vigor and leaf area. Similarly, MN135 and MN157 excelled in plant height and the number of leaves per plant, highlighting their potential for increasing structural biomass. MN136 demonstrated its superiority in plant height and tassel length, emphasizing its utility in improving both traits simultaneously. For leaf length and the number of ears, MN115 and MN83 were identified as outstanding genotypes, while MN2 and MN40 were notable for their excellence in leaf length and leaf area, making them critical for enhancing traits associated with photosynthetic efficiency. Additionally, MN4 proved to be highly versatile, excelling in both leaf length and the number of cobs, making it an excellent resource for targeting multiple traits simultaneously. MN48 also demonstrated its significance by performing well in both leaf length and the number of leaves, contributing to improvements in these important traits. Among the genotypes associated with leaf breadth, MN29, MN5, and MN25 emerged as strong candidates. MN20 further distinguished itself by excelling in leaf breadth and number of ears, making it a valuable resource for improving vegetative and reproductive traits. Collectively, these genotypes provide essential opportunities for breeding programs to enhance multiple traits effectively, paving the way for the development of high-performing maize varieties.

Conclusion

This study highlights the potential of *Zea nicaraguensis* introgression in improving fodder-related traits in maize. By evaluating 166 BC1F3 lines derived from a biparental cross between maize and *Zea nicaraguensis*, significant variability was observed across critical morphological traits, including plant height, tassel length, leaf length, leaf breadth, number of leaves, and number of ears. These findings underscore the genetic diversity introduced through interspecific hybridization and its contribution to enhancing vegetative biomass and fodder quality. The positive correlations among traits such as plant height, leaf length, and leaf breadth demonstrate their combined role in improving biomass production, which is essential for fodder yield. The identification of genotypes with superior characteristics, such as high plant height, larger leaf area, and greater leaf production, provides a strong foundation for targeted breeding strategies. Furthermore, traits like tassel length and cob production, which showed limited correlations with other traits, present opportunities for independent trait-specific improvements.

The study reaffirms the value of wild relatives like *Zea nicaraguensis* as genetic resources for sustainable crop improvement. By leveraging the unique traits of wild germplasm, such as robust plant structure, prolific leaf production, and abiotic stress tolerance, breeding programs can address the challenges of fodder shortages and enhance the productivity of maize in diverse agro-ecological conditions. Future research should focus on advanced genomic studies to further unravel the genetic mechanisms underlying these traits and optimize the integration of wild species into cultivated maize germplasm. These efforts will contribute to the development of high-yielding, stress-tolerant maize varieties, ensuring sustainable fodder production to support India's large livestock population.

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Declaration of Competing Interest

The authors Declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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